Handbook of Plant Breeding

Johannes Novak
Wolf-Dieter Blüthner *Editors*

Medicinal, Aromatic and Stimulant Plants

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Handbook of Plant Breeding

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Medicinal, Aromatic and Stimulant Plants

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Preface

Medicinal and aromatic plants (MAPs) and stimulant plants include many thousand plant species with specific physiological effects of their plant secondary compounds on health, taste of food, and well-being. They can be found in all plant families worldwide, and their use is not only restricted to humans but also extended more and more to animal husbandry and plant protection.

The detection and use of these effects by humans date back to ancient times based on trial and error and are region-specific. However, during history, important plants and their application were exchanged between cultures and have been incorporated into world knowledge.

A major characteristic of MAPs is their richness in species. Whereas less than 5 food plants (sugarcane, rice, wheat, corn, potato) (Joy et al. 1998) save more than half of the world harvested yield, MAPs comprise between 52,000 species (out of 422,000 flowering plant species). In Germany, for example, 1,543 species are traded, but only 50–100 of these are exclusively sourced from cultivation. Extrapolating from the figure, only a few hundred species are under cultivation (Schippmann et al. 2002). With increasing pressure on natural populations, domestication is promoted, and the number of cultivated species will increase. Therefore, breeding different wild MAP species will increase in the short term.

In food plants, only a few chemical groups with a modest number of substances are used, namely, carbohydrates, fatty oils, and proteins, while in MAPs, several hundred thousand different products of the secondary metabolism are of value. However, with increasing popularity of polyphenols as antioxidants, breeding is trying to enrich food, vegetables, and fruits with beneficial compounds for healthier and tastier food. So, the borders between food and MAPs are blurred:

- Aromatic plants (herbs and spices) can still be distinguished from "flavor-improved" food by their property of not having nutritional value. They are in a strict sense not "food" but change the properties of food.
- Stimulant plants, like nicotine, coffee, cocoa, and tea, are also not food but estimated due to their stimulating effect, often bringing also complex flavors into our diet.
- Medicinal plants improve or maintain health but are sometimes used because of their flavor as food (e.g., herbal teas).

Medicinal plants are used (a) as traditional medicine; (b) as phytomedicines, registered according to pharmaceutical regulatory requirements; and (c) as food supplements (botanicals), products based on medicinal plants targeting primarily health maintenance, which are known from traditional medicine to be safe and effective (but regulated by food law). So, also here, the borders between medicinal and food use are blurred.

In legislation, however, we need to think in black and white resulting in pharmaceutical law and regulations that are dominantly a "negative law" (to say it bold and simple, "Everything is forbidden that is not allowed.") and food law and regulations, a "positive law" ("Everything is allowed that is not forbidden."), which can become quite challenging for breeding, if quality criteria – differently defined for medicine and food – need to be considered.

Only a few species, where seed sales are able to refinance breeding investments, are in intensive and continuous breeding programs of professional breeding companies. As a result, only a few cultivars are registered and protected, most selections are used in closed production systems, and maintenance breeding is restricted to the product life cycle. For most of the MAP species, breeding is project-based, so time-restricted and as part of a complex optimization of agricultural production. Here, product sales are refinancing optimization investments. For this group, academia, farmer associations and sometimes even raw material processors, traders, and product producers are practically breeding in a pragmatic way. This form of breeding is often performed with only simple breeding techniques like mass selection. This approach, however, can lead in many cases to a fast, significant improvement in just a few generations based on the wide natural variability in the starting materials collected from the wild.

In this book, a few important genera or species are covered in 17 specific chapters. Three general chapters reflect the particularities of MAP research and breeding. Two chapters summarize information on over 2,000 MAP species on available genetic resources, DNA particularities, and pollination biology. Two chapters can help entering more efficiently in more detailed research and breeding work. Another chapter focuses on peculiarities of chemical analysis of plant secondary compounds and some approaches to adapt analytics to breeding requirements.

Under the plethora of available literature on MAPs, breeding is severely neglected. We hope that this compilation contributes to a wider view on MAPs.

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Wien, Austria — Johannes Novak

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Chapter 1
Genetic Resources of Medicinal and Aromatic Plants

Ulrike Lohwasser and Stephan Weise

1.1 Medicinal and Aromatic Plants of the World

In the State of the World's Plants Report (Kew 2016; Willis 2017), 391,000 vascular plant species from 452 plant families are described worldwide. At least 28,187 plant species are currently recorded as being of medicinal use (Willis 2017). Information on medicinal and aromatic plants can be found in many different databases and scientific information systems (Bartol and Baricevic 2015). Around 5000 species are cited in regulatory medicinal publications but much more are in use based on traditional knowledge. A total of 12 plant families have a high proportion of medicinal plants (e.g., 22.5% of the Moraceae species are used for medicinal purpose (Willis 2017)). Other estimations by the World Health Organization (WHO) report 21,000 plant taxa for medicinal purpose (Groombridge 1992). Farnsworth and Soejarto (1991) speak about 70,000 species used for folk medicine. Another question is how strict the term medicinal and aromatic plants (MAPs) is defined. If we cover not only medicinally used plants sensu *strictu* but also the whole range including cosmetics, condiments, and food, we end up with more than 72,000 plant species (Table 1.1) (Schippmann et al. 2006). Plants with known medicinal uses have been a source of vital pharmaceutical drugs for the treatment of many diseases. For example, artemisinin (discovered in *Artemisia annua* L.) and quinine (from *Cinchona officinalis* L.) remain among the most important weapons against malaria. Since 1980, 15 drugs have been registered for the treatment of cancer. For example, paclitaxel has been isolated from the yew tree (*Taxus* spp.), camptothecin from the

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Table 1.1 MAPs used medicinally worldwide with some country examples

Country	Plant species	Medicinal plant species
Bulgaria	3567	750
China	32,200	4941
France	4630	900
Hungary	2,24	270
India	18,664	3000
Jordan	2100	363
Korea, Rep. of	2898	1000
Malaysia	15,500	1200
Nepal	6973	900
Pakistan	4950	1500
Philippines	8931	850
Sri Lanka	3314	550
Thailand	11,625	1800
USA	21,641	2564
Vietnam	10,500	1800
World	**422,000**	**72,000**

Schippmann et al. 2006, modified

happy tree (*Camptotheca acuminata* Decne.), and podophyllotoxin from the mayapple (*Podophyllum hexandrum* Royle and *P. peltatum* L.). Another study documents 656 flowering plant species used traditionally for diabetes, representing 437 genera and 111 families. For example, *Galega officinalis* L. (goat's rue) provided a useful compound for the design of the antidiabetic drug metformin, while another plant used traditionally for diabetes, *Stevia rebaudiana* (Bertoni) Bertoni (sweetleaf), is a source of sweetener compounds used in the food industry (Willis 2017).

For thousands of years, medicinal plants have been used in various cultures. They play an important role in human history, culture, and tradition. Cave paintings dating back to 13,000–25,000 BC depict the use of medicinal plants (Cooper and Deakin 2016). The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old (Petrovska 2012). Different regions of the world have different preferences in the use and consumption of medicinal plants also based on historical and traditional knowledge. Probably the oldest, richest, and most diverse cultural traditions in the use of medicinal plants are in India (Lange 2004). About 7500–8000 plant species are used in ethnomedicines (Chandra 2016; Shankar and Majumdar 1997) which is half of the country's 17,000 Indian native plant species. The well-known ancient Indian medicine system, which is called Ayurveda, is a set of guidelines to maintain balance and harmony and to ensure a long and happy life. Famous Ayurvedic medicinal plants include *Azadirachta indica* A. Juss (neem, nimtree), *Centella asiatica* (L.) Urb. (Asiatic pennywort, gotu kola), *Cinnamomum verum* J. Presl (cinnamon), *Elettaria cardamomum* (L.) Maton (cardamom), *Rauvolfia serpentina* (L.) Benth. ex Kurz (Indian snakeroot), *Santalum album* L. (sandalwood),

Terminalia chebula Retz. (black myrobalan) and other *Terminalia* species, and *Withania somnifera* (L.) Dunal (Indian ginseng) (van Wyk and Wink 2017). Also, the traditional Chinese medicine is believed to be more than 5000 years old (van Wyk and Wink 2017). The total number of plant species here is up to 6000 according to Xiao (1991) and more than 10,000 according to He and Sheng (1997). Examples of famous Chinese medicinal plants are *Angelica sinensis* (Oliv.) Diels (dang gui, Chinese angelica), *Artemisia annua* L. (qing hao, sweet wormwood), *Ephedra sinica* Stapf (ma huang, Chinese ephedra), *Paeonia lactiflora* Pall. (bai shao yao, Chinese peony), *Panax ginseng* C. A. Mey. (ren shen, Chinese ginseng), and *Rheum palmatum* L. (da huang, Chinese rhubarb) (van Wyk and Wink 2017). However, not only India and China have important MAPs, but also the other Asian countries have a long history in well-documented traditional knowledge and a long-standing practice of traditional medicine (ICS-UNIDO 2003; Handa et al. 2006; Yaniv and Dudai 2014). Especially from the Middle East, many important plants are known such as *Allium cepa* L. (onion), *Astracantha gummifera* (Labill.) Podlech (tragacanth), *Carthamus tinctorius* L. (safflower), *Ferula assa-foetida* L. (asafetida), *Juniperus phoenicea* L. (Phoenician juniper), *Lawsonia inermis* L. (henna), *Myrtus communis* L. (myrtle), *Papaver somniferum* L. (opium poppy), *Peganum harmala* L. (Syrian rue), *Pinus halepensis* Mill. (Aleppo pine), *Pistacia lentiscus* L. (mastic), *Prunus dulcis* (Mill.) D. A. Webb (almond), *Punica granatum* L. (pomegranate), *Rosa* x *damascena* Herrm. (Damask rose), *Ricinus communis* L. (castor), *Salvadora persica* L. (toothbrush tree), *Senna alexandrina* Mill. (senna), *Sesamum indicum* L. (sesame), *Trachyspermum ammi* (L.) Sprague (ajowan), *Trigonella foenum-graecum* L. (fenugreek), and *Vitis vinifera* L. (grape) (van Wyk and Wink 2017; Yaniv and Dudai 2014).

African traditional medicine is perhaps one of the most diverse medicine systems in the world but unfortunately poorly recorded. The biological and cultural diversity of Africa that constitutes the cradle of mankind shows many regional differences in healing practices (Gurib-Fakim 2006; van Wyk and Wink 2017). About 80% of the population relies on traditional medicine for healthcare needs. Within the rich African flora with a high rate of endemic species, around 3000 MAPs are recorded from Southern Africa and 7000 from Northern Africa. In Eastern Africa, many biological resources are used for obtaining pharmaceuticals. Western Africa is the home of large rainforests constituting many MAPs (Vasisht and Kumar 2004). Other references speak about more than 5000 African plant species that are in use for medicinal purpose (Iwu 1993; Lange 2004; Neffati et al. 2017). The most important and well-known African MAPs are listed in van Wyk (2017). Some very famous plants should be mentioned here such as *Agathosma betulina* (P. J. Bergius) Pillans (buchu), *Aloe ferox* Mill. (bitter aloe, Cape aloe), *Aloe vera* (L.) Burm. f. (true aloe, of North African origin), *Artemisia afra* Jacq. ex Willd. (African wormwood), *Aspalathus linearis* (Burm. F.) R. Dahlgren (rooibos tea), *Boswellia sacra* Flueck. (frankincense), *Catha edulis* (Vahl) Endl. (khat), *Commiphora myrrha* (Nees) Engl. (myrrh), *Harpagophytum procumbens* (Burch.) DC. ex Meisn. (devil's claw), *Hibiscus sabdariffa* L. (roselle), *Hypoxis hemerocallidea* Fisch., C. A. Mey. &

Avé-Lall. (African potato), *Prunus africana* (Hook. f.) Kalkman (African cherry), and *Senegalia senegal* (L.) Britton) (gum arabic) (van Wyk and Wink 2017).

The European healing system originated with Hippocrates (460–377 BC) and Aristotle (384–322 BC). Their ideas go back to ancient beliefs from India and Egypt (van Wyk and Wink 2017). In the ancient Western world, the Greeks contributed significantly to the rational development of the use of herbal drugs. Europe has a long tradition in the use of botanicals. A large number of traditional herbal remedies have become widely known as a result of commercialization, and a number of active compounds have been isolated from medicinal plants and are used today as single chemical entities (Gurib-Fakim 2006). About 2000 MAPs are used on a commercial basis of which 1200–1300 are native in Europe (Barata et al. 2016; Lange 2004). Examples can be listed as follows: *Arnica montana* L. (arnica), *Atropa belladonna* L. (deadly nightshare), *Drimia maritima* (L.) Stearn (squill), *Foeniculum vulgare* Mill. (fennel), *Matricaria chamomilla* L. (chamomile), *Silybum marianum* (L.) Gaertn. (milk thistle), *Urtica dioica* L. (nettle), and *Valeriana officinalis* L. (valerian) (van Wyk and Wink 2017).

For North and South America, 2564 medicinal plants are listed in the literature (Mamedov 2012; Moerman 2009). The region is very rich in the diversity of plant resources; the number of plants used for medicines in different ways by different people is probably endless. The American flora represents one of the world's wealthiest sources of material with pharmacological activity. Detailed information about the status of MAPs in the different countries can be found in Gupta et al. (2014). Especially in South but also in North America, the rich but diverse healing cultures based on indigenous healer or shaman approaches are poorly recorded but will be a source of many new herbal remedies. Rural people in many American countries still use traditional Indian herbal medicine (Gurib-Fakim 2006; van Wyk and Wink 2017). Some important MAPs for North America are *Digitalis purpurea* L. (foxglove), *Echinacea purpurea* (L.) Moench (purple coneflower), *Hydrastis canadensis* L. (goldenseal), *Lobelia inflata* L. (Indian tobacco), and *Taxus canadensis* Marshall (Pacific yew). For South America, famous examples are *Cinchona pubescens* Vahl (Peruvian bark), *Erythroxylum coca* Lam. (coca), *Handroanthus impetiginosus* (Mart. ex. DC.) Mattos (lapacho), *Ilex paraguariensis* A. St.-Hil. (maté), *Myroxylon balsamum* (L.) Harms (Tolu balsam), *Paullinia cupana* Kunth (guaraná), *Peumus boldus* Molina (boldo), *Psidium guajava* L. (guava), *Spilanthes acmella* (L.) L. (Brazilian cress), and *Uncaria tomentosa* (Willd. ex Schult.) DC. (cat's claw) (Gupta et al. 2014; van Wyk and Wink 2017).

As a result of geographic isolation, Australia is home to a large variety of unique and distinct flora not found elsewhere in the world. Herbal medicines have played an important role in the health, culture, and traditions of Australian Aboriginal people. Old literature speaks about 9000 flowering plant species used as medicinal plants (von Mueller 1889). Unfortunately, most Aboriginal knowledge of plant usage is not systematically reported (Gurib-Fakim 2006). Nevertheless, some information about the used plant species is available. Later on with the arrival of the European settlers, the plant usage is much better documented (Cock 2011; Lassak

and McCarthy 2011; Williams 2010). Among others, some very important MAPs from Australia are *Backhousia citriodora* F. Muell. (Australian lemon myrtle), *Duboisia hopwoodii* (F. Muell.) F. Muell. (pituri), *Eucalyptus globulus* Labill. (bluegum), and *Melaleuca alternifolia* (Maiden & Betche) Cheel (tea tree) (Cock 2011; van Wyk and Wink 2017).

Determining an exact number of MAPs worldwide is very difficult. However, the number of used species as medicinal and spice plants is quite impressive.

1.2 Conservation Strategies

Globally, an estimated 70,000 species are used for their medicinal, nutritional, and aromatic properties. Every year more than 500,000 tons of materials of such species are traded (Farnsworth and Soejarto 1991; WHO 2015). The increased global interest in the use of MAPs and the increasing demand on raw materials by various processing industries (pharmaceutical, food, cosmetic, perfume, etc.) have resulted in the increasing demand for MAPs. This places pressure on natural resources, since most species used are still collected in the wild (Barata et al. 2016). Overharvesting, habitat alteration, and climate change are among major drivers of declines in commercially important wild plant resources used for medicinal purposes (Canter et al. 2005; WHO 2015). There is no reliable estimate for the number of MAPs that are globally threatened (Hamilton 2004). An extrapolation based on the total number of threatened species leads to estimate that at least 15,000 MAP species are threatened at least to some degree (Chen et al. 2016; Schippmann et al. 2006). In the European Red List of Medicinal Plants, 400 taxa are described as endangered (Allen et al. 2014). Looking into the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN 2018), 1500 species (filtered for medicine, poison, manufacturing chemicals, and other chemicals) are mentioned as threatened or endangered, but the list includes only 25,452 plant species. The principal tool for monitoring or restricting trade of species threatened by overexploitation is the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES). In total, 30,345 plant species are protected by CITES (CITES 2018) from which, based on literature, only 17 species are MAPs (Schippmann et al. 2002; Hamilton 2004). Several declarations and sets of recommendations calling for the conservation and sustainable use of biodiversity including also medicinal plants exist. Among these, the Convention on Biological Diversity (CBD) has implemented three major goals since its adoption in 1992: (1) conservation of biological diversity, (2) sustainable use of its components, and (3) fair and equitable sharing of the benefits from the use of genetic resources. All of them are fully applicable to MAP resources (Máthé 2015; Schippmann et al. 2002). In April 2002, the CBD adopted the Global Strategy for Plant Conservation, which was updated in a strategy 2011–2020 (CBD 2018). Following the CBD goals and strategy, it is necessary to have a concept of sustainability. This means for MAPs that

conservation strategies are essential in order to avoid loss of natural resources. For example, the revised Guidelines on the Conservation of Medicinal Plants (WHO/IUCN/WWF/TRAFFIC forthcoming) and the WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants provide general recommendations (Kathe 2006; Leaman 2006; MPSG 2007). Beside a sustainable system for harvesting MAPs in the wild, various conservation strategies including both in situ and ex situ conservation are necessary. In situ conservation involves protection and establishment of plants in the location of their natural occurrence. In addition, identification of ecosystems is very essential (Okigbo et al. 2008). Ex situ conservation aims to cultivate MAP species to ensure their continued survival (Chen et al. 2016). MAP genetic resources conservation can be considered from both points of view: On the one hand, in situ conservation involves the establishment and/or maintenance of natural reserves where species are allowed to remain in optimal ecosystems. On the other hand, ex situ conservation involves the use of botanical gardens, field plantations, seed stores, and gene banks. In gene banks, seeds can be safely stored at low moisture contents (5–8% RH) and at low temperatures (−18 °C) (Ford-Lloyd et al. 2014). Plant in vitro technology and cryopreservation in liquid nitrogen (−196 °C) offer potential solutions for the long-term conservation of difficult to store germplasm categories (Carlen and Simonnet 2015). MAPs represent a consistent part of the natural biodiversity; effective conservation strategies should take place (Okigbo et al. 2008).

1.3 Genetic Resources of Medicinal and Aromatic Plants

Many countries have recognized the need for a complete national inventory of cultivated plant genetic resources, wild relatives, ecosystems, and the traditional knowledge associated with them. In situ conservation efforts worldwide have mostly focused on establishing protected areas and taken an ecosystem-oriented rather than a species-oriented approach. Many priority sites for conserving crop wild relatives in situ have been identified around the world. In some cases, new protected areas have been proposed for conserving a particular genus or even species (FAO 2010). Some examples of successful programs for in situ conservation of MAPs are described in Heywood and Dulloo (2005) and Labokas and Karpavičienė (2018). However, the focus of this paragraph will be on ex situ conservation because it is rather difficult to give exact figures about in situ conservation of MAPs.

Ex situ approaches to conserve and maintain MAPs involve the preservation of samples mainly in botanical gardens and gene banks (Shahidulla and Haque 2015). Some 1800 medicinal plant taxa are represented in botanical garden collections globally (FAO 2010). Detailed information about the holdings of MAPs in botanical gardens worldwide can be found in a plant conservation network, which is called

Botanic Gardens Conservation International (BGCI). BGCI provides a database of living plant, seed, and tissue collections. In the database, 1,384,852 collection records representing 548,973 taxa at 1100 contributing institutions are searchable including Red List and CITES status; 1786 medicinal plant species are listed here together with the number of botanical gardens in which the species are conserved (BGCI 2018).

From the 1920s, several countries initiated collection missions in order to accumulate and store genetic resources in ex situ gene banks (Börner 2006). Today, worldwide, more than 7.4 million accessions are conserved in ex situ collections. The largest groups are cereals (3.2 million) and food legumes (1.1 million). Looking into literature, only 160,050 accessions of medicinal, aromatic, spice, and stimulant crops are available in global germplasm holdings (FAO 2010).

Based on a list of 12,235 important vascular plant species, 3578 are defined as MAPs sensu *lato* (Wiersema and León 2013). The information about the potential usage of this plant species are obtained from the Germplasm Resources Information Network (GRIN 2018) and comprise the use as medicine, food additives, materials, or for social purposes, respectively. Out of the 3578 identified MAP species, 2507 could be found in ex situ collections with at least one accession. The accession passport data used are obtained from various information systems and give an overview on gene bank holdings from all over the world. Data on accessions maintained in European gene banks are retrieved from the European Search Catalogue for Plant Genetic Resources (EURISCO). This information system is being maintained by the European Cooperative Programme for Plant Genetic Resources (ECPGR) and provides information about almost two million accessions maintained at more than 370 institutions (Weise et al. 2017). A second important source is the information system Genesys, which is being funded by the Global Crop Diversity Trust. Genesys provides information about the US National Plant Germplasm System and about the CGIAR gene banks. Moreover, Genesys also provides data about different Embrapa collections from Brazil and some Australian gene banks. Further information was downloaded from different databases or provided by curators (Table 1.2). However, not from all countries English websites or contacts are available so that some countries with large gene bank collections are missing.

In total, 770,701 MAP accessions could be found in worldwide gene bank collections based on a total number of 4,302,721 available passport data over all continents. The largest holdings of MAPs are in Europe (373,555 accessions) and in North America (170,169) followed by Latin America (87,077) and Asia (86,548). The smallest collections are in Oceania (28,669) and in Africa (24,683) (Table 1.3). The species with the most accessions (133,037) all over the world is *Zea mays* L. which is not only known as cereal. Used parts for medicinal purpose are styles and stigmas, containing some essential oil (carvacrol and other terpenes) and having a diuretic and anti-inflammatory effect; the other used part of corn is pollen as extract for a urological purpose and raw pollen as appetite stimulant based on the presence of sterols (van Wyk and Wink 2017). Number

Table 1.2 Data sources of gene bank holdings

Region	Country	Institute	No of accs.	Data source
Africa	Ivory Coast	West African Rice Development Association	19,868	Genesys[a]
	Ivory Coast	Station de Recherche Marc Delorme, Centre National de Recherches Agronomiques	147	Genesys[a]
	Ethiopia	International Livestock Research Institute	18,640	Genesys[a]
	Kenya	Genetic Resources Unit, ICRAF	5391	Genesys[a]
	Kenya	Genetic Resources Research Institute	50,885	Genesys[a]
	Nigeria	International Institute of Tropical Agriculture	33,713	Genesys[a]
	Tunisia	Banque national de gènes de Tunisie	3166	Genesys[a]
	Zambia	SADC Plant Genetic Resources Centre	11,326	Genesys[a]
Asia	India	International Crop Research Institute for the Semi-Arid Tropics	126,830	Genesys[a]
	India	ICAR - National Bureau of Plant Genetic Resources	5593	Provided by NBPGR[b]
	Philippines	International Rice Research Institute	130,175	Genesys[a]
	Syria	International Centre for Agricultural Research in Dry Areas	155,414	Genesys[a]
	Taiwan	World Vegetable Center	59,954	Genesys[a]
	Republic of Korea	National Agrobiodiversity Center	172,352	Download from web information system[c]
	Japan	Genetic Resources Center, National Agriculture and Food Research Organization	101,160	Download from web information system[d]
Europe	various	372 institutes throughout Europe	1,983,324	EURISCO[e]
Latin America	Brazil	Embrapa	192,356	Genesys[a]
	Colombia	Centro Internacional de Agricultura Tropical	67,770	Genesys[a]
	Costa Rica	Centro Agronómico Tropical de Investigación y Enseñanza	1990	Genesys[a]
	Mexico	Centro Internacional de Mejoramiento de Maíz y Trigo	165,240	Genesys[a]
	Peru	Centro Internacional de la Papa	17,898	Genesys[a]
North America	Canada	Plant Gene Resources of Canada	23,948	Provided by PGRC[f]
	USA	US National Plant Germplasm System	639,764	Genesys[a]
Oceania	Australia	South Johnstone Research Station Queensland Department Primary Industries	282	Genesys[a]
	Australia	Australian Grains Genebank, Department of Economic Development Jobs Transport and Resources	138,016	Genesys[a]

(continued)

Table 1.2 (continued)

Region	Country	Institute	No of accs.	Data source
	Australia	Australian Pastures Genebank	83,838	Genesys[a]
	Australia	Australian PlantBank	10,609	Australian Seedbank Partnership[g]
	Australia	South Australian Seed Conservation Centre	1322	Australian Seedbank Partnership[g]
	Australia	Australian National Botanic Gardens Seedbank	11,788	Australian Seedbank Partnership[g]
	Australia	Brisbane Botanic Gardens Conservation Seedbank	918	Australian Seedbank Partnership[g]
	Australia	Tasmanian Seed Conservation Centre	1755	Australian Seedbank Partnership[g]
	Australia	Victorian Conservation Seedbank	1289	Australian Seedbank Partnership[g]
	Australia	Western Australia Seed Technology Centre	11,687	Australian Seedbank Partnership[g]
	Australia	Threatened Flora Seed Centre	4544	Australian Seedbank Partnership[g]
	Fiji Islands	Centre for Pacific Crops and Trees	2163	Genesys[a]
	New Zealand	Margot Forde Germplasm Centre	46,606	Download from web information system[h]

[a]Genesys, https://www.genesys-pgr.org, provided from Genesys at 2018-10-10
[b]ICAR – National Bureau of Plant Genetic Resources of India (NBPGR, http://www.nbpgr.ernet.in/) provided information about 5593 Indian MAP accessions at 2018-10-30
[c]Information about 172,352 accessions was downloaded from the National Agrobiodiversity Center, Republic of Korea (http://genebank.rda.go.kr/, 2018-09-24)
[d]Information about 101,160 accessions maintained in Japan was downloaded from the Genetic Resources Center, National Agriculture and Food Research Organization (http://www.gene.affrc.go.jp/databases-plant_search_en.php, 2018-09-25)
[e]EURISCO, European Search Portal for Plant Genetic Resources, http://eurisco.ecpgr.org, provided from EURISCO at 2018-11-07
[f]Plant Gene Resources of Canada (PGRC, http://pgrc3.agr.gc.ca, 2018-10-01) provided information about 23,948 Canadian MAP accessions
[g]Information about 43,912 additional Australian gene bank accessions was available from the Australian Seedbank Partnership (Atlas of Living Australia occurrence download at https://biocache.ala.org.au/occurrences/search?&q=*%3A*&qc=data_hub_uid%3Adh4 accessed on Fri Aug 03 21:23:40 AEST 2018)
[h]Information about 46,606 accessions maintained in New Zealand was downloaded from the Margot Forde Germplasm Centre (https://www.agresearch.co.nz/margot-forde-forage-germplasm-centre/, 2018-08-07)

Table 1.3 Overview of accession passport data available for comparison

Region	No of acc.	No of MAPs
Africa	143,136	24,683
Asia	751,478	86,548
Europe	1,983,324	373,555
Latin America	445,254	87,077
North America	663,712	170,169
Oceania	315,817	28,669
Total	**4,302,721**	**770,701**

two in the species list is *Avena sativa* L. (61,070) which is also well known as cereal. However, the grains of oat are used for dietary aid; the straw (added to a bath) has antipruritic effects for the relief of inflammatory and seborrheic skin disease because of containing high levels of soluble silica and minerals (iron, manganese, zinc) (van Wyk and Wink 2017). The species with the third highest numbers of accessions is *Vitis vinifera* L. (29,741). Grape vine is not only the base of a famous alcoholic drink, but also even red wine is considered to be healthy if taken in moderation. In addition, extracts from grape seeds have an antioxidative effect; the active ingredients in grape seed oil are non-hydrolyzable or condensed tannins (van Wyk and Wink 2017). Number four is *Malus domestica* Borkh. (28,165). Apples are not only popular fruits, but also they are used traditionally against diarrhea and dyspepsia and have antioxidant effects. Active ingredients are polyphenols, fruit acids (malic acid), pectin, sucrose, amines, vitamins, and mineral salts (van Wyk and Wink 2017). Position five in the top five ranking has *Linum usitatissimum* L. with 27,556 accessions. Flax has cultivars grown for stem fibers, others for seeds or seed oil. The active ingredients are mucilage in the outer cell layer of the seed coat. The main compounds in the seed are oil including linoleic acid and α-linolenic acid, proteins, and fibers having a bulk laxative effect. The mucilage is also beneficial in cases of gastritis and enteritis (van Wyk and Wink 2017). Meanwhile, 1071 MAP species are not listed in any gene bank collection; 433 out of 2507 species have just one accession available in ex situ collections, and 1408 species have a number lower than 10. Detailed information about the holdings can be found in Table 1.4. About 48 species of the 2507 have an entry in the different appendices of CITES, one in appendix I, 45 in appendix II, and two in appendix III. Appendices I (all parts or derivatives are always regulated and cannot be exempted), II, and III (exemptions of certain commodities and products are possible) to the convention are lists of species afforded different levels or types of protection from overexploitation (CITES 2018; Schippmann 2018). In the IUCN Red List, 475 MAP species of the 2507 have an entry, from "data deficient" to "extinct in the wild." Three species, all belonging to the genus *Brugmansia*, are listed as "extinct in the wild," four species (*Castanea dentata* (Marshall) Borkh., *Commiphora wightii* (Arn.)

Bhandari, *Fraxinus americana* L., *Fraxinus nigra* (Marshall)) have a status as "critically endangered," and 17 a status of "endangered" (IUCN 2018). Further details about CITES and/or Red List status can be found in Table 1.4.

1.4 Use of MAP Genetic Resources for Breeding Purpose

MAPs comprise a huge number of plant species. Compared with other groups of cultivated food plants, they utilize only a very small cultivation area. Plant breeding offers the opportunity to adapt these most diverse species to the specific demands of their users. Breeding for increased yield of valuable compounds, for elimination of unwanted compounds, for tolerance against abiotic and biotic stresses, and for better homogeneity of the cultivars is an important issue. Gene banks, botanical gardens, and other institutions collect and maintain a wide diversity of different accessions of the great variety of MAP species (Carlen 2012; Pank 2007). Especially gene banks play an important role for the long-term conservation of MAPs. Thereby, the focus is not only on the aspect of pure conservation. Screening of gene bank material can lead to new chemical compounds as shown in sage (*Salvia officinalis* L.) where a new viridiflorol chemotype could be described (Lamien-Meda et al. 2010). Often, the beginning of a breeding program starts with screening and selection of accessions coming from different gene banks. The large genetic diversity stored in gene banks is also used to provide new impulses to traditional breeding (e.g., by adding new alleles to existing breeding stocks) (Hoisington et al. 1999). An older literature survey over the last three decades of the last century shows more than 300 scientific publications about resistance research and breeding in MAPs (Gabler 2002). However, in many cases, it is not known which sources were used for breeding purposes. Examples for successful breeding including gene bank accessions are available from peppermint (*Mentha x piperita* L.) for yield, constituents and sensory quality, bitter fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *vulgare*) for essential oil content, St. John's wort (*Hypericum perforatum* L.) for high constituent content, and summer savory (*Satureja hortensis* L.) for essential oil content (Pank 2010). Nevertheless, gene bank accessions can have a high potential for breeding efforts.

With more than 770,000 MAP samples in ex situ collections worldwide, it seems that a good base for long-term conservation and breeding of medicinal and aromatic plant genetic resources is available. However, from more than 1000 species, no sample exists in any collection; and many species have a very low number of samples. More than one-third (36%) of all MAP accessions come from the top five which are, with partly exception of flax, not primarily medicinal plants. Some of the species are extinct in the wild or critically endangered. In conclusion, further strategies are urgently necessary to conserve and maintain the rich biodiversity of medicinal and aromatic plants.

Table 1.4 List of MAP species with gene bank holdings

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Abelmoschus esculentus (L.) Moench	Medicine	Folklore	72	29	1197	2484	198	5	3985		
Abelmoschus moschatus Medik.	Additive, material, medicine	Flavoring, essential oils, folklore	3		15	68		1	87		
Abies alba Mill.	Material	Essential oils	6			1			7		Least concern
Abies amabilis Douglas ex J. Forbes	Social	Religious/secular	10						10		Least concern
Abies balsamea (L.) Mill.	Material, medicine, social	Essential oils, folklore, religious/secular	8			2			10		Least concern
Abies concolor (Gordon & Glend.) Lindl. ex Hildebr.	Social	Religious/secular	10			2			12		Least concern
Abies fraseri (Pursh) Poir.	Medicine, social	Folklore, religious/secular	14			2			16		Endangered
Abies grandis (Douglas ex D. Don) Lindl.	Social	Religious/secular	7						7		Least concern
Abies nordmanniana (Steven) Spach	Social	Religious/secular	5			9			14		Least concern
Abies procera Rehder	Social	Religious/secular	3						3		Least concern
Abies religiosa (Kunth) Schltdl. and Cham.	Medicine	Folklore	1						1		Least concern

Abies sachalinensis (F. Schmidt) Mast.	Material	Essential oils	4			1			5		Least concern
Abies veitchii Lindl.	Social	Religious/secular	4						4		Least concern
Abroma augustum (L.) L. f.	Medicine	Folklore			13				13		
Abrus precatorius L.	Medicine	Folklore	20	6	129	4	21	9	189		
Abutilon indicum (L.) Sweet	Medicine	Folklore	5		59	1		7	72		
Acacia confusa Merr.	Material	Tannin/dyestuff				1			1		
Acacia cyclops A. Cunn. ex G. Don	Material	Tannin/dyestuff		4		2		36	42		
Acacia dealbata Link	Material	Essential oils, tannin/dyestuff	6	1		3		31	41		
Acacia decurrens Willd.	Material	Tannin/dyestuff	1	4		2		15	22		
Acacia elata A. Cunn. ex Benth.	Material	Tannin/dyestuff	1					3	4		
Acacia mearnsii De Wild.	Material	Tannin/dyestuff	1	7		1		7	16		
Acacia saligna (Labill.) H. L. Wendl.	Material	Tannin/dyestuff	2	4		4	1	40	51		
Acaciella angustissima (Mill.) Britton and Rose	Medicine	Folklore	15			17		9	41		
Acalypha fruticosa Forssk.	Medicine	Folklore		1					1		
Acalypha hispida Burm. f.	Medicine	Folklore				1			1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Acalypha indica L.	Medicine	Folklore	1						1		
Acanthosicyos horridus Welw. ex Benth. and Hook. f.	Medicine	Folklore	2						2		
Acanthospermum australe (Loefl.) Kuntze	Medicine	Folklore	2						2		
Acanthospermum hispidum DC.	Medicine	Folklore	2	3					5		
Acanthus mollis L.	Medicine	Folklore	1						1		
Acer rubrum L.	Medicine	Folklore	6			5			11		Least concern
Acer spicatum Lam.	Medicine	Folklore	1			8			9		
Achillea alpina L.	Medicine	Folklore	2			4			6		
Achillea erba-rotta All.	Additive	Flavoring	9						9		
Achillea fragrantissima (Forssk.) Sch. Bip.	Medicine	Folklore	16						16		
Achillea millefolium L.	Additive, medicine	Flavoring, folklore	394		7	73		15	489		Least concern
Achillea ptarmica L.	Medicine	Folklore	46			1			47		Least concern
Achyranthes aspera L.	Medicine	Folklore	17	6	35	2		5	65		
Achyranthes bidentata Blume	Medicine	Folklore	2			1			3		

Achyranthes fauriei H. Lev. and Vaniot	Medicine	Folklore	1		4				5		
Achyranthes japonica (Miq.) Nakai	Medicine	Folklore			4				4		
Achyrocline satureioides (Lam.) DC.	Medicine	Folklore					12		12		
Acmella oleracea (L.) R. K. Jansen	Medicine	Folklore	3						3		
Acokanthera schimperi (A. DC.) Benth. and Hook. f. ex Schweinf.	Medicine	Source of ouabain		3					3		
Aconitum carmichaelii Debeaux	Medicine	Folklore	1						1		
Aconitum columbianum Nutt.	Medicine	Folklore	1			6			7		
Aconitum coreanum (H. Lev.) Rapaics	Medicine	Folklore				2			2		Least concern
Aconitum falconeri Stapf	Medicine	Folklore			2				2		
Aconitum ferox Wall. ex Ser.	Medicine	Folklore			2				2		
Aconitum heterophyllum Wall. ex Royle	Medicine	Folklore	1		8				9		Endangered
Aconitum kusnezoffii Rchb.	Medicine	Folklore				1			1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Aconitum nagarum Stapf	Medicine	Folklore	1						1		
Aconitum napellus L.	Medicine	Folklore	27			1			28		Least concern
Aconogonon alpinum (All.) Schur	Material	Tannin/dyestuff	1			1			2		
Acorus calamus L.	Material, medicine	Essential oils, folklore	32			1			33		Least concern
Acorus gramineus Sol. ex Aiton	Medicine	Folklore	2			5			7		Least concern
Actaea asiatica H. Hara	Medicine	Folklore	1			3			4		
Actaea cimicifuga L.	Medicine	Folklore			1				1		
Actaea heracleifolia (Kom.) J. Compton	Medicine	Folklore				3			3		
Actaea pachypoda Elliott	Medicine	Folklore	1						1		
Actaea racemosa L.	Medicine	Folklore	1			53			54		
Actaea rubra (Aiton) Willd.	Medicine	Folklore	10			10			20		
Actaea spicata L.	Medicine	Folklore	20						20		
Actinidia arguta (Siebold & Zucc.) Planch. ex Miq.	Material	Potential as chemicals	25		2	221			248		

Actinidia deliciosa (A. Chev.) C. F. Liang and A. R. Ferguson	Medicine	Folklore	62		4	54			120		
Actinidia polygama (Siebold & Zucc.) Maxim.	Medicine	Folklore	2			18			20		
Adansonia digitata L.	Material, medicine	Tannin/dyestuff, folklore	8	182		2			192		
Adansonia za Baill.	Medicine	Folklore	4			1			5		Lower risk/ near threatened
Adenanthera pavonina L.	Medicine	Folklore	2	1	12	4	1		20		
Adenia venenata Forssk.	Medicine	Folklore		1		1			2		
Adenium obesum (Forssk.) Roem. and Schult.	Medicine	Folklore		1					1		
Adenophora liliifolia (L.) Besser	Medicine	Folklore	3						3		
Adenophora stricta Miq.	Medicine	Folklore	1						1		
Adenophora triphylla (Thunb.) A. DC.	Medicine	Folklore	4						4		
Adenostemma viscosum J. R. Forst. and G. Forst.	Medicine	Folklore	1						1		
Adiantum capillus-veneris L.	Medicine	Folklore				1			1		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Adiantum pedatum L.	Medicine	Folklore				1			1		
Adonis amurensis Regel & Radde	Medicine	Folklore				8			8		
Adonis vernalis L.	Medicine	Source of adoniside	69			1			70	II	
Aegle marmelos (L.) Correa	Material, medicine	Essential oils, folklore	1		13	5			19		
Aegopodium podagraria L.	Medicine	Folklore	44						44		
Aerva javanica (Burm. f.) Juss. ex Schult.	Medicine	Folklore	12	6	1			3	22		
Aerva lanata (L.) Juss. ex Schult.	Medicine	Folklore		5	3				8		
Aesculus glabra Willd.	Medicine	Folklore	1			4			5		Least concern
Aesculus hippocastanum L.	Medicine	Source of aescin	18						18		Vulnerable
Aethusa cynapium L.	Medicine	Folklore	12			1			13		
Afzelia xylocarpa (Kurz) Craib	Medicine	Folklore	1						1		Endangered
Agastache foeniculum (Pursh) Kuntze	Additive, medicine	Flavoring, folklore	22			15			37		
Agastache rugosa (Fisch. & C. A. Mey.) Kuntze	Material, medicine	Essential oils, folklore	19		10	16			45		

Agathosma betulina (P. J. Bergius) Pillans	Additive, medicine	Flavoring, folklore	1						1		
Agathosma crenulata (L.) Pillans	Additive, medicine	Flavoring, folklore	1						1		
Agave americana L.	Medicine	Folklore	3			1			4		
Agave angustifolia Haw.	Medicine	Folklore	1						1		
Agave sisalana Perrine	Medicine	Folklore	2				5		7		
Ageratum conyzoides L.	Material, medicine	Potential as essential oils, folklore	14	3			1		18		
Agrimonia eupatoria L.	Material, medicine	Tannin/dyestuff, essential oils, source of agrimophol	155			1		4	160		
Agrimonia gryposepala Wallr.	Medicine	Folklore	1			1			2		
Agrimonia pilosa Ledeb.	Medicine	Folklore	3		3	2			8		
Agrimonia procera Wallr.	Medicine	Folklore	9						9		
Agrostemma githago L.	Medicine	Folklore	306						306		
Ailanthus altissima (Mill.) Swingle	Medicine	Folklore	6	1		3			10		
Ajuga chamaepitys (L.) Schreb.	Medicine	Folklore	12						12		
Ajuga decumbens Thunb.	Medicine	Folklore, potential source of pharmaceutical agent	1						1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Ajuga reptans L.	Medicine	Folklore	21						21		
Akebia quinata (Thunb. ex Houtt.) Decne.	Medicine	Folklore	2			2			4		
Akebia trifoliata (Thunb.) Koidz.	Medicine	Folklore	1						1		
Alangium salviifolium (L. f.) Wangerin	Medicine	Folklore	1						1		
Albizia adianthifolia (Schumach.) W. Wight	Medicine	Folklore	2	1		1			4		Least concern
Albizia ferruginea (Guill. & Perr.) Benth.	Medicine	Folklore		2					2		Vulnerable
Albizia julibrissin Durazz.	Medicine	Folklore	8	3		6			17		
Albizia lebbeck (L.) Benth.	Medicine	Folklore	8	5		3		9	25		
Alcea rosea L.	Medicine	Folklore	60		7	15			82		
Alchemilla xanthochlora Rothm.	Medicine	Folklore	4					1	5		Least concern
Alchornea cordifolia (Schumach.) Mull. Arg.	Material, medicine	Tannin/dyestuff, folklore	1						1		
Alepidea amatymbica Eckl. and Zeyh.	Medicine	Folklore	1						1		

Aletris farinosa L.	Medicine	Folklore	1			4			5		
Aleurites moluccanus (L.) Willd.	Additive, medicine	Flavoring, folklore	1			2			3		Least concern
Alhagi graecorum Boiss.	Medicine	Folklore	10						10		
Alhagi maurorum Medik.	Medicine	Folklore	3			3		1	7		
Alisma plantago-aquatica L.	Medicine	Folklore	38			2		4	44		Least concern
Alkanna tinctoria Tausch	Material, medicine	Tannin/dyestuff, folklore	4						4		
Alliaria petiolata (M. Bieb.) Cavara and Grande	Additive, medicine	Flavoring, folklore	130			45			175		
Allium ampeloprasum L.	Additive	Flavoring	630		60	217			907		Least concern
Allium carolinianum Redoute	Additive	Flavoring	28			1			29		
Allium cepa L.	Additive, medicine	Flavoring, folklore	5378		1211	1363	757	2	8711		
Allium cernuum Roth	Additive	Flavoring	22			19		1	42		
Allium chinense G. Don	Medicine	Folklore	8		7				15		Least concern
Allium f istulosum L.	Additive, medicine	Flavoring, folklore	424		280	156		17	877		
Allium macrostemon Bunge	Medicine	Folklore	3			1			4		Least concern
Allium neapolitanum Cirillo	Additive	Flavoring	39			1			40		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Allium oleraceum L.	Additive	Flavoring	15						15		
Allium oschaninii O. Fedtsch.	Additive	Flavoring	14		1	6			21		Data deficient
Allium porrum L.	Additive	Flavoring	552		72	2		1	627		
Allium proliferum (Moench) Schrad. ex Willd.	Additive	Flavoring	4						4		
Allium roylei Stearn	Additive	Flavoring	5			1			6		Near threatened
Allium sativum L.	Additive, medicine	Flavoring, folklore	2611		209	575	90	16	3501		
Allium schoenoprasum L.	Additive, medicine	Flavoring, folklore	276		14	34		34	358		Least concern
Allium scorodoprasum L.	Additive	Flavoring	27		1	4			32		
Allium tricoccum Aiton	Additive	Flavoring	4			3			7		
Allium tuberosum Rottler ex Spreng.	Additive, medicine	Flavoring, folklore	97		27	14		23	161		
Allium ursinum L.	Medicine	Folklore	64			1		2	67		
Allophylus cobbe (L.) Raeusch.	Medicine	Folklore	1						1		
Alnus acuminata Kunth	Material	Tannin/dyestuff	3	3					6		Least concern
Alnus glutinosa (L.) Gaertn.	Medicine	Folklore	64			7			71		Least concern

Alnus incana (L.) Moench	Medicine	Folklore	11	1		19			31		Least concern
Alnus jorullensis Kunth	Medicine	Folklore	1						1		Least concern
Alnus rubra Bong.	Medicine	Folklore	2	1		8			11		Least concern
Alnus serrulata (Aiton) Willd.	Medicine	Folklore				5			5		Least concern
Alnus viridis (Chaix) DC.	Medicine	Folklore	1			10			11		
Aloe africana Mill.	Medicine	Folklore	2						2	II	
Aloe arborescens Mill.	Medicine	Source of aloesin, aloenin, aloesone	4						4	II	
Aloe ferox Mill.	Medicine	Folklore	2						2	II	
Aloe littoralis Baker	Medicine	Folklore	3						3	II	
Aloe spicata L. f.	Medicine	Folklore	1						1	II	
Aloysia citrodora Palau	Additive, material, medicine	Flavoring, essential oils, folklore	1						1		
Alphitonia zizyphoides (Spreng.) A. Gray	Medicine	Folklore	3						3		
Alpinia conchigera Griff.	Medicine	Folklore	1						1		
Alpinia galanga (L.) Willd.	Additive, medicine	Flavoring, folklore				1			1		
Alpinia nigra (Gaertn.) B. L. Burtt	Medicine	Folklore			1				1		
Alpinia zerumbet (Pers.) B. L. Burtt & R. M. Sm.	Medicine	Folklore	1			3			4		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Alstonia constricta F. Muell.	Medicine	Folklore	2					2	4		
Alstonia macrophylla Wall. ex G. Don	Medicine	Folklore	1			1			2		Lower risk/ least concern
Alstonia scholaris (L.) R. Br.	Medicine	Folklore	2			1			3		Lower risk/ least concern
Alternanthera pungens Kunth	Medicine	Folklore	1	1		1			3		
Alternanthera sessilis (L.) DC.	Medicine	Folklore	2				1		3		Least concern
Althaea officinalis L.	Medicine	Folklore	98			3		3	104		
Amaranthus albus L.	Medicine	Folklore	40		1	7		1	49		
Amaranthus caudatus L.	Medicine	Folklore	169	4	104	577	536	32	1422		
Amaranthus dubius Mart. ex Thell.	Medicine	Folklore	22	24	106	44		2	198		
Amaranthus hybridus L.	Medicine	Folklore	256	50	21	212		10	549		
Amaranthus hypochondriacus L.	Medicine	Folklore	208	6	336	1606	1446	65	3667		
Amaranthus spinosus L.	Medicine	Folklore	31	12	48	24		3	118		
Amaranthus tricolor L.	Medicine	Folklore	37	2	317	200	2	9	567		
Amaryllis belladonna L.	Medicine	Folklore	1						1		

Ambrosia artemisiifolia L.	Medicine	Folklore	5						5		
Amelanchier alnifolia (Nutt.) Nutt. ex M. Roem.	Medicine	Folklore	19	1		216			236		
Ammi majus L.	Medicine	Source of xanthotoxin	46			18		3	67		
Ammi visnaga (L.) Lam.	Medicine	Source of khellin	42			15			57		
Amomum subulatum Roxb.	Additive, medicine, social	Flavoring, folklore, masticatory			3				3		
Amorphophallus paeoniifolius (Dennst.) Nicolson	Medicine	Folklore	1			2			3		Least concern
Ampelopsis japonica (Thunb.) Makino	Medicine	Folklore				2			2		
Amphipterygium adstringens (Schltdl.) Schiede ex Standl.	Medicine	Folklore	3						3		
Anabasis aphylla L.	Medicine	Source of anabasine	1						1		
Anacardium occidentale L.	Medicine	Folklore		1	3	1	605		610		
Anacyclus officinarum Hayne	Medicine	Folklore	2						2		
Anacyclus pyrethrum (L.) Link	Medicine	Folklore	6		1				7		Vulnerable
Anadenanthera colubrina (Vell.) Brenan	Social	Hallucinogen	5			5			10		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Anadenanthera peregrina (L.) Speg.	Social	Hallucinogen	2						2		
Anagallis arvensis L.	Medicine	Folklore	34					3	37		
Ananas comosus (L.) Merr.	Medicine	Source of bromelain	20			154	845	7	1026		
Anaphalis contorta (D. Don) Hook. f.	Additive, material	Potential as flavoring, potential as essential oils	2						2		
Anaphalis margaritacea (L.) Benth. and Hook. f.	Medicine	Folklore	5			10			15		
Anchusa azurea Mill.	Material, medicine	Tannin/dyestuff, folklore	14						14		
Anchusa officinalis L.	Material, medicine	Tannin/dyestuff, folklore	13			4		1	18		
Andira inermis (W. Wright) Kunth ex DC.	Medicine	Folklore	2			1			3		Least concern
Andrographis echioides (L.) Nees	Medicine	Folklore			3				3		
Andrographis paniculata (Burm. f.) Wall. ex Nees	Medicine	Folklore			122				122		
Andropogon gayanus Kunth	Medicine	Folklore	6	105		1	91	43	246		

Anemone nemorosa L.	Medicine	Folklore	8			3			11		
Anemone patens L.	Medicine	Folklore	1			8			9		
Anemone pulsatilla L.	Medicine	Folklore	2			2			4		
Anemone rivularis Buch.-Ham. ex DC.	Medicine	Folklore	2						2		
Anemopsis californica (Nutt.) Hook. and Arn.	Medicine	Folklore	2			1			3		
Anethum graveolens L.	Additive, material, medicine	Flavoring, essential oils, folklore	1098		145	106		7	1356		
Angelica acutiloba (Siebold & Zucc.) Kitag.	Medicine	Folklore				1			1		
Angelica anomala Ave-Lall.	Medicine	Folklore				4			4		
Angelica archangelica L.	Additive, material, medicine	Flavoring, essential oils, folklore	124			14		5	143		
Angelica atropurpurea L.	Medicine	Folklore	2			3			5		Least concern
Angelica dahurica (Hoffm.) Benth. and Hook. f. ex Franch. & Sav.	Medicine	Folklore	2		1	5			8		
Angelica decursiva (Miq.) Franch. and Sav.	Medicine	Folklore	1		1	5			7		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Angelica gigas Nakai	Medicine	Folklore	3		1	5			9		
Angelica glauca Edgew.	Additive, medicine	Flavoring, folklore	1						1		Endangered
Angelica pubescens Maxim.	Medicine	Folklore				2			2		
Angelica sinensis (Oliv.) Diels	Medicine	Folklore				1			1		
Angelica sylvestris L.	Medicine	Folklore	28			12			40		Least concern
Angelica ursina (Rupr.) Maxim.	Medicine	Folklore	1						1		
Anisomeles indica (L.) Kuntze	Material, medicine	Essential oils, folklore	2		1				3		
Anisomeles malabarica (L.) R. Br. ex Sims	Medicine	Folklore	2					4	6		
Annickia polycarpa (DC.) Setten and Maas	Medicine	Folklore	1						1		
Annona cherimola Mill.	Medicine	Folklore	257			6			263		
Annona glabra L.	Medicine	Folklore	2			1			3		
Annona muricata L.	Medicine	Folklore	1			6	1		8		
Annona reticulata L.	Medicine	Folklore	2			16			18		

Annona squamosa L.	Medicine	Folklore	3			6			9		
Anoda cristata (L.) Schltdl.	Medicine	Folklore	4			1			5		
Anredera cordifolia (Ten.) Steenis	Medicine	Folklore					1		1		
Antennaria dioica (L.) Gaertn.	Medicine	Folklore	14						14		
Anthemis cotula L.	Medicine	Folklore	12		1				13		
Anthocleista grandiflora Gilg	Medicine	Folklore		1					1		
Anthoxanthum nitens (Weber) Y. Schouten and Veldkamp	Medicine, social	Folklore, religious/ secular				12			12		
Anthoxanthum odoratum L.	Material, medicine	Essential oils, folklore	114			9		230	353		
Anthriscus cerefolium (L.) Hoffm.	Additive, medicine	Flavoring, folklore	46		1			3	50		
Anthyllis vulneraria L.	Medicine	Folklore	300		3	49		96	448		
Antiaris toxicaria (Pers.) Lesch.	Medicine	Folklore	2						2		
Antidesma bunius (L.) Spreng.	Medicine	Folklore	2			4			6		
Apium graveolens L.	Additive, material, medicine	Flavoring, essential oils, folklore	838	1	32	527	26		1424		Least concern
Apocynum androsaemifolium L.	Medicine	Folklore	2			6			8		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Apocynum cannabinum L.	Medicine	Folklore	2			7			9		
Apocynum venetum L.	Medicine	Folklore	3						3		
Aquilegia vulgaris L.	Medicine	Folklore	91			2			93		
Aralia californica S. Watson	Medicine	Folklore	1			1			2		
Aralia continentalis Kitag.	Medicine	Folklore	4			3			7		
Aralia elata (Miq.) Seem.	Medicine	Folklore	8		1	3			12		
Aralia nudicaulis L.	Additive, medicine	Flavoring, folklore	2						2		
Aralia racemosa L.	Additive, medicine	Flavoring, folklore	4			1			5		
Aralia spinosa L.	Medicine	Folklore	3			1			4		
Araucaria bidwillii Hook.	Material	Essential oils				1		1	2		Least concern
Arbutus unedo L.	Medicine	Folklore	9			2			11		Least concern
Arctium lappa L.	Medicine	Folklore	83		35	2	1	2	123		
Arctium minus (Hill) Bernh.	Medicine	Folklore	20			1			21		
Arctium tomentosum Mill.	Medicine	Folklore	9			2			11		

Arctopus echinatus L.	Medicine	Folklore	3						3		
Arctostaphylos pungens Kunth	Medicine	Folklore	7			3			10		
Arctostaphylos uva-ursi (L.) Spreng.	Medicine	Folklore	52			10			62		
Ardisia japonica (Thunb.) Blume	Medicine	Source of bergenin				1			1		
Areca triandra Roxb. ex Buch.-Ham.	Social	Masticatory, stimulant	1						1		
Arenaria serpyllifolia L.	Medicine	Folklore	22					1	23		
Arenga pinnata (Wurmb) Merr.	Additive	Flavoring	1			3			4		
Argania spinosa (L.) Skeels	Medicine	Folklore	2	1					3		
Argemone mexicana L.	Medicine	Folklore	23	2	26		1		52		
Argyreia cuneata (Willd.) Ker Gawl.	Medicine	Folklore			1				1		
Argyreia nervosa (Burm. f.) Bojer	Medicine, social	Folklore, hallucinogen			9				9		
Arisaema amurense Maxim.	Medicine	Folklore				3			3		
Arisaema erubescens (Wall.) Schott	Medicine	Folklore	2						2		
Arisaema heterophyllum Blume	Medicine	Folklore	2			3			5		Least concern
Arisaema serratum (Thunb.) Schott	Medicine	Folklore	1			1			2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Arisaema triphyllum (L.) Schott	Medicine	Folklore	1						1		
Aristolochia acuminata Lam.	Medicine	Folklore			6				6		
Aristolochia bracteolata Lam.	Medicine	Folklore	1	2	5				8		
Aristolochia clematitis L.	Medicine	Folklore	5						5		
Aristolochia contorta Bunge	Medicine	Folklore				3			3		
Aristolochia indica L.	Medicine	Folklore			20			1	21		
Aristolochia manshuriensis Kom.	Medicine	Folklore				1			1		
Arivela viscosa (L.) Raf.	Additive, medicine	Flavoring, folklore			47	1			48		
Armoracia rusticana G. Gaertn. et al.	Additive, material, medicine	Flavoring, chemicals, folklore	170				1		171		Least concern
Armoracia sisymbroides (DC.) Cajander	Additive	Flavoring	1						1		
Arnebia euchroma (Royle) I. M. Johnst.	Material, medicine	Tannin/dyestuff, folklore	1						1		
Arnebia hispidissima (Sieber ex Lehm.) A. DC.	Material, medicine	Tannin/dyestuff, folklore	3		1				4		

Arnica angustifolia Vahl	Medicine	Folklore	3						3		
Arnica chamissonis Less.	Medicine	Folklore	5			1			6		
Arnica cordifolia Hook.	Medicine	Folklore	4			4			8		
Arnica fulgens Pursh	Medicine	Folklore				1			1		
Arnica latifolia Bong.	Medicine	Folklore	3			5			8		
Arnica montana L.	Medicine	Folklore	91			1		2	94		Least concern
Arnica sororia Greene	Medicine	Folklore	2			5			7		
Aronia melanocarpa (Michx.) Elliott	Medicine	Folklore	11		3	48			62		
Aronia prunifolia (Marshall) Rehder	Medicine	Folklore				24			24		
Artemisia abrotanum L.	Additive, medicine	Flavoring, folklore	14						14		
Artemisia absinthium L.	Additive, material, medicine	Flavoring, essential oils, folklore	87			10			97		
Artemisia afra Jacq. ex Willd.	Medicine	Folklore	5	2					7		
Artemisia annua L.	Medicine	Source of artemisinin	15		13				28		
Artemisia anomala S. Moore	Medicine	Folklore	1						1		
Artemisia balchanorum Krasch.	Material	Essential oils	2						2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Artemisia californica Less.	Medicine	Folklore				5			5		
Artemisia capillaris Thunb.	Medicine	Folklore	2		10				12		
Artemisia cina O. Berg	Medicine	Source of santonin	1						1		
Artemisia douglasiana Besser ex Hook.	Medicine	Folklore	2			2			4		
Artemisia dracunculus L.	Additive, material, medicine	Flavoring, essential oils, folklore	35		1	8			44		
Artemisia genipi Stechm.	Additive	Flavoring	3						3		Least concern
Artemisia gmelinii Weber ex Stechm.	Material	Potential as essential oils				1			1		
Artemisia ludoviciana Nutt.	Medicine	Folklore	2			19			21		
Artemisia maritima L.	Medicine	Folklore	5		1				6		
Artemisia montana (Nakai) Pamp.	Medicine	Folklore			1				1		
Artemisia pallens Wall. ex DC.	Material	Essential oils	1		1				2		
Artemisia pontica L.	Additive	Flavoring	3						3		
Artemisia princeps Pamp.	Additive	Flavoring						1	1		

Artemisia scoparia Waldst. and Kit.	Medicine	Folklore	6		1	1			8		
Artemisia sphaerocephala Krasch.	Medicine	Folklore	1						1		
Artemisia tridentata Nutt.	Medicine	Folklore	2	3		100			105		
Artemisia umbelliformis Lam.	Additive	Flavoring	10						10		Least concern
Artemisia vulgaris L.	Additive, medicine	Flavoring, folklore	79		6	4		5	94		
Artocarpus lacucha Buch.-Ham.	Medicine	Folklore				2			2		
Arum maculatum L.	Medicine	Folklore	41						41		
Arundo donax L.	Medicine	Folklore	5			10		1	16		Least concern
Asarum canadense L.	Material, medicine	Essential oils, folklore	1			1			2		
Asarum caudatum Lindl.	Medicine	Folklore	1						1		
Asarum europaeum L.	Medicine	Folklore	61						61		
Asarum sieboldii Miq.	Medicine	Folklore				2			2		
Asclepias asperula (Decne.) Woodson	Medicine	Folklore	1			3			4		
Asclepias curassavica L.	Medicine	Folklore	3		7	1			11		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Asclepias incarnata L.	Medicine	Folklore	8			7			15		Least concern
Asclepias linaria Cav.	Medicine	Folklore	5						5		
Asclepias syriaca L.	Medicine	Folklore	16			13		1	30		
Asclepias tuberosa L.	Medicine	Folklore	3			28			31		
Asimina triloba (L.) Dunal	Medicine	Folklore	34			58			92		
Asparagus adscendens Roxb.	Medicine	Folklore			2				2		
Asparagus cochinchinensis (Lour.) Merr.	Medicine	Folklore			1	3			4		Data deficient
Asparagus officinalis L.	Medicine	Folklore	202		13	96	1		312		Least concern
Asparagus racemosus Willd.	Medicine	Folklore	3	2	56	8			69		
Asplenium scolopendrium L.	Medicine	Folklore	1						1		
Aster tataricus L. f.	Medicine	Folklore				3			3		
Asteromyrtus symphyocarpa (F. Muell.) Craven	Material	Essential oils	2					7	9		
Astragalus cicer L.	Medicine	Folklore	97	2	56	187		94	436		

Astragalus glycyphyllos L.	Medicine	Folklore	159		2	34		59	254		
Astragalus gummifer Labill.	Medicine	Folklore	2			1			3		
Astragalus henryi Oliv.	Medicine	Folklore	1						1		
Astragalus mongholicus Bunge	Medicine	Folklore	4			5		6	15		Least concern
Astragalus sinicus L.	Medicine	Folklore	11		194	65		1	271		
Astronium urundeuva (Allemao) Engl.	Medicine	Potential source of pharmaceutical agent					246		246		Data deficient
Atalantia monophylla (L.) DC.	Medicine	Folklore				5			5		
Athyrium filix-femina (L.) Roth ex Mert.	Medicine	Folklore	2						2		
Atractylodes lancea (Thunb.) DC.	Medicine	Folklore	1						1		
Atropa acuminata Royle ex Lindl.	Medicine	Folklore	1						1		
Atropa belladonna L.	Medicine	Source of atropine	32		1				33		
Aucklandia costus Falc.	Additive, material, medicine	Flavoring, essential oils, folklore			15				15		
Aucuba japonica Thunb.	Medicine	Folklore	4			11			15		
Avena abyssinica Hochst. ex A. Rich.	Medicine	Folklore	114		5	495	64		678		Least concern
Avena fatua L.	Medicine	Folklore	417	9	41	1973	2	29	2471		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Avena sativa L.	Medicine	Folklore	25176	4310	7058	23653	421	452	61070		
Averrhoa carambola L.	Medicine	Folklore	15		1	84			100		
Azadirachta indica A. Juss.	Medicine	Folklore	1	1		3			5		Least concern
Azorella compacta Phil.	Medicine	Folklore	1						1		
Baccharis pteronioides DC.	Medicine	Folklore	5			1			6		
Baccharis salicifolia (Ruiz & Pav.) Pers.	Medicine	Folklore	5						5		
Baccharoides anthelmintica (L.) Moench	Medicine	Folklore			10	33			43		
Backhousia citriodora F. Muell.	Material	Essential oils	1					2	3		
Bacopa monnieri (L.) Pennell	Medicine	Folklore	3			1			4		Least concern
Bactris gasipaes Kunth	Medicine	Folklore	1			29	137		167		
Baeckea frutescens L.	Material	Essential oils	2					3	5		
Balanites aegyptiacus (L.) Delile	Additive, material, medicine	Flavoring, essential oils, tannin/dyestuff, potential source of pharmaceutical agent		14	39				53		

Balanites maughamii Sprague	Medicine	Folklore	2	1					3		
Ballota nigra L.	Medicine	Folklore	10						10		
Bambusa bambos (L.) Voss	Medicine	Folklore				2			2		
Bambusa textilis McClure	Medicine	Folklore				6			6		
Bambusa tuldoides Munro	Medicine	Folklore				3			3		
Bambusa vulgaris Schrad. ex J. C. Wendl.	Medicine	Folklore				2			2		
Baphia nitida hort. Lodd.	Material, medicine	Tannin/dyestuff, folklore		1					1		Least concern
Baptisia australis (L.) R. Br.	Material	Tannin/dyestuff	3			19		3	25		
Baptisia tinctoria (L.) R. Br.	Material, medicine	Tannin/dyestuff, folklore	3			4			7		
Barkleyanthus salicifolius (Kunth) H. Rob. and Brettell	Medicine	Folklore	11						11		
Barleria prionitis L.	Medicine	Folklore			4				4		
Basella alba L.	Medicine	Folklore	26	1	142	5	1	1	176		
Bassia scoparia (L.) A. J. Scott	Medicine	Folklore	18	1	7	2		1	29		
Bauhinia divaricata L.	Medicine	Folklore	2						2		
Bauhinia forficata Link	Medicine	Folklore				1			1		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Bauhinia racemosa Lam.	Medicine	Folklore	2						2		
Bauhinia variegata L.	Medicine	Folklore	2	1		7			10		Least concern
Begonia fimbristipula Hance	Medicine	Folklore	1						1		
Bellis perennis L.	Medicine	Folklore	148			3		2	153		
Benincasa hispida (Thunb.) Cogn.	Medicine	Folklore	33		326	35			394		
Berberis aquifolium Pursh	Medicine	Folklore				3			3		
Berberis aristata DC.	Medicine	Folklore	2		2				4		
Berberis asiatica Roxb. ex DC.	Medicine	Folklore	2						2		
Berberis chitria Ker Gawl.	Medicine	Folklore			2				2		
Berberis japonica (Thunb.) R. Br.	Material, medicine	Tannin/dyestuff, folklore	1						1		
Berberis nervosa Pursh	Medicine	Folklore	1			3			4		
Berberis repens Lindl.	Medicine	Folklore				5			5		
Berberis thunbergii DC.	Medicine	Folklore	11			1			12		

Berberis vulgaris L.	Additive, medicine	Flavoring, source of berberine	21			2			23		
Bergenia ciliata (Haw.) Sternb.	Medicine	Folklore			1				1		
Bergenia crassifolia (L.) Fritsch	Material, medicine	Tannin/dyestuff, folklore	5			4			9		
Bergera koenigii L.	Additive, material, medicine	Flavoring, essential oils, folklore				7			7		
Bertholletia excelsa Humb. & Bonpl.	Medicine	Folklore				1	18		19		Vulnerable
Betula lenta L.	Additive, medicine	Flavoring, folklore	3			8			11		Least concern
Betula papyrifera Marshall	Medicine	Folklore	5			11			16		Least concern
Betula pendula Roth	Medicine	Folklore	89		1	13			103		Least concern
Betula pubescens Ehrh.	Medicine	Folklore	69			3			72		Least concern
Betula pumila L.	Medicine	Folklore	1			4			5		Least concern
Betula utilis D. Don	Medicine	Folklore	5			5			10		Least concern
Bidens bipinnata L.	Medicine	Folklore	6		4				10		
Bidens biternata (Lour.) Merr. and Sherff	Medicine	Folklore		1					1		
Bidens frondosa L.	Medicine	Folklore	6			1			7		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Bidens pilosa L.	Medicine	Folklore	30	2	1	1	7		41		
Bidens tripartita L.	Medicine	Folklore	11		1				12		Least concern
Bistorta officinalis Delarbre	Medicine	Folklore	6						6		
Bistorta vivipara (L.) Delarbre	Medicine	Folklore	3			2			5		
Bixa orellana L.	Material, medicine	Tannin/dyestuff, folklore	3		39	1	30		73		
Blainvillea acmella (L.) Philipson	Medicine	Folklore	11	2					13		
Blepharis maderaspatensis (L.) B. Heyne ex Roth	Medicine	Folklore	5	1					6		
Bletilla striata (Thunb.) Rchb. f.	Medicine	Folklore	1						1	II	
Blighia sapida K. D. Koenig	Medicine	Folklore				1			1		
Blumea balsamifera (L.) DC.	Additive, medicine	Flavoring, folklore	1						1		
Blumea lanceolaria (Roxb.) Druce	Medicine	Folklore			1				1		
Bocconia arborea Watson	Material, medicine	Tannin/dyestuff, source of pharmaceutical agent	1						1		
Bocconia frutescens L.	Medicine	Folklore	5						5		

Boehmeria clidemioides Miq.	Medicine	Folklore	1						1		
Boehmeria tricuspis (Hance) Makino	Medicine	Folklore			2				2		
Boerhavia diffusa L.	Medicine	Folklore	2		19				21		
Boesenbergia rotunda (L.) Mansf.	Additive	Flavoring				1			1		Least concern
Bolboschoenus fluviatilis (Torr.) Sojak	Medicine	Folklore	2			1		1	4		
Bolboschoenus maritimus (L.) Palla	Medicine	Folklore	17	1		9			27		Least concern
Bombax ceiba L.	Medicine	Folklore	1			2		1	4		
Borago officinalis L.	Additive, medicine	Flavoring, folklore	127		1	5		3	136		
Borassus flabellifer L.	Medicine	Folklore				1			1		
Borojoa patinoi Cuatrec.	Medicine	Folklore				1			1		
Boswellia neglecta S. Moore	Material	Essential oils		2					2		
Bougainvillea glabra Choisy	Medicine	Folklore	3			2			5		
Bougainvillea spectabilis Willd.	Medicine	Folklore				2			2		
Bowiea volubilis Harv. ex Hook. f.	Medicine	Folklore	3						3		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Brachylaena huillensis O. Hoffm.	Material	Essential oils	1	1					2		Lower risk/ near threatened
Brassica carinata A. Braun	Additive, medicine	Flavoring, folklore	386	6	234	170		129	925		
Brassica juncea (L.) Czern.	Additive, medicine	Flavoring, folklore	2435	19	1540	1034	1	1366	6395		
Brassica napus L.	Medicine	Folklore	5373	96	2488	997	591	1453	10998		
Brassica nigra (L.) W. D. J. Koch	Additive, medicine	Flavoring, folklore, source of allyl isothiocyanate	370		90	124		232	816		
Brassica oleracea L.	Medicine	Folklore	11459	15	639	2928	60	459	15560		Data deficient
Brassica rapa L.	Medicine	Folklore	3983	25	4209	1815	52	1210	11294		
Breynia vitis-idaea (Burm. f.) C. E. C. Fisch.	Medicine	Folklore			2				2		
Brickellia veronicifolia (Kunth) A. Gray	Medicine	Folklore	5						5		
Bridelia micrantha (Hochst.) Baill.	Medicine	Folklore	2	1					3		Least concern
Bridelia stipularis (L.) Blume	Medicine	Folklore	1		7				8		
Brosimum alicastrum Sw.	Medicine	Folklore				4			4		
Broussonetia papyrifera (L.) Vent.	Medicine	Folklore	3			2			5		

Brucea javanica (L.) Merr.	Medicine	Folklore	1		1				2		
Brugmansia arborea (L.) Lagerh.	Medicine	Folklore	3						3		Extinct in the wild
Brugmansia aurea Lagerh.	Medicine, social	Folklore, hallucinogen	3						3		Extinct in the wild
Brugmansia candida Pers.	Medicine	Folklore				1			1		
Brugmansia sanguinea (Ruiz & Pav.) D. Don	Medicine	Source of hyoscine	1						1		Extinct in the wild
Brunfelsia uniflora (Pohl) D. Don	Medicine	Folklore	3						3		
Bryonia alba L.	Medicine	Folklore	15			6			21		
Bryonia cretica L.	Medicine	Folklore	29					1	30		
Bryonia dioica Jacq.	Medicine	Folklore	28						28		
Buddleja asiatica Lour.	Material	Essential oils	1						1		
Buddleja globosa Hope	Medicine	Folklore	2						2		
Buddleja lindleyana Fortune	Medicine	Folklore	2						2		
Buddleja officinalis Maxim.	Material, medicine	Essential oils, folklore	1						1		
Buddleja perfoliata Kunth	Medicine	Folklore	2						2		
Bulbostylis hispidula (Vahl) R. W. Haines	Medicine	Folklore	9	5					14		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Bunium bulbocastanum L.	Medicine	Folklore	8		3	1			12		Least concern
Bunium persicum (Boiss.) B. Fedtsch.	Additive, medicine	Flavoring, folklore				2			2		
Bupleurum exaltatum M. Bieb.	Medicine	Folklore	1						1		
Bupleurum falcatum L.	Medicine	Folklore	13						13		
Bupleurum longifolium L.	Medicine	Folklore	2			1			3		
Burkea africana Hook.	Medicine	Folklore	5			1			6		
Bursera fagaroides (Kunth) Engl.	Medicine	Folklore	3						3		
Bursera microphylla A. Gray	Medicine	Folklore	1						1		
Bursera simaruba (L.) Sarg.	Medicine	Folklore				1			1		
Butea monosperma (Lam.) Taub.	Medicine	Folklore	1			2			3		
Buxus sempervirens L.	Medicine	Folklore	44			63			107		Least concern
Byrsonima spicata (Cav.) DC.	Material	Tannin/dyestuff	1						1		
Byttneria herbacea Roxb.	Medicine	Folklore			1				1		

Caesalpinia bonduc (L.) Roxb.	Medicine	Folklore	9	1	38	2		2	52		
Caesalpinia coriaria (Jacq.) Willd.	Material	Tannin/dyestuff	2		2				4		
Caesalpinia crista L.	Medicine	Folklore			7				7		
Caesalpinia decapetala (Roth) Alston	Medicine	Folklore	3	2		1			6		
Caesalpinia digyna Rottler	Medicine	Folklore			3				3		
Caesalpinia echinata Lam.	Material, medicine	Tannin/dyestuff, folklore					5		5	II	Endangered
Caesalpinia pulcherrima (L.) Sw.	Medicine	Folklore	2	3	4	5			14		
Caesalpinia sappan L.	Medicine	Folklore			5				5		
Caesalpinia spinosa (Molina) Kuntze	Material	Tannin/dyestuff	4	1		2			7		
Calea ternifolia Kunth	Medicine	Folklore	2						2		
Calendula officinalis L.	Additive, medicine	Flavoring, folklore	254			39		8	301		
Calia secundiflora (Ortega) Yakovlev	Medicine	Folklore	1			13			14		
Calliandra grandiflora (L'Her.) Benth.	Medicine	Folklore				1			1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Callicarpa americana L.	Medicine	Folklore	1			11			12		
Callicarpa pedunculata R. Br.	Medicine	Folklore	2					4	6		
Calluna vulgaris (L.) Hull	Medicine	Folklore	36						36		
Caloncoba echinata (Oliv.) Gilg	Medicine	Folklore	1						1		
Calophyllum inophyllum L.	Medicine	Folklore			3	3			6		Lower risk/ least concern
Calotropis gigantea (L.) W. T. Aiton	Additive, medicine	Flavoring, folklore		1	7				8		
Calotropis procera (Aiton) W. T. Aiton	Medicine	Folklore	13	229	16			1	259		
Caltha palustris L.	Medicine	Folklore	47			2		1	50		Least concern
Calycanthus floridus L.	Additive	Flavoring	2			5			7		
Calystegia sepium (L.) R. Br.	Medicine	Folklore	21			1		3	25		Least concern
Camellia sinensis (L.) Kuntze	Additive, medicine, social	Flavoring, folklore, stimulant	1		42	21			64		Data deficient
Camphorosma monspeliaca L.	Medicine	Folklore	6			5			11		

Campsis grandiflora (Thunb.) K. Schum.	Medicine	Folklore				1			1		
Camptotheca acuminata Decne.	Medicine	Source of camptothecin	1						1		
Cananga odorata (Lam.) Hook. f. and Thomson	Additive, material, medicine	Flavoring, essential oils, folklore			1	1			2		
Canarium album (Lour.) Raeusch.	Medicine	Folklore				2			2		
Canarium indicum L.	Material, medicine	Essential oils, folklore				2			2		
Canarium ovatum Engl.	Material, medicine	Essential oils, folklore				5			5		Vulnerable
Canarium strictum Roxb.	Medicine	Folklore				1			1		
Canavalia ensiformis (L.) DC.	Medicine	Folklore	11	22	3	25	23	36	120		
Canella winterana (L.) Gaertn.	Additive, medicine	Flavoring, folklore	1						1		
Canna indica L.	Medicine	Folklore	8	1		2	51		62		
Cannabis sativa L.	Additive, medicine, social	Flavoring, folklore, psychoactive, source of tetrahydrocannabinol, stimulant	1477		54				1531		
Canscora alata (Roth) Wall.	Medicine	Folklore	1						1		
Canscora diffusa (Vahl) R. Br. ex Roem. and Schult.	Medicine	Folklore	1					1	2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Capparis decidua (Forssk.) Edgew.	Medicine	Folklore	3						3		
Capparis spinosa L.	Additive, medicine	Flavoring, folklore	17			4		6	27		
Capparis tomentosa Lam.	Medicine	Folklore	10	2					12		
Capraria biflora L.	Medicine	Folklore	1						1		
Capsella bursa-pastoris (L.) Medik.	Medicine	Folklore	94		1	4		17	116		
Capsicum annuum L.	Additive, medicine	Flavoring, folklore, source of capsaicin	10200	14	10306	3727	658	3	24908		Least concern
Capsicum baccatum L.	Additive, medicine	Flavoring, folklore	159		718	446	339		1662		
Capsicum cardenasii Heiser and P. G. Sm.	Additive	Flavoring	3			1			4		
Capsicum chacoense Hunz.	Additive	Flavoring	29		34	29			92		
Capsicum chinense Jacq.	Additive, medicine	Flavoring, folklore	207		875	569	973		2624		
Capsicum flexuosum Sendtn.	Additive	Flavoring	1			4	4		9		
Capsicum frutescens L.	Additive, medicine	Flavoring, folklore	351	8	1009	313	221	1	1903		Least concern
Capsicum pubescens Ruiz and Pav.	Additive	Flavoring	52		32	93	10		187		

Cardamine flexuosa With.	Medicine	Folklore	5						5		
Cardiospermum halicacabum L.	Medicine	Folklore	21		15	1		4	41		
Carex arenaria L.	Medicine	Folklore	28						28		
Careya arborea Roxb.	Medicine	Folklore	1						1		
Carica papaya L.	Material, medicine	Chemicals, source of chymopapin, papain	3		5	165	147		320		Data deficient
Carissa spinarum L.	Medicine	Folklore	7	2	1	1		1	12		
Carlina acaulis L.	Medicine	Folklore	24						24		
Carnegiea gigantea (Engelm.) Britton and Rose	Social	Religious/secular	2			5			7	II	Least concern
Carpesium abrotanoides L.	Medicine	Folklore	1		2				3		
Carpobrotus edulis (L.) N. E. Br.	Medicine	Folklore	1						1		
Carthamus tinctorius L.	Material, medicine	Tannin/dyestuff, folklore	984	3	303	2479	1863	843	6475		
Carum carvi L.	Additive, material, medicine	Flavoring, essential oils, folklore	443		84	32		12	571		
Caryota urens L.	Additive	Sweetener	1			1			2		Least concern
Casearia graveolens Dalzell	Medicine	Folklore			1				1		
Casearia sylvestris Sw.	Medicine	Folklore	3						3		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Casimiroa edulis La Llave and Lex.	Medicine	Folklore	5			4			9		
Cassia fistula L.	Medicine	Folklore	2		20	3			25		
Cassia grandis L. f.	Medicine	Folklore	1						1		
Cassia javanica L.	Medicine	Folklore	1	1	1	2		2	7		
Cassytha filiformis L.	Material, medicine	Tannin/dyestuff, folklore	4	2	4				10		
Castanea dentata (Marshall) Borkh.	Additive, medicine	Flavoring, folklore				5			5		Critically endangered
Castanea sativa Mill.	Medicine	Folklore	605		3	18			626		Least concern
Castanospermum australe A. Cunn. and C. Fraser ex Hook.	Medicine	Potential source of pharmaceutical agent				1			1		
Castilleja tenuiflora Benth.	Medicine	Folklore	5						5		
Catalpa bignonioides Walter	Medicine	Folklore	5			4		1	10		Data deficient
Catha edulis (Vahl) Forssk. ex Endl.	Medicine, social	Folklore, masticatory, stimulant	2						2		Lower risk/ least concern
Catharanthus roseus (L.) G. Don	Medicine	Source of vinblastine, vincristine	13		23	45			81		
Catunaregam spinosa (Thunb.) Tirveng.	Medicine	Folklore	5		6				11		
Caulophyllum robustum Maxim.	Medicine	Folklore				3			3		

Caulophyllum thalictroides (L.) Michx.	Medicine	Folklore				1			1		
Cayratia trifolia (L.) Domin	Medicine	Folklore	1						1		
Ceanothus americanus L.	Medicine	Folklore	4			5			9		
Ceanothus cuneatus (Hook.) Nutt.	Medicine	Folklore	1			10		1	12		
Ceanothus integerrimus Hook. and Arn.	Medicine	Folklore	1			8		2	11		
Ceanothus velutinus Douglas	Medicine	Folklore	1			8		1	10		
Cecropia obtusifolia Bertol.	Medicine	Folklore	2						2		Lower risk/ least concern
Cecropia peltata L.	Medicine	Folklore				1			1		
Cedrela odorata L.	Material, medicine	Essential oils, folklore	3			1			4	III	Vulnerable
Cedrus atlantica (Endl.) G. Manetti ex Carriere	Material	Essential oils	3						3		Endangered
Cedrus deodara (Roxb. ex D. Don) G. Don	Material, medicine	Essential oils, folklore	7					1	8		Least concern
Cedrus libani A. Rich.	Material, medicine	Essential oils, folklore	8			2		1	11		Vulnerable
Ceiba pentandra (L.) Gaertn.	Medicine	Folklore	3	1	7	1			12		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Celastrus paniculatus Willd.	Medicine	Folklore	2		31				33		
Celastrus scandens L.	Medicine	Folklore	3			8			11		
Celosia argentea L.	Medicine	Folklore	41	3	59	29		1	133		
Centaurea aspera L.	Medicine	Folklore	5						5		
Centaurea benedicta (L.) L.	Medicine	Folklore	13			3			16		
Centaurea cineraria L.	Medicine	Folklore	4						4		
Centaurea cyanus L.	Medicine	Folklore	41		1	11			53		
Centaurium erythraea Rafn	Additive, medicine	Flavoring, folklore	68					6	74		Least concern
Centella asiatica (L.) Urb.	Medicine	Source of asiaticoside	2		5				7		Least concern
Centranthus ruber (L.) DC.	Medicine	Folklore	10			1		3	14		
Centrapalus pauciflorus (Willd.) H. Rob.	Medicine	Folklore				65			65		
Ceratonia siliqua L.	Additive, medicine	Flavoring, sweetener, folklore	85	7	1	2		13	108		Least concern
Ceratostigma willmottianum Stapf	Medicine	Folklore	1			1			2		

Cercis chinensis Bunge	Medicine	Folklore	1			6			7		Least concern
Cestrum nocturnum L.	Medicine	Folklore	6						6		
Chaenomeles speciosa (Sweet) Nakai	Medicine	Folklore	7			17			24		
Chamaebatiaria millefolium (Torr.) Maxim.	Medicine	Folklore	1			3			4		
Chamaecrista absus (L.) H. S. Irwin and Barneby	Medicine	Folklore	11	15	2	1	1	6	36		Least concern
Chamaecyparis lawsoniana (A. Murray) Parl.	Material	Essential oils	90		2	2			94		Near threatened
Chamaecyparis obtusa (Siebold & Zucc.) Endl.	Material	Essential oils	23			3			26		Near threatened
Chamaelirium luteum (L.) A. Gray	Medicine	Folklore				1			1		
Chamaemelum nobile (L.) All.	Additive, material, medicine	Flavoring, essential oils, folklore	23			2			25		Least concern
Chamaenerion angustifolium (L.) Scop.	Medicine	Folklore				21			21		
Cheilocostus speciosus (J. Koenig) C. D. Specht	Medicine	Folklore			30	1			31		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Chelidonium majus L.	Medicine	Folklore	43		2	1			46		
Chelone glabra L.	Medicine	Folklore	1						1		Least concern
Chenopodiastrum murale (L.) S. Fuentes et al.	Medicine	Folklore				2		2	4		
Chenopodium album L.	Medicine	Folklore	87	10	1	33		9	140		
Chenopodium vulvaria L.	Medicine	Folklore	18	1		1			20		
Chimaphila maculata (L.) Pursh	Medicine	Folklore	1						1		
Chimaphila umbellata (L.) W. P. C. Barton	Additive, medicine	Flavoring, folklore	2			2			4		
Chimonanthus praecox (L.) Link	Material, medicine	Essential oils, folklore	3		3	3			9		
Chionanthus virginicus L.	Medicine	Folklore	2			13			15		
Chromolaena odorata (L.) R. M. King and H. Rob.	Medicine	Folklore	13		1				14		
Chrysanthemum boreale (Makino) Makino	Medicine	Folklore			1				1		
Chrysanthemum indicum L.	Medicine	Folklore	12		13	4			29		

Chrysanthemum morifolium Ramat.	Medicine	Folklore	41		144	5			190		
Chrysophyllum cainito L.	Medicine	Folklore				3			3		
Chrysopogon aciculatus (Retz.) Trin.	Medicine	Folklore					2		2		
Chrysopogon zizanioides (L.) Roberty	Material, medicine	Essential oils, folklore		1	9	11		1	22		
Cichorium intybus L.	Additive, medicine	Flavoring, folklore	783	1	60	174		405	1423		
Cicuta maculata L.	Medicine	Folklore	1			4			5		Least concern
Cicuta virosa L.	Medicine	Folklore	5			1			6		Least concern
Cinchona calisaya Wedd.	Additive, medicine	Flavoring, source of quinidine, quinine	1						1		
Cinchona officinalis L.	Medicine	Source of quinine	1						1		
Cinnamomum aromaticum Nees	Additive, material, medicine	Flavoring, essential oils, folklore				1			1		
Cinnamomum camphora (L.) J. Presl	Material, medicine	Essential oils, source of camphor			1	1			2		
Cinnamomum verum J. Presl	Additive, material, medicine	Flavoring, essential oils, folklore	1			1			2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Cinnamomum yabunikkei H. Ohba	Material	Essential oils				1			1		
Cirsium arvense (L.) Scop.	Medicine	Folklore	5					6	11		
Cirsium japonicum Fisch. ex DC.	Medicine	Folklore	1		1				2		
Cirsium mexicanum DC.	Medicine	Folklore	2						2		
Cissampelos pareira L.	Medicine	Source of cissampeline	1	2	4				7		
Cissus quadrangularis L.	Medicine	Folklore	5	1	3				9		
Cistanche salsa (C. A. Mey.) Beck	Medicine	Folklore	4						4		
Cistus creticus L.	Material	Essential oils	35						35		
Cistus ladanifer L.	Additive, material, medicine	Flavoring, essential oils, folklore	12						12		
Citrofortunella microcarpa (Bunge) Wijnands	Additive, medicine	Flavoring, folklore				8			8		
Citrullus colocynthis (L.) Schrad.	Medicine	Folklore	69	5	2	25	1		102		
Citrullus lanatus (Thunb.) Matsum. and Nakai	Medicine	Folklore	1649	87	1145	2193	1358	6	6438		

Citrus amblycarpa (Hassk.) Ochse	Additive	Flavoring	1		1	2			4		
Citrus aurantiifolia (Christm.) Swingle	Additive, material, medicine	Flavoring, essential oils, folklore				38			38		
Citrus aurantium L.	Additive, material, medicine	Flavoring, essential oils, folklore	32		8	98			138		
Citrus bergamia Risso and Poit.	Additive, material, medicine	Flavoring, essential oils, folklore	6			6			12		
Citrus deliciosa Ten.	Material	Essential oils	8		1	6			15		
Citrus hongheensis Y. M. Ye et al.	Additive, medicine	Flavoring, folklore				1			1		
Citrus hystrix DC.	Additive, material	Flavoring, essential oils	2		2	8			12		
Citrus inflata hort. ex Tanaka	Material	Potential as essential oils			1				1		
Citrus iyo hort. ex Tanaka	Material	Potential source of chemicals			2	7			9		
Citrus junos Siebold ex Tanaka	Additive	Flavoring	1		6	5			12		
Citrus latifolia (Yu. Tanaka) Tanaka	Additive	Flavoring	18			15			33		
Citrus limetta Risso	Material	Essential oils	2			12			14		
Citrus limettioides Tanaka	Material	Essential oils	2			25			27		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Citrus limon (L.) Burm. f.	Additive, material, medicine	Flavoring, essential oils, folklore	157		17	225			399		
Citrus limonia Osbeck	Additive, medicine	Flavoring, folklore	3		4	42			49		
Citrus maxima (Burm.) Merr.	Material	Essential oils	8			208			216		
Citrus medica L.	Additive, medicine, social	Flavoring, folklore, religious/secular	14		1	128			143		
Citrus myrtifolia Raf.	Additive	Flavoring	4		2	8			14		
Citrus natsudaidai Hayata	Additive	Flavoring			16	2			18		
Citrus paradisi Macfad.	Additive, material, medicine	Flavoring, essential oils, folklore	42		5	120			167		
Citrus pennivesiculata (Lush.) Tanaka	Additive	Flavoring				2			2		
Citrus reticulata Blanco	Additive, medicine	Flavoring, folklore	78		9	298			385		
Citrus sinensis (L.) Osbeck	Additive, material, medicine	Flavoring, essential oils, folklore	356		102	605			1063		
Citrus sudachi hort. ex Shirai	Additive	Flavoring	1		4	2			7		
Citrus tardiva hort. ex Shirai	Material	Potential as chemicals			1	2			3		

Citrus tumida hort. ex Tanaka	Material	Potential as chemicals			1				1		
Citrus ujukitsu Tanaka	Material	Potential as chemicals			2	2			4		
Citrus wilsonii Tanaka	Additive, medicine	Flavoring, folklore			1	2			3		
Clausena anisata (Willd.) Hook. f.	Material	Potential as essential oils	5	2		2			9		
Clausena lansium (Lour.) Skeels	Medicine	Folklore	2			5			7		
Clematis aethusifolia Turcz.	Medicine	Folklore				1			1		
Clematis apiifolia DC.	Medicine	Folklore	1		1				2		
Clematis armandii Franch.	Medicine	Folklore	1			2			3		
Clematis chinensis Osbeck	Medicine	Folklore	2						2		
Clematis drummondii Torr. and A. Gray	Medicine	Folklore	9			1			10		
Clematis florida Thunb.	Medicine	Folklore	1						1		
Clematis gouriana Roxb. ex DC.	Medicine	Folklore	2						2		
Clematis hexapetala Pall.	Medicine	Folklore	2			4			6		
Clematis peterae Hand.-Mazz.	Medicine	Folklore	1			3			4		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Clematis potaninii Maxim.	Medicine	Folklore				3			3		
Clematis recta L.	Medicine	Folklore	6						6		
Clematis tangutica (Maxim.) Korsh.	Medicine	Folklore	3			1			4		
Clematis virginiana L.	Medicine	Folklore				9			9		
Clematis vitalba L.	Medicine	Folklore	70			2			72		
Clerodendrum bungei Steud.	Medicine	Folklore	2						2		
Clerodendrum indicum (L.) Kuntze	Medicine	Folklore			1				1		
Clerodendrum infortunatum L.	Medicine	Folklore			8				8		
Clerodendrum trichotomum Thunb.	Medicine	Folklore	4		3	3			10		
Clinopodium nepeta (L.) Kuntze	Additive, medicine	Flavoring, folklore	6						6		
Clitoria ternatea L.	Medicine	Folklore	10	506	38	28	150	150	882		
Cnidium officinale Makino	Medicine	Folklore	1						1		
Coccinia grandis (L.) Voigt	Medicine	Folklore	2	7	111		1		121		
Coccoloba uvifera (L.) L.	Medicine	Folklore	2						2		
Cocculus hirsutus (L.) Diels	Medicine	Folklore	1	1	3				5		

Cocculus orbiculatus (L.) DC.	Medicine	Folklore	2						2		
Cochlearia officinalis L.	Medicine	Folklore	26						26		
Cochlospermum vitifolium (Willd.) Spreng.	Medicine	Folklore	5			4			9		
Cocos nucifera L.	Material, medicine	Essential oils, folklore		147		21	27		195		
Codiaeum variegatum (L.) A. Juss.	Medicine	Folklore	5			1			6		
Codonopsis lanceolata (Siebold & Zucc.) Trautv.	Additive, medicine	Flavoring, folklore	1		5	2			8		
Codonopsis pilosula (Franch.) Nannf.	Medicine	Folklore	2			4			6		
Coffea arabica L.	Additive, material, medicine, social	Flavoring, tannin/ dyestuff, folklore, stimulant	3		1	3	1826		1833		
Coffea canephora Pierre ex A. Froehner	Additive, medicine, social	Flavoring, folklore, stimulant					73		73		Least concern
Coffea congensis A. Froehner	Social	Stimulant					4		4		Least concern
Coffea liberica W. Bull ex Hiern	Social	Stimulant					24		24		Least concern
Coffea stenophylla G. Don	Social	Stimulant					1		1		Vulnerable

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Coix lacryma-jobi L.	Medicine	Folklore	46	3	435	18	6	4	512		
Cola acuminata (P. Beauv.) Schott and Endl.	Additive, medicine, social	Flavoring, folklore, masticatory, religious/ secular, stimulant				1			1		
Colchicum autumnale L.	Medicine	Source of colchicine, colchicine amide, demecolcine	93						93		Least concern
Collinsonia canadensis L.	Medicine	Folklore	1						1		
Colocasia esculenta (L.) Schott	Medicine	Folklore	3		29			1165	1197		Least concern
Colophospermum mopane (Kirk ex Benth.) Kirk ex J. Leonard	Medicine	Folklore	6	3		3			12		
Combretum apiculatum Sond.	Medicine	Folklore	3	3					6		
Combretum indicum (L.) DeFilipps	Medicine	Source of quisqualic acid				1			1		
Combretum micranthum G. Don	Medicine	Folklore	6	3					9		
Combretum roxburghii Spreng.	Medicine	Folklore			1				1		
Commelina benghalensis L.	Medicine	Folklore	3						3		Least concern
Commelina coelestis Willd.	Medicine	Folklore	2						2		

Commiphora africana (A. Rich.) Engl.	Medicine	Folklore		6					6		
Commiphora caudata (Wight & Arn.) Engl.	Medicine	Folklore			2				2		
Commiphora habessinica (O. Berg) Engl.	Additive, material, medicine	Flavoring, essential oils, folklore		1					1		
Commiphora myrrha (Nees) Engl.	Additive, material, medicine	Flavoring, essential oils, folklore		1					1		
Commiphora schimperi (O. Berg) Engl.	Medicine	Folklore		1					1		
Commiphora wightii (Arn.) Bhandari	Medicine	Source of guggulsterone			3				3		Critically endangered
Comptonia peregrina (L.) J. M. Coult.	Medicine	Folklore				1			1		
Conium maculatum L.	Medicine	Folklore	66		1	3			70		
Conocarpus erectus L.	Material	Tannin/dyestuff	4						4		Least concern
Conopodium majus (Gouan) Loret and Barrandon	Additive	Flavoring	8						8		
Consolida ajacis (L.) Schur	Medicine	Folklore	11		2	4			17		
Consolida orientalis (J. Gay) Schrodinger	Medicine	Folklore	5			2			7		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Consolida regalis Gray	Medicine	Folklore	47			3			50		
Convallaria majalis L.	Material, medicine	Essential oils, source of convallatoxin	159			6			165		
Convolvulus arvensis L.	Medicine	Folklore	19		1	1			21		
Convolvulus floridus L. f.	Material	Essential oils	2						2		Least concern
Convolvulus prostratus Forssk.	Medicine	Folklore			4				4		
Convolvulus scammonia L.	Medicine	Folklore	1						1		
Convolvulus scoparius L. f.	Material	Essential oils	1						1		
Conzattia multiflora (B. L. Rob.) Standl.	Medicine	Folklore	7						7		
Copaifera langsdorffii Desf.	Medicine	Folklore					2		2		Least concern
Copernicia prunifera (Mill.) H. E. Moore	Medicine	Folklore				1			1		
Coptis trifolia (L.) Salisb.	Medicine	Folklore	1						1		
Corallocarpus epigaeus (Rottler) C. B. Clarke	Medicine	Folklore	2						2		
Corchorus capsularis L.	Additive	Flavoring	38	1	132	10	19	2	202		

Corchorus olitorius L.	Medicine	Folklore	153	86	143	48	3	4	437		
Cordia curassavica (Jacq.) Roem. and Schult.	Medicine	Folklore	1				1		2		
Cordia myxa L.	Medicine	Folklore	5						5		
Cordia sebestena L.	Medicine	Folklore	2			1			3		Least concern
Cordyline australis (G. Forst.) Endl.	Additive	Sweetener	4						4		
Coriandrum sativum L.	Additive, material, medicine	Flavoring, essential oils, folklore	1158	1	148	286		44	1637		
Coriaria ruscifolia L.	Medicine	Folklore	3						3		
Coridothymus capitatus (L.) Rchb. f.	Additive, material	Flavoring, essential oils	26						26		
Cornus canadensis L.	Medicine	Folklore	1			5			6		
Cornus capitata Wall.	Material, Medicine	Tannin/dyestuff, folklore	3			5			8		
Cornus florida L.	Medicine	Folklore	3		1	48			52		
Cornus oblonga Wall.	Medicine	Folklore	1						1		
Cornus officinalis Siebold and Zucc.	Medicine	Folklore	2		2	8			12		
Corydalis cava (L.) Schweigg. and Korte	Medicine	Folklore	22						22		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Corydalis solida (L.) Clairv.	Medicine	Folklore	3						3		
Corylus avellana L.	Material, medicine	Essential oils, folklore	808			552			1360		Least concern
Corylus cornuta Marshall	Medicine	Folklore	2			19			21		Least concern
Corymbia citriodora (Hook.) K. D. Hill and L. A. S. Johnson	Material	Essential oils	4			1	4	11	20		
Cosmos bipinnatus Cav.	Material, medicine	Essential oils, folklore	25		2	2			29		
Cosmos sulphureus Cav.	Material	Essential oils, tannin/dyestuff	18	1	2	3			24		
Costus afer Ker Gawl.	Additive, medicine	Flavoring, folklore	1						1		
Costus spicatus (Jacq.) Sw.	Medicine	Folklore				1			1		
Cota tinctoria (L.) J. Gay	Material	Tannin/dyestuff			1	1			2		
Cotinus coggygria Scop.	Material	Tannin/dyestuff	14			9			23		Least concern
Crambe hispanica L.	Medicine	Folklore	66			235			301		
Crataegus azarolus L.	Medicine	Folklore	19			3			22		Least concern
Crataegus chrysocarpa Ashe	Medicine	Folklore, potential natural health product use	1			3			4		

Crataegus cuneata Siebold and Zucc.	Medicine	Folklore	1						1		
Crataegus douglasii Lindl.	Medicine	Folklore	2			8			10		
Crataegus laevigata (Poir.) DC.	Medicine	Folklore	23						23		Least concern
Crataegus mexicana DC.	Medicine	Folklore				3			3		
Crataegus monogyna Jacq.	Medicine	Folklore	99			17			116		Least concern
Crataegus pinnatifida Bunge	Medicine	Folklore	2		3	18			23		
Crataegus rivularis Nutt.	Medicine	Folklore				1			1		
Crateva religiosa G. Forst.	Medicine	Folklore			1	2			3		
Cratoxylum cochinchinense (Lour.) Blume	Additive, medicine	Flavoring, folklore	1						1		Lower risk/ least concern
Crescentia alata Kunth	Medicine	Folklore	3	1		1	1		6		
Crescentia cujete L.	Medicine	Folklore	1			2			3		
Crinum asiaticum L.	Medicine	Folklore			1				1		
Crithmum maritimum L.	Additive, medicine	Flavoring, folklore	24			2			26		
Crocanthemum canadense (L.) Britton	Medicine	Folklore				1			1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Crocus sativus L.	Additive, medicine	Flavoring, folklore	143						143		
Crossandra infundibuliformis (L.) Nees	Medicine	Folklore				1			1		Least concern
Crotalaria juncea L.	Medicine	Folklore	3	32	25	24	5	51	140		
Crotalaria sessiliflora L.	Medicine	Source of monocrotaline				1			1		
Crotalaria verrucosa L.	Medicine	Folklore				5	2	16	23		
Croton cajucara Benth.	Medicine	Folklore					15		15		
Croton dioicus Cav.	Medicine	Folklore	1			1			2		
Croton tiglium L.	Medicine	Folklore			8				8		
Cryptolepis buchananii Roem. and Schult.	Medicine	Folklore	1						1		
Cryptomeria japonica (L. f.) D. Don	Material	Essential oils	12			2	9		23		Near threatened
Cryptostegia grandiflora R. Br.	Medicine	Folklore	1			1			2		
Cryptotaenia japonica Hassk.	Additive, medicine	Flavoring, folklore	2		5				7		
Cucumis melo L.	Medicine	Folklore	4052	4	2532	4216	628	29	11461		
Cucumis prophetarum L.	Medicine	Folklore	24	27	1	4			56		

Cucumis sativus L.	Medicine	Folklore	5666	4	1483	2098	23	2	9276		
Cucurbita ficifolia Bouche	Medicine	Folklore	171	2	15	96	17		301		
Cucurbita foetidissima Kunth	Medicine	Folklore	16	1	12	42	28		99		
Cucurbita maxima Duchesne	Medicine, social	Folklore, religious/ secular	2042	24	407	1210	1457	2	5142		
Cucurbita moschata Duchesne	Medicine	Folklore	781	183	1419	1014	3677	7	7081		
Cucurbita pepo L.	Medicine	Folklore	4091	16	438	1432	156	7	6140		
Cullen corylifolium (L.) Medik.	Medicine	Folklore	5	1	65			1	72		
Cuminum cyminum L.	Additive, material, medicine	Flavoring, essential oils, folklore	65		12	35		3	115		
Cunila origanoides (L.) Britton	Medicine	Folklore	1						1		
Cuphea aequipetala Cav.	Medicine	Folklore				14	12	1	27		
Cuphea carthagenensis (Jacq.) J. F. Macbr.	Medicine	Folklore				52	8	1	61		
Cuphea ignea A. DC.	Medicine	Folklore	1			8		1	10		
Cupressus arizonica Greene	Social	Religious/secular	7			45			52		Least concern
Cupressus cashmeriana Carriere	Social	Religious/secular	1			2			3		Near threatened

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Cupressus funebris Endl.	Material	Essential oils	1			1			2		Data deficient
Cupressus lusitanica Mill.	Medicine	Folklore	5	1			43		49		Least concern
Cupressus nootkatensis D. Don	Material	Essential oils	2			1			3		
Cupressus sempervirens L.	Material, medicine	Essential oils, folklore	5	1		1		1	8		Least concern
Cupressus torulosa D. Don	Material	Essential oils	1						1		Least concern
Curculigo orchioides Gaertn.	Medicine	Folklore			1				1		
Curcuma aeruginosa Roxb.	Medicine	Folklore	3						3		
Curcuma amada Roxb.	Medicine	Folklore	1						1		
Curcuma longa L.	Additive, material, medicine	Flavoring, essential oils, source of curcumin	1			2			3		
Curcuma phaeocaulis Valeton	Medicine	Folklore				1			1		
Cuscuta americana L.	Material, medicine	Tannin/dyestuff, folklore	1						1		
Cuscuta chinensis Lam.	Medicine	Folklore	1		1				2		
Cuscuta epithymum (L.) L.	Medicine	Folklore	3						3		

Cuscuta europaea L.	Medicine	Folklore	8						8		
Cyamopsis tetragonoloba (L.) Taub.	Medicine	Folklore	5	11	83	2190	84	463	2836		
Cyanthillium cinereum (L.) H. Rob.	Medicine	Folklore	10			7		1	18		
Cyanthillium patulum (Aiton) H. Rob.	Medicine	Folklore				1			1		
Cyathula capitata Moq.	Medicine	Folklore	1						1		
Cybistax antisyphilitica (Mart.) Mart.	Medicine	Folklore	1				3		4		
Cycas revoluta Thunb.	Medicine	Folklore	1						1	II	Least concern
Cyclamen purpurascens Mill.	Medicine	Folklore	4						4	II	Least concern
Cyclanthera pedata (L.) Schrad.	Medicine	Folklore	37		3	14	2		56		
Cyclopia genistoides (L.) R. Br.	Medicine	Folklore	1						1		
Cydonia oblonga Mill.	Medicine	Folklore	472		9	229			710		
Cymbidium goeringii (Rchb. f.) Rchb. f.	Additive	Flavoring	1			2			3	II	
Cymbopogon caesius (Nees ex Hook. & Arn.) Stapf	Material	Essential oils	1	14				10	25		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Cymbopogon citratus (DC.) Stapf	Additive, material, medicine	Flavoring, essential oils, folklore	2	1		3			6		
Cymbopogon flexuosus (Nees) Will. Watson	Additive, material, medicine	Flavoring, essential oils, folklore			3	1			4		
Cymbopogon jwarancusa (Jones) Schult.	Material, medicine	Essential oils, folklore	1		3	1			5		
Cymbopogon martini (Roxb.) Will. Watson	Material, medicine	Essential oils, folklore			10	3			13		
Cymbopogon nardus (L.) Rendle	Additive, material, medicine	Flavoring, essential oils, folklore		16			1		17		
Cymbopogon schoenanthus (L.) Spreng.	Material, medicine	Essential oils, folklore	7	21		2			30		
Cynanchum acutum L.	Medicine	Folklore	4						4		Least concern
Cynanchum officinale (Hemsl.) Tsiang and H. D. Zhang	Medicine	Folklore	1						1		
Cynanchum viminale (L.) Bassi	Medicine	Folklore	3					1	4		
Cynanchum wilfordii (Maxim.) Hook. f.	Medicine	Folklore	1		1				2		

Cynara cardunculus L.	Medicine	Source of cynarin	186			49			235		
Cynodon dactylon (L.) Pers.	Medicine	Folklore	49	161	4	338		178	730		
Cynoglossum officinale L.	Medicine	Folklore	52					3	55		
Cyperus articulatus L.	Material, medicine, social	Essential oils, folklore, religious/secular	12	1		1			14		Least concern
Cyperus compressus L.	Material, medicine	Essential oils, folklore	3	2				1	6		Least concern
Cyperus cyperoides (L.) Kuntze	Medicine	Folklore	7					1	8		Least concern
Cyperus esculentus L.	Medicine	Folklore	26				1		27		Least concern
Cyperus haspan L.	Medicine	Folklore	6	3				3	12		
Cyperus involucratus Rottb.	Medicine, social	Folklore, religious/ secular	5			1			6		
Cyperus iria L.	Medicine	Folklore	13					2	15		Least concern
Cyperus longus L.	Medicine	Folklore	6						6		Least concern
Cyperus malaccensis Lam.	Medicine	Folklore	1						1		
Cyperus rotundus L.	Material, medicine	Essential oils, folklore	19	7					26		Least concern
Cypripedium calceolus L.	Medicine	Folklore	1			1			2	II	Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Cypripedium parviflorum Salisb.	Medicine	Folklore	1						1	II	Least concern
Cyrtocarpa procera Kunth	Medicine	Folklore	2						2		
Cytisus scoparius (L.) Link	Medicine	Source of sparteine	31			1		8	40		
Dactylorhiza maculata (L.) Soo	Medicine	Folklore	6						6	II	
Dactylorhiza majalis (Rchb.) P. F. Hunt and Summerh.	Medicine	Folklore	4						4	II	
Dahlia coccinea Cav.	Medicine	Folklore	5						5		
Dalbergia lanceolaria L. f.	Medicine	Folklore	2			1			3	II	
Dalbergia nigra (Vell.) Allemao ex Benth.	Medicine	Folklore					2		2	I	Vulnerable
Dalbergia sissoo Roxb. ex DC.	Medicine	Folklore		2		1			3	II	
Dalea bicolor Humb. and Bonpl. ex Willd.	Medicine	Folklore	2						2		Least concern
Daphne genkwa Siebold and Zucc.	Medicine	Source of yuanhuacine, yuanhuadine				1			1		
Daphne mezereum L.	Medicine	Folklore	13			2			15		
Datisca cannabina L.	Material	Tannin/dyestuff	3						3		

Datura inoxia Mill.	Medicine	Folklore	73		10	12			95		
Datura metel L.	Medicine	Source of hyoscine (scopolamine)	64	1	43	18	1		127		
Datura stramonium L.	Medicine	Folklore	310	6	24	159	2	1	502		
Daucus carota L.	Additive, medicine	Flavoring, folklore	4364		289	1615	25	4	6297		
Deeringia amaranthoides (Lam.) Merr.	Medicine	Folklore	1					2	3		
Delonix regia (Bojer) Raf.	Medicine	Folklore	4	30		10		1	45		Least concern
Delphinium semibarbatum Bien. ex Boiss.	Material, medicine	Tannin/dyestuff, folklore	3						3		
Delphinium staphisagria L.	Medicine	Folklore	1			1			2		
Delphinium tatsienense Franch.	Medicine	Folklore	1						1		
Dendrobium loddigesii Rolfe	Medicine	Folklore	2						2	II	
Dendrobium nobile Lindl.	Medicine	Folklore	2						2	II	
Dendrocalamus strictus (Roxb.) Nees	Medicine	Folklore				3			3		
Derris trifoliata Lour.	Medicine	Folklore			1				1		
Descurainia sophia (L.) Webb ex Prantl	Additive, medicine	Flavoring, folklore	17			1			18		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Desmodium adscendens (Sw.) DC.	Medicine	Folklore	2	26		6	16	12	62		Least concern
Desmodium gangeticum (L.) DC.	Medicine	Folklore	6		36	2	268	53	365		
Desmodium microphyllum (Thunb.) DC.	Medicine	Folklore					2	1	3		Least concern
Desmodium sequax Wall.	Medicine	Folklore	2				15	11	28		
Desmodium styracifolium (Osbeck) Merr.	Medicine	Folklore					99	9	108		
Desmodium triflorum (L.) DC.	Medicine	Folklore					73	17	90		
Desmostachya bipinnata (L.) Stapf	Medicine	Folklore	3			1			4		Least concern
Deverra tortuosa (Desf.) DC.	Additive	Flavoring	9						9		
Dianthus caryophyllus L.	Material, medicine	Essential oils, folklore	48		5	9			62		
Dianthus chinensis L.	Medicine	Folklore	26		4	22			52		
Dianthus superbus L.	Medicine	Folklore	10		3	5			18		
Dicentra canadensis (Goldie) Walp.	Medicine	Folklore	1						1		

Dichondra micrantha Urb.	Medicine	Folklore						1	1		
Dichroa febrifuga Lour.	Medicine	Folklore				1			1		
Dichrocephala integrifolia (L. f.) Kuntze	Medicine	Folklore	1					1	2		
Dictamnus albus L.	Medicine	Folklore	28			1			29		
Digera muricata (L.) Mart.	Medicine	Folklore	2	1					3		
Digitalis lanata Ehrh.	Medicine	Source of acetyldigoxin, deslanoside, digoxin, lanatosides A, B, C	39		7				46		
Digitalis purpurea L.	Medicine	Source of digitalin, digitoxin, gitalin	215		6	3			224		
Digitaria exilis (Kippist) Stapf	Medicine	Folklore	1	23	2	1		17	44		
Digitaria iburua Stapf	Medicine	Folklore				1			1		
Dillenia indica L.	Additive, material	Flavoring, tannin/ dyestuff	3		1	1			5		
Dimocarpus longan Lour.	Medicine	Folklore	8			33			41		Lower risk/ near threatened
Dimorphandra mollis Benth.	Medicine	Potential source of pharmaceutical agent					1		1		
Dionaea muscipula J. Ellis	Medicine	Folklore				3			3	II	Vulnerable

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Dioscorea alata L.	Medicine	Folklore	8	1258	12			231	1509		
Dioscorea bulbifera L.	Medicine	Folklore	5	71	4		1	8	89		
Dioscorea communis (L.) Caddick and Wilkin	Medicine	Folklore	14						14		
Dioscorea dregeana (Kunth) T. Durand and Schinz	Medicine	Folklore	1						1		Least concern
Dioscorea nipponica Makino	Medicine	Source of steroid precursors	3			1			4		
Dioscorea polystachya Turcz.	Medicine	Folklore	6						6		
Dioscorea villosa L.	Medicine	Folklore	1			1			2		
Dioscoreophyllum cumminsii (Stapf) Diels	Additive	Sweetener	1						1		
Diospyros digyna Jacq.	Medicine	Folklore	1			6			7		
Diospyros kaki Thunb.	Medicine	Folklore	259		344	289			892		
Diospyros lotus L.	Medicine	Folklore	14			29			43		Least concern
Diospyros melanoxylon Roxb.	Material, social	Tannin/dyestuff, smoking material			10				10		
Diospyros virginiana L.	Medicine	Folklore	3		1	19			23		

Dipsacus asper Wall. ex DC.	Medicine	Folklore	1						1		
Dipsacus fullonum L.	Medicine	Folklore	52						52		
Dipsacus japonicus Miq.	Medicine	Folklore	1						1		
Dipsacus sativus (L.) Honck.	Medicine	Folklore	8						8		
Dodonaea viscosa Jacq.	Medicine	Folklore	54	1		2		102	159		
Dolichandra unguis-cati (L.) L. G. Lohmann	Medicine	Folklore	3			2		1	6		
Dorstenia contrajerva L.	Additive, medicine	Flavoring, folklore	2						2		
Dracaena draco (L.) L.	Medicine	Folklore	4						4		Vulnerable
Dracocephalum moldavica L.	Material, medicine	Essential oils, folklore	45			1			46		
Drimia indica (Roxb.) Jessop	Medicine	Folklore	1	1					2		
Drimia maritima (L.) Stearn	Medicine	Source of scillarin A	24						24		Least concern
Drimys winteri J. R. Forst. and G. Forst.	Medicine	Folklore	4						4		
Drosera anglica Huds.	Medicine	Folklore	4						4		
Drosera intermedia Hayne	Medicine	Folklore	7						7		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Drosera peltata Sm. ex Willd.	Medicine	Folklore	4					6	10		Least concern
Drosera rotundifolia L.	Medicine	Folklore	27			1			28		Least concern
Dryas octopetala L.	Medicine	Folklore	23			6			29		
Drymaria cordata (L.) Willd. ex Schult.	Medicine	Folklore	5	1				1	7		
Dryopteris crassirhizoma Nakai	Medicine	Folklore				2			2		
Dryopteris filix-mas (L.) Schott	Medicine	Folklore	4					2	6		
Duboisia hopwoodii (F. Muell.) F. Muell.	Medicine, social	Folklore, masticatory	2					4	6		
Duboisia leichhardtii (F. Muell.) F. Muell.	Medicine	Source of hyoscine	1					1	2		
Duboisia myoporoides R. Br.	Medicine	Source of hyoscine	2					3	5		
Duchesnea chrysantha (Zoll. & Moritzi) Miq.	Medicine	Folklore			2	1			3		
Duchesnea indica (Andrews) Teschem.	Medicine	Folklore	6			15			21		
Duranta erecta L.	Medicine	Folklore	4	1					5		
Dysphania ambrosioides (L.) Mosyakin & Clemants	Additive, material, medicine	Flavoring, essential oils, folklore	18	1		4		1	24		

Echeveria gibbiflora DC.	Medicine	Folklore	1						1		
Echinacea angustifolia DC.	Medicine	Folklore	32			60	1		93		
Echinacea pallida (Nutt.) Nutt.	Medicine	Folklore	15			56			71		
Echinacea purpurea (L.) Moench	Medicine	Folklore	67		1	30			98		
Echinacea tennesseensis (Beadle) Small	Medicine	Folklore	3			4			7		
Echium vulgare L.	Medicine	Folklore	22			3			25		
Eclipta prostrata (L.) L.	Medicine	Folklore	15	2	20				37		Least concern
Ehretia acuminata R. Br.	Medicine	Folklore	1					3	4		
Elaeis guineensis Jacq.	Medicine	Folklore		4		19	329		352		Least concern
Elaeodendron croceum (Thunb.) DC.	Medicine	Folklore	1						1		
Elephantopus mollis Kunth	Medicine	Folklore	5						5		
Elephantopus scaber L.	Medicine	Folklore	3						3		
Elettaria cardamomum (L.) Maton	Additive, material, medicine	Flavoring, essential oils, folklore	2						2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Eleutherococcus lasiogyne (Harms) S. Y. Hu	Medicine	Folklore	1						1		
Eleutherococcus senticosus (Rupr. & Maxim.) Maxim.	Medicine	Folklore	3			8			11		
Eleutherococcus sessiliflorus (Rupr. & Maxim.) S. Y. Hu	Medicine	Folklore	4		3	1			8		
Eleutherococcus sieboldianus (Makino) Koidz.	Medicine	Folklore	1			1			2		
Elsholtzia ciliata (Thunb.) Hyl.	Medicine	Folklore	11		5				16		
Elsholtzia splendens Nakai ex F. Maek.	Medicine	Folklore	1		3				4		
Elymus repens (L.) Gould	Additive, medicine	Flavoring, folklore	41		8	281		34	364		
Embelia ribes Burm. f.	Medicine	Folklore	2		15				17		
Embelia schimperi Vatke	Medicine	Folklore	1	1					2		
Embelia tsjeriam-cottam (Roem. & Schult.) A. DC.	Medicine	Folklore			1				1		
Enicostema axillare (Lam.) A. Raynal	Medicine	Folklore		1					1		

Entada phaseoloides (L.) Merr.	Medicine	Folklore			2				2		
Entada rheedei Spreng.	Medicine	Folklore	4		21				25		
Enterolobium cyclocarpum (Jacq.) Griseb.	Medicine	Folklore	6	9		8	1		24		
Ephedra californica S. Watson	Medicine	Folklore				4			4		Least concern
Ephedra distachya L.	Medicine	Folklore	4			1			5		Least concern
Ephedra equisetina Bunge	Medicine	Source of ephedrine, pseudoephedrine	3					1	4		
Ephedra gerardiana Wall. ex Stapf	Medicine	Folklore	3						3		
Ephedra intermedia Schrenk & C. A. Mey.	Medicine	Folklore, source of ephedrine, pseudoephedrine	2						2		Least concern
Ephedra major Host	Medicine	Folklore	1			1			2		Least concern
Ephedra nevadensis S. Watson	Medicine	Folklore				1			1		Least concern
Ephedra sinica Stapf	Medicine	Source of ephedrine, pseudoephedrine	1						1		Least concern
Ephedra torreyana S. Watson	Medicine	Folklore	1			1			2		Least concern
Ephedra trifurca Torr. ex S. Watson	Medicine	Folklore	1			1			2		Least concern

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Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Ephedra viridis Coville	Medicine	Folklore	1	2		3			6		Least concern
Epigaea repens L.	Medicine	Folklore	1			1			2		
Epilobium collinum C. C. Gmel.	Medicine	Folklore	3						3		
Epilobium parviflorum Schreb.	Medicine	Folklore	21						21		Least concern
Epilobium roseum Schreb.	Medicine	Folklore	2						2		
Epimedium alpinum L.	Medicine	Folklore	2						2		
Epimedium grandiflorum C. Morren	Medicine	Folklore				3			3		
Epimedium pubescens Maxim.	Medicine	Folklore				1			1		
Epimedium sagittatum (Siebold & Zucc.) Maxim.	Medicine	Folklore				1			1		
Epimedium sempervirens Nakai ex F. Maek.	Medicine	Folklore				4			4		
Epipremnum pinnatum (L.) Engl.	Medicine	Folklore				2			2		
Equisetum arvense L.	Medicine	Folklore	4						4		Least concern

Equisetum hyemale L.	Medicine	Folklore	2						2		Least concern
Equisetum ramosissimum Desf.	Medicine	Folklore	1						1		
Equisetum telmateia Ehrh.	Medicine	Folklore	4						4		Least concern
Eranthemum roseum (Vahl) R. Br.	Medicine	Folklore			1				1		
Erigeron annuus (L.) Pers.	Medicine	Folklore	2						2		
Erigeron canadensis L.	Medicine	Folklore	10			2		1	13		
Erigeron philadelphicus L.	Medicine	Folklore	1						1		
Erigeron strigosus Muhl. ex Willd.	Medicine	Folklore	2			2			4		
Eriobotrya japonica (Thunb.) Lindl.	Medicine	Folklore	160		46	78			284		
Eriocaulon buergerianum Korn.	Medicine	Folklore	1						1		
Eriodictyon californicum (Hook. & Arn.) Torr.	Additive, medicine	Flavoring, folklore				10			10		
Eriodictyon crassifolium Benth.	Medicine	Folklore	1						1		
Eriodictyon tomentosum Benth.	Medicine	Folklore				1			1		
Eriodictyon trichocalyx A. Heller	Medicine	Folklore				1			1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Eriogonum fasciculatum Benth.	Medicine	Folklore	3	1		25		1	30		
Eriophyllum confertiflorum (DC.) A. Gray	Medicine	Folklore	3			7			10		
Erodium cicutarium (L.) L'Her.	Medicine	Folklore	10			1			11		
Eruca vesicaria (L.) Cav.	Additive	Flavoring	163		145	251			559		
Eryngium campestre L.	Medicine	Folklore	41			10			51		
Eryngium foetidum L.	Additive, medicine	Flavoring, folklore	3		32	2	1		38		
Eryngium maritimum L.	Medicine	Folklore	21			4			25		
Eryngium planum L.	Medicine	Folklore	9			8			17		
Eryngium yuccifolium Michx.	Medicine	Folklore	2			16			18		
Erysimum cheiri (L.) Crantz	Material, medicine	Essential oils, folklore	56			7			63		
Erysimum diffusum Ehrh.	Medicine	Source of erysid	6			3			9		
Erythrina americana Mill.	Medicine	Folklore	1			2			3		
Erythrina coralloides DC.	Medicine	Folklore	2						2		

Erythrina fusca Lour.	Medicine	Folklore	1	2		1	17		21		
Erythrina stricta Roxb.	Medicine	Folklore	1						1		
Erythrina variegata L.	Medicine	Folklore	1	3		4			8		Least concern
Erythrophleum suaveolens (Guill. & Perr.) Brenan	Medicine	Folklore	4	5					9		
Eschscholzia californica Cham.	Medicine	Folklore	23		1	6			30		
Etlingera elatior (Jack) R. M. Sm.	Additive	Flavoring				2			2		
Eucalyptus cinerea F. Muell. ex Benth.	Material	Tannin/dyestuff	3					8	11		
Eucalyptus cordata Labill.	Material	Tannin/dyestuff	2					111	113		
Eucalyptus globulus Labill.	Additive, material, medicine	Flavoring, essential oils, folklore	9				4	15	28		
Eucalyptus gunnii Hook. f.	material	Tannin/dyestuff	4					16	20		
Eucalyptus macrorhyncha F. Muell. ex Benth.	Medicine	Source of rutin	4					21	25		
Eucalyptus piperita Sm.	Medicine	Folklore	1					9	10		
Eucalyptus polybractea R. T. Baker	Material	Potential as essential oils	3					11	14		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Eucalyptus radiata Sieber ex DC.	Material	Essential oils	3					28	31		
Eucalyptus tereticornis Sm.	Material	Chemicals	5				58	15	78		
Euclea divinorum Hiern	Material	Potential as tannin/dyestuff	3	3					6		
Eucomis autumnalis (Mill.) Chitt.	Medicine	Folklore	6						6		
Eucommia ulmoides Oliv.	Medicine	Source of aucubin	2			2			4		Lower risk/near threatened
Eugenia uniflora L.	Medicine	Folklore	3			1	9		13		
Euonymus atropurpureus Jacq.	Medicine	Folklore	1			9			10		
Eupatorium cannabinum L.	Medicine	Folklore	119			4		1	124		
Eupatorium chinense L.	Medicine	Folklore	1		2	1			4		
Eupatorium fortunei Turcz.	Material, medicine	Essential oils, folklore	1						1		
Eupatorium perfoliatum L.	Medicine	Folklore	2			5			7		Least concern
Euphorbia cyathophora Murray	Material, medicine	Tannin/dyestuff, folklore	3		1	4			8		
Euphorbia cyparissias L.	Medicine	Folklore	3			1			4		

Euphorbia dracunculoides Lam.	Medicine	Folklore				1			1		
Euphorbia hirta L.	Medicine	Folklore	4	1	15				20		
Euphorbia humifusa Willd.	Medicine	Folklore	2						2		
Euphorbia hypericifolia L.	Medicine	Folklore	1			1			2		
Euphorbia hyssopifolia L.	Medicine	Folklore	2						2		
Euphorbia lathyris L.	Medicine	Folklore	85		1	13		2	101		
Euphorbia pekinensis Rupr.	Medicine	Folklore	1						1		
Euphorbia peplis L.	Medicine	Folklore	3						3		
Euphorbia prostrata Aiton	Medicine	Folklore	1						1	II	
Euphorbia pulcherrima Willd. ex Klotzsch	Medicine, social	Folklore, religious/ secular	1						1		
Euphorbia thymifolia L.	Medicine	Folklore			1				1		
Euphorbia tirucalli L.	Medicine	Folklore	3			1			4	II	Least concern
Euploca procumbens (Mill.) Diane and Hilger	Medicine	Folklore				1			1		
Euryale ferox Salisb.	Medicine	Folklore			109				109		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Euterpe oleracea Mart.	Medicine	Folklore				2	1		3		
Eutrochium purpureum (L.) E. E. Lamont	Medicine	Folklore				3			3		
Evolvulus alsinoides (L.) L.	Medicine	Folklore	5	2	3			2	12		
Eysenhardtia polystachya (Ortega) Sarg.	Medicine	Folklore	6			1			7		Least concern
Fabiana imbricata Ruiz & Pav.	Medicine	Folklore	2						2		
Fagonia cretica L.	Medicine	Folklore	2		4				6		
Fagopyrum dibotrys (D. Don) H. Hara	Medicine	Folklore	1						1		
Fagopyrum esculentum Moench	Medicine	Source of rutin	2981		718	562	77		4338		
Fagus grandifolia Ehrh.	Medicine	Folklore				6			6		Least concern
Faidherbia albida (Delile) A. Chev.	Medicine	Folklore	25	840		6			871		
Falcaria vulgaris Bernh.	Medicine	Folklore	13						13		
Fatoua villosa (Thunb.) Nakai	Medicine	Folklore	1						1		
Ferula assa-foetida L.	Additive, material, medicine	Flavoring, essential oils, folklore, veterinary	8			2			10		

Ferula foetida (Bunge) Regel	Additive, medicine	Flavoring, veterinary	2						2		
Ferula gummosa Boiss.	Additive, medicine	Flavoring, folklore				3			3		
Ferula kirialovii Pimenov	Medicine	Folklore	1						1		
Ferula kuhistanica Korovin	Medicine	Folklore	3						3		
Ferula narthex Boiss.	Additive, medicine	Flavoring, folklore	1			1			2		
Ferula penninervis Regel and Schmalh.	Medicine	Folklore	8						8		
Ferula persica Willd.	Additive, medicine	Flavoring, folklore	1			1			2		
Ficaria verna Huds.	Medicine	Folklore	8						8		
Ficus benghalensis L.	Medicine, social	Folklore, religious/ secular	1			5			6		
Ficus carica L.	Medicine	Folklore	904		63	648			1615		Least concern
Ficus copiosa Steud.	Medicine	Folklore						1	1		
Ficus exasperata Vahl	Medicine	Folklore	3						3		
Ficus hispida L. f.	Medicine	Folklore	1					1	2		
Ficus insipida Willd.	Medicine	Folklore	1			3			4		
Ficus microcarpa L. f.	Medicine	Folklore	1			1		2	4		
Ficus pumila L.	Medicine	Folklore	1			5			6		
Ficus racemosa L.	Medicine	Folklore	1			5		1	7		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Ficus religiosa L.	Medicine, social	Folklore, religious/ secular	2			3			5		
Ficus retusa L.	Medicine	Folklore	2						2		
Ficus rumphii Blume	Medicine	Folklore	1			1			2		
Ficus septica Burm. f.	Medicine	Folklore				2		2	4		
Filipendula ulmaria (L.) Maxim.	Additive, medicine	Flavoring, folklore	108			2		5	115		Least concern
Flacourtia indica (Burm. f.) Merr.	Medicine	Folklore	6	6	1	2			15		
Flacourtia jangomas (Lour.) Raeusch.	Medicine	Folklore	1						1		
Flacourtia rukam Zoll. and Moritzi	Medicine	Folklore				1			1		
Flaveria bidentis (L.) Kuntze	Medicine	Folklore	4						4		
Flemingia macrophylla (Willd.) Merr.	Material	Tannin/dyestuff	1	20	6		114	12	153		
Flueggea suffruticosa (Pall.) Baill.	Medicine	Folklore	1		1	9			11		
Foeniculum vulgare Mill.	Additive, material, medicine	Flavoring, essential oils, folklore	696	1	22	91	3	26	839		
Forsythia suspensa (Thunb.) Vahl	Medicine	Folklore	3			1			4		

Fortunella margarita (Lour.) Swingle	Additive	Flavoring	4		1	9			14		
Fouquieria splendens Engelm.	Medicine	Folklore	5			2			7		
Fragaria vesca L.	Medicine	Folklore	158			249			407		
Fragaria virginiana Mill.	Medicine	Folklore	14			548	1		563		
Frangula alnus Mill.	Medicine	Folklore	33			1			34		Least concern
Frangula californica (Eschsch.) A. Gray	Medicine	Folklore	4			10			14		
Frangula purshiana (DC.) A. Gray	Additive, medicine	Flavoring, folklore	1			2			3		Least concern
Frasera speciosa Douglas ex Griseb.	Medicine	Folklore	1			14			15		
Fraxinus americana L.	Medicine	Folklore	28			844			872		Critically endangered
Fraxinus bungeana DC.	Medicine	Folklore				9			9		Least concern
Fraxinus chinensis Roxb.	Medicine	Folklore, source of aesculetin	3			25			28		Least concern
Fraxinus excelsior L.	Medicine	Folklore	108			15			123		Near threatened
Fraxinus griffithii C. B. Clarke	Medicine, social	Folklore, smoking material, stimulant	1						1		Least concern
Fraxinus nigra Marshall	Medicine	Folklore	14			336			350		Critically endangered
Fraxinus ornus L.	Medicine	Folklore	16			11			27		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Fraxinus stylosa Lingelsh.	Medicine	Folklore				2			2		Least concern
Fraxinus uhdei (Wenz.) Lingelsh.	Medicine	Folklore	1			1			2		Least concern
Fumaria officinalis L.	Medicine	Folklore	9					3	12		
Fumaria parviflora Lam.	Medicine	Folklore	5		1				6		
Funtumia africana (Benth.) Stapf	Medicine	Folklore	1						1		
Funtumia elastica (P. Preuss) Stapf	Medicine	Folklore				1			1		
Galanthus nivalis L.	Medicine	Folklore	3						3	II	Near threatened
Galanthus woronowii Losinsk.	Medicine	Source of galantamine	1						1	II	
Galega officinalis L.	Medicine	Folklore	101			16		40	157		
Galeopsis segetum Neck.	Medicine	Folklore	3						3		Data deficient
Galium aparine L.	Medicine	Folklore	31					1	32		
Galium boreale L.	Medicine	Folklore	7			2			9		
Galium odoratum (L.) Scop.	Additive, medicine	Flavoring, folklore	58						58		
Galium verum L.	Material, medicine	Tannin/dyestuff, folklore	94		1	1		1	97		
Gamblea ciliata C. B. Clarke	Medicine	Folklore	1						1		

Garcinia afzelii Engl.	Medicine	Folklore	2						2		Vulnerable
Garcinia kola Heckel	Social	Masticatory	1						1		Vulnerable
Garcinia mangostana L.	Medicine	Folklore			1	4			5		
Gardenia jasminoides J. Ellis	Medicine	Folklore	1			1			2		
Gardenia resinifera Roth	Medicine	Folklore			1				1		
Garrya flavescens S. Watson	Medicine	Folklore				1			1		
Gaultheria procumbens L.	Additive, medicine	Flavoring, source of methyl salicylate	1			6			7		
Gelsemium sempervirens (L.) J. St.-Hil.	Medicine	Folklore				1			1		
Genista tinctoria L.	Material, medicine	Tannin/dyestuff, folklore	70			8		1	79		
Gentiana lutea L.	Additive, medicine	Flavoring, folklore	60						60		
Gentiana macrophylla Pall.	Medicine	Folklore	5						5		
Gentiana olivieri Griseb.	Medicine	Folklore	1						1		
Gentiana rigescens Franch.	Medicine	Folklore	1						1		
Geranium macrorrhizum L.	Material	Essential oils	6						6		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Geranium maculatum L.	Medicine	Folklore	1						1		
Geranium nepalense Sweet	Material	Tannin/dyestuff	1		4				5		
Geranium robertianum L.	Medicine	Folklore	19			1		1	21		
Geum aleppicum Jacq.	Material, medicine	Essential oils, folklore	9		3	2			14		
Geum japonicum Thunb.	Medicine	Folklore	3		5				8		
Geum macrophyllum Willd.	Medicine	Folklore	3			4			7		
Geum rivale L.	Medicine	Folklore	14						14		Least concern
Geum urbanum L.	Additive, medicine	Flavoring, folklore	151					6	157		
Gillenia stipulata (Muhl. ex Willd.) Nutt.	Medicine	Folklore				2			2		
Ginkgo biloba L.	Material, medicine	Tannin/dyestuff, folklore	9			4			13		Endangered
Gladiolus dalenii Van Geel	Medicine	Folklore	5						5		
Glaucium flavum Crantz	Medicine	Source of glaucine	50		6	3			59		
Glechoma hederacea L.	Medicine	Folklore	28						28		

Gleditsia sinensis Lam.	Medicine	Folklore	1			4		2	7		
Gleditsia triacanthos L.	Medicine	Folklore	10	5	1	16		73	105		
Glehnia littoralis F. Schmidt ex Miq.	Additive, medicine	Flavoring, folklore	2			2			4		
Glinus lotoides L.	Medicine	Folklore	17	5				6	28		
Gliricidia sepium (Jacq.) Kunth	Medicine	Folklore	5	344		1	32	45	427		
Gloriosa superba L.	Medicine	Folklore	3	1	7				11		Least concern
Glossocardia bidens (Retz.) Veldkamp	Medicine	Folklore	2					8	10		
Glossocardia bosvallia (L. f.) DC.	Medicine	Folklore			1				1		
Glycyrrhiza echinata L.	Medicine	Folklore	40			3			43		
Glycyrrhiza glabra L.	Additive, material, medicine	Flavoring, essential oils, source of glycyrrhizin	60		11	23		36	130		
Glycyrrhiza inflata Batalin	Medicine	Folklore	1						1		
Glycyrrhiza uralensis Fisch. ex DC.	Medicine	Folklore	16		16	10		13	55		
Gmelina arborea Roxb.	Medicine	Folklore	1	2	9	1			13		
Gnaphalium affine D. Don	Medicine	Folklore	2		1				3		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Gnaphalium uliginosum L.	Medicine	Folklore	3					1	4		
Gomphocarpus fruticosus (L.) W. T. Aiton	Medicine	Folklore	9					1	10		
Gomphocarpus physocarpus E. Mey.	Medicine	Folklore	4	1					5		
Grangea maderaspatana (L.) Poir.	Medicine	Folklore	1						1		Least concern
Gratiola officinalis L.	Medicine	Folklore	10						10		Least concern
Grewia asiatica L.	Medicine	Folklore	1		2	2			5		
Grindelia camporum Greene	Medicine	Folklore	4			8			12		
Grindelia hirsutula Hook. and Arn.	Medicine	Folklore	8			3			11		
Grindelia squarrosa (Pursh) Dunal	Medicine	Folklore	11			10			21		
Grindelia stricta DC.	Medicine	Folklore	2			1			3		
Guaiacum officinale L.	Medicine	Folklore	5						5	II	Endangered
Guaiacum sanctum L.	Medicine	Folklore	6						6	II	Near threatened
Guarea guidonia (L.) Sleumer	Medicine	Folklore	1						1		Least concern
Guazuma ulmifolia Lam.	Medicine	Folklore	11	2		1	1		15		

Guizotia abyssinica (L. f.) Cass.	Medicine	Folklore	8		4	22	1	106	141		
Guizotia scabra (Vis.) Chiov.	Medicine	Folklore	1	6					7		
Gymnanthemum amygdalinum (Delile) Sch. Bip.	Medicine	Folklore				1			1		
Gymnanthemum coloratum (Willd.) H. Rob. and B. Kahn	Medicine	Folklore				1			1		
Gymnema sylvestre (Retz.) Schult.	Medicine	Folklore			7				7		
Gymnosporia senegalensis (Lam.) Loes.	Medicine	Folklore	5						5		
Gynandropsis gynandra (L.) Briq.	Additive, medicine	Flavoring, folklore				31			31		
Gynostemma pentaphyllum (Thunb.) Makino	Medicine	Folklore	2			1			3		
Gypsophila acutifolia Steven ex Spreng.	Material	Chemicals	1			1			2		
Gypsophila arrostii Guss.	Material, medicine	Chemicals, folklore	1						1		
Gypsophila oldhamiana Miq.	Medicine	Folklore			1	4			5		
Gypsophila paniculata L.	Medicine	Folklore	30			5			35		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Haematoxylum brasiletto H. Karst.	Material	Tannin/dyestuff	1	1		5			7		
Haematoxylum campechianum L.	Material, medicine	Tannin/dyestuff, folklore	4						4		
Hamamelis virginiana L.	Material, medicine	Tannin/dyestuff, folklore	2			24			26		Least concern
Hamelia patens Jacq.	Medicine	Folklore	3			2			5		
Handroanthus heptaphyllus (Vell.) Mattos	Medicine	Folklore				2			2		
Handroanthus impetiginosus (Mart. ex DC.) Mattos	Medicine	Folklore	1			9			10		
Haplophyllum tuberculatum (Forssk.) A. Juss.	Medicine	Folklore	16						16		
Harpagophytum procumbens (Burch.) DC. ex Meisn.	Medicine	Source of harpagoside	12						12		
Harpagophytum zeyheri Decne.	Medicine	Source of harpagoside	5						5		
Harungana madagascariensis Lam. ex Poir.	Medicine	Folklore	6	1					7		
Hedeoma pulegioides (L.) Pers.	Additive, medicine	Flavoring, folklore	2						2		
Hedera helix L.	Medicine	Folklore	32						32		

Hedychium spicatum Sm.	Material, medicine	Essential oils, folklore	3			3			6		
Hedysarum neglectum Ledeb.	Medicine	Folklore	1						1		
Heimia salicifolia Link	Medicine	Folklore	8						8		
Helianthus annuus L.	Medicine	Folklore	5931	81	561	4103	1881	1362	13919		Least concern
Helianthus tuberosus L.	Medicine	Folklore	193		126	124	5	4	452		Least concern
Helichrysum arenarium (L.) Moench	Medicine	Folklore	57			3			60		
Helichrysum italicum (Roth) G. Don	Additive, material, medicine	Flavoring, essential oils, folklore	87						87		
Helicteres angustifolia L.	Medicine	Folklore	1					1	2		
Helicteres isora L.	Medicine	Folklore			30				30		
Heliotropium arborescens L.	Material	Essential oils	2			1			3		
Heliotropium curassavicum L.	Medicine	Folklore	3			7		2	12		Least concern
Heliotropium europaeum L.	Medicine	Folklore	5			1		1	7		
Heliotropium indicum L.	Medicine	Folklore	4		3	2			9		
Helleborus niger L.	Medicine	Folklore	7						7		
Helleborus viridis L.	Medicine	Folklore	4						4		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Hemerocallis citrina Baroni	Medicine	Folklore	1			1			2		
Hemerocallis fulva (L.) L.	Additive, medicine	Flavoring, folklore	6		4	18			28		
Heracleum candicans Wall. ex DC.	Medicine	Source of xanthotoxin	2		14				16		
Heracleum dissectum Ledeb.	Medicine	Folklore	2			3			5		
Heracleum grande (Dalzell & A. Gibson) P. K. Mukh.	Additive, medicine	Flavoring, folklore			1				1		
Heracleum pastinacifolium K. Koch	Additive	Flavoring	3						3		
Heracleum sosnowskyi Manden.	Medicine	Folklore	8			1			9		
Heracleum sphondylium L.	Medicine	Folklore	100		1	18			119		
Herniaria glabra L.	Medicine	Folklore	14						14		
Hesperoyucca whipplei (Torr.) Trel.	Medicine	Folklore	7			3			10		
Heteromorpha arborescens (Spreng.) Cham. and Schltdl.	Medicine	Folklore		1					1		

Heterotheca grandiflora Nutt.	Medicine	Folklore	3			3			6		
Heterotheca inuloides Cass.	Medicine	Folklore	4						4		
Heuchera micrantha Douglas ex Lindl.	Medicine	Folklore	1			4			5		
Hibiscus radiatus Cav.	Medicine	Folklore			1	9			10		
Hibiscus rosa-sinensis L.	Medicine	Folklore	4			2			6		
Hibiscus sabdariffa L.	Additive, medicine	Flavoring, folklore	17	7	346	199	16	95	680		
Hibiscus syriacus L.	Medicine	Folklore	16		1	2			19		
Hintonia latiflora (DC.) Bullock	Medicine	Folklore	5						5		
Hippophae rhamnoides L.	Medicine	Folklore	70		173	15			258		
Holarrhena pubescens Wall. ex G. Don	Medicine	Folklore	2		24	1			27		Least concern
Holigarna arnottiana Hook. f.	Medicine	Folklore			1				1		
Hoslundia opposita Vahl	Medicine	Folklore	8	4					12		
Houttuynia cordata Thunb.	Medicine	Folklore	5		2				7		
Hovenia dulcis Thunb.	Medicine	Folklore	3			1			4		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Hoya carnosa (L. f.) R. Br.	Medicine	Folklore	1						1		
Humulus japonicus Siebold and Zucc.	Material, medicine	Essential oils, folklore				24			24		
Humulus lupulus L.	Additive, medicine	Flavoring, folklore	1115			1567			2682		
Hura crepitans L.	Medicine	Folklore	2						2		
Hyacinthus orientalis L.	Material	Essential oils	7						7		
Hybanthus enneaspermus (L.) F. Muell.	Medicine	Folklore	1						1		
Hydnocarpus pentandrus (Buch.-Ham.) Oken	Medicine	Folklore			2				2		Vulnerable
Hydrangea anomala D. Don	Medicine	Folklore	4			2			6		
Hydrangea arborescens L.	Medicine	Folklore	6			26			32		
Hydrangea paniculata Siebold	Medicine	Folklore	4			6			10		
Hydrangea serrata (Thunb.) Ser.	Medicine	Source of phyllodulcin	2			4			6		
Hydrastis canadensis L.	Medicine	Source of hydrastine				12			12	II	Vulnerable
Hydrocotyle sibthorpioides Lam.	Additive	Potential as flavoring	1						1		Least concern

Hygrophila auriculata (Schumach.) Heine	Medicine	Folklore	4	2					6		Least concern
Hymenaea courbaril L.	Medicine	Folklore	6				18		24		Least concern
Hyoscyamus albus L.	Medicine	Source of atropine, hyoscine, hyoscyamine	66			1			67		
Hyoscyamus muticus L.	Medicine, social	Source of atropine, hyoscine, hyoscyamine, narcotic	14		3	2			19		
Hyoscyamus niger L.	Medicine	Source of atropine, hyoscine, hyoscyamine	137		24	13		3	177		
Hypericum japonicum Thunb.	Medicine	Folklore	2					2	4		
Hypericum monogynum L.	Medicine	Folklore	1			3			4		
Hypericum perforatum L.	Material, medicine	Essential oils, source of hypericin	584		7	110			701		
Hypericum sampsonii Hance	Medicine	Folklore	1						1		
Hypoxis hemerocallidea Fisch. and C. A. Mey.	Medicine	Folklore	9						9		
Hyssopus officinalis L.	Additive, material, medicine	Flavoring, essential oils, folklore	139		7	6		14	166		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Iberis amara L.	Medicine	Folklore	12			9			21		
Ilex aquifolium L.	Medicine, social	Folklore, religious/ secular	84						84		Least concern
Ilex colchica Pojark.	Medicine	Folklore	3			2			5		
Ilex cornuta Lindl. and Paxton	Medicine	Folklore	1			6			7		
Ilex opaca Aiton	Medicine	Folklore				5			5		Least concern
Ilex paraguariensis A. St.-Hil.	Additive, material, medicine, social	Flavoring, essential oils, folklore, stimulant					39		39		VLower risk/near threatened
Ilex vomitoria Sol. ex Aiton	Medicine	Folklore	1			4			5		Least concern
Impatiens balsamina L.	Medicine	Folklore	25		5	4			34		
Impatiens capensis Meerb.	Medicine	Folklore	1			1			2		Least concern
Impatiens pallida Nutt.	Medicine	Folklore	1			1			2		
Imperata conferta (J. Presl) Ohwi	Medicine	Folklore	1						1		
Imperata cylindrica (L.) P. Beauv.	Medicine	Folklore	17	2		1		1	21		
Indigofera arrecta Hochst. ex A. Rich.	Material, medicine	Tannin/dyestuff, folklore	7	60		7	1	3	78		
Indigofera hendecaphylla Jacq.	Material	Tannin/dyestuff	3						3		

Indigofera heterantha Wall. ex Brandis	Material	Tannin/dyestuff	2						2		
Indigofera suffruticosa Mill.	Material, medicine	Tannin/dyestuff, folklore	18	34		13	73	25	163		
Indigofera tinctoria L.	Material, medicine	Tannin/dyestuff, folklore	30	18	27	9	1	24	109		
Intsia bijuga (Colebr.) Kuntze	Medicine	Folklore	5						5		Vulnerable
Inula britannica L.	Medicine	Folklore	8						8		
Inula helenium L.	Additive, medicine	Flavoring, folklore	87		1	3			91		
Inula linearifolia Turcz.	Medicine	Folklore	1						1		
Inula racemosa Hook. f.	Medicine	Folklore	1		2				3		
Ipomoea aquatica Forssk.	Medicine	Folklore	1		76	6	8		91		Least concern
Ipomoea batatas (L.) Lam.	Medicine	Folklore	157		571	871	8272	315	10186		
Ipomoea cairica (L.) Sweet	Medicine	Folklore	9	2		10	6		27		Least concern
Ipomoea hederacea Jacq.	Medicine	Folklore	1			13			14		
Ipomoea mauritiana Jacq.	Medicine	Folklore	2			2			4		
Ipomoea muricata (L.) Jacq.	Medicine	Folklore	3			3			6		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Ipomoea nil (L.) Roth	Medicine	Folklore	5		2	17	40		64		
Ipomoea pes-caprae (L.) R. Br.	Medicine	Folklore	8	1	1	5			15		
Ipomoea purga (Wender.) Hayne	Medicine	Source of jalap				1			1		
Ipomoea purpurea (L.) Roth	Medicine	Folklore	8			22	98		128		
Ipomoea sagittifolia Burm. f.	Medicine	Folklore			1				1		
Ipomoea tricolor Cav.	Social	Hallucinogen	2			10	3		15		
Ipomoea violacea L.	Medicine	Folklore	3			2	1		6		
Iresine diffusa Humb. and Bonpl. ex Willd.	Medicine	Folklore	3						3		
Iris domestica Goldblatt and Mabb.	Medicine	Folklore	4			12			16		
Iris germanica L.	Additive, material, medicine	Flavoring, essential oils, folklore	7						7		
Iris pallida Lam.	Material, medicine	Essential oils, folklore	8						8		
Iris tectorum Maxim.	Medicine	Folklore	1			2			3		
Iris versicolor L.	Medicine	Folklore	2			1			3		Least concern
Iris virginica L.	Medicine	Folklore	1			1			2		Least concern

Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) Baill.	Medicine	Folklore		1					1		Lower risk/ near threatened
Isatis tinctoria L.	Material, medicine	Tannin/dyestuff, folklore	164			8		4	176		
Ixora coccinea L.	Medicine	Folklore				1			1		
Jacaranda caroba (Vell.) DC.	Medicine	Folklore					1		1		
Jacaratia mexicana A. DC.	Medicine	Folklore	1						1		
Jacobaea maritima (L.) Pelser and Meijden	Medicine	Folklore	3						3		
Jacobaea vulgaris Gaertn.	Medicine	Folklore	2						2		
Jasminum multiflorum (Burm. f.) Andrews	Medicine, social	Folklore, religious/ secular				1			1		
Jasminum odoratissimum L.	Medicine	Folklore	1						1		
Jasminum officinale L.	Additive, material, medicine	Flavoring, essential oils, folklore	2						2		
Jasminum sambac (L.) Aiton	Additive, material, medicine	Flavoring, essential oils, folklore	1						1		
Jateorhiza palmata (Lam.) Miers	Medicine	Folklore		2					2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Jatropha curcas L.	Material, medicine	Tannin/dyestuff, folklore	3	10		1	139	2	155		
Jatropha dioica Sesse	Medicine	Folklore	1						1		
Jatropha gossypiifolia L.	Medicine	Folklore	2	1					3		
Jeffersonia diphylla (L.) Pers.	Medicine	Folklore	2						2		
Juglans cinerea L.	Medicine	Folklore	3			97			100		
Juglans major (Torr.) A. Heller	Medicine	Folklore	1			54			55		
Juglans mandshurica Maxim.	Material	Tannin/dyestuff	3			40			43		
Juglans neotropica Diels	Material	Tannin/dyestuff				8			8		Endangered
Juglans nigra L.	Medicine	Folklore	6		1	41			48		Least concern
Juglans regia L.	Material, medicine	Tannin/dyestuff, folklore	1888		18	750			2656		Least concern
Juncus effusus L.	Medicine	Folklore	38	1	1	6		1	47		Least concern
Juniperus communis L.	Additive, material, medicine	Flavoring, essential oils, folklore	97			39			136		Least concern
Juniperus deppeana Steud.	Medicine	Folklore	7			1			8		Least concern

Juniperus monosperma (Engelm.) Sarg.	Medicine	Folklore	1			3			4		Least concern
Juniperus osteosperma (Torr.) Little	Medicine	Folklore	3			8			11		Least concern
Juniperus oxycedrus L.	Material, medicine	Essential oils, folklore	14			13			27		Least concern
Juniperus phoenicea L.	Material	Essential oils	7			4			11		Least concern
Juniperus procera Hochst. ex Endl.	Material	Essential oils	3	5					8		Least concern
Juniperus sabina L.	Material, medicine	Essential oils, folklore	12		1	5			18		Least concern
Juniperus semiglobosa Regel	Medicine	Folklore	1						1		Least concern
Juniperus squamata Buch.-Ham. ex D. Don	Material, medicine	Essential oils, folklore	3			1			4		Least concern
Juniperus virginiana L.	Material, medicine, social	Essential oils, folklore, religious/secular	12			46			58		Least concern
Justicia adhatoda L.	Medicine	Source of vasicine			5				5		
Kadsura japonica (L.) Dunal	Medicine	Folklore				4			4		
Kaempferia galanga L.	Additive, medicine	Flavoring, folklore				1			1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Kaempferia parviflora Wall. ex Baker	Medicine	Folklore	1						1		
Kaempferia rotunda L.	Medicine	Folklore				1			1		
Kalanchoe crenata (Andrews) Haw.	Medicine	Folklore	3	1					4		
Kalanchoe pinnata (Lam.) Pers.	Medicine	Folklore	1						1		
Kalanchoe spathulata DC.	Medicine	Folklore						1	1		
Kalmia angustifolia L.	Medicine	Folklore	2			3			5		
Kalmia latifolia L.	Medicine	Folklore	5			10			15		Least concern
Kalopanax septemlobus (Thunb.) Koidz.	Medicine	Folklore	3			7			10		
Karwinskia humboldtiana (Schult.) Zucc.	Medicine	Folklore	11						11		
Kerria japonica (L.) DC.	Medicine	Folklore	8						8		
Kigelia africana (Lam.) Benth.	Medicine	Folklore	3	3		3			9		Least concern
Knautia arvensis (L.) Coult.	Medicine	Folklore	13			1			14		

Kopsia fruticosa (Roxb.) A. DC.	Medicine	Folklore				1			1		
Krameria lanceolata Torr.	Medicine	Folklore	1						1		
Kummerowia stipulacea (Maxim.) Makino	Medicine	Folklore	1			31		6	38		
Kummerowia striata (Thunb.) Schindl.	Medicine	Folklore	1	1		39		10	51		
Kyllinga brevifolia Rottb.	Medicine	Folklore	3			1			4		Least concern
Kyllinga nemoralis (J. R. Forst. & G. Forst.) Dandy ex Hutch. & Dalziel	Medicine	Folklore	1						1		Least concern
Lablab purpureus (L.) Sweet	Medicine	Folklore	89	740	505	170	152	271	1927		
Laburnum anagyroides Medik.	Medicine	Folklore	4			3			7		Least concern
Lachnanthes caroliniana (Lam.) Dandy	Medicine	Folklore	1						1		
Lactuca quercina L.	Medicine	Folklore	8			10			18		
Lactuca serriola L.	Medicine	Folklore	1755		3	359			2117		
Lactuca virosa L.	Medicine	Folklore	304		1	96		4	405		
Laennecia filaginoides DC.	Medicine	Folklore	1						1		
Lagenaria siceraria (Molina) Standl.	Medicine	Folklore	306	288	808	467	332	14	2215		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Lagoecia cuminoides L.	Additive	Flavoring	17						17		
Lamium album L.	Medicine	Folklore	41			2			43		
Landolphia heudelotii A. DC.	Medicine	Folklore	1						1		
Lansium domesticum Correa	Medicine	Folklore				1			1		
Lappula squarrosa (Retz.) Dumort.	Medicine	Folklore	4						4		
Larix decidua Mill.	Medicine	Folklore	8						8		Least concern
Larix laricina (Du Roi) K. Koch	Medicine	Folklore	1			2			3		Least concern
Larix occidentalis Nutt.	Medicine	Folklore	2						2		Least concern
Larrea divaricata Cav.	Medicine	Folklore	1						1		
Larrea tridentata (DC.) Coville	Medicine	Folklore	5			36			41		
Laser trilobum (L.) Borkh.	Medicine	Folklore	8						8		
Latua pubiflora (Griseb.) Baill.	Medicine, social	Folklore, hallucinogen, psychoactive, religious/secular	2						2		
Laurelia sempervirens (Ruiz & Pav.) Tul.	Additive	Flavoring	2						2		Near threatened

Laurus nobilis L.	Additive, material, medicine	Flavoring, essential oils, folklore	13			1			14		Least concern
Lavandula angustifolia Mill.	Material, medicine	Essential oils, folklore	84		5	3		1	93		Least concern
Lavandula bipinnata (Roth) Kuntze	Medicine	Folklore	1		1				2		
Lavandula dentata L.	Medicine	Folklore	2						2		
Lavandula latifolia Medik.	Material, medicine	Essential oils, folklore	29					1	30		Least concern
Lavandula stoechas L.	Additive, medicine	Flavoring, folklore	64			2			66		
Lawsonia inermis L.	Material, medicine	Tannin/dyestuff, folklore	14	9	109				132		
Leea asiatica (L.) Ridsdale	Medicine	Folklore			2				2		
Leea macrophylla Roxb. ex Hornem.	Medicine	Folklore			3				3		
Lemna minor L.	Medicine	Folklore	2						2		Least concern
Leonotis leonurus (L.) W. T. Aiton	Medicine	Folklore	2		1				3		
Leonotis nepetifolia (L.) W. T. Aiton	Medicine	Folklore	9	2	10	1	1		23		
Leonurus cardiaca L.	Medicine	Folklore	62			2		4	68		
Leonurus japonicus Houtt.	Medicine	Folklore	8		1				9		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Leonurus sibiricus L.	Medicine	Folklore	10		5		1		16		
Leonurus tataricus L.	Medicine	Folklore	2						2		
Lepidium latifolium L.	Additive, medicine	Flavoring, folklore	14		3	2			19		
Lepidium meyenii Walp.	Medicine	Folklore	4		1	1	157		163		
Lepidium sativum L.	Medicine	Folklore	304		33	89		1	427		
Lepidium virginicum L.	Medicine	Folklore	5			5	1		11		
Leptactina senegambica Hook. f.	Material	Essential oils	1						1		
Leptadenia reticulata (Retz.) Wight and Arn.	Medicine	Folklore			1				1		
Leptopyrum fumarioides (L.) Rchb.	Medicine	Folklore	1			1			2		
Leptospermum liversidgei R. T. Baker and H. G. Sm.	Material	Potential as essential oils	2					10	12		
Leptospermum petersonii F. M. Bailey	Additive, material	Flavoring, essential oils	1				1	3	5		
Leptospermum scoparium J. R. Forst. and G. Forst.	Medicine	Folklore	12					121	133		

Lespedeza bicolor Turcz.	Medicine	Folklore	5	2	70	16		14	107		
Lespedeza capitata Michx.	Medicine	Folklore	1			58		8	67		
Lessertia frutescens (L.) Goldblatt and J. C. Manning	Medicine	Folklore				3		5	8		
Leucaena esculenta (DC.) Benth.	Medicine	Folklore	3	33		18	4	3	61		
Leucaena leucocephala (Lam.) de Wit	Material	Potential as tannin/ dyestuff	28	842	20	421	157	527	1995		
Leucanthemum vulgare Lam.	Medicine	Folklore	33			88		1	122		
Leucas aspera (Willd.) Link	Additive, medicine	Flavoring, folklore			10				10		
Leucas cephalotes (Roth) Spreng.	Medicine	Folklore			2				2		
Leucas lavandulifolia Sm.	Medicine	Folklore	2						2		
Leucojum aestivum L.	Medicine	Folklore	16			2			18		Least concern
Leucojum vernum L.	Medicine	Folklore	8						8		Least concern
Levisticum officinale W. D. J. Koch	Additive, medicine	Flavoring, folklore	67			10		1	78		
Liatris spicata (L.) Willd.	Medicine	Folklore	4			15			19		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Ligularia fischeri Turcz.	Medicine	Folklore	1			1			2		
Ligusticum mutellina (L.) Crantz	Medicine	Folklore	2						2		
Ligusticum porteri J. M. Coult. and Rose	Medicine	Folklore	1			2			3		
Ligusticum scoticum L.	Medicine	Folklore	7			8			15		
Ligustrum japonicum Thunb.	Medicine	Folklore				1			1		
Ligustrum lucidum W. T. Aiton	Medicine	Folklore	1			1			2		
Ligustrum sinense Lour.	Medicine	Folklore				1			1		
Ligustrum vulgare L.	Medicine	Folklore	16			30			46		
Lilium brownii F. E. Br. ex Miellez	Medicine	Folklore	1			1			2		
Lilium canadense L.	Medicine	Folklore				5			5		
Lilium candidum L.	Medicine	Folklore	7			1			8		
Lilium lancifolium Thunb.	Medicine	Folklore				5			5		
Lilium martagon L.	Medicine	Folklore	22		1	12			35		
Lilium pumilum Redoute	Medicine	Folklore	1		1	10			12		
Limonia acidissima L.	Medicine	Folklore	1			2			3		

Limonium carolinianum (Walter) Britton	Medicine	Folklore	2						2		
Limonium gmelinii (Willd.) Kuntze	Material	Tannin/dyestuff	2						2		
Limonium sinense (Girard) Kuntze	Medicine	Folklore	2						2		
Limonium vulgare Mill.	Medicine	Folklore	3					1	4		
Linaria vulgaris Mill.	Medicine	Folklore	34					4	38		
Lindera aggregata (Sims) Kosterm.	Medicine	Folklore, veterinary				1			1		
Lindera benzoin (L.) Blume	Additive, medicine	Flavoring, folklore	3		1	13			17		
Lindera communis Hemsl.	Medicine	Folklore	2						2		
Lindera glauca (Siebold & Zucc.) Blume	Material, medicine	Essential oils, folklore	2			6			8		
Lindera megaphylla Hemsl.	Material	Essential oils	1						1		
Lindera obtusiloba Blume	Medicine	Folklore			1	20			21		
Lindernia crustacea (L.) F. Muell.	Medicine	Folklore	1						1		Least concern
Linum catharticum L.	Medicine	Folklore	10			10		2	22		
Linum usitatissimum L.	Medicine	Folklore	19871	3	661	6364	4	653	27556		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Lippia alba (Mill.) N. E. Br. ex Britton and P. Wilson	Medicine	Folklore	1						1		
Lippia graveolens Kunth	Additive, medicine	Flavoring, folklore	1						1		
Lippia javanica (Burm. f.) Spreng.	Material, medicine	Essential oils, folklore	7	3					10		
Liquidambar formosana Hance	Medicine	Folklore	1						1		Least concern
Liquidambar orientalis Mill.	Material, medicine	Essential oils, folklore	1						1		Endangered
Liquidambar styraciflua L.	Material, medicine	Essential oils, folklore	9						9		Least concern
Liriodendron tulipifera L.	Medicine	Folklore	11			5			16		Least concern
Liriope minor (Maxim.) Makino	Medicine	Folklore				2			2		
Liriope muscari (Decne.) L. H. Bailey	Medicine	Folklore	1			6			7		
Litchi chinensis Sonn.	Medicine	Folklore	39			87			126		
Lithospermum erythrorhizon Siebold and Zucc.	Material, medicine	Tannin/dyestuff, folklore	1						1		
Lithospermum officinale L.	Medicine	Folklore	23						23		

Lithospermum ruderale Douglas ex Lehm.	Medicine	Folklore	3			5			8		
Litsea cubeba (Lour.) Pers.	Additive, material, medicine	Flavoring, essential oils, folklore	2						2		
Litsea glutinosa (Lour.) C. B. Rob.	Medicine	Folklore	1		1				2		
Lobelia cardinalis L.	Medicine	Folklore	5			6			11		Least concern
Lobelia inflata L.	Medicine	Source of alpha-lobeline	5			1			6		
Lobelia laxiflora L.	Medicine	Folklore	2						2		
Lobelia siphilitica L.	Medicine	Folklore	5		1	7			13		Least concern
Lobelia tupa L.	Medicine	Folklore	4						4		
Lobularia maritima (L.) Desv.	Additive	Flavoring	15		2	9			26		
Lolium temulentum L.	Medicine	Folklore	223	1	2	61		51	338		
Lomatium dissectum (Nutt.) Mathias and Constance	Medicine	Folklore	1			53			54		
Lonicera caprifolium L.	Medicine	Folklore	3						3		
Lonicera japonica Thunb.	Medicine	Folklore	1						1		
Lophatherum gracile Brongn.	Medicine	Folklore	1						1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Lophira lanceolata Tiegh. ex Keay	Medicine	Folklore	1						1		
Lophophora williamsii (Lem. ex Salm-Dyck) J. M. Coult.	Medicine, social	Folklore, hallucinogen, masticatory, religious/secular	2						2	II	Vulnerable
Luffa acutangula (L.) Roxb.	Medicine	Folklore	16		341	72	61		490		
Luffa aegyptiaca Mill.	Medicine	Folklore	29		416	96		17	558		
Luffa operculata (L.) Cogn.	Medicine	Folklore	3						3		
Lupinus luteus L.	Medicine	Folklore	3181	8	3	348		363	3903		Least concern
Lycium barbarum L.	Medicine	Folklore	7			9			16		
Lycium chinense Mill.	Medicine	Folklore	17		3	2			22		
Lycopodium clavatum L.	Medicine	Folklore	2						2		
Lycopus americanus Muhl. ex W. P. C. Barton	Medicine	Folklore	2						2		Least concern
Lycopus europaeus L.	Medicine	Folklore	17						17		Least concern
Lycopus virginicus L.	Medicine	Folklore	1						1		Least concern
Lycoris squamigera Maxim.	Medicine	Source of galanthamine				1			1		

Lysiloma latisiliquum (L.) Benth.	Material	Tannin/dyestuff	2			1			3		
Lysimachia christinae Hance	Medicine	Folklore	1						1		
Lysimachia nummularia L.	Medicine	Folklore	3						3		Least concern
Lysimachia vulgaris L.	Material, medicine	Tannin/dyestuff, folklore	8						8		Least concern
Lythrum salicaria L.	Medicine	Folklore	73			1		3	77		Least concern
Macaranga indica Wight	Medicine	Folklore	1						1		
Maclura pomifera (Raf.) C. K. Schneid.	Material	Tannin/dyestuff	4			6			10		Least concern
Maclura tinctoria (L.) D. Don ex Steud.	Material	Tannin/dyestuff	2						2		Least concern
Macrotyloma uniflorum (Lam.) Verdc.	Medicine	Folklore	9	24	76	37	5	47	198		
Madhuca longifolia (L.) J. F. Macbr.	Medicine	Folklore				1			1		
Magnolia acuminata (L.) L.	Medicine	Folklore				11			11		Least concern
Magnolia champaca (L.) Baill. ex Pierre	Material, medicine	Essential oils, folklore				1			1		Least concern
Magnolia denudata Desr.	Medicine	Folklore	1			3			4		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Magnolia grandiflora L.	Medicine	Folklore	4						4		Least concern
Magnolia liliiflora Desr.	Medicine	Folklore	1						1		Data deficient
Magnolia obovata Thunb.	Medicine	Folklore	1		1	2			4		Least concern
Magnolia officinalis Rehder and E. H. Wilson	Medicine	Folklore				1			1		Endangered
Magnolia virginiana L.	Medicine	Folklore	3			4			7		Least concern
Mallotus philippensis (Lam.) Mull. Arg.	Medicine	Folklore	2		11	1		5	19		
Malpighia emarginata DC.	Medicine	Folklore	2			8	1		11		
Malpighia glabra L.	Medicine	Folklore				7			7		
Malpighia mexicana A. Juss.	Medicine	Folklore				1			1		
Malus domestica Borkh.	Medicine, social	Folklore, religious/ secular	25064			3101			28165		
Malus pumila Mill.	Medicine	Folklore	217		583	22			822		
Malus sylvestris (L.) Mill.	Medicine	Folklore	158			92			250		Data deficient
Malva alcea L.	Medicine	Folklore	14			6			20		
Malva arborea (L.) Webb and Berthel.	Medicine	Folklore	8			2		2	12		

Malva neglecta Wallr.	Medicine	Folklore	22		1	3			26		
Malva parviflora L.	Medicine	Folklore	23	1		8			32		
Malva pusilla Sm.	Medicine	Folklore	5						5		
Malva sylvestris L.	Medicine	Folklore	124			12			136		
Malva verticillata L.	Medicine	Folklore	81	3	22	12			118		
Mammea americana L.	Medicine	Folklore	1			9			10		
Mammea suriga (Buch.-Ham. ex Roxb.) Kosterm.	Material, medicine	Tannin/dyestuff, folklore			4				4		
Mandragora officinarum L.	Medicine	Source of scopolamine	4			1			5		
Mangifera indica L.	Medicine	Folklore	89			343	136		568		Data deficient
Manihot esculenta Crantz	Additive, medicine	Flavoring, folklore		3748		21	9483	23	13275		
Manilkara kauki (L.) Dubard	Medicine	Folklore				1			1		
Manilkara zapota (L.) P. Royen	Medicine	Folklore	1			53			54		
Mansoa alliacea (Lam.) A. H. Gentry	Medicine	Folklore				1			1		
Maranta arundinacea L.	Medicine	Folklore	2			1	1		4		
Marrubium vulgare L.	Additive, medicine	Flavoring, folklore	78		1	3		2	84		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Matricaria chamomilla L.	Additive, material, medicine, social	Flavoring, essential oils, folklore, stimulant	42		11	3			56		
Matricaria discoidea DC.	Medicine	Folklore	5						5		
Mauritia flexuosa L. f.	Medicine	Folklore				2			2		
Maytenus ilicifolia Mart. ex Reissek	Medicine	Folklore					199		199		
Mediasia macrophylla (Regel ex Schmalh.) Pimenov	Additive, medicine	Flavoring, folklore	2						2		
Medicago sativa L.	Medicine	Folklore	7954	459	988	5086	257	4506	19250		Least concern
Melaleuca alternifolia (Maiden & Betche) Cheel	Material, medicine	Essential oils, folklore	1				1	43	45		
Melaleuca cajuputi Powell	Material, medicine	Essential oils, folklore	2				7	3	12		
Melaleuca ericifolia Sm.	Material	Essential oils	1				1	4	6		
Melaleuca linariifolia Sm.	Material, medicine	Essential oils, folklore	2				1	9	12		
Melaleuca quinquenervia (Cav.) S. T. Blake	Material, medicine	Essential oils, folklore	2				3	9	14		

Melaleuca uncinata R. Br.	Material	Potential as essential oils	2				1	32	35		
Melastoma malabathricum L.	Medicine	Folklore	5						5		
Melia azedarach L.	Medicine	Folklore	8	4	12	1		4	29		Least concern
Melicope pteleifolia (Champ. ex Benth.) T. G. Hartley	Medicine	Folklore	1						1		
Melilotus albus Medik.	Medicine	Folklore	594	92	44	790	2	284	1806		
Melilotus altissimus Thuill.	Additive	Flavoring	16	5		46		13	80		
Melilotus indicus (L.) All.	Medicine	Folklore	45	17	12	163		97	334		
Melilotus officinalis (L.) Lam.	Additive, medicine	Flavoring, folklore	410	24	69	735		185	1423		
Melissa officinalis L.	Additive, material, medicine	Flavoring, essential oils, folklore	177		6	1		7	191		
Melochia corchorifolia L.	Medicine	Folklore	3	2					5		
Menispermum canadense L.	Medicine	Folklore	1						1		
Mentha aquatica L.	Additive, material, medicine	Flavoring, essential oils, folklore	57		5	30			92		Least concern
Mentha arvensis L.	Material, medicine	Essential oils, folklore	26		59	5			90		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Mentha canadensis L.	Material, medicine	Essential oils, folklore	77			53			130		
Mentha gracilis Sole	Additive, material, medicine	Flavoring, essential oils, folklore				26			26		
Mentha longifolia (L.) Huds.	Additive, medicine	Flavoring, folklore	209		2	27			238		Least concern
Mentha piperita L.	Additive, material, medicine	Flavoring, essential oils, folklore	55		1	48			104		
Mentha pulegium L.	Material, medicine	Essential oils, folklore	133		3	16			152		Least concern
Mentha spicata L.	Additive, material, medicine	Flavoring, essential oils, folklore	117		34	117		1	269		Least concern
Mentha suaveolens Ehrh.	Additive	Flavoring	37			35			72		Least concern
Mentha verticillata L.	Medicine	Folklore	2			3			5		
Mentha villosa Huds.	Additive, medicine	Flavoring, folklore	4			15			19		
Mentha villosonervata Opiz	Additive	Flavoring	1						1		
Mentzelia pumila (Nutt.) Torr. and A. Gray	Medicine	Folklore	1			1			2		
Menyanthes trifoliata L.	Medicine	Folklore	11					1	12		Least concern

Mercurialis annua L.	Medicine	Folklore	27			2			29		
Merremia discoidesperma (Donn. Sm.) O'Donell	Medicine	Folklore	1						1		
Merremia hederacea (Burm. f.) Hallier f.	Medicine	Folklore	8			1		5	14		
Mesembryanthemum crystallinum L.	Medicine	Folklore	8						8		
Mesosphaerum suaveolens (L.) Kuntze	Medicine	Folklore	12		6	1			19		
Mesua ferrea L.	Medicine	Folklore			12				12		
Mikania glomerata Spreng.	Medicine	Folklore					1		1		
Mikania scandens (L.) Willd.	Medicine	Folklore	3						3		
Milla biflora Cav.	Medicine	Folklore	1						1		
Millettia pinnata (L.) Panigrahi	Medicine	Folklore	3		1	2		1	7		
Mimosa pudica L.	Medicine	Folklore	7	2	30	2	2	3	46		Least concern
Mimosa tenuiflora (Willd.) Poir.	Medicine	Folklore	1						1		
Mimusops elengi L.	Medicine	Folklore	2			3		4	9		
Mirabilis jalapa L.	Medicine	Folklore	25	1	3	1			30		
Mirabilis multiflora (Torr.) A. Gray	Medicine	Folklore				2			2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Miscanthus floridulus (Labill.) Warb. ex K. Schum. and Lauterb.	Medicine	Folklore	3			6			9		
Mitchella repens L.	Medicine	Folklore	1			1			2		
Mollugo cerviana (L.) Ser.	Medicine	Folklore	4	1				1	6		
Mollugo pentaphylla L.	Medicine	Folklore			1				1		
Mollugo stricta L.	Medicine	Folklore	1						1		
Momordica balsamina L.	Medicine	Folklore	20		2	16			38		
Momordica charantia L.	Medicine	Folklore	14	5	497	31	11	7	565		
Momordica cochinchinensis (Lour.) Spreng.	Medicine	Folklore			5	1			6		
Monarda citriodora Cerv. ex Lag.	Material	Essential oils	13			5			18		
Monarda clinopodia L.	Medicine	Folklore				2			2		
Monarda didyma L.	Medicine	Folklore	20			3			23		
Monarda fistulosa L.	Medicine	Folklore	33			59			92		
Monarda pectinata Nutt.	Medicine	Folklore	3						3		
Monarda punctata L.	Medicine	Folklore	4			5			9		

Mondia whitei (Hook. f.) Skeels	Medicine	Folklore	1	1					2		
Moneses uniflora (L.) A. Gray	Medicine	Folklore	5			1			6		
Monodora myristica (Gaertn.) Dunal	Additive, medicine	Flavoring, folklore	1			1			2		
Monodora tenuifolia Benth.	Additive, medicine	Flavoring, folklore	2						2		
Monodora undulata (P. Beauv.) Couvreur	Additive	Flavoring	1						1		
Monotropa uniflora L.	Medicine	Folklore	1						1		
Montanoa tomentosa Cerv.	Medicine	Folklore	4						4		
Morella cerifera (L.) Small	Medicine	Folklore				3			3		
Morella pensylvanica (Mirb.) Kartesz	Medicine	Folklore	1			3			4		
Morinda citrifolia L.	Material, medicine	Tannin/dyestuff, folklore	1		174			3	178		
Moringa oleifera Lam.	Medicine	Folklore	1	30	75	1	33	4	144		
Moringa peregrina (Forssk.) Fiori	Medicine	Folklore	2		1				3		
Moringa stenopetala (Baker f.) Cufod.	Medicine	Folklore		28	1				29		
Morus alba L.	Medicine	Folklore	213		55	51			319		
Morus nigra L.	Medicine	Folklore	29			15			44		
Morus rubra L.	Medicine	Folklore	2			1		1	4		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Mucuna pruriens (L.) DC.	Medicine	Folklore, source of L-dopa	5	85	11	35	26	17	179		
Murraya paniculata (L.) Jack	Medicine	Folklore	3		1	9			13		
Musa acuminata Colla	Additive	Flavoring	170	268		157	11	26	632		Least concern
Musa basjoo Siebold and Zucc. ex Iinuma	Medicine	Folklore	4	1			1		6		
Musa paradisiaca L.	Medicine	Folklore				14			14		
Myosotis arvensis (L.) Hill	Medicine	Folklore	8					2	10		
Myosoton aquaticum (L.) Moench	Medicine	Folklore	3						3		
Myrciaria dubia (Kunth) McVaugh	Medicine	Folklore					55		55		
Myrica gale L.	Additive, material, medicine	Flavoring, potential as essential oils, folklore	11			3			14		Least concern
Myristica fragrans Houtt.	Additive, material, medicine, social	Flavoring, essential oils, folklore, hallucinogen				1			1		Data deficient
Myrrhis odorata (L.) Scop.	Additive, medicine	Flavoring, folklore	37						37		
Myrsine africana L.	Medicine	Folklore	2	2	1				5		
Myrtus communis L.	Material, medicine	Essential oils, tannin/dyestuff, folklore	19						19		Least concern

Nageia nagi (Thunb.) Kuntze	Medicine	Folklore				3			3		Near threatened
Nandina domestica Thunb.	Medicine	Folklore	1			1			2		
Narcissus jonquilla L.	Material	Essential oils	1						1		Data deficient
Narcissus poeticus L.	Material	Essential oils	5			1			6		Least concern
Narcissus pseudonarcissus L.	Medicine	Folklore	11			1			12		
Narcissus tazetta L.	Material, medicine	Essential oils, folklore	24					1	25		
Naringi crenulata (Roxb.) Nicolson	Additive, medicine	Flavoring, folklore				2			2		
Nasturtium officinale W. T. Aiton	Medicine	Folklore	73			4		1	78		Least concern
Nelumbo nucifera Gaertn.	Medicine, social	Folklore, religious/ secular			1			1	2		
Nepeta cataria L.	Additive, medicine	Flavoring, folklore	44		3	5			52		
Nerium oleander L.	Medicine	Folklore	17						17		Least concern
Neurolaena lobata (L.) Cass.	Medicine	Folklore	1						1		
Nicotiana alata Link and Otto	Social	Smoking material	48			5		2	55		
Nicotiana glauca Graham	Medicine	Folklore	30	4		9		3	46		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Nicotiana rustica L.	Medicine, social	Folklore, smoking material, stimulant	513			89	2	6	610		
Nicotiana suaveolens Lehm.	Medicine	Folklore	12			14		53	79		
Nicotiana tabacum L.	Medicine, social	Folklore, masticatory, smoking material, stimulant	6526	1	1	2180	107	643	9458		
Nigella damascena L.	Medicine	Folklore	87			2		1	90		
Nigella sativa L.	Additive, medicine	Flavoring, folklore	88		5	29			122		
Notobubon galbanum (L.) Magee	Medicine	Folklore	2						2		
Nymphaea alba L.	Medicine	Folklore	1						1		Least concern
Nymphaea nouchali Burm. f.	Medicine	Folklore		1					1		Least concern
Nypa fruticans Wurmb	Additive	Potential as sweetener				1			1		Least concern
Ochrosia borbonica J. F. Gmel.	Medicine	Folklore	1						1		Endangered
Ochrosia elliptica Labill.	Medicine	Folklore				1			1		
Ocimum africanum Lour.	Medicine	Folklore	4		2	11			17		

Ocimum americanum L.	Additive, medicine	Potential as flavoring, folklore	41	8	106	7	3		165		
Ocimum basilicum L.	Additive, material, medicine	Flavoring, essential oils, folklore	672	5	353	74	6	3	1113		
Ocimum gratissimum L.	Material, medicine	Essential oils, folklore	50	12	73	10	3	1	149		
Ocimum kilimandscharicum Baker ex Gurke	Material	Essential oils	10	9	10				29		
Ocimum minimum L.	Additive	Flavoring	22		1				23		
Ocimum tenuiflorum L.	Additive, medicine	Flavoring, folklore	31		273	10		6	320		
Odontosoria chinensis (L.) J. Sm.	Medicine	Folklore				1			1		
Oenanthe aquatica (L.) Lam.	Medicine	Folklore	5						5		Least concern
Oenanthe crocata L.	Medicine	Folklore	14						14		
Oenanthe javanica (Blume) DC.	Additive, medicine	Flavoring, folklore	2		1				3		Least concern
Oenothera biennis L.	Medicine	Folklore	110		2	176		21	309		
Oenothera pubescens Willd. ex Spreng.	Medicine	Folklore	1			5			6		
Oenothera rosea L'Her. ex Aiton	Medicine	Folklore	14			2		2	18		
Oldenlandia biflora L.	Medicine	Folklore	1						1		
Oldenlandia corymbosa L.	Medicine	Folklore	2	1					3		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Oldenlandia diffusa (Willd.) Roxb.	Medicine	Folklore	1						1		Least concern
Olea europaea L.	Medicine	Folklore	1624	3		299		1	1927		
Ononis spinosa L.	Medicine	Folklore	30		1	1		7	39		
Onopordum acanthium L.	Medicine	Folklore	37					5	42		
Onosma echioides L.	Medicine	Folklore	2						2		
Operculina macrocarpa (L.) Urb.	Medicine	Folklore	1						1		
Operculina turpethum (L.) Silva Manso	Medicine	Folklore	4		12				16		
Ophiopogon japonicus (Thunb.) Ker Gawl.	Medicine	Folklore	2			8			10		
Oplopanax elatus (Nakai) Nakai	Medicine	Folklore				1			1		
Oplopanax horridus (Sm.) Miq.	Medicine	Folklore	2			12			14		
Opuntia ficus-indica (L.) Mill.	Medicine	Folklore	3			152			155	II	Data deficient
Opuntia stricta (Haw.) Haw.	Material, medicine	Tannin/dyestuff, folklore	4						4	II	Least concern
Orchis mascula (L.) L.	Medicine	Folklore	5						5	II	
Origanum majorana L.	Additive, material, medicine	Flavoring, essential oils, folklore	27		3	2		1	33		

Origanum onites L.	Additive	Flavoring	6					1	7		
Origanum syriacum L.	Additive	Flavoring	3						3		
Origanum vulgare L.	Additive, medicine	Flavoring, folklore	543		35	22		3	603		
Ornithogalum umbellatum L.	Medicine	Folklore	3			1			4		
Oroxylum indicum (L.) Vent.	Medicine	Folklore	1		42				43		
Orthosiphon aristatus (Blume) Miq.	Medicine	Folklore	2						2		
Osbeckia chinensis L.	Medicine	Folklore	1						1		
Osyris quadripartita Salzm. ex Decne.	Medicine	Folklore	2						2		
Otholobium glandulosum (L.) J. W. Grimes	Medicine	Folklore	1			1			2		
Oxalis acetosella L.	Additive, medicine	Flavoring, folklore	28		1				29		
Oxalis corniculata L.	Medicine	Folklore	2	6	1				9		
Pachira aquatica Aubl.	Material	Tannin/dyestuff				3			3		
Packera aurea (L.) A. Love & D. Love	Medicine	Folklore	1			2			3		
Paederia foetida L.	Medicine	Folklore	2		1				3		
Paeonia anomala L.	Medicine	Folklore	6		1				7		
Paeonia lactiflora Pall.	Medicine	Folklore	15			2			17		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Paeonia obovata Maxim.	Medicine	Folklore				1			1		
Paeonia officinalis L.	Medicine	Folklore	195						195		Least concern
Paeonia suffruticosa Andrews	Medicine	Folklore	10						10		
Panax pseudoginseng Wall.	Medicine	Folklore	1						1		
Panax quinquefolius L.	Additive, medicine	Flavoring, folklore				1			1	II	
Pandanus fascicularis Lam.	Additive, material, medicine	Flavoring, essential oils, folklore			2				2		
Pandanus tectorius Parkinson	Medicine	Folklore				2			2		
Panicum miliaceum L.	Medicine	Folklore	16941	7	1920	730		258	19856		
Papaver bracteatum Lindl.	Medicine	Folklore	37			254			291		
Papaver rhoeas L.	Medicine	Folklore	173			15		4	192		
Papaver somniferum L.	Additive, medicine, social	Flavoring, folklore, source of codeine, morphine, noscapine, papaverine, narcotic	4473		427	21			4921		
Parietaria judaica L.	Medicine	Folklore	16						16		
Parietaria officinalis L.	Medicine	Folklore	3						3		

Parietaria pensylvanica Muhl. ex Willd.	Medicine	Folklore	1			1			2		
Paris polyphylla Sm.	Medicine	Folklore	1						1		
Paris quadrifolia L.	Medicine	Folklore	6						6		
Parmentiera aculeata (Kunth) Seem.	Medicine	Folklore	2			3			5		
Parnassia palustris L.	Medicine	Folklore	13			7			20		Least concern
Paronychia argentea Lam.	Medicine	Folklore	16		6				22		
Parthenium integrifolium L.	Medicine	Folklore	1						1		
Parthenocissus quinquefolia (L.) Planch.	Medicine	Folklore	7		1	11			19		
Paspalum scrobiculatum L.	Medicine	Folklore	21	58	678	338	17	22	1134		Least concern
Passiflora caerulea L.	Medicine	Folklore	3			1	2		6		
Passiflora coriacea Juss.	Medicine	Folklore	1			1			2		
Passiflora edulis Sims	Medicine	Folklore	2	2	1	25	20		50		
Passiflora foetida L.	Medicine	Folklore	8	2	18		2	3	33		
Passiflora incarnata L.	Additive, medicine	Flavoring, folklore	1			1	1		3		
Passiflora laurifolia L.	Medicine	Folklore	1				2		3		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Passiflora quadrangularis L.	Medicine	Folklore	1		1	2	2		6		
Pastinaca sativa L.	Medicine	Folklore	322		1	143		1	467		
Patrinia villosa (Thunb.) Juss.	Medicine	Folklore	1						1		
Paullinia cupana Kunth	Additive, medicine, social	Flavoring, folklore, stimulant					307		307		
Paullinia pinnata L.	Medicine	Folklore	3	1					4		
Pedalium murex L.	Medicine	Folklore		2	20				22		
Pedicularis bracteosa Benth.	Medicine	Folklore	2			3			5		
Pedicularis canadensis L.	Medicine	Folklore	2						2		
Pedicularis centranthera A. Gray	Medicine	Folklore	1						1		
Pedicularis densiflora Benth.	Medicine	Folklore	1			2			3		
Pedicularis groenlandica Retz.	Medicine	Folklore	2			6			8		Least concern
Pedicularis racemosa Douglas ex Hook.	Medicine	Folklore	1			1			2		
Peganum harmala L.	Material, medicine	Tannin/dyestuff, folklore	34		19	5		1	59		
Pelargonium capitatum (L.) L'Her.	Medicine	Folklore	8			1		1	10		
Pelargonium crispum (P. J. Bergius) L'Her.	Additive	Flavoring	1			1			2		

Pelargonium graveolens L'Her.	Additive, material, medicine	Flavoring, essential oils, folklore			1	4			5		
Pelargonium hybr.	Material	Essential oils				5			5		
Pelargonium odoratissimum (L.) L'Her.	Material	Essential oils				1			1		
Pelargonium reniforme Curtis	Medicine	Folklore				1			1		
Pelargonium sidoides DC.	Medicine	Folklore				1			1		
Pelargonium tomentosum Jacq.	Material	Potential as essential oils				1			1		
Pentaclethra macrophylla Benth.	Medicine	Folklore	1						1		
Peperomia pellucida (L.) Kunth	Medicine	Folklore	3		2				5		
Peperomia tetraphylla (G. Forst.) Hook. and Arn.	Medicine	Folklore	3					3	6		
Pergularia daemia (Forssk.) Chiov.	Medicine	Folklore	6	3	6				15		
Periandra mediterranea (Vell.) Taub.	Additive, medicine	Potential as flavoring, folklore					1		1		Least concern
Perilla frutescens (L.) Britton	Additive, medicine	Flavoring, folklore	45		2118	26		8	2197		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Periploca forrestii Schltr.	Medicine	Folklore	1						1		
Persea americana Mill.	Medicine	Folklore	53			329			382		Least concern
Persicaria capitata (Buch.-Ham. ex D. Don) H. Gross	Medicine	Folklore	2			1			3		
Persicaria chinensis (L.) H. Gross	Medicine	Folklore	1		3				4		
Persicaria hydropiper (L.) Delarbre	Medicine	Folklore	17		1			1	19		Least concern
Persicaria tinctoria (Aiton) Spach	Material, medicine	Tannin/dyestuff, folklore	1		6				7		
Petasites frigidus (L.) Fr.	Medicine	Folklore	1			1			2		Least concern
Petasites hybridus (L.) G. Gaertn. et al.	Medicine	Folklore	18			1			19		
Petiveria alliacea L.	Medicine	Folklore	1						1		
Petroselinum crispum (Mill.) Fuss	Additive, material, medicine	Flavoring, essential oils, folklore	1209		28	195		12	1444		
Peucedanum formosanum Hayata	Medicine	Folklore				1			1		
Peucedanum officinale L.	Medicine	Folklore	10						10		
Peucedanum oreoselinum (L.) Moench	Medicine	Folklore	13						13		

Peucedanum ostruthium (L.) W. D. J. Koch	Additive, medicine	Flavoring, folklore, veterinary	9						9		
Peucedanum praeruptorum Dunn	Medicine	Folklore	1						1		
Peucedanum ruthenicum M. Bieb.	Medicine	Folklore	2						2		
Pfaffia glomerata (Spreng.) Pedersen	Medicine	Folklore					1		1		
Phalaris canariensis L.	Medicine	Folklore	60			101		19	180		
Phellodendron amurense Rupr.	Medicine	Folklore	11			9			20		
Philenoptera cyanescens (Schumach.) Roberty	Material	Tannin/dyestuff	2	1					3		
Phlebodium aureum (L.) J. Sm.	Medicine	Folklore	1						1		
Phoenix dactylifera L.	Medicine	Folklore	188			217			405		
Phoenix sylvestris (L.) Roxb.	Additive	Sweetener	31			20			51		
Phragmites australis (Cav.) Trin. ex Steud.	Medicine	Folklore	34		1	8		9	52		Least concern
Phyla dulcis (Trevir.) Moldenke	Medicine	Folklore	1						1		
Phyla nodiflora (L.) Greene	Medicine	Folklore	10	1		1			12		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Phyllanthus acidus (L.) Skeels	Additive, medicine	Flavoring, folklore			2	2			4		
Phyllanthus amarus Schumach.	Medicine	Folklore	1				6		7		
Phyllanthus cochinchinensis Spreng.	Medicine	Folklore	1						1		
Phyllanthus emblica L.	Medicine	Folklore	2		13				15		
Phyllanthus fraternus G. L. Webster	Medicine	Folklore	2		2				4		
Phyllanthus maderaspatensis L.	Medicine	Folklore	5	1					6		
Phyllanthus niruri L.	Medicine	Folklore			3		5		8		
Phyllanthus reticulatus Poir.	Medicine	Folklore		2	1			1	4		
Phyllanthus tenellus Roxb.	Medicine	Potential source of pharmaceutical agent					11		11		
Phyllanthus urinaria L.	Medicine	Folklore	2				3		5		
Phyllanthus virgatus G. Forst.	Medicine	Folklore						1	1		
Phyllodium pulchellum (L.) Desv.	Medicine	Folklore	1	11			19	3	34		Least concern

Phyllolobium chinense Fisch. ex Spreng.	Medicine	Folklore				2		1	3		
Phyllostachys nigra (Lodd. ex Lindl.) Munro	Medicine	Folklore	2			14			16		
Physalis alkekengi L.	Medicine	Folklore	82		4	4			90		
Physalis minima L.	Medicine	Folklore	2		2	1			5		
Physalis peruviana L.	Medicine	Folklore	81	1	1	7			90		
Physalis philadelphica Lam.	Medicine	Folklore	55			225			280		Least concern
Physostigma venenosum Balf.	Medicine	Source of physostigmine		1					1		
Phytolacca acinosa Roxb.	Medicine	Folklore	13		4				17		
Phytolacca americana L.	Medicine	Folklore	21		1	2		1	25		
Phytolacca dodecandra L'Her.	Medicine	Folklore	1	3					4		
Phytolacca icosandra L.	Medicine	Folklore	4		1				5		
Phytolacca rivinoides Kunth and C. D. Bouche	Medicine	Folklore	1						1		
Picea abies (L.) H. Karst.	Material, medicine, social	Essential oils, folklore, religious/secular	46						46		Least concern
Picea glauca (Moench) Voss	Material, medicine, social	Essential oils, folklore, religious/secular	9			6			15		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Picea jezoensis (Siebold & Zucc.) Carriere	Material	Essential oils	2			7			9		Least concern
Picea mariana (Mill.) Britton et al.	Material, social	Essential oils, religious/secular	2			1			3		Least concern
Picea obovata Ledeb.	Social	Religious/secular	1						1		Least concern
Picea pungens Engelm.	Social	Religious/secular	17			1			18		Least concern
Picea rubens Sarg.	Social	Religious/secular	2						2		Least concern
Picrasma quassioides (D. Don) Benn.	Medicine	Folklore			1				1		
Picrorhiza kurrooa Royle ex Benth.	Medicine	Folklore			2				2	II	
Pilea pumila (L.) A. Gray	Medicine	Folklore	2						2		
Pilocarpus microphyllus Stapf ex Wardleworth	Medicine	Source of pilocarpine					52		52		
Pilosella officinarum Vaill.	Medicine	Folklore	9						9		
Pimenta dioica (L.) Merr.	Additive, medicine	Flavoring, folklore				1			1		
Pimenta racemosa (Mill.) J. W. Moore	Additive, material, medicine	Flavoring, essential oils, folklore				1			1		

Pimpinella anisum L.	Additive, material, medicine	Flavoring, essential oils, folklore	102		5	34		7	148		
Pimpinella major (L.) Huds.	Medicine	Folklore	17			5			22		
Pimpinella saxifraga L.	Medicine	Folklore	36			13			49		
Pinguicula vulgaris L.	Medicine	Folklore	6						6		Least concern
Pinus banksiana Lamb.	Medicine	Folklore	4						4		Least concern
Pinus elliottii Engelm.	Medicine	Folklore	1			5			6		Least concern
Pinus halepensis Mill.	Additive	Flavoring	4			1			5		Least concern
Pinus koraiensis Siebold and Zucc.	Material, medicine	Tannin/dyestuff, folklore	2			44			46	III	Least concern
Pinus massoniana Lamb.	Material, medicine	Tannin/dyestuff, folklore				1			1		Least concern
Pinus mugo Turra	Additive, medicine	Flavoring, folklore	7			4			11		Least concern
Pinus nigra J. F. Arnold	Medicine	Folklore	17			20			37		Least concern
Pinus palustris Mill.	Additive, medicine	Flavoring, folklore				34			34		Endangered
Pinus patula Schltdl. and Cham.	Medicine	Folklore	16	1		1			18		Least concern

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Pinus pinaster Aiton	Medicine	Folklore	1			9			10		Least concern
Pinus roxburghii Sarg.	Medicine	Folklore			1	1			2		Least concern
Pinus strobiformis Engelm.	Medicine	Folklore	1			111			112		Least concern
Pinus strobus L.	Medicine, social	Folklore, religious/secular	8			5			13		Least concern
Pinus sylvestris L.	Additive, material, medicine, social	Flavoring, tannin/dyestuff, folklore, religious/secular	36		1	455			492		Least concern
Pinus tabuliformis Carriere	Material, medicine	Tannin/dyestuff, folklore			1	4			5		Least concern
Pinus virginiana Mill.	Social	Religious/secular	1			6			7		Least concern
Pinus yunnanensis Franch.	Material	Tannin/dyestuff	13						13		Least concern
Piper aduncum L.	Medicine	Folklore	1				742		743		
Piper hispidinervum C. DC.	Material	Potential as essential oils					1714		1714		
Piper nigrum L.	Additive, medicine	Flavoring, folklore	5			2	6		13		
Piper umbellatum L.	Additive, medicine	Flavoring, folklore	2	1					3		
Piscidia piscipula (L.) Sarg.	Medicine	Folklore	3			1			4		

Pistacia atlantica Desf.	Material	Tannin/dyestuff	10	2		72			84		Near threatened
Pistacia integerrima J. L. Stewart	Material, medicine	Tannin/dyestuff, folklore	1			22			23		
Pistacia lentiscus L.	Medicine, social	Folklore, masticatory	42	1		10			53		Least concern
Pistacia terebinthus L.	Material	Tannin/dyestuff	15			40			55		Least concern
Pithecellobium dulce (Roxb.) Benth.	Medicine	Folklore	5	2	6	3			16		
Pittosporum undulatum Vent.	Material	Essential oils	1					12	13		
Plantago afra L.	Medicine	Folklore	31			1		15	47		
Plantago arenaria Waldst. and Kit.	Medicine	Folklore	14		6	8		8	36		
Plantago asiatica L.	Medicine	Folklore	4		13				17		
Plantago depressa Willd.	Medicine	Folklore	1		1	2		1	5		
Plantago lanceolata L.	Medicine	Folklore	303	1	41	4	1	279	629		
Plantago major L.	Medicine	Folklore	151	1	3	3	2	65	225		Least concern
Plantago media L.	Medicine	Folklore	52					18	70		
Plantago ovata Forssk.	Medicine	Folklore	18		77	48	1	7	151		
Platycladus orientalis (L.) Franco	Medicine	Folklore	5		1	7			13		Near threatened

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Platycodon grandiflorus (Jacq.) A. DC.	Medicine	Folklore	10		2	4			16		
Plectranthus amboinicus (Lour.) Spreng.	Additive, material, medicine	Flavoring, essential oils, folklore	1	2					3		
Plectranthus barbatus Andrews	Medicine	Folklore	4	3	1	1			9		
Plectranthus scutellarioides (L.) R. Br.	Medicine	Folklore	1			26		4	31		
Pluchea odorata (L.) Cass.	Medicine	Folklore	4			2			6		
Plumbago zeylanica L.	Medicine	Folklore	5	2	27				34		
Plumeria alba L.	Medicine	Folklore	2			1			3		
Plumeria obtusa L.	Medicine	Folklore				7			7		
Plumeria rubra L.	Medicine	Folklore	2			3			5		
Podophyllum hexandrum Royle	Medicine	Folklore	1		6				7	II	
Podophyllum peltatum L.	Medicine	Source of etoposide, podophyllotoxin, teniposide	2						2		
Podophyllum pleianthum Hance	Medicine	Folklore				1			1		
Pogostemon benghalensis (Burm. f.) Kuntze	Material	Essential oils			1				1		

Pogostemon cablin (Blanco) Benth.	Additive, material, medicine	Flavoring, essential oils, folklore	4					1	5		
Polemonium caeruleum L.	Medicine	Folklore	19			7			26		
Polemonium reptans L.	Medicine	Folklore	1						1		
Poliomintha incana (Torr.) A. Gray	Additive	Flavoring	1			1			2		
Pollia japonica Thunb.	Medicine	Folklore	1						1		
Polycarpaea corymbosa (L.) Lam.	Medicine	Folklore	8					3	11		
Polygala amara L.	Medicine	Folklore	1						1		
Polygala japonica Houtt.	Medicine	Folklore			1			2	3		
Polygonatum biflorum (Walter) Elliott	Medicine	Folklore	2			5			7		
Polygonatum cirrhifolium (Wall.) Royle	Medicine	Folklore			1	1			2		
Polygonatum multiflorum (L.) All.	Medicine	Folklore	8			2			10		
Polygonatum odoratum (Mill.) Druce	Medicine	Folklore	24		1	9			34		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Polygonatum sibiricum Redoute	Medicine	Folklore				2			2		
Polygonum aviculare L.	Medicine	Folklore	10			1			11		
Polypodium vulgare L.	Medicine	Folklore	4						4		
Poncirus trifoliata (L.) Raf.	Medicine	Folklore	31		7	139			177		
Populus alba L.	Material	Tannin/dyestuff	136			2			138		Least concern
Populus balsamifera L.	Additive, medicine	Flavoring, folklore	1			1			2		Least concern
Populus deltoides W. Bartram ex Marshall	Medicine	Folklore	3			1			4		
Populus fremontii S. Watson	Medicine	Folklore				1			1		
Populus nigra L.	Medicine	Folklore	647						647		Data deficient
Populus tremula L.	Medicine	Folklore	7			1			8		Least concern
Populus tremuloides Michx.	Medicine	Folklore				1			1		
Populus trichocarpa Torr. and A. Gray	Medicine	Folklore	1			1			2		Least concern
Poraqueiba sericea Tul.	Medicine	Folklore					1		1		

Porophyllum ruderale (Jacq.) Cass.	Medicine	Folklore	11			2			13		
Portulaca grandiflora Hook.	Medicine	Folklore	17		2				19		
Portulaca oleracea L.	Medicine	Folklore	71	2	20	10		11	114		
Portulaca pilosa L.	Medicine	Folklore	6	2		3		1	12		
Potentilla anserina L.	Medicine	Folklore	13			7			20		Least concern
Potentilla chinensis Ser.	Medicine	Folklore	1		3	2			6		
Potentilla erecta (L.) Raeusch.	Medicine	Folklore	57			5			62		
Potentilla fragarioides L.	Medicine	Source of catechin				2			2		
Potentilla reptans L.	Medicine	Folklore	7			3			10		
Pothos scandens L.	Medicine	Folklore	2						2		
Prangos ferulacea (L.) Lindl.	Additive	Flavoring	15						15		
Prangos pabularia Lindl.	Medicine	Folklore	5						5		
Prangos uloptera DC.	Medicine	Folklore	3						3		
Premna herbacea Roxb.	Medicine	Folklore			1				1		
Premna serratifolia L.	Medicine	Folklore	1						1		
Primula veris L.	Medicine	Folklore	98		2	4		6	110		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Prinsepia uniflora Batalin	Medicine	Folklore				1			1		
Prosopis cineraria (L.) Druce	Medicine	Folklore	39	1	1	2			43		
Prosopis juliflora (Sw.) DC.	Material, Medicine	Tannin/dyestuff, folklore	10	22	1	3	13		49		
Prosopis laevigata (Humb. & Bonpl. ex Willd.) M. C. Johnst.	Material, Medicine	Tannin/dyestuff, folklore	6			3			9		Lower risk/ least concern
Prosopis pallida (Humb. & Bonpl. ex Willd.) Kunth	Material	Tannin/dyestuff	33	3					36		
Prosopis strombulifera (Lam.) Benth.	Medicine	Folklore	1						1		
Prunella vulgaris L.	Medicine	Folklore	112		6	81		8	207		Least concern
Prunus africana (Hook. f.) Kalkman	Medicine	Folklore	2			2			4	II	Vulnerable
Prunus apetala (Siebold & Zucc.) Franch. and Sav.	Medicine	Folklore	1						1		
Prunus armeniaca L.	Material, medicine	Essential oils, folklore	2428		60	661	1		3150		
Prunus avium (L.) L.	Medicine	Folklore	5932		53	349			6334		Least concern
Prunus cerasoides D. Don	Additive	Flavoring	1	1		4			6		

Prunus cerasus L.	Medicine	Folklore	1025		2	268	2		1297		
Prunus domestica L.	Additive, medicine	Flavoring, folklore	4715		10	440			5165		Data deficient
Prunus dulcis (Mill.) D. A. Webb	Additive, medicine	Flavoring, folklore	761			308			1069		
Prunus ilicifolia (Nutt. ex Hook. & Arn.) D. Dietr.	Medicine	Folklore	1			4			5		
Prunus japonica Thunb.	Medicine	Folklore				4			4		
Prunus laurocerasus L.	Additive, medicine	Flavoring, folklore	22			56			78		
Prunus mahaleb L.	Additive	Flavoring	32			32			64		Least concern
Prunus mume Siebold & Zucc.	Medicine	Folklore	6		86	51			143		
Prunus padus L.	Medicine	Folklore	32		1	21			54		Least concern
Prunus persica (L.) Batsch	Medicine	Folklore	2758		218	994	181		4151		
Prunus salicina Lindl.	Medicine	Folklore	224		82	146	147		599		Least concern
Prunus serotina Ehrh.	Additive, medicine	Flavoring, folklore	12			33			45		
Prunus spinosa L.	Medicine	Folklore	82		1	31			114		Least concern
Prunus virginiana L.	Medicine	Folklore	24			26			50		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Pseudocydonia sinensis (Thouin) C. K. Schneid.	Medicine	Folklore				4			4		
Pseudognaphalium obtusifolium (L.) Hilliard and B. L. Burtt	Medicine	Folklore	1						1		
Pseudotsuga menziesii (Mirb.) Franco	Medicine, social	Folklore, religious/secul	15			5			20		Least concern
Psidium guajava L.	Medicine	Folklore	13			54	17		84		
Psilocaulon coriarium (Burch. ex N. E. Br.) N. E. Br.	Material	Chemicals	1						1		
Psophocarpus tetragonolobus (L.) DC.	Medicine	Folklore	3	58	314	185	17	174	751		
Psychotria asiatica L.	Medicine	Folklore	1						1		
Psydrax subcordata (DC.) Bridson	Medicine	Folklore	2						2		
Ptelea trifoliata L.	Medicine	Folklore	10			8			18		
Pteridium aquilinum (L.) Kuhn	Medicine	Folklore	1						1		
Pterocarpus indicus Willd.	Material, medicine	Tannin/dyestuff, folklore	1			2			3		Vulnerable

Pterocarpus soyauxii Taub.	Material	Tannin/dyestuff	1						1		
Pteroceltis tatarinowii Maxim.	Material	Essential oils	1			2			3		
Pterodon emarginatus Vogel	Medicine	Folklore	1				2		3		
Pueraria montana (Lour.) Merr.	Medicine	Folklore		3		15	17		35		
Pulicaria dysenterica (L.) Bernh.	Medicine	Folklore	18						18		
Pulmonaria officinalis L.	Medicine	Folklore	20						20		Least concern
Punica granatum L.	Medicine	Folklore	687		3	485			1175		Least concern
Putranjiva roxburghii Wall.	Medicine	Folklore	1		3	1			5		
Pycnanthemum virginianum (L.) T. Durand and B. D. Jacks. ex B. L. Rob. and Fernald	Additive	Flavoring	1			12			13		
Pycnanthus angolensis (Welw.) Warb.	Medicine	Folklore	1						1		
Pyrola asarifolia Michx.	Medicine	Folklore	2			2			4		
Pyrola rotundifolia L.	Medicine	Folklore	3						3		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Pyrostegia venusta (Ker Gawl.) Miers	Medicine	Folklore				1			1		
Pyrus communis L.	Medicine	Folklore	12979		128	1365			14472		Least concern
Quercus alba L.	Medicine	Folklore				6			6		Least concern
Quercus petraea (Matt.) Liebl.	Medicine	Folklore	7			10			17		Least concern
Quercus robur L.	Medicine	Folklore	26			10			36		Least concern
Quillaja saponaria Molina	Additive, material, medicine	Flavoring, essential oils, folklore	1						1		
Radermachera xylocarpa (Roxb.) K. Schum.	Medicine	Folklore			1				1		
Ranunculus acris L.	Medicine	Folklore	53					27	80		
Ranunculus bulbosus L.	Medicine	Folklore	9						9		
Ranunculus cantoniensis DC.	Medicine	Folklore	2						2		
Ranunculus chinensis Bunge	Medicine	Folklore			2				2		
Ranunculus japonicus Thunb.	Medicine	Folklore	1			3			4		
Ranunculus sceleratus L.	Medicine	Folklore	5					1	6		Least concern

Raphanus raphanistrum L.	Medicine	Folklore	223		11	6		39	279		
Raphanus sativus L.	Medicine	Folklore	3460		2671	1178	1	14	7324		
Rauvolfia caffra Sond.	Medicine	Folklore		2					2		
Rauvolfia serpentina (L.) Benth. ex Kurz	Medicine	Source of ajmalicine, rescinnamine, reserpine	1		16	1			18	II	
Rauvolfia tetraphylla L.	Medicine	Source of deserpidine	2		16				18		
Rauvolfia verticillata (Lour.) Baill.	Medicine	Folklore	2						2		
Rauvolfia vomitoria Afzel.	Medicine	Source of reserpine	1						1		
Reissantia indica (Willd.) N. Halle	Medicine	Folklore	2						2		
Reseda luteola L.	Material	Tannin/dyestuff	87			1			88		
Reseda odorata L.	Material	Essential oils	9						9		
Reynoutria japonica Houtt.	Medicine	Folklore	2						2		
Rhamnus cathartica L.	Medicine	Folklore	14			4			18		Least concern
Rhaponticum carthamoides (Willd.) Iljin	Medicine	Folklore	7			4			11		
Rheum australe D. Don	Medicine	Folklore	6		2				8		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Rheum officinale Baill.	Additive, medicine	Flavoring, folklore	13			3			16		
Rheum palmatum L.	Additive, medicine	Flavoring, folklore	12			7			19		
Rheum tanguticum (Maxim. ex Regel) Maxim. ex Balf.	Medicine	Folklore	2			1			3		
Rhinacanthus nasutus (L.) Kurz	Medicine	Folklore	1						1		
Rhodiola kirilowii (Regel) Maxim.	Medicine	Folklore	7						7		
Rhodiola rosea L.	Medicine	Folklore	38			1			39		
Rhododendron campanulatum D. Don	Medicine	Folklore	13						13		
Rhododendron ferrugineum L.	Medicine	Folklore	20			2			22		Least concern
Rhododendron groenlandicum (Oeder) Kron and Judd	Medicine	Folklore	6			1			7		
Rhododendron molle (Blume) G. Don	Medicine	Source of rhomitoxin	7			1			8		
Rhododendron ponticum L.	Medicine	Folklore	23			3			26		
Rhododendron subarcticum Harmaja	Medicine	Folklore				2			2		

Rhododendron tomentosum Harmaja	Medicine	Folklore	4			5			9		
Rhodotypos scandens (Thunb.) Makino	Medicine	Folklore			1	6			7		
Rhus aromatica Aiton	Medicine	Folklore	2			30			32		
Rhus chinensis Mill.	Material, medicine	Tannin/dyestuff, folklore	3		1	4			8		
Rhus coriaria L.	Additive, medicine	Flavoring, folklore	34			1			35		Vulnerable
Rhus glabra L.	Medicine	Folklore	4			12			16		
Rhus integrifolia (Nutt.) Benth. & Hook. f. ex W. H. Brewer and S. Watson	Medicine	Folklore	1						1		
Rhynchosia pyramidalis (Lam.) Urb.	Medicine	Folklore	1	6	1	2	3	6	19		Least concern
Rhynchosia volubilis Lour.	Medicine	Folklore	1	1	3			1	6		
Ribes nigrum L.	Additive, material, medicine	Flavoring, essential oils, folklore	1430		2	376			1808		
Ribes rubrum L.	Medicine	Folklore	628			65			693		
Ribes uva-crispa L.	Medicine	Folklore	628			340			968		
Ricinodendron heudelotii (Baill.) Heckel	Medicine	Folklore	1						1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Ricinus communis L.	Additive, medicine	Flavoring, folklore	1729	45	91	1716	1287	1	4869		
Ritchiea reflexa (Thonn.) Gilg and Benedict	Medicine	Folklore	1						1		
Robinia pseudoacacia L.	Medicine	Folklore	17	3		23		5	48		Least concern
Rorippa indica (L.) Hiern	Medicine	Source of roritone	5		1				6		
Rosa alba L.	Medicine	Folklore	12						12		
Rosa arkansana Porter	Additive	Flavoring	4			2			6		
Rosa bella Rehder and E. H. Wilson	Material, medicine	Essential oils, folklore				1			1		
Rosa canina L.	Medicine	Folklore	72			7			79		
Rosa centifolia L.	Additive, material, medicine	Flavoring, essential oils, folklore	8						8		
Rosa chinensis Jacq.	Medicine	Folklore	1						1		
Rosa damascena Herrm.	Additive, material, medicine	Flavoring, essential oils, folklore	10						10		
Rosa davurica Pall.	Medicine	Folklore	1			6			7		
Rosa gallica L.	Material, medicine	Essential oils, folklore	14			1			15		
Rosa laevigata Michx.	Material, medicine	Tannin/dyestuff, folklore	1						1		

Rosa lucieae Franch. and Rochebr. ex Crep.	Medicine	Folklore				2			2		
Rosa multiflora Thunb.	Medicine	Folklore	4		1	4			9		
Rosa roxburghii Tratt.	Medicine	Folklore	6			1			7		
Rosa rugosa Thunb.	Material, medicine	Essential oils, folklore	10		2	15			27		
Rosmarinus officinalis L.	Additive, material, medicine	Flavoring, essential oils, folklore	114		3	1			118		
Rotheca serrata (L.) Steane and Mabb.	Medicine	Folklore			5				5		
Rubia cordifolia L.	Material, medicine	Tannin/dyestuff, folklore	3	2	13	2			20		
Rubia tinctorum L.	Material, medicine	Tannin/dyestuff, folklore	31						31		
Rubus allegheniensis Porter	Additive	Flavoring	2		4	45			51		
Rubus chamaemorus L.	Additive	Flavoring	2			46			48		Least concern
Rubus chingii Hu	Medicine	Folklore				8			8		
Rubus corchorifolius L. f.	Material, medicine	Tannin/dyestuff, folklore	1			14			15		
Rubus cuneifolius Pursh	Additive	Flavoring				1			1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Rubus fruticosus auct.	Medicine	Folklore	38			1			39		
Rubus ichangensis Hemsl. anand Kuntze	Material, medicine	Tannin/dyestuff, folklore				1			1		
Rubus idaeus L.	Medicine	Folklore	630			522			1152		
Rubus laciniatus Willd.	Medicine	Folklore	2			10			12		
Rubus parvifolius L.	Material, medicine	Tannin/dyestuff, folklore	4			46		5	55		
Rudbeckia fulgida Aiton	Medicine	Folklore	7			71			78		
Rumex acetosa L.	Medicine	Folklore	228		1	5	1		235		
Rumex acetosella L.	Medicine	Folklore	30	1		1		17	49		
Rumex alpinus L.	Medicine	Folklore	14						14		
Rumex aquaticus L.	Medicine	Folklore	13						13		
Rumex crispus L.	Medicine	Folklore	83		6	3			92		
Rumex hymenosepalus Torr.	Material, medicine	Tannin/dyestuff, folklore	1						1		
Rumex obtusifolius L.	Medicine	Folklore	44		1			21	66		
Rumex vesicarius L.	Medicine	Folklore	6					7	13		
Rungia pectinata (L.) Nees	Medicine	Folklore	1						1		
Ruscus aculeatus L.	Medicine	Folklore	33			2			35		
Ruta chalepensis L.	Medicine	Folklore	46			1			47		

Ruta graveolens L.	Additive, material, medicine	Flavoring, essential oils, folklore	100		3	3		4	110		
Saccharum arundinaceum Retz.	Medicine	Folklore	1			124			125		
Saccharum bengalense Retz.	Medicine	Folklore				17			17		
Saccharum officinarum L.	Additive, medicine	Flavoring, folklore		303	9	1102	35		1449		
Saccharum spontaneum L.	Medicine	Folklore	7	1	253	748	4		1013		Least concern
Sagina japonica (Sw.) Ohwi	Medicine	Folklore			1				1		
Salix acutifolia Willd.	Material	Tannin/dyestuff	2						2		Least concern
Salix alba L.	Medicine	Source of salicin	10			4			14		Least concern
Salix babylonica L.	Medicine	Folklore	7		1				8		
Salix caprea L.	Material, medicine	Tannin/dyestuff, folklore	7			1			8		Least concern
Salix cinerea L.	Material	Tannin/dyestuff	5			2			7		Least concern
Salix daphnoides Vill.	Medicine	Folklore	2						2		Least concern
Salix discolor Muhl.	Medicine	Folklore	1						1		
Salix fragilis L.	Medicine	Source of salicin	2			1			3		
Salix integra Thunb.	Material	Tannin/dyestuff	1			1			2		
Salix lucida Muhl.	Medicine	Folklore				3			3		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Salix pentandra L.	Material, medicine	Tannin/dyestuff, folklore	6			3			9		Least concern
Salix phylicifolia L.	Material	Tannin/dyestuff	1						1		
Salix purpurea L.	Medicine	Folklore	5			5			10		Least concern
Salix triandra L.	Material	Tannin/dyestuff	6			3			9		Least concern
Salsola collina Pall.	Medicine	Folklore	2			3			5		
Salvadora persica L.	Medicine	Source of chlorhexidine gluconate	3	1	4				8		
Salvia apiana Jeps.	Medicine	Folklore	4			5			9		
Salvia coccinea Buc'hoz ex Etl.	Medicine	Folklore	9			1			10		
Salvia columbariae Benth.	Medicine	Folklore	7			23			30		
Salvia elegans Vahl	Additive, medicine	Flavoring, folklore	2						2		
Salvia fruticosa Mill.	Additive, medicine	Flavoring, folklore	71						71		
Salvia hispanica L.	Additive, medicine	Flavoring, folklore	8		3	2		1	14		
Salvia lavandulifolia Vahl	Material	Essential oils	23						23		
Salvia lyrata L.	Medicine	Folklore	5			7			12		
Salvia mellifera Greene	Medicine	Folklore	3			6			9		

Salvia microphylla Kunth	Medicine	Folklore	6			1			7		
Salvia miltiorrhiza Bunge	Medicine	Folklore	1						1		
Salvia officinalis L.	Additive, material, medicine	Flavoring, essential oils, folklore	381		1	10		5	397		Least concern
Salvia plebeia R. Br.	Medicine	Folklore	2						2		
Salvia sclarea L.	Additive, material, medicine	Flavoring, essential oils, folklore	241		9	5			255		
Sambucus australis Cham. and Schltdl.	Medicine	Folklore				2			2		
Sambucus ebulus L.	Medicine	Folklore	28			8			36		
Sambucus javanica Reinw. ex Blume	Medicine	Folklore	2			3			5		
Sambucus nigra L.	Additive, medicine	Flavoring, folklore	314			127			441		
Sambucus racemosa L.	Medicine	Folklore	13			85			98		
Sambucus williamsii Hance	Medicine	Folklore			1	6			7		
Sanguinaria canadensis L.	Material, medicine	Tannin/dyestuff, source of sanguinarine	1			3			4		
Sanguisorba officinalis L.	Additive, medicine	Flavoring, folklore	55		2	32		29	118		Least concern
Sanicula europaea L.	Medicine	Folklore	34					3	37		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Santalum album L.	Additive, material, medicine	Flavoring, essential oils, folklore		1					1		Vulnerable
Santalum spicatum (R. Br.) A. DC.	Material	Essential oils	1	1				6	8		
Santalum yasi Seem.	Material	Essential oils	1						1		
Santolina chamaecyparissus L.	Medicine	Folklore	12			1			13		
Sanvitalia procumbens Lam.	Medicine	Folklore	7			13			20		
Sapindus mukorossi Gaertn.	Material	Chemicals			5	1			6		
Sapindus rarak DC.	Material	Chemicals	1						1		
Sapindus saponaria L.	Material, medicine	Chemicals, folklore	6			4	1		11		
Sapindus trifoliatus L.	Medicine	Folklore			7	2			9		
Saponaria officinalis L.	Medicine	Folklore	119					1	120		
Saposhnikovia divaricata (Turcz.) Schischk.	Medicine	Folklore	3			4			7		
Saraca indica L.	Medicine	Folklore		4	4				8		
Sarcocephalus latifolius (Sm.) Bruce	Medicine	Folklore	2						2		

Sarracenia purpurea L.	Medicine	Folklore	1			1			2	II	
Saruma henryi Oliv.	Medicine	Folklore	3			1			4		Endangered
Sassafras albidum (Nutt.) Nees	Additive, material, medicine	Flavoring, essential oils, folklore				3			3		Least concern
Satureja hortensis L.	Additive, medicine	Flavoring, folklore	240		4	16		5	265		
Satureja montana L.	Additive, medicine	Flavoring, folklore	184					4	188		
Saussurea involucrata (Kar. & Kir.) Sch. Bip.	Medicine	Folklore	1						1		
Saussurea obvallata (DC.) Sch. Bip.	Medicine	Folklore	1		1				2		
Saxifraga stolonifera Curtis	Medicine	Folklore	1						1		
Sceletium tortuosum (L.) N. E. Br.	Medicine	Folklore	2						2		
Schefflera arboricola (Hayata) Merr.	Medicine	Folklore	2						2		
Schefflera heptaphylla (L.) Frodin	Medicine	Folklore	1						1		
Schefflera venulosa (Wight & Arn.) Harms	Medicine	Folklore	2						2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Schinus molle L.	Additive, medicine	Flavoring, folklore	1			1			2		
Schinziophyton rautanenii (Schinz) Radcl.-Sm.	Medicine	Folklore	10						10		
Schisandra chinensis (Turcz.) Baill.	Medicine	Folklore	4			11		1	16		
Schisandra sphenanthera Rehder and E. H. Wilson	Medicine	Folklore				2			2		
Schizonepeta tenuifolia (Benth.) Briq.	Medicine	Folklore			1				1		
Scindapsus officinalis (Roxb.) Schott	Medicine	Folklore			1				1		
Sclerocarya birrea (A. Rich.) Hochst.	Medicine	Folklore	11	5		1			17		
Scoparia dulcis L.	Medicine	Folklore	8	2	4	1			15		
Scopolia carniolica Jacq.	Medicine	Source of hyoscine (scopolamine)	8						8		
Scrophularia buergeriana Miq.	Medicine	Folklore			1				1		
Scrophularia californica Cham. and Schltdl.	Medicine	Folklore	2			5			7		
Scrophularia marilandica L.	Medicine	Folklore	1						1		

Scrophularia ningpoensis Hemsl.	Medicine	Folklore	1						1		
Scrophularia nodosa L.	Medicine	Folklore	62						62		
Scutellaria baicalensis Georgi	Medicine	Folklore	14		1	2			17		
Scutellaria indica L.	Medicine	Folklore	1						1		
Scutellaria lateriflora L.	Medicine	Folklore	2			1			3		Least concern
Sechium edule (Jacq.) Sw.	Medicine	Folklore	6						6		
Sedum acre L.	Medicine	Folklore	29						29		
Semecarpus anacardium L. f.	Medicine	Folklore	1						1		
Semiaquilegia adoxoides (DC.) Makino	Medicine	Folklore	2						2		
Sempervivum tectorum L.	Medicine	Folklore	12					1	13		
Senecio nemorensis L.	Medicine	Folklore	1			1			2		
Senecio ovatus (G. Gaertn. et al.) Willd.	Medicine	Folklore	5						5		
Senecio vulgaris L.	Medicine	Folklore	10					2	12		
Senegalia ataxacantha (DC.) Kyal. and Boatwr.	Medicine	Folklore				2			2		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Senegalia catechu (L. f.) P. J. H. Hurter and Mabb.	Additive, material, medicine	Flavoring, tannin/dyestuff, folklore				1			1		
Senegalia modesta (Wall.) P. J. H. Hurter	Medicine	Folklore				2			2		
Senegalia pennata (L.) Maslin	Material, medicine	Tannin/dyestuff, folklore	1						1		
Senegalia rugata (Lam.) Britton and Rose	Medicine	Folklore			3				3		
Senegalia senegal (L.) Britton	Medicine	Folklore				6			6		
Senna alata (L.) Roxb.	Medicine	Folklore	8	2	12	4	1	2	29		
Senna alexandrina Mill.	Material, medicine	Tannin/dyestuff, source of sennosides A and B	4	1	23	2			30		
Senna auriculata (L.) Roxb.	Material, medicine	Tannin/dyestuff, folklore	3		18	1			22		
Senna obtusifolia (L.) H. S. Irwin and Barneby	Medicine	Folklore	25	23	9	5		15	77		
Senna occidentalis (L.) Link	Medicine, social	Folklore, stimulant	41	27	35	24	3	17	147		
Senna septemtrionalis (Viv.) H. S. Irwin and Barneby	Medicine	Folklore	7	2		1			10		

Senna sophera (L.) Roxb.	Medicine	Folklore	5	1	5	1		2	14		
Senna surattensis (Burm. f.) H. S. Irwin and Barneby	Medicine	Folklore	2						2		
Senna tora (L.) Roxb.	Medicine	Folklore	11	4	23			2	40		
Serenoa repens (W. Bartram) Small	Medicine	Folklore				1			1		
Sesamum indicum L.	Additive, medicine	Flavoring, folklore	2220	1749	6457	1283	2372	625	14706		
Sesbania grandiflora (L.) Poir.	Medicine	Folklore	1	63	85	1	3	11	164		
Sesbania sesban (L.) Merr.	Medicine	Folklore	24	276		10	20	66	396		
Seseli libanotis (L.) W. D. J. Koch	Medicine	Folklore	19						19		
Seseli tortuosum L.	Medicine	Folklore	2						2		
Sida acuta Burm. f.	Medicine	Folklore	5	6	7			1	19		
Sida cordata (Burm. f.) Borss. Waalk.	Medicine	Folklore			1				1		
Sida cordifolia L.	Medicine	Folklore	14	2	6				22		
Sida rhombifolia L.	Medicine	Folklore	18	5	8	1		3	35		
Sideritis hirsuta L.	Medicine	Folklore	8						8		
Sideritis syriaca L.	Medicine	Folklore	13						13		
Sigesbeckia pubescens (Makino) Makino	Medicine	Folklore	1						1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Silene banksia (Meerb.) Mabb.	Medicine	Folklore				1			1		
Silene conoidea L.	Medicine	Folklore	11						11		
Silphium laciniatum L.	Medicine	Folklore	1			9			10		
Silphium perfoliatum L.	Medicine	Folklore	13			25		1	39		
Silybum marianum (L.) Gaertn.	Additive, medicine	Flavoring, source of silymarin	105		2	2		3	112		
Simarouba amara Aubl.	Medicine	Folklore	2						2		
Simarouba glauca DC.	Medicine	Source of glaucarubin				2			2		
Simmondsia chinensis (Link) C. K. Schneid.	Medicine	Folklore	2	1	2	508		11	524		
Sinapis alba L.	Additive, medicine	Flavoring, folklore	1297		106	266		83	1752		
Siraitia grosvenorii (Swingle) A. M. Lu and Zhi Y. Zhang	Additive, medicine	Sweetener, folklore				2			2		
Sison amomum L.	Additive	Flavoring	3						3		
Sisymbrium officinale (L.) Scop.	Medicine	Folklore	59			5		20	84		
Sium sisarum L.	Medicine	Folklore	13			7			20		

Sium suave Walter	Medicine	Folklore	1			3			4		Least concern
Smallanthus sonchifolius (Poepp. & Endl.) H. Rob.	Medicine	Folklore	1		1		50		52		
Smilax china L.	Medicine	Folklore			2				2		
Smilax glauca Walter	Medicine	Folklore	1						1		
Smithia conferta Sm.	Medicine	Folklore	1						1		
Solanum aculeatissimum Jacq.	Medicine	Folklore	12	1	48	3			64		
Solanum aethiopicum L.	Medicine	Folklore	184	4	480	85	67		820		
Solanum anguivi Lam.	Medicine	Folklore	62	8	24	5		1	100		
Solanum aviculare G. Forst.	Medicine	Source of solasodine	13		2	2		6	23		
Solanum capsicoides All.	Medicine	Folklore	16		3	4		1	24		
Solanum carolinense L.	Medicine	Folklore	6			1			7		
Solanum dulcamara L.	Medicine	Folklore	232		1	3			236		
Solanum erianthum D. Don	Medicine	Folklore	6		1				7		
Solanum laciniatum Aiton	Medicine	Source of solasodine	20		4	3		7	34		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Solanum lasiocarpum Dunal	Additive, medicine	Flavoring, folklore	5		31	3			39		
Solanum marginatum L. f.	Medicine	Potential source of solasodine	11						11		
Solanum mauritianum Scop.	Medicine	Potential source of solasodine	12			1			13		
Solanum nigrescens M. Martens and Galeotti	Medicine	Folklore	6		2				8		
Solanum nigrum L.	Medicine	Folklore	163	69	28	9		3	272		
Solanum paniculatum L.	Medicine	Folklore					11		11		
Solanum rostratum Dunal	Medicine	Folklore	18		1	2			21		
Solanum torvum Sw.	Medicine	Folklore	13		118	1	3		135		
Solanum viarum Dunal	Medicine	Source of solasodine	26		16	5	22		69		
Solanum violaceum Ortega	Medicine	Folklore	9		63	1			73		
Solanum virginianum L.	Medicine	Potential source of solasodine	36		9	7			52		
Solidago canadensis L.	Medicine	Folklore	26			12			38		
Solidago gigantea Aiton	Medicine	Folklore	16			2			18		
Solidago multiradiata Aiton	Medicine	Folklore	4			5			9		
Solidago odora Aiton	Medicine	Folklore	1						1		

Solidago virgaurea L.	Medicine	Folklore	86			1		1	88		
Sonchus oleraceus L.	Medicine	Folklore	30	2	1	7	1		41		
Sophora flavescens Aiton	Medicine	Folklore	1		2	5			8		Least concern
Sophora macrocarpa Sm.	Material	Potential as essential oils	2			1			3		
Sophora pachycarpa Schrenk ex C. A. Mey.	Medicine	Source of pachycarpine	2			1			3		
Sophora tomentosa L.	Medicine	Folklore	8			1		5	14		
Sopubia delphiniifolia (L.) G. Don	Medicine	Folklore			1				1		
Sorbus americana Marshall	Medicine	Folklore	2			6			8		
Sorbus aucuparia L.	Medicine	Folklore	132			69			201		Least concern
Sorbus domestica L.	Medicine	Folklore	93			12			105		Least concern
Sorbus torminalis (L.) Crantz	Medicine	Folklore	31			21			52		Least concern
Sparganium erectum L.	Medicine	Folklore	17						17		Least concern
Sparganium eurycarpum Engelm.	Medicine	Folklore	1			1			2		Least concern
Spartium junceum L.	Additive, material	Flavoring, essential oils	28	2	1	3			34		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Spergularia rubra (L.) J. Presl and C. Presl	Medicine	Folklore	6					2	8		
Sphaeralcea angustifolia (Cav.) G. Don	Medicine	Folklore	8						8		
Sphaeranthus indicus L.	Medicine	Folklore	2		5			1	8		Least concern
Spigelia anthelmia L.	Medicine	Folklore	1						1		
Spinacia oleracea L.	Medicine	Folklore	1449	1	225	524	15	2	2216		
Spondias mombin L.	Medicine	Folklore	1			1	35		37		
Stachys affinis Bunge	Medicine	Folklore	4						4		
Stachys officinalis (L.) Trevis.	Medicine	Folklore	36						36		
Stachys palustris L.	Medicine	Folklore	35			2			37		Least concern
Stellaria dichotoma L.	Medicine	Folklore			1	1			2		
Stellaria media (L.) Vill.	Medicine	Folklore	19	1	1			3	24		
Stephania japonica (Thunb.) Miers	Medicine	Folklore	4					5	9		
Stephania tetrandra S. Moore	Medicine	Source of tetrandine	1						1		
Sterculia foetida L.	Medicine	Folklore	1			1			2		

Stevia rebaudiana (Bertoni) Bertoni	Additive, medicine	Sweetener, source of stevioside	4		4	2	1		11		
Stevia serrata Cav.	Medicine	Folklore	5						5		
Stewartia pseudocamellia Maxim.	Medicine	Folklore	1			3			4		Least concern
Strobilanthes callosa Nees	Medicine	Folklore			4				4		
Strophanthus divaricatus (Lour.) Hook. and Arn.	Medicine	Folklore	1						1		
Strophanthus eminii Asch. and Pax	Medicine	Folklore	1						1		
Strophanthus hispidus DC.	Medicine	Source of pseudostrophanthin	1						1		
Strophanthus kombe Oliv.	Medicine	Source of strophanthin		1					1		
Strophanthus sarmentosus DC.	Medicine	Folklore	1						1		
Strychnos lucida R. Br.	Medicine	Folklore	1					2	3		
Strychnos minor Dennst.	Medicine	Folklore						1	1		
Strychnos nux-vomica L.	Medicine	Source of strychnine			39				39		
Strychnos potatorum L. f.	Medicine	Folklore	4		10				14		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Strychnos wallichiana Steud. ex A. DC.	Medicine	Folklore			1				1		
Stryphnodendron adstringens (Mart.) anon.	Material, medicine	Tannin/dyestuff, folklore	1						1		
Styphnolobium japonicum (L.) Schott	Medicine	Source of rutin	2	1	1	1		1	6		
Suaeda glauca (Bunge) Bunge	Material	Essential oils	2						2		
Succisa pratensis Moench	Medicine	Folklore	30						30		
Swertia chirayita (Roxb.) H. Karst.	Additive, medicine	Flavoring, folklore			9				9		
Swietenia humilis Zucc.	Medicine	Folklore	2			1			3	II	Vulnerable
Swietenia macrophylla King	Medicine	Folklore	1			6	2		9	II	Vulnerable
Symphytum asperum Lepech.	Medicine	Folklore	4			1			5		
Symphytum officinale L.	Medicine	Folklore	57			8		1	66		
Symplocos lucida (Thunb.) Siebold and Zucc.	Medicine	Folklore	2						2		
Symplocos paniculata Miq.	Medicine	Folklore	7		2	7			16		

Syringa reticulata (Blume) H. Hara	Material	Essential oils	2			15			17		Least concern
Syringa vulgaris L.	Medicine	Folklore	109						109		Least concern
Syzygium cumini (L.) Skeels	Medicine	Folklore			1	8			9		
Syzygium jambos (L.) Alston	Medicine	Folklore	2						2		
Tabernaemontana alternifolia L.	Medicine	Folklore			5				5		
Tabernaemontana crassa Benth.	Medicine	Folklore	1						1		
Tabernaemontana divaricata (L.) R. Br. ex Roem. and Schult.	Medicine	Folklore	1			1			2		
Tacazzea conferta N. E. Br.	Medicine	Folklore, veterinary	1						1		
Tacca chantrieri Andre	Medicine	Folklore	2						2		
Tacca leontopetaloides (L.) Kuntze	Medicine	Folklore	6	5				6	17		Least concern
Tagetes erecta L.	Material, medicine, social	Tannin/dyestuff, folklore, religious/secula	155		1	24	2		182		
Tagetes lucida Cav.	Additive, medicine	Flavoring, folklore	6			5			11		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Tagetes micrantha Cav.	Medicine	Folklore	1			1			2		
Tagetes minuta L.	Additive, material, medicine	Flavoring, essential oils, folklore	8	1		1	1		11		
Tagetes patula L.	Material	Tannin/dyestuff	194			50			244		
Talinum paniculatum (Jacq.) Gaertn.	Medicine	Folklore	8		2	2	1		13		
Tamarindus indica L.	Additive, medicine	Flavoring, folklore	9	12	3	42	1		67		Least concern
Tamarix gallica L.	Medicine	Folklore	2						2		Least concern
Tamilnadia uliginosa (Retz.) Tirveng. and Sastre	Medicine	Folklore	2		1				3		
Tanacetum balsamita L.	Additive	Flavoring	12			5			17		
Tanacetum cinerariifolium (Trevir.) Sch. Bip.	Medicine	Folklore	137		6	8			151		Least concern
Tanacetum parthenium (L.) Sch. Bip.	Medicine	Folklore	45		1	9			55		
Tanacetum vulgare L.	Additive, material, medicine	Flavoring, essential oils, folklore	141		2	9			152		

Taraxacum erythrospermum Andrz. ex Besser	Medicine	Folklore	2						2		
Taraxacum mongolicum Hand.-Mazz.	Medicine	Folklore			1				1		
Taraxacum officinale F. H. Wigg.	Additive, medicine	Flavoring, folklore	59		9	3	2	8	81		
Tasmannia lanceolata (Poir.) A. C. Sm.	Additive	Flavoring	6					19	25		
Taxodium mucronatum Ten.	Medicine	Folklore				1			1		Least concern
Taxus baccata L.	Medicine	Folklore	61			1			62		Least concern
Taxus brevifolia Nutt.	Medicine	Source of taxol	2			8			10		Near threatened
Taxus canadensis Marshall	Medicine	Folklore				1			1		Least concern
Taxus cuspidata Siebold and Zucc.	Material, medicine	Tannin/dyestuff, folklore	3			10			13	II	Least concern
Taxus wallichiana Zucc.	Medicine	Potential source of pharmaceutical agent	1			1			2	II	Endangered
Tecoma stans (L.) Juss. ex Kunth	Medicine	Folklore	13			2			15		
Tecomella undulata (Sm.) Seem.	Medicine	Folklore	3		341				344		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Tectona grandis L. f.	Medicine	Folklore	2	2		3			7		
Telosma cordata (Burm. f.) Merr.	Material	Essential oils	1		2				3		
Tephrosia purpurea (L.) Pers.	Medicine	Folklore	31	47	18	11	12	32	151		
Tephrosia villosa (L.) Pers.	Medicine	Folklore	2	12		27	3	4	48		Least concern
Tephrosia virginiana (L.) Pers.	Medicine	Folklore	1			4			5		
Teramnus labialis (L. f.) Spreng.	Medicine	Folklore	15	207		20	88	148	478		
Terminalia arjuna (Roxb. ex DC.) Wight and Arn.	Medicine	Folklore	1		29	2			32		
Terminalia bellirica (Gaertn.) Roxb.	Material, medicine	Tannin/dyestuff, folklore	1		49	3			53		
Terminalia catappa L.	Medicine	Folklore	2		1	4			7		
Terminalia chebula Retz.	Material, medicine	Tannin/dyestuff, folklore	1		46	1			48		
Terminalia laxiflora Engl. and Diels	Material, medicine	Tannin/dyestuff, folklore	1						1		
Terminalia sericea Burch. ex DC.	Medicine	Folklore	4						4		
Tetraclinis articulata (Vahl) Mast.	Medicine	Folklore	1			1			2		Least concern

Tetradenia riparia (Hochst.) Codd	Medicine	Folklore	2	2					4		
Tetradium ruticarpum (A. Juss.) T. G. Hartley	Medicine	Folklore	1			1			2		
Tetragonia tetragonoides (Pall.) Kuntze	Medicine	Folklore	33		1	3		3	40		
Teucrium canadense L.	Medicine	Folklore	2			4			6		
Teucrium chamaedrys L.	Medicine	Folklore	43			3			46		
Teucrium polium L.	Medicine	Folklore	31		1	6			38		
Teucrium scordium L.	Material, medicine	Essential oils, folklore	10			1			11		
Teucrium scorodonia L.	Medicine	Folklore	40			1		3	44		
Thalictrum alpinum L.	Medicine	Folklore	4						4		
Thalictrum dasycarpum Fisch. and Ave-Lall.	Medicine	Folklore	2			18			20		
Thalictrum dioicum L.	Medicine	Folklore	1			1			2		
Thalictrum petaloideum L.	Medicine	Folklore				3			3		
Thelesperma filifolium (Hook.) A. Gray	Medicine	Folklore	1			3			4		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Theobroma bicolor Humb. and Bonpl.	Additive	Flavoring				1	1		2		
Theobroma cacao L.	Additive, medicine, social	Flavoring, folklore, source of theobromine, religious/secular, stimulant				428			428		
Theobroma grandiflorum (Willd. ex Spreng.) K. Schum.	Additive, medicine	Flavoring, folklore				2	445		447		
Thermopsis lanceolata R. Br.	Medicine	Folklore	5			3			8		
Thespesia populnea (L.) Sol. ex Correa	Material, medicine, social	Tannin/dyestuff, folklore, religious/secular	5					2	7		Least concern
Thladiantha dubia Bunge	Medicine	Folklore	2						2		
Thottea siliquosa (Lam.) Ding Hou	Medicine	Folklore	1						1		
Thuja occidentalis L.	Material, medicine	Essential oils, folklore	36			5			41		Least concern
Thuja plicata Donn ex D. Don	Material, medicine	Essential oils, folklore	26		2	2			30		Least concern
Thujopsis dolabrata (Thunb. ex L. f.) Siebold and Zucc.	Material	Essential oils	1			1			2		Least concern
Thymus caespititius Brot.	Additive	Flavoring	17						17		

Thymus citriodorus (Pers.) Schreb.	Additive, medicine	Flavoring, folklore	2						2		
Thymus herba-barona Loisel.	Additive	Flavoring	1						1		
Thymus mastichina L.	Additive	Flavoring	29						29		Least concern
Thymus praecox Opiz	Material, medicine	Essential oils, folklore	29			1			30		
Thymus pulegioides L.	Material, medicine	Essential oils, folklore	81						81		
Thymus quinquecostatus Celak.	Additive	Flavoring			1				1		
Thymus serpyllum L.	Medicine	Folklore	79			6		2	87		
Thymus vulgaris L.	Additive, material, medicine	Flavoring, essential oils, source of thymol	119		1	1		1	122		Least concern
Thymus zygis L.	Additive, material, medicine	Flavoring, essential oils, folklore	26						26		
Tiarella cordifolia L.	Medicine	Folklore				1			1		
Tilia americana L.	Medicine	Folklore	7			5			12		Least concern
Tilia cordata Mill.	Medicine	Folklore	29			8			37		Least concern
Tilia europaea L.	Medicine	Folklore	1						1		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Tilia platyphyllos Scop.	Medicine	Folklore	14		1	1			16		Least concern
Tillandsia recurvata (L.) L.	Medicine	Folklore	9			1	1		11		
Tillandsia usneoides (L.) L.	Medicine	Folklore					1		1		
Tinospora cordifolia (Willd.) Hook. f. and Thomson	Medicine	Folklore			5				5		
Toona ciliata M. Roem.	Material, medicine	Tannin/dyestuff, folklore	1					7	8		Least concern
Toona sinensis (A. Juss.) M. Roem.	Medicine	Folklore	2						2		
Torilis japonica (Houtt.) DC.	Medicine	Folklore	13			8			21		
Trachycarpus fortunei (Hook.) H. Wendl.	Medicine	Folklore	4						4		
Trachyspermum ammi (L.) Sprague ex Turrill	Additive, medicine	Flavoring, folklore	3		20	4			27		
Trachyspermum roxburghianum (DC.) Craib	Additive, medicine	Flavoring, folklore			1				1		
Triadica sebifera (L.) Small	Material, medicine	Tannin/dyestuff, folklore	1		2	1			4		

Trianthema portulacastrum L.	Medicine	Folklore	2	1	5				8		
Tribulus terrestris L.	Medicine	Folklore	10	1	31			1	43		
Trichosanthes cucumerina L.	Medicine	Folklore	10		74	9	1	4	98		
Trichosanthes dioica Roxb.	Medicine	Folklore			1				1		
Trichosanthes kirilowii Maxim.	Medicine	Source of trichosanthin	2		1	7			10		
Trichosanthes tricuspidata Lour.	Medicine	Folklore				1			1		
Trifolium pratense L.	Additive, medicine	Flavoring, folklore	10147	111	326	1541	11	2614	14750		Least concern
Trigonella caerulea (L.) Ser.	Additive	Flavoring	103			39		8	150		
Trigonella foenum-graecum L.	Additive, material, medicine	Flavoring, essential oils, folklore	311	2	91	277		558	1239		
Trillium ovatum Pursh	Medicine	Folklore	1			1			2		
Tripterygium wilfordii Hook. f.	Medicine	Folklore	1						1		
Triumfetta rhomboidea Jacq.	Medicine	Folklore	7	4					11		
Trollius chinensis Bunge	Medicine	Folklore	3			6			9		
Tropaeolum majus L.	Medicine	Folklore	47			18	1	2	68		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Tropaeolum minus L.	Material, medicine	Essential oils, folklore	1						1		
Tsuga canadensis (L.) Carriere	Material, medicine	Essential oils, folklore	8			226			234		Near threatened
Turbina corymbosa (L.) Raf.	Medicine, social	Folklore, hallucinogen	5			1			6		
Turnera diffusa Willd.	Additive, medicine	Flavoring, folklore	1			2			3		
Tussilago farfara L.	Additive, medicine	Flavoring, folklore	69						69		
Tylophora indica (Burm. f.) Merr.	Medicine	Folklore			1				1		
Typha angustifolia L.	Medicine	Folklore	5			1			6		Least concern
Typha domingensis Pers.	Medicine	Folklore	13	1		1		5	20		Least concern
Typha latifolia L.	Medicine	Folklore	41	1		6			48		Least concern
Typha orientalis C. Presl	Medicine	Folklore	5					3	8		Least concern
Ugni molinae Turcz.	Additive	Flavoring	1						1		
Ulmus minor Mill.	Medicine	Folklore	8			3			11		Data deficient
Ulmus rubra Muhl.	Medicine	Folklore	1						1		Least concern
Umbellularia californica (Hook. & Arn.) Nutt.	Medicine	Folklore	5			2			7		

Uraria rufescens (DC.) Schindl.	Medicine	Folklore					12		12		
Urena lobata L.	Aaterial	Essential oils	19	5		12	11	2	49		
Urochloa brizantha (Hochst. ex A. Rich.) R. D. Webster	Medicine	Folklore				23		30	53		
Urtica dioica L.	Medicine	Folklore	107			3		7	117		Least concern
Urtica urens L.	Medicine	Folklore	17						17		
Utricularia vulgaris L.	Medicine	Folklore	2						2		Least concern
Uvaria chamae P. Beauv.	Medicine	Folklore	2						2		
Vaccaria hispanica (Mill.) Rauschert	Medicine	Folklore	25			6			31		
Vaccinium angustifolium Aiton	Medicine	Folklore	2			74			76		
Vaccinium corymbosum L.	Medicine	Folklore	120		22	344			486		
Vaccinium macrocarpon Aiton	Medicine	Folklore	1			166			167		Least concern
Vaccinium myrtillus L.	Material, medicine	Tannin/dyestuff, folklore	106			54			160		
Vaccinium ovalifolium Sm.	Material	Tannin/dyestuff	2			115			117		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Vaccinium ovatum Pursh	Medicine	Folklore	2			39			41		
Vaccinium oxycoccos L.	Medicine	Folklore	11			91			102		Least concern
Vaccinium pallidum Aiton	Medicine	Folklore	1			37			38		
Vaccinium uliginosum L.	Medicine	Folklore	21		1	129			151		
Vaccinium vitis-idaea L.	Medicine	Folklore	41			132			173		Least concern
Vachellia amythethophylla (Steud. ex A. Rich.) Kyal. and Boatwr.	Medicine	Folklore				2			2		
Vachellia caven (Molina) Seigler and Ebinger	Material	Essential oils				10			10		
Vachellia farnesiana (L.) Wight and Arn.	Additive, material, medicine	Flavoring, essential oils, tannin/dyestuff, folklore				13		6	19		
Vachellia karroo (Hayne) Banfi and Galasso	Material, medicine	Tannin/dyestuff, folklore				10		1	11		
Vachellia nilotica (L.) P. J. H. Hurter and Mabb.	Material, medicine	Tannin/dyestuff, folklore				6			6		

Vachellia seyal (Delile) P. J. H. Hurter	Medicine	Folklore				1			1		
Valeriana jatamansi Jones ex Roxb.	Additive, material, medicine	Flavoring, essential oils, folklore			4				4		
Valeriana officinalis L.	Additive, material, medicine	Flavoring, essential oils, folklore, source of valepotriates	139			10		2	151		
Valeriana sitchensis Bong.	Medicine	Folklore	3			1			4		
Vanda tessellata (Roxb.) G. Don	Medicine	Folklore	1						1	II	Least concern
Vanilla planifolia Andrews	Additive, material, medicine	Flavoring, essential oils, folklore				2			2	II	Endangered
Vanilla pompona Schiede	Additive	Flavoring				2			2	II	Endangered
Ventilago denticulata Willd.	Medicine	Folklore			1				1		
Ventilago madraspatana Gaertn.	Material	Tannin/dyestuff			2				2		
Veratrum album L.	Medicine	Folklore, source of protoveratrines A and B	6						6		
Veratrum viride Aiton	Medicine	Folklore	1			4			5		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Verbascum densiflorum Bertol.	Medicine	Folklore	30						30		
Verbascum phlomoides L.	Medicine	Folklore	38						38		
Verbascum thapsus L.	Medicine	Folklore	63		3	1			67		
Verbena carolina L.	Medicine	Folklore	1						1		
Verbena hastata L.	Medicine	Folklore	2			8			10		
Verbena lasiostachys Link	Medicine	Folklore	1			3			4		
Verbena officinalis L.	Medicine	Folklore	90	3		5		1	99		
Veronica anagallis-aquatica L.	Medicine	Folklore	21	1					22		Least concern
Veronica beccabunga L.	Medicine	Folklore	12						12		Least concern
Veronica officinalis L.	Medicine	Folklore	25					2	27		
Veronica undulata Wall.	Medicine	Folklore	1						1		
Veronicastrum virginicum (L.) Farw.	Medicine	Folklore	1			8			9		
Viburnum opulus L.	Medicine	Folklore	55		1	47			103		
Viburnum prunifolium L.	Additive, medicine	Flavoring, folklore	2			17			19		
Vinca major L.	Medicine	Folklore	5						5		
Vinca minor L.	Medicine	Source of vincamine	7						7		

Vincetoxicum atratum (Bunge) C. Morren and Decne.	Medicine	Folklore				1			1		
Vincetoxicum forrestii (Schltr.) C. Y. Wu and D. Z. Li	Medicine	Folklore				1			1		
Vincetoxicum hirundinaria Medik.	Medicine	Folklore	12						12		
Viola alba Besser	Additive, material	Flavoring, essential oils	3						3		
Viola cucullata Aiton	Medicine	Folklore				4			4		
Viola odorata L.	Additive, material, medicine	Flavoring, essential oils, folklore	17						17		
Viola philippica Cav.	Medicine	Folklore	1						1		
Viola sororia Willd.	Medicine	Folklore	1			22			23		
Viola suavis M. Bieb.	Additive	Flavoring	2						2		
Viola tricolor L.	Medicine	Folklore	38			8			46		
Viscum album L.	Medicine, social	Folklore, religious/ secular	8		1				9		
Vitex agnus-castus L.	Medicine	Folklore	43						43		Data deficient
Vitex negundo L.	Medicine	Folklore	1		18	6			25		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Vitex quinata (Lour.) F. N. Williams	Medicine	Folklore	1			1			2		
Vitex rotundifolia L. f.	Medicine	Folklore				5		1	6		
Vitex trifolia L.	Medicine	Folklore	2						2		
Vitis rotundifolia Michx.	Medicine	Folklore	2		8	240			250		
Vitis vinifera L.	Additive, medicine, social	Flavoring, folklore, religious/secular	26183		296	3091	171		29741		Least concern
Voacanga africana Stapf	Medicine	Folklore, source of tabersonine	1						1		
Voacanga thouarsii Roem. and Schult.	Medicine	Folklore, source of tabersonine	1						1		
Warburgia ugandensis Sprague	Medicine	Folklore		1					1		
Withania somnifera (L.) Dunal	Medicine	Folklore	28	9	314			1	352		
Woodfordia fruticosa (L.) Kurz	Medicine	Folklore	3						3		Lower risk/ least concern
Wrightia pubescens R. Br.	Medicine	Folklore	1					8	9		
Xanthium strumarium L.	Medicine	Folklore	31	1	10	2			44		
Xanthoceras sorbifolium Bunge	Medicine	Folklore	2			8			10		

Xanthorhiza simplicissima Marshall	Medicine	Folklore	1			3			4		
Xysmalobium undulatum (L.) R. Br.	Medicine	Folklore	2						2		
Yucca aloifolia L.	Medicine	Folklore	1			1			2		
Yucca baccata Torr.	Medicine	Folklore	1			1			2		
Yucca brevifolia Engelm.	Additive, medicine	Flavoring, folklore	1			2			3		
Yucca filamentosa L.	Medicine	Folklore	3						3		
Yucca glauca Nutt.	Medicine	Folklore	3			7			10		
Yucca schidigera Roezl ex Ortgies	Additive, medicine	Flavoring, folklore	2						2		
Zanthoxylum acanthopodium DC.	Additive, material, medicine	Flavoring, essential oils, folklore	2						2		
Zanthoxylum americanum Mill.	Additive, medicine	Flavoring, folklore	2			2			4		
Zanthoxylum armatum DC.	Material, medicine	Essential oils, folklore	1		12	2			15		
Zanthoxylum bungeanum Maxim.	Additive, medicine	Flavoring, folklore	1			1			2		
Zanthoxylum clava-herculis L.	Additive, medicine	Flavoring, folklore	3			1			4		

(continued)

Table 1.4 (continued)

Taxon	Usage code	Usage type	Europe	Africa	Asia	North America	Latin America	Oceania	Total	CITES appendix	IUCN Red List status
Zanthoxylum nitidum (Roxb.) DC.	Medicine	Folklore				1			1		
Zanthoxylum piperitum (L.) DC.	Additive, medicine	Flavoring, folklore	1		2	6			9		
Zanthoxylum rhetsa (Roxb.) DC.	Additive, medicine	Flavoring, folklore			5				5		
Zanthoxylum schinifolium Siebold & Zucc.	Medicine	Folklore	3		2	3			8		
Zanthoxylum simulans Hance	Additive, medicine	Flavoring, folklore	2						2		
Zanthoxylum zanthoxyloides (Lam.) Zepern. and Timler	Medicine	Folklore	3						3		
Zea mays L.	Additive, medicine	Sweetener, folklore	47142	4943	11106	32099	35829	1918	133037		
Zingiber mioga (Thunb.) Roscoe	Additive	Flavoring	1			2			3		
Zingiber montanum (J. Koenig) Link ex A. Dietr.	Medicine	Folklore	1						1		
Zingiber officinale Roscoe	Additive, material, medicine	Flavoring, essential oils, folklore				2		1	3		
Zingiber zerumbet (L.) Sm.	Additive, medicine	Flavoring, folklore				1			1		

Ziziphus jujuba Mill.	Material, medicine	Chemicals, folklore	130	4		1			135		Least concern
Ziziphus mauritiana Lam.	Medicine	Folklore	22			6			28		
Ziziphus spina-christi (L.) Desf.	Medicine	Folklore	25	3					28		
Zosima absinthifolia (Vent.) Link	Additive	Flavoring	5						5		

CITES and Red List status are described in separate columns, respectively

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Chapter 2
Analysis of Secondary Metabolites in Breeding Research and Plant Breeding

Hartwig Schulz

2.1 Introduction

In most cases, active principles of medicinal and aromatic plants occur in relatively complex mixtures and are not distributed uniformly throughout the plant, but are preferably found in the roots, leaves, flowers, fruits, or in the bark. In addition, the content of individual plant substances is considerably influenced not only by the genetic background of the respective species (e.g., chemotype or variety) but also by the environment. Finally, the harvesting technique, drying, and storage also affect the content of the respective valuable components. For analytical characterization of medicinal and spice plants, usually different chromatographic separation methods are applied, which ensure a sufficient separation of the most relevant analytes. Even today, thin-layer chromatography (DC) is still of some importance, since numerous medicinal and aromatic plants provide characteristic DC fingerprints and in this way usually allow reliable and fast identification (Wagner et al. 1983; Pachaly 2002). Using suitable detectors, also quantitative results can be obtained for many chemical compound classes. The limits of DC are reached when complex multicomponent mixtures have to be separated or unknown plant substances have to be identified. On the one hand, high-performance thin-layer chromatography (HPTLC) still offers a significant improvement over the classical DC techniques due to the automatic sample application and the integrated detection unit, there are also limits in identifying unknown components. On the other hand, high-performance liquid chromatography (HPLC) offers significantly better possibilities both for the identification and quantification of plant constituents. Due to the wide range of different HPLC columns and the option to use two-dimensional

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HPLC, today most separation problems of nonvolatile plant substances can be solved satisfactorily. The separated substances can be detected electrochemically by means of a conductivity detector, spectroscopically applying UV-Vis, fluorescence, or MS, and by refractometry.

For the determination of volatile substances, especially gas chromatographic methods are applied. In this context, the analysis can be carried out after appropriate sample preparation in a relatively short time. For enrichment of trace components, adsorptive sample enrichment methods such as solid phase microextraction (SPME) (Pawliszyn 2011) or stir bar sorptive extraction (SBSE) (Sánchez-Rojas et al. 2009) are available. Those plant substances associated with a certain medicinal efficacy or a characteristic aroma can be related to the following chemical classes: mono-, sesqui-, and diterpenes, polyacetylenes, phenylpropanes, saponins, alkaloids, anthracenes, flavonoids, coumarins, tannins, lipids, glucosinolates, and numerous glycosidic compounds.

Usually, quality assessment of herbal drugs is performed in accordance with the European Pharmacopoeia or the existing DIN/ISO standards, provided that the specifications for the plant material to be examined are valid. If no standardized specifications must be fulfilled, then it is up to the individual companies processing herbal drugs to decide which methods should be used for the incoming goods inspection. In this context, it can be observed that more and more rapid methods such as near-infrared spectroscopy (NIRS) and other rapid vibrational spectroscopy methods are used to increase the efficiency in plant breeding, breeding research, and quality control of herbal drugs and the related extracts. Sophisticated coupling techniques such as gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography–mass spectrometry (LC–MS) are available to obtain initial information on the structural composition of unknown analytes. For further investigations, it is necessary to isolate these components from the plant matrix and analyze them by nuclear magnetic resonance (NMR). This technique provides especially important additional information about the steric arrangement of certain plant substances within their molecular structure.

Recent technological advances allow for large-scale GC–MS or LC–MS profiling of medicinal and aroma plants. These high-throughput methods are generally termed as "metabolomics." The applicability and significance of particularly GC–MS metabolite profiling in functional genomics has been specially regarded for the plant model systems *Arabidopsis thaliana* and potato (*Solanum tuberosum*) (Fiehn et al. 2000; Roessner et al. 2000).

The most popular analytical methods in metabolomics are those based on nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). NMR allows the rapid, high-throughput and automated analysis of crude extracts, and the quantitative detection of many different groups of metabolites (Kim et al. 2010, 2011), providing also structural information including the necessary stereochemical details (Seger and Sturm 2007). However, NMR is less sensitive than MS-based approaches (Kim et al. 2011), and NMR data have been metaphorically compared to "the tip of the iceberg," with LC–MS providing details of the much larger, submerged portion (Sumner et al. 2003).

Between these extremes, GC–MS is particularly suitable for the detection of thermally stable volatile compounds (or compounds with volatile derivatives). Generally, LC–MS is more sensitive than GC–MS, and furthermore it allows the analysis of thermally labile nonvolatile compounds (Hegeman 2010). The molecules that can be detected by LC–MS range from polar sugars and nonaromatic organic acids (Wishart 2008) through to various lipids (Hummel et al. 2011), as discussed in recent reviews (Kim et al. 2011; Hegeman 2010; Boccard et al. 2010). Applying untargeted metabolomics LC–MS can be used to characterize the composition of complex plant tissues and their extracts. Nevertheless, the ability to analyze various kinds of metabolites by LC–MS depends strongly on the ionization source and the chromatographic method used for analyte fractionation and elution. Therefore, LC–MS-based untargeted metabolomics combines high sensitivity and an untargeted approach to provide an ideal procedure for the analysis of a wide range of nonvolatile metabolites.

2.2 Sampling and Cleanup

In order to obtain representative plant samples, it is extremely important to ensure that the sample taken for analysis corresponds to the average quality of the plant part to be analyzed. Especially for research and development projects, it must be ensured that the samples are suitable in terms of quantity and homogeneity to reflect the specific ingredient characteristics of the analyzed plant tissue as realistically as possible. In this context, it has been demonstrated for fennel, that at least 50–60 fennel fruits per analysis are necessary to limit the relative standard deviation to values below 2 % (Krüger and Schulz 2007). For breeding research, it is also necessary to perform the individual cultivation experiments with a sufficient number of single plants and repetitions to minimize the influence of environmental effects and to guarantee reliable statistical statements. In industry, there is a particular interest in controlling the uniformity of individual batches of incoming raw materials. Because complete inspection of all the packages in a shipment may be time-consuming and costly, it is usually based on appropriate ISO rules that provide a basis for calculating the necessary number of samples. For example, ISO 948-1980 informs on the specific aspects to be considered when sampling spice deliveries, and ISO 212-1973 describes the precise sampling of essential oils. Before instrumental analysis techniques are applied, in most cases it is first necessary to clean up the herbal material with special regard to the analytes to be determined. If the intention is to investigate nonvolatile substances in freshly harvested medicinal and aromatic plants, these substances must be isolated from the plant matrix by means of solvent extraction and should be then analyzed as soon as possible. If an immediate analysis of the fresh material is not possible, the samples should be dried in order to reduce possible biochemical processes in the plant material as far as possible. In order to reproduce the metabolomic profile as authentically as possible, freeze-drying is the method of choice, since in this way thermal influences can be minimized. For

characterization of medicinal and aromatic drugs, however, the usual air-drying is preferred, which leads to samples with a residual moisture content of about 6–12%. Then, the dried plant material has to be further processed (e.g., separating of seeds, leaves, and flowers, grinding, and homogenizing of the drug) before the individual analytical method can be applied. If volatile plant substances are to be analyzed, hydro-distillation described in the individual pharmacopoeia monographs, which is based on the volumetric method developed by Clevenger, is the appropriate method (Clevenger 1928; DAB 1996). The distilled essential oil can be used directly for subsequent gas chromatographic analyses. If only small sample amounts are available, microdistillation may be a suitable alternative. For this, sample amounts of approximately 200–250 mg are sufficient (Pfannkuche 2000). However, it has to be mentioned here that the small sample size does not always provide representative results and should therefore be used only for orientation studies (e.g., to classify a certain chemotype).

2.3 Chromatography Techniques

Thin Layer Chromatography (DC) and High-Performance Thin Layer Chromatography (HPTLC)

The method of thin-layer chromatography was first introduced in 1959 but has been continuously developed in accordance to the increased requirements (Jork et al. 1987; Hahn-Deinstrop 2007). Even today, DC remains an important tool in the identification and quality control of drugs and plant extracts. The special advantages of HPTLC consist in particular in the easy handling of this technology, its speed, and comparatively low cost. Commercially available HPTLC plates are disposable, which is particularly suitable for routine use. Furthermore, consumption of solvents in HPTLC is also low: with a few milliliters of mobile phase, up to 70 plant samples can be simultaneously analyzed in a linear chamber next to the related authentic reference substances. Various physical, microchemical, and biological–physiological detection methods can be used for the detection of the finished plates (Jork et al. 1987). Of these three methods, the biological–physiological detection method provides the highest specificity, because only those analytes that have a certain biological activity are detected. This detection method has already been successfully used to detect various plant ingredients (e.g., alkaloids (Verpoorte et al. 1982, 1983), bitter substances (Telek et al. 1974; Baur et al. 1977), and saponins (Yoon and Wrolstad 1984)). In order to perform (semi-)quantitative HPTLC studies, both external and internal standard methods are principally used in practice.

Attimarad et al. (2011) highlight the special advantages of HPTLC for analysis of natural products such as medicinal and spice plants. Even today, HPTLC is frequently used as a rapid, comparatively simple, robust, and extremely versatile analytical technique in this field. It is not only applied to confirm the identity of certain drugs but also is used as a screening tool for adulterations and is highly suitable for the evaluation and monitoring of cultivation, harvesting, and extraction processes

and testing of stability. Many reports present the evidence of utilization of HPLTC in fingerprinting analysis of drugs of natural origin, and hence, the increasing acceptance of natural products is well suited to provide the core scaffolds for future drugs (Jadhav et al. 2009; Hong et al. 2009; Patel et al. 2010; Hamrapurkar et al. 2011).

Recently, Taha et al. (2015) demonstrated the special advantages of HPTLC hyphenated with bioassays and mass spectrometry to discover and quantify the activity of certain bioactive components. They present this approach exemplary for turmeric (*Curcuma longa*) and milk thistle (*Silybum marianum*), applying three selected bioassays, the *Aliivibrio fischeri* bioassay, the *Bacillus subtilis* bioassay, and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. As a proof of principle, the bioactive components found in the extracts were confirmed by HPTLC–MS. Then, the mentioned bioassays in combination with planar chromatography were directly linked to the known, single effective compounds such as curcumin and silibinin. Furthermore, also several unknown bioactive components could be discovered and exemplarily characterized, which demonstrates the strength of this method. The authors highlight that this streamlined methodology comprises a nontargeted, effect-directed screening first, followed by a highly targeted characterization of the discovered bioactive compounds. Therefore, they strongly recommend this approach for bioactivity profiling of phytochemicals and for detecting bioactive degradation products.

Gas Chromatography (GC)

Volatile plant substances such as mono- and sesquiterpenoids, aliphatic alcohols, aldehydes, and aromatic compounds in essential oils and solvent extracts are usually determined by gas chromatography. Today, numerous fused silica capillary columns with different stationary phases are available for the respective separation problem such as the nonpolar dimethyl polysiloxanes (e.g., DB-1, DB-5, CPSil 5) and more polar polyethylene glycol polymers (e.g., Carbowax 20 M, DB-Wax) (Schreier 1984; Sandra and Bicchi 1987). The samples are directly injected as solvent extracts into the heated injector. Usually, the separated substances are recorded by means of a flame ionization detector (FID). For this, the carbon-containing analytes are first fragmented in a small flame, which is fed by pure hydrogen and synthetic air. The ions formed cause an overall voltage drop across the electrode of the detector, which is accordingly recorded as a signal and electronically amplified. For selective and sensitive detection of those plant substances which contain nitrogen or phosphorus (such as pyrazines), NPD detectors provide best results. A more reliable identification, in particular based on library spectra, can be performed applying various hyphenated techniques such as GC–MS, GC–IR, and GC–AED. The major advantage of GC is the large number of plant substances that can be separated in one single analysis run. GC–MS has been used already for more than 50 years for characterization of various plant secondary substances, and consequently today extensive MS databases such as NIST or AMDIS are available for first tentative identifications of unknown substances. Especially the individual composition of numerous essential oil plants has been analyzed already since the early 1960s and thus contributed significantly to the enormous knowledge in this special field of

research. In spite of the fact that sample preparation for GC measurements is quite elaborate, this technique has been commonly used in breeding projects which aimed to improve the content of certain valuable plant substances (Franz and Koehler 1992).

Since the absolute retention times for individual GC signals depend on the experimental conditions such as column temperature, type and velocity of the carrier gas, type and amount of stationary phase, and the individual column dimensions, relative retention indices are used to reliably compare peaks recorded under different experimental conditions (Kovats 1958). The qualitative characterization of plant extracts serves primarily to classify a particular drug or plant extract (e.g., whether it corresponds to a certain "reference chromatogram"). In such a way, it is principally possible to detect very quickly whether a sample contains adulterations or shows a GC profile deviating from a previously agreed specification. Also, for plant breeding, it is sometimes sufficient to be able to distinguish the existing chemotypes of a plant species by means of qualitative GC analysis (Schulz et al. 2003a, b).

In order to accumulate the analytes, solid phase microextraction (SPME), solid phase dynamic extraction (SPDE), and stir bar sorptive extraction (SBSE) are increasingly used for GC measurements as a monitoring technique for volatile compounds in medicinal and aromatic plants (Belliardo et al. 2006; Jirovetz et al. 2003). All three mentioned microtechniques follow the same principle: the analyte is fixed by a special adsorbent material such as polydimethylsiloxane (PDMS). For SPDE and SBSE, generally a higher amount of polymer is used, and thus a higher capacity and a lower detection limit for the individual analytes can be obtained. The theory, technology, evolution, and applications of SPME have been reported by Arthur and Pawliszyn (1990). Some practical application examples for SPME and SBSE in the field of medicinal and aromatic plants are described by Bicchi et al. (Bicchi et al. 2000). Furthermore, purge and trap methods (dynamic headspace sampling) have been described as closed-loop stripping or as "pull" or "push–pull" systems. For these sampling methods, a continuous air stream is passed through the sample vial as a carrier gas. While the analytes are trapped on adsorbents, the carrier gas is circulated (closed loop) or purged out of the sample vial (pull). Today, a large number of different adsorbent materials are available, and several reviews present the advantages of this approach (Tholl et al. 2006; Dettmer and Engewald 2002; Boland et al. 1984). Recently, Killeen et al. (2017) analyzed the volatile monoterpenes and sesquiterpenes of hops which are sequestered in lupulin glands (extracellular trichomes) applying a high-throughput method by headspace–SPME–GC. The chromatographic data were subjected to principal component analysis (PCA) and the results compared with other analytical techniques (Raman and NMR). Based on these data, a rapid discrimination of different hops chemotypes could be successfully achieved, and those plants with highest potential for new flavor cultivars could be selected. As can be seen in Fig. 2.1, the score plots separate the GC traces into genotype-specific clusters, and the chemical origins of the genotypic clustering is presented in two-dimensional loadings plots (Belliardo et al. 2006).

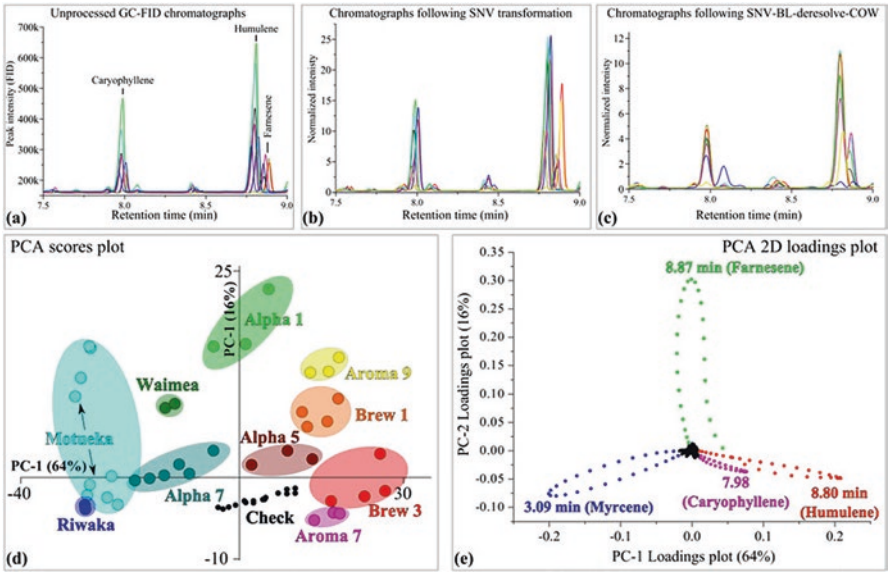

Fig. 2.1 (**a**–**c**) Effects of preprocessing of selected HS–SPME–GC–FID chromatographs of eight random lupulin glands samples in the range 7.5–9.0 min; (**d**) and (**e**) summary of PCA applied to preprocessed chromatographs of lupulin glands from 10 hops cultivars and the check sample (Killeen et al. 2017)

High-Performance Liquid Chromatography (HPLC)

The basics of high-performance liquid chromatography are described in numerous books and review articles, of which only a few can be mentioned here (Eppert 2000; Meyer 2004; Galensa et al. 1995). Plant substances that cannot be vaporized without decomposition are usually analyzed by HPLC. For this separation technique, beside columns with normal phases (silica gel), mainly columns equipped with chemically bonded phases are used (e.g., octylsilane, C-8 and octadecylsilane, C-18). In addition, amino, cyano, and diol phases are also used in plant analysis. Over the last years, the development of small adsorbent particles has significantly improved the separation performance of HPLC and at the same time shortened the analysis times. Usually, the separated components of a plant extract are detected by UV-Vis or fluorescence detection depending on the individual chemical properties of the analytes. For substances that do not show any absorption bands in the UV-Vis range and cannot be detected by fluorescence (e.g., sugars and saponins), refractometric detection may be another option; however, in most cases, this technique demonstrates a significantly lower selectivity. Based on retention times and absorption spectra, a first classification of separated plant components is generally possible, but nevertheless it is recommended to chromatograph a reference substance under the same HPLC conditions. Since in most cases suitable reference substances are not available or very expensive, more and more LC–MS is used for the tentative identification of unknown plant substances.

Today, numerous separation techniques, specific stationary phases, and detectors are available to achieve the necessary selectivity, sensitivity, and speed for nearly any separation problem. More than 40 years after the introduction of LC–MS, this analytical technique is well established even in routine labs. Nevertheless, there is a steady advancement in terms of instrumentation (ultra-high-performance LC, ion mobility MS, etc.) and hyphenation of different techniques (supercritical fluid chromatography–mass spectrometry, two-dimensional techniques, etc.) (Ganzera and Sturm 2018). Furthermore, the commercialization of LC–TOF-MS allows today the use of gentle electrospray ionization while providing accurate mass and retention time data to identify individual analytes (Polettini et al. 2008). The ability of LC–TOF-MS to acquire accurate mass measurements provides data that will provide confidence in the detection of target compounds. The feature of all-scan, all-the-time TOF data not only allows the detection of known target compounds but also provides a retrospective searching for compounds that may be present but not originally identified.

2.4 Vibrational Spectroscopy Techniques

In addition to mass spectroscopy, which is usually used in combination with GC and HPLC, vibrational spectroscopic methods for rapid identification and quantification of chemical compounds in medicinal and spice plants have been increasingly used in recent years. The spectra of the components or mixtures to be analyzed (e.g., fresh plant constituents, pharmaceutical drugs, essential oils, plant extracts) can generally be registered in a short time with comparatively little experimental effort. In particular, the molecular vibrations to be observed in the mid-infrared wavelength range (400–4000 cm^{-1}) provide important structure-specific information which can either be interpreted directly or evaluated with the aid of statistical methods, possibly including also reference data.

Infrared (IR) and Raman Spectroscopy
IR is based on direct vibrational excitation caused by an IR radiation source (e.g., a Nernst pin). Generally, an interaction with the electric field of the IR light is always observed when the electric dipole moment changes during the vibration of the analyte molecule. In this case, the analyte molecule absorbs from the radiated infrared energy exactly those components that resonate with the natural frequencies of the bonds present in the molecule. By contrast, Raman spectroscopy is an indirect vibrational excitation using a monochromatic laser. Here, especially those vibrations of individual functional groups showing high polarizability can be detected very sensitively. Moreover, double and triple bonds in hydrocarbons very often cause intense signals in the Raman spectrum (e.g., carotenoids and polyacetylenes). Raman scattering is observed whenever monochromatic excitation radiation in the visible, UV, or NIR region interacts with molecules. Overall, IR and Raman spectroscopy provide a comprehensive fingerprint of the different vibrational modes,

performed by the affected analyte molecules. Therefore, Raman and infrared spectroscopy are called generally as "complementary spectroscopy techniques." The theory as well as the details of the different spectrometer systems will not be discussed here in detail, but only referred further literature (Schrader 1995; Schrader 1997; Siesler et al. 2002; Smith and Dent 2005; Siebert and Hildebrandt 2008). Applications of Raman spectroscopy in plant analysis came to a successful breakthrough comparatively late. This is due in particular to the fact that very often considerable fluorescence is superimposed on the Raman bands, and as a result no interpretation of the spectra obtained is possible. To minimize those unwanted fluorescence effects, NIR diode lasers providing an excitation at 780 or 830 nm were first used to produce Raman scattering. Today, virtually fluorescence-free spectra are obtained when applying so-called neodymium-YAG laser with excitation at 1064 nm. However, due to the lower excitation energy in the NIR range, a lower detection sensitivity is usually achieved as well.

First applications of Raman microspectroscopy have been described more than 30 years ago. Today, micro-Raman mapping or imaging techniques are widely used in medicine and plant analysis and provide the unique advantage that the spatial distribution of several components can be detected simultaneously even on a cellular scale (Salzer and Siesler 2014). While for Raman mapping, the sample to be analyzed is moved stepwise on an xy-table and spectra are recorded at each position, data acquisition can be performed in a very short time when using Raman or infrared imaging. Applying two-dimensional detectors (so-called focal plane detectors), even larger areas can be detected with one measurement. By means of the "image-mosaicking technique," this spectral information is then put together to form a larger, high-resolution overall picture. The currently available microscope spectrometer systems usually have a software-controlled *xy* table, and the resulting "maps" or "images" can be directly compared with the corresponding video image of the light microscope. While micro-Raman measurements can usually be performed directly on the plant material without any time-consuming sample preparation, tissue sections of the sample to be examined with a layer thickness of approx. 10–20 µ must generally be prepared for the micro-infrared spectroscopy prior to each measurement. Usually, a cryomicrotome is used for this purpose, because embedding material can cause additional IR bands which may superimpose the signals of the plant tissue. Alternatively, it is also possible to use ATR infrared spectroscopy to further characterize the chemical composition of tissue surfaces (e.g., wax layers on fruits and leaves, adhering pesticide residues) (Schulz et al. 2002a, 2003a; Sakhteman et al. 2015; Clara et al. 2016; Wulandari et al. 2016). Nevertheless, it has to be taken into account that the spectroscopic techniques discussed here hardly offer any possibilities for trace analysis. Most spectral bands are usually produced by plant substances occurring in amounts of higher than 1%. There exist a few exceptions such as the so-called "Resonance Raman Effect," which allows detecting certain analytes such as carotenoids in much lower concentrations. There is also the option to use the so-called "surface-enhanced Raman scattering" (SERS) technique (Baia et al. 2008; Schlücker 2011). By adding certain metal colloids (silver or gold) to the sample, a considerable increase of the Raman scattering produced

by the analyte can sometimes be achieved with this methodology. However, the SERS technique significantly alters the relative intensities in the Raman spectrum, making it more difficult to interpret the individual signals in individual cases.

Some studies of carrot roots (*Daucus carota*) have been performed by Krähmer et al. (2016) aiming to quantify certain biochemical quality parameters. Within a single FT–Raman experiment, carbohydrates, carotenoids, and polyacetylenes could be reliably quantified achieving high coefficients of determination. Thus, it was possible to quantify the individual representatives of each compound class with comparably high quality which can be seen by the statistical parameters ($R^2 = 0.97$ and $R^2 = 0.96$ for α-carotene and β-carotene, $R^2 = 0.90$ for falcarindiol, and $R^2 = 0.99$, $R^2 = 0.98$, and $R^2 = 0.96$ for fructose, glucose, and sucrose). Contrary to that, the authors were unable to quantify two laserine-type phenylpropanoids due to low concentration and Raman response. Nevertheless, applying principle component analysis revealed metabolic variety of carrot root composition depending on root color and botanical relationship.

Mid-Infrared Spectroscopy (MIR)

Until recently, mid-infrared spectroscopy (MIR) was primarily used in agricultural research as a qualitative technique for identification and verification of unknown pure substances isolated from extracts or distillates (Colthup et al. 1990). Usually infrared spectra obtained from plant samples are very complex because each functional group in a molecule contributes more or less to the spectral output. The net result is a spectrum in which band assignments may be difficult due to the fact that overlapping and mixing of various vibrational modes occur. Therefore, the MIR has been limited for a long time. Only a few years ago FT-IR spectroscopy has become a powerful tool for elucidating the structure, physical properties, and interactions of various carbohydrates, including commercial sugars, cellulose, pectins, starch, hemicellulose, carrageenans, and others. Applications in the area of systematic fingerprinting, quantification, and IR microspectroscopy to monitor cell wall constituents such as pectins, proteins, aromatic phenols, cellulose, and hemicellulose have been reviewed by Kačuráková et al. (2001).

Liquid samples are usually measured using a quartz glass cell or a horizontal attenuated total reflection (H-ATR) unit. For ATR analysis, the sample is placed on the ATR crystal (consisting of diamond or zinc selenide), and the absorbance of the sample can be easily determined within a few seconds. Alternatively, also solid samples such as powdered plant material can be measured if the sample is pressed on the surface of the ATR crystal.

In order to increase the amount of plant volatiles and to reduce spectral influences of the plant matrix, microdistillation may provide a useful approach especially for analytical characterization of single plants or selected plant parts (Briechele et al. 1997). For this, approx. 200 mg of the sample is hydro-distilled for 1 hour, and tetrachloromethane is placed in the receiving tube of the microdistillation unit as solvent. Only small sample amounts of about 5–10 µl are usually necessary to obtain well-resolved spectra dominated by the individual main essential oil components. Applying statistical methods such as PCA, even different chemotypes can be

identified as clearly separated clusters. Generally, onsite-IR measurements (e.g., to perform measurements directly on the field) can be also performed applying ATR-IR spectroscopy. A new generation of mobile IR spectrometers can be powered by a car battery and is already available for a comparatively small amount of money.

Near-Infrared Spectroscopy (NIR)

For quality control of medicinal and aromatic plants, various microbiological tests, determination of dry matter content, and various chromatographic techniques are used. In particular, chromatographic separations performed by TLC, HPLC, HPTLC, and GC are time-consuming and require highly skilled technical personnel. Therefore, over the last years, new reliable and easy-to-perform rapid methods for the analytical characterization of herbal drugs have been developed. In this context, numerous applications of NIR spectroscopy for both qualitative and quantitative evaluation of various plant species have been described in recent years (Schulz 2004). Although the concentration of some of the plant constituents to be determined is close to the detection limit of the NIR detectors, it is often possible to develop reliable NIR calibration functions for these analytes even in such cases. In NIR spectroscopy, the respective herbal sample is illuminated in the wavelength range between 780 and 2500 nm, and the absorption bands that occur are registered. These NIR signals are overtone and combination vibrations of the fundamental vibrations to be observed in the mid-infrared range. Compared to MIR spectra, NIR bands are significantly less well resolved, and in most cases, it is very difficult to interpret the individual vibrational modes. Nevertheless, they contain important structural information which can be used for statistical (chemometric) calculations. Depending on the nature of the sample, the NIRS measurements are carried out in transmission, transflection, or in diffuse reflection. By contrast, NIR spectra of essential oils and plant extracts are measured either in transmission or in transflection. NIR spectroscopy is an indirect method of analysis. Before quantification of individual components can be obtained, it is first necessary to establish correlations with reference data of the parameters to be determined. The prediction for each parameter is usually described by suitable statistical values such as the coefficient of determination (R^2) and the standard error of the calibration (SEC) (Watt 1999; Siesler et al. 2002; Ozaki et al. 2006). The particular advantages of NIR spectroscopy consist in the fact that, compared with measurements in the MIR range, a greater penetration depth into the plant sample can be achieved. Furthermore, very sensitive and fast photodetectors are available (e.g., indium gallium arsenide (InGaAs) detectors), and cost-effective fiber optic fibers for sample presentation can be used. Because it is a nondestructive analytical rapid method, NIR spectroscopy can be used successfully, especially in the conventional breeding of medicinal and aromatic plants. For this purpose, the individual plants to be investigated (e.g., caraway or fennel fruits, chamomile flowers, peppermint leaves, devil's claw roots, cinnamon bark) is transferred to a measuring cell, and the spectroscopic data are recorded within several seconds. Finally, the individual plant parameters are predicted on the basis of the related reference data.

The applicability of NIR spectra has been demonstrated for numerous medicinal and aromatic plant species (Schulz 2004; Huck 2014; Lafhal et al. 2016; Ercioglu et al. 2018a, b). In this context, main areas of application are plant breeding (selection of high-performance plants), prediction of the optimal harvest time, and quality control.

A clear separation of red and white clover species (*Trifolium pratense* L. and *Trifolium repens* L.) could be achieved applying NIR or ATR-IR spectroscopy (Krähmer et al. 2013). As can be seen in Fig. 2.2, chemometrical calculation of the individual spectral data presents more or less characteristic differences in the NIR dendrogram for various red and white clover cultivars which have been cultivated for 2 years and which have been partly exposed to mechanical stress. The presented cluster analysis shows a strict separation between samples of red and white clover (heterogeneity 0.6) with two subclusters separating the samples according to the two investigated years (black and gray color, heterogeneity level 0.38). In between these subclusters, a grouping of samples in reference to the harvest date can be observed, whereas the different cultivars are completely mixed. The application of mechanical stress also leads to a certain clustering but on a very low level of heterogeneity (less than 0.1) (Krähmer et al. 2013).

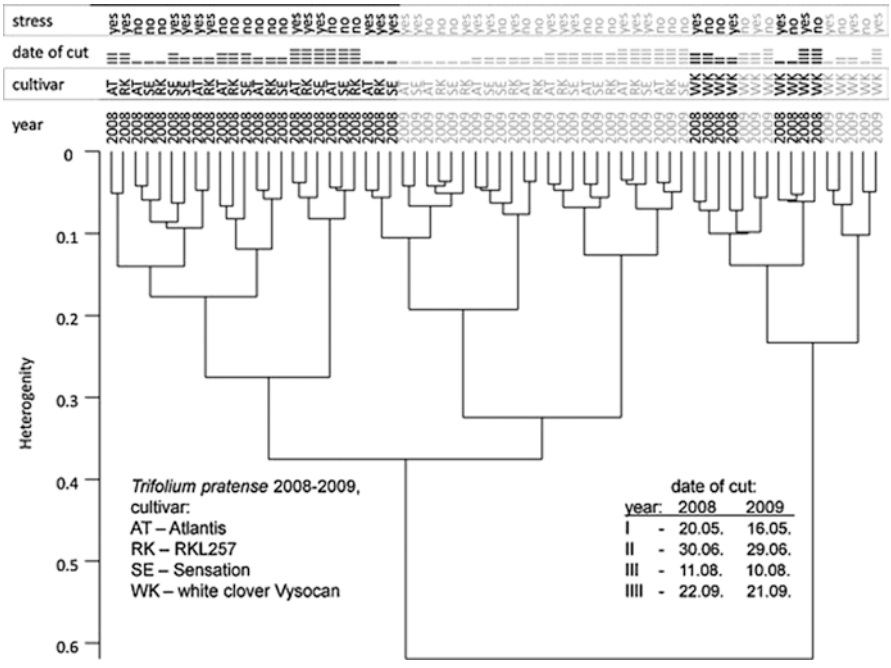

Fig. 2.2 Hierarchical cluster analysis for the NIR spectra of three red and one white clover cultivars collected during two cultivation periods in 2008 (black) and 2009 (gray), at four different seasons and with or without mechanical stress application (Krähmer et al. 2013)

NIR and ATR-IR spectroscopy methods have been also used to classify different fennel chemotypes according to their individual profile of volatile substances. Thus, intact fennel fruits of different chemotypes and the related solvent extract and essential oils could be successfully discriminated (Gudi et al. 2014). Figure 2.3 shows the hierarchical cluster analysis of different fennel accessions investigated in a study according to the GC–FID data (A), compared with the ATR–FTIR spectra of the fennel extracts (B) and the intact fruits (C), as well as the Raman spectra of the extracts (D). According to the qualitative and quantitative composition of their volatile fraction, the 10 analyzed fennel genotypes were classified into four chemotypes: the *trans*-anethole type with accessions cv. Berfena, FOE 16, FOE 18, cv. Chumen, and cv. Feniks; the estragole type with FOE 43 and FOE 48; the γ-asarone type with FOE 86 and FOE 87; and the piperitenone oxide type FOE 25. A very similar classification is obtained by ATR–FTIR for both the fruits and the extract. Additionally, for the fruits and the extracts, a strict separation of sweet and bitter fennel, as demanded by the European Pharmacopoeia, can be observed. The two accessions, cv. Berfena and FOE 16, contain much more than 15% of fenchone and simultaneously a content of *trans*-anethole of about 60% (Gudi et al. 2014).

In recent years, hyperspectral NIR spectroscopy has also been increasingly used in the quality control of medicinal and aromatic plants (Sandasi et al. 2018). This technique combines conventional spectroscopy and digital imaging to gather chemical information and visualize spatial distribution of chemical constituents within a plant matrix. Tankeu et al. (2017) applied shortwave infrared hyperspectral imaging (SWIR-HSI) in tandem with chemometric data analysis as a fast alternative method for the discrimination of four cohosh species (*Actaea racemosa*, *Actaea podocarpa*, *Actaea pachypoda*, *Actaea cimicifuga*). From SWIR-HSI data (920–2514 nm), the range containing the most discriminating information of the four species was identified as 1204–1480 nm. Using vector machine discriminant analysis (SVM-DA), seven out of 36 commercial products were recognized as being true black cohosh, while 29 products indicated adulteration. Analysis of the UHPLC-MS data demonstrated that six commercial products could be authentic black cohosh.

The same group applied SWIR hyperspectral imaging and image analysis as a rapid quality control method to distinguish between *Illicium anisatum* and *Illicium verum* whole dried fruit (Vermaak et al. 2013). PCA was used to reduce the high dimensionality of the data, to remove unwanted background information, and to visualize the data. The new model was able to accurately predict the identity of *I. anisatum* (98.42%) and *I. verum* (97.85%). The authors highlight that this method has also the potential to detect *I. anisatum* whole dried fruits within large batches of *I. verum* through upscaling to a conveyor belt system.

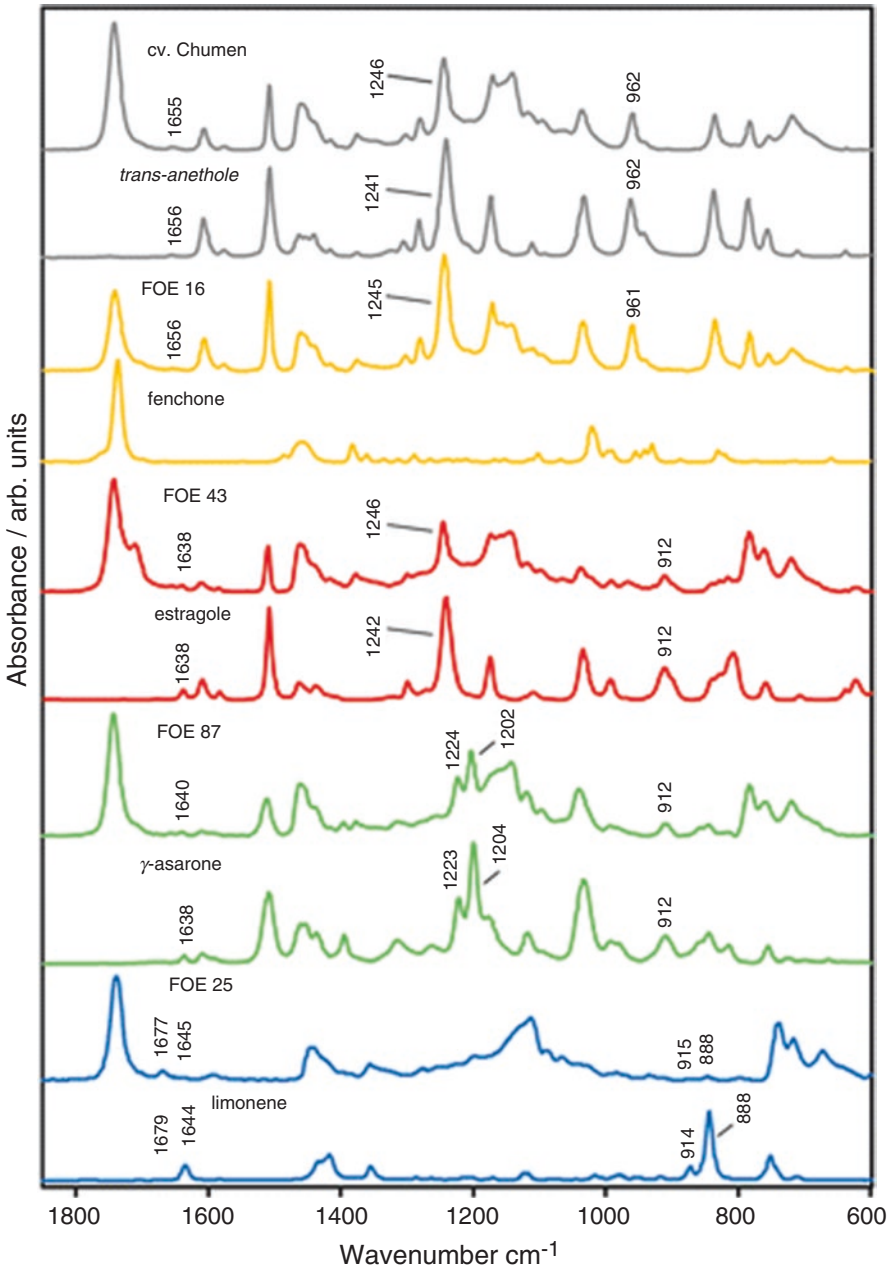

Fig. 2.3 ATR-FTIR spectra obtained from CCl_4 extracts of different fennel genotypes and the corresponding standards for the most valuable components in the volatile fraction (Gudi et al. 2014)

2.5 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy has contributed widely to the study of plant primary or secondary metabolism in very different ways. Although NMR is less sensitive than mass spectrometry, it offers some major advantages by being reproducible, nondestructive, and nonspecific. Furthermore, this technique can be also used for quantitative measurements. NMR can be used alone or in combination with other analytical technologies such as MS, as demonstrated, for instance, for metabolomic studies (Sumner et al. 2015). Indeed, NMR and MS can be applied as complementary techniques: the advantages of NMR include a very high reproducibility and the access to unambiguous structural information, whereas the major advantage of MS is its high sensitivity. NMR has been proven to be an important tool for the global characterization of sample composition within plant metabolomic studies and presents several examples of its use for targeted phytochemistry with a special focus on compound identification and quantification. In such cases, NMR approaches are often used to provide characteristic fingerprints of plant sample composition.

Applying ^{1}H NMR metabolomic studies, Frédérich et al. (2010) succeeded to discriminate three *Echinacea* species (*E. purpurea*, *E. pallida*, and *E. simulata*) which are used worldwide as medicinal plants. The powdered plant samples were extracted with a mixture of phosphate buffer in D_2O and methanol-d4 and subsequently measured by NMR. Based on multivariate data analysis of the whole NMR spectra, a good discrimination between aerial parts and roots, predominantly influenced by sugars and fatty acids, could be obtained. A complete separation of the three *Echinacea* species was achieved using only the aromatic part (δ 5.8–10.0) of the NMR spectra. The authors found that various caffeic acids and here particularly echinacoside and cichoric acid were mainly responsible for this discrimination.

NMR spectroscopy presents much less sensitivity, but provides clearly more structural information, reproducibility, and can be also easily applied for quantitative analyses. Furthermore, in comparison with MS sample preparation is simpler, and a short analysis time makes NMR the ideal high-throughput technique for fast metabolite fingerprinting (Lommen et al. 1998; Dixon et al. 2006). The quality of most medicinal and aromatic plants is directly connected with the presence of certain plant metabolites, contributing especially to texture, nutritional and aroma value, and shelf life. At the same time, many agronomic traits such as yield, disease resistance, and stress tolerance are influenced and regulated by certain plant metabolites. Aiming to improve specific traits, very often wild species are used to reintegrate special genetic functions which have been lost during the last decades. In this context, metabolite profiling is a very useful tool to identify metabolite traits (QTL) in wild genotypes or breeding lines resulting from crossing experiments. Based on the individual profiles, genetic regions related to certain biochemical pathways, which are responsible for the production of various plant substances, can be identified. Furthermore, metabolite profiling can speed up early selection of breeding processes by using suitable biomarkers.

Metabolite profiling via NMR is a quick, easy-to-perform, and efficient tool to distinguish between various substances of a natural product group, which is attributed to detect of very important regions of a spectrum. Among the individual isotopes, ^{1}H proton is the most sensitive one and therefore preferred for the analysis of medicinal and aromatic plants. Another appropriate isotope is ^{31}P isotope which can be applied to profile phosphate esters that are frequently occurring in herbal extracts. Furthermore, ^{13}C isotope is frequently used for mapping of amino acids, carbohydrates, lipids, and organic acids in herbal extracts. NMR identification of the main components in essential oils or herbal extracts clarifies the possible chemotaxonomic differences between plant species. For example, the identification of limonene-10-al, one of the main chemical markers among *Dracocephalum* species, was carried out by NMR, while it was not successful detecting this major compound in several GC/MS studies (Saeidnia et al. 2007).

2.6 Metabolomics

Plant metabolites represent intermediary and final products of biochemical processes taking place in the individual cells. It is assumed that more than 200,000 secondary metabolites exist in plant kingdom possessing various biological functions. The chemical nature of these substances can be very different and may include a broad range of different chemical classes such as mono-, sesqui-, di-, tri-, and polyterpenes, phenylpropanes, amaroides, glycosides, saponins, alkaloids, glucosinolates, anthracenes, flavonoids, coumarins, tannins, lipoids, lectins, and several others.

After introduction of special techniques for DNA sequencing (genomics), gene expression analysis (transcriptomics), and protein analysis (proteomics), the expression of plant metabolites (metabolomics) as a missing link in functional genomic strategies has been described only a few years ago (Dunn et al. 2005; Hall 2006).

The term “metabolomics” has been introduced for the analysis of intra- and extracellular metabolites in simple biological systems such as plants. In this context, the main goal is to determine the metabolic response of living systems to genetic modification and physiological stimuli. Generally, metabolomic experiments have been employed to identify more or less all detectable plant metabolites. However, realistically, in most cases a range of different strategies using various analytical techniques must be applied aiming to identify and semiquantify individual metabolites related to specific biochemical pathways (metabolite profiling). A more global approach is to perform high-throughput analyses for a tentative sample classification (metabolic fingerprinting) (Warwick et al. 2005).

Today, metabolite profiling of medicinal and aroma plants is performed to study more or less all small molecules that are built in the individual metabolic pathways and that are required for the maintenance, growth, and normal function of a cell (Schripsema 2010). This approach is focused on the complete analysis of all detectable metabolites occurring inside a certain sample. Usually, metabolomic

fingerprinting is performed on the basis of the individual MS or NMR pattern analysis in combination with chemometric algorithms aiming to identify efficient discriminating factors. These metabolite patterns represent all changes which may occur in response of a disease and environmental or genetic perturbations. Furthermore, metabolomics may provide a better understanding to compare different cultivation strategies (e.g., conventional and ecological cultivation) or different storage conditions including postharvest processes.

Generally, different spectroscopy techniques such as MS, NMR, UV, and IR and also chromatography methods such as GC or HPLC, sometimes hyphenated with MS or NMR, are used for metabolic fingerprinting (Schauer and Fernie 2006). Beside NMR spectroscopy, also vibrational spectroscopy methods offer the option to rapidly analyze plant tissues, in some cases even without destruction of the sample material. Special advantages of these techniques exist for breeding research and applied breeding, because after analysis seeds are still available for further breeding processes. Furthermore, techniques such as near-infrared spectroscopy (NIRS) or attenuated total reflection infrared spectroscopy (ATR-IR) can be used to evaluate the metabolic profile even of those plant substances which are insoluble in water or certain organic solvents. Several applications underlining the particular use of these sophisticated vibrational spectroscopy methods (NIRS, MIRS, Raman) have been reviewed (Baranska et al. 2004, 2005a, b, c, d, 2006; Daferera et al. 2002; Krähmer et al. 2013; Lasme et al. 2008; Schulz 1998, 2004, 2014a, b; Schulz et al. 2002b, 2003b, 2005a, b, 2014; Schulz and Baranska 2007; Siatis et al. 2005; Strehle et al. 2005; Zalacain et al. 2005).

Thus, infrared and Raman spectroscopy methods may provide a holistic fingerprint of the present metabolic status. By contrast to the classical approach applying various chemical methods, the special advantage exists of using all available spectral information obtained from the analyzed plant tissue for detailed evaluation. The combination of spectroscopic methods such as IR, NIR, Raman, and NMR with statistical tools such as principal component analysis (PCA) allows identifying clusters or outliers and may provide important information for various studies related to the plant metabolomic.

From the early 1990s, the increased resolution in NMR spectra by higher field magnets provided the option to perform metabolic fingerprinting of various plant materials (Schripsema and Verpoorte 1991). However, also the development of improved separation power of GC and HPLC techniques with higher separation power permitted a comprehensive profiling of various samples (Fiehn et al. 2000). Furthermore, advances in chemometrics also contributed significantly to the increasing interest to use metabolomics in plant analysis aiming to evaluate large data sets and to characterize changes in relation to specific parameters.

The main disadvantage of both metabolomics techniques MS and NMR is that they need tissue extraction prior to analysis. Furthermore, some compounds should be derivated before being injected to GC/MS, because they cannot be evaporated without decomposition. Applying the hyphenated techniques LC/MS and LC/NMR equipped with a solid phase extraction (SPE) system located between the HPLC and the detector increases the sensitivity in detecting natural compounds from herbal

extracts. Today, LC–UV–SPE–NMR–MS are widely used in genobiotic metabolism studies and related researches in natural compounds (Krishnan et al. 2005; Saeidnia and Gohari 2012).

Wang et al. (2017) applied metabolomics to demonstrate how to screen for specific biomarkers of *Panax ginseng*. The roots of this medicinal plant are widely used not only in Asian countries but also in many Western countries. To avoid confusion with other common species of this genus, the authors analyzed the individual metabolite profiles of *Panax notoginseng*, *Panax quinquefolium*, *Panax japonicus var*., and *Panax ginseng* using UHPLC–TOF–MS. On the first view, the obtained total ion chromatograms (TICs) for the four ginseng species show very similar (Fig. 2.4). Most major peaks registered in the TICs appeared in almost all herb samples. Then, the authors further processed the MS data set with Mass Hunter software to recognize the ion peaks and extract the chemical metabolites. Finally, three metabolites, including chikusetsusaponin IVa, ginsenoside Rf, and ginsenoside Rc, were selected as the most representative specific biomarkers of *Panax ginseng* via correlation analysis. Thus, they were able to differentiate *Panax ginseng* from the other species of the same genus.

Plant metabolomics is an efficient approach for evaluation and quality control of traditional and folk medicine, especially large-scale analysis of herbal medicines as a complementary to common quality control methods. The future innovative goal would be developing an evaluation system for medicinal plants and phytopharma-

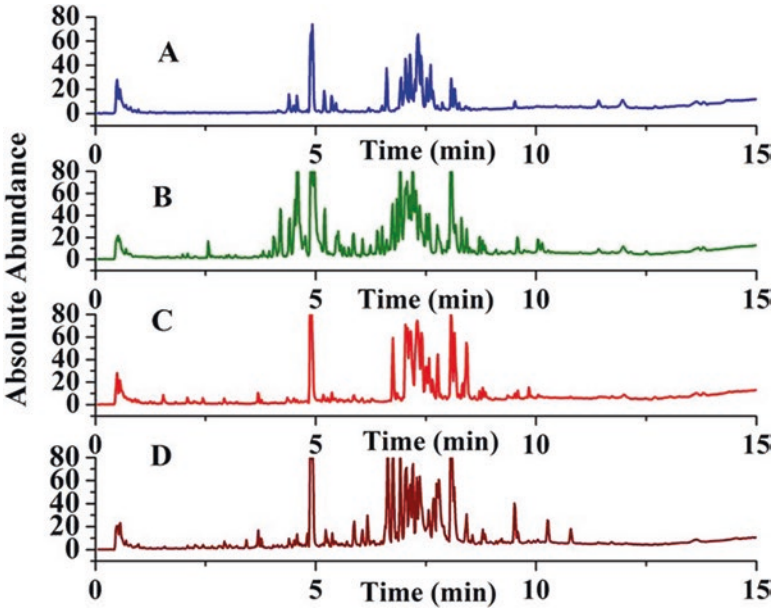

Fig. 2.4 Metabolite profiling of the medicinal herbs in negative-ion mode: *Panax ginseng* (**a**), *Panax notoginseng* (**b**), *Panax quinquefolium* (**c**). and *Panax japonicus var.* (**d**) applying UHPLC-TOF-MS (Wang et al. 2017)

ceutical products based on the analytical results. Although rapid advances in analytical techniques have occurred, interpretation of plant metabolomic profiles remains difficult. This is mainly attributed to the complexity of natural product mixtures which makes the evaluation of their function incomprehensive. In order to be successful here in the near future, recognition of bioinformatics, computational databases, and metabolic networks needs to develop among the researchers in various fields of breeding, cultivation, and phytochemical evaluations of medicinal and aromatic plants.

2.7 Summary and Outlook

In the near future, modern spectroscopy techniques will become more and more important for an efficient production of medicinal and aromatic plants. Beside selection of high-throughput analysis of single plants, also reliable and easy-to-perform prediction of the right harvest time will be extremely helpful for breeders and farmers. In this context, especially nondestructive techniques which can be applied directly on the field will provide new options to simplify and cheapen the individual processes. Overall, it can be assumed that the improved analytical methods will result in a higher level of product quality and safety in the sense of the consumer. By applying hyperspectral NIR spectroscopy, it will be possible to sort out unwanted weeds automatically after harvesting.

Infrared and Raman spectroscopy have already gained wide acceptance for routine analysis of a wide range of medicinal and aromatic plants, although these techniques generally cannot measure molecules with low concentration. Nevertheless, the individual fingerprint of plant species can be used to elucidate particular compositional characteristics which cannot be easily detected by traditional targeted chemical analysis. Calibration development is, by far, the most critical aspect in order to develop a successful method based in IR spectroscopy. This step requires a high level of expertise, particularly in chemometrics or multivariate data analysis, in order to make the practical application successful.

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Chapter 3
Cannabis sativa L. –Cannabis

Pawel Rodziewicz and Oliver Kayser

3.1 The Plant *Cannabis sativa* L.

3.1.1 A Brief History of Cannabis sativa *L. Medicinal Use*

Cannabis sativa L. is a remarkable herbaceous species with a long and interesting history of its use for medicinal, recreational, and religious purposes (Kalant 2001; Zuardi 2006). Although this species is best known for its unique pharmacological properties, it is also a source of high-quality fiber and valuable seed oil (Andre et al. 2016). *Cannabis* sp. originates from Central Asia (Kazakhstan/Mongolia), but it grows in most of the regions of the world. It is also one of the earliest cultivated plants with first documented evidence of its use tracing back to ancient civilizations of China, Egypt, Mesopotamia, and Summers (Long et al. 2017; Russo 2007). Roughly, *Cannabis sativa* L. grows in the subtropical areas; and in northern and southern temperate zones, *Cannabis indica* Lam. is present. In the past, the plant's healing properties were mostly recognized among South Asian, Middle Eastern, and African cultures, whereas the Europeans and East Asians utilized this species mainly to produce strong fibers and seeds for nutritional purposes (Clarke and Watson 2007). As early as 2600 BC, cannabis preparations were recommended by Chinese Emperor Huang Ti as a remedy for cramps and rheumatic and menstrual pain. The first written evidence of the medicinal use of cannabis recorded in Chinese pharmacopeia comes from the fifteenth century BC (Brand and Zhao 2017). In Western medicine, cannabis was introduced and popularized in the mid of nineteenth century by Irish physician William Brooke O'Shaughnessy. During his fellowship in India, he validated the folk use of cannabis – one of the sacred plants of that country – and

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also found new therapeutic applications for the extracted cannabis resin (O'Shaughnessy 1843). Shortly after, the herb was adopted as medicine in British and other European pharmacopeias, as well as in the US Dispensary in the form of dried flowers, extracts, and tinctures (Fig. 3.1), where it remained for over 100 years (Piomelli 2000). However, in the mid of twentieth century and starting in the USA, the use of cannabis flowers became controversial, and ultimately, its possession and cultivation for various purposes (including medicinal use) was penalized in most countries. After the US government introduced the Marihuana Tax Act in 1937 (Marihuana Tax Act 1937), cultivation of cannabis became economically unjustified, and therefore, also its use as a medicine greatly diminished. Ultimately, and against the advice of the American Medical Association, the prohibition led to the classification of *Cannabis sativa* L.-derived products as containing substances of high potential for abuse and without any therapeutic effects (Aggarwal et al. 2009). The factors that played the main role in establishing the US federal cannabis prohibition policy were mostly related to the end of prohibition, ethnic and social problems in the USA, and the expansion of polymer and synthetic fiber industry at that time.

After the discovery of the endocannabinoid system and the recognition of its functions in physiological processes in the human body, the pharmacological potential of cannabis started to attract both the scientific community and pharmaceutical industry. Cannabinoids became promising therapeutic agents to treat cancer (Guzmán 2003), inflammations (Klein 2005), appetite disorders (Parker et al. 2011), and also neurological diseases, including multiple sclerosis (Lorente Fernández et al. 2014). Nowadays, most of the states in the USA legalized the medicinal application of cannabis flowers again. Also, in Europe, this trend is observed, as more countries authorize flowers as a medicine. Several cannabinoid-based drugs have

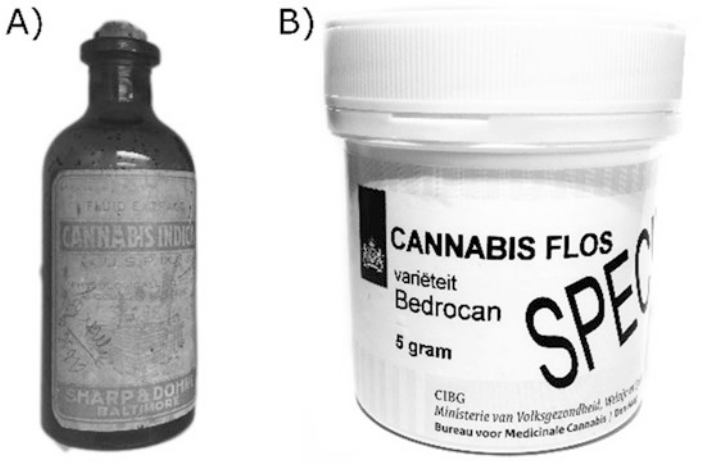

Fig. 3.1 *Cannabis indica* Lam. extract extemporaneous bottle (**a**). *Cannabis sativa* L. (var. Bedrocan) flos (**b**)

already been developed and are currently available in the market, for example, Sativex® (GW Pharmaceuticals) and Marinol® (AbbVie), or undergo clinical trials, for example, Epidiolex® (GW Pharmaceuticals).

3.1.2 Taxonomy, Morphology, and Anatomy

A majority of cannabis strains exist as dioecious (separate sexes) plants and are wind-pollinated. Under normal conditions, it is an annual herb, although longer-living *Cannabis sativa* L. has also been observed. Some cannabis strains appear as monoecious (containing both male and female parts) cultivars, such as the Ukrainian USO31 cultivar (Hazekamp and Fischedick 2012), or as hermaphrodites. In optimal environmental conditions, *Cannabis sativa* L. can grow up to 6 m in a 4–6 month growing season. Together with hops (*Humulus*) *Cannabis* belongs to the Cannabaceae family. The taxonomic organization of *Cannabis*, whether it is a monotypic *Cannabis sativa* L. species with further division into subspecies or a polytypic species, is still a matter of a debate. In principle, the real genetic background is not clear in currently available *Cannabis* strains due to the random and nonrational crossing and breeding (McPartland and Guy 2017). However, the results from allozyme variation analysis in combination with morphological traits of various *Cannabis* accessions of different geographic origins demonstrate significant morphological and chemotaxonomic differences between the genotypes and support the recognition of *Cannabis sativa* L., *Cannabis indica* Lam., and putatively *Cannabis ruderalis* Janisch, as separate species (Hillig 2005a, Hillig and Mahlberg 2004). *Cannabis indica* Lam. is usually shorter than *Cannabis sativa* L. and with broader leaves. Both species are rich in cannabinoids and are used for medicinal/recreational purposes. *Cannabis sativa* L. is also utilized for fiber production, and its seeds serve nutritional purposes. *Cannabis ruderalis* Janisch has a low content of cannabinoids and appears mostly in Central and Eastern Europe and also in Central Asia. It often grows at roadsides or fallow grounds. *Cannabis* is usually a short-day plant, which requires a defined period of uninterrupted darkness to start its generative phase. In *Cannabis ruderalis* Janisch, however, the flowering is independent from photoperiodism and can start as soon as 3 weeks after germination (Clarke 1981). The "autoflowering" trait has been introduced into many commercially available *Cannabis sativa* L. and *C. indica* Lam. strains and their hybrids. Depending on the light duration of the inductive photoperiod, first flower primordia may appear after 1 week (12 h of light), but it can take even up to 90 days to switch to generative phase (16 h of light). The flowers of male and female plants differ significantly. Male flowers are small bell-shaped organs (staminate), which hang loosely in clusters and open releasing pollen. Female plants produce tear-shaped calyxes with two pollen-catching pistils (pistillate), densely present between the top floral leaves. The calyx and closest leaves are covered abundantly with glandular trichomes, which produce cannabinoids, terpenes, and other secondary metabolites. Male flowers tend to mature earlier than female flowers, and soon after the pollen is released, the

male plant dies, whereas unfertilized female plants may mature even up to several months and continue to develop glandular trichomes and biosynthesis of secondary metabolites (Fig. 3.2). After female plants have been pollinated, they stop to invest the resources in secondary metabolite production and start to form the seeds instead (Clarke 1981). Seeds are oval in shape and, however, depending on the variety, may greatly vary in size (Russo 2007). The mature floral clusters, commonly known as buds, are harvested, dried, and used in various ways and for various purposes, whether medicinal or recreational.

3.1.3 Cannabinoids as Main Constituents

Cannabis sativa L. has been studied intensively for its metabolic profile with special emphasis on the terpenophenolic cannabinoids. Over 500 metabolites have already been identified in this species, including terpenes, sugars, hydrocarbons, steroids, flavonoids, nitrogenous compounds, non-cannabinoid phenols, and amino acids (ElSohly et al. 2017; ElSohly and Slade 2005). In total, around 109 different

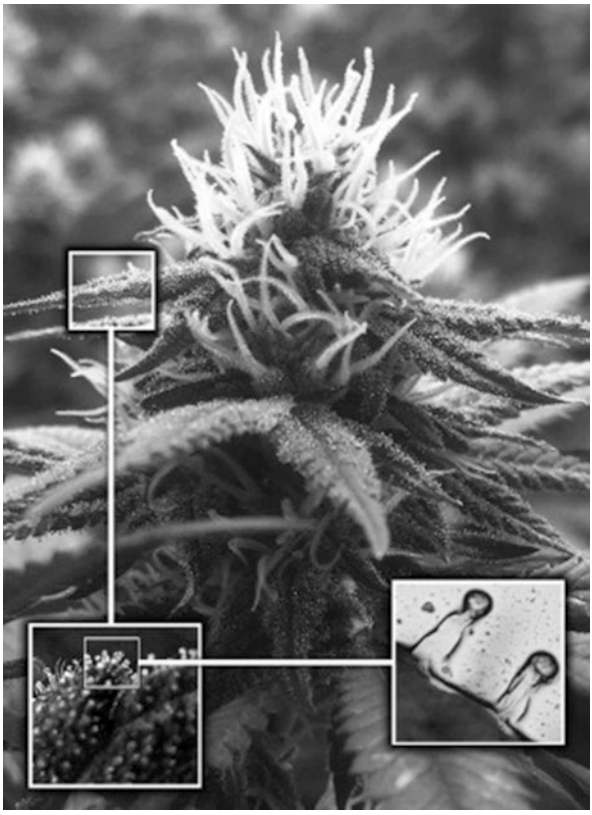

Fig. 3.2 Unfertilized female *Cannabis sativa* L. at seventh week of the flowering stage with marked capitate-stalked glandular trichomes

cannabinoids have been identified, with tetrahydrocannabinolic acid, cannabidiolic acid, cannabigerolic acid, and cannabichromenic acid being the most dominant constituents (ElSohly et al. 2017). Although the medicinal effect of the cannabinoid content, both pure and mixed isolated, has been studied most intensively, other constituents have also been found to be medicinally active (Tuner et al. 2017).

Cannabinoid biosynthesis (Fig. 3.3) takes place in the glandular trichomes: capitate-stalked, capitate-sessile, and bulbous (Dayanandan and Kaufman 1976; Kim and Mahlberg 1997b; Turner et al. 1978). The capitate-stalked glandular trichomes contain the largest quantities of cannabinoids and are abundantly found on female inflorescences (pistillates) (Hammond and Mahlberg 1973; Happyana et al. 2013; Mahlberg and Kim 2004). The precursors of cannabinoids, olivetolic acid (OA) or alternatively divarinolic acid (DA), and geranyl diphosphate (GPP) originate from polyketide pathway and the plastidal 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, respectively (Gagne et al. 2012; Phillips et al. 2008). Prenylation of OA or DA with GPP leads to the formation of central cannabinoid precursors – cannabigerolic acid (CBGA) or cannabigerovarinic acid (CBGVA), respectively (Fellermeier and Zenk 1998). The unique oxidative cyclization of

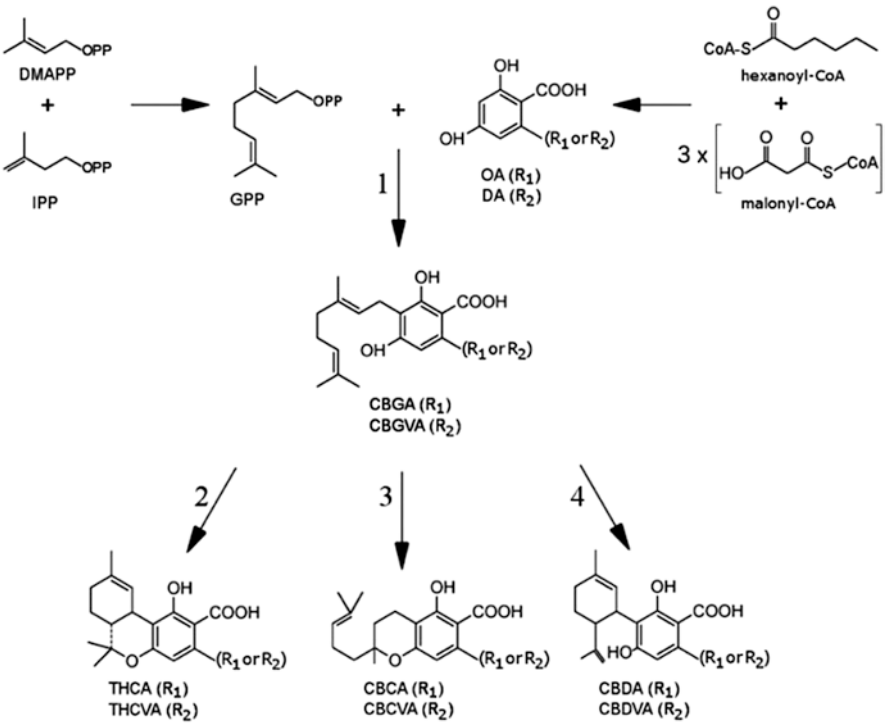

Fig. 3.3 Simplified biosynthesis of cannabinoids in *Cannabis sativa* L., according to Degenhardt et al. (2017). 1, aromatic prenyltransferase; 2, THCA synthase; 3, CBCA synthase, CBDA synthase; R_1, C_5H_{11}; R_2, C_3H_7; DMAPP, dimethylallyl pyrophosphate; IPP, isoprenyl pyrophosphate

CBGA or CBGVA by respective synthases, for example, tetrahydrocannabinolic acid synthase or cannabidiolic acid synthase, leads to the biosynthesis of the final cannabinoids in their acidic form (Onofri et al. 2015; Taura et al. 1995, 2007). Due to increased temperature, storage, light, and oxidation, cannabinoids undergo non-enzymatic decarboxylation, which gives rise to their neutral forms, for example, Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) (Andre et al. 2016). Cannabinol (CBN), which is a product of THC oxidation, was the first isolated cannabinoid from *Cannabis sativa* L. extract at the end of nineteenth century (Wood et al. 1899). The first resolved structure together with partial synthesis was elucidated for Δ9-tetrahydrocannabinol (THC) (Gaoni and Mechoulam 1964). Several numbering systems of cannabinoids can be found in the literature, with the two most commonly used, either dibenzopyrane or terpene moiety, as a reference (Fig. 3.4) (Hanuš et al. 2016).

3.1.4 Genomics

Cannabis sativa L. is a diploid species with 10 chromosome pairs (*2n* = 20). Nine pairs are autosomal and two are sex-linked chromosomes (XY). The genome was measured in both female (XX) as well as in male plants (XY). It must be stated that dioecious plants are able to change sex during their development (Freeman et al. 1980). For example, by the apical application of silver nitrate and thiosulfate anionic complex to female cannabis plants, it is possible to induce fertile male flowers (Mohan Ram and Sett 1982; Hillig 2005a, b). The size of the cannabis haploid genome is 534 Mb, and its transcriptome contains approximately 30,000 genes (Bakel et al. 2011). Later, from the same group, a detailed genetic structural analysis of the drug-type and low-THC fiber-type cannabis was published, revealing similarities and differences between C. *sativa* L., *C. indica* Lam., and C. *ruderalis* Janisch (Sawler et al. 2015). The complete chloroplast and mitochondrial genomes were reported to be 415.5 and 154 kbp in size, respectively (Vergara et al. 2016; White et al. 2016). Further analysis of the chloroplast genome revealed conservative

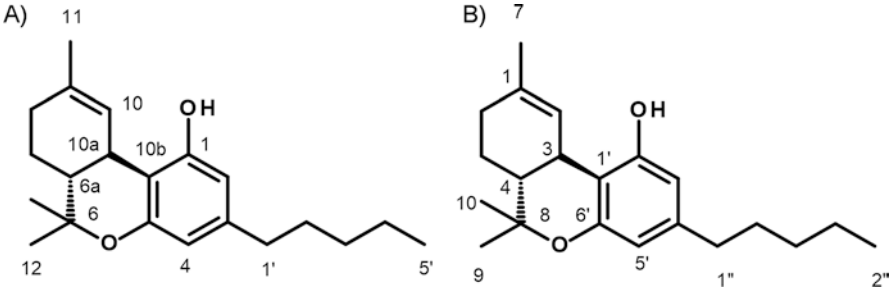

Fig. 3.4 Δ9-Tetrahydrocannabinol (THC) and its common numbering systems according to dibenzopyrane (**a**) and terpene moiety (**b**)

features typical for order Rosales and also a close phylogenetic relation with the family Moraceae (Oh et al. 2015).

3.1.5 Chemotypes and Genetic Basics for Cannabis Cultivar Development

Cannabis chemical phenotypes (chemotypes) are determined based on the content and composition of produced cannabinoids. On this basis, three main cannabis chemotypes were specified – the fiber type (CBD > THC), the intermediate type (CBD ≈ THC), and the drug type (CBD < THC) (Small and Beckstead 1973; de Meijer et al. 2003; Pacifico et al. 2006). The distribution of CDB/THC ratio observed in cannabis populations underlies the genetic mechanism of chemotype inheritance. Recently, a genetic model postulating the existence of two codominant alleles at locus B determining all three chemotypes was developed (de Meijer et al. 2003). Locus B shows simple Mendelian inheritance of the two alleles B_D and B_T, which were suggested to code two isoforms of the same synthase, responsible for CBDA and THCA synthesis, respectively (Pacifico et al. 2006). Cannabis plants homozygous at locus B with B_D/B_D or B_T/B_T genotype exhibit correspondingly CBD- or THC-dominant profiles, whereas the intermediate chemotype is heterogeneous at the locus B (B_D/B_T) (de Meijer et al. 2003). However, it must be noted that plants homozygous at locus B can still produce both cannabinoids, i.e., THCA or CBDA. For instance, in the fiber-type cannabis, expressing B_D/B_D genotype, low amounts of THCA are still present. It is thus possible that the B_D/ B_D homozygote either carries the THCA synthase gene, which is however not detected due to its polymorphisms or the enzymes are promiscuous to some degree and each isoform can synthesize also the second cannabinoid isomer (Kojoma et al. 2002, 2006; Geissler et al. 2018). The third variant of a minimally functional allele B_0, which possibly represents a mutated version of the B_D allele, is also postulated (de Meijer and Hammond 2005). Plants expressing B_0/B_0 genotype were found among fiber-type cultivars and displayed a rare CBGA-dominant chemotype, in which this compound constituted 85–94% of the total cannabinoid fraction (Fournier et al. 1987; Virovets 1996). A fixed locus C encoding CBCA synthase, which determines the CBCA production, was also proposed (De Meijer et al. 2009a). A substantial fraction of CBCA was identified in a "prolonged juvenile chemotype" of cannabis, which was associated with a reduced number of floral bracts, bracteoles, and capitate-stalked glandular trichomes. In further studies, recessive allele o in the locus O was suggested as cannabinoid knockout factor, which blocks cannabinoid production in homozygotes (o/o) and strongly suppresses it in heterozygotes (O/o), whereas the O/O expressing plants are high cannabinoid content chemotypes. Locus O operates upstream of locus B and C and segregates independently from them (De Meijer et al. 2009b). Studies on the regulation of propyl/pentyl cannabinoids ratio further expanded the genetic inheritance model of cannabis. A polygenic locus A described

as A^1, A^2, …, A^n was suggested to determine which cannabinoid precursors is formed. Codominant alleles of that locus contribute additively but with various effects to the formation of propyl ($A_{pr}{}^{1\text{-}n}$) and pentyl ($A_{pe}{}^{1\text{-}n}$) cannabinoid precursors – divarinolic and olivetolic acids, respectively. The recent cannabis breeding program enabled generation of chemotypes with significant fractions of propyl cannabinoids reaching up to 96% of the total cannabinoid content and led to their commercial availability (de Meijer and Hammond 2016). The established genetic model of chemical phenotype inheritance regarding major cannabinoids explains the basics for cannabis genotype crossing and aids in the prediction of the frequency of chemotypes in the progeny. It is also an indispensable tool for breeding cannabis with desired cannabinoid profiles. It must be noted that for medicinal purposes the most important components in the plants are THC and CBD. Until now, these two cannabinoids have the most important practical and verifiable effects in treating various conditions. The content of THC in different plant varieties increased in the last years extremely. For example, cannabis strain Bedrocan® (Bedrocan International BV) developed for medicinal purposes contains up to 22% of THC (in flower dry weight). However, medicinal strains with high CBD content are also available. For example, Charlotte's Web™ (CW™ Hemp) contains up to 17% of CBD and less than 0.5% of THC.

3.2 Basics for Growing Cannabis

3.2.1 Abiotic Factors

As any other plant, cannabis is sensitive to various abiotic environmental factors, and the most important are humidity, macro- and micronutrients, light, and temperature. Proper use of these abiotic influences can increase glandular density and cannabinoid content. Environmental factors have also been shown to induce sex change in cannabis. Moreover, when some chemotypes are grown in different environments, their cannabinoid content seems to change (Russo 2007). With genetic analysis, it should be possible to determine and later to predict if a strain is indeed a fiber or intermediate type that has been suppressed for its cannabinoid content due to the environment of cultivation. Some of the major abiotic environmental factors influencing the cannabinoid content are described below.

Dehydration Cannabis is considered to be a drought-resistant plant, which grows well on arid areas. This species requires a well-drained soil and standing water to promote rotting of the roots. In higher humidity, plants tend to have longer internodes, whereas in dry conditions internodes are shorter (Clarke 1981). The optimal temperatures for growing cannabis are 24–29 °C. However, in drought conditions combined with elevated temperatures, flowering was found to be accelerated, but at the same time, the plant biomass was largely decreased (Amaducci et al. 2008). In times of low water accessibility, cannabinoid content seems to increase. It was

suggested that cannabis plants cover themselves with oily cannabinoids to prevent water evaporation, especially around the flowers. For instance, Sharma (1975) found that glandular trichomes are longer and more abundant on the leaves of cannabis grown under dry circumstances, which suggest the adaptability to arid areas.

Macro- and micronutrients The nutrients in the soil are important for plant development, and their right supply results in healthy plants. Fertilizers high in nitrogen are recommended for the vegetative growing of cannabis. Many manufacturers, as well as breeders, recommend the use of formulations that contain equal amounts of nitrogen, potassium, and phosphorus, for example, N10-P10-K10. According to the recent study, the highest yield, cannabinoid content, and plant growth were achieved at the rate of 389 mg/L of N for liquid organic fertilizer (N4.0-P1.3-K1.7) together with two coir-based organic substrates (Caplan et al. 2017). In the flowering stage, fertilizers rich in phosphorus are recommended for better yield, although no scientific data exist on the matter. However, the additional information can be found in many cannabis grower guides. The availability of the secondary nutrients is also a limiting factor in plant growth. Calcium, magnesium, and sulfur are equally important to primary nutrients (N, P, K) but come in smaller quantities. Other essential elements are micronutrients, such as boron, chlorine, copper, iron, manganese, molybdenum, and zinc. Their deficiencies are rarely a problem unless their bioavailability is reduced, for example, due to precipitation. It is also important to control the pH of the nutrient solution as it determines the bioavailability of the nutritional elements. The optimal pH for growing cannabis plants should be kept at 5.5–6.5. Nowadays, complete fertilizers containing all necessary elements are available from many vendors, as well as other organic products, which may enhance plant growth and vitality.

Light Light has a major influence on plants, and for cannabis, photoperiod has a crucial impact on vegetative and generative stages. Long daylight induces strong vegetative growth and shorter daylight leads to the flowering of the plants (Teramura and Sullivan 1994). For indoor cultivation, very popular setups are 24 h/0 h or 18 h/6 h photoperiod during the vegetative stage and 12 h/12 h during the generative stage. Cannabis is a light-demanding plant and requires at least 2500 lumens when grown indoor, but higher values are usually recommended. The popular light sources used in indoor cultivations are high-intensity discharge bulbs (HID). HID lamps are divided into metal halide (MH), mercury vapor (MV), and high-pressure sodium (HPS) lamps. An HID 400 W bulb emits up to 56,000 lumens and is usually sufficient to cover the growing area of $1m^2$ (4–6 plants). Nowadays, it is also possible to grow cannabis under LED lighting. The advantages of LED lights are lower energy requirements, reduction of heat generated by lamps, and also the robustness of the system. The correlated color temperature of the lighting also has an impact on growing cannabis. In general, warm colors (2000 K–3000 K) promote flowering, whereas cold colors (4500 K–6500 K) enhance plant growth (Green 2017). Also, it has been also shown that the UV-B radiation increases the level of THC, but not CBD in plants (Lydon et al. 1987).

3.2.2 Biotic Factors

Interactions with microorganisms, both symbiotic and pathogenic, greatly influence plant growth and development (Lugtenberg et al. 2002). Cannabis diseases are caused by multiple organisms, including fungi, bacteria, viruses, nematodes, and also arthropods. Disease prevalence strongly depends on the geographic region of cannabis and the type of cultivation (indoor or outdoor), and fluctuates as the plant develops. Also, growing plants in large monocultures increases the probability of infections (Burdon and Chilvers 1982). Various diseases may strongly reduce the yield, lower its quality, or even destroy the whole crop (Peterson and Higley 2001). The most common cannabis diseases were reviewed by McPartland and McEno (McPartland 1996a; McEno 1998). Recently, nonpathogenic endophytic fungi, potentially displaying beneficial effects on plants, were identified in various *Cannabis sativa* L. tissues (Kusari et al. 2013).

Pathogenic fungi Most of the plant diseases are caused by fungi, which negatively affect their growth and development. More than 80 cannabis-specific and nonspecific pathogenic species were already identified (McPartland 1996a). The most threatening cannabis disease is gray mold, caused by the necrotrophic fungus *Botrytis cinerea*. The symptoms of the disease develop on mature and senescent tissues, but the fungus usually infects the plant in earlier developmental stages and thrives in latent form. Because of several modes of attack, this pathogen is hard to control (Williamson et al. 2007). *Botrytis cinerea* affects cannabis flowers, leaves, stems, and branches. Soon after the fungus starts to spread on plants, a gray mycelium is formed, which eventually turns into a gray-brown slime. High humidity and moderate and cool temperatures favor the development of the disease, and in such conditions, plants may be destroyed even within a week (Barloy and Pelhate 1962). *Botrytis cinerea* and other fungi, such as *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Fusarium* species, are responsible for damping-off the seeds or young seedlings, which may lead to their death. Hemp canker is caused by *Sclerotinia sclerotiorum* and may account for big losses in cannabis crops. The symptoms develop as lesions on stems and branches and soon become darky cankers covered with white mycelium. Plants affected by this disease may wilt and fall over. *Trichothecium roseum* affects cannabis leaves and flowers and causes "pink rot" disease, which is especially a threat for plants grown under greenhouse conditions (McPartland 1991). Other fungal infections include yellow and brown leaf spot diseases caused by *Septoria*, *Phoma*, and *Ascochyta* species and also root diseases caused, for example, by *Fusarium solani* and *Rhizoctonia solani* (McPartland 1996a).

Viruses Viruses, such as the hemp streak virus and hemp mosaic virus, may severely impact cannabis yield. However, nonspecific viruses also affect cannabis, for example, alfalfa mosaic virus and cucumber mosaic virus. Various arthropods, which feed on cannabis, for instance, whiteflies, thrips, aphids, and spider mites,

and therefore cause wounding and weakening of the plants, may also transmit viral infections (McPartland 1996b).

Nematodes Nematodes constitute another group of pathogens, which mainly attack the roots of the cannabis. The symptoms include the formation of knots or galls on roots and severe yield reduction as well as wilting of the plant. Several species infecting cannabis are known, for example, nematodes belonging to *Meloidogyne* ssp. and the aboveground nematode *Ditylenchus dipsaci*, which attacks the stems, branches, and leaf petioles (McPartland 1996a).

Bacteria Pathogenic bacteria infecting cannabis were also identified. The symptoms of bacterial blight caused by *Pseudomonas syringae* pv. *cannabis* are similar to brown leaf spot disease, and it is the most serious disease among bacterial infections. Other known pathogenic species include *Striatura ulcerosa*, *Agrobacterium tumefaciens*, *Erwinia tracheiphila*, and *Xanthomonas campestris* (McPartland 1996a).

Arthropods are pests that feed on various plant parts, which lead to the injuries of the leaves, stems, flowers, and others. The weakened plant is also more prone to microbial infections, as well as viruses, which additionally may be transmitted by arthropods. Although in the past cannabis was regarded as a pest-free species due to insecticide properties of the produced cannabinoids and other compounds, nearly 300 various pests have been described for that species until today. Arthropods such as spider mites (Tetranychidae), aphids (Aphidoidea), thrips (Thysanoptera), or white flies (Aleyrodidae) may cause serious damages on cannabis crops, especially in indoor cultivations, where they do not have their natural enemies (McPartland 1996b). Spider mites are less than 1 mm in size and thrive on the abaxial side of the leaves. In hot and dry conditions, spider mites reproduce very fast and therefore may quickly adapt to the used pesticides (Green 2017). The most common are the two-spotted spider mite (*Tetranychus urticae*) and the carmine spider mite (*T. cinnabarinus*), which may, however, represent one polymorphic species (Auger et al. 2013). The symptoms of the mite attack appear as white spots on the leaves, which arise from sucking out the cell content by the pest, ultimately leading to wilting. Spider mites may also create a web around the affected areas of the plant. Aphids are another piercing–sucking pests, which severely affect cannabis in both outdoor and indoor conditions. These tiny insects feed on plant sap from leaves, branches, and stems. Six species have been reported so far, including bhang aphid (*Phorodon cannabis*) and hop aphid (*Phorodon humuli*), which feed specifically on cannabis (McPartland 1996b). Apart from causing tissue damage, aphids excrete a sugary waste material, called "honeydew," which attracts ants. The latter insects use that material as a nutrient, and in exchange, they support aphids' growth by setting up new aphid colonies and warding off predatory insects (Pasteels 2007). Thrips are other common pests that feed on cannabis sap. These dark-colored tiny insects were found to be more common in soil-free cultivations, whereas in bioferitilized soil, their growth can be diminished by biocontrol agents, such as the soil fungus *Entomophthora thripidum*,

which infects and kills these insects (Samson et al. 1979). Known species of thrips include the greenhouse thrips (*Heliothrips haemorrhoidalis*), western flower thrips (*Frankliniella occidentalis*), onion thrips (*Thrips tabaci*), and also *Oxythrips cannabensis*, which is specific to cannabis (McPartland 1996b). In outdoor growing conditions, pests have many natural enemies, such as ladybugs, beetles, and wasps, as well as other bugs. However, in indoor conditions, the use of pesticides may be inevitable. However, it is necessary to provide strict regulations and guidelines for pesticide use on cannabis intended for medicinal purposes. Of note, in the recent study, it was reported that various pesticides were identified on 64% of all tested samples from illicit cannabis cultivations, and therefore, using the material from unknown sources may pose a threat to human health (Cuypers et al. 2017).

Endophytic fungi Endophytic microorganisms are bacteria or fungi that colonize plant tissues without causing symptoms of infection and thrive in a symbiotic relation with the host for at least a part of its life cycle. Endophytes produce a variety of metabolites that are useful for the host plant and may act as growth promoters or defensive compounds (Nair and Padmavathy 2014). In cannabis, a total number of 30 fungal isolates from various plant parts were characterized. The highest number of species was identified in flowers, followed by leaves and twigs. Most of the identified species belonged to *penicillium with the most dominant species Penicillium copticola* (Kusari et al. 2013). In other study, the highest colonization frequency was found in stem parts of cannabis, and the dominant genera was *Aspergillum* with three identified species, *A. niger*, *A. flavus*, and *A. nidulans*, followed by *Penicillium* with two identified species (Gautam et al. 2013). The identified endophytes individually or in combinations displayed various antagonistic effects on pathogenic fungal strains, which make them the potential biocontrol agents. For example, the endophyte *Paecilomyces lilacinus* completely inhibited the growth of *Botrytis cinerea* and depending on the tested medium also *Trichothecium roseum* (Kusari et al. 2013). Also, the isolates of *A. niger* and *A. flavus* showed antifungal potential against common plant pathogens *Colletotrichum gloeosporioides* and *Curvularia lunata* (Gautam et al. 2013).

Although most of cannabis pathogens are strictly plant pathogens, microbes related to postharvest and decay of the material may also be harmful to humans. They may cause respiratory tract infections or produce toxins. In the study of microbiome of cannabis dispensary products, several mycotoxic fungi were identified, mostly belonging to *Penicillium* ssp. (McKernan et al. 2015). Bacterial species were also identified, including *Salmonella*, *Klebsiella*, *Enterobacter*, *Streptococcus*, and *Bacillus* (Taylor et al. 1982; Ungerlerder et al. 1982). Since cannabis is often used by patients with weakened immune system, it is necessary to provide medicinal products free of any microbial contaminations (McPartland 1994). Gamma radiation is currently the only recommended method used to ensure microbiological safety of cannabis medicinal preparations described in pharmaceutical standards. This method does not influence the overall composition of cannabinoids, although it may slightly reduce the content of terpenoids (Hazekamp et al. 2005).

3.2.3 *Cultivation of* Cannabis sativa *L.*

The main legal purpose of *Cannabis sativa* L. cultivation is currently the production of hemp fibers and pulp (Sankari 2000; Prade 2011; Russell et al. 2015). From these raw materials, paper, textiles, clothes, insulation, and ropes are mostly made. In many Western countries, the cultivation of *C. sativa* L. for industrial purposes has been made legal, although special permissions may be required. For research purposes and forensic studies, such as determination of the chemotypes, it is also possible to cultivate the drug-type of *C. sativa* L. under special licenses. In Europe, growing cannabis for medicinal purposes is hardly performed. However, there are several companies eligible to cultivate cannabis under strictly controlled regulations, for example, Bedrocan BV and GW Pharmaceuticals.

The optimization of THC yield, as well as other cannabinoids, is mostly performed through breeding programs. Because of the illegality of the plant in most countries, it is performed on either small scale or through criminal activities. In the previous section, we have already discussed the fact that cannabinoid production is mostly genetically determined, and although further studies are needed to expand the genetic model of cannabinoid profile inheritance, the already acquired knowledge is helpful in breeding new varieties with the desired cannabinoid profile (De Meijer et al. 2003; Pacifico et al. 2006).

Cannabinoid content varies significantly in different cannabis strains. Not only THCA but also the amounts of CBDA and even their precursor CBGA are under fluctuations. It is still a question as to what extent THCA production can be increased in the plant by breeding programs and genetic modifications. Even the discussion of making illegal transgenic plants is under debate (Cascini 2012). The yield of THCA in THCA-dominant plants can be increased by abiotic and biotic environmental influences as discussed above. It must be also noted that female cannabis plants are desired when cultivating plants for their pharmacological properties, whereas the male plants are used for generating pollen for seed production.

Genetic modifications seem to be an option for increased yield since THCA production is mainly dependent on genetic factors. However, THCA yield is not purely determined by the B_T allele and also depends on the amount of plant biomass, glandular trichome density, and the production of precursor compounds, which all together indicate a complex spectrum of different possibilities for professional plant breeders. In principle, breeders could think about (i) increasing glandular trichome densities, (ii) increasing precursor production, or (iii) increasing enzyme activity involved in THCA biosynthesis and knocking out competitive enzymes that use CBGA to produce other cannabinoids. Within cannabis, there are many phenotypes; for example, while one has the ability to grow over a few meters high, another stay small. Furthermore, variations in glandular trichome densities have also been observed with regard to THCA content and ratios (Happyana et al. 2013). By combining various cannabis phenotypes, it is possible to breed a new strain that will be, for example, tall with high glandular trichome density and/or high THCA content. Through breeding techniques, plants with high CBGA content and propyl

cannabinoids have been already obtained (Onofri et al. 2015; De Meijer et al. 2009a, b). In the drug culture, plants were mainly bred to achieve high THCA content. Nowadays, the content of this cannabinoid in cannabis preparations can exceed 20% of the dried flower weight.

3.2.4 Many Cultivars: Is There a Best Drug?

Seeds of drug-type cannabis can be obtained nowadays from local shops and over the Internet. Currently, 9007 cannabis strains used for medicinal and recreational purposes are reported (https://www.cannabisreports.com/, accessed 15.05.2018). However, a closer look at EU genetic resources reveals a limited number of only 17 *C. sativa* L. fiber hemp lines (Community Plant Variety Office 2018). Besides that, at the German departmental gene bank in Gatersleben and at the Russian NI Vavilov Research Institute of Plant Genetic Resources, about 55 and 491 *C. sativa* L. strains, respectively, are deposited. It is clear that no regulation exists that defines the best drug and best variety of medicinal hemp. In the future, we can expect at least three major lines, which are already recognized by Dutch, German, and Austrian authorities.

- THC high, CBD low variety with a content of THC from 18% to 21% and a low CBD content of about 5–8% in average
- THC and CBD balanced at concentrations of 8–14% for both
- CBD high, THC low variety with a content of CBD between 8% and 14% and a low THC content of 1% or even lower.

When it comes to cultivar development, the main focus is currently put on achieving plants with desired cannabinoid profile. However, in the future, the profile of terpenoids may also play an important role. For example, the main variation between strains and market competition may be determined by the content of various terpenoids like myrcene, β-caryophyllene, limonene, α-pinene, β-pinene, terpinolene, and α-humulene. Apart from their synergistic pharmacological effect, they also largely contribute to the flavor and aroma of cannabis (Bertoli et al. 2010; Gulluni et al. 2018). For example, bicyclic sesquiterpene β-caryophyllene, which was found to act as an agonist of cannabinoid receptor 2, was also suggested as a useful compound for treating anxiety and depression (Bahi et al. 2014). It is not in the scope of this book chapter to review pharmacological literature, but it can be expected that in the future breeding new *Cannabis sativa* L. lines will be shifted toward optimizing the profile of terpenoids.

3.3 Rational Concepts for Industrial Production

The production of *Cannabis sativa* L. has been optimized since centuries for fiber production in outdoor cultivations. Fiber-type cannabis (hemp) with THC content below 0.3% can be cultivated legally in Europe, and farmers have already intro-

duced various innovative growing techniques to optimize the biomass, fibers, and oil content (Ranalli 2004). Unlike in the USA, the outdoor growing of high-quality medicinal cannabis in Europe is strongly limited, mostly due to law regulations.

The most appropriate way to produce medicinal cannabis on an industrial scale is indoor growing. The main advantage of this type of cultivation is the control of growth parameters such as humidity, temperature, the amount of water and fertilizer, and especially light regime, which enable precise and synchronized induction of the flowering stage. From the industrial perspective, indoor cultivation is ideal for continuous production, and three to four harvest cycles per year can be achieved. A special cultivation form is the "Sea-Of-Green" (SOG) method (Hammersvik et al. 2012), where an intentional early flowering is induced to keep plants at low size (80–120 cm) (Sitt and Wright 2000). The plants will have only been in vegetative state for about 2 weeks. The SOG technique is especially useful when working with large quantities of cuttings obtained from mother cannabis plant. This technique was developed by cannabis grower community to save time, space, and energy. In principle, the harvest is possible every second month, but no scientific reports on yield quality and cannabinoid content have been published so far. It is therefore unclear if the industrial cultivators, who are obliged to follow strict pharmacopeia standards, can benefit from that concept of cannabis growing.

Indoor production facilitates the cultivation of cannabis plants derived from cuttings while avoiding growing from the seeds. The genetic uniformity of the mother plant–derived clones and the control over growth parameters ensure predictable profile of the synthesized natural products as well as limit fluctuations in biomass, which all together guarantee a standardized industrial process. It is very important to keep appropriate number of mother plants for sustainable cutting production. It is possible to obtain 20 to 40 cuttings from one mother plant. However, it also has to be calculated that around 10% of the whole cultivation cannot be used for commercialization (see Sect. 3.1).

3.3.1 Economics

The legal situation of cannabis has changed drastically in the last 10 years. *Cannabis* made a turn from an illicit drug to a promising herbal medicine with an extreme economic growth rate of more than 10–15% per year, and the total economic impact of legal marijuana sales rises from $16–$18 billion in 2016 to $48–$68 billion by 2021 in the USA, which accounts for 241% increase (McVey 2017). This trend will stabilize in the upcoming year, but an increase in patients eligible for cannabis use can be expected (Romanow 2017). Approximately 35 million Americans use marijuana on a monthly basis, and from this group, about 20% use it out of medical reason (seven million). This is about 2% of the total US population and stands in line with Canada (1.4%). In other countries like Israel and Germany, a significant increase of medicinal cannabis users can also be observed (Table 3.1). Based on the assumption that 2% of a population may use cannabis chronically, for instance, in Germany, which is one of highest populated countries in Europe, the expected number can reach 1.2–1.6 million patients.

Long-term consumption and its development are not easy to estimate. The total amount of dried cannabis flowers per patients can be predicted by analysis of figures for illicit cannabis use or by legal limitations as known in Germany. The UNDOC report in 2010 on drugs and crime mentions 1.3 kg/person per year, and German law limits the amount of prescribed cannabis flowers per patient per year to 1.2 kg (United Nations 2010). To forecast the amount of dried cannabis flowers that must be industrially produced is a challenge and economic risk. Chronic patients tend to consume in average up to 1.3 kg per year and some even up to 2.4–3 kg per year. It depends on disease status, titration effects, and THC/CBD concentration in the plant itself. Chronic pain patients tend to use larger amounts (9 g per day), while acute and terminal patients may use less (2–3 g per day). Looking at the figures of the amount of dried medicinal marijuana authorized per client in Canada, it is obvious that in 2017 the average amount was rather low at 2.3 g/ patient per day (Ian McDowell University, 2018). If we keep this number in mind, we can calculate that about 1 ton (1002 kg) per day and 365 tons per year is needed in 2020 for the expected number of patients (Table 3.1). Health Canada reported recently that from January to September 2017 153 tons dried cannabis flowers and 39.5 tons of cannabis oil were already on the market (Canada Health 2018). *Cannabis* flos production is a complex process due to law restrictions and also due to criteria set for medicinal plants in Good Agriculture and Collection Practise (GACP) (Committee on herbal medicinal products (HMPC) 2006) and other national (e.g., AGES, BfArM) and supranational guidelines (EMA 2005).

When calculating production costs of dried cannabis flowers grown under indoor conditions on an industrial scale, it must be considered that this type of cultivation is a demanding process in terms of energy and water consumption, staff effort, and to some extend also to specific safety issues. No reports on economic calculations for the production of medicinal cannabis have been published so far, although some data for hemp cultivation are available (Riegler-Nurnscher 2009). Therefore, cost calculations have to consider the biology of the plant and the technical parameters of the indoor facility. The estimated values are rather conservative and are mostly based on public data from catalogues and published reports (Table 3.2):

Table 3.1 Development of number of legal cannabis user and cost per gram dried *Cannabis sativa* L. flowers, [a]based on assumption of 2% of patients (see text)

Country	Legal users	Estimated in 2020[b]	Costs per gram in US$	Reference
USA	7,000,000	–	6.50 (CA) 6.0–8.0 (US)	Telgheder aaa (2018)
Israel	26,000 (2016)	–	25–30	Israel (2018)
Canada	235,621 (2017)	501,381	5.9–12.9	Canada Health (2018)
Germany	1300 (2017)	800,000	12.5–20.0	Telgheder (2018)
Uruguay	2743	70,000	1.3	Goni (2017)

Table 3.2 Legal binding or guiding documents from various drug authorities for growing and quality control of cannabis for medicinal use

Title	Country	Reference
Cannabis inflorescence: standards of identity	US	American Herbal Pharmacopeia (2013)
Conforming with TGO 93 (standard for medicinal Cannabis)	AU	Australian Government Department of Health (2017)
DAC monography "Cannabisblüten" Cannabidiol (C-052, since 2015/2) Cannabisblüten (C-053, since 2015/2) Eingestelltes raffiniertes Cannabisölharz (C-054, since 2016/2) Dronabinol (D-100, seit 2001)	DE	Bundesministerium für Gesundheit (2016)

Biological Parameters

Size of plant:	120–180 cm
Growth cycle	8 weeks
Plants/m^2	6 plants
Flower to be harvested (g):	70–90 g (calculations with 80 g)
THC content (%, g):	18%, 180 g/kg dried flowers
CBD content (%, g):	7%, 70 g/kg dried flowers

For our cost calculations, the average plant size, growth cycle, and planting density were estimated on the basis of the information obtained from various publications as well as our own experimental data. The authors are aware about plant densities in illegal indoor plantations, which may reach up to 20 plants per m^2, but for high-quality breeding and cultivation, this number is not realistic. In consideration of these assumptions, six plants will produce in one generation cycle per m^2 approximately 480 g fresh flowers. During drying process, around 90% of the weight is lost, which results in 48 g dried flowers. Cleaning, maintenance, and handling of all plants may limit the number of growth cycles to five per year. In total, 240 g of final product can be obtained and commercialized per m^2 per year. The yield can be increased by cultivating plants in higher densities, which is a commonly met practice at illegal farms, but this greatly increases the risk of pathogen infections and may lower the quality of the final product. The legal producers invest more in space and less in extreme biomass production. To cover the requested daily production rate estimated by Health Canada of 1 ton/day in 2017, about 4178 m^2 indoor cultivation space is needed (Canada Health 2018). This gigantic number alone shows the economic potential but also a logistic challenge (e.g., vertical farming).

Besides the above-suggested biological parameters, technical aspects and costs have to be taken into consideration as well (Coffman and Gentner 1979):

Technical Parameters

Water consumption/plant	0.5 l
Nursery: LED, electric power/m^2	20 W/m^2
Production: Hg, electric power/m^2	450 W/m^2
Cost for electricity	0.08 US$/kW/h
Nursery: temperature	22–24 °C (day), 16–19 °C (night)
Production: temperature	24–28 °C
Nursery: humidity	80–95%
Production: humidity	60–70%

Without going into details, given the growth area, the final costs calculation can be done easily. If the total produced biomass have to be considered, the cost can be broken down. In Table 3.3, a first and simple calculation is presented for the indoor production of plants on 1000 m^2. In total, one growth cycle over 8 weeks (57 days) will deliver plants for 1.91 US$ each. We apportion the costs for seedlings to 0.82 US$. In this rough calculation, no investments for infrastructure (housing, HEPA filter, etc.) are included, which may, however, even double the costs.

3.3.2 Standardization of Industrial Cultivation

The industrial production of medicinal cannabis must reach the goal of the standardized cultivation to guarantee the same high quality of the final product over time (Fig. 3.5). Depending on the cannabis chemotype and strain, various parameters, such as growing temperature, day/night rhythm, and humidity, but also planting density, cultivation, and harvest time, have to be considered and adopted. However, none of the above was described in national and supranational guidelines. Therefore, the quality assurance largely depends on the experience of the farmers, who have to meet the demanded THC and CBD concentrations according to pharmacopeia. However, in general, the industrial propagation process will follow simi-

Table 3.3 Cost calculation for cultivation of *Cannabis sativa* L on 1000 m^2 indoor ground for 8 week (57 days) growth cycle (calculation on German cost base in 2017; fte, full time equivalent; pm, person month)

	Unit	Price US$	Total	Cost US$
Number of plants	6/m^2	0.14/plant	6000	840
Water consumption	0.5 l	6/1000 l	162,000 l	972
Fertilizer in water	1 g/l	0.4/g	3000 g	1200
Total electric nursery	240 W/day	0.08/kWh	13,680/day	1094
Electricity, production	450 W/h	0.08/kWh	25,650 W/day	2052
Staff	1 fte	3000/month	2 pm	6000
Total amount				**12,158**

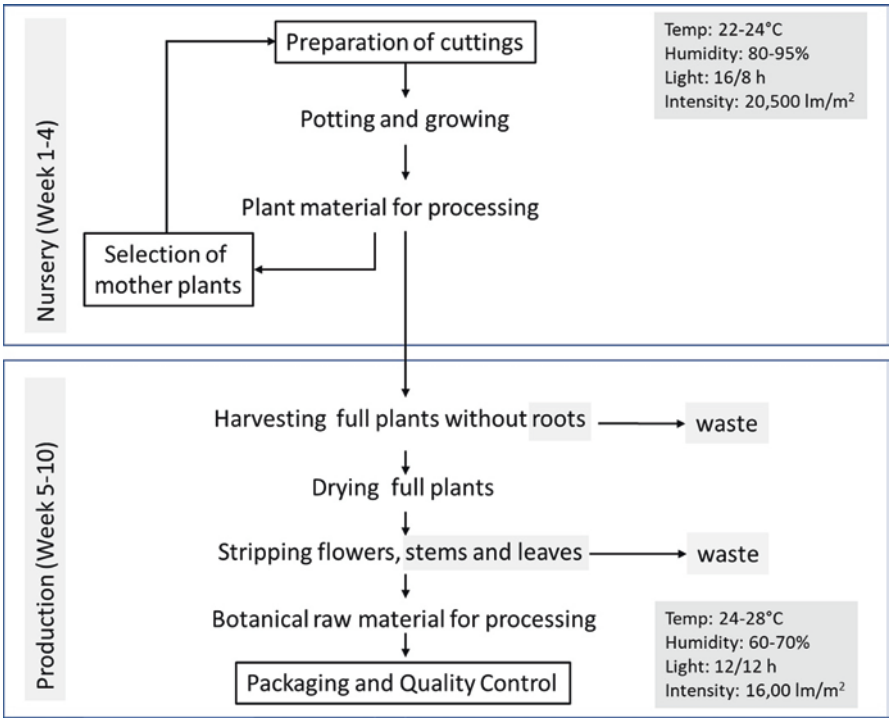

Fig. 3.5 Generalized workflow for propagation of *Cannabis sativa* L. plants

lar scheme as depicted on the Fig. 3.4, which include, for example, characterization of the mother plant, light intensity, humidity, and plant handling.

As mentioned before, in indoor cultivations of medicinal cannabis, plants are propagated from the cuttings obtained from mother plants. Usually, growing from seeds is not favored due to genetic interchanges and variability, whereas cuttings allow reproduction of the plant material with the same genetic background. Cuttings are placed in rockwool, foam, or ceramic correls usually under hydroponic conditions or in simple agar medium with a phytohormone cocktail to induce growing and rooting under continuous light conditions (24 h) at rather high humidity (70–80%) and 25–27 °C for 2–4 weeks (Mansouri et al. 2009). Maturated plants of a height of 40–50 cm with first branching are taken out of the nursery and transferred to a production unit for the next 4–12 weeks at slightly lower temperatures (23–25 °C) and reduced humidity (50–70%). The generative phase is induced by changing the photoperiod to 8 h or 12 h of light per day. Depending on the strain and chemotype, the highest concentrations of THC and CBD are achieved from seventh week of the flowering stage (Muntendam et al. 2012). Light intensity depends on plant size and distance to lamps. The initial plant distance to light source is set to 1.5 m. From the fourth week of the flowering stage, the distance is constantly decreasing to even 0.2 m at the late flowering stages. In the initial phase, the quan-

tity of light is set to 20,550 lumens, but later, it must be reduced to prevent heating up or even burning the plants. Best temperatures for growing cannabis have been identified to be around 35 °C but are less favored due to increase in the overall cultivation costs, pests, and fungal infections (McPartland and Russo 2001; McPartland 1984) and are also less preferred for the staff.

3.4 Analytics

The chemical composition of cannabis extracts is very complex, and more than 500 compounds have are already been identified. While cannabinoids are odorless, the complex mixture of about 120 mono- and sesquiterpenes is responsible for the characteristic smell of cannabis plants. Interestingly, one of those terpene compounds, caryophyllene oxide, is used as a leading substance for detecting cannabis material by trained dogs (Riva et al. 2012). The qualitative and quantitative analyses of cannabinoids present in cannabis or its extracts enable discrimination between various chemotypes and determination of cannabinoid profile, which may be helpful in forensics or in quality control if the variety is intended for medicinal use. For example, the ratio between Δ9-THC and CBN can be used to determine the age of stored cannabis samples (Vanhove et al. 2011, 2012).

3.4.1 Analytical Methods for Detection of Δ9-THC and Related Cannabinoids in C. sativa *L.*

Various methods for determination of cannabinoids in plant material have been developed and are excellently summarized by Klein (2015). In the past decades, thin-layer chromatography (TLC) was used for cannabinoid separation, and colorimetric tests or UV detection under 254 nm were applied for their identification (dos Santos et al. 2016; Hazekamp et al. 2005). Nowadays, commonly applied techniques are High Pressure Liquid Chromatography (HPLC) and GC and very often in tandem with mass spectrometric detector. Molecular techniques are also available to detect these compounds and will be discussed in this section.

Sample preparation Usually, the first step of metabolite analysis is their extraction from plant material. Depending on the applied analytical technique, cannabinoid extraction can be performed with different solvents, for example, methanol, *n*-hexane, and petroleum ether, or with mixtures such as methanol/chloroform or methanol/acetonitrile (Raharjo and Verpoorte 2004). After the extraction with nonpolar solvent, the application of a second liquid–liquid extraction (LLE) with the addition of 0.1 M NaOH enables a separate analysis of acidic cannabinoids, present in the water phase in the form of salts (Fischedick et al. 2010). These methods are useful for the analysis of flowers or leaves, in which cannabinoid content is relatively

high. However, when analyzing seeds, which usually contain low amounts of cannabinoids, a solid phase extraction (SPE) is preferred to concentrate secondary compounds (Stefanis 1978). The extracts are commonly used directly for analysis; however, a dilution may be required. If gas chromatography (GC)-based methods are used for the evaluation of acidic cannabinoids, derivatization of the sample prior to analysis is necessary (Hazekamp et al. 2004a).

Gas chromatographic (GC) methods GC is commonly used for the analysis of cannabinoids, mostly in combination with mass spectrometry (GC-MS). Despite the fact that a lot of different cannabinoids are known, almost all of them can be separated by using silica-fused nonpolar columns. However, due to similarities in structure and chemical properties, some problems related to incomplete separation may occur. It also has to be noted that the use of GC-based methods for cannabinoid profiling of cannabis samples is not a straightforward approach. High temperatures used in GC cause decarboxylation of acidic cannabinoids, and therefore, only neutral forms can be observed. To detect acidic cannabinoids, e.g., THCA, a derivatization process is required. This procedure increases the stability of the compounds by converting polar groups to nonpolar, whereas the required volatility of the compound is maintained. The employment of established detectors such as flame ionization detector (FID) or electron capture detector (ECD) gives information about the quantity of the analyzed compounds, whereas their qualification is limited to the number of known chemicals used as standards. In case of cannabinoids three-, six-, or even tenfold deuterated compounds are often used as internal standards (Aizpurua-Olaizola et al. 2014). On the other hand, combining GC with mass spectrometric detector enables fragmentation of the analyzed compounds and therefore provides additional structural information. The electron ionization (EI) or chemical ionization (CI) techniques used in mass spectrometers combined with GC produce reproducible fragmentation spectra (comparable between different laboratories) and thus enable identification of the analyzed compounds in databases, such as NIST Standard Reference Database. Mass spectrometric cannabinoid fragmentation patterns were extensively described by Harvey (1987). Further data on free, derivative, and deuterated compounds with typical mass fragmentations were reported by Raharjo and Verpoorte (2004).

Liquid chromatographic methods (HPLC) In comparison to GC, one advantage of using HPLC is that there is no decomposition of the acidic forms of cannabinoids. Reversed-phase (hydrophobic) materials are commonly used as the stationary phase fort cannabinoid analysis, and the octadecyl carbon chain (C18) is the most popular. Furthermore, the employment of a guard cartridge containing the same material as used for the stationary phase is normally recommended. The typical mobile phases are the mixtures of methanol and water or acetonitrile and water, acidified with phosphoric acid or formic acid. While for the analysis of the main cannabinoids, such as Δ9-THC, CBD, and CBN, an isocratic method is usually sufficient, and the separation of all cannabinoids requires a gradient elution (De Backer et al. 2009; Gul et al. 2015). The use of a photodiode array (PDA) detector is recommended for

the identification of cannabinoids because of their characteristic UV spectra. If a PDA is used for the detection of cannabinoids, Δ8-THC can be employed as an internal standard (De Backer et al. 2009). According to the law of Lambert–Beer, a quantification of cannabinoids based on the strength of the absorption signal is possible. An excellent summary of the most important cannabinoids with their UV spectra and other specific analytical data can be found in Hazekamp et al. (2005). As described in the section on GC-based methods, the employment of mass spectrometry provides additional structural information of the analyzed compounds and increases the limit of detection (LOD), whereas the use of only a UV detector does not offer such sensitivity. Coupling HPLC with NMR is another possibility of obtaining structural information of the analyzed compounds. The interpretation of [^{1}H] signals specific for different substances can also be used for their quantification (Hazekamp et al. 2004a).

Immunologically Based Techniques The enzyme-linked immunosorbent assay (ELISA) is a popular method used for protein detection. However, it is also possible to employ this technique to detect small organic molecules. This assay is based on antibodies that bind with high affinity to certain molecular structures. Testing of cannabinoids with antibodies has been under investigation since the 1970s. The first attempts were performed with radiolabeled antibodies raised in sheep by immunization with conjugates from THC, THC-hemisuccinate, and bovine serum albumin (Teale et al. 1975). It was found that the antibody was able to detect cannabinoids and its metabolites from urine and plasma collected from rabbits, which were earlier administered intravenously with cannabinoids. Feng et al. (2000) provided antibodies specific for cannabinoids and related metabolites. Furthermore, they tested the antibodies against cannabinoid metabolites excreted via urine in humans. They also showed that the developed antibodies were highly specific for plant cannabinoids and did not bind to any of the non-cannabinoid phenolics. In the past decades, these studies were performed with polyclonal antibodies, and later monoclonal antibodies were developed providing similar results (Feng et al. 2000). Apart from cannabinoid detection, the antibodies may also be used for other research purposes. For instance, labeled antibodies were used against THC and related compounds to show that cannabinoids accumulate in the glandular trichomes. Moreover, with this technique, it was also possible to detect the specific location of cannabinoid accumulation within the trichomes (Kim and Mahlberg 1997a, b). The advantage of using antibody-based methods for cannabinoid analysis is their detection in situ. It is also possible to use these tests with enzymatic, fluorescent, and radioactive labels.

Molecular markers and PCR These molecular techniques cannot readily detect small organic molecules. However, it is possible to employ them to analyze plant material on a genetic basis and make assumptions about the cannabinoid profile. For instance, it was possible to discriminate the major cannabis chemotypes by the use of only three nucleotide primers and PCR technique (Cascini et al. 2013). Within the analyzed cannabis groups, PCR analysis allowed 100% identification of the chemotypes (Cascini et al. 2013). Furthermore, by a simple PCR technique and

only two primers, it was possible to discriminate drug-type and fiber-type plants (Kojoma et al. 2006). However, it must be stated that a very small number of plants was used, and even then, polymorphism on the THCA synthase gene was found. The PCR technique cannot be used to detect cannabinoids directly, but it may be useful in plant breeding and cultivation because of its simplicity and relatively low cost of analysis. Furthermore, it may also find its place in the detection of illegal drug-type cannabis within a population of legal fiber-type cultivated plants. This technique relies on a genetic data to allow reliable information of the differences between male and female individuals as well as drug- and fiber-type plants. Therefore, the design of genetic markers for specific traits detection will result in the increase of data robustness and facilitate its interpretation.

3.4.2 Pharmacopeia Analytics

Like any other herbal drug, cannabis medicinal preparations have to follow pharmaceutical standards to provide the safest possible medicine for the patients (see also Table 3.2). The precise knowledge about the amount of the drug components and the assurance of its uniformity as well as purity is essential for the development of robust medicine. In general, the purity tests for cannabis preparations (flowers) follow the specification included in the international pharmacopeia regarding herbal medicines (Apotheker 2016; American Herbal Pharmacopeia 2013). This include testing herbal medicines for foreign matters, mass loss on drying, water, pesticides, heavy metals, total ash, ash insoluble in hydrochloric acid, extractable matter, aflatoxin B1, ochratoxin A, radioactive contamination, and microbial contamination. In case of cannabis, the determination of the mass loss must be carried out under mild temperature conditions since cannabinoids as well as terpenoids are volatile compounds. For example, drying the material under standard conditions (2 h in 100–105 °C) will result in more than 10% of the cannabinoids loss. The determination of cannabinol, which is a breakdown product of THC oxidation, serves as a quality marker, and its high content may, for example, indicate improper storage or age of cannabis flowers. Over time at room temperature, the THC content will be reduced by 2–5% per month. The highest loss of THC is observed within the first year of storage. In the subsequent years, around 7% of THC loss can be determined (Ross and Elsohly 1997). The isomeric nature of cannabinoids and temperature instability make their analysis a demanding task. Currently, the method recommended for cannabinoid content determination is HPLC using silica-based C18 reverse-phase column. HPLC combined with UV/VIS detector enables determination of both cannabinoid acids at 306 nm as well as their decarboxylated forms at 225 nm. Currently, a six-point calibration is used in the range of 1–22% for THC/THCA and 1–10% for CBD/CBDA, although one-point calibration methods are being tested. The total content of a particular cannabinoid is given as a sum of the acidic form and its neutral counterpart (Hoernig 2017).

3.5 What Is the Future Trend for Breeding *Cannabis sativa* L.?

3.5.1 Micropropagation

Several reports on the growth of cannabis callus and cell suspension cultures can be found already (Lata et al. 2016; Honda et al. 2001; Iliev et al. 2010). However, in most of the attempts, cannabinoids could not be detected, although in one study it was shown that variations in media components may induce cannabinoid secretion (Heitrich and Binder 1982). Until now, no second report confirmed these results. Cannabis callus and cell suspension cultures are induced by standard in vitro techniques used in plant cell manipulation. The efficiency of callus induction seems to be dependent on cannabis variants (Feeney and Punja 2003). Callus-derived cell suspension cultures are grown in the same media used for callus growth with the exception of agar as solidifier. An example of cannabis callus culture is depicted in Fig. 3.6. In one study, the cell suspensions made from cannabis callus were used for bioconversion purposes. It was described that cannabis cell suspension culture was able to convert CBD and THC into cannabielsoins and cannabicoumaronon, respectively (Braemer and Paris 1987). However, when designing cell cultures for cannabinoid production, it must be considered that these compounds are highly toxic to the plant cells and induce apoptotic response (Schachtsiek et al. 2018). Thus, for the successful continuous cannabinoid production in in vitro cultures, it is important to develop techniques that will enable the extraction of these metabolites from the growth media.

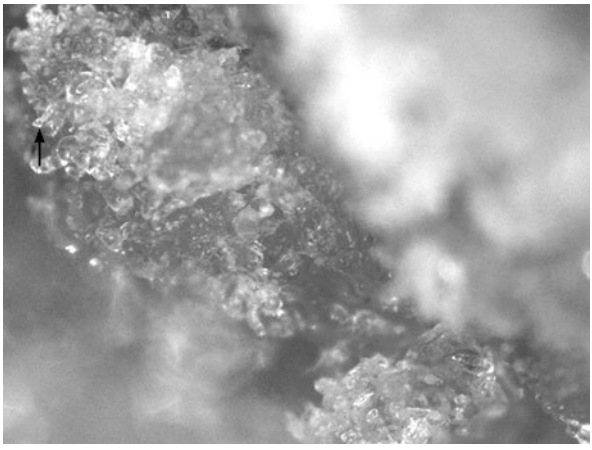

Fig. 3.6 *Cannabis sativa* L. callus culture. The arrow indicates a juvenile glandular trichome-like structure

3.5.2 Transgenic Plants

The use of transgenic plants is not generally accepted for medicinal herbal preparations. However, genetic modifications could be used to express certain preferable traits in cannabis (Schachtsiek et al. 2018). For example, the yield of THCA or any other cannabinoid could be increased by overexpressing or making knockouts of particular biosynthetic genes. Also, knocking out the THCA production in cannabis could provide a plant used as a placebo for clinical testing. Furthermore, by genetic manipulations, it would be also possible to increase plant resistance to pathogens or abiotic stresses (Hilder and Boulter 1999; Wang et al. 2003). Until now, no data on genetically modified cannabis has been reported, although the hypothesis that some of the extremely high THCA-producing strains may have been the result of gene manipulation has been discussed recently (Cascini 2012). Nevertheless, the first strategies to create transgenic cannabis plants are being currently developed (Schachtsiek et al. 2018; Geissler et al. 2018).

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Chapter 4
Coriandrum sativum L. – Coriander

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4.1 Main Use and Production of Coriander

The coriander plant has two very distinct uses: (1) mature fruits are used as a spice or for essential and/or fatty oil production and (2) leaves and occasionally roots are used as a herb or vegetable. Coriander can be found all over the world as a cultivated plant or as a plant escaped from cultivation as ruderal or segetal plant (CABI 2018). Commonly, the entire globular coriander fruit is referred to as seed, which is botanically not fully correct, because each enitre globular fruit contains two seeds (Fig. 4.1).

The mature and dry fruits are marketed whole or as a ground spice, or essential oil is extracted from them (Purseglove et al. 1981). Apart from the culinary utilization, the fatty oil of coriander fruit, which is rich in petroselinic acid, can also be used for industrial purposes. Although industrial applications have been explored for the fatty oil, a stable market has not been developed (McKeon 2016). An important utilization of coriander fruits worldwide is as a major constituent of curry powder. Curry powder is mostly produced in India. In western countries, ground mature coriander fruits are occasionally used to flavour bread, and the extracted essential oil is used in liquors (Purseglove et al. 1981). While production of coriander for fruit use is very common in India, Pakistan and Bangladesh, significant production of coriander for fruit use is also found in Southeast Asia (China, Burma, Thailand), Southeastern Europe (Ukraine, Russia, Bulgaria, Romania, Hungary), the Near East (Turkey, Israel), the Mediterranean (Egypt, Morocco) and the Americas (Argentina,

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Fig. 4.1 Fruit size and shape characteristics for the three subspecies of coriander (accessions at Plant Gene Resources of Canada): top, *C. sativum* L. subsp. *sativum* (CN 19172); middle, *C. sativum* L. subsp. *microcarpum* DC. (CN 19174); bottom, *C. sativum* L. subsp. *indicum* Stolet. ex Diederichsen (CN 19173). The two channels (*vittae*) containing the essential oil in the mature fruits can be seen on the inner seed surface of the split fruits. The globular fruit is a schizocarpium, containing two seeds

Mexico, Canada) (Blade et al. 2016). Based on the volume of production, coriander is globally the leading essential oil crop.

Use of coriander leaves (Fig. 4.2) in salads or as a vegetable is very popular in certain regions and, due to recent global migration, has spread from the major centres of coriander leaf consumption, such as Southeast and Central Asia, the Indian subcontinent, the Near East, North Africa and Mexico, to many other regions. Notably, the use of coriander leaves has become popular in North America (Smith et al. 2011). Coriander for leaf use is often referred to by the Spanish name *cilantro*. In the Caucasus, the Georgian name *kinza* is used for the leafy vegetable, and this name is also used in Russia and Ukraine. Large-scale production of cilantro occurs in Mexico and the United States of America. In Thailand, utilization of the tap root is common, which also has a specific aroma (Oulton 2009) (Fig. 4.3).

Coriander fruits and also the herb are widely used in traditional medicine with many positive effects on the digestive tract and the immune system, which have been well confirmed by recent research (Prachayasittikul et al. 2018; Nadeem et al. 2013). Antifungal and antimicrobial effects of coriander have also been confirmed, and antioxidants are present in both leaves and fruits (Silva and Domingues 2017).

Coriander is most commonly grown as a summer annual. However, production as a winter annual is practised in southern Russia and Turkey (Romanenko and Nevkrytaj 1988; Alborishvili 1984; Unlukara et al. 2016). Small-scale production of coriander for fruit and/or vegetable use can be found in many countries

Fig. 4.2 Genotypic variation in shape and length of the longest basal leaf in coriander. Accession numbers at Plant Gene Resources of Canada. From left to right: CN 120242 (Oman), CN 19165 (Bhutan), CN 114089 (Turkey), CN 114317 (Ukraine), CN 114316 (Ukraine), CN 114091 (Ethiopia), CN 19168 (Syria)

Fig. 4.3 Tap root of coriander: CN 31477 (India)

reaching from short season circumpolar Alaska of the United States (Holloway et al. 2014) to tropical Kenya (Martins 2018). Seed production of coriander in tropical areas does not appear to be possible because the plant needs dry heat for producing high-quality fruits. The content of essential oil in the mature fruits is lower under humid conditions. High humidity during maturation also promotes fungal colonization of the fruits. The production of coriander leaves (cilantro) has potential in many areas as neither humidity nor a short-growing season prohibits production of good-quality leaves. In the province Abkhazia of Georgia, four leaf harvests (cuts) after winter seeding are possible (Alborishvili 1971), and in India, five to seven leaf cuts are envisioned (Giridhar et al. 2015).

4.2 Flower and Pollination Biology

Coriander belongs to the Apiaceae (syn. Umbelliferae) family, also referred to as the carrot family. The inflorescence of coriander consists of compound umbels that are characteristic of the family (Fig. 4.4). The primary umbel terminates the primary stem of the plant and is the first to flower and mature. Most genotypes of coriander produce several side branches of secondary and higher order, all of which terminate in an inflorescence. Plants of some genotypes of coriander that produce many side branches with terminating seed-setting flowers can thus be planted at distances of 40 cm or more from each other without loss of productivity. In other genotypes, the branching capacity is reduced, resulting in a limited amount of seed produced from a single plant. A higher seeding density is therefore required for such genotypes. Genotypes with many side branches and flowers have an extended

Fig. 4.4 Marginal umbellets of coriander flowers without any anthocyanin (white petals, left) and, more common, with some anthocyanin (pink petals, right)

duration of flowering. The flower organs as well as the fruits produced by the early flowers are slightly larger in size than those of later-flowering umbels of higher order.

Within an umbel and also within the umbellets, the marginal florets are the first to flower. The central florets within an umbellet are pistillate, i.e., they have only the female flower organs, but no stamina with anthers, and depend on pollen from neighbouring florets/umbellets/umbels of the same plant (geitonogamy) or on pollen from other plants (allogamy) for seed set. The marginal florets in an umbellet are protandrous, i.e. the anthers are mature and start shedding pollen 2–3 days before the stigma of the same floret is receptive. Each floret has five petals. The marginal florets of an umbellet, in particular those at the margin of an umbel, have asymmetric petals with the petals pointing to the outside of the umbel being enlarged (Fig. 4.4). The central florets of an umbellet have petals that are bent inwards.

The flower features of coriander support a high degree of outcrossing. However, coriander is not an obligate outcrosser, and a high degree of self-pollination based on geitonogamy occurs. Under isolation, a single floret will not set seeds, while isolated umbels and isolated single plants do set seeds but at a reduced rate (Diederichsen 1997). Many insects including honeybees are attracted to coriander, and their pollination increases seed setting and hence fruit yield (Martins 2018; Patil and Pastagia 2016). Coriander yields a lot of high-quality honey (Anonymous 2018; Dinkov and Ivanov 2010). For maintaining the genetic integrity of a breeding line or a genebank accession, it is required to isolate different populations of coriander from each other during flowering. If pollination is incomplete, some florets do not produce fruits, and it can occur that none or only one of the two seeds in a fruit (*schizocarpium*) develops normally, thus impacting yield and reducing the quality of the fruits. A considerable proportion of such incomplete fruit was observed by the author when coriander was grown in an environment deprived of pollinating insects. The flowering features of coriander promote a high degree of outcrossing. However, coriander produces seeds when self pollinated and insects are not obligatory for pollination and seed set.

4.3 Crossing of Coriander

A recent study describing the pollination biology and crossing techniques for coriander was published from India (Giridhar et al. 2016), and there are several older studies on that subject available in Russian (Palamarja and Chotina 1953). The marginal florets of the primary or secondary umbels are preferred for crossing because their florets as well as their flower organs are larger in size than those of later-appearing umbels, hence easier to work with. During the early flowering stage, it is easy to remove the anthers because the filaments are bent outwards and can be seen between the two neighbouring petals (Fig. 4.4). At this stage, the anthers have not yet shed pollen and are stuck in the centre of the floret. The five anthers can be pulled out one by one by grasping their filaments with forceps. Due to their size, the marginal florets are preferably targeted for emasculation, and the central florets of

the umbellet need to be removed. Emasculated umbels must be isolated and pollinated by brushes with pollen collected from the father line or by brushing with flowering umbels from the father line 2–3 days after emasculation. The seeds mature normally in glassine paper bags. Coriander has a high degree of self-fertility, and it is possible to isolate single plants in a field plot by placing glassine paper bags over single umbels or the entire inflorescence. However, seed set is improved if insects support pollination, and it is advantageous to supply pollinating insects if cages are used for isolating populations.

4.4 Breeding Objectives for Coriander

Depending on the intended utilization of coriander, main breeding objectives vary (Table 4.1). In addition to utilization purposes, the production system and the environment that is targeted are critical for determining target traits for breeding. In certain environments, coriander can be grown as a winter crop, but it reduces the quality of the fruits and leaf material (Telci and Hışıl 2008; Chaulagain et al. 2011).

When fruit production is the objective, fruit yield will be the breeding objective of highest relevance. Physical properties of the fruits are important. For example, fruit size is a yield component and a wide range of variation exists for this character (Table 4.2). Even maturity is a relevant breeding objective to obtain homogenous fruits. Only fully matured fruits have optimal quality, and desiccation to accelerate maturation or to obtain even maturity may reduce the fruit quality. Spontaneous shattering of the mature fruits needs to be reduced by breeding. Genotypes with fruits that do not separate spontaneously into the two single seeds are preferred because round fruits that maintain their integrity are easier to clean and optically more appealing. In entire fruits, the oil channels inside the fruits are less exposed to physical damage, so the essential oils are better protected from being lost. When marketing the entire fruit for use as spice, the colour of the mature fruit, which can vary from pale beige to dark brown, is a relevant feature, although it is only an optical quality. The fruit shape can vary from being globular to pointed oval and preferences may exist. Notably, coriander from India has oval-shaped fruits (Fig. 4.1).

Coriander is a specialty crop, so the relationship between buyers and producers is not as anonymous as in major crops that are sold in bulk. As a result, favourable quality of the fruits can considerably impact the price. Hence, fruit quality attributes, such as the content and composition of the essential oil, are relevant breeding objectives. The aromatic properties of the mature fruit are based on monoterpenes, of which linalool is the dominating component (Table 4.2). Other monoterpenes and aldehyde components may occur in lower quantity, and some can cause distinct flavours that are not desired, such as camphor. The composition of the essential oil in the fruit is strongly influenced by the genotype allowing breeding to impact this trait very efficiently (Diederichsen 1996a). Immature fruits have similar aromatic flavours as the green leaves, which are based on aldehyde components, in particular aliphatic aldehydes (Nurzyńska-Wierdak 2013). If coriander fruits are harvested too

Table 4.1 Breeding objectives for coriander (*Coriandrum sativum*)

Intended use	Main target	Components
Fruit use	Fruit yield	Yield per area
		Fruit yield per plant
		No seed shattering
		Lodging resistance
	Physical fruit quality	Fruit shape
		Homogenous fruits
		Colour of mature fruit
		No spontaneous splitting of fruits
		Easy separation of fruit wall from seed
	Chemical fruit quality	Content of essential oil
		High linalool content
		Composition of the essential oil
		Content of fatty oil
		Composition of fatty oil
	Plant development	Even maturity
		No lodging
		Adapted to vegetation period
Leaf use	Leaf yield	Fresh matter production
	Plant features	Slow bolting
		Many basal leaves
		Basal leaves growth habit not prostrate
		Leaf shape appealing
		Leaf colour appealing
		Aromatic features
		Small fruits
Root use		Root shape
All usages	Disease resistances	Fungal diseases (*Ramularia*, *Fusarium*)
		Bacterial diseases (*Pseudomonas syringae*)

early, these flavours are still present and the aromatic quality of the spice is reduced. Parthasarathy and Zachariah (2008) reported that the essential oil located in the periphery of the globular fruit wall facing outside is made up of aldehyde components, while the monoterpenes, including the dominating linalool, are located in two oil tubes (*vittae*) on each seed, which are on the surfaces facing the inside of the fruit (Fig. 4.1). These oil tubes remain intact during maturation while the periphery oil tubes collapse. This explains the aforementioned change in flavour of the fruits during maturation. Telci et al. (2006) showed that the content of essential oil in the fruits decreases towards full maturity, while the proportion of linalool increases. Despite this decrease in content of essential oil, the yield per area of linalool increases during maturation (Diederichsen 1996b).

The use of the fatty oil from coriander has been explored because of the high content of petroselinic acid (Uitterhaegen 2014), but also because of many bioactive

Table 4.2 Ranges of variation in coriander (*Coriandrum sativum*) in important characteristics (spring seeding of a world collection in central Germany) (Diederichsen 1997)

Character	Min.	Average	Max.
Phenology traits (n = 290)			
Start of stem elongation (number of days from seeding)	44	51	81
Start of flowering (number of days from seeding)	51	74	93
End of flowering (number of days from seeding)	85	114	123
Days until maturity (number of days from seeding)	90	119	123
Vegetative plant parts (n = 290)			
Number of basal leaves (at start of flowering)	1	5	>10
Length of the longest basal leaf (at start of flowering) (cm)	4	18	41
Plant height (flowering) (cm)	20	105	150
Generative plant parts (n = 290)			
Weight of 1000 fruits (g)	3.1	8.1	18.9
Volatile and fatty oil in the fruits (n = 289)			
Volatile oil content in the air-dried fruits (%)	0.01	0.4	1.73
Linalool in the volatile oil (%)	18.8	68.8	86.4
Camphor in the volatile oil (%)	0	3.6	19.6
Fatty oil content in the air-dried fruits (%)	8.8	15.3	28.8

Source: Diederichsen and Hammer (2003)

components found in oil extracts (Kozłowska et al. 2016). Variation in oil content and fatty acid composition exists (Diederichsen 1997; Diederichsen and Hammer 2003). Combined use of essential and fatty oil is possible. Mechanical separation of the fruit wall from the seed in mature fruits can be done to enhance the fatty oil content in the raw material prior to extraction (Evangelista et al. 2015). Genotypic differences in this trait exist, and it is thus a trait of interest for plant breeding.

The breeding objectives for leaf use (cilantro) are very different from those for fruit use. The term *slow bolt coriander* refers to types suitable for leaf usage indicating that the plants have a prolonged juvenile phase in which they produce a sedentary rosette of basal stem leaves, which are soft and aromatic. The aromatic qualities of the leaves of coriander are due to essential components that are chemically different from the essential oil of the mature fruits. Although these components have been described (Potter and Fagerson 1990; Nurzyńska-Wierdak 2013), only preliminary studies on genetic variation for these aromatic substances in coriander have been conducted to date (e.g. Rao et al. 2004), so it can only be assumed that genetic variation is present. Also, the chemical compositions of the volatile substances in the vegetative plant parts may change during the vegetation period. The number of basal leaves and the growth habit of the leaves are important features for technical reasons, because they impact seeding density and harvesting. Variation for these features is enormous in coriander (Diederichsen 1996a) (Fig. 4.2). The basal leaves can be prostrate or erect, and a great genotypic diversity exists. Some coriander landraces from Syria have clearly been developed for leaf use because their mature fruits are devoid of essential oil (Diederichsen 1996a). They produce large leafy plants that can be planted at large distances. For commercial purposes, the

shape, colour, texture and flavour of the leaves must be appealing to the consumer and are all traits plant breeding can address. In home gardens, the dual use of leaves and fruits is common.

No distinct types for root use have been reported in the literature, although root consumption is reported from Thailand (Oulton 2009). Given the law of homologous variation proposed by Vavilov (1920), it may be possible to greatly impact the root characteristics in coriander by breeding, as it belongs to the Apiaceae family, which includes important root crops such as carrot and parsnip.

Winter annual production of coriander is possible in temperate climates when selecting genotypes that have a prolonged juvenile period. For other areas with cold, prolonged winters, summer annual cultivation is the only option and more rapid plant development is required. In subtropical climates, there are fewer seasonal restrictions on cultivating coriander, in particular, when leaves are targeted for use.

For all types of utilization of coriander, resistance to a range of diseases is important. In India, resistance to stem gall disease (*Protomyces macrosporus* Unger) has been identified in coriander where this disease is of economic importance (Singh et al. 2003). Wilting of the umbels (blossom blight) at or shortly after flowering is a phenomenon ascribed to various pathogens including the bacterium *Pseudomonas syringae* pv. *coriandricola* (Toben et al. 1994; Toben and Rudolph 1996; Cerkauskas 2009) and the fungus *Ramularia coriandri*, resulting in fruit yield and quality loss. *Pseudomonas syringae* pv. *coriandricola* can also affect the leaf quality of cilantro (Cerkauskas 2009). Resistance to ramularia was targeted by plant breeding in Russia (Bochkarev et al. 2014). However, the causal agents for blossom blight are not well understood, and fungi other than *Ramularia* have been associated with blossom blight. Surveys in 2000 and 2001 in the Canadian Province of Saskatchewan identified *Aureobasidium* sp., *Ascochyta* sp., *Fusarium* sp., *Botrytis cinerea* and *Scelrotinia scleriotiorum* as causal organisms of coriander blossom blight (Duczek and Slinkard 2003). Surveys in 2015–2017 showed significant variability in disease severity from year to year and also in the organisms recovered from diseased flowers (unpublished data). In Saskatchewan, severe disease outbreaks resulting in crop losses were associated with high incidence of an undescribed fungus tentatively identified as belonging to the genus *Heterosphaeria* (K. Seifert, Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, personal communication). Isolates of *Aureobasidium* previously associated with coriander blossom blight in this region (Duczek and Slinkard 2003) were only rarely recovered during the recent survey and were shown to be nonpathogenic in growth chamber tests (Armstrong-Cho and Banniza 2020). It is plausible that previously reported *Aureobasidium* isolates were misidentified isolates of *Heterosphaeria*. Fields with low to moderate disease symptoms correlated with high incidence of *Didymella cari*, *Botrytis* or *Fusarium* spp. or with low incidence of *Heterosphaeria*. The pathogenicity of *Heterosphaeria* was confirmed in growth chamber tests, as well as that of *Fusarium avenaceum*, *F. graminearum*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*.

The potential for multiple pathogens to infect coriander flowers along with local and seasonal variations in disease outbreaks makes disease management and

breeding for disease resistance a formidable challenge. Breeding for disease resistance in coriander is hampered by a lack of clarity regarding the pathogens and the biological processes involved, which is required for a systematic breeding approach. Similar wilt diseases as in coriander are observed in other umbelliferous crops, e.g. dill (*Anethum graveolens* L.), caraway (*Carum carvi* L.) and cumin (*Cuminum cyminum* L.). It would be worthwhile to study the parallelisms regarding the wilt symptoms in the many plant species of the carrot family.

4.5 Breeding Progress in Coriander

Coriander breeding was most actively conducted at the research station Alekseev in the Voronezh area of the Soviet Union, about 500 km south of Moscow, where the first larger-scale extraction of essential oil was already established around 1830 (Stoletove 1931). Significant production and breeding efforts started there in the 1920s and were continued after World War II. The methods applied were either mass selection or, more often, line selection (Palamarja and Chotina 1953). Cross-breeding was only initiated recently (Bochkarev et al. 2014). Although research into breeding methods was conducted and male sterility with its potential to generate F_1 hybrid cultivars was described, such sophisticated breeding strategies have not yet been pursued in coriander. Gametocides for enforcing hybridization (Giridhar et al. 2009) and mutation breeding (Bochkarev et al. 2014; Ramkrishna 2008) have been proposed, and polyploidization has been applied to generate tetraploid coriander (Mozhenko et al. 2009). Publications from the last 20 years point at active breeding efforts in coriander in India, Russia, Turkey, Brazil and Canada (Bochkarev et al. 2014; Giridhar et al. 2016; Inan et al. 2014; Unlukara et al. 2016; Oliveira et al. 2015; Slinkard et al. 2000). From 1938 to the 1990s, breeding of coriander was also actively pursued in the Ukraine (Simferopol, Crimea). After the disintegration of the Soviet Union, research and breeding of coriander ceased for a while in Eastern Europe. In the Russian Federation, the revitalized breeding work on coriander at Alekseev is now headed by the 'All-Russian Research Institute of Oil Crops by the name of Pustovoit VS', located in Krasnodar, from where new cultivars for fruit use have been released continuously (Bochkarev et al. 2014). Older cultivars that were produced at Alekseev are still in use in Russia and Ukraine. Formerly, coriander breeding occurred also in Bulgaria, Romania and Hungary. However, there are no reports on recent breeding activities in any of these countries. In the 1990s, coriander breeding efforts were made in Germany and Canada (Toben et al. 1994; Slinkard et al. 2000), which have since ceased. Nevertheless, the Canadian cultivars are still used in western Canada and Alaska (Blade et al. 2016; Holloway et al. 2014).

Publications describing systematic assessments of diverse coriander genetic resources for leaf use are rare. A recent study from Nepal points at coriander cultivars bred for dual purpose, i.e. fruit and leaf use, and assessed various Indian cultivars for leaf use (Chaulagain et al. 2011). For vegetable use, many types are marketed in

North America under the name ‘slow bolt coriander’. Harvest of leaf material yields of 18–25 t/ha is reported (Alborishvili 1971; Smith et al. 2011).

In Europe, cultivar ‘Jantar’ developed in the Soviet Union in 1976 and other cultivars of the Alekseevskij series are used for fruit production. In western Canada, cultivars ‘CDC Minor’ and ‘CDC Major’, released in the late 1990s by the University of Saskatchewan, are popular for fruit production (Government of Saskatchewan 2018; Blade et al. 2016). Reported fruit yields vary significantly. For example, fruit yields of cultivar ‘Jantar’ are 1.6 t/ha in Russia (Bochkarev et al. 2014), whereas up to 4.6 t/ha were reported for the Turkish cultivar ‘Arlsan’ (Inan et al. 2014), although such high yield may be exceptional. Studies in Germany showed that under optimal conditions yields of up to 2.7 t/ha can be achieved by many genebank accessions (Diederichsen 1996a).

Reasons for the absence of breeding efforts in coriander from many regions may be related to the high levels of fluctuation in demand for coriander, the predominately small-scale production systems and the common practise of using farm saved seeds. In the main production and consumption areas for coriander such as India, landraces are still widely used. Seed markets in coriander are not regulated, and even in industrialized countries such as Canada, reseeding farm-produced seeds is common practise. As a consequence, there is no market incentive to invest in systematic and expensive plant breeding efforts.

4.6 Genetic Diversity and Genetic Resources in Coriander

The enormous phenotypic and genetic diversity of coriander has been documented in several studies (Luzina and Michelson 1937; Diederichsen 1996a; Diederichsen 1997; Lopez et al. 2008; Dyulgerov and Dyulgerova 2013; Fufa 2013) (Fig. 4.5; Table 4.2). The studies were primarily based on descriptive assessments of morphological, agronomical and chemical features. For example, an evaluation of the essential oil composition of the fruits allowed for identification of certain chemotypes associated with geographic origin and particular morphological features (Diederichsen and Hammer 2003). Studies of this nature are of great relevance for plant breeders to identify germplasm as sources for particular traits of interest, in particular considering that molecular studies on coriander are rare. Molecular traits evaluated to date could not be associated with relevant phenotypic characters (Furan and Geboloğlu 2017; Galata et al. 2014; Tomar Rukam et al. 2014). Table 4.2 presents the ranges of variation observed in systematic field trials conducted at the national German genebank. The Food and Agriculture Organization of the United Nations lists more than 3100 coriander accessions stored in various national genebanks with the most extensive collections in Germany, India, Vietnam, Pakistan, Portugal, Romania, Russia, Ukraine and the United States of America (WIEWS 2018). However, the use of the wide diversity available from genebank collections for plant breeding has been limited (Chislova 2001).

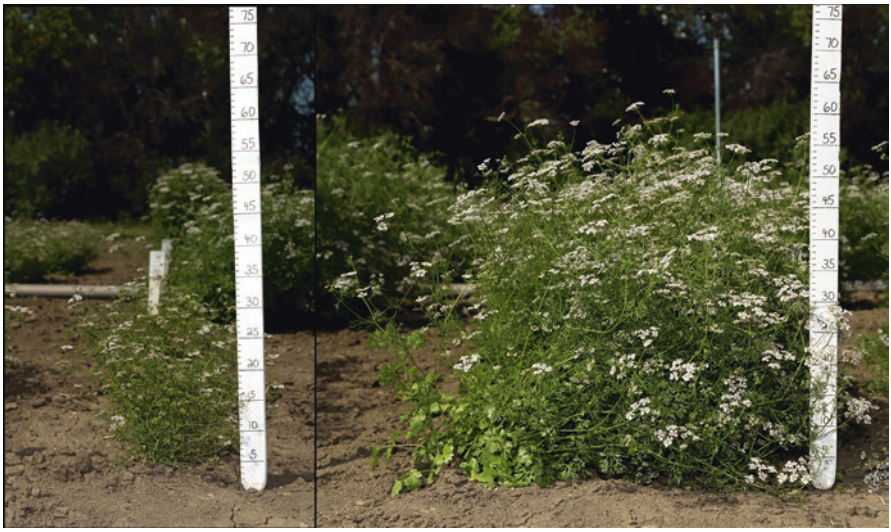

Fig. 4.5 Two types of coriander (accessions at Plant Gene Resources of Canada): dwarf type from Egypt (left, CN 19162) and Hungarian cultivar 'Budakalaszi Rekord' (right, CN 19172)

A crop wild relative in the sense of being a wild progenitor for *C. sativum* does not exist. The species closest related in the tribus Coriandreae are *Coriandrum tordylium* (Fenzl) Bornm., *Bifora radians* M.-Bieb. and *B. testiculata* (L.) Spreng ex Schultes. The *Bifora* species share the strong presence of volatile secondary products of metabolism in the vegetative plant parts, and they also contain fatty oil that resembles the composition of the fatty oil in coriander (Diederichsen 1996a). These species are not of relevance for breeding of coriander but could become cultivated plants in their own right due to their fatty oil content in the seeds. The *Bifora* species are weeds and are threatened by extinction, so genebanks play an important role in their conservation.

4.7 Genetic and Genomic Studies of Coriander

As breeding has primarily been focused on line selections, there is not much information available regarding the genetic control of relevant traits in coriander based on segregating populations. Monogenic recessive inheritance has been demonstrated for the absence of anthocyanins from all plant parts (Diederichsen 1997) (Fig. 4.6). The feature of having entire primary leaves is also monogenic recessive to the rarely found feature of having multipinnate primary leaves (Silchenko 1981; Diederichsen 1997) (Fig. 4.7).

Genetic material from coriander has been introduced into tobacco and rapeseed for academic research (Cahoon et al. 1992; Murphy et al. 1994). The transcriptome for monoterpene synthase in coriander has been investigated (Galata et al. 2014). Practical applications of these techniques have not occurred.

Fig. 4.6 Parental lines (left and right, accessions at IPK, Gatersleben, Germany) and F_1 plants from crosses in both directions (middle) showing the dominant inheritance of anthocyanin presence in vegetative plant parts. This relates also to the presence or absence of anthocyanins in the petals (Fig. 4.3)

Fig. 4.7 Parental lines (left and right, accessions at IPK, Gatersleben, Germany) and F_1 plants from crosses in both directions (middle) showing the dominant inheritance of multipinnate primary leaves

4.8 Conclusions

Coriander is globally the most relevant essential oil crop and the leafy vegetable use is increasingly popular. The enormous genetic diversity of the species accessible from genebanks offers ample opportunities to develop cultivars adapted to specific usage and growing conditions. The diseases affecting coriander and other umbelliferous plants are not well understood.

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Chapter 5
Duboisia sp. – Corkwood Tree

Julia Sparke

5.1 Introduction to the Genus *Duboisia*

The genus *Duboisia* was named by Robert Brown in honor of the French botanist Dubois (Brown 1810). Based on molecular phylogenetic analyses, the genus *Duboisia* belongs to the subfamily Nicotianoideae within the family of the Solanaceae (Olmstead et al. 2008). Within Nicotianoideae, the tribe Anthocercideae comprises all solanaceous genera including *Duboisia*, which are endemic to Australia (Garcia and Olmstead 2003; Olmstead et al. 2008). Four *Duboisia* species were described: *Duboisia arenitensis* Craven, Lepschi and Haegi (1995), *D. hopwoodii* F. Muell. (Mueller 1876–1877), *D. leichhardtii* F. Muell. (Mueller 1867–1868), and *D. myoporoides* R. Br. (Brown 1810). The haploid chromosome number of *D. leichhardtii* and *D. myoporoides* was determined at meiosis to be $n = 30$, and the diploid number was observed at $2n = 60$ in mitotic root-tip divisions (Barnard, 1949). Although Ikenaga et al. (1979) stated a haploid chromosome number of $n = 28$ for *D. leichhardtii*, no further studies of the chromosome number were reported until to date. The average nuclear DNA content for the species *D. hopwoodii*, *D. leichhardtii*, and *D. myoporoides* was estimated at 4.5 pg/2C with a standard deviation of 0.5 pg/2C by flow cytometry with the use of up to five different solanaceous internal reference standards (Dietrich 2013).

The species are distributed to different regions of Australia. They grow like shrubs or trees, which can reach a height of up to 14 m. *Duboisia hopwoodii* is most widespread in the outback and desert regions of Australia, whereas the distribution of *D. arenitensis* is highly restricted to Mount Gilruth on Arnhem Land in the Northern Territory (Williams 2013). *Duboisia myoporoides* is distributed along the Australian east coast from Cape York to Southern New South Wales. It is the only

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species that also exists outside of Australia in New Caledonia (Williams 2013). *Duboisia leichhardtii* grows in the South Burnett region in Queensland (Williams 2013). The areas of distribution of *D. myoporoides* and *D. leichhardtii* overlap, and intermediate forms of both species, which are likely to be hybrids between *D. myoporoides* and *D. leichhardtii*, were reported (Barnard 1952).

Duboisia hopwoodii, *D. leichhardtii*, and *D. myoporoides* differ in leaf and flower morphology (Figs. 5.1, 5.2, and 5.3). While leaves of *D. hopwoodii* are narrowly elliptical (Fig. 5.1a, d), *D. leichhardtii* leaves are lanceolate (Fig. 5.1b, e) and *D. myoporoides* leaves are elliptical to ovate (Fig. 5.1c, f).

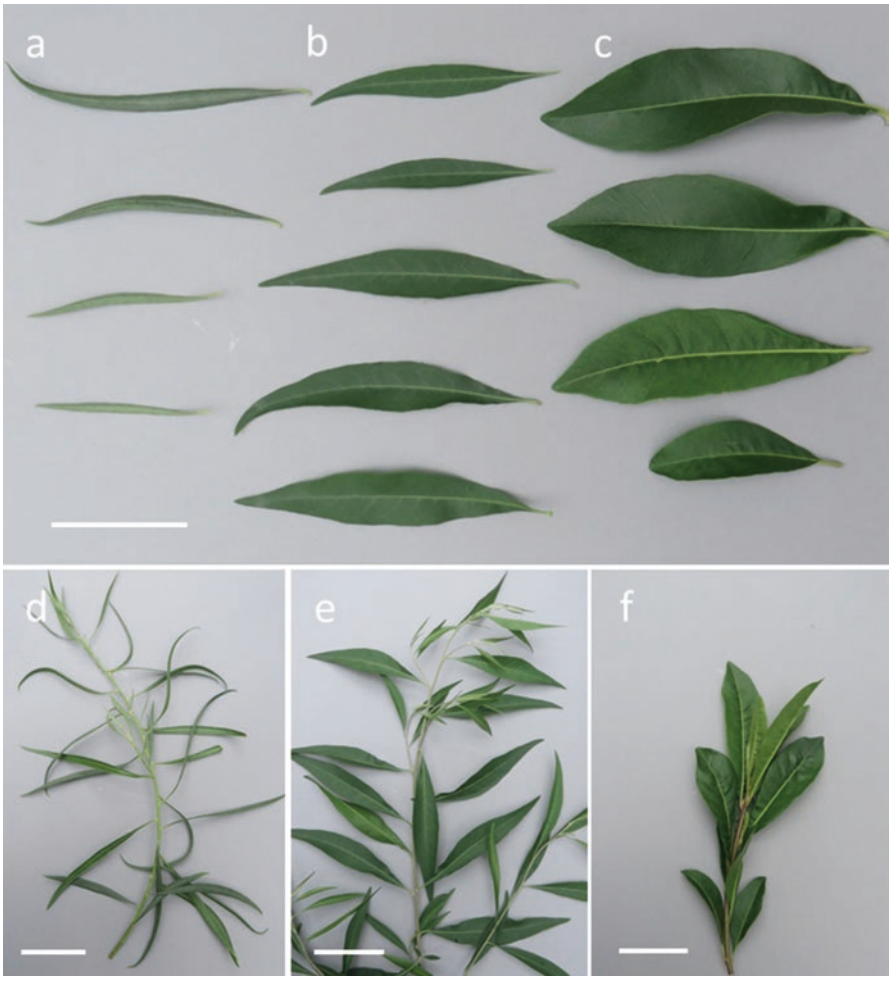

Fig. 5.1 Leaves and shoots of representative *D. hopwoodii* (**a**, **d**), *D. leichhardtii* (**b**, **e**), and *D. myoporoides* (**c**, **f**) genotypes. Bar = 5 cm

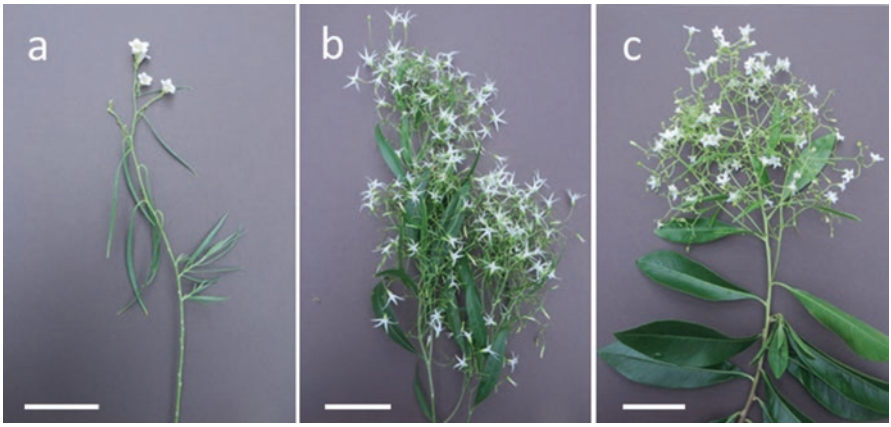

Fig. 5.2 Inflorescences of *D. hopwoodii* (**a**), *D. leichhardtii* (**b**), and *D. myoporoides* (**c**). Bar = 5 cm

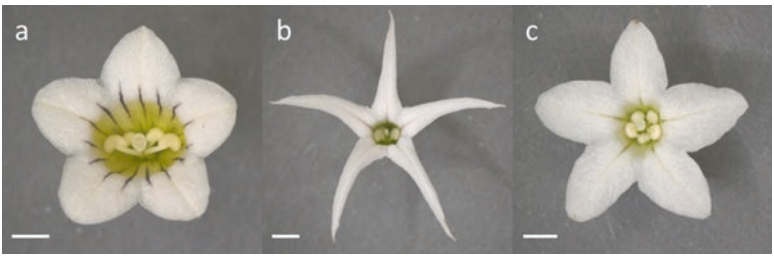

Fig. 5.3 Flowers of *D. hopwoodii* (**a**), *D. leichhardtii* (**b**), and *D. myoporoides* (**c**). Bar = 2 mm

Duboisia flowers naturally appear in spring. They are white and disposed in large terminal panicles (Fig. 5.2a–c). Single flowers are bisexual and protogynous (Fig. 5.3a–c). The flower symmetry is bilateral (Knapp 2010). The number of anthers ranges from four to six between the different species. The corolla diameter varies between 9 and 15 mm depending on the species (Hiltrop et al. 2016). Naturally, flowers are pollinated by insects indicating that *Duboisia* is a cross-pollinated plant. The seed development from pollen germination to fertilization and the ripe seed was described by Barnard (1949).

5.2 Economic Importance of *Duboisia*

Species belonging to the genus *Duboisia* contain tropane alkaloids of which some of them are of great economic importance in medicinal use. *Duboisia myoporoides* and *D. leichhardtii* plants contain scopolamine and hyoscyamine in quite higher concentrations than other solanaceous genera, such as *Atropa*, *Datura*, or

Hyoscyamus. In addition to these tropane alkaloids, the rapid plant growth is basically the reason for the high interest in the genus *Duboisia*. The determination of tropane alkaloid contents in different plant organs of *D. myoporoides* revealed scopolamine as one of the dominant alkaloids in all studied organs with the highest concentrations in the leaves (Coulson and Griffin 1967). A comprehensive summary of alkaloids that were identified in *D. myoporoides* is given by Foley (2006). Nicotine and nornicotine are the most prominent tropane alkaloids in *D. hopwoodii*. *Duboisia arenitensis* was not object of any further studies since Craven et al. (1995), probably because of the lower content of tropane alkaloids in comparison to the other *Duboisia* species. Tropane alkaloids have a long tradition in medicinal use due to their anticholinergic properties and are still of great significance today. In a recent review of tropane alkaloids, chemical and pharmacological properties as well as known and unknown steps in the biosynthesis were described (Kohnen-Johannsen and Kayser 2019). Tropane alkaloids are chemically classified by their bicyclic saturated structure (*N*-methyl-8-azabicyclo [3.2.1] octane), the tropane ring, which is a characteristic of a class of approximately 200 alkaloids that naturally occur in, e.g., solanaceous plants (Grynkiewicz and Gadzikowska 2008).

The production of scopolamine for commercial purposes started in 1941, while that of hyoscyamine/atropine in 1942 (Barnard 1952). In the beginning of the 1940s, *Duboisia* leaves for extraction were collected from the wild. During the Second World War, the interest in *Duboisia* as a source of scopolamine, which was predominantly used for surgical anesthesia and to manage sea sickness, increased strongly (Foley 2006). The demand of *Duboisia* leaf was so great that the extinction of the species was feared. Furthermore, the natural plant variation and the variation of tropane alkaloid quantity were reasons for the establishment of commercial plantations (Kelenyi 1949), and the first trials with the focus of commercial *Duboisia* production were initiated. The first large-scale plantations of transplanted seedlings that germinated in the wild did occur in the late 1950s (Ohlendorf 1996). Initially, plantations of *D. leichhardtii* with naturally set seedlings were established as the seed was very difficult to germinate. Seed pretreatment with gibberellic acid improved germination, but still the results were genetically heterogeneous plantations. Vegetative propagation of *D. leichhardtii* was not successful (Luanratana and Griffin 1980a). Then, the cultivation of hybrids started. In the beginning, cultivation practices were developed from rather extensive practices with wide spacing and little fertilizer input. With the launch of Buscopan® by Boehringer Ingelheim in 1951, the demand for scopolamine increased further (Ohlendorf 1996). Scopolamine was and is still used as a substrate for scopolamine-*N*-butyl bromide as the active pharmaceutical ingredient in Buscopan®, but also for other partially synthetic scopolamine derivatives such as tiotropium bromide or scopolamine-*N*-methyl bromide. In order to secure the supply chain with *Duboisia* raw leaf material for scopolamine extraction, Boehringer Ingelheim developed its own plantations in 1976 in Queensland, Australia, where the red volcanic soils, which are preferred by *Duboisia*, occur (Kelenyi 1949) and started its own research and development work on *Duboisia* (Ohlendorf 1996). Systematic trials were carried out to improve *Duboisia* cultivation in order to increase yield. Nowadays, Boehringer Ingelheim

Fig. 5.4 *Duboisia* hybrids in plantation: hybrid plant (**a**), plantation rows (**b**)

owns and leases a total area of around 1400 ha in Queensland (Fig. 5.4), Australia, to guarantee a stable and robust supply of herbal raw material to produce pharmaceutical active ingredients (https://www.boehringer-ingelheim.com.au/duboisia-farms14.03.2019).

Chemical synthesis of scopolamine, in particular, is possible, but the routes are long and expensive and the yields are low (Nocquet and Opatz 2016). Therefore, it is not competitive to the extraction from *Duboisia* leaf material. Biotechnological approaches, which will be mentioned in another chapter, have also not been successful so far in the sense of having an alternative and competitive way of producing hyoscyamine or scopolamine, which is superior to the extraction from plant material. Probable reasons are complex metabolism interactions and unknown steps in tropane alkaloid biosynthesis.

5.3 *Duboisia* Breeding

5.3.1 Pollination Biology

A simple pollination procedure for *Duboisia* was described by Grozsmann et al. (1949), which has been developed further. In order to avoid cross-pollination in *Duboisia* breeding, flowers must be isolated before bud-opening. After bud-opening, the flowers are emasculated with the help of a small crochet hook as long as the stamens are still closed. Since the stigma is receptive at the same time, pollination with respective father pollen can be done immediately after emasculation. Pollinated flowers must be isolated again to prevent unwanted cross-pollination. Small green berries develop after successful fertilization. At the end of the fruit ripening process after some weeks, small, globular, black, juicy berries with a diameter of about

6 mm developed, which contain the seeds. One berry may contain up to 20 seeds, which may differ depending on the cross combination.

5.3.2 Propagation Strategies

In the beginning, the *Duboisia* propagation faced several difficulties such as low seed germination rates and the insufficient rooting rates of *Duboisia* cuttings. In order to improve seed propagation, trials were initiated to optimize seed germination through dormancy-breaking seed pretreatments. Heat treatments were not successful, but a gibberellic acid treatment turned out to improve germination. Furthermore, a large percentage of sterile seed was discovered (Barnard 1949; Kelenyi 1949). The seeds were either empty or contained shriveled material and must be separated from fertile seeds before sowing. The rooting of cuttings was improved by selection of suitable plant parts, optimization of substrate, climate conditions, and the application of plant growth regulators to promote rooting. In the 1940s, a vegetative propagation method for *D. leichhardtii* and *D. myoporoides* via cuttings was reported with the use of softwood cuttings at 60–70 °C and a root-promoting hormone treatment (Hills 1945). Different rooting media, high humidity and open cultivation, with and without hormone treatment, and different times of the year were studied. Furthermore, the rooting ability of cuttings is a very important factor during plant selection. Genotypes must root fast and achieve high rooting rates throughout the whole year. Vegetative propagation via cuttings is nowadays the method of choice for clonal mass propagation.

5.3.3 Cultivation Process and Its Influence on Yield

Biomass yield was improved through the optimization of cultivation techniques. Plant spacing was trialed, and 1 m × 1 m was mentioned to maximize yield in field cultivation in South India (Ram et al. 1996). Other reports of alkaloid variation or composition indicated the cultivation or commercial production and study of *Duboisia* in India (Shukla and Thakur 1992), Thailand (Luanratana et al. 1990a), Japan (Ikenaga et al. 1977), and Brazil (Foley 2006; Fioretto et al. 2016). Nowadays, *Duboisia* harvest is performed with special harvesting machines, which cut off complete branches and chop them. The separation of leaf and wood is done in an optimized process immediately before the leaves are dried in an industrial dryer. The *Duboisia* stumps regrow in about 10–12 months and can be harvested several times in this way again. The regrowth potential after harvest is paid attention during genotype selection, and the plants must follow a certain upright growth habit to be suitable for mechanical harvest (Ohlendorf 1996).

Studies of nutritional effects were carried out in hydroponic culture (Luanratana and Griffin 1980a; Ullrich et al. 2017a, b). More nitrogen resulted in a decrease of

total alkaloid content, but in an increase of the plant size. Higher potassium levels seemed to increase the scopolamine percentage (Luanratana and Griffin 1980a). The effect of plant growth regulators was also tested (Luanratana and Griffin 1980a). While cytokinin treatment resulted in total alkaloid increase, scopolamine levels did not increase. However, the application of seaweed, which is known to have cytokinin activity in strong solution, increased scopolamine percentage, but had no effect on plant growth. Plant growth substances containing gibberellic acid or auxins were tried as well. Elongated leaves were observed after the use of gibberellic acid (Luanratana and Griffin 1980a). Recently, the study of young *Duboisia* plants in hydroponic culture in climate chambers revealed that high light intensity and long daily illumination time influenced the scopolamine content negatively (Ullrich et al. 2017a, b). However, biomass increased with the application of a high light intensity of 300–350 µmol/m^2∗s and a temperature of 28 °C. Regarding the macronutrients, scopolamine level was influenced negatively by nitrogen and calcium, whereas biomass was positively affected by nitrogen only (Ullrich et al. 2017a, b), which was in accordance with Luanratana and Griffin (1980a). Potassium neither affected biomass nor scopolamine production within the tested concentration range (0.05–4 mM) in these experiments (Ullrich et al. 2017a, b). In a commercial plantation, fertilization did only show a little or no effect on tropane alkaloid contents (Luanratana and Griffin 1980b). Hyoscyamine contents in young plants were higher than in older plants revealing the plant age as a significant factor in tropane alkaloid pattern and yield. A connection between phosphorus and alkaloid biosynthesis was assumed, but not examined (Luanratana and Griffin 1980b). However, the amount of mineral nitrogen fertilization had a strong influence on leaf biomass in field trial plots (Oster et al. 2016).

Since the seasonal variation of the tropane alkaloid content resulted in lower yields especially in the cooler months from May to August, different attempts at optimizing cultivation techniques to keep the tropane alkaloid content on a stable level with, e.g., seaweed extract (Luanratana and Griffin 1982c), were followed, and studies continued. Although climatic factors were considered as important, soil type, nutrition and light intensity, and drought were not regarded as fundamental causes of alkaloid fluctuations (Hills et al. 1954d). If moisture, fertility and temperature were at an optimum, the alkaloid content would be usually higher during active growth periods (Hills et al. 1954d). Mishra and Sangwan (1997) also described the quite high tropane alkaloid contents in periods of growth and a strong decrease during senescence in the winter months in *D. myoporoides*. Moreover, in an evaluation of *Duboisia* hybrid plants over three years in a commercial plantation, stable scopolamine contents between 1.2 % and 1.6 % were determined during the summer months from November to February, followed by a decrease in May and June, but increase of hyoscyamine and 6-hydroxy-hyoscyamine (Luanratana and Griffin 1980b). Accordingly, if only the seasonal fluctuations were considered, the best would be to harvest only in the months with high scopolamine yields and good vegetative growth. In a recent evaluation of *Duboisia* growth and nutrient uptake in a commercial plantation by Fioretto et al. (2016), *Duboisia* plants reached a height

of 187 cm, and the dry weight per plant after 324 days of cultivation in the field was composed of 77 % branches and 23 % leaves. The increase of dry weight in the leaves was almost zero from the 250th day onward, and the dry weight as well as the nutrient accumulation was greater in the branches than in the leaves from day 250 until day 324 after planting. The nutritional demand of macronutrients was N > K > Ca > P > Mg > S and Fe > Mn > Zn > B > Cu for micronutrients, respectively (Fioretto et al. 2016). As a consequence out of this study, the harvesting schedules would have to be rethought.

5.3.4 Scopolamine Metabolism in Duboisia

In order to gain insight into tropane alkaloid biosynthesis and metabolism in *Duboisia*, several trials were reported. In 1982 and 1992, *Duboisia* hybrids were analyzed regarding their alkaloid metabolism (Luanratana and Griffin 1982a; Gritsanapan and Griffin 1992a). Young vegetative propagated *Duboisia* hybrid plants were observed to contain hyoscyamine as the major alkaloid. Though, after about one year of cultivation, scopolamine was the dominant alkaloid (Luanratana and Griffin 1982a). Scopolamine was further metabolized to aposcopolamine (Gritsanapan and Griffin 1992a). It was ruled out that hyoscyamine and 6-hydroxyhyoscyamine could be direct metabolites of scopolamine (Luanratana and Griffin 1982a; Gritsanapan and Griffin 1992a). The highest concentrations of scopolamine were found in the leaves, whereas the highest recovered activity was found in the side stem bark leading to the assumption that scopolamine metabolism occurred in the bark.

To date, it is known that tropane alkaloids are synthesized in the roots followed by rapid translocation to the leaves. Hyoscyamine 6β-hydroxylase (H6H) was identified to catalyze the metabolism from hyoscyamine to scopolamine (Hashimoto et al. 1991). It has been localized in the pericycle of the roots and in low concentrations in the shoots of *D. myoporoides*. The alkaloid translocation was assumed to proceed through the xylem. In a recent study, different tropane alkaloids were localized in roots, stems, and leaves of young *D. myoporoides* plants with MALDI-MSI (Kohnen et al. 2018). The results confirmed that biosynthesis of tropane alkaloids was localized in the roots with subsequent accumulation in the leaves. Even though scopolamine accumulated in leaves, no cell compartments for alkaloid storage were detected. Within the roots, the amounts of littorine, which is a precursor in scopolamine and hyoscyamine biosynthesis, and scopolamine remained constant. The scopolamine concentrations in the xylem were low (Kohnen et al. 2018). Accordingly, the transport of scopolamine from the roots to the leaves is still not understood to date. It is likely to be transported as scopolamine, hyoscyamine, and 6-hydroxy hyoscyamine rather than as scopolamine glucoside (Kohnen et al. 2018), but further investigations would be necessary to clarify the transport question.

5.3.5 The Development of Breeding Activities

Basic knowledge about tropane alkaloid variation in *Duboisia* species and genotypes was obtained since the 1950s. The composition and the quantity of the total alkaloid content from the same tree varied considerably depending on the season as well as the origin of plant material for *D. hopwoodii* (Luanratana and Griffin 1982b), *D. leichhardtii* (Hills et al. 1954b), *D. myoporoides* (Hills et al. 1954a; Gritsanapan and Griffin 1991), and in different *D. myoporoides* × *D. leichhardtii* genotypes (Ikenaga et al. 1985). Hills and Rodwell (1951) studied *D. myoporoides* genotypes from the northern type from different locations with regard to alkaloid pattern and content. Scopolamine was found to be the major alkaloid in these northern types, but a certain variation in the scopolamine level was observed between the genotypes (Hills and Rodwell 1951). This was in accordance with a later study of northern and southern types, in which again the variability of the tropane alkaloids hyoscyamine, scopolamine, and norhyoscyamine was greater in the northern plant material than in the southern (Hills et al. 1954a). The variation in the *Duboisia* plant material with regard to tropane alkaloid content and pattern, biomass yield, and tolerances and resistances was the most important prerequisite for *Duboisia* improvement via breeding.

During the 1970s, Boehringer Ingelheim built up its own *Duboisia* breeding program in Ingelheim, Germany. Since then, it followed a classical breeding scheme for clonally propagated crops. Parental *Duboisia* genotypes were selected based on their characteristics and were cross-pollinated. Single superior offspring plants were selected based on systematically collected data about their trait expression. These genotypes were propagated clonally and were evaluated in bigger numbers and in different areas until they were mass propagated for production. Different biotechnological methods supported the conventional breeding scheme (Keil 2002; Hiltrop 2016; Hiltrop et al. 2016).

Duboisia breeding targets have not changed a lot since *Duboisia* cultivation and improvement started, but breeding for disease resistance and stress tolerances is getting more important. In plant disease management crop rotation and selection of resistant genotypes are the methods of choice. Important diseases are flea beetle (*Psylliodes parilis*), which causes shot hole appearance in the leaves, a stem borer; looper caterpillars; red spider mite; nematodes, such as *Heterodera marioni* or *Meliodogyne* species; and the beetle *Cyrtomon luridus* Boheman (Kelenyi 1949; Ohlendorf 1996; Tironi et al. 2005; Machado et al. 2014). Abiotic factors of attention are drought tolerance, frost tolerance, and tolerance against wind toppling. Still, the main point of attention is the productivity increase of certain tropane alkaloids, especially scopolamine. Therefore, many attempts such as interspecific (Grozsmann et al. 1949; Hills et al. 1954c; Ikenaga et al. 1979) and intergeneric hybridizations (El Imam et al. 1991; Endo et al. 1988; Endo et al. 1991), the application of molecular markers for species discrimination (Mizukami et al. 1993; Keil 2002; Hiltrop et al. 2016), ploidy modification (Hiltrop 2016), in vitro culture techniques (Endo and Yamada 1985; Khanam et al. 2001; Kitamura et al. 1985; Kukreja

and Mathur 1985; Lin and Griffin 1992; Luanratana et al. 1990a, b; Yoshimatsu et al. 1990), and genetic transformations of different plant tissues (Celma et al. 2001; Deno et al. 1987; Hashimoto et al. 1993a, b; Keil 2002; Mano et al. 1989; Mano 1993; Moyano et al. 2002; Muranaka et al. 1999; Palazón et al. 2003; Rahman et al. 2006; Singh et al. 2018; Yoshimatsu et al. 2004), which are all summarized in the following paragraphs, were made to improve and use plants in order to maximize tropane alkaloid yield.

5.3.6 Application of Different Breeding Methods

5.3.6.1 Interspecific Hybridization

Probably the most successful breeding method in order to produce robust, high-yielding genotypes for commercial production was the interspecific hybridization. First studies of interspecific hybrids between *D. leichhardtii* and *D. myoporoides* were carried out in the late 1940s and in the 1950s (Grozsmann et al. 1949; Hills et al. 1954a, b, c). Hybridizations were done in both parental directions with only a very small number of offspring (Grozsmann et al. 1949). However, all hybrid offspring expressed a total alkaloid content, which was either comparable or superior to the parental species regardless of the crossing direction. Offspring from *D. leichhardtii* × *D. myoporoides* had scopolamine as the dominant alkaloid in almost all individuals. With regard to abiotic factors, the hybrids were tolerant against frost, such as *D. leichhardtii*, and superior to *D. leichhardtii* in survival under waterlogging conditions. They demonstrated better growth in general under suboptimal conditions. The morphological characteristics regarding flowers were described as being intermediate between both parental species, and those of the leaves of the interspecific hybrids showed higher similarity to *D. leichhardtii* (Groszmann et al. 1949). Subsequently, the effort in selecting high-potential genotypes for hybridization increased. In 1954, the alkaloid content depending on genotypes from progenies that were collected from different locations and seasonal and environmental factors of *D. leichhardtii* and *D. myoporoides* were studied (Hills et al. 1954a, b, d). Variations in the alkaloid pattern and leaf yield of different genotypes were identified and were the prerequisite for a first selection of genotypes that were superior to others. Extreme hyoscyamine types and extreme scopolamine types within *D. leichhardtii* were identified and verified regarding their alkaloid pattern in a study over four years (Hills et al. 1954b).

Moreover, naturally occurring intermediate forms between both species *D. leichhardtii* and *D. myoporoides* were selected for further studies, and interspecific hybrids between *D. leichhardtii* and *D. myoporoides* as well as their F2 progeny were created and examined (Hills et al. 1954c). Within *D. myoporoides*, southern and northern types were distinguished and characterized (Hills et al. 1954a). Hybridizations with *D. leichhardtii* were performed with both types. Generally,

F1 hybrids were found to grow more vigorously, and some of them produced higher alkaloid yields than both parental species. While Hills et al. (1954c) focused on crosses with *D. leichhardtii* as the female parent, Ikenaga et al. (1979) studied interspecific hybrids in both crossing directions. The choice of the parental genotypes in a certain cross combination determined whether the leaf shape was similar to *D. myoporoides* and *D. leichhardtii* or intermediate between both. In most hybrid genotypes, the alkaloid contents were higher than that of their parental plants.

The superiority of interspecific *Duboisia* hybrids regarding tropane alkaloid content and genotype-dependent pattern as well as plant growth and tolerances to abiotic growth conditions, such as frost, waterlogging, or drought, were the basis for their establishment in all commercial *Duboisia* plantations. Although *D. hopwoodii* contains no relevant scopolamine and hyoscyamine contents, it could be interesting for breeding purposes due to its tolerance against drought and heat because of its natural habit. However, no hybridizations with other *Duboisia* species were reported until to date.

5.3.6.2 Usage of Molecular Markers for Species Discrimination

Species discrimination between *D. leichhardtii* and *D. myoporoides* and their hybrid was achieved by analyzing restriction fragment length polymorphism (RFLP) profiles with the usage of ribosomal rice DNA as a probe (Mizukami et al. 1993), but no further applications of RFLPs are known. Random amplified polymorphic DNA (RAPD) markers were established for genotype identification and quality control during clonal propagation (Keil 2002). Furthermore, these RAPD markers were used for determination of genetic distances between *D. hopwoodii*, *D. leichhardtii*, and *D. myoporoides* genotypes (Hiltrop et al. 2016). Data of 70 polymorphic RAPD bands were converted into a 0–1 matrix to calculate pairwise genetic distances (Nei and Li 1979) between *D. hopwoodii*, *D. leichhardtii*, and *D. myoporoides*. Two genotypes per species were included in the analysis. Intraspecific genetic distances ranged from 0.036 to 0.073, while interspecific genetic distances were between 0.141 and 0.186. Selected sequences of three plastid genes, *psbA* (photosystem II protein D1), *ndhF* (NADH dehydrogenase F), and *matK* (maturase K) (Shapcott et al. 2015), have also been proven to be suitable for species discrimination of *D. hopwoodii*, *D. leichhardtii*, and *D. myoporoides* (Hiltrop et al. 2016).

5.3.6.3 Intergeneric Hybridization

Intergeneric hybridization could be an interesting breeding approach in order to produce hybrids that are sterile and are characterized by even higher tropane alkaloid contents and/or an interesting pattern or better growth characteristics than their parental plants. Three naturally occurring intergeneric hybrids between plants of genera of the tribe Anthocercideae were studied, of which two included a *Duboisia*

species (El Imam et al. 1991). For *Cyphanthera albicans* × *D. myoporoides*, the total alkaloid content of the hybrid was intermediate between the parental species, and plants were characterized by reduced pollen fertility. *D. hopwoodii* × *Grammosolen dixonii* hybrids were pollen sterile, and the tropane alkaloid content was a component mixture of both parental species. Both intergeneric hybrids were morphologically intermediate between the parental species (El Imam et al. 1991), but they were not used for further breeding activities.

In addition to intergeneric hybridization, somatic hybridization could be interesting to speed up the breeding process and overcome natural crossing barriers. A protoplast isolation and culture protocol for cell suspension cultures, regenerated shoots, and cultured roots including callus induction and plant regeneration, which would be a prerequisite, was reported by Kitamura (1993). Intergeneric somatic hybrids between *D. hopwoodii* and *Nicotiana tabacum* (Endo et al. 1988) and *D. leichhardtii* and *Nicotiana tabacum* (Endo et al. 1991) were successfully obtained via protoplast fusion. *Duboisia* cell suspension protoplasts were fused with *N. tabacum* mesophyll protoplasts in both cases. Chromosome elimination of *N. tabacum* chromosomes was observed in fusion products with *D. hopwoodii*. After two years of callus culture of subclone hybrid cell lines of *D. hopwoodii* and *N. tabacum*, two shoots were obtained, but the growth did not go beyond root initiation. Nuclear DNA contents were different, and generally, somatic hybrid cell lines were genetically unstable (Endo et al. 1988). For the *D. leichhardtii* and *N. tabacum* hybrid, six shoot cultures were obtained and characterized regarding their genetic constitution and their alkaloid composition. The dominant alkaloid in all shoots was nicotine; only two of them contained small amounts of scopolamine and hyoscyamine (Endo et al. 1991). Most hybrid plants died when they were transferred to pots (Endo et al. 1991).

5.3.6.4 Ploidy Modification

Polyploid *Duboisia* genotypes have been developed recently from one *D. myoporoides* genotype and one *Duboisia* hybrid, which is grown in commercial plantations (Hiltrop 2016). Nodal segments of *D. myoporoides* from greenhouse grown plants were surface disinfected, followed by an oryzalin treatment that was carried out in liquid medium for two days at a concentration of 20 mg/l (Hiltrop 2016). Nodal segments were cultured in vitro in a climate chamber at 24 °C and 16 h light (~49 µmol m^{-2} s^{-1}). Ploidy level was analyzed simultaneously with diploid control plants via flow cytometry and polyploid plants were identified (Hiltrop 2016). The same concentration of oryzalin either in solid or liquid medium with an incubation time of 1–3 days was also successful to create polyploid plants of a *Duboisia* hybrid, although in vitro shoots were used as explants (Hiltrop 2016). No information about the field performance of the polyploid genotypes in comparison to the control plants has been available so far.

5.3.6.5 Application of In Vitro Culture Techniques

Since the commercial cultivation of *Duboisia* is limited to tropical areas because of its restricted distribution and seasonal factors have an impact on the tropane alkaloid content, several efforts were made to establish *Duboisia* in vitro culture techniques in order to speed up the breeding process and to produce tropane alkaloids independently from climatic conditions. Some of them are summarized in the following paragraphs.

A stem node culture protocol for clonal propagation of *D. myoporoides* was established (Kukreja and Mathur 1985) as an alternative to cutting propagation. For axillary bud growth of nodal explants on solid medium, 3 mg/l kinetin and 1 mg/l indole acetic acid (IAA) were suitable (Kukreja and Mathur 1985). 1-Naphthaleneacetic acid (NAA) turned out to be suitable for root initiation of shoots, but only in liquid medium (Kukreja and Mathur 1985). A general plant regeneration procedure via organogenesis from shoot tips or young seeds as explants via callus induction and shoot and root regeneration to field-cultivated plants was reported in 1992 (Lin and Griffin 1992). Callus was induced from either shoot tips or young seeds (Lin and Griffin 1992), but was also possible from leaf or stem explants (Luanratana et al. 1990b). Callus from young seeds was superior to that from shoot tips to induce buds on medium with 22 μM 6-benzylaminopurine (BAP) and form shoots on medium with 5 μM BA and 0.5 μM 1-naphthaleneacetic acid (NAA) (Lin and Griffin 1992). Roots were induced with 25 μM indolebutyric acid (IBA). Plantlets were transplanted and cultured in the field for three years, reached a height of three meters, and flowered and fruited normally (Lin and Griffin 1992).

The tropane alkaloid content at different developmental stages of *D. myoporoides* shoots and roots that regenerated from non-organogenic and organogenic calli was investigated by Khanam et al. (2001). Tropane alkaloids were only occasionally found in non-organogenic and organogenic calli. Hyoscyamine and scopolamine were detected in low quantities in young regenerated shoots and roots in comparison to the contents in roots and leaves of mature plants (Khanam et al. 2001). Moreover, the distribution of the main tropane alkaloids in *D. myoporoides* was studied in plants regenerated from stem callus cultures at the age of 1, 6, and 20 months in leaves, stem, and roots in comparison to plants from seedlings (Kitamura et al. 1985). Only at the age of 20 months, the alkaloid levels in the leaves of the regenerated plants were comparable to the contents of the leaves of the reference seedlings. Total alkaloid contents were lower in regenerated plants at the age of one and six months, and some tropane alkaloids were completely absent (Kitamura et al. 1985). Accordingly, regenerated plants were not competitive to conventionally bred *Duboisia* hybrids.

Neither in callus cultures nor in *D. leichhardtii* shoots were tropane alkaloids detected (Yamada and Endo 1984). However, shoots were able to convert hyoscyamine, which was added to the medium, to scopolamine (Yamada and Endo 1984; Gritsanapan and Griffin 1992b). Only after root formation, tropane alkaloids were synthesized and found in the regenerated shoots (Yamada and Endo 1984). As a consequence, root cultures were established to improve tropane alkaloid

production. For this reason, callus cultures were established from leaf segments of *D. hopwoodii*, *D. leichhardtii*, and *D. myoporoides* (Endo and Yamada 1985). Adventitious roots formed on medium supplemented with NAA and BA for all three species. Root cultures of all three species produced tropane alkaloids but in lower concentrations than intact *Duboisia* plants, which were cultivated in greenhouses. The application of auxin derivatives enhanced the tropane alkaloid production in root cultures of a *Duboisia* hybrid (Yoshimatsu et al. 1990). However, the tropane alkaloid yield has never been comparable to that of field-cultivated plants.

5.3.6.6 Genetic Modification

Since cell suspension cultures, callus cultures, and shoot cultures produced only low amounts of tropane alkaloids, root cultures were the only chance to create an in vitro system for tropane alkaloid production. The most reported technique was the regeneration of transgenic plants from hairy roots (Celma et al. 2001; Deno et al. 1987; Hashimoto et al. 1993a, b; Mano et al. 1989; Mano 1993; Moyano et al. 2002; Muranaka et al. 1999; Palazón et al. 2003; Rahman et al. 2006; Singh et al. 2018; Yoshimatsu et al. 2004) in order to increase scopolamine content, but also *A. tumefaciens*-mediated gene transfer was used (Keil 2002).

Genetic transformation of *Duboisia* by the Ri T-DNA of *Agrobacterium rhizogenes* in order to produce hairy root clones from stem cultures or leaf segments as explants has been reviewed by Mano (1993). Shoots occurred either spontaneously (Celma et al. 2001) or were induced through the application of plant growth regulators to the culture medium (Yoshimatsu et al. 2004). One important factor in hairy root culture was the selection of high-yielding hairy root clones, e.g., Deno et al. (1987) found that hairy roots had a lower scopolamine content than untransformed roots of *D. myoporoides*, whereas Mano et al. (1989) reported considerable variation in growth rate, alkaloid content, and productivity of 45 hairy root clones and selected hairy root clones from *D. leichhardtii* of which one clone contained 2.1 % scopolamine on a dry weight basis. Precise culture conditions and a comparison between adventitious and hairy root cultures regarding tropane alkaloid production as well as the role of indole-3-acetic acid (IAA) of a *D. myoporoides* × *D. leichhardtii* hybrid were studied by Yoshimatsu et al. (2004). Even shoot induction at root segments for the purpose of generating intact plants was described and possible. In order to obtain results about the agronomical performance of plants, which were derived from hairy roots, hairy root clones were cultivated in the greenhouse and in the field and were evaluated regarding their morphological characteristics and their scopolamine content (Celma et al. 2001). Plants that demonstrated the strongest hairy root syndrome symptoms, which were associated with the expression of the *rol*-genes of *A. rhizogenes*, had a significantly higher scopolamine content than the untransformed hybrid plants. But due to their hairy root phenotype, which resulted in a lower leaf biomass yield than in the untransformed plants, the total scopolamine yield was not competitive to field-cultivated conventionally bred *Duboisia* hybrids (Celma et al. 2001).

Further attempts were made to create high productive root cultures and to genetically improve plants with regard to the scopolamine content. With the aim of overexpression of certain genes that are involved in tropane alkaloid biosynthesis, protocols for binary vector construction and plant transformation via *A. rhizogenes*-mediated gene transfer were established and reported by Muranaka et al. (1999). One gene of interest to improve tropane alkaloid yield was the putrescine N-methyltransferase (*pmt*) gene, which catalyzes the conversion from putrescine to N-methylputrescine, an early step in scopolamine biosynthesis (Moyano et al. 2002). Although the levels of N-methylputrescine increased up to four times in the hairy roots transformed with *pmt* in comparison to wild-type hairy roots, the tropane alkaloids hyoscyamine and scopolamine did not increase significantly. Another approach had the aim to silence the quinolinic acid phosphoribosyl transferase (*qpt*) gene in the nicotine biosynthesis pathway via RNAi in *D. leichhardtii* hairy root cultures (Singh et al. 2018). The QPT enzyme is responsible for the conversion of quinolinic acid to nicotinate mononucleotide, which subsequently forms nicotine through combination with methylpyrrolinium cation (Singh et al. 2018). The methylpyrrolinium cation is the common precursor for the nicotine and tropane alkaloids (Kohnen-Johannsen and Kayser 2019). The downregulation of the nicotine production in order to divert the methylpyrrolinium cation towards scopolamine production resulted in an increased scopolamine amount in these hairy root cultures and inhibited nicotine biosynthesis (Singh et al. 2018). The gene of most interest for genetic modification was the hyoscyamine 6β-hydroxylase (*h6h*), which catalyzes the conversion from hyoscyamine to scopolamine (Hashimoto et al. 1993a, b). Palazón et al. (2003) transformed a *D. myoporoides* × *D. leichhardtii* hybrid via an *A. rhizogenes* binary vector system containing the *h6h* gene from *Hyoscyamus niger* under the control of the CaMV 35S promoter. Transformed hairy root cultures were established and shoots were regenerated. The scopolamine contents of the transformed hairy roots were up to three times higher than in the wild-type hairy roots, but in the transformed regenerated plants, there was no clear scopolamine increase compared to plants that were regenerated from wild-type hairy roots (Palazón et al. 2003). In comparison to untransformed plants, the transgenic plants either from wild-type hairy roots or hairy roots that contained the *h6h* gene exhibited the same morphological abnormalities that were reported by Celma et al. (2001). Even a further report about *A. rhizogenes*-mediated gene transfer of *h6h* in *Duboisia* (Rahman et al. 2006) did not improve plants to that extent that they were competitive to *Duboisia* hybrids, which were obtained via conventional breeding methods.

Genetic modification of *Duboisia* via *Agrobacterium tumefaciens*-mediated transformation of leaf discs was reported by Keil (2002). With the usage of a binary vector system with an intron-containing β-glucuronidase (*gus*) gene and neomycin-phosphotransferase II (*npt*II) gene as selectable marker in different *A. tumefaciens* strains, a transformation protocol was established successfully. Shoots were regenerated from leaf discs and rooted and plants that expressed the GUS gene constitutively were obtained. The plants were morphologically not differing from the untransformed control plants (Keil 2002). Thus, they did not exhibit morphological abnormalities such as the plants obtained from hairy roots. No further genetic modifications via *A. tumefaciens* have been reported since then.

5.3.7 Economical Transfer of Breeding Results

Since most of the *Duboisia* growing industry is located in Australia, it seems obvious to register cultivars or apply for patents on certain genotypes in Australia. Three *Duboisia* hybrids were patented in Australia. Two of the patents were filed in 1986 for *Duboisia* hybrid cultivars, AU1986057498 and AU1986057499, and are meanwhile expired. The patent owner was Boehringer Ingelheim International GmbH (Ingelheim, Germany). Another patent was filed in 2012, AU2012244129, about an interspecific hybrid of *D. myoporoides* and *D. leichhardtii*, which is capable of producing more than 4 % (w/w) (scopolamine/hybrid). The patent owner in this case is also Boehringer Ingelheim Pharma GmbH & Co. KG (Ingelheim, Germany). This patent is still granted.

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Chapter 6
Lavandula angustifolia Mill. and *Lavandula x intermedia* Emeric ex Loisel: Lavender and Lavandin

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6.1 Introduction

Lavender and lavandin belong to the Lamiaceae family and more specifically to the genus *Lavandula* which includes over 30 different species (Lis-Balchin 2003). The various species of *Lavandula* vary in appearance and aromatic quality (Upson and Andrews 2004); several members of the genus are used for ornamental purposes, but three main members are cultivated on an industrial scale (Harborne and Williams 2002, Guenther 1954, Lesage-Meessen et al. 2015).:

- True lavender (*Lavandula angustifolia* Mill. = *Lavandula officinalis* Chaix)
- Spike lavender (*Lavandula latifolia* Medik.)
- *Lavandin,* the hybrids of these two species

These three species are all herbaceous perennial dwarf shrubs that reach heights between 40 and 80 cm. Spikes that rise above the foliage bear whorls of flowers that vary in color from lilac, blue, and violet. The calyx and the corolla are tubular, usually with five lobes. Aside from the difference in quality and composition of their essential oils (Muñoz-Berto et al. 2007), various morphological criteria can be used to distinguish between the three species. A clear difference between true lavender and lavandin can be observed concerning the size and the vigor of the plants and broader leaves, which are much larger in the case of lavandin. The stems of true lavender have a length that varies from 15 to 20 cm. The plant carries short and thin flowering spikes or inflorescence stalks that bear only one group of flowers. The stems of spike lavender and lavandin are longer and superior in number, and all

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flowering spikes are forked, woody, and glabrous. Finally, the hybrid lavandin is sterile; it does not produce seeds (Lis-Balchin 2003, Heather et al. 2005). In this chapter, we will concentrate on true lavender and the hybrid lavandin.

Lavandula angustifolia (true lavender) is thought to originate from the mountainous regions of countries bordering the western half of Mediterranean at altitudes between 600 m and 1200 m above sea level on well-drained calcareous soils. *Lavandula latifolia* (known as spike lavender) will only occur naturally from 300 m to 800 m in the same area (Lis-Balchin 2003).

Lavandin (the sterile hybrid) is encountered when the two parent species are simultaneously present.

In plants, essential oil is produced by the conical cells lining the petals and sepals, where the oil-secreting glandular trichomes are found (Biswas et al. 2009; Hallahan 2000). The main components of the oils from the species are mono- and sesquiterpenes, with the latter being significantly less abundant than the former. More specifically, the exact composition of the essential oil varies depending on the species and on distillation process. In the case of *Lavandula angustifolia* (true lavender), the main components are linalool and linalyl acetate (Harborne and Williams 2002) which give to this species its unique fine fragrance (high ratio of linalool over camphor content). On the other hand, Lavandin is characterized by the generally higher levels of camphor and eucalyptol (=1,8-cineole). Lavandin varieties (*Lavandula* x *intermedia*) produce both the highest yields of flowers and highest concentrations of essential oil per acre.

6.2 Economical Use

The main uses of lavender and lavandin productions are as follows:

- Essential oil
- Concrete
- Hydrolate
- Ornamental bunch

Main tonnages are made with the essential oil. Worldwide, slightly more than 1000 kg of lavandin essential oil is produced when 250 kg of oil are extracted from lavender. Lavandins are way more productive than lavender (100 kg of essential oil/ha vs 15–40 kg of essential oil/ha for lavender) resulting in lower cost of production. On the other hand, the sensory profile of lavandin essential oil is less sophisticate (mainly explained by the high level of camphor in lavandin) and cannot be used in fine perfumery. Lavandin essential oil is intended for industrial perfumery; it is used to give fragrance to detergents and air fresheners, while lavender essential oil is intended for perfumery, cosmetics, aromatherapy, and medicinal uses (Guenther 1954; Cavanagh and Wilkinson 2002, 2005; Lis-Balchin 2002). In France, this sector generates a direct turnover for growers estimated around 30 million euros annually. In addition, it is also of importance to highlight that these crops have a strong impact on the tourism and honey production in their producing areas.

6.3 Cultivation

In the case of *Lavandula angustifolia* (true lavender), two types of varieties are cultivated:

1. Clonal varieties multiplied by cuttings
2. "Population" multiplied through seeds

In France traditionally, local populations used to be grown, but since the emergence of stolbur disease, only tolerant varieties are recommended. There are three tolerant populations bred by iteipmai—Rapido, Carla and Saralia—accounting for 80% of the acreages grown with populations. The essential oil of these varieties is incorporated into the recipes of fine perfumes of the most famous brands. In France, the rest of the acreage (i.e., 70%) is grown with clonal material. Maillette, Matherone, and Diva are the most famous varieties for their high production of essential oil

With almost 60% of the world production, Bulgaria has reached the number one position in the production of essential oil of lavender over the past decade. In this country, the growing material is clonal with some high-performing varieties that have become famous such as Hemus, Druzhba, Karlovo, Sevtopolis, Yubileina, Raya, and Hebar (Stanev et al. 2016). The other lavender producer countries are Moldavia and China (Stanev et al. 2016).

Today almost 90% of the world production of lavandin originates from the southeast regions of France. The planting material is only clonal; Abrial, Grosso, and Super are the leading varieties grown in France (Lis-Balchin 2002).

Currently, the main enemy of lavender and lavandin is the phytoplasma of stolbur, inoculated by the bite of a leafhopper, *Hyalesthes obsoletus*. This disease is commonly called "lavender dieback." The first symptoms are often confused with other diseases, nutrient deficiencies, or the misusage of phytopharmaceutical products. The first visible symptoms are yellowing of the aerial parts and curving of the leaves that eventually become brittle (Cousin et al. 1970; Danet et al. 2010). These symptoms are explained by the obstruction of the vessels of the phloem tissue; this often leads to the death of the whole plant. Between 2005 and 2010, the stolbur phytoplasma destroyed 50% of the French lavender crop, reducing it to between 25 and 30 tonnes in 2011. To a less extent, stolbur phytoplasma can attack lavandin (Yvin et al. 2017; Cousin et al. 1970; Moreau et al. 1974; Danet et al. 2010). Interestingly, the prevalence of this disease varies significantly from country to country: while it is a major treat for French growers, it is still rare in countries like Bulgaria.

The vector, a leafhopper (*Hyalesthes obsoletus*), bites the plant to suck its sap from the vessels of the phloem. The phytoplasma resides in this plant vessels, and the mouthparts of *Hyalesthes obsoletus* are inoculated while biting the plant. Due to the mobility of this insect vector, the disease is often seen to appear sporadically in lavender fields, but its emergence forces the grower to renew the entire fields as no curative control is possible so far. The larvae of *Hyalesthes obsoletus* are present in soil during the winter, and the adults are only present during the flowering period

that coincides with the presence of various pollinating insects forbidding insecticide sprays. During the past decade, many studies and trials had been carried out to control the expansion of the disease. So far no drastic solution has been found; some prophylactic measures are now recommended such as the following:

- Propagation of healthy plantlets along with a list of good agricultural practices (GAP) recommended to plant raisers in a "charter of certified healthy plantlets"
- Intercropping with cereals that act as a physical barrier impeding the dispersion of the leafhopper into the lavender rows
- Spraying the lavender plants with clay as a repellant (color and physical barrier)

Finally, breeding programs that have given rise to the creation of various varieties with good levels of tolerance to the disease (such as Carla, Rapido, and Saralia) have also greatly contributed toward finding a solution to this problem; however, much work remains to be done.

The other major pest is the midge (*Resseliella lavandulae*). Between January and April, adults emerge from the ground; females lay eggs in the cracks of the bark. The eggs develop into larvae that feed off the plant, which gives rise to symptoms of dieback (Coutin 1982). The symptoms rarely appear before the second and third year of cultivation and should be controlled before they affect more than 5–10% of the plants and field. In most countries, plant protection products are available and assure a good control of the cycle of the pest.

6.4 Plant Breeding

Breeding goals are the same for both lavender and lavandin and can be summarized as followed:

- Improved yield explained by the quantity of flowers produced and their contents in essential oil
- Chemical profile of the essential oil
- Resistance to biotic and abiotic stresses

6.4.1 Breeding Lavender

Over the past decades in major producing countries, many works have been carried out to breed new varieties of lavender. The main activities were conventional plant breeding to seek for outstanding individuals within wild and cultivated population. Some innovative work has also been done to generate genetic diversity such as intraspecific hybridization, gamma irradiation, or chemical mutagenesis. All these activities mainly led in Eastern Europe resulted in the development of more than ten

productive clonal varieties, producing good quality of essential oil (Dimitrova 1959; Topalov 1962, 1969; Raev and Boyadzhieva 1988; Staikov and Boyadzhieva 1989). Currently, seven varieties are used in Bulgaria as explained earlier. The introduction of such improved material replacing the traditional population may explain the improvement of yield and the quality of its essential oil (Stanev et al. 2016). Clonal material is quite homogeneous and stable which makes crop management easier and allows the prediction of the performances; that explains the success of such a material with growers. But for the use in fine perfumery, perfumers look for complex chemotypes and sensory profile of the essential oil. These profiles cannot be produced by only one genetic material (so-called clone); therefore, monoclonal crop is a limiting factor for such "high-quality" production of lavender. To tackle this issue, selection of polyclonal varieties was at one point considered but never seriously put into practice. The preferred method is the use of population.

At iteipmai, choice was made to breed synthetic varieties. This breeding method keep a good level of heterozygosity but focusses on a small number of parents selected for the intrinsic value and ability to transfer these traits to their offsprings. The breeding strategy applied is the same as the one described for clary sage. The work was started in the late 1980s; it was sponsored by the French government and the growers' association to select productive varieties with improved level of tolerance against stolbur. The breeding process has started in production fields that had been heavily attacked, and individuals with various levels of tolerance were selected. This first run led to the selection of 400 clones which were multiplied and further evaluated for essential oil content, quality, and yield. This work was continued, and after several years of evaluation, 60 superior clones were characterized. Theses clones were introduced into intercrossed to follow the breeding process of synthetic variety. Rapido was the first commercial variety that came out of this selection program followed by Carla. The parent clones of these two varieties were screened for their tolerance against drought. The selected material led to the selection of Salaria, an improved version of Carla more adapted to dry conditions. This material became famous for the quality of their essential oils (both chemotypes and sensory profile preferred by the luxury perfumery), their yield, and their tolerance against dieback; currently these three varieties account for 80% of the acreage grown with "population" material.

6.4.2 Breeding Lavandin

The lavandin is a sterile plant resulting from spontaneous crosses between *Lavandula angustifolia* Mill. and *Lavandula latifolia*. The genetic diversity available in the wild is very limited. Most, if not all, of the traditionally cultivated clones were obtained through massal selection collected from natural spread area. "Abrial" was the clone largely grown in France between 1950 and 1970; however, it was heavily attacked by dieback. The clone "Grosso" rapidly replaced Abrial because

of its tolerance to the disease; however, its essential oil profile is quite different (Cousin et al. 1971).

In order to induce genetic variability into lavandins, various projects were attempted:

1- *Polyploids.* As described by Urwin (2014), the fertility of lavandins was restored following treatment with colchicine leading to tetraploid plants (4x chromosomes) which produced viable seeds. This track has also been investigated by iteipmai since the 1990s. 4x plants were cross-pollinated with true lavender to produce triploid plants (3x chromosomes). From this study, nine clones have been selected. Further agronomical characterization is ongoing. For the time being, this material is not grown at a commercial scale.
2- *Artificial interspecific crosses* between *Lavandula angustifolia* Mill. and *Lavandula latifolia*. This strategy has been attempted several times by different teams. The number of interspecific hybrids obtained has been quite low, and the few individuals obtained have poor performances compared to the current varieties (Rabotyagov and Yakovlev 1980).
3- *Genetic engineering*. Some intentions were reported in the late 1990s to generate diversity through genetic engineering. At that time, the goals were to introduce stolbur resistance or to boost some biochemical pathways using *Agrobacterium* as a vector (Dronne et al. 1999). None of these concepts were concretely applied. The emergence of the so-called new breeding techniques (NBT) may open the door of a new era. This technique could be considered provided that the genes controlling the traits of interest are known and the regulatory locks are lifted.

6.4.2.1 Toward Molecular-Marker-Assisted Selection Applied to Lavender and Lavandin

Several projects aimed at developing molecular tools to better understand the lavender genome and to develop tools to speed up the breeding process.

Next-generation sequencing (NGS) technologies, by reducing the cost and increasing the throughput of sequencing, have opened doors to generate genomic data in a range of previously poorly studied species. To date, few molecular resources have been developed on *Lavandula angustifolia*. Indeed, nucleotide sequences had been deposited in the NCBI's GenBank database for *Lavandula* species, with more than 50% being sequences of *Lavandula angustifolia*. Most of them (~25%) are related to synthesis or regulation of essential oils or are isolated from the chloroplast genome (~30%). Moreover, EST-derived simple sequence repeat (SSR) molecular markers have been recently developed and successfully tested for transferability between *Lavandula* species (Adal et al. 2015). Recently, Malli et al. (2019) deciphered the genome of *Lavandula angustifolia* which is estimated to be 870 megabase large. Iteipmai, in partnership with INRA and the lavender producers, also contributed to the development of genomic resources for lavender. A project founded by the French Minister of Agriculture and grower association and led by the iteipmai

allows to build about obtained 8, 030 sequences of lavender that were functionally in silico annotated genes (Fopa-Fomeju et al., in prep). A total of 359,000 putative molecular markers of type single-nucleotide polymorphism (SNP) were reported for the first time on lavender. This large amount of data allowed to identify a high SNP frequency (mean of 1 SNP per 90 bp) and a high level of heterozygosity (more than 60% of heterozygous SNP per genotype). This study also indicated that genetically, the current cultivated materials are relatively similar to each other at a marker level. A study is being performed on various wild and cultivated populations. Because the range of accessions tested is widen, it is expected that the results will give more polymorphism.

6.5 Conclusion

Conventional breeding methods have led to the selection of improved material in lavender. Significant progress concerns yield, quality, and quantity of essential oil and tolerance to dieback (Singh et al. 1989). Current work making uses of the latest progress in molecular biology should lead to the development of tools that will enhance the breeding techniques applicable in the future for this species.

Much work remains to be accomplished, notably in the case of lavandin, where the genetic diversity is quite narrow.

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Chapter 7
Matricaria recutita L.: True Chamomile

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7.1 Introduction

7.1.1 Origin and Domestication

True chamomile (*Matricaria recutita* L., syn. *Matricaria chamomilla* L., syn. *Chamomilla recutita* (L.) Rauschert, Asteraceae) is one of the most common medicinal plants worldwide. It was already mentioned by Hippocrates in the fifth century BC (Franke and Schilcher 2005) currently with an estimated worldwide consumption of a minimum of 8000 tons per year (Franke and Hannig 2012). As True or German chamomile classified flowers (*Chamomillae flos*) or flower extracts (*Chamomillae aetheroleum*) has been used in herbal remedies, known in ancient Near East, Egypt, Greece, and Italy (Singh et al. 2011). Besides, as a raw product for food industries especially as tea drug, it is one of the most important specialty crops for pharmaceutical and cosmetic purposes in Germany (Wagner et al. 2005). Its origin could be located in the Near East and South and Eastern Europe (Franke and Schilcher 2007) residential to the Mediterranean Sea. Chamomile is an archaeophyte (Heeger 1956) and is to be found almost all over Europe, Asia Minor, North and South America, New Zealand, and Australia (Fig. 7.1; Franke and Schilcher 2005).

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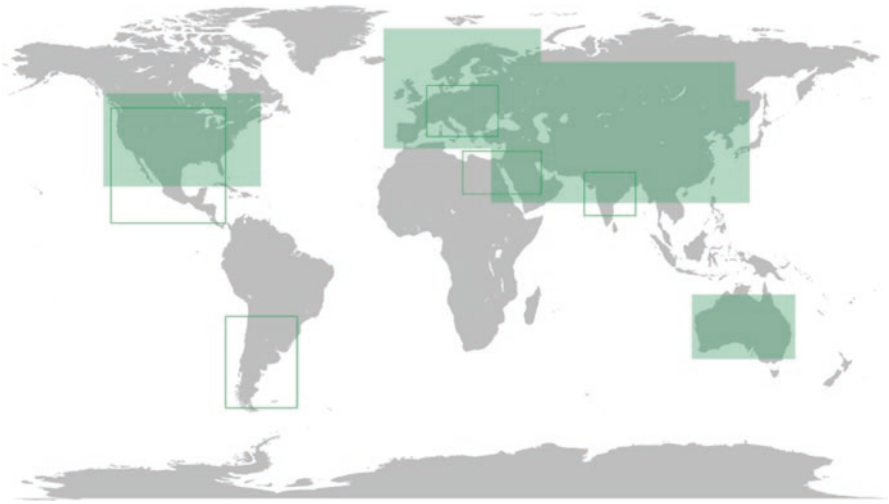

Fig. 7.1 Natural distribution of German chamomile (*Matricaria chamomilla* L.) worldwide (filled rectangles) and centers of cultivation (rectangles unfilled)

Chamomile is an annual, herbaceous plant of 10–90 cm height and natively a diploid species (2n = 2× = 18) with tri- (2n = 3× = 27) and tetraploids (2n = 4× = 36) occurring. The thousand kernel weight (TKW) is 0.02–0.06 g in diploid forms and 0.04–0.12 g in tetraploid forms (Franke in Franke and Schilcher (ed.) 2005). It has an unlike wide ecological range and is therefore widespread all over the world in different climate regions and ecological niches. As a resilient weed, it is found in canopies of common main crops worldwide.

The existence of wild types described as *chemodems* (Schilcher 1973) with distinct, often contrasting chemical compound composition shows the clear relation to genotype occurrence in different locations. With regard to the sesquiterpene alcohols, original forms of chamomile mostly show bisabololoxides (Types A and B) in the essential oil (Franke and Schilcher 2005). As further described by the authors, the origin of chamomile flowers from wild collections can therefore easily be determined by means of the chemical composition (Schilcher 1973; Franz 1982; Schilcher 1987). For example, an (−)-α-bisabolol-rich genotype is of Spanish origin (Carle and Isaac 1985), while in Egypt and Central Europe (CZ, SK, HUN) the bisabololoxide A-types are native, and in South America, the bisabololoxide B-types are found (Weldt 2006). Bisabolon types are found in Southeastern Europe and Turkey. Also forms with low or no matricin were found in Southeastern Europe.

7.1.2 Cultivation

Raw material for industrial uses in pharmaceutical and food production comes from chamomile cultivation all over the world. The use of special varieties and favorable chemotypes depend on the plant parts (flowers, herb) used and the application. The

terms of cultivation like sowing date, fertilization, weed control, and harvest date must be well adapted to the selected cultivar that is to be grown if certain genotypic features of yield and quality will be exhausted to the maximum (Franke and Hannig 2012). The adaption to cultivation practices took place in Germany in the mid of the twentieth century while the demand for tea drugs and later for phytopharmaceuticals increased. With this increase, the requirements for the agronomic performance increased, and so breeding efforts were necessary (Heeger 1956). Recently, in most of the countries with agricultural focus, the cultivation of chamomile is mechanized in aspects of sowing, cultivation, harvesting, drying, and postharvest processing. Main countries of current production are Argentina, Egypt, Poland, Czech and Slovak Republic, Germany, and Balkan states like Bulgaria and Hungary. In smaller but continual amount also Austria, Chile, Croatia, China, Brazil, India, Iran, Italy, Serbia, the USA and Russia count for an established chamomile production.

In Germany, the most common cultivation method is autumn- and spring-sowing with serial sowing dates, often carried out as combination in the same location to enable progressive harvest dates by growing chamomile in large-scale areas of up to 300–600 ha. In the last years, pre-summer drought caused yield losses especially in spring-sown canopies, which therefore begin to loose relevance. The demand for (new) cultivars with different and especially early flowering dates increases to realize consecutive harvest dates of the cultivated canopies (Schnelle 2016, pers. comm.). The acreage where chamomile is grown is organized as perennial/multiannual areas with a high amount of self-seeding by seed shedding and reseeding to some extent (Franke and Hannig 2012).

The sowing in German cultivation is mostly realized with 2.0–2.5 kg/ha with a row spacing of commonly 0.25 m (ranging between 0.16 and 0.40 m). Chamomile is an obligate light-dependent germinator and is therefore sown on the surface of a consolidated soil. For germination and a continuous development, a well-moisturized soil after sowing is obligatory. Good soil and weather condition for growing chamomile are mainly achievable with sowing dates before September 15th for autumn-sown chamomile and the end of March for spring-sown chamomile (Franke and Hannig 2012).

As the flowers in full bloom are picked by picking machines or by hand as harvest products, the postharvest technology must be customized for a quick and gentle separation and drying process immediately after the harvest. Thereby, the chamomile flower drug remains its morphological appearance and the quality of their active compounds will be conserved.

7.1.3 Uses

Chamomile and its products are a rich source of pharmaceutical active compounds for therapeutic uses. The most important classes are found in the essential oil and the flavonoid fraction (Schilcher in Franke and Schilcher (ed.) 2005). Chamomile compounds have anti-inflammatory, antiphlogistic, and spasmolytic effects (Agga and Yousef 1972, Franke in Franke and Schilcher (ed.) 2005), and also antiseptic

and antimicrobial effects could be ascribed (Okoń et al. 2013). It is used in the form of chamomile tea, used as a drink for well-being or more active against painful gastric and intestinal complaints like diarrhea and flatulence. It is externally used as hot compresses or ointment for dermatological indications like badly healing wounds (Schilcher in Franke and Schilcher (ed.) 2005) and used as mouthwash solution or as a steam inhalation containing chamomile products to cure oropharyngeal complaints such as inflammations. Therapeutically, the utilization and the benefits are objects of numerous clinical studies and publications (Schilcher 1987; Letchamo and Marquard 1993; Das 2015).

7.2 Genetics and Breeding History

Although the common use of medicinal and aromatic plants (MAPs) is known since ancient times and widely spread, currently, the field of breeding work has only a brief history. Almost all MAPs with common use were sourced from wild collection and breeding efforts established gradually, especially in the most important plants like chamomile. The history of chamomile breeding in Germany started around the 1950s with the development of the "Quedlinburger Großblütige" and the "Erfurter Kleinblütige" populations as commercially used varieties (Heeger and Brückner 1952). After this initiation, systematic breeding of chamomile in Germany started a rapid development with a widespread outcome. However, in other countries, mainly in Eastern Europe, further breeding has resulted in improved populations and registered varieties.

Today, a large number of different varieties with agricultural use are known worldwide, which are mostly old cultivars, landraces, or mixed populations without a current right protection. Only a few cultivars with high contents of (−)-α-bisabolol and chamazulene, e.g., "Manzana" (1986) and "Mabamille" (1995) are dominating the German medicinal and aromatic plant cultivation. Currently, only individuals of known cultivars are registered in the EU with protected owner rights, mostly in the framework of contractual growing. The difficulty is to purchase true cultivars, which are often sold by nurseries multiplied without mandatory maintenance breeding.

The breeding of the tetraploid variety "Bodegold" (1962) was a breakthrough for cultivation practice in Germany. This cultivar, which was bred in the 1950s in Quedlinburg, is still widespread in production because of its exemplary sensorial qualities (Otto et al. 2015). Other, mainly tetraploid (4×) and also diploid (2×), breeding varieties are available. In general, these are special cultivars bred with a certain focus for active compounds and often reveal deficits in agronomic traits, e.g., regarding their harvest ability or flowering homogeneity. Parameters like a homogenous picking height or a homogeneous flowering time still need improvement. Also, their yield capability under machine harvest conditions is still unexploited.

Beneath focusing on yield and agronomic feasibility (technological processing), the amount and composition of essential oil is in particular attention of breeding efforts on chamomile. Beneath the essential oil components that show high heritabilities (Massoud and Franz 1990a, b) and therefore strong genetic determination, the amount of essential oil in the flower drug (a very important parameter in production) is strongly influenced by environmental factors (e.g., drought, high temperatures, rainfall) and by postharvest processing. High amounts of essential oil can be reached by correct cultivation and the choice of a cultivar. However, the essential oil content shows considerable variation of up to 0.7% in the drug even not only within varieties and cultivation methods but also within small geographical distances (Franke in Franke and Schilcher (ed.) 2005). Particular cultivars reach a maximum value up to 1.3% essential oil in the drug under optimal conditions.

7.2.1 Gene Pools, Germplasm, and Use of Genetic Resources

Chamomile flowers originated previously all from wild collections while breeding work was established in the nineteenth century. Based on the native diploid form, landraces and populations were bred mostly through selection and other methods. Centers of breeding efforts on chamomile since the 1950s were the Czech and Slovak Republic, Poland (Seidler-Lozykowska 2016), Hungary and Germany. A wide range of diploid and tetraploid cultivars originated from universities and research institutes of these countries mostly before 1990. A short, fragmentary list (modified after Franke (in Franke and Schilcher (ed.) 2005) is shown in Table 7.1.

7.2.2 Varietal Groups/Classes

As mentioned in the introduction, the clarification of the occurrence of genetically determined chemotypes by Schilcher (1973) and their classification into four chemodems lead to a better understanding of the different origins and their compound composition. The origins could be classified by the means of their bisaboloid fractions and more specifically also selected by their matricin/chamazulene fraction. All origins with high bisabololoxide A content in the essential oil are classified as chemotype "Type A," while "Type B" comprises all origins with high bisabololoxide B, and origins of the "Type D" have the bisabololoxides A and B in equal shares. The origins with high percentage of (−)-α-bisabolol in the essential oil are named "Type C." The validation of these types gave rise to an improvement of the breeding processes of new varieties by then focusing on preselected parents and special traits. A short, fragmentary list of early cultivars belonging to certain compound pattern is given in Table 7.2. In the last decade, new bred cultivars mostly originate from Slovak Republic (SK) and the Russian Federation (RUS).

Table 7.1 Cultivars of chamomile with origin and ploidy level

Country	Cultivar	Ploidy	Year	Parental population
Austria	Manzana	4×	1986	
Bulgaria	Lazur	4×	1980	
	Sregez	n.a.		
Brazil	Mandirituba	n.a.		
Chile	Manzanilla Primavera Puelche	n.a.		
Czech Republic	Bohemia	2×	1952	
	Pohorelicky Velkokvety	2×	1957	
France	MA.VS.1			
Germany	Bodegold	4×	1962	
	Camoflora	2×	1997	
	Chamextrakt	2×		
	Degumill	2×	1977	
	Euromille	n.a.	2002	
	Germania	2×		
	Mabamille	4×	1995	
	Robumille	4×	2002	
Hungary	Budakalászi 2 (BK-2)	4×	1970	
	Soroksari 40	2×	1970	
Italy	Minardi	2×		
	Olanda	n.a.		
Poland	Promyk	2×	1992	
	Tonia	n.a.	1977	
	Zloty Lan	4×	1970	
Rumania	Margaritar	4×		Selection from Zloty Lan (PL)
	Flora	4×	1989	Selection from Zloty Lan (PL)
Slovak Republic	Bona	2×	1984	(Spanish origin x Bohemia) ∗ polyploidization
	Goral (Kosice II)	4×	1990	(Spanish origin x Bohemia) ∗ polyploidization
	Lutea	4×	1997	Selection from Goral
	Novbona	2×	1997	Selection from Bona
	Lianka	2×	2016	
Slovenia	Tetra	n.a.		
Spain	Adzet	2×	1993	

Modified after Franke and Schilcher 2005

Table 7.2 Affiliation of cultivars to a certain type of compound pattern in the chamomile flower drug

Flower drug with high amount of	Cultivar/origin	Flower drug with low amount of
Matricin/chamazulene and bisabololoxides	Bodegold, Bohemia, Budakalászi 2, Camoflora, Flora, MA.VS.1, Pohorelicky Velkokvety, Promyk, Soroksari 40, Tetra, Tonia, Vallery, Zloty Lan, Argentina, Egypt, Mexico, Chile	
Matricin/chamazulene and (-)-α-bisabolol	Adzet, Bona, Chamextrakt, Degumill, Dukat, Goral, Lutea, Mabamille, Manzana, Margaritar, Mastar, Novbona, Robumille	
Matricin/chamazulene and bisabolon	Lazur, Turkey, Bulgaria	
Matricin/chamazulene	Olanda, Sregez, Turkey	
	Egypt, Turkey	Matricin/ chamazulene

7.3 Current Goals of Chamomile Breeding

Before systematic breeding of chamomile, wild populations were not delivering sufficient harvest quantity and quality. As in most of the medicinal and aromatic plants, the increase of medicinally valuable components and the improvement for mechanical harvesting are the objectives of breeding (Pank and Bluethner 2009). As Franke (in Franke and Schilcher (ed.) in 2005) quotes, in early populations, important traits regarding the stability of the genotype, the germinability of seed, the flower yield, the homogeneity of flowering and ripening and in the end the suitability for mechanical harvest were insufficiently realized. As a further phenotypic trait, the stability of the flowers after drying is of vital interest. With systematic breeding, the attention and selection of certain chemotypes with varying patterns of essential oil content, the amount of matricin/chamazulene, spiroethers, flavonoids, and the bisaboloids were elaborated. Reasoned by special demands, the differentiation and breeding of flowering types in groups of small and large blooming types were also targeted (Table 7.3).

7.3.1 Flower Drug Yield and Machine Harvesting

Flower drug yield is the most important yield component. Flower drug yield is a rather complex trait and consists of a homogenous and narrow picking height caused by a narrow flower horizon, a homogeneous flowering and ripening, large flower heads with good stability, and a high regeneration capacity of the plants after each harvest stage. They have to be combined in one chamomile cultivar. In the breeding process, the traits are measured in each plot on a representative number of individual plants. Yield measurement must be ascertained by reliable methods like standardized machine harvest on small plots or a standardized hand-picking (overestimating the practical yield).

Table 7.3 Breeding targets in chamomile (overview, modified and supplemented after Franke and Schilcher (2005))

Breeding targets
Agronomic traits
High yield
Large flowers
Compact flower heads
Stability of the flowers for mechanical harvest
Regular growth with branching and many flowers
Narrow, homogeneous flowering horizon
Homogeneous flowering time and simultaneous ripening
Early flowering for more than two harvest cycles
Good shooting capacity and good recovering after first harvest
Firm flower head with little inclination of disintegration (flower drug quality)
High germination capacity of the seeds
Resistance to powdery mildew and other fungal diseases
Resistance to common pests
Stable stand, no lodging of canopies
Quality traits
High content of essential oil (0.7–1.0% and more)
High content of chamazulene (>25% of the essential oil)
Hight content of (−)-α-bisabolol (>50% of the essential oil)
Absence or low contents of bisaboloxides A and B
High content of total flavonoids (>3%)

7.3.2 Active Compounds

The main characteristic compound group of *M. recutita* L. flower is the essential oil. The active constituents of the essential oil and of particular interest in pharmaceutical applications are (−)-α-bisabolol (levomenol), a monocyclic sesquiterpene alcohol, matricin, and flavone derivates (Schilcher in Franke and Schilcher (ed.) in 2005). More medicinally active chemical compounds in chamomile are also the derivates of the (−)-α-bisabolol, namely, bisabololoxide A, bisabololoxide B, and bisabolon. Other important ones are matricin (its active form chamazulene is

formed by distillation), flavonoids, farnesene, and spiroethers. Beside the terpenes, important flavonoids are apigenin (and its glycosides) and luteolin. Breeding work focuses on the increase of most of these compounds except the bisabololoxides A and B, which are less effective than bisabolol. The analytical determination of these substances is complex and expensive. Thus, the analytical possibilities are a limiting factor in chamomile breeding programs.

7.3.3 Resistance to Biotic/Tolerance to Abiotic Stress

In chamomile breeding focusing on yield, active compounds, and sensory quality, the adaption and selection of the material concerning resistance against plant diseases were long enough pushed into the background. To enhance most important traits, the breeding material, which is mostly derived from single plants with a low amount of seeds, should be maintained healthy, which is done by plant protection agents. Reasoned by this, a parallel screening of plant disease susceptibility and resistance traits is mostly not well established. The most important plant diseases of chamomile are caused by fungal diseases like *Fusarium* spp., which causes chamomile wilt and stem rot primarily caused by *Fusarium culmorum* (WG SM.). The infections by powdery mildew (*Erysiphe cichoracearum* DC ex Merat and *E. polyphaga* Hamm.) or downy mildew (*Peronospora radii* De By., *Peronospora danica* Gäum., and *Peronospora leptosperma* [De By.] Gäum.) are of high relevance in high-intensity growing locations (Stojanovic et al. 2012; Brandenburger and Hagedorn 2006). The mildew could be recognized by white powdery patches of fungal growth on the leaves. Chamomile flowers could also be affected by *Alternaria* spp., and the whole plants could be infected with rust by *Puccinia matricariae* Syd., as well as with white rust caused by *Albugo tragopigonis* [Pers.] Schroet. The leaves and shoots could be infected by a leaf spot disease, which is caused by *Stemphylium botryosum* (Wallr.). In German cultivation, also a previously undiscovered fungal disease was detected in the last decade. The fungi affect the stems of chamomile with a formation of dark-brown to black spots on the stem. It could cause lodging, but notably, the regeneration after the first harvest is remarkable decreased and therefore yield-effective. Currently the pathogenic fungus is identified as *Rhexocercosporidium matricariae* spec. nov. (Gaerber et al., pers. comm. 2020).

Chamomile is more threatened by pests (Mielke and Schöber-Butin 2007). The flower heads are affected by the herbivore damage of the shining flower beetle (*Olibrus aeneus*), and on young shoots and leaves, different species of aphids (*Aphidoidea*) cause damages. Also, species of thrips (*Thysanoptera*) affect the flowers and the flower drug quality by pollution. The infestation of flowers with weevil (*Ceutorhynchus rugulosus*) is affecting yield vigorous. A sustainable plant protection by the choice of cultivars is up to now not achievable; thus, the infestations are subject to chemical plant protection by insecticides (Dachler and Pelzmann 1999). Finding and breeding of cultivars with native resistances against common pest would outline a step forward also for integrated pest management.

Chamomile, originating from locations with lower latitude near the Mediterranean Sea, is presumably equipped with a native tolerance level to drought and water deficit, assuming the plant is well established (BBCH 10-12). Until now, for the European growing area, the selection for drought and heat tolerance as well as water use efficiency as breeding targets were not of particular interest but are conceivable.

7.4 Breeding Methods and Techniques

With the continuous demand for chamomile flower drug of tea and pharmaceutical industries, the growers attempt to cultivate improved cultivars with higher, more stable yield and assessable compound patterns. Also, the sensory qualities are of high importance by producing a high-quality flower drug. Chamomile exists as most of the medicinal and aromatic plants in a near wild-type stage with low number of selection cycles; therefore, breeding efforts could result in effective and distinct breeding success by selection of particular donors from distinct locations. In the former German Democratic Republic (GDR) and other countries of Central and Eastern Europe, the breeding efforts on chamomile were federally funded and coordinated. In Germany, after 1990, only a few private businesses pursued the further breeding work for chamomile.

In breeding, chamomile is treated as an outcrossing species (Massoud 1988; Letchamo 1992). In literature, not very much about special breeding methods on chamomile are published (Fejer and Salamon 2016; Oravec Sr. et al. 2007; Otto et al. 2015; Tsivelika et al. 2018). Conventional methods of population breeding used in breeding schemes for outcrossing main crops (species) are well adapted and therefore right of first choice.

7.4.1 Individual Plant Selection

The process of selection on chamomile could only be done after flowering because most of the traits will be apparent in this stage. On one side, the selection power could be shortened on behalf of the mother plants by continuing only mother plants pollinated by a donor mixture. On the other hand, there is the possibility to select mother plants and finish blooming by cutting all first flush flowers of the population and using the second flush of female and male plants. By this, the selection also for male flowers for pollination will accomplish the influence of the pollinator. The process of positive selection will be repeated for several years, because the plants will only be similar regarding the phenotype in the first place not for the genotypes. The isolated flowering of the breeding population is crucial for the success of a positive selection process.

7.4.2 Directed Cross-Breeding

Starting with preselected populations or selected genotypes (inbred lines or clones as components), a direct crossing could be carried out when a selection process is not successful. By crossing of, preferably diverging, genotypes, a widened genetic variability could be generated. By using more than two donors, it results in a synthetic population (Syn_1). In chamomile, the outcrossing character is leading, although a self-pollinating character is preserved. Synthetic populations of chamomile could be created with characterized inbred lines (self-compatibility provided) or clone plants (in vitro plants). Advantages of a synthetic population are the consolidation out of characterized components, which could be maintained separately from the population. The synthetic population itself could be generated de novo, also with modified components in later breeding cycles.

7.4.3 Polyploidization

In chamomile, diploid forms (2×) are doubled to a tetraploid (4×) form, mainly by colchicine. Mitsuoka (1963) showed that polyploidization by colchicine resulted in a frequency of eutetraploids of 41% (C_2 generation). By the isolation of eutetraploids in every generation, the frequency was increased to 72% in the C_4 generation. Tetraploid chamomile has larger flower heads than diploids, but the weight of dry flowers per plant is almost equal to diploid plants, because the numbers of tiller and flower heads are smaller in 4× compared to 2× plants. Pollen fertility decreased from 97% to 80%, while pollen diameter increased from 7.7 to 9.5 µm (Mitsuoka 1963). Additionally, tetraploid plants can be indirectly recognized by stoma length, number of chloroplasts per guard cell, and seed weight (Seidler-Lozykowska 2003).

7.4.4 In Vitro Cultivation

Cultivation of certain genotypes is of vital interest in breeding work as in the preservation of germplasm (genetic resources) in general. There are established protocols to adopt and propagate selected chamomile plants especially in the stage before flowering to preserve them in vitro as clones (Sato et al. 2006). With the propagation in vitro, the multiplication of genotypes of interest is possible and can be used for providing the same genotype for crossings or seed production, e.g., as the clones are components of a synthetic population. This strategy in breeding is costly and mainly used to react very quickly on a demand for supplying mostly low quantities of plant material (Carlen and Simmonet 2015) and is therefore only remunerative by preserving exceptional genotypes or plants where seed production is not possible or very expensive too.

7.4.5 Additional Breeding Methods in Chamomile

Breeding a synthetic variety of chamomile is a sophisticated pattern of a hybridization of donor lines in an outcrossing species. Another breeding method on chamomile that was outlined by Salamon et al. (2016) is the method of "the middle seedbed." The best plants of selection of a population are established in the center of the breeding plot as mother plants while a selected paternal donor are surrounding them as a tester. Performing this method for a minimum of four generations, the impact of the pollinator will be monitored and controlled through every generation. As a result, a population of the beginning will result in different families of half siblings with assorted father lines, which were pollinating the selected mother line in the middle seedbed.

An additional breeding method, which extends breeding work with a spatial component, is the method of an R-7 honeycomb arrangement. With this method, cited by Vlachostergios et al. (2011) and applied by Tsivelika et al. (2018) in chamomile breeding following a systematic array, one can eliminate adverse influences of interplant competition (shadowing, water and nutrient stress) and soil heterogeneity.

The honeycomb design with a basic form of a hexagon allocates each test entry (with 1.2 plants/m^2) systematically in the entire field as the authors describe in detail. This should allow a single plant evaluation to characterize and to keep apart specific genotypes. As each entry is surrounded by the plants of the control plants (same entry), the process of comparison and selection will be facilitated. This method is used and adapted for phenotype screening by realizing a most unbiased environment. Primarily, it aims at keeping seeds of each entry for further breeding work, e.g., crossing of two selected entries.

7.5 Major Breeding Achievements

7.5.1 Yield

Since the beginning of chamomile breeding, several cultivars with increasing yield levels originated in the decades beginning in the 1960s. The exceptional challenge of this process is to create genotypes with high quality regarding the amount of essential oil and the active compounds while harvesting as many and as big flowers with the objective of a high yield level. Current flower drug yield of high-quality cultivars reaches 4.0–4.5 dt/ha in average by picking flowers particularly without the leaves. By extensive grow cycles or different harvest technology, a lower yield level is corresponding within one and the same genotype. Meeting the demands for higher economic efficiency by growing chamomile, in 2009, a federal funded project in Germany reestablished breeding work on chamomile. The aim of the project is breeding an open-pollinating variety (non-hybrid) or a synthetic line (artificial population emerging of selected component lines), which is capable to exceed yield

performance of current German cultivars (4.5 dt/ha) by use of machine processed harvesting. The project will be finalized in 2020 with one or more appropriate candidate genotypes for a high-yielding new cultivar.

7.5.2 Quality

Another aim in this above mentioned breeding framework is the refinement of chamomile flower contents of essential oil to a minimum of 0.8% under most conditions and the consolidation of the content of matricin/chamazulene to a minimum of 25%. The general composition of compounds must accomplish to the standards of the Pharmacopoeia Europaea (Ph. Eur.) in its latest version. In conclusion of different efforts there is already a wide set of bred cultivars with specific chemotypes. Solely the maintenance breeding and therefore the availability of seeds are strongly limited.

7.5.3 Resistance and Tolerance

The cultivars in current cultivation have only subtle differences in the susceptibility and tolerance to common diseases. As the European gene pool and its mostly tetraploid cultivars are presumably descending of a small number of parental breeding lines, the genetic components for a wide range of resistance and tolerance are not given in a desired extent. Searching for exotic alleles in native forms and the simultaneous development of reliable resistance tests is crucial for a stable cultivation process further on. Several forthcoming research projects in Germany will address this topic. The improvement in abiotic stress tolerance is very complex and can rarely be selected with rapid determination tests, e.g., at a seedling stage (Gabler 2002).

7.6 Integration of Novel Technologies in Breeding Programs

7.6.1 Flower Biology and Flowering Time

The flowers of chamomile are organized, as typical for the plant family Asteraceae, in a flower head, which display a radial symmetry. The flower head consists of an outer circle of 11–24 ray florets and the inner 400–500 disc florets (Fig. 7.2), causing the flower head to resemble a single flower. The disc florets are penta-dentate (Fig. 7.3). Whereas the ray florets are male sterile and female fertile, the disc florets are both male and female fertile. The fruit is an achene of about 0.8–1.5 mm length. The thousand kernel weight (TKW) lies between 0.02 and 0.12 g. The seeds can lay dormant in the soil up to 15 years before germination (Dachler and Pelzmann 1999).

Fig. 7.2 Flower head with white outer ray florets and approx. Four circles of disc florets flowering (from outside toward inside)

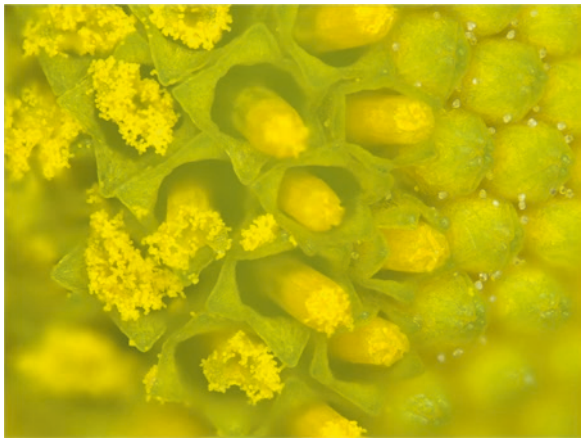

Fig. 7.3 Yellow-green penta-dentate disc florets, which begin to flower (yellow pollen is released)

Chamomile is mainly an outcrossing species (Massoud 1988; Letchamo 1992), whereby most of the plants are to some extend self-compatible with full self-incompatibility being rare (Dachler and Pelzmann 1999; Faehnrich et al. 2013). Based upon first investigations, a strong genotypic effect and additionally a moderate environmental effect on the outcrossing ratio can be hypothesized (Woytowich and Otto 2020, pers. communication).

Diploid cultivars tend to show stronger self-incompatibility (Faehnrich et al. 2013). High temperatures above 30 °C did not overcome the self-incompatibility

mechanism in chamomile (Otto, unpublished data), unlike in rye (*Secale cereale* L.; Gertz and Wricke 1991) or perennial ryegrass (*Lolium perenne* L.; Wilkins and Thorogood 1992). Specifically, early- or late-flowering varieties could extend the choice of the farmers. With early flowering varieties, the drought period in the summer could be at least partially avoided. Varieties flowering at different times could also help to avoid working peaks in the harvesting season, leading to a more even need of machines and workers. The flowering time in chamomile depends on the ploidy level, with tetraploid plants flowering significantly later than diploid ones, but also on the genetics (Otto et al. 2017). Thus, the breeding of new varieties with specific flowering time as one of the objectives is possible.

7.6.2 Ploidy

The wild populations of chamomile are diploid. By artificial polyploidization, tetraploid varieties were generated, which provide a major part of the commercially cultivated chamomile. The tetraploids are characterized by a higher seed weight and larger flower heads (Seidler-Lozykowska 2003; Otto et al. 2015) and tend to flower later (Otto et al. 2017). Nowadays, most cultivated chamomile is tetraploid, which is also hypothesized to be better protected against unwanted hybridization with diploid wild chamomile. However, with technical proper seed propagation also for diploid varieties, this risk can be minimized and is negligible. Many cultivated chamomile varieties and populations display a certain degree of heterogeneity and phenotypic variability. Unlike diploid populations, many tetraploid varieties show ploidy variation and contain a significant percentage of individuals with a different ploidy (di-, tri, and aneuploidy) (Otto et al. 2015; Faehnrich et al. 2019). The ploidy of chamomile proved to be stable during the life time of the genotypes, also after in vitro maintenance and propagation (Faehnrich et al. 2019). The cultivar "Mabamille" is a synthetic tetraploid variety, of which the parents are maintained and propagated by in vitro culture.

7.6.3 Genetics and Genomics

The use of molecular methods and genomics for the breeding and analysis also of minor crops is rapidly increasing, as the costs are constantly going down. Being valid also for medicinal, aromatic and stimulant plants, efforts are under way to apply these methods in chamomile.

Genetic diversity in chamomile at the DNA level was evaluated in a few studies using a variety of different genetic markers (summarized in Das 2015), including amplified fragment-length polymorphisms (AFLP) (Taviana et al. 2003; Wagner et al. 2005), randomly amplified polymorphic DNA (RAPD) (Wagner et al. 2005; Pirkhezri et al. 2010), and Inter simple sequence repeat polymorphisms (ISSR)

(Okoń et al. 2013). Wagner et al. (2005) identified several AFLP markers linked to the (−)-α-bisabolol locus and to the chamazulene content. However, in the subsequent years, no application of the identified molecular markers in the breeding of chamomile was described. No reference genome is for chamomile described yet, like for most medicinal and spice plants. Related reference genomes from crop plants of the same family Asteraceae are available for sunflower (SUNRISE consortium: https://www.heliagene.org/HanXRQ-SUNRISE/) and globe artichoke (Scaglione et al. 2016: http://www.artichokegenome.unito.it and http://gviewer.gc.ucdavis.edu/fgb2/gbrowse/Artichoke_v1_1/). These data could help to understand economically important traits in chamomile and to identify their underlying genes.

Recently, a next-generation sequencing (NGS) approach, the genotyping by sequencing (GBS), was applied to analyze the genetic structure and diversity within cultivated chamomile (Otto et al. 2017), providing a far higher marker density than with the former methods and revealing several diploid populations being genetically distinct from the others. Overall, the tetraploid populations showed lower genetic diversity than the diploids, which might reflect their generation by artificial polyploidization from a limited set of genetic backgrounds. Model-based cluster analysis revealed one large group of 39 tetraploid genotypes (out of 91 analyzed genotypes) from 14 different varieties and traded populations with low genetic differentiation.

Using data generated by the GBS, SNP markers were identified, which are highly significantly associated with the traits flowering time and (−)-α-bisabolol (Otto et al. 2017). As already described above, (−)-α-bisabolol is one of the most important medicinally active chemical compounds in chamomile but is not always present in the plants/populations, as different chemotypes exist in chamomile (Schilcher 1973, 1987; Franke and Schilcher 2005). Often, only one out of the four bisaboloids ((−)-α-bisabolol, bisabololoxide A, bisabololoxide B, bisabolon) is present in high amounts (Otto et al. 2017). Identified molecular markers could serve as starting point to apply marker-assisted selection (MAS) in chamomile, e.g., to detect genotypes with a high level of (−)-α-bisabolol. MAS enables the selection of plant material at an early stage (young plants or even seedlings), saving time and money, and could thus enhance the breeding process in chamomile.

Based upon segregation analysis, Wagner et al. (2005) describe a monogenic recessive mode of inheritance of the (−)-α-bisabolol and chamazulene content. The gene most likely responsible for (−)-α-bisabolol formation is the (−)-α-bisabolol synthase McTPS2 identified in chamomile (Zhu et al. 2015). In many other plant species, the expression of this kind of a terpene synthase was found to be dominant (Köllner et al. 2004; Köllner et al. 2008). Overall, several terpene synthases are identified in chamomile (Irmisch et al. 2012).

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Chapter 8
Nasturtium officinale R.Br.: Watercress

Wolf-Dieter Blüthner

8.1 Botany (Taxonomy, Origin, Distribution, Cytology, Plant Description)

Nasturtium officinale R.Br. belongs to the family Brassicaceae, tribe Cardaminae. Its synonyms are *Sisymbrium nasturtium-aquaticum* L., *Cardamine fontana* Lam., *Sisymbrium nasturtium* Thunb., *Cardaminum nasturtium* Moench, *Nasturtium fontanum* (Lam.) Asch., *Rorippa nasturtium* Beck, and *Rorippa nasturtium-aquaticum* (L.) Hayek.

In the genus *Nasturtium*, 14 species have been reported (Klimek-Szczykutowicz et al. 2018; Rasheed et al. 2018):

Europe:	*N. officinale* R. Br.
	N. microphyllum Boenn. ex Reich.
	N. x sterile (Airy Shaw) Oefelein (hybrid *N. officinale* x *N. microphyllum*)
South and Central America:	*N. gambelii* O. E. Schulz
	N. erectum Trevir. ex Sweet
	N. patens Phil.
	N. pubescens Poir.
	N. tanacetifolium Hook. and Arn.
	N. floridanum Al-Shebaz and Rollins
	N. tweediei O. E. Schulz

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Africa:	*N. africanum* Braun-Blanc
	N. munbyanum Boiss. Et Reut.
	N. barbaraefolium Baker
Asia:	*N. sordidum* (A. Gray) Kuntze
Australia:	*N. groenlandicum* (Hornem.) Kuntze

Nasturtium officinale, *N. microphyllum* (syn: *N. uniseratium* How. & Mant., *Rorippa microphylla* (Boeningh.) Hyl., and *N. x sterile* (Airy Shaw) Oefelein (Hanelt 2001; Morozowska et al. 2010) are used commercially.

The original indigenous area of watercress is not known. It is native in the northern hemisphere but also in the tropic mountain regions. Due to cultivation, watercress is distributed worldwide.

- *Nasturtium officinale* is a natural tetraploid plant with 2n = 4x = 32 chromosomes, of which the diploid progenitors are not known (Bleeker et al. 1999). In cultivation, it was propagated by seeds and shoot cuttings.
- *Nasturtium microphyllum* is a natural allo-octoploid species between *N. officinale* and an unknown *Rorippa* species with 2n = 8x = 64 chromosomes (Bleeker et al. 1999). It is distributed in northern parts of Central Europe and is not in cultivation.
- *Nasturtium* x *sterile* is a hexaploid natural hybrid with 2n = 6x = 48 between *N. officinale* and *N. microphyllum*, confirmed by crossing experiments (Howard and Manton 1946) and common in regions where these two species grow together. Bleeker et al. (1999) validated this origin by analyzing six isozymes. In cultivation, this hybrid is known as "brown cress" or "winter cress" and can only be propagated vegetatively.

The taxonomic identification of *Nasturtium* species is based on seed and fruit morphology (Al-Shebaz and Price 1998). Recently, genome sizes were analyzed by flow cytometry. In *N. officinale*, the 2C DNA content/1C genome size ranged from 0.760 to 0.776 pg/377 Mbp., in *N. microphyllum* from 1121 to 1140 pg/551 Mbp, and in *N.* x *sterile* from 1.121 to 1.140 pg/714 Mbp (Morozowska et al. 2015).

Nasturtium officinale is a very plastic plant. It is a perennial plant, emergently growing in lakes, ponds, streams, and rivers. Watercress is a creeping plant, rooting easily at the nodes in water. The leaves are lyrate to pinnate, with lower petiolate having a length of the petiole of 12 mm. The upper leaves are sessile, auricled in the base with 3–7 leaflets where the terminal leaflet is suborbicular or broadly cordate and lateral leaflets entire to sinuate toothed. The inflorescence is a raceme, up to 5 cm long, with 10–20 flowers. The flowers are white, pedicellate, up to 13 mm long in fruit, and the petals are longer than the sepals. The fruit is silique, up to 16 mm long and 1 mm broad. The inflorescence produces many single-row seeds (Figs. 8.1 and 8.2) (Rasheed et al. 2018, Figure 1, 2).

Fig. 8.1 Watercress in a natural waterbed. (Picture, R Schmidt)

Fig. 8.2 (**a**) Shoot with adventive roots, (**b**, **c**) flowering plant with young siliques, (**d**) watercress seeds. (All pictures, R. Schmidt)

8.2 Economical Uses (History, Domestication, Cultivation, Main Production Areas, Economical Significance, Valuable/Undesired Plant Secondary Compounds)

8.2.1 History: Domestication

Watercress' history started with the Persians, Greeks, and Romans. It has been used as both food and medicine. When Hippocrates founded the first hospital around 400 BC, he used wild-growing watercress to treat blood disorders. The Greeks had a saying that "Eating watercress makes one witty." In the folk and the traditional medicine, watercress has been considered as diuretic, expectorant, purgative, stimulant, aphrodisiac, stomachic, and tonic.

All green parts are useable. Wild collection dominated up to the eighteenth century. Cultivation started in Erfurt, Germany, about 1750, initiated by Chr. Reichart, 1808 in England, and 1811 in France (Manton 1934). Two species were used in Europe, North America, and later in New Zealand, green watercress (*N. officinale*) and brown watercress (*N.* x *sterile*). In Germany and France, only green watercress was used. In England, the cultivation of brown watercress was replaced in the nineteenth century by green watercress due to the easier propagation by seeds and a lower susceptibility against the main fungal disease crook root, *Spongospora subterranea* f. sp. *nasturtii*.

8.2.2 Cultivation: Main Production Areas

Watercress is a minor crop and is grown locally. The production area included 246 ha in the USA (Florida, California, Hawaii) (Palaniswamy and McAvoy 2001), 160 ha in France, 36 ha in England, 18 ha in Spain, and less than 5 ha in Germany and Switzerland. In Australia, New Zealand, and Belgium, the watercress area is increasing.

Watercress can be propagated generatively from seeds or vegetatively by shoot tip cuttings. Today, mainly generative propagation is used because virus diseases (e.g., turnip mosaic virus) spread in the production areas where vegetative propagation is used and may lead to a complete breakdown of the production.

Currently, micro-propagation methods and tissue culture are also available (Klimek-Szczykutowicz et al. 2018).

Seeds are small with a thousand seed mass of about 2–3 g. Young seeds are pale yellow and older one changes more and more to brown. Young seeds have a weak dormancy, and older seeds (brown) or pale seeds germinate better after 2 years of storage. The best conditions for germination are temperatures between 10 and 15 °C and the absence of light. Transplanting healthy plantlets in beds is better than direct sowing. Watercress conventionally can be produced in beds with running

water, in pods, or in hydroponic systems. Watercress is known as a cool season crop and will be harvested in Europe from September to April. The dominant production system is in beds with soil bottom and moving water. Beds of watercress can be used up to 10 years without resowing or replanting. Harvest is possible one time per month. Covers protect the beds in the winter month against frost damages. In the summer month, a net protects against birds and other animals. Plants were cut at a height of about 20 cm. Watercress should be immediately used after harvest. Fresh leaves should be precooled before packing in containers. Best temperature for transport and marketing is 0 °C and 95% relative humidity. These conditions can be safe with crushed ice. Cooled watercress in plastic bags remains marketable for up to 4 weeks.

The plants take up most of nitrogen and other nutrients from the water. A daily uptake of nitrogen from 1.14 g N/m^2 was measured (Howard-Williams et al. 1982). In case of nutrient deficit in the water, the plants can use nutrients from the soil.

8.2.3 Economical Significance: Valuable/Undesired Plant Secondary Compounds

8.2.3.1 Food Use

Watercress has a long history as a salad vegetable. Like other Brassicaceae, watercress is tasteful, fresh, and somewhat bitter. Leaves are used as salad greens or can be steamed and consumed as a normal processed vegetable. The recommended daily uptake is 80–100 g. The best way to use watercress is fresh eating or drinking as smoothie. Beside this, several products with watercress are offered on the market, e.g., juices, mustard, salt, noodles, and pesto.

In ancient time, watercress was used by the Persians, Greeks, and Romans. Watercress soup became very popular in Britain in the seventeenth century when it was claimed to cleanse the blood. *Nasturtium officinale* contains a large amount of vitamins C and provitamin A, folic acid, iodine, iron, protein, calcium, and sulfur compounds (Blüthner et al. 2016). Compared to other Brassicaceae, *Nasturtium* has the highest concentration of nutrients in relation to the energy content (Di Noia 2014).

Due to its health-promoting effects, watercress can be used as a supplement in animal feeding (D'Agaro 2005).

8.2.3.2 Medicinal Use

Traditional uses were connected to diuretic, expectorant, purgative stimulant, stomachic, and tonic effects. More recently, chemopreventive effects had been analyzed in several investigations. A review was given by Klimek-Szczykutowicz et al. (2018).

Anticancer Activity

2-Phenylethyl isothiocyanate (PEICT, nasturtiin) inhibited cancers in rats and mice caused by tobacco carcinogens. It acts as inhibitor and a blocker of tumor initiation (Meyer et al. 1995; Hecht et al. 1995; Boyd et al. 2006; Gupta et al. 2014; Chai et al. 2015). The content of nasturtiin varies from 2 to 7 mg/g leaf dry weight (Palaniswamy and McAvoy 2001). Nasturtiin originates from a hydrolytic reaction of glucosinolates (gluconasturtiin) and the enzyme myrosinase. It is also responsible for the bitter and peppery-hot taste.

Antioxidant activity: Fogarty et al. (2013) found out that short- and long-term watercress ingestion had potential antioxidant effects against exercise-induced DNA damage and lipid peroxidation.

Antibacterial Activity

Nasturtium officinale showed a high activity (MIC = 100 mu g/mL) against the sensitive *Mycobacterium tuberculosis* (Camacho-Corona et al. 2008). Watercress is also an excellent source of the antioxidant α-tocopherol with 0.34 mg/g fresh weight (Hadas et al. 1994).

The essential oil extracted from all watercress parts has also an antioxidant activity. GC-MS analyses of the essential oils of leaves, stems, and flower of *N. officinale* resulted in the main compounds of the oil of leaves, which were myristicin (57.6%), alpha-terpinolene (8.9%), and limonene (6.7%). Caryophyllene oxide (37.2%), p-cymene-8-ol (17.6%), alpha-terpinolene (15.2%), and limonene (11.8%) were the main components in stems, whereas limonene (43.6%), alpha-terpinolene (19.7%), p-cymene-8-ol (7.6%), and caryophyllene oxide (6.7%) were the major constituents in the oil of flowers (Hamzeh 2012).

Klimek-Szczykutowicz et al. (2018) summarized positive effects on different diseases and disorders, e.g., antipsoriatic activity, anti-inflammatory activity, antiallergic activity, antibacterial activity, hypolipemic and cardioprotective activity, antidiabetic activity, and effects on hormone activity.

8.2.3.3 Use in Phytoremediation and Industrial Processes

Phytoremediation is a method to remove contaminants from water, soil, or air by the help of plants. Numerous research works was done in the last 40 years. The uptake of nitrate, phosphate, and ammonium by watercress was analyzed by Vincent and Downes (1980). *Nasturtium officinale* was emphasized as a biofilter for purification of eutrophic water (Abe et al. 1993) and is useful for a passive biofilter monitoring of heavy metals (Baldantoni and Alfani 2016).

Nasturtium officinale is able to absorb numerous heavy metals and to accumulate them in all plant parts. However, this works at low and moderate concentrations only. Numerous research works were done to study the phytoremediation ability of the following:

- Copper (Cu), nickel (Ni), and zinc (Zn) (Kara 1988, 2002; Kara et al. 1999; Duman and Ozturk 2010).
- Cobalt (Co), chromium (Cr), and cadmium (Cd) (Duman et al. 2009; Aslan et al. 2003).
- Arsen (As) (Ozturk et al. 2010) and lead (Pb) (Keser and Saygideger 2010).

8.3 Diseases and Pests

The main problem in production areas is crook root caused by the plasmodia fungus *Spongospora subterranea* f. sp. *nasturtii*. The infected plant shows deformations of all organs like swollen brittle roots and chlorosis. The disease appears mainly from October up to April. The yield decreases seriously.

The fungus belongs to the obligate parasites and spread by zoospores. The soil can stay infected for up to 10 years. The presence of this fungus is proven by a PCR test. The only known method of prevention is the treatment with zinc (zinc sulfate).

Beside the direct negative effects, *Spongospora* is a transmitter of the following viruses: watercress chlorotic leaf spot virus (WCLV) and watercress yellow spot virus (WYSV).

Leaf spot (*Cercospora nasturtii*) infection results in unmarketable plants. The infection was proved in greenhouses in the warm months not only in several states in the United States but also in Africa, Asia, New Zealand, and South America. In gene sequence comparison, watercress isolates were identical to *C. armoraciae* (Koike et al. 2016). Leaf spot disease can be controlled with copper sulfate.

In Florida, pathogenic and nonpathogenic strains of the bacterial disease *Xanthomonas* were found and became the species name *X. nasturtii* in case of the pathogenic species and *X. floridensis* for the nonpathogenic species (Vicente et al. 2017).

Fascioliasis is a zoonosis caused by the liver flukes *Fasciola hepatica* found in Europe, Africa, Asia, and America and *F. gigantica* found in Africa and Asia. About 17 million people are infected worldwide (Mas-Coma et al. 2017). Freshwater wild plants, namely, watercress, are an important source for human infection, but water, juices, and smoothies can also transfer the disease.

Besides, the pests are notable duckweed (*Lemna* and *Spirodela*), diamondback moth (*Plutella xylostella*), and the terrestrial arthropod (*Gammarus pulex*).

8.4 Breeding

8.4.1 Flower and Pollination Biology

Nasturtium officinale is self-compatible. Seed setting and seed germination are successful after isolation. In natural situations, both self- and cross-pollination are common. In case of wet situations with much rainfall, the flowers stay closed and

self-pollination is dominant. The flowers possess two nectars and are attractive to insects resulting in cross-pollinated seeds. Artificial crossings within the different *Nasturtium* species are possible. Crossing *N. officinale* with *N. microphyllum* produces only viable seeds with *N. microphyllum* as the female parent (Howard and Lyon 1952). The seeds germinate immediately after harvesting and the capacity of germination remains for about 5 years. Sometimes, dormancy up to the late spring of the following year was described (Kratky 2015). Crossings between closely related genera are in most cases unsuccessful (Walsh 1998).

8.4.2 Propagation Strategies

Cuttings and/or seeds are the standard propagating material. Until 1955, shoot tip cuttings were prevalent, and in the present production systems, direct seeding or planting of plantlets after greenhouse pre-cultivation is preferred. Seeds as propagation material also prevent virus transfer. Germination is best at 10–15 °C. Possible dormancy can break down by storing the seeds at 40 °C for 3 days (Biddington and Ling 1983).

Watercress is a successful example for a production in hydroponic systems (Kratky 2015).

Plant biotechnology, in particular in vitro culture, opened possibilities to maintain plant species. The micro-propagation from plant nodes of watercress started in 1985 and was demonstrated by Wainwright and Marsh (2017). Different groups showed the possibility of micro-propagation through callus culture (Gilby and Wainwright 1989; Jin et al. 1999).

8.4.3 Breeding Methods Applied

Watercress is a significant nutrient plant worldwide. However, little systematic breeding work was done. The growers use their own propagation material in the form of seeds but mostly as cuttings. The commonly used strains or clones are often not optimized for the different traits. For example, the most used watercress strain in England was also the most susceptible for crook root and virus (WYSV) infections (Walsh 1998). The large seed companies such as Stokes Seed Co., Johnny's Seed Co., and Kitazawa Seed Co. deliver unnamed watercress seed material that shows differences in habit and yield.

Nevertheless, some attempts were done to collect the natural variability and to generate new variability. The commercially available genetic resources show differences in agronomic, in contents of secondary plant compounds, and in resistance traits (Jafari and Hassandokht 2012).

In England, 132 watercress accessions were tested for resistance to crook root and watercress yellow spot virus (WYSV) and for agronomic traits (Walsh 1998).

Payne et al. (2015) analyzed 48 accessions for agronomic and molecular traits. Three accessions were selected for a transcriptome analysis. They found variation in gene expression of the phenylpropanoid pathway. A dwarf type with a high antioxidative potential was the most different accession, compared to the control. Out of the highlighting of commercial interesting genes, a molecular method for an examination of many genes was developed.

The possibilities of in vitro culture also lead to selection attempts on the cell culture level. Du et al. (1999) selected salt-tolerant genotypes after callus culture in salt-containing MS medium. Claxton et al. (1998) tried to select crook root-resistant genotypes in in vitro culture but without success.

Recently, various experiments were done in genetic transformation by *Agrobacterium rhizogenes* (Li et al. 2000; Wielanek et al. 2009; Park et al. 2011). The production of secondary plant compounds with the help of in vitro culture was tried. Different researcher groups used genetically transformed hairy root culture for an in vitro production of glucosinolates (Wielanek et al. 2009; Park et al. 2011; Klimek-Szczykutowicz et al. 2018).

Walsh (1998) tried intra- and interspecific crossings with the related genera *Cardamine*, *Rorippa*, and *Barbarea verna.* The intraspecific crossings resulted in quantitative F2 segregations for crook root and WYSV. The crossing experiments with related genera were unsuccessful.

A red-colored mutant line, found in natural variation, was tested for a commercial use and is protected by US patents (US Patent 2010, 2014).

8.4.4 Breeding Results Achieved/Economical Transfer (Registered Cultivars/Patents, Trial Results)

Nasturtium officinale is a long-day species. A late-flowering strain of *N. officinale* was selected (Bleasdale 1964).

The only known named strain is “Sylvasprings.” It is the description for a watercress line from England and grown in Hawaii. It is also variable in many traits. The large enterprises have done some efforts in germplasm collection and in simple selection breeding. The results were used only in closed production systems. Different traits show the plantings from seeds of various large seed suppliers. The public research work is more or less directed on the description of the natural variation and the effects of watercress for health and nutrition.

The US enterprise B&W Growers had protected a natural mutant with two US patents. In a green watercress production area, a single plant with red-colored leaves and stems was found. This mutation was described as BWRW genotype. With a patent, B&W Growers protected the red plant, their seeds, and hybrids (US 2010). In 2014, a new red-colored variety was introduced and protected with a patent (US 2014). This variety was described as red pigmented, with a stem diameter of 3.5–5.5 mm and a nutritional antioxidant capacity of at least 2.27 times greater than the green standard varieties.

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Chapter 9
Nicotiana tabacum L.: Tobacco

Ramsey S. Lewis

9.1 Botany

Tobacco is sometimes casually used to refer to multiple species of *Nicotiana* that are used for ornamental, ceremonial, or smoking purposes. In botanical classification, however, tobacco is the common name for *Nicotiana tabacum* L., the most economically important member of the genus *Nicotiana*. *Nicotiana tabacum* is a member of the Solanaceae family, and the most recent taxonomic revision of the genus recognizes 76 naturally occurring species subdivided into 13 sections (Knapp et al. 2004). The genus *Nicotiana* is believed to have initially evolved east of the Andes Mountains in southern South America, with the majority of current species (50) being native to North and South America and associated islands. Twenty-five species of section *Suaveolentes* are indigenous to either Australia or its archipelago. Only a single species, *Nicotiana africana*, has been found on the continent of Africa (Merxmüller and Buttler 1975).

Nicotiana tabacum is the only member of section *Nicotiana* and is believed to have evolved on the eastern slopes of the Andes Mountains near Bolivia or northern Argentina (Goodspeed 1954; Gerstel and Sisson 1995) less than 200,000 years ago (Kovarik et al. 2008). Although the species can occasionally be found growing without apparent human intervention in some parts of South America, such plants are usually considered to be naturalized escapes from cultivation, and the species is not thought to currently exist in a true wild state (Goodspeed 1954). *Nicotiana tabacum* is a classic amphidiploid species ($2n = 48$) with the "S" nuclear genome and cytoplasm being contributed by *N. sylvestris* ($2n = 24$) (Bland et al. 1985; Olmstead and Palmer 1991; Aoki and Ito 2000; Yukawa et al. 2006) and the "T" genome being contributed by a member of section *Tomentosae*. Although a role for *N. otophora*

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has been suggested in the evolution of *N. tabacum* (Goodspeed 1954; Kenton et al. 1993), recent genomic sequence information strongly supports *N. tomentosiformis* ($2n = 24$) as the "T" genome donor to modern-day *N. tabacum* (Sierro et al. 2014; Edwards et al. 2017). *Nicotiana tabacum* could have arisen by union of two unreduced parental gametes or by chance hybridization between the two $2n = 24$ progenitor species followed by spontaneous chromosome doubling. It is not known if multiple hybridization events may have occurred in the evolution of *N. tabacum* or if there may have been subsequent gene flow with diploid relatives post-speciation. Recent genome mapping and assembly information suggests the importance of inter-genomic exchanges between chromosomes of the S and T genomes post-polyploidization (Bindler et al. 2011; Edwards et al. 2017). The vast majority of tobacco strains exhibit strict bivalent pairing, although some variations in chromosome structural differences may exist among diverse *N. tabacum* accessions (Mallah 1943; Kenton et al. 1993; Lim et al. 2004), which could affect chromosome pairing in some crosses. An array of additional genomic changes such as sequence deletion/amplification, epigenetic adjustments, and structure and organization of ribosomal DNA also likely occurred post-polyploidization (Kovarik et al. 2008; Lim et al. 2004, 2007), and the S and T genomes have become interdependent as evidenced by observations that most nullisomics do not survive.

9.2 Economic Use

Tobacco is one of the most economically important nonfood crops grown around the world. The plant is cultivated on small to large hectarages on well-drained soils, in temperate and tropical regions, as a source of leaves that are subsequently cured in various ways for ultimate use in the manufacture of an array of tobacco products consumed by greater than 1 billion persons globally. *Nicotiana rustica* has also been used for similar purposes, although on a more localized and minor scale. China is the largest tobacco-producing country, followed by Brazil, India, and the United States. Manufactured products using cured tobacco leaves include combustible cigarettes, cigars, pipe tobacco, and smokeless tobacco products such as dry snuff, moist stuff, and snus. Due to toxicant exposure associated with the use of combustible cigarettes, there is also a growing interest in the marketing of heat-not-burn products that are similar in form to traditional cigarettes but where the cigarette tobacco is heated to a high temperature, but not burned to reduce exposure to toxicants that are primarily the by-products of high-temperature combustion.

Cured tobacco leaves are an internationally traded commodity that can fall into one of several market classes that are the result of combinations of cultivar type and leaf curing methodology. Different market types are used in different ways in the manufacture of various tobacco products. The major tobacco market types are flue-cured, burley, Maryland, dark air-cured, dark fire-cured, sun-cured, cigar filler, cigar wrapper, cigar binder, and Oriental. By volume, flue-cured and burley tobacco are the two most important types used in the production of cigarettes and are

consequently the most widely grown. Oriental and Maryland tobaccos are also used in more limited amounts in the manufacture of cigarettes. Cigar tobaccos are almost exclusively used in the production of cigars. Dark air-cured and dark fire-cured tobaccos are the primary raw material used in smokeless tobacco products.

Although tobacco can be asexually propagated by rooting of axillary buds or branches, commercial cultivation is carried out exclusively using plants propagated by seed. The species has the potential for perennial growth under environmentally permissive conditions but is cultivated as an annual. Seeds are typically germinated in outdoor plant beds or greenhouse float tray systems, and 5- to 10-week-old plants are subsequently transplanted by hand or via machine into rows in well-drained fields. Varied production and curing regimes are used to produce cured leaf that falls into one of the aforementioned market types. The general objective is to produce leaves, usually 14–50, on a central stalk that can be conveniently harvested by hand or by using mechanized systems. In most production regimes, plants are decapitated at the flowering stage in a process known as "topping," followed by control of axillary branch (also known as sucker) formation through chemical application supplemented with hand removal. This practice results in increased leaf yields and accentuated accumulation of alkaloids and other chemical constituents in cured leaves. Flower removal is not practiced during the production of Oriental and some styles of cigar tobacco. For some market types, leaves are harvested sequentially as they reach a desired state of maturity and ripeness. Such leaves may be tied on strings or sticks and subjected to air-curing or sun-curing for more than 3 weeks. Flue-cured tobacco is subjected to a "flue-curing" process of 5–7 days in an enclosed structure where precise control of humidity and temperature results in the production of a bright style of cured leaf with high levels of reducing sugars. For other market types, entire plants are stalk-cut at the ground level and speared onto sticks for air-curing under structures protected from rainfall for at least 3 weeks. In the case of dark fire-cured tobacco, harvested stalks are exposed to smoke from smoldering wood and sawdust to impart a smoke flavor to the cured leaf.

9.3 Breeding

9.3.1 Flower and Pollination Biology

Under field conditions, tobacco cultivars typically flower between 100 and 190 days after germination, depending on tobacco type and area of adaptation. The species is generally reported to be photoperiod insensitive, although some genotypes known as "mammoth" or "nonflowering" flower only under short-day conditions (Allard 1919; Garner and Allard 1920). This phenotype is believed to be under simple control (Smith 1979; Wernsman and Matzinger 1980a) and can be utilized by growers to produce tobacco with a high number of leaves in conducive environments (Mann and Chaplin 1957; Chaplin 1963). Tobacco is primarily a self-pollinated crop

species, although insects and hummingbirds can facilitate cross-pollination to varying degrees depending upon their activity. Using visible plant marker systems, outcrossing rates have been found to be between 1.3% and 11.3% (McMurtrey et al. 1960; Litton and Stokes 1964). Pollen transfer by wind is believed to be minimal or nonexistent.

For a number of reasons, tobacco is one of the easiest self-pollinated crop species to manipulate from a reproductive standpoint. First, as a perennial species, tobacco has the potential to continue flowering for several years in permissive environments (such as greenhouse settings) once flowering is initiated. The primary inflorescence is a terminal panicle with the potential to produce dozens of flowers (Fig. 9.1a), and each leaf axil has the potential to produce flowering branches. Hundreds of flowers can therefore be produced per plant. Once flowering is initiated, these

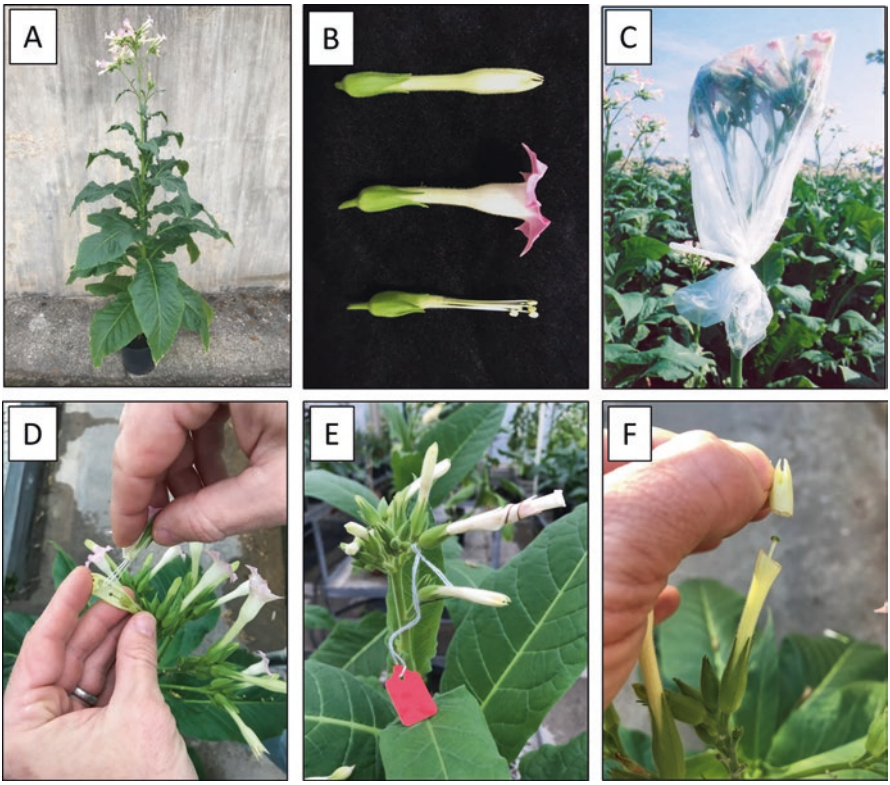

Fig. 9.1 Tobacco inflorescence and pollination techniques. (**a**) Plant with developed primary inflorescence. (**b**) From top to bottom: flower stage suitable for pollination as a female, opened flower, and flower with corolla removed to reveal five anthers and a central pistil and stigma. (**c**) Pollination bag for protection against undesired outcrossing. (**d**) Technique for manual self- or cross-fertilization. (**e**) Pollinated flower with straw and wire used to protect the stigma from undesired fertilization. (**f**) Simple removal of flower tip to expose stigma on cytoplasmic male-sterile plant

characteristics allow for pollinations to be made any time of day and any time of the year, temperature permitting. Second, the large size (~ 6 to 10 cm) of the tobacco flower enables simple manipulation for manual self- or cross-pollinations. Flowers are perfect with five anthers that are easily removed and a single central stigma that is easily pollinated by hand (Fig. 9.1b). Finally, each tobacco flower has the potential to develop into a seed capsule containing up to ~ 3000 seeds. This level of fecundity combined with the large number of flowers per plant permits for large amounts of seed to be produced per plant.

To produce large amounts of seed through enforced self-pollination, perforated bags can be placed over the tobacco inflorescence (Fig. 9.1c) to exclude transfer of foreign pollen by insect or hummingbird pollinators. To exclude the possible harvesting of seeds resulting from undesired cross-fertilization, bags are placed over the inflorescence after removal of all opened flowers, previously pollinated flowers, and developing seed capsules. Pollination bags can be secured around the base of the inflorescence using string or plastic zip ties. Self-pollinations can also be carried out by manually transferring pollen from opened flowers onto the stigmas of flowers from the same plant (Fig. 9.1d). A paper straw, folded on one end, can be placed over the stigma and style to exclude pollen from alternative sources. The straw can be secured in place until fertilization occurs by using thin wire to wrap the corolla around the paper straw (Fig. 9.1e).

Manual cross-pollinations can also be carried out by collecting flowers or anthers from a desired male parent and pollinating unopened flowers of a chosen female parental individual. Pollen can be sourced from opened flowers in a greenhouse environment where insect-mediated pollen transfer can be assumed to be minimal or nonexistent. In a field environment, pollinations are best made using male flowers that were collected 1 or 2 days prior to opening and stored in small cups or beakers with small amounts of water in a protected environment for up to 4 days. Pollen can also be isolated and stored for longer periods of time in containers with desiccant or stored in bottles under freezing conditions (Wernsman and Matzinger 1980b). Pollen should be stored with care, as improper storage can result in offtype progeny (Jack and Krahner 1994; Berbec and Jack 1996). Hand cross-pollinations are best carried out 1 or 2 days prior to anther dehiscence of the female flower, which usually corresponds with flower opening (Fig. 9.1b). The stigma typically becomes receptive to pollen 24–48 hours prior to anthesis, although this can be genotype dependent.

A flower of the female parent is prepared by making an incision with a forceps (sterilized with 70% ethanol) along the length of the unopened corolla to expose the reproductive parts. The anthers of the female flower are removed with a forceps. Upon anther dehiscence of a flower collected from a male parental individual, the anthers can be made to be more accessible for cross-pollination by making a similar incision along the length of the corolla. The corolla can then be folded back, and pollen can easily be applied to the stigma of the female plant (Fig. 9.1d), followed by placement of a folded paper straw over the stigma (Fig. 9.1e). Labeled pollination tags looped around the flower pedicel can be used to indicate details of the particular pollination. In some field environments, control of chewing insects via

insecticide application can be of extreme importance as complete destruction of developing seed capsules can otherwise occur.

Seed capsules turn from green to brown approximately 25 days post-pollination and can be harvested and shelled for collection of mature seed. Because tobacco seeds are small (<1 mm in diameter), careful cleaning and packaging is important to maintain seed purity. Separation of trash and nonviable seeds can be carried out using sieves, gravity tables, or seed blowers. In countries with large-scale tobacco hectarages, tobacco seeds are pelleted to increase the diameter of the seeded unit to assist in mechanized seeding of float trays.

Cytoplasmic male sterility (*Cms*) can be used to facilitate the production of commercial hybrid seed since male-sterile female parental plants do not require emasculation prior to hand-pollination with pollen collected from a male parental line. *Cms* is sometimes observed following transfer of the *N. tabacum* nuclear genome to the cytoplasm of another *Nicotiana* species, particularly those from section *Suaveolentes* (Gerstel 1980; Nikova et al. 1997; Berbec 2001; Berbec and Laskowska 2005). Disharmony between the nuclear and cytoplasmic genomes causes an absence or distortion of male reproductive parts, resulting in no pollen production (Fig. 9.2). Fertility restoration systems are not required because only vegetative parts (leaves) are harvested during tobacco production. Some alien cytoplasms can have negative effects on agronomic performance (Mann et al. 1962; Aycock et al. 1963; Chaplin and Ford 1965; Hosfield and Wernsman 1974). Currently, the most

Fig. 9.2 Fertile and cytoplasmic male-sterile (*Cms*) flowers of tobacco. The lack of well-developed male reproductive parts is illustrated for plants with cytoplasmic elements derived from *N. suaveolens* and *N. glauca*

commonly used sources of *Cms* used in F_1 hybrid seed production are those from *N. suaveolens* and *N. glauca*. *Cms* is introduced to a female parent of a new F_1 hybrid by using five to seven backcrosses to transfer the nuclear genome of the female parental line into an alien cytoplasm. Seed of the *Cms* parental line is maintained through simple pollination with the fertile version. Because insect-mediated pollen transfer is low, hand-pollination is still required for F_1 hybrid seed production using *Cms* systems. This is easily done, however, as emasculation of female plants is not required and exposure of the stigma is efficiently accomplished by rapid pinching off the tip of female flowers (Fig. 9.1f).

9.3.2 Breeding Methods Applied

Prior to the 1900s, tobacco varieties used by farmers were the result of mass selection and were genetically heterogeneous to varying degrees. Early varietal strains within each market class are best described by Garner et al. (1936). After establishment of the laws of inheritance and development of science-based plant breeding methodologies in the first part of the twentieth century, target tobacco cultivars became those with a high degree of genotypic and phenotypic uniformity. This can be achieved through the development of stable, highly inbred lines that do not segregate for morphological characteristics or for alleles controlling other traits of interest.

Alternatively, two different stable inbred lines can be hybridized to produce a genetically uniform but somewhat heterozygous F_1 hybrid. F_1 hybrids are desired for several reasons and the majority of cultivars commercialized since the 1990s are of this form. First, although the degree of heterosis is not exceedingly high in tobacco (Matzinger et al. 1962, 1971; Matzinger and Mann 1962; Chaplin 1966; Marani and Sachs 1966; Legg et al. 1970; Dean 1974; Wilkinson and Rufty 1990), some F_1 hybrids significantly outperform the best-performing parental line. Second, *Cms* F_1 hybrids do not produce any seed which therefore provides a mechanism of intellectual property protection. Male sterility forces growers to purchase seed from reputable seed sources, which contributes to crop uniformity and an increased level of predictability of the style of cured leaf procured by manufacturers. Finally, F_1 hybrids can be used to more rapidly and effectively deploy certain trait combinations, particularly disease resistance traits conferred by genes contained within introgressed foreign chromosome segments. Linkage drag effects often associated with such alien chromosome segments can cause significant reductions in yields and/or quality when present in homozygous condition in inbred lines (Lewis and Rose 2010). In contrast, when such chromosome segments are deployed in heterozygous condition in F_1 hybrids, undesirable negative corresponding effects are often ameliorated.

Approaches for tobacco cultivar improvement therefore focus on the generation of novel inbred lines with improved trait combinations, followed by strategic

development of new F_1 hybrids. Conventional methodology typically involves multiple steps:

1. identification of two or more parental materials that, in combination, possess the traits desired in a new inbred line
2. hybridization of such materials followed by creation of segregating populations
3. exercising phenotypic or genotypic selection for traits of interest during multiple generations of inbreeding
4. extensive multi-environment, replicated testing of stable experimental inbred lines and hybrid combinations for yield, cured leaf quality, and disease resistance, and
5. determining which, if any, experimental materials should be released commercially

Initial segregating populations may be derived from biparental or three-way crosses or possibly be inter-mated populations from recurrent selection schemes. Multiple methods of conventional inbreeding can subsequently be used by tobacco breeders and may include single seed descent, bulk breeding, pedigree selection, or some combination thereof. Selection for some plant characteristics under simple genetic control (such as certain disease resistance and leaf chemistry traits) and several other traits with high heritability (such as disease resistance, flowering time, and plant architecture) can be carried out on single plants during initial generations of inbreeding with little error. Some of the most economically important traits such as yield and quality are quantitatively inherited, however, and subject to high environmental influence. Most investigations of quantitative characters in segregating populations of tobacco indicate a predominance of additive genetic variance, with dominance and epistatic types of variance being low, but not always zero (Matzinger and Wernsman 1979). Selection for complex traits with lower heritability is carried out in later generations of inbreeding using replicated testing over multiple environments. Backcross breeding is also frequently used in tobacco cultivar development when the goal is to transfer a simply inherited characteristic controlled by one or a few genes from source material into recipient germplasm, usually an existing elite inbred line. The number of generations of conventional inbreeding carried out per year is dependent upon the traits under selection and availability of off-season nursery or greenhouse space. Once desirable inbred lines are identified, they can be evaluated in F_1 hybrid combination with other elite lines to identify superior hybrid combinations.

The time required from an initial breeding cross to the commercialization of a new tobacco cultivar using conventional approaches is typically between 8 and 12 years. Up to one-half of this time might be spent on the development of inbred lines. The majority of the time, however, is allocated to multi-environment and multiyear testing to determine which of hundreds of candidate cultivars might possess trait combinations and agronomic performance to justify commercial release. Little can be done to obviate the time required for extensive testing prior to a commercialization decision. Several approaches can be used to reduce the time required for inbred line development, however. For example, the application of doubled haploid

breeding can reduce the time required to develop completely inbred lines to less than 2 years. Anther culture can be used to quickly produce large numbers of androgenic haploid plants from heterozygous individuals derived from breeding crosses (Nitsch and Nitsch 1969; Tanaka and Nakata 1969; Burk et al. 1972). Alternatively, gynogenic haploid plants can be isolated by pollinating heterozygous *N. tabacum* individuals with pollen from *N. africana*. Abundant seeds are produced from this interspecific cross, but the vast majority of true interspecific F_1 hybrids die at the cotyledonary stage. A fraction of surviving individuals are gynogenic haploids (~ 2 haploids per 1000 seed) that can be distinguished from surviving aneuploid F_1 hybrids with experience or by using transgenic seedling marker technologies (Burk et al. 1979; Hancock et al. 2015). Haploids have also been isolated at low frequencies of 2.7 per 10,000 seed in progeny from *N. tabacum* × *N. tabacum* crosses using a transgenic seedling color marker system (Lewis and Rose 2011). Isolation of gynogenic haploids from seed is preferred by many breeding programs, as the anther culture method has been shown to introduce deleterious genetic variation (reviewed by Wernsman 1992). Haploid individuals can be chromosome doubled to produce doubled haploid lines using either colchicine treatment of young seedlings (Burk et al. 1972) or tissue culture of leaf midveins (Kasperbauer and Collins 1972). Marker-assisted or phenotypic selection for traits such as resistance to tobacco mosaic virus (TMV) or root knot nematodes (Rufty et al. 1987) can be carried out on haploid plants prior to chromosome doubling in order to improve breeding efficiency. The primary advantages to doubled haploid breeding are

1. reduced time required to create new inbred lines
2. increased frequency of desirable genotypes within haploid populations (Nei 1963)
3. theoretical increased genetic gain over multiple generations of selection (Choo and Kannenberg 1978, 1988), and
4. certainty regarding homozygosity of alleles in newly established inbred lines

Some disadvantages are the requirement for specialized laboratory equipment and technical expertise and a reduced ability to select for complex traits during the one-step inbreeding process.

Since inbred line development is largely dependent upon generation time, methods that reduce days to flowering can also be used to decrease the time required to develop new inbred lines. Transgenic overexpression of genes involved in the stimulation of flowering, such as the *Arabidopsis thaliana* gene *Flowering Locus T* (*FT*), can be used to reduce days to flowering in tobacco to approximately 39 days. This mechanism can be used to dramatically reduce the time required to complete a backcross trait conversion (Lewis and Kernodle 2009) or develop new inbred lines coupled with selection for disease resistance (Eickholt and Lewis 2013). In these modified breeding schemes, a constitutively expressed *FT* transgene is held in heterozygous condition during the inbreeding process but is segregated away near the end of the scheme to result in non-transgenic breeding outcomes that can be developed, in many cases, in less than half the time than that ordinarily required.

Application of DNA marker-assisted selection (MAS) can also increase the efficiency of inbred line development. In the case of MAS, breeding programs can

screen for the presence of desired DNA markers which, due to close linkage with underlying genes, can predict certain traits of interest. This is of particular value for traits subject to high environmental influence or that are not easily measured due to high costs or special phenotyping requirements. MAS can be carried out on laboratory- or greenhouse-grown plants anytime of the year. The technique has been used most extensively in tobacco breeding programs for disease resistance traits, although markers for genes affecting leaf chemistry and morphological traits are also becoming increasingly available. A table of traits and genes amenable for MAS in a tobacco breeding program is provided in Table 9.1.

9.3.3 Sources of Genetic Variation

9.3.3.1 Naturally Occurring Variation

As long as a desired trait can be found within *N. tabacum*, the within-species gene pool is usually the preferred source of genetic variability for a tobacco breeding program due to accessibility and ease of crossing. Parental germplasm must be

Table 9.1 Simply controlled traits amenable to DNA marker-based selection in tobacco

Trait	References
N-mediated tobacco mosaic virus (TMV) resistance	Lewis et al. (2005)
va-mediated potyvirus resistance	Dluge et al. (2018), Julio et al. (2015)
N. africana-derived potato virus Y (PVY) resistance	Lewis (2005)
N. longiflora-derived *Pseudomonas syringae* pv. *tabaci* (Race 0) and *Pseudomonas syringae* pv. *angulata* resistance	Yi et al. (1998a)
N. plumbaginifolia and *N. longiflora*-derived *Phytophthora nicotianae* (Race 0) resistance (*Php* and *Phl* genes)	Johnson et al. (2002)
N. rustica-derived *P. nicotianae* resistance (*Wz* gene)	Drake and Lewis (2013)
Phn 7.1 and *Phn15.1* partial *P. nicotianae* resistance QTL	Ma (2017)
N. plumbaginifolia-derived *Globodera tabacum* resistance	Johnson et al. (2002), Johnson et al. (2009)
Peronospora tabacina resistance	Milla et al. (2005)
N. debneyi-derived *Thielaviopsis basicola* resistance	Bai et al. (1995)
Meloidogyne incognita (races 1 and 3) resistance (*Rk* gene)	Yi et al. (1998b)
Tomato spotted wilt virus (TSWV) resistance	Moon and Nicholson (2007)
Increased leaf number and delayed flowering time	Eickholt and Lewis (2014)
Reduced nicotine demethylation	Li et al. (2012)
nic1 + *nic2*-based reduced nicotine accumulation	Adams et al. (2016)
Z-abienol accumulation	Sallaud et al. (2012)
Sucrose ester accumulation	Vontimitta et al. (2010)
Burley trait (chlorophyll deficiency)	Edwards et al. (2017)

chosen carefully, however, if the intent is to develop a new cultivar that continues to satisfy the unique quality requirements of a given market class. Stringent requirements of modern market types seem to limit the degree of tolerance for use of diverse germplasm from alternative market types in breeding programs. Consequently, genetic variability among current cultivars within major market classes such as flue-cured and burley tobacco is substantially less than that for the species as a whole (Moon et al. 2009b). Lower amounts of genetic interrelatedness are present among cigar tobacco cultivars as compared to that for flue-cured and burley tobacco cultivars (Fricano et al. 2012). Diverse germplasm has generally only been used in low amounts in flue-cured and burley tobacco breeding, usually as sources of disease resistance, and application of conservative breeding approaches to improvement of flue-cured tobacco has led to an erosion of genetic variability over decades of breeding (Moon et al. 2009a).

Nevertheless, genetic variability is essential for continued improvement of any crop species, and several *Nicotiana* germplasm collections around the world serve as repositories for genetic variability within *N. tabacum* (Lewis and Nicholson 2007; McCoy et al. 2018). Abundant variation exists within these collections for plant growth habit and architecture, leaf chemistry traits, and levels of disease resistance. Although diverse germplasm has great potential value for improvement of the major tobacco market classes, long-term commitment is needed for ultimate success. Use of modern genetic approaches to identify favorable allelic variability in diverse source material and application of marker-assisted selection to preferentially transfer corresponding genomic regions to elite genetic backgrounds may aid in the exploitation of such material (Ma 2017).

9.3.3.2 Genetic Variation from *Nicotiana* Relatives

Alternative species can also be used as sources of valuable genetic variability. Although wild *Nicotiana* relatives have been investigated for their potential to positively affect complex traits such as yield in cultivated tobacco, corresponding deleterious effects on cured leaf quality have made their use difficult for this purpose (Mann and Weybrew 1958; Matzinger and Wernsman 1967; Wernsman et al. 1976). Consequently, most commercially successful efforts have focused on the use of wild relatives as sources of simply inherited traits, particularly disease resistance (Table 9.2).

It is generally not straightforward to directly transfer genetic material from most *Nicotiana* wild relatives because of barriers to hybridization, hybrid sterility, and lack of donor-recipient chromosome pairing in interspecific hybrids. These impediments become increasingly problematic as the taxonomic distance between *N. tabacum* and the trait donor species increases. The conventional transfer of genes of interest from wild *Nicotiana* relatives to tobacco is comprised of multiple steps: (1) identification of a species possessing the desired trait, (2) hybridization with the cultivated species, (3) generation of fertile offspring, and (4) backcrossing to

Table 9.2 Traits introgressed into cultivated tobacco from *Nicotiana* relatives

Trait	Donor species	Reference(s)
Tobacco mosaic virus (TMV) resistance	*N. glutinosa*	Holmes (1938), Ternovsky (1945), Gerstel (1945), Kostoff (1948), Valleau (1952)
Potato virus Y (PVY) resistance	*N. tomentosiformis*	Legg and Smeeton (1999)
Potato virus Y tolerance	*N. africana*	Lewis (2005)
Tomato spotted wilt virus (TSWV) resistance	*N. alata*	Gajos (1987)
Phytophthora nicotianae (Race 0) resistance	*N. longiflora*	Valleau et al. (1960)
Phytophthora nicotianae (Race 0) resistance	*N. plumbaginifolia*	Apple (1962), Chaplin (1962)
Phytophthora nicotianae resistance	*N. rustica*	Woodend and Mudzengerere (1992)
Thielaviopsis basicola resistance	*N. debneyi*	Clayton (1969)
Peronospora tabacina resistance	*N. velutina*	Clayton (1967), Clayton et al. (1967), Lea (1963)
Peronospora tabacina resistance	*N. debneyi*	Wark (1963, 1970)
Peronospora tabacina resistance	*N. goodspeedii*	Wark (1963, 1970)
Erysiphe cichoracearum resistance	*N. debneyi*	Smeeton and Ternouth (1992)
Erysiphe cichoracearum resistance	*N. glutinosa*	Smeeton and Ternouth (1992)
Erysiphe cichoracearum resistance	*N. tomentosiformis*	Smeeton and Ternouth (1992)
Pseudomonas syringae pv. *tabaci* (Race 0)	*N. longiflora*	Clayton (1947)
Pseudomonas syringae pv. *tabaci* (Race 0 and 1) and *Pseudomonas syringae* pv. *angulata* resistance (Race 1)	*N. rustica*	Stavely and Skoog (1976), Woodend and Mudzengerere (1992)
Meloidogyne incognita (Race 1 and 3) resistance	*N. tomentosa*	Clayton et al. (1958), Yi et al. (1998b)
Meloidogyne javanica resistance	*N. longiflora*	Schweppenhauser (1968, 1975), Ternouth et al. (1986)
Meloidogyne javanica resistance	*N. repanda*	Ternouth et al. (1986)
Globodera tabacum resistance	*N. plumbaginifolia*	Apple (1962), Chaplin (1962), Johnson et al. (2009)
Increased leaf number and delayed flowering time	*N. tomentosa*	Clausen and Cameron (1944), Eickholt and Lewis (2014)

cultivated tobacco with hope of transferring the gene(s) controlling the desired phenotype.

Very few direct F_1 hybrids between *N. tabacum* and other *Nicotiana* species exhibit sufficient male or female fertility to permit a subsequent backcross to *N. tabacum*. Chromosome doubling techniques are thus used to generate partially fertile interspecific hybrid intermediates that can hopefully be backcrossed to *N. tabacum*. For transfer of genetic material from diploid species closely related to

N. tabacum (such as *N. sylvestris*, *N. tomentosiformis*, or *N. otophora*), production of synthetic amphidiploids might be first produced, followed by direct crossing with *N. tabacum* (Hancock and Lewis 2017). The breeder may also double the chromosome number of initial interspecific F_1 hybrids using colchicine treatment to produce fertile allopolyploids. As an alternative, fertile pentaploids can be produced directly by chromosome doubling the *N. tabacum* parental line to generate an 8*x* allooctoploid prior to hybridization with the *Nicotiana* donor species of interest. If sufficient fertility is exhibited by such breeding intermediates, the materials can be used as starting points for subsequent backcrossing to *N. tabacum* with selection for the trait or gene(s) of interest. During the backcrossing process, the breeder hopes for a chance recombination or translocation event between the donor chromosome and a recipient chromosome of the *N. tabacum* genome. In some cases, the use of a bridge species may be necessary, whereby a gene of interest is preliminarily transferred to the genetic background of an intermediate species prior to efforts for ultimate transfer to *N. tabacum* (Burk 1967).

Major limiting factors to ultimate success in breeding with wild relatives are sequence and structural differences between gene donor and recipient chromosomes that can operate to limit chromosome pairing. The ultimate commercial objective is usually to obtain the smallest genomic introgression in a position with minimal disturbance to the genetic constitution of an elite *N. tabacum* genetic background. Many alien gene introgressions can be difficult to deploy in commercially successful varieties because of deleterious linkage drag effects on yield and/or cured leaf quality characteristics. Examples include black root rot from *N. debneyi* (Legg et al. 1981), black shank resistance from *N. longiflora* and *N. plumbaginifolia* (Valleau et al. 1960; Johnson 1999), and TMV resistance from *N. glutinosa* (Chaplin et al. 1966; Chaplin and Mann 1978; Lewis and Rose 2010). Such unfavorable linkages can be exceedingly difficult to break using conventional backcrossing because of a paucity of recombination within introgressed chromosome segments (Johnson et al. 2002; Drake and Lewis 2013). As mentioned previously, negative linkage drag effects can be ameliorated in F_1 hybrids where dominant disease resistance genes are deployed in heterozygous condition (Lewis and Rose 2010).

9.3.3.3 Gene Transfer via Genetic Engineering

The desired genetic variation to achieve a particular breeding objective may often not naturally exist within the genus *Nicotiana*. In some cases, desired genetic variation might be introduced into a tobacco breeding program through the use of recombinant DNA technology which circumvents sexual incompatibilities and essentially permits the entire living world to serve as a potential source of genetic variation. Because *N. tabacum* has been used as a model species in the development of tissue culture and plant transformation protocols, introduction of foreign genes via genetic engineering is usually technically straightforward. Methods of tobacco plant transformation include classical *Agrobacterium tumefaciens*-based gene transfer (Horsch et al. 1985), biolistic approaches (Sanford 1990), and protoplast-based systems

(Negrutiu et al. 1987). Application of recombinant DNA technology allows for the overexpression or silencing of target genes or ectopic expression of genes introduced from a foreign source. Plant transformation research in tobacco is extensive, but several relevant examples have included the use of posttranscriptional gene silencing methods to mediate plant virus resistance (Tanzer et al. 1997; Levin et al. 2005), RNA interference to alter tobacco metabolic pathways affecting leaf chemistry (Wang and Wagner 2003; Lewis et al. 2008, 2015; Wang et al. 2008; Steede et al. 2017), and transfer of disease resistance mechanisms from other species to *N. tabacum* (Spassova et al. 2001; Lewis et al. 2007).

Although tremendous potential exists for novel tobacco trait introduction in tobacco using the aforementioned approaches, only a single transgenic event has ever been deregulated for cultivation in the United States. In this case, transgenic burley tobacco cultivar "Vector 21–41" was engineered to carry an antisense construct designed to reduce expression of the gene *NtQPT1* for the purpose of reducing nicotine content in cured leaves (Xie et al. 2004). This variety is grown on an exceedingly low hectarage, however, and the mainstream tobacco industry is currently reluctant to adopt transgenic tobacco cultivars because of issues related to consumer perception and barriers associated with international regulation of genetically engineered plants and derived products.

9.3.3.4 Introduction of De Novo Variability via Induced Mutation

Novel genetic variability can also be introduced into tobacco breeding programs via conventional mutagenesis. Treatment of seeds with chemical mutagens such as ethyl methanesulfonate (EMS) tend to produce single base-pair mutations, while various types of irradiation such as X-rays or gamma rays tend to create genomic deletions or structural rearrangements. Examples of successful development of new traits using mutation breeding include potyvirus resistance generated via X-ray treatment of seeds (Koelle 1961) and modified alkaloid profiles (Lewis et al. 2010, 2015) and reduced heavy metal uptake (Liedschulte et al. 2017) produced by exposure of seeds to EMS.

Although valuable genetic variation can be induced using classical mutation-based approaches, such genomic changes are largely random and substantial effort can be required to identify and separate desired mutations from undesirable genetic changes. Gene editing approaches such as CRISPR/Cas9, TALENS, zinc finger nucleases, and meganuclease technology (Gaj et al. 2013; Baltes and Voytas 2015) have been developed within the last 10 years that permit for targeted gene mutations with minimal disturbance to the rest of the genome. Such approaches typically involve temporary expression of a foreign transcript that, based upon its design, mediates the creation of mutations at a strategically chosen target site within the tobacco genome. Although the breeding outcomes of gene editing are generally more easily achieved as compared to those produced using nontargeted mutation, the regulatory status of resulting cultivars is currently unclear in some global jurisdictions.

9.3.4 Breeding Targets

Target cultivars of tobacco breeding programs are those containing novel trait combinations that collectively influence the economy of tobacco growing and quality characteristics that affect tobacco product manufacture and the consumer experience. It is critical that new cultivars be developed without degrading quality characteristics to unacceptable levels, as such cultivars will not be accepted by the industry. Specific objectives are outlined below.

9.3.4.1 Agronomic and Disease Resistance Characters

Most tobacco-growing operations require high-yielding cultivars to maintain profitability. Cultivars must also possess some level of disease resistance for most growing areas, as some tobacco pathogens can cause near-complete crop loss on susceptible genotypes. The most important disease resistance traits vary according to region and include those caused by oomycete, fungal, bacterial, nematode, and viral pathogens (Table 9.3). Other important characteristics for growers include appropriate maturity, lodging resistance, low suckering potential, uniform ripening of individual leaves, retention of leaf integrity in the field and after harvest, and curability. Although some disease resistance traits such as TMV and PVY resistance are under simple genetic control, most agronomically important traits are complex and controlled by numerous genes.

Published analyses of historical yields gains for tobacco of any type are few, but Bowman et al. (1984) estimated an annual yield increase of 49.5 kg/ha per year for US flue-cured tobacco, of which 32% was attributed to improved genetics. Examination of North Carolina Official Variety Test (OVT) data from 1954 to 2017 suggests a plateauing of US flue-cured tobacco yields since the study of Bowman et al. (Fig. 9.3). Reasons for these observations may include (1) declining genetic variability in US flue-cured germplasm pools (Moon et al. 2009a), (2) reduced number of research programs engaged in tobacco breeding, or (3) constraining effects of a growing season of limited length. Despite a deceleration in the rate of yield increase for US flue-cured tobacco, modern cultivars are superior to those from decades past because of their high-yielding ability combined with increased levels of resistance to multiple pathogens.

9.3.4.2 Quality Traits

Leaf buyers and tobacco product manufacturers are highly concerned with cured leaf quality characteristics because of their importance in the cost-effective manufacture of products with desired sensory attributes. Quality characteristics are also important to growers, as quality affects the price paid per kilogram upon sale. Cured leaf quality can sometimes be hard to define but can be described in terms of

Table 9.3 Important diseases affecting tobacco production

Disease	Causal organism
Viral diseases	
Tobacco mosaic virus infection	Tobacco mosaic virus (TMV)
Tomato spotted wilt virus infection	Tomato spotted wilt virus (TSWV)
Potato virus Y infection	Potato virus Y (PVY)
Tobacco etch virus infection	Tobacco etch virus (TEV)
Tobacco vein mottle virus infection	Tobacco vein mottle virus (TVMV)
Cucumber mosaic virus infection	Cucumber mosaic virus (CMV)
Oomycete diseases	
Black shank	*Phytophthora nicotianae*
Blue mold	*Peronospora tabacina* DB Adam
Damping off/root and stem rot	*Pythium* spp.
Fungal diseases	
Black root rot	*Thielaviopsis basicola*
Powdery mildew	*Erysiphe cichoracearum* DC
Fusarium wilt	*Fusarium oxysporum*
Brown spot	*Alternaria alternata* (Fr. ex Fr.) Kiessl.
Target spot	*Thanatephorus cucumeris* (AB Frank) Donk
Nematode diseases	
Root knot nematode infection	*Meloidogyne incognita*
Root knot nematode infection	*Meloidogyne javanica*
Root knot nematode infection	*Meloidogyne arenaria*
Tobacco cyst nematode	*Globodera tabacum*
Bacterial diseases	
Bacterial wilt or Granville wilt	*Ralstonia solanacearum*
Wildfire/angular leaf spot	*Pseudomonas syringae* pv. *tabaci*

physical, visual, chemical, and sensory properties (Smeeton 1987). Physical quality traits of importance to buyers may include filling value, stem to lamina ratio, leaf shape, leaf elasticity, and leaf vein patterns. Visual quality can be described by the degree of maturity and ripeness, body, texture, color, and uniformity. This aspect of tobacco quality can be reported by using standard grading systems (Bowman et al. 1988; United States Department of Agriculture 1989). Chemical quality is measured by analyses of certain chemical components of the cured leaf (see the following section). Although a large number of compounds are important to manufacturers, the most commonly measured are percent nicotine, percent total alkaloids, percent secondary alkaloids, percent reducing sugars, and percent total nitrogen. An appropriate balance of chemical constituents contributes to favorable smoke organoleptic characteristics. Smoke sensory characteristics are not easily quantified and often difficult to describe. Industry-associated smoke panels are used to judge acceptability of smoke characteristics for a given manufacturing purpose. While genes controlling some chemical traits have been characterized (see Sect. 9.3.4.3), the genetic

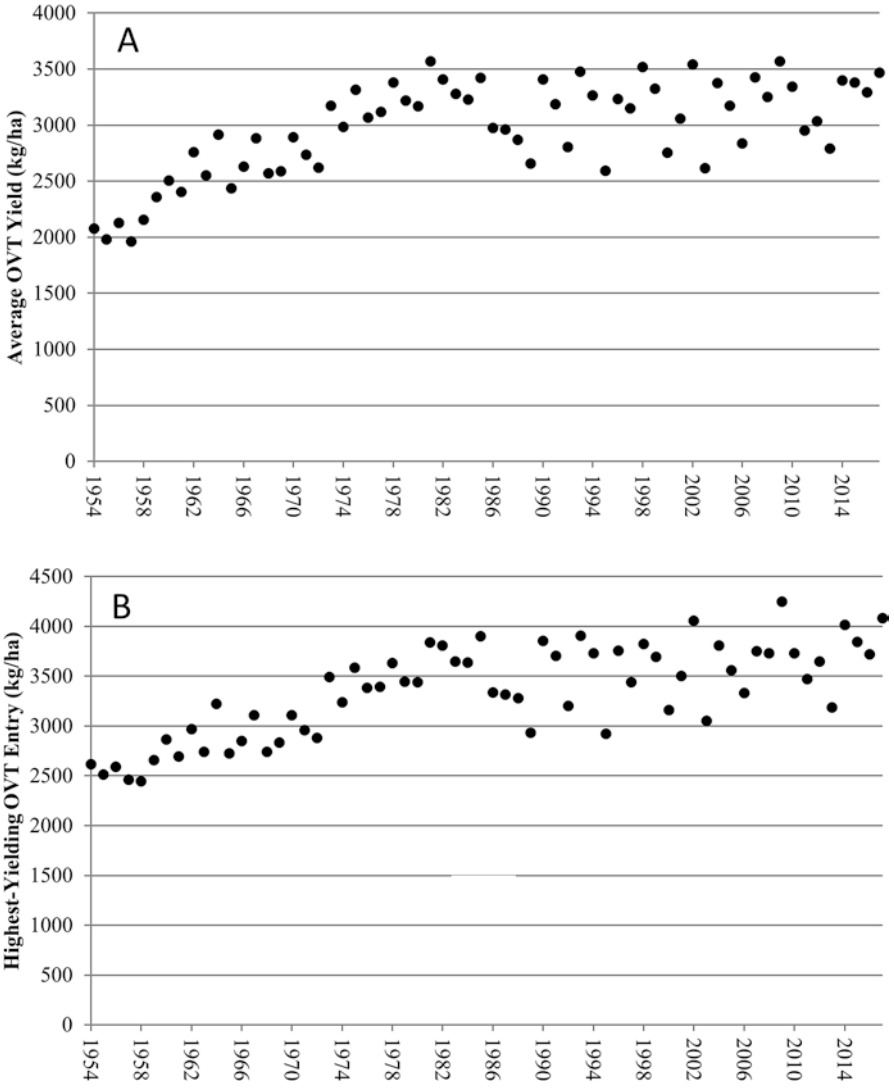

Fig. 9.3 Historical cured leaf yield data for the North Carolina (USA) Official Variety Test (OVT) for flue-cured tobacco from 1954 to 2017. (**a**) Average yields for all OVT entries. (**b**) Yield of highest-yielding OVT entry

control of most quality attributes is not easily studied because of complex inheritance and high environmental influence (See Sect. 9.3.4.4 and Sect. 9.3.4.5).

For some market types in some countries, collaborative regional testing programs such as the United States Minimum Standards Program for Flue Cured Tobacco exist to ensure that experimental tobacco cultivars meet specific physical and chemical requirements prior to commercialization (Bowman 1996). In these

programs, candidate cultivars must display chemistry profiles within boundaries defined by specific check cultivars and have a minimum level of usability as judged by industry cooperators. Such programs may also include a smoke panel component to ensure that the experimental variety in question produces cured leaf that exhibits desired sensory attributes upon smoking. Such programs not only ensure a certain degree of uniformity and expectation for buyers of cured leaf but also restrict the use of diverse germplasm in breeding programs that could lead to the development of cultivars with unique combinations of quality traits that could be used in the innovation of novel tobacco products.

9.3.4.3 Chemical Traits

Nicotiana species are well known for their production of diverse plant secondary metabolites, and substantial variation exists among and within species for the types and amounts of compounds produced. Plant secondary products are of tremendous importance in tobacco as they (1) affect plant interactions with insects and microorganisms during cultivation, (2) impart flavor and aroma characteristics to tobacco products, (3) can be associated with pharmacological effects experienced by users of tobacco products, and (4) can be direct or indirect sources of compounds recognized to have undesirable effects on human health when introduced to the body.

9.3.4.4 Alkaloid Composition

Due to their addictive and pleasurable properties, alkaloids are perhaps the most well-known plant natural products synthesized by tobacco. Alkaloids are also important because their correlation with the accumulation of toxic nitrosated derivatives, the tobacco-specific nitrosamines (TSNAs) (Bush et al. 2001). The major alkaloids produced by *N. tabacum* include nicotine, nornicotine, anatabine, and anabasine. In most tobacco cultivars, nicotine is by far the predominant alkaloid and comprises greater than 90% of the total alkaloid pool, although nornicotine can predominate in certain breeding materials. Knowledge of the genetic control of alkaloid accumulation is important in connection with efforts to develop cultivars with desired nicotine levels (including lower levels potentially mandated by regulatory agencies) and reduced potential for TSNA accumulation.

Wide variation exists for alkaloid accumulation among diverse tobacco materials in the US *Nicotiana* Germplasm Collection, ranging from 0.2 to 65.5 mg/g dry weight (Sisson and Saunders 1982). A large number of unknown genes likely have small effects on alkaloid accumulation, as evidenced by the successful application of recurrent selection to continually increase nicotine levels in narrow breeding populations (Matzinger et al. 1972, 1989). However, several naturally occurring genetic factors are known to be associated with large effects on alkaloid accumulation or composition. The most well known are the *Nic1* and *Nic2* (also called *A* and *B*) loci, which globally regulate expression of a number of genes involved in

alkaloid biosynthesis (Hibi et al. 1994; Riechers and Timko 1999; Reed and Jelesko 2004) and affect accumulation of all four major tobacco alkaloids. Recessive alleles at both of these loci can reduce total alkaloid levels from between 1.5% and 4.5% to approximately 0.2% on a dry weight basis (Legg et al. 1969; Legg and Collins 1971; Chaplin and Weeks 1976). The recessive alleles are associated with reduced yields and quality, however, which complicates their use in breeding programs where low alkaloid accumulation is an objective (Chaplin and Weeks 1976).

An additional naturally occurring genetic mechanism that can be used to affect alkaloid composition in *N. tabacum* is one which controls the biochemical conversion of nicotine to nornicotine via enzymatic demethylation. Three genes designated as *CYP82E4*, *CYP82E5*, and *CYP82E10* are known to encode for active nicotine demethylase enzymes in tobacco leaves (Lewis et al. 2010). The *CYP82E4* locus is unstable and usually exists in a low state of transcriptional activity (Siminszky et al. 2005; Gavilano et al. 2007). The locus can become spontaneously reactivated through unknown means, however, to result in the majority of nicotine being converted to nornicotine and causing the latter to become the predominant alkaloid. This occurs most frequently in burley tobacco and is considered undesirable as nornicotine is easily converted to its corresponding TSNA, *N*′-Nitrosonornicotine, under conducive curing and storage conditions. A screening method called the "LC Protocol" (Jack et al. 2007) can be applied to tobacco seed production to reduce nornicotine accumulation in commercial tobacco seed lots. In this method, Foundation seed is not collected from plants in which greater than 3% of nicotine is converted to nornicotine.

Considerable knowledge has been gained in the last 15 years regarding the biochemical pathways involved in tobacco alkaloid biosynthesis, and the majority of genes directly involved in the pathway have been characterized (reviewed by Dewey and Xie 2013). Such gene sequence information permits modification of alkaloid levels and composition via genetic engineering or induced mutation (Xie et al. 2004; Chintapakorn and Hamill 2003; Lewis et al. 2008, 2010, 2015; Lewis 2018). A major breeding challenge still exists, however, to introduce any variability affecting reduced nicotine content into a breeding program without sacrificing yield and/or quality (Chaplin and Weeks 1976; Lewis et al. 2015; Lewis 2018).

9.3.4.5 Chemical Traits Affecting Taste and Aroma

Hundreds of tobacco plant natural products and their derivatives likely influence organoleptic properties of tobacco products. Classes of chemistry of known favorable importance to tobacco flavor and aroma include carotenoids, sugars, and polyphenols, which accumulate within the leaves, and diterpenes and sugar esters that are exuded onto the leaf surface by glandular trichomes. With bans on tobacco product additives in many parts of the world, there is increased interest in using genetic variability within the tobacco plant to develop novel chemical profiles that could positively affect sensory characteristics.

The primary diterpenes in most tobacco germplasm are the cembranoids α- and β-cembratriene-diols. The labdanoid *Z*-abienol can also accumulate to significant amounts on the leaf surfaces of Oriental and some cigar tobaccos to influence smoke properties of these market classes. Many genes likely influence absolute accumulation of these compounds, but genes involved in the direct biosynthesis of tobacco cembranoids and labdanoids have been characterized (Wang et al. 2001; Wang and Wagner 2003; Ennajdaoui et al. 2010; Sallaud et al. 2012), which might permit for marker-based selection of desired allelic variants to influence their accumulation. Sucrose esters also accumulate to significant levels on the leaf surfaces of Oriental tobacco and some cigar types. An example of using selective breeding to develop a novel tobacco market type is the transfer of active genes influencing the accumulation of the sucrose esters and *Z*-abienol from a Galpao type of tobacco to the genetic background of a US burley cultivar. This method produces a unique air-cured tobacco style designated as "American Aromatic" with leaf surface chemistry characteristics similar to that from Oriental tobacco. Nielsen et al. (2017) also describe methodology for reducing levels of cembratriene-diols for the purpose of reducing bitterness of smokeless tobacco products. The successful application of recurrent selection to increase levels of carotenoids has also been demonstrated (Beatson et al. 1984), although this material has never been used in the manufacture of tobacco products.

9.3.4.6 Harmful Constituents

Because of recognized health risks associated with the use of tobacco products, a large group of compounds is considered to be undesirable in cured tobacco leaves or in derived smoke. To date, over 60 compounds found in cigarette smoke and up to 25 contained in unburned tobacco products have been associated with carcinogenesis in laboratory animals (Hoffmann and Djordjevic 1997; Hecht 2003, 2006). The United States Food and Drug Administration (FDA) lists 93 harmful and potentially harmful chemical constituents associated with tobacco products and tobacco smoke due to their association with addiction, carcinogenesis, or respiratory, cardiovascular, reproductive, or developmental toxicity (US Food and Drug Administration 2012). A list of toxicants suggested for lowering in cigarette smoke has already been outlined by the World Health Organization (WHO) (Burns et al. 2008) and includes tobacco-specific nitrosamines (TSNAs), acetaldehyde, acrolein, benzene, benzo[a]pyrene, 1,3-butadiene, carbon monoxide, and formaldehyde. While not a carcinogen per se, nicotine is also being suggested for mandated lowering in conventional cigarettes in order to reduce addiction and smoke toxicant exposure (World Health Organization 2015).

Many harmful constituents of tobacco smoke are by-products that would occur from incomplete combustion of organic compounds from any plant species, but modification of plant genetics can be used as part of an overall strategy to reduce levels of some harmful constituents associated with tobacco products. As is the case

for morphological and disease resistance traits, significant genetic variation exists among tobacco germplasm for leaf accumulation of chemical compounds directly associated with toxicity or that are converted to toxic compounds upon combustion. For example, Rathkamp et al. (1973) observed genetic variability for the smoke constituents total particulate matter, CO, CO_2, acetaldehyde, acrolein, gas phase hydrogen cyanide, phenols, benzo[a]pyrene, and benz[a]anthracene. Matzinger et al. (1978) explored the potential of using recurrent mass selection to reduce particulate matter index (PMI). Five cycles of selection for lower PMI in a synthetic flue-cured tobacco population reduced PMI by an average 1.5% per cycle (Matzinger et al. 1984), with a correlated decrease in total alkaloids of 6.1% per cycle, however. A selection index was later applied in the same population to decrease PMI while maintaining alkaloid accumulation at the initial population mean. After seven cycles of restricted index selection, the PMI:TA ratio was reduced by an average of 3% per cycle (Matzinger et al. 1984). While not resulting in commercial varieties, this work demonstrated the effect that genetic selection can have on levels of declared toxicants in tobacco smoke.

A recent success story on the utilization of modified tobacco plant genetics to achieve reduction of a tobacco toxicant is that related to efforts to reduce accumulation of *N′*-Nitrosonornicotine (NNN) in cured tobacco leaves. NNN is a recognized carcinogenic compound in tobacco that has been recommended for regulation and mandated lowering (Burns et al. 2008; US Food and Drug Administration 2017). NNN is formed via the nitrosation of nornicotine during curing, storage, and pyrolysis of tobacco (Bush et al. 2001). Genetic methods to reduce levels of the nornicotine precursor were thus devised as a strategy to reduce levels of NNN in cured tobacco leaves. As mentioned previously, nornicotine results from the enzymatic demethylation of nicotine, and three active tobacco genes encoding for these enzymes have been identified and characterized (Lewis et al. 2010). Based upon gene sequence information, RNA interference was used to produce transgenic tobacco plants in which transcription of the nicotine demethylase gene family was repressed (Siminszky et al. 2005). Cured leaf from transgenic lines exhibited highly significant reductions in nornicotine and NNN formation (Lewis et al. 2008). Non-transgenic breeding materials with similar levels of nornicotine reduction were subsequently produced by introducing deleterious mutations into each of three nicotine demethylase genes (Lewis et al. 2010). This genetics-based strategy has been designated as ZYVERT™ technology and functions to reduce NNN in cured leaves and in tobacco smoke (Lusso et al. 2017).

Similar efforts have been carried out to reduce uptake and leaf accumulation of cadmium (Cd), a heavy metal included on the FDA's list of harmful and potentially harmful constituents. Several authors have reported on the impact of transgenic expression of mammalian metallothionein genes (*mMTI* or *hMTII*) to entrap Cd in tobacco root tissues through cytosolic chelation (Yeargan et al. 1992; Dorlhac de Borne et al. 1998), resulting in 14–73% reductions in leaf Cd accumulation in field-grown plants. Expression of vacuole cation exchange genes has also been evaluated for the potential to sequester Cd in root vacuoles. Transgenic field-grown tobacco plants expressing *CAX2* or *CAX4* Cd antiporter genes from *Arabidopsis thaliana*

under the control of root-specific promoters accumulated 15–25% less Cd in the lamina as compared to control plants (Korenkov et al. 2009). Combining the two transgenic mechanisms (*mMT* and *CAX*2) into single tobacco genotypes indicates that additive reductions in lamina Cd contents might be achieved (Wagner et al. 2009). A third documented strategy for reducing leaf Cd accumulation involves the use of RNA interference or induced mutation to reduce expression of *N. tabacum* heavy metal ATPase (*HMA*) genes that play a role in Cd translocation from roots to shoots (Hernand et al. 2014; Liedschulte et al. 2017). Fifty to ninety percent reductions in Cd leaf accumulation have been reported through the use of these approaches.

9.4 Conclusions and Outlook

Since the first published reports on tobacco varietal improvement slightly more than 100 years ago, much has been accomplished through increased insight into the genetic control of important traits in tobacco, development and application of new breeding methodologies, and the generation of new cultivars that affect the efficiency of tobacco growing and the economics of tobacco product manufacture. Modern tobacco cultivars of the major market classes possess trait combinations affecting yield, disease resistance, and quality characteristics that were not available in the early part of the twentieth century.

A major impediment to continued tobacco improvement is the finding that many desired characteristics exhibit unfavorable genetic correlations. For example, multigenic partial resistance to soilborne disease appears to be inversely correlated with yield and cured leaf quality in flue-cured tobacco. Likewise, cured leaf yields tend to be inversely correlated with nicotine content (Matzinger et al. 1960; Legg et al. 1965). Such genetic relationships, along with complex inheritance, serve as impediments to the simultaneous improvement of multiple traits or the improvement of one trait while keeping a second trait at an acceptable level. Although trade-offs often exist between yield, disease resistance, and quality characteristics, negative associations with cured leaf quality are perhaps the most important. Achievements in terms of increased yield or disease resistance can be nullified if cured leaf quality is compromised to the point that it becomes unacceptable to industry standards.

Perhaps the greatest issues facing the tobacco industry relate to increased governmental regulation of smoking per se and of levels of harmful and addictive substances in tobacco products. There is consequently an industry desire to innovate nicotine-containing tobacco-based products with reduced toxicant exposure characteristics. Product design and engineering will play major roles in the creation of novel products. However, modification of tobacco plant genetics can aid in the development of the primary raw materials with desired chemical composition. This work could be advanced by utilization of diverse *Nicotiana* germplasm to influence chemical constituents or sensory characteristics. Minimum standards programs for certain market types in some countries currently operate to restrict the utilization of

diverse germplasm in tobacco breeding programs. With the development of varied new tobacco products, however, industry-wide minimum standards programs could become irrelevant as different manufacturers may desire contrasting styles of cured leaf for use in their own unique products in a competitive environment.

Introduction of commercially valuable genetic variation into future tobacco cultivars can also be carried out through genetic engineering or gene editing. Despite the fact that tobacco has been used as a model system in the development of such technologies, a curious relationship exists between the tobacco industry and varietal outcomes from these breeding approaches. Tobacco was the first plant species to be genetically engineered, and dozens of transgenic traits have been documented that could increase production efficiency or alter chemical profiles in a desirable way. Genetically engineered varieties of other major crop species have been widely planted in many parts of the world. To date, however, the mainstream tobacco industry has been reluctant to use cured leaf from transgenic tobacco cultivars. The only transgenic tobacco cultivar to be commercialized thus far is transgenic low-nicotine burley tobacco variety "Vector 21–41," which has been grown on an exceedingly limited basis. The scientific risks associated with genetic engineering or gene editing per se appear to be minimal or nonexistent. The reluctance of the tobacco industry to utilize transgenic tobacco likely relates to issues associated with public perception but may also be due to differing global policies on regulation of Genetically Modified Organism (GMO) plants. Varying regulations in different countries complicate growing and trade of genetically engineered tobacco as well as international marketing of derived products. The regulatory status of products of gene editing is currently unclear in many parts of the world. Under increasingly stringent levels of tolerance for harmful constituents that may develop over time, it may become exceedingly difficult to comply with regulations without the use of such new breeding methodologies.

A further issue facing future tobacco cultivar development is a decline in the number of public-sector investigators in the fields of tobacco breeding, genetics, plant pathology, and chemistry. The contributions of public researchers to tobacco trait research and cultivar development have been tremendous over the past 70 years. The number of current public research programs involved in tobacco breeding worldwide is believed to be only four, however. Despite a decline in this activity at the public level, most major tobacco companies employ workers in the areas of tobacco breeding, genomics, and molecular biology. Such researchers work to develop proprietary technologies and cultivars for their own internal purposes. Plant breeding, in general, is probably evolving more rapidly at the current time than at any era in the past. The outlook for further genetic improvement of tobacco is great because of the evolution of techniques to cheaply characterize individuals at the DNA level. Although relating DNA sequence variability to observed phenotypic variability for many important characteristics in tobacco can be difficult, application of modern genomic approaches will play a role in future tobacco cultivar improvement.

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Chapter 10
Ocimum basilicum L. (Basil)

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10.1 Botany and Characteristics

Basil is native to tropical and subtropical regions in Asia and acclimatized in South and North America, Africa, and Europe. It is classified to the genus *Ocimum* in the *Lamiaceae* family and is comprised of up to 160 different species, the majority of which are aromatic and produce essential oils (Paton et al. 1999; Vieira et al. 2003b; Dudai and Belanger 2016). The most familiar species, sweet basil (*Ocimum basilicum* L.), is commercially used as a fresh and dry herb. Most commonly used species are characterized by green leaves and white flowers and are typically used for culinary purposes, such as the renowned pesto sauce in Italian cuisine, as ornaments in home gardens, and in traditional medicine (Simon et al. 1984; Javanmardi et al. 2002; Dudai and Belanger 2016). Basil demonstrates a wide variety of morphologies and essential oil profiles, making the taxonomy of the genus complex (Grayer et al. 1996; Paton et al. 1999). Such diversity in the species is likely due to human interference with the genus, interspecies hybridization, polyploidization, the presence of multiple cultivars, and chemotype heterogeneity in morphologically dissimilar species (Simon et al. 1999).

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10.1.1 Morphological Characteristics

Basil species vary in plant height and width, leaf size, leaf shape, leaf and flower color, inflorescence size, time to flowering, seed size, color, and time to germination (Darrah 1974, 1980; Simon et al. 1984, 1999; Grayer et al. 1996; Srivastava et al. 2002). The height of mature basil plants at the time of flowering may be divided into tall plants, which are between 45 and 75 cm, and small, compact plants, which are between 15 and 38 cm (Darrah 1974; Srivastava et al. 2002). Plant width or lateral spread may range between 31 and 55 cm (Simon et al. 1999). Leaves have been documented to be an area of 2.7–9.2 cm^2, with flat, spoonlike, or concaved shape and smooth or wrinkled surfaces (Srivastava et al. 2002). Seeds of basil varieties were described to be gray, brown, or black, with a length of 1.1–2.9 mm and a width of 0.7–1.9 mm with a pitted surface. The time from sowing to germination ranges between 4 and 11 days (Darrah 1974; Simon et al. 1999; De Masi et al. 2006). The number of weeks from sowing to flowering ranges between 8 and 19 weeks (Darrah 1974; Simon et al. 1999) and the time from seed development until seed maturation varies between 6 and 28 days. Basil color is the outcome of leaf, stem, spike, and inflorescence pigmentation. Leaf and stem color may be variations of green, purple, or purple-green; and flowers and spike colors may be white, off-white, gray, dark purple, purplish, pink, or violet (Simon et al. 1999; Srivastava et al. 2002; De Masi et al. 2006).

10.1.2 Cytogenetics and Genome Size

Ocimum spp. have been recorded to have chromosome numbers of $2n = 24$, 26, 32, 36, 48, 52, 56, and 72 (Pushpangadan and Sobti 1982; Khosla 1995; Grayer et al. 1996; Mukherjee and Datta 2006). This information suggests that processes such as aneuploidy and polyploidy have been involved in defining chromosome number of basil species (Carović-Stanko et al. 2010). Other reports suggested that chromosomes in *Ocimum* spp. have undergone cytological diploidization from x = 6, 8, 10, 12, 16 (Khosla and Sobti 1985; Mukherjee et al. 2005; Mukherjee and Datta 2006). Carović-Stanko et al. (2010) counted the chromosomes in 28 basil varieties, with *O. basilicum* consisting of 22 of them. They found that in 20 of the *O. basilicum* varieties, $2n = 48$ and in two *O. basilicum* var. *purpurascens* Benth. $2n = 72$. They also recorded that in *O. americanum* L. and *O. africanum* Lour. $2n = 72$, in *O. gratissimum* L. $2n = 40$, and in *O. tenuiflorum* L. (*O. sanctum* L.) $2n = 36$. These chromosome counts were in a good agreement with DNA content measured by flow cytometry (Carović-Stanko et al. 2010). Recent research by Dash et al. (2017) cytogenetically explored *O. basilicum*, *O. gratissimum*, and green and purple varieties of *O. tenuiflorum*. They identified $2n = 54$ chromosomes in *O. basilicum* and $2n = 40$ in *O. gratissimum*. The number of chromosomes identified in the green and purple varieties of *O. tenuiflorum* was $2n = 36$ and $2n = 34$, respectively. Rastogi

et al. (2014) found $2n = 48$ for *O. basilicum* and $2n = 16$ for *O. tenuiflorum*. Overall, it seems that the multiple cultivars, especially within *O. basilicum*, and the high level of morphological variation prevented researchers from having a clear decision on chromosome number for basil. Despite the cytogenetic differences among *Ocimum* species, interspecies hybridizations have been documented (Khosla 1988; Paton and Putievsky 1996; Putievsky et al. 1999; Vieira et al. 2003a). Intraspecies crosses of *O. basilicum* have been shown to produce fertile pollen (Putievsky et al. 1999).

Several attempts were done to estimate the size of the basil's genome based on total DNA content. One study found that DNA content (2C value) of *O. basilicum* among 20 genotypes ranged from 4.17 to 4.75 pg DNA/nucleus with 2 genotypes of *O. basilicum* var. *purpurascens* having 2C values of 7.13 and 7.43 pg (Carović-Stanko et al. 2010). Outgroups of *O. americanum* and *O. africanum* showed 2C values of 6.45 and 7.08, respectively, and *O. gratissimum* and *O. minimum* were similar to *O. basilicum*. The holy basil, *O. tenuiflorum*, seemed to have the smallest genome with 0.76 pg DNA/nucleus. Based on these data a rough estimation of the haploid genome size of *O. basilicum* is 2.2 Gbp and of *O. tenuiflorum* is 370 Mbp. Another work found more variable values (Koroch et al. 2010), where the 2C values of *O. basilicum* (7 genotypes) ranged from 2.92 to 4.74 pg and of *O. americanum* (6 genotypes) from 1.8 to 5.64 pg. Interestingly, *O. gratissimum* (4 genotypes) displayed low 2C values of 1.34 to 1.88 pg. Moreover, *O. tenuiflorum* (1 genotype) had a 2C of 2.91 pg (Koroch et al. 2010). Recently, the published genome of the holy basil spanned over a haplotype assembly of 386 Mbp (Rastogi et al. 2015), representing, roughly, a 2C value of 0.75 pg. Another sequencing effort assembled the genome of the holy basil to 374 Mbp and estimated its total size in 612 Mbp based on k-mer analysis (Upadhyay et al. 2015). Recently, in effort to reveal the genome sequence and size of *O. basilicum*, paired-end, mate-pair, and 10X Genomics™ Chromium™ DNA libraries were assembled with DeNovoMagic™ assembly tool. The total haplotype genome size of the cultivar "Perrie" was found to be 2.13 Gbp (Dudai et al. 2018). That size represents a 2C value of roughly 4.2 pg. Furthermore, in a subsequent BUSCO analysis of this assembly with 1440 single-copy ortholog genes (Simao et al. 2015), it was found that 74.4% of the genes are in duplicated state (Dudai et al. 2018) indicating the tetraploid nature of *O. basilicum*.

10.1.3 Volatiles and Aroma Profiles

Aroma profiles in *Ocimum* spp. reveal substantial heterogeneity (Grayer et al. 1996). The most frequently encountered constituents with the highest relative content of essential oils are linalool (1.9–85%), 1,8-cineole (<1–20%), methyl chavicol (<1–90%), methyl cinnamate (<1–52%), eugenol (0–68%), germacrene D (1.13–5.17%), and *t*-cadinol (3.12–8.73%) (Grayer et al. 1996; Simon et al. 1999; Özcan and Chalchat 2002; Srivastava et al. 2002; Koutsos et al. 2009; Pandey et al. 2014; Chenni et al. 2016). Additional constituents encountered at lesser frequencies

are geranyl acetate, β-caryophyllene, *p*-cymene, camphor, citral, β-bisabolene, thymol, methyl eugenol, β-bergamotene, and geraniol (Marotti et al. 1996; Simon et al. 1999; Sengul and Sezen 2000; Özcan and Chalchat 2002; Pascual-Villalobos and Ballesta-Acosta 2003; Vieira et al. 2003b; Klimankova et al. 2008; Hanif et al. 2011; Pandey et al. 2014; Chenni et al. 2016; Dudai and Belanger 2016; Saran et al. 2017). The major constituent of most essential oil chemotypes identified in basil was recorded to be linalool (Dudai and Belanger 2016). However, many additional chemotypes have been reported, including methyl cinnamate, methyl eugenol, methyl chavicol, citral, and combinations of linalool/methyl chavicol, linalool/methyl cinnamate, linalool/methyl eugenol, linalool/1, 8-cineole, and citral/methyl chavicol (Marotti et al. 1996; Simon et al. 1999; Sengul and Sezen 2000; Telci et al. 2006; Varga et al. 2017). Chemical types were also associated with geographical regions (Simon et al. 1999) and thus given names such as European, Greek, Turkish, German, Egyptian, Reunion, and Java (Marotti et al. 1996; Simon et al. 1999). The topic of volatile composition and aroma is further elaborated in Sect. 10.4, in the context of the breeding.

10.2 Breeding Basil for Commercial Use

The utmost familiarity with sweet basil in the western world is culinary, as the source of pesto sauce in Italian cuisine. Nonetheless, basil has diverse commercial applications, which include as a fresh-cut herb, a dried spice, a source of essential oils and flavoring compounds for the food and beverage industries, a source of fragrance for cosmetics and hygienic products, a source of biologically active substances, and an ornament for home gardening (Simon et al. 1990; Dudai and Belanger 2016). As with other crops, the goals of basil breeding are primarily concerned with economic improvement of the crop, which includes:

(a) Improvement of yield and quality of the plant product for the intended use.
(b) Acclimation and adaptation of the crop to new environmental and cultivation conditions, such as soil, climate, and agro-technical methods, or adjustment of the crop to new cropping systems and harvest methods and technologies.
(c) Solutions to cultivation limiting factors, such as biotic and abiotic stresses, or control of harvest timing to achieve the best time to market.

Consequently, sweet basil breeding efforts must focus on a myriad of traits that are industry specific and can be generalized into the following main qualities:

(a) *Performance*, which is primarily important in fresh-cut as well as in potted basil and is characterized by leaf size and shape, length and thickness of internodes, number of stems, branching, color, etc. The fresh-cut industry requires small-sized, smooth leaves with inverted, spoonlike form, delicate stems with short internodes that should be soft for culinary proposes, and vivid colors that are eye-catching and appealing to consumers.

(b) *Resistance to diseases*, caused by bacteria, fungi, or viruses in the field and during postharvest that reduce quality and quantity.
(c) *Tolerance to chilling injury*, which is a significant parameter in areas where basil is produced during the winter and also of immense implication for fresh-cut during postharvest in storage and freight.
(d) *High yield quantity and quality of volatile composition and aroma*, which are the principal traits for dry spice sweet basil, pesto, and essential oil/extract production of valuable compounds – where large leaf size, the ability to withstand drying without losing oil and aroma quality, and the levels of constituents of interest are the leading variables in the breeding process.

With the aforementioned qualities in mind, sweet basil breeding strategy must be goal specific, rather than a "one-fits-all" approach. The sweet basil breeder must focus breeding efforts to fulfill all of the specific downstream industry requirements. The key is to manage the balance between the grower's necessities for high weight yield, pre- and postharvest diseases or late blooming varieties, and the consumers' requirement for high-quality fresh-cut with the sought-for aroma or for the extract producer for high quantity and the desirable composition of essential oil and compounds. In order to achieve these innumerable goals, the sweet basil breeder needs to acquire a wide spectrum of understanding of the *Ocimum* spp., its botany and genetic background, the intended use of the variety, and the cultivation conditions that are expected at the growing region (i.e., soil type, climate, agro-techniques). Thus, breeding programs should establish and maintain an *Ocimum* spp. gene bank with substantial phenotypic variation, as phenotypic dissimilarity is a key for developing varieties with optimal trait combinations. Genetic variability can be attained by combining the following approaches: collecting germplasm from the wild, breeding for specific parental lines, preserving seed or clones of intermediate genotypes, conventional mass and line selection, introgression of new traits via intra- or interspecific outcrossing, hybridization for hybrid vigor, in vitro tissue culture procedures, application of selection pressure, and smart breeding methods (e.g., genetic markers and mapping). All of the aforementioned are accepted tools that may be used to fulfill the task and ultimately deliver a genotype with the sought-for qualities.

10.2.1 Breeding Basil for Performance

The fresh-cut market was strongly influenced by the Italian cuisine that originally used the *O. basilicum* "Genovese Gigante" for pesto sauce (Dudai and Belanger 2016). The variety, with its small- to medium-sized leaves and convex form, set the bar for all future varieties bred for the fresh market including for aroma. In Liguria, Italy, where basil-based pesto sauce originated, the cultivation is performed in greenhouses and the harvest is carried out manually by selectively uprooting young plants. Then, the plants are grouped into bunches with the roots wrapped in paper.

Under this harvesting system, the leaves are small and convex and have a bright greenish color.

In contrast, the harvesting system in the rest of the basil-producing regions in the world removes fresh basil young shoots from mature plants. This enables multi-harvest cycles per season and is more economically viable. However, the common "Genovese" varieties are unsuitable for multi-harvesting systems because mature plants produce large and wrinkled leaves that are unacceptable by the fresh-cut market. Furthermore, the large leaf and thick shoot do not fit the size of commonly used packaging for fresh-cut herbs (Dudai and Belanger 2016). Therefore, when breeding for performance, one of the goals is to develop varieties with small convex leaves and compact internodes that would fit both the intensive multi-harvest cropping system and the requirements of the fresh-cut market. In Israel, this goal is achieved by increasing the diversity of performance traits by open pollination, hence crossing germplasm with multiple other morphological types. Then, germplasm demonstrating suitable performance traits are selected and self-pollinated for three generations (Dudai et al. 2018). Using this breeding approach the first common varieties were developed during the 1990's (Dudai and Belanger 2016).

Breeding basil as an edible ornamental is another example of a performance goal. Basil varieties offer an assortment of morphological forms, aroma, and fragrance, making them an edible as well as an ornamental herb. Breeding programs focusing on ornamental basil select for varieties that are visually and olfactometrically attractive. Hence, vivid leaf color (green to dark purple), flower and inflorescence colors (white, red, lavender, or purple), and pleasant fragrance are parameters of interest. The cultivar "Magical Michael" is an example of a green leaf basil that is edible and rich in essential oils for cooking or in salads. As an edible ornamental "Magical Michael" has small flowers with purple calices and white corollas, making it visually very attractive.

The variety "Cardinal" (Dudai et al. 2000, 2002) is another example of an outdoor ornament with an anise aroma characterized by purple stems and large, compact inflorescences with deep red bracts (Fig. 10.1). In work conducted in Israel, the crossing of "Cardinal" with a commercial variety yielded an intermediate hybrid. Upon self-pollination, a wide spectrum of diverse F_2 genotypes was attained (Fig. 10.1). These are used as a source of multiple unique ornamental varieties and traits (Dudai et al. 2018).

Another well renowned ornamental variety is "Sweet Dani," developed by Morales and Simon (Morales and Simon 1997; Simon et al. 1999). This variety is a classic example of mass selection followed by line selection for breeding basil with an intense lemon fragrance that can be used as an ornament or cultivated as a fresh herb. Basil varieties with purple features are also favorable as ornaments. For example, a very popular variety in home gardens is "Dark Opal" (Gardner and Dougherty 2005), a tall, upright basil that can grow to approximately 50 cm in height with purple leaves and pinkish flowers. The purple foliage makes it a vivid addition to container arrangements.

The special types of basil varieties that are important in home gardens and for container cultivation are the "dwarf" and "compact types," also known as "bush"

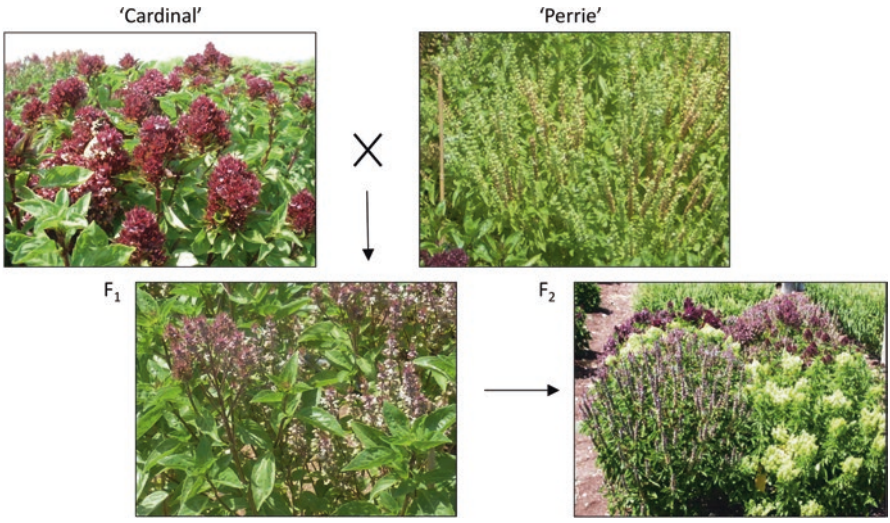

Fig. 10.1 A cross between "Cardinal," a variety with compact inflorescence, purple bract, and calix and pink corolla, and "Perrie," a variety with a spike, green bract, and calix and white corolla. Images present parental lines, F_1, and several F_2 progeny phenotypes, demonstrating the wide-spectrum phenotypes segregating from the cross

basil. This performance trait relates to growth characteristics such as plant height, lateral spread, and compactness (Morales and Simon 1996; Makri and Kintzios 2008). "Bush" varieties tend to be approximately 15 cm in height and are used ornamentally as border plants in gardens or in pots and containers. An example is the variety "Pluto," a bush-forming cultivar, producing small, even dome-shaped plants of approximately 20 cm. "Pluto" has fine, mid-green leaves that are excellent for use in cooking (Society 2018).

10.2.2 Breeding for Disease Resistance

The need to breed for resistance to diseases indicates that under the cropping conditions the cropped variety is susceptible to a plethora of disease-causing agents. Thus, the downstream goal of breeding for resistance is to develop a commercial variety with better resistance traits than the ones currently available on the market. Therefore, breeding for resistance to disease requires an understanding of the following components:

(a) *The disease causal agent and the disease cycle*: knowledge of the etiology, epidemiology, and environmental conditions that promote disease development, the disease symptoms, and the pathogen's signs will promote an understanding to the disease causal agent and the disease cycle. These characteristics are crucial for developing protocols that assist in correctly inoculating

the plant and for developing phytopathological and agronomic quantification scales to determine the plant's reaction. Furthermore, the genetic variation in the pathogen's population (i.e., physiological races, vegetative compatibility groups, pathotypes, *forma specialis*, etc.) should be taken into consideration, as this diversity directly affects the gene-for-gene interaction, hence pathogenicity and virulence. A pathogen collection from field-diseased plants must be established and all isolates should be tested for pathogenicity by completion of Koch postulates. It is suggested that all field isolates would be micromanipulated into mono-conidial or hyphal tip cultures to assure work with a single fungal genotype. In the breeding process, a set of pathogen reference isolates/genotypes should be used for germplasm mass selection. In the presence of pathotypes or races of the pathogen, inoculations with a cocktail of isolates/genotypes should be considered.

(b) *The plant host*: a diverse germplasm collection that represents, at best, the population of the crop species is required. These should include commercial varieties with a known reaction to the disease that can be used as susceptible and resistant references for comparisons (Nitzan et al. 2008, 2009, 2010). In the absence of a resistant reference variety, germplasm that are less susceptible to the disease than the commercial variety/varieties should advance for further breeding. If case reference varieties are unavailable, the breeder may use the disease severity/incidence grand mean score of germplasm population as a baseline, and the germplasm, whose reaction to the disease is lesser than this baseline score, should be further advanced in the breeding process.

(c) *The resistance mechanism and inheritance*: disease resistance may be monogenic, controlled by a single gene, or polygenic, controlled by many genes and quantitative trait loci (QTL). Therefore, in a cross between a resistant and a susceptible parent, two types of disease reaction should be looked for: (i) a progeny population with both resistant and susceptible types, which suggest the involvement of a single gene (monogenic or vertical resistance), or (ii) the reaction to the disease in the progeny that can be quantified into a continuum, suggesting the involvement of multiple genes (polygenic or horizontal resistance). An additional parameter, which is not exclusive to resistance, is the genetic stability of the trait, or genetic × environment interaction (G × E), which needs to be examined at early stages of the breeding process under different cropping conditions and locations.

Sweet basil is susceptible to a variety of plant pathogens (Garibaldi et al. 1997). Among the most important diseases are the soil- and airborne fungal pathogens, *Fusarium oxysporum* f. sp. *basilici*, which is the causal agent of Fusarium wilt (FOB); *Botrytis cinerea*, the causal agent of gray mold; *Sclerotinia sclerotiorum*, the causal agent of white mold (Elad et al. 2015); and in recent years *Peronospora belbahrii*, the causal agent of downy mildew. For many years, the management of these diseases was primarily chemical, which included fungicide application or soil fumigation (Garibaldi et al. 1997). The ban of methyl bromide and the severe regulation of fungicides' residue in the fresh-cut crop limited the available chemical

control means. Additionally, crop rotations, which can reduce initial inoculum, are usually limited under the intensive sweet basil production that requires high planting densities and continuous cropping throughout the year. Furthermore, overuse of fungicides increases pathogen resistance buildup, reducing their efficacy (Georgopoulos and Skylakakis 1986; Karaoglanidis et al. 2000; Ajouz et al. 2011; Lucas et al. 2015), while soil disinfection has been demonstrated to harm the gentle balance of the microflora in the soil (Gamliel et al. 2000). These limitations are the motivation to identify and implement integrated pest management (IPM) approaches, among which breeding of resistant varieties is conceivably the most agronomically flexible and economically suitable. In the next subsections, we discuss the breeding of commercial varieties resistant to FOB and downy mildew, as well as the ongoing attempts to select for resistance to gray and white molds.

10.2.2.1 Breeding for Resistance to Fusarium Wilt

Fusarium wilt of sweet basil (FOB) is caused by the fungal pathogen *Fusarium oxysporum* f. sp. *basilici*. Basil plants diseased with FOB exhibit stunting, browning of vascular tissues, and severe wilting without chlorosis and/or defoliation (Garibaldi et al. 1997). The disease was first reported in the Soviet Union in 1959 (Kvartskhava 1959; Dzidzariya 1963), but since then has been reported in many additional sweet basil production regions (Gamliel et al. 1996). FOB initial inoculum is soilborne and later in the season infected plants become the source of secondary, airborne inoculum (Gamliel et al. 1996). Seed transmission is also recorded as a tactic of disease spread (Reuveni et al. 1997). These possible transmission modes coupled with the prohibition of methyl bromide and the requirement to eliminate pesticide residue make the nature of the disease quite complex and a motivation to breed basil varieties with resistance.

In 1992, Dudai et al. (2002) observed individual plants of the local commercial sweet basil variety "Chen" growing in a field with history of severe FOB that did not exhibit disease symptoms. These putatively resistant plants were removed from the field and were self-crossed for seed. They were rigorously examined for resistance to FOB by artificial inoculations with a FOB isolate (preemptively confirmed as causing FOB following completion of Koch's postulates). Subsequently, mass selections were carried out for five generations under controlled environment by growing the progeny generations in soil artificially infested with FOB using the isolated pathogen (Fig. 10.2). As a result "Nufar," the first commercial variety with resistance to FOB was developed and registered (Dudai et al. 2002). "Nufar" was further crossed with FOB susceptible basil genotypes yielding resistant F_1 individuals, which indicated that the inheritance of FOB resistance was the outcome of a single dominant allele (Dudai et al. 2002; Chaimovitsh et al. 2006). This led to a line selection strategy that enabled the rapid development of resistant varieties with sought-for aroma traits, as described in Fig. 10.3. In case resistance is not present in the population of interest, crosses may be undertaken with resistant populations from other, previous breeding processes. The line selection strategy, in which a

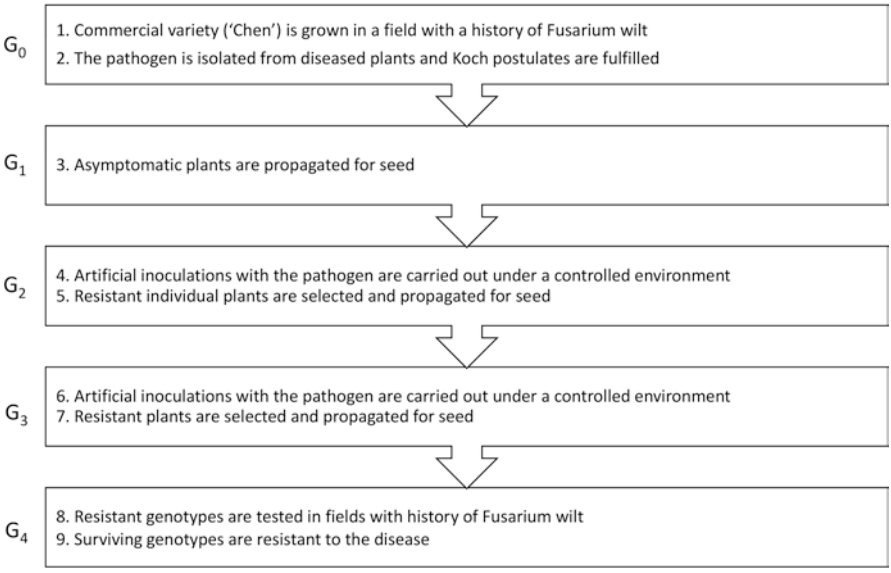

Fig. 10.2 A schematic representation of mass selection for germplasm with resistance to sweet basil *Fusarium* wilt. G_0–G_4 represents germplasm generations in the selection process

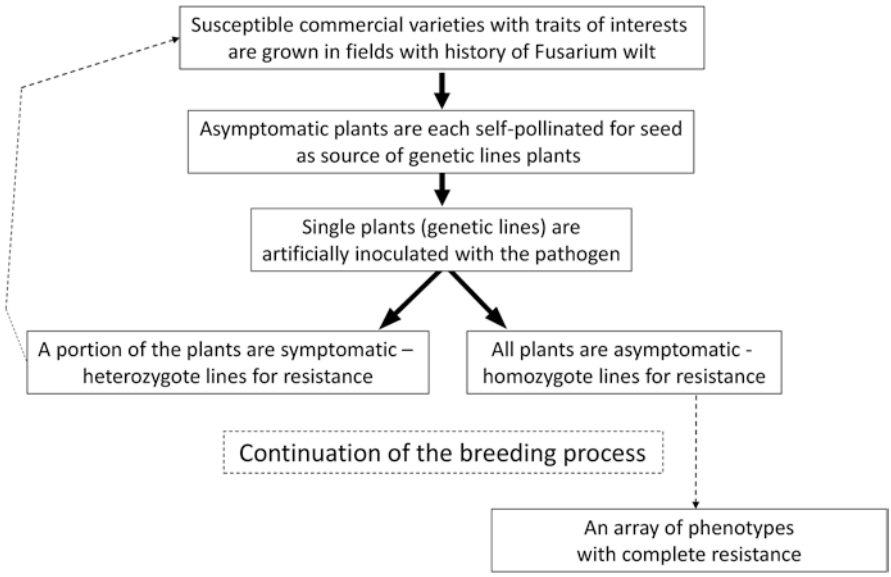

Fig. 10.3 A schematic representation of line selection for resistance to Fusarium wilt of sweet basil. Dashed lines represent continuation of the breeding process

susceptible variety with a trait of interest is crossed with any resistant variety, is also an excellent approach for developing a gene pool of parental plant material that can be further used to select and/or breed for desirable traits. From a commercial breeder's point of view, crossings of susceptible lines with resistant ones also can be used for F_1 protected commercial seed production. Today, most fresh-cut varieties with aroma quality, good yields, acceptable leaf size, shape, internode length, and post-harvest storability are being developed with resistance to FOB using the line selection approach (Dudai et al. 2002; Chaimovitsh et al. 2006). Nevertheless, as stated by E.C. Stakman, "plant diseases are shifty enemies" (Stakman 1947), the work of Reis et al. (2008) suggests the potential presence of FOB physiological races. Hence, additional sources of wide-spectrum FOB resistance are continuously vital to keep FOB under control.

10.2.2.2 Breeding for Resistance to Gray Mold

Gray mold is caused by the fungal pathogen *Botrytis cinerea*. In Israel, *B. cinerea* attacks basil during the winter and early summer seasons, when temperature of around 20 °C and relative humidity of approximatly 80% are optimal for disease development (Sharabani et al. 1999; Shamai 2004). The disease may be present during the summer season, though without a significant economic effect (Shamai 2004). All parts of the sweet basil plant are susceptible to infection by the pathogen, yet wounded stems following harvest are primarily predisposed (Sharabani et al. 1999; Shamai 2004; Yermiyahu et al. 2006). With this in mind, screening efforts to identify variability among basil germplasm in response to infection by *B. cinerea* were carried out by Shamai (2004). In this work, stems were removed from 5-month-old basil plants that were grown from seeds in walk-in tunnels. The wounded stems were inoculated with *B. cinerea* conidial suspensions and were placed in humidity chambers at 20 °C. The rates of lesion expansion and lesion length at 7 days post-inoculation were recorded. Twenty-two germplasm were examined, and the work identified significant ($P < 0.05$) variation within and among the germplasm. As a result, nine basil lines with reduced susceptibility to the disease were identified, suggesting potential for the presence of sources for resistance.

10.2.2.3 Breeding for Resistance to White Mold

White mold is caused by the fungal pathogen *Sclerotinia sclerotiorum*. The fungus is active under cool environments and prevalent in Israel during the winter season. The pathogen infects the bases of basil stems grown indoors and may also directly infect the shoots (Elad et al. 2015). Attempts to identify germplasm less susceptible to the disease than the commercial variety 'Perrie' were made at the Unit of Aromatic and Medicinal Plant located at Newe Ya'ar, Israel, by Nitzan et al. (2012). Basil bunches of the commercial varieties "Perrie" and "Hagar" and additional advanced

breeding lines were inoculated with *S. sclerotiorum* strain that was isolated from a diseased sweet basil plant in a commercial greenhouse. The isolate was validated for pathogenicity via completion of Koch postulates and was subcultured onto rice grains as inoculum carriers. Basil plants were inoculated at the soil surface and disease development was monitored. Disease was visible 3 days post infestation and was characterized by a water-soaked lesion at the base of the plants. Statistically significant ($P \leq 0.05$) differences in susceptibilities among the examined germplasm were recorded (Fig. 10.4). Of notable interest was the advanced breeding line "22," which demonstrated slower disease development than "Perrie." In an additional experiment using the straw inoculation technique (Miklas et al. 2001), the advanced breeding line "4×4" demonstrated reduced rate of lesion expansion than "Perrie" (Nitzan et al. 2012). In contrast to *Fusarium* wilt where complete qualitative resistance was identified, here the outcome suggests the presence of general, quantitative resistance. Furthermore, line "22" (Fig. 10.4) also has been recorded to be drought tolerant and with reduced activity of the enzyme polyphenol oxidase (Shafran et al. 2007). This may direct to involvement of the enzyme in white mold resistance. The outcome calls for exploring additional *Ocimum* species for the presence of reduced susceptibility to the disease.

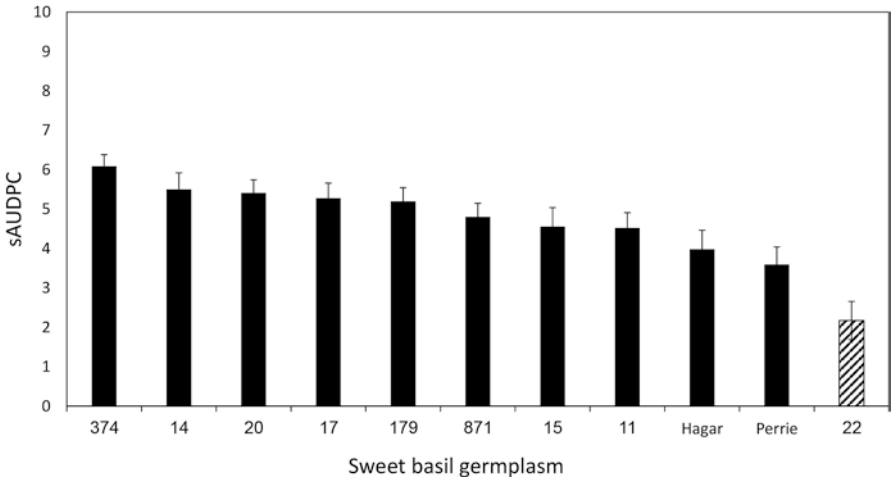

Fig. 10.4 Standardized area under the disease progress curve (means AUDPC±SEM) scores depicting the response of two commercial sweet basil varieties and nine advanced select germplasm to infection by the white mold fungal pathogen *Sclerotinia sclerotiorum* in a trial carried out in 2011 at the Newe Ya'ar Research Center of the Agriculture Research Organization, Israel. Germplasm "22" had significantly ($P = 0.04$) less white mold than the commercial standard "Perrie" following a single degree of freedom ($df = 1$) contrast

10.2.2.4 Breeding for Resistance to Basil Downy Mildew

The oomycete *Peronospora belbahrii* is a destructive foliar pathogen causing downy mildew of sweet basil. The pathogen is prevalent in many commercial basil-growing regions around the world, causing severe epidemics. Wyenandt et al. (2010) reported for the first time about the presence of resistance to the disease following field trials that were conducted in New Jersey in 2009. Among the 30 different germplasm and commercial varieties, the cultivars "Nofima," "Nufar," and "Puppy Joe" were susceptible, whereas cultivars of *O. x citriodorum* and *O. americanum* had less disease, while the cultivars "Spice," "Blue spice," and "Blue spice fil" had no disease recorded on them (Wyenandt et al. 2010). In a following study, Pyne et al. (2014) reported a rapid technique for mass screening of sweet basil susceptibility to downy mildew that was correlated with field observations. As part of this research the authors screened for resistance in 36 accessions of *O. basilicum* from the "US National Plant Germplasm System" (USDA-NPGS) and recorded four accessions with partial resistance that had minute levels of sporulation, but symptoms of chlorosis and necrosis and one accession (PI 652053) with no visible signs or symptoms associate with the disease. Resistance to downy mildew was also identified in the work of Farahani-Kofoet et al. (2014), who screened 236 basil germplasm under controlled conditions optimal for disease development. The authors reported that *O. americanum* var. *americanum*/*O. canum*, *O. americanum* × *basilicum* "Blue Spice," *O. americanum* var. *pilosum*, *O. campechianum*/*O. micranthum* "Peruvian basil," *O. gratissimum*, and *O. tenuiflorum* "Tulsi" had resistance to the disease. Furthermore, the authors indicated that the resistant germplasm are exotic basil species with substantial variation in plant characteristics aroma and taste.

In 2015, two works shed light on the inheritance of resistance to sweet basil downy mildew (Ben-Naim et al. 2015; Pyne et al. 2015). Ben-Naim et al. (2015) screened 113 accessions of *Ocimum* species in growth chambers and in 3 years of field trials, identifying germplasm of *O. americanum*, *O. kilimandscharicum*, *O. gratissimum*, *O. campechianum*, or *O. tenuiflorum* to be resistant to the disease. Later, individual resistant plants were crossed with the susceptible commercial variety "Perrie" and the F_1 progeny distributed into two crosses with resistance consisting of 24 crosses with moderate resistance and a single cross with susceptibility. This confirmed the potential resistance of sweet basil to downy mildew, suggesting complete or partial dominance of the resistance trait. Ben-Naim et al. (2018) reported the transfer of a resistance gene designated *Pb1* from the resistant tetraploid wild basil *O. americanum* var. *americanum* (PI 500945, $2n = 4x = 48$) to the tetraploid susceptible *O. basilicum* "Sweet basil" ($2n = 4x = 48$). F_1 progeny of the cross between the two germplasm were all sterile and resistant, indicating that the gene *Pb1* conferring resistance is dominant. Subsequent crosses suggested that two dominant genes, *Pb1A* and *Pb1A'*, located on two homeologous chromosomes, are responsible for the resistance of germplasm PI 500945 against the basil downy mildew pathogen *P. belbahrii*. Pyne et al. (2015) attempted to introgress resistance into commercial varieties by crossing the commercial basil cultivar "Mrihani" that was identified as resistant with SB22 with a susceptible sweet basil inbred line from

Rutgers University, hence generating a complete-sibling family. Following 2 years of field trials at two locations in New Jersey, the researchers observed that the F_1 progenies and all generations of backcrosses with the resistant parent "Mrihani" were resistant to the disease, hence indicating that the inheritance of resistance from "Mrihani" is due to dominant alleles. Chi-square goodness of fit analysis of the F_2 and backcross to the susceptible parent were congruent with segregation ratios that fitted the two-gene complementary and recessive epistatic models. Additional studies of the data pointed out to significant ($P < 0.001$) nonallelic additive-additive and additive-dominant gene effects that were resistance reducing. Recently, Pyne et al. (2017) used the same cross to construct the first highly saturated linkage map based on EST-SSR and ddRADseq markers. They used 1847 markers mapped to 26 linkage groups (LG) and based on the markers segregation determined that *O. basilicum* is an allotetraploid with disomic inheritance. Then, they mapped three QTLs for downy mildew resistance. One major QTL, *dm11.1*, acts in dominant fashion, and another two minor QTLs, *dm9.1* and *dm14.1*, were additive to *dm11.1*. A downstream development of molecular markers for downy mildew resistance and validating them in different germplasm will assist to develop resistant elite cultivars. This study demonstrates the importance and feasibility of marker-assisted selection in basil breeding strategies and hopefully is shaping the future of basil breeding.

10.2.2.5 Breeding for Resistance to Chilling Injury

Sweet basil is susceptible to chilling injuries caused by exposure to temperatures below 12 °C during growth, storage, and transport (Ribeiro and Simon 2007; Aharoni et al. 2010). Symptoms of chilling injuries appear as brown spots on the leaves that develop into necrosis. As a result, the leaves may abscise, lose glossiness, and become prone to decay, causing soft rot from bacteria and fungi such as *Botrytis cinerea*. Work conducted by Dudai et al. (2004) during the winter season in a non-heated greenhouse identified variation in response to chilling injury in commercial cultivars. Of the tested germplasm, the variety "Hagar" was the most tolerant, demonstrating 11.5% less ($P < 0.0001$) chilling injured leaves than the standard industry cultivar "Perrie," which is considered moderately tolerable (Figs. 10.5 and 10.6).

Breeding sweet basil for postharvest requires a different screening approach than in the field. Aharoni et al. (2010) demonstrated that chilling injury symptoms were more pronounced upon transfer of sweet basil bunches from cold storage (6–12 °C) to room temperature (17 °C). Therefore, protocols to identify germplasm with reduced sensitivity to the harmful effect of transfer to ambient conditions were devised. In 2001, Dudai et al. (2004) replicated a postharvest protocol in which two-week-old basil bunches in 96-well trays were exposed to 4 °C for 7 days followed by 2 days of shelf life at 17 °C, to screen germplasm for chilling injury tolerance (Fig. 10.5). The results identified several varieties that were significantly ($P \leq 0.05$) more tolerable than the commercial cultivar "Perrie" (Fig. 10.6). In 2007, the inheritance of chilling tolerance was investigated by Ribeiro and Simon (2007), who

Fig. 10.5 Mass screening of sweet basil germplasm for chilling tolerance (right). The plants were exposed to multiple cycles of storage at 4 °C for 7 days followed by 2 days at 17 °C in the greenhouse. Leaf browning (left) is an indicative symptom of chilling injury

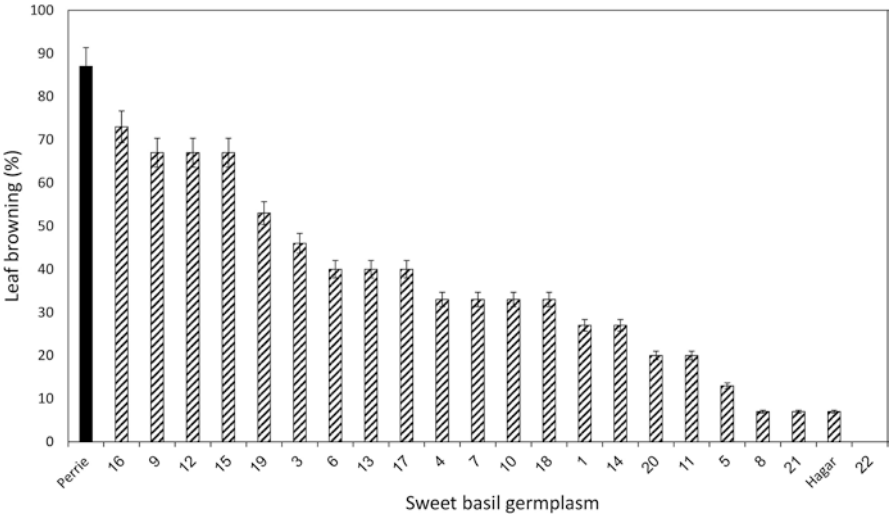

Fig. 10.6 Leaf browning (mean % ± SEM) due to chilling injury in advanced selected germplasm depicting greater ($P \leq 0.05$) tolerance than the commercial variety "Perrie." The plants were exposed to multiple cycles of storage at 4 °C for 7 days followed by 2 days at 17 °C in the greenhouse. Among these germplasm, the variety "Hagar" was commercialized

examined more than 6000 individual sweet basil plants for the trait. In their research, the plants were exposed to various cold temperatures for different durations and 46 individual plants were identified with tolerance. F_2 progenies from a cross of a tolerant line with a sensitive line resulted with 18 of 23 plants with tolerance, hence suggesting a 1:3 segregation ratio and single gene dominance for the trait. The mechanism involved with resistance to chilling injury may be associated with polyphenol oxidase activity that was also recorded to be associated with drought tolerance (Shafran et al. 2007).

10.3 Breeding Basil for Flavor, Aroma, and Chemical Quality

10.3.1 Characteristics of Basil Chemical Quality

The quality of basil is determined mainly by its essential oil content and composition. Being cultivated for various culinary uses and consumed by diverse populations, basil does not have one "right" aroma. The aroma of basil is determined by the various compounds comprising its essential oil, which are classified into two main chemical groups: terpenoids (mainly linalool, 1,8-cineole, and citral) and phenylpropanoids (mainly eugenol, methyl chavicol, and methyl cinnamate) (Dudai and Belanger 2016). The "Genovese" basil used in Italian cuisine, either for pesto sauce or as a fresh herb, contains high levels of eugenol/methyl eugenol and linalool but almost no methyl chavicol (Elementi et al. 2006). Alternatively, some anise-like flavor varieties with high levels of methyl chavicol and linalool, such as "Nufar," are very popular in the United States. Thai basil, used in Southeast Asian cuisine, which is known as "exotic basil," also has an anise-like flavor with high levels of methyl chavicol, and is also used for industrial essential oil production (Simon et al. 1999). Lemon basil, used as a culinary herb mainly in Thai, Cambodian, Indonesian, and Persian cuisines, contains high citral levels and low linalool and phenylpropanoid levels (Morales and Simon 1997; Dudai and Belanger 2016). Cinnamon basil, which is used for various culinary purposes, such as baking and cooking, is rich in methyl cinnamate (Simon et al. 1999; Dudai and Belanger 2016). While these descriptions might sound fixed, there is no clear cut for what the "correct" levels or ranges of each compound in the essential oil for a specific type of basil are. In addition, minor constituents of the essential oil, sometimes having very low odor thresholds, might have an important effect on the overall flavor of the basil, contributing to an even greater aroma complexity.

Essential oil production has a dynamic nature that is affected by various environmental parameters (Chang et al. 2015), by the age of the plant and leaves, as well as by the specific position of the leaf on the plant (Lewinsohn et al. 2000). For example, it was found that the order of the emergence of the leaves, regardless of their age or distance from the apex, determines eugenol versus methyl eugenol levels in Genovese basil type (Fischer et al. 2011). Moreover, the method that is used to measure the aroma, either water distillation, solvent extraction, or headspace analysis, can also have a profound impact on the profile obtained (Dudai and Belanger 2016). Finally, morphological characteristics such as leaf color and shape have additional important impacts on product acceptance by the consumer, contributing to the overall quality of the product. Altogether, this makes the task of breeding basil for high quality, especially for a desired aroma, challenging and not straightforward. Many works have characterized the essential oil composition of various basil varieties and some of them have also tried to determine a genetic and molecular bases for the variation observed. To some degree, experimental crosses, reporting also on aroma, were published.

10.3.2 Characterization of Germplasm Collections

The chemical composition of basil essential oil can be classified into several major chemotypes. These classifications are based on the major components of the essential oil, some of which followed the classification system suggested by Grayer et al. (1996). This system suggests naming each chemotype based on all compounds that exceed 20% of the total essential oil composition. Roughly five major chemotypes were found: (i) linalool rich, (ii) linalool/eugenol (or methyl eugenol) rich, (iii) linalool/methyl chavicol rich, (iv) methyl chavicol rich, and (v) methyl cinnamate rich (Lawrence 1992; Grayer et al. 1996; Telci et al. 2006; Vieira and Simon 2006; Liber et al. 2011; Dudai and Belanger 2016; Varga et al. 2017). Studies also report of a lemon basil, which contain citral as main constituents. Nevertheless, lemon basil is not *O. basilicum*, but rather *O. x citriodorum* (Vieira et al. 2003a). It is a hybrid of *O. basilicum* and *O. americanum* (Paton and Putievsky 1996) with the latter being the origin for the citral compounds (Carović-Stanko et al. 2011a). While the aroma of a given basil is mainly determined by its major essential oil components, minor components have the potential to considerably affect the noted aroma. Advancements in chromatography and spectrometry methods, as well as the availability of GC-MS instruments in many laboratories, permit more studies reporting on these minor components (Vieira and Simon 2006; Liber et al. 2011; Dudai and Belanger 2016; Maggio et al. 2016). However, since they are reported in percentage of total essential oil, it is hard to estimate their effect because the absolute concentration is unknown. Regardless, some of these compounds often affect the aroma undesirably even at low concentrations (Dudai and Belanger 2016).

Essential oil characterization is a laborious task and it could be useful to find common characteristics for visual parameters and essential oil composition. Yet, when essential oil was characterized together with agronomic traits such as yield, leaf size and shape, or plant height, no correlation was found to any morphologic or yield-related trait (Marotti et al. 1996; Vieira and Simon 2006; Carović-Stanko et al. 2011a, b; Liber et al. 2011; Varga et al. 2017). Another method to avoid comprehensive essential oil characterization is to correlate a genetic pattern for the observed variation. DNA fingerprinting techniques, such as amplified fragment length polymorphism (AFLP) or random amplified polymorphic DNA (RAPD), were used to distinguish among several different *Ocimum* species (Carović-Stanko et al. 2011a) and among different varieties (e.g., cv. "Genovese" vs. cv. "Dark Opal") (Singh et al. 2004; Carovic et al. 2007; Carović-Stanko et al. 2010; Moghaddam et al. 2011; Rewers and Jedrzejczyk 2016). In one study (Liber et al. 2011), DNA fingerprinting clearly distinguished between green and purple basil cultivars and to a certain degree between green and purple wild Iranian genotypes (Aghaei et al. 2012). Studies on purple basils and their reverted green varieties showed that they did not differ in their aroma profiles (Koroch et al. 2017), but the purple phenotype was genetically unstable (Phippen and Simon 2000). Some DNA fingerprinting studies tried to find the genetic basis of different basil chemotypes, suggesting a genetic

basis for basil chemotypes with high methyl chavicol (De Masi et al. 2006; Carović-Stanko et al. 2011a; Liber et al. 2011). The origin of lemon basil, accumulating high citral levels, was demonstrated in several studies to carry a genetic basis (Vieira et al. 2003a; De Masi et al. 2006; Carović-Stanko et al. 2011a), validating that it is not a true *O. basilicum* spp. as mentioned above. Based on AFLP analysis, cinnamon basil seems to harbor a certain genetic basis as was demonstrated in a work analyzed six cinnamon basil cultivars in a set of 27 basil genotypes (Liber et al. 2011). Yet, in a different work it was shown that two cultivars of cinnamon basil did not cluster together based on RAPD analysis in a set of 37 *Ocimum* genotypes (Vieira et al. 2003a). Unlike lemon basil, cinnamon basil varieties share common cytological features with other *O. basilicum* genotypes, such as DNA content and chromosome number (Koroch et al. 2010; Rewers and Jedrzejczyk 2016). However, in most of these studies, the number of genotypes used was too small to have a clear call.

Finally, *Ocimum* species other than *O. basilicum* show greater variation in chemotypes and chemical composition. Some of these species are rich with different sets of phenylpropanoids, some show different monoterpenoid composition, and some are rich with sesquiterpenes. While highly variable some generalizations can be made. *O. kilimandscharicum* accumulates high camphor levels, *O. gratissimum* accumulates high thymol and *p*-cymene levels or high α-bergamotene levels, *O. tenuiflorum* accumulates β-bisabolene, and *O. americanum* varieties show large versatility accumulating citral or methyl cinnamate or even anisole (Martins et al. 1999; Vieira and Simon 2006; Carović-Stanko et al. 2011a). One must bear in mind that aroma profiling of *Ocimum* species other than *O. basilicum* is often analyzed as outgroups for *O. basilicum* genotypes and the data do not represent the entire chemotypes of a given species. *O. canum* is a good example for multiple chemotypes detected in different studies that include a linalool type (Ravid et al. 1997; Ngassoum et al. 2004), a eugenol type (Ekundayo et al. 1989), a methyl cinnamate type (Martins et al. 1999), a camphor type, and even a limonene type (Ngassoum et al. 2004). Another chemical variation that is more predominant among species than within a species is the presence of various optical isomers (enantiomers). For example, *O. canum* and *O. sanctum* accumulate mainly (*S*)(+)-linalool, while various *O. basilicum* cultivars accumulate mainly (*R*)(−)-linalool (Ravid et al. 1997). It has been shown that enantiomers of the same compound can have different aroma notes in human perception (Brookes et al. 2009). Crop wild relatives, as well as relative cultivated species, are abundant sources for desired phenotypes in plant breeding (Gur and Zamir 2004; Zhang et al. 2017) and interspecific crossing can be a useful strategy also for basil breeding (Ben-Naim et al. 2018). Considering the variation in essential oil composition among basil cultivars and species, an outcome of undesired aroma originated in one of these species might be dragged a by-product during a breeding process that is trying to fix another important trait. Breeders must bear in mind the importance of the desired aroma and control it during the breeding process.

10.3.3 Experimental Crosses

Given the high variation in aromas of various basil germplasm, experimental crosses carry the potential to elucidate the inheritance and genetic nature of this complex trait of aroma profiles. Putievsky et al. (1999) crossed several cultivars and followed the essential oil composition in the F_1 and F_2 generations. In some of the crosses, the progenies showed transgressive phenotypes in which linalool or methyl chavicol composed more than 90% of the essential oils while only present in moderate levels in the parental lines. Interestingly, the inheritance of the essential oil composition was cross dependent. When eugenol chemotypes were crossed with methyl chavicol chemotypes, some progeny showed dominancy of high eugenol, some showed dominancy of high methyl chavicol, and others showed codominancy. Moreover, in one instance, in which two low eugenol/high methyl chavicol lines were crossed, progenies were high in eugenol and low in methyl chavicol indicating epistasis interaction. Low linalool phenotypes became dominant in two different crosses. Intermediate geraniol levels, which are at low levels in most cultivars, came out to be recessive in two crosses. In a different study, using a series of test crosses investigating the inheritance of phenylpropanoid volatiles of basil essential oil, it was found that methyl chavicol was dominant over eugenol, which is dominant over camphor (Gupta 1994). This might partially explain the low number of cultivars showing camphor chemotype. Analysis of the F_2 generagion of a different cross between the eugenol chemotype, "Perrie," and the methyl chavicol chemotype, "Cardinal," showed a codominancy between eugenol and methyl chavicol with a single biallelic locus determining a 1:2:1 segregation ratio (Dudai et al. 2018). Interestingly, the progeny also accumulated chavicol that was not accumulated in the parental lines. The difference in the observed inheritance mechanism in these two studies might reflect the advancement in volatile sampling and analysis techniques between 1994 (Gupta 1994) and 2018 (Dudai et al. 2018). Another explanation may be different inheritance mechanisms recorded in each of the studies since different parental lines were used for the different chemotypes. The "Perrie" × "Cardinal" cross (Dudai et al. 2018) also provided evidence for epistatic interaction where the minor component fenchone, which was not found in any of the parents, was accumulated in some of the progeny (Dudai et al. 2010). This "silent metabolism" phenomenon, that was also reported in other species (Lewinsohn and Gijzen 2009), should be taken into consideration in breeding processes as the outcome might be an undesirable aroma. Many breeding efforts are allocated to develop cultivars with resistance or tolerance to various biotic and abiotic stresses. Since in many cases the breeding line that donates the resistance phenotype is not of a similar chemotype, an undesired aroma might be a significant obstacle. Furthermore, even if the chemotypes are the same, a "silent metabolism" phenomenon might have a crucial effect on the final aroma, especially when minor components are ignored.

10.3.4 Biosynthesis Directs Chemical Diversity

The biosynthetic pathways are the heart of the genetics dictating the chemical quality of basil. The essential oil of basil is accumulated in its glandular trichomes located on the surface of the leaves (Werker et al. 1993; Gang et al. 2002b). The ability to isolate these trichomes and specifically check gene expression and enzymatic activities (Lewinsohn et al. 2000; Iijima et al. 2004b) has enabled the characterization of important genes and enzymes in the biosynthetic pathways. Homologous genes or allelic variants may cause different metabolic profiles and can be utilized for breeding proposes. Two major groups of compounds compose basil aroma: (i) phenylpropanoids and (ii) terpenoids. Research to elucidate the active genes and enzymes of these pathways in basil benefited from the extensive studies that have been performed on other plant species, like mint and clarkia (Bohlmann et al. 1998; Wang and Pichersky 1998).

The phenylpropanoids, eugenol and methyl chavicol, which account for two different basil chemotypes, are biosynthesized in parallel pathways originating from the same precursor, L-phenylalanine (Fig. 10.7). Two homologous *O*-methyl transferase (OMT) genes isolated from basil (line EMX-1) encode for enzymes with opposite substrate preferences; EOMT1 utilizes eugenol for methyl eugenol production, whereas CVOMT1 prefers chavicol for methyl chavicol production

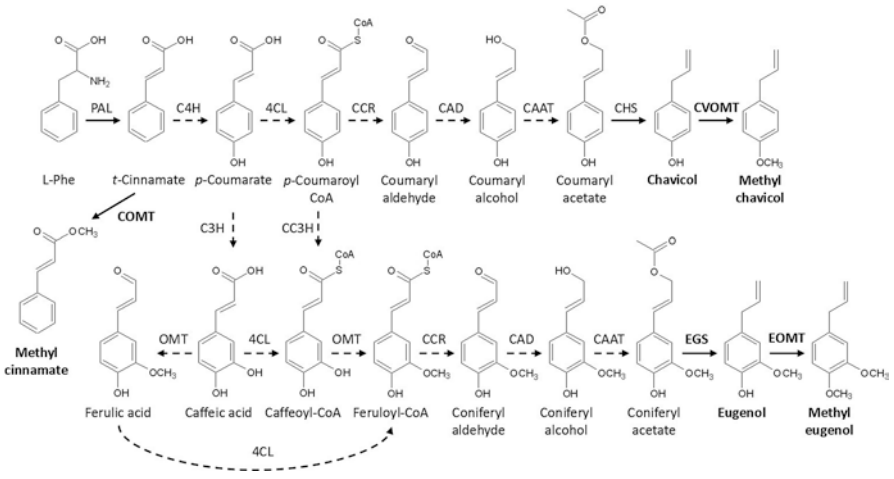

Fig. 10.7 Proposed biosynthetic pathways for phenylpropanoid biosynthesis in basil. Solid arrows represent reactions demonstrated in basil. Bold enzyme names depict enzymes which their encoding genes where characterized from basil. Bold compound names depict volatiles found in basil essential oil. PAL phenylalanine ammonia lyase, C4H *t*-cinnamate 4-hydroxylase, 4CL *p*-coumarate CoA ligase, CST *p*-coumaroyl-CoA:shikimic acid *p*-coumaroyl transferase, CS3'H *p*-coumaroyl-CoA shikimate 3'-hydroxylase, CCR cinnamoyl-CoA reductase, CAD cinnamyl alcohol dehydrogenase, CAAT coniferyl alcohol acetyltransferase, EGS eugenol synthase, EOMT eugenol *O*-methyltransferase, CHS chavicol synthase, CVOMT chavicol *O*-methyltransferase, OMT *O*-methyltransferase, COMT *t*-cinnamate *O*-methyltransferase, CoA coenzyme A

(Gang et al. 2002a). A single C ➔ T nucleotide substitution, which is the most common mutation in DNA, converted EOMT1 activity into CVOMT activity. This evolutionary mechanism might provide the basis for methyl chavicol basil chemotypes. Absence of CVOMT activity might shift the metabolic flux toward eugenol synthesis as was observed between methyl chavicol accumulating lines and eugenol accumulating lines (Lewinsohn et al. 2000; Gang et al. 2001). Methyl chavicol levels decreased in matured basil leaves, following the reduction of CVOMT gene expression and enzymatic activity (Deschamps et al. 2006). Gang et al (2002c) showed that eugenol chemotype basil harbors *p*-coumaroyl-CoA:shikimic acid *p*-coumaroyl transferase (CST) activity, and *p*-coumaroyl-CoA shikimate 3'-hydroxylase (CS3'H) activity (Fig. 10.7) suggesting these enzymes mediate eugenol biosynthesis, yet a coding gene was isolated only for the later enzyme. Another important gene in the pathway, eugenol synthase (EGS), was also characterized from basil (Koeduka et al. 2006) with its structure and mechanism of action determined by X-ray crystallography (Louie et al. 2007). Enzymatic production of chavicol from *p*-coumaryl esters also was demonstrated in basil (Vassão et al. 2006). A certain degree of correlation between EGS expression levels and eugenol/methyl eugenol levels was observed across different *Ocimum* species (Anand et al. 2016). Another phenylpropanoid common in cinnamon basil is methyl cinnamate in both *cis* and *trans* forms. The gene encoding the enzyme responsible for methyl cinnamate production, *t*-cinnamate *O*-methyltransferase (Fig. 10.7), has been identified and characterized from basil variety accumulating high levels of methyl cinnamate (Kapteyn et al. 2007). Its expression in this line was at least 50-fold higher than in the lines accumulating low or no methyl cinnamate.

The major terpenoids accumulating in basil are linalool, 1,8-cineole, and citral (a mixture of geranial and neral). Basil genes encoding various terpene synthase enzymes, including linalool synthase (LIS) and geraniol synthase (GES), have been functionally characterized (Iijima et al. 2004a; Iijima et al. 2004b). The balance between linalool and citral in the different chemotypes was shown to be driven by differential expression levels of LIS and GES genes, as well as by allelic variation (Iijima et al. 2004a). A 1-bp insertion caused a premature stop codon in the LIS gene from the citral chemotypes in comparison to the LIS gene from the linalool chemotype (Iijima et al. 2004a). A certain degree of balance between the phenylpropanoid pathway and the terpenoid pathway was noticed in both transcriptional, proteomic, and enzymatic levels of the enzyme phenylalanine ammonia lyase (PAL). The citral accumulating cultivars, which also show low levels of phenylpropanoids, demonstrated low PAL activity and gene expression (Iijima et al. 2004a; Xie et al. 2008). Other *Ocimum* species accumulate other terpenoids such as thymol, *p*-cymene, and several sesquiterpenes indicating the involvement of additional genes. For example, a β-caryophyllene synthase gene was isolated from *O. kilimandscharicum*, and its expression levels were in agreement with β-caryophyllene levels among five various *Ocimum* spp. (Jayaramaiah et al. 2016). Currently, the genetic mechanisms preventing the accumulation of these compounds in *O. basilicum* are unclear. Another field that needs further scientific exploration is the genetic

basis for the various enantiomers and their effect on basil product acceptability. Unraveling the genetic and biochemical mechanisms across basil species and retracing the history of basil breeding that led to the common chemotypes we are familiar with today would be of a tremendous benefit for future basil breeders and scientists.

10.4 Crop Improvement by Transgenic Means

Genetic engineering bears the potential to pinpoint a specific target to improve crop performance and quality (Ashraf and Akram 2009). Rice plants have been engineered to accumulate β-carotene, a pro-vitamin A, in the grains, hence elevating its nutritional value to address vitamin A deficiency in third world (Ye et al. 2000; Paine et al. 2005). The flavor of tomato fruits was manipulated by overexpressing a gene from lemon basil, geraniol synthase (GES), introducing new rose and lemongrass notes in the fruit (Davidovich-Rikanati et al. 2007). There are multiple potential targets in basil for manipulation including precise aroma profile, resistance to stresses, and control of leaf size and shape. One obstacle in genetic engineering is the need for reliable transformation and regeneration system (Altpeter et al. 2016). In basil, only few reports exist of successful transformations. In 2002, Simon and Deschamps (2002) reported on *Agrobacterium tumefaciens*-mediated transformation of a GUS reporter gene into two *O. basilicum* genotypes and two *O. x citriodorum* genotypes. They showed that the transgenic plants, regenerated from leaf explants, had similar aroma profile as the non-transgenic control. Another successful *A. tumefaciens*-mediated transformation of a GUS gene was performed with *O. gratissimum* using cotyledon node explants (Khan et al. 2015). While the latter work used the canonical cauliflower mosaic virus 35S promoter, the former work used a costumed designed promoter in the pBISN1 plasmid that was successful in mint transformation (Niu et al. 1998). Beyond proof-of-concept work was recently published by Wang et al. (2016), in which they used embryo rescue in combination with a cocultivation method to transform the *O. basilicum* embryos using *A. tumefaciens*. GFP and kanamycin were used for selection and a mint transcription factor, *MsYABBY5*, was overexpressed under the 35S promoter. The resulted transgenic basil accumulated lower levels of monoterpenes, sesquiterpenes, and eugenol similarly to the results achieved in mint (Wang et al. 2016). This promising direction with the recent advances in genome editing techniques by the CRISPR:Cas9 system (Brooks et al. 2014) and novel transformation methods (Zhao et al. 2017) can accelerate research and breeding of basil in the near future.

10.5 Summary

In their book *Breeding Field Crops* (Poehlman et al. 1995), the authors Poehlman and Sleeper stated that "Plant breeding is the art and science of changing the genetics of plants in order to produce desired characteristics." This statement could not be more precise in the case of sweet basil, in which the creativity of the breeder is required to account for the complex commercial uses of the crop and to achieve the harmonization of innumerable phenotypic traits. These include primarily volatile composition and aroma combined with resistance to diseases and pests; tolerances to heat or chilling injuries; appropriate leaf size, shape, color, and aesthetics; time to maturity; and other general or industry-specific traits that contribute to yield and quality. Thus, breeders of sweet basil seldom can emphasize their efforts on a single phenotypic trait. Rather, a wide-spectrum strategy is required to have sweet basil fulfil its various industrial and commercial purposes. The recently published genome of *O. tenuiflorum* (Rastogi et al. 2015) and the forthcoming publication of *O. basilicum* genome (Dudai et al. 2018) will provide the necessary resolution and assist to better understand the genetic basis of multiple important traits and will stimulate many breeding efforts. Lastly, the majority of the breeding programs aim for the growers' needs, such as increased yield weight, resistance to diseases and pests, time from flowering to harvest, etc. However, despite the industrial requirements, breeders should not forget the consumer needs as a factor in the breeding process, who seeks flavor, aroma, and other relevant quality traits on his dinner plate.

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Chapter 11
Origanum majorana L. (Marjoram)

Brigitte Lukas and Johannes Novak

11.1 Botany (Taxonomy, Origin, Distribution, Cytology, Plant Description)

A short description of family relationship, genus taxonomy and further characteristics of the genus *Origanum* is already given in the oregano chapter. *Origanum majorana* L. (syn. *Majorana hortensis* Moench) is a member of section Majorana (group B), together with three other *Origanum* taxa of commercial importance as oregano: *O. onites* L., *O. syriacum* L. and *O. dubium* Boiss. Section Majorana constitutes a prime example of the taxonomic difficulties within *Origanum*. The species boundaries of *O. syriacum*, *O. majorana* and *O. dubium* are not entirely clear and their differentiation relies on subtle morphological differences like indumentum and size and shape of leaves. *O. majorana* and *O. dubium* were treated as one species in Ietswaart's genus revision (Ietswaart 1980) and in the Flora of Turkey (Ietswaart 2008), but then as separate species in the Flora of Cyprus (Ietswaart 1985). The two-species concept was further supported by the results of Lukas et al. (2013).

O. majorana s. s. has a very specific aromatic quality characterized by volatiles containing high percentages of bicyclic monoterpenoids (sabinyl compounds, mainly sabinene, *cis*- and *trans*-sabinene hydrate and *cis*-sabinene hydrate acetate). *O. majorana* s. s. is endemic to Cyprus where it grows often on dry limestone, in garigue or open *Pinus* forests, from sea level up to 1000 m. The bushy shrub is up to 100 cm high. Leaves (2–25 mm long and 2–15 mm wide) are distinctly petiolate, oblong, obovate, suborbicular or subspathulate, with rounded or subacute apices and entire margins. Leaf lamina is usually thinly or densely grey pubescent with short, adpressed, crispate hairs. The inflorescences are composed of spikes consisting

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of flowers with a one-lipped, sheathlike, gland-dotted and tomentellous calyx (2 mm and 1.5 mm wide) and a white, pubescent, thinly gland-dotted corolla (about 2 mm long and 1.5 mm wide at apex) (Ietswaart 1985). Ietswaart (1985) distinguished two varieties of marjoram: var. *tenuifolium*, the wild crop relatives, and var. *majorana*, the cultivated sweet or knotted marjoram that differs to some degree from the wild type (e.g. larger and more greenish leaves (Novak et al. 2008b)). For culinary and pharmaceutical purposes, various cultivars of sweet marjoram are cultivated all over the world.

11.2 Economical Use (Plant Parts Used, Wild Collection/ Cultivation, Domestication, Valuable/Undesired Plant Secondary Compounds, Main Production Areas, Economical Valuation/Parameters)

11.2.1 Bioactivity

Origanum majorana L., sweet marjoram, has been used for a variety of purposes in traditional medicine. For its essential oils a wide range of pharmacological activities, besides other antioxidant, antibacterial, antifungal, insecticidal, anti-inflammatory, cardioprotective, hepatoprotective, gastroprotective, antineurodegenerative, antimetastatic or antitumor activities, have been reported (reviewed in Hänsel 1993; Bina and Rahimi 2017). Infusions of Majoranae herba were described to have antiviral activity (Hänsel 1993). However, due to the taxonomic difficulties within the *Origanum* section *Majorana* and the different species concepts of *O. majorana* used, the correct assignment of the plant material investigated is at least sometimes doubtful. In case essential oils of investigated plants were characterized as carvacrol rich, or test plants were described as purple flowering, it can be assumed that the respective investigation was based rather on plant material of *O. dubium* or a hybrid with *O. vulgare* (*O.* x *applii* Boros or *O.* x *majoricum* Cambessedes) than on plant material of *O. majorana* in sensu stricto.

11.2.2 Valuable Plant Secondary Compounds

Essential oil compounds: within *Origanum*, *O. majorana* exhibits an exceptional chemotype almost lacking the oregano typical cymyl compounds and usually accumulating large amounts of sabinyl compounds, namely, *cis-*/*trans*-sabinene hydrate and *cis-sabinene* hydrate acetate (Fischer et al. 1987; Novak et al. 2008b; Nowak and Ogonowski 2010). *Cis*-sabinene hydrate and its acetate are responsible for the

typical 'marjoramy' aroma, while *trans*-sabinene hydrate has no typical 'marjoramy' properties' (Fischer et al. 1988; Lossner 1968). Component rearrangements during distillation ore drying process are responsible for qualitative and quantitative changes in the essential oil (e.g. higher contents of terpinen-4-ol, α-terpinene and γ-terpinene) (Fischer et al. 1988; Hänsel 1993).

In natural populations of Cyprus three chemotypes were described, besides the standard marjoram sabinyl type, a rare α-terpineol chemotype and a mixed sabinyl/α-terpineol chemotype were present (Novak et al. 2008b). Elevated α-terpineol content, compared to the essential oils of the different cultivars, may be a special characteristic of the natural marjoram populations on Cyprus.

Literature survey indicates the presence of various flavones and flavone glycosides (e.g. diosmetin, vitexin, orientin, dinalin, thymonin, luteolin-7-O-glucoside, apigenin-7-O-glucuronide, scutellarein-6,4′-dimethylether). Further compound groups identified in *O. majorana* are Lamiaceae tannins and phenol carbon acids (e.g. caffeic acid depsides, caffeic acid, rosmarinic acid chlorogenic acids) and triterpenes and sterols (e.g. oleanolic acid, ursolic acid, sitosterol) (Hänsel 1993)).

11.2.3 Undesired Compounds

Arbutin is due to its high biological activity undesired in food (McDonald et al. 2001). It was found in *O. majorana* at significant levels of up to 118 mg/g dry mass in Cypriot wild populations of *O. majorana* and up to 40 mg/g dry mass in commercial samples of cultivated marjoram (Lukas et al. 2010).

11.2.4 Uses and Production

Fresh or dried leaves and flowers of the plants are used for culinary purposes or essential oil production. Due to the limited geographical distribution of the native species on Cyprus, all of the material in trade derives from cultivation. Germany imported in 1990 about 500 t, the Netherlands 400 t, France 500 t, the United Kingdom 250 t and the USA 450 t (Maftei 1992). Those figures are already quite old and import may have increased rather than decreased. However, the figures show the importance of this species. Egypt is by far dominating marjoram production with a production volume of 3800 t in 2011, of which North America imported 30% and Europe 65% (Trumpy 2012). Further production areas are in Tunisia, Morocco, Germany, Hungary, the Russian Federation, the USA, China and India. The essential oil of marjoram (CAS# 8016339) has a lower market importance as the herb. Main producing countries are Egypt, Morocco and Turkey. The flavour is described as herbal, minty, with a green curry note (Mosciano 1991).

11.3 Breeding

11.3.1 Flower and Pollination Biology

Marjoram is – as oregano – a long-day plant. All plants flowered at 16 h day length. At 13 h day length, all genotypes with the exception of one out of five breeding lines flowered, but plants were less vigorous than under 16 h day length. At 10 h day length, marjoram did not flower. Associated to this flowering behaviour, also the essential oil composition was significantly influenced by the length of the daylight (Circella et al. 1995).

11.3.1.1 Hybridizations

Within the genus *Origanum*, hybridizations are quite frequent. Natural species hybrids with marjoram described in the revision of the genus *Origanum* (Ietswaart 1980) are *O.* x *applii* (Domin) Boros (*O. majorana* x *O. vulgare* ssp. *vulgare*) and *O.* x *majoricum* Cambessedes (*O. majorana* x *O. vulgare* ssp. *virens*). *O.* x *majoricum* has commercial importance in cultivation in South America. However, due its high carvacrol content in the essential oil *O.* x *majoricum* is used as oregano, not as marjoram (Tabanca et al. 2004; González et al. 2017).

Appl (1928) found in 1923 a plant in a marjoram field, which he recognized as a hybrid between white flowering *O. majorana* and dark red flowering *O. vulgare*. After overwintering a vegetative cutting and multiplying it vegetatively to 50 plants, he obtained seeds for 20,000 plants on which he studied the Mendelian inheritance of growth habit, flower colour, colour of the stem, shape of the calyx, hairiness, winter hardiness, length of the twigs and length of the inflorescences. The flower colour showed a segregation ration of 1:2:1 (white/light red/dark red). Colours of bracts and stems were coupled to flower colour. Winter hardiness was determined by a recessive gene. Although promising, no further breeding works to obtain a winter hardy marjoram were undertaken.

A good morphological tracer for species hybrids is the shape of the calyx, since most *Origanum* species have a calyx with 5 equal teeth, while *O. majorana* has a 1-lipped calyx (3 teeth grown together to the ‘lip’ and 2 teeth reduced). Five genes were identified responsible for this peculiar calyx shape (Novak et al. 2002b). Hybrids show many different intermediate forms. Therefore, they are easy to identify.

11.3.1.2 Gynodioecy, Male Sterility

Origanum is a gynodioecious genus, which often has female (male-sterile) and hermaphrodite individuals together in a population ranging in natural populations between 1% and 62% (Kheyr-Pour 1980) (confer also to the oregano chapter). In marjoram, the percentage of male-sterile plants is even higher ranging in 20 accessions, foreseen as pollinators in a hybrid-breeding project, from 3% to 100% with 16 accessions over a male sterility of over 80% (Langbehn et al. 2001).

11.3.2 Propagation Strategies

11.3.2.1 Vegetative Propagation

Propagation by cuttings Propagation of marjoram by stem cuttings is similar to oregano. Rooting powders with indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) increase root formation. After treatment with growth hormone, cuttings can be directly put into soil when kept subsequently under humid condition. A division of the rootstock, preferentially in spring, is also possible.

In vitro propagation Rapid multiplication of marjoram from callus and nodal stem explants is possible on Murashige and Skoog's medium supplemented with 2 mg/l benzylaminopurine and maltose as source of carbon and yields up to 40 shoots. In the presence of 0.2 mg/l, indole-3-butyric acid shoots produced roots. Callus can be induced in stem explants when supplemented with 0.4 mg/l 2,4-dichlorophenoxy acetic acid. The callus itself can be differentiated in MS medium with 3 mg/l benzylaminopurine and 0.2 mg/l indole-3-butyric acid (Pai and Iyer 2000).

11.3.2.2 Generative Propagation

The spikes contain on average 6 (range 2–30) pairs of flowers and are arranged in groups of 3–5 at a branch. They are globose, ovoid or quadrigonus cylindrical 6 (range 3–20) mm long and approximately 3 mm wide (Ietswaart 1980). The nutlets (commonly called 'seeds') are small, egg shaped to spherical, 1.1 mm long and 0.5 mm wide with a thousand seed weight around 0.2 g and an optimum germination temperature of 20 °C (Heeger 1956). The insertion at the base is a little bit protruding. Seed colour changes during maturation from white over light brown to dark brown. White seeds are unripe and do not germinate, while light brown and dark brown seeds have the same germinability. A maximum of four seeds per flowers is possible. The number of seeds per spike is influenced by variety and harvest time as well as the interaction between these two factors (D'Andrea et al. 2001). Seed yields vary between 50 and 100 kg/ha under Central European conditions but can reach 400–800 kg/ha in Mediterranean countries, where marjoram can be kept as perennial plant (Heeger 1956).

11.3.3 Breeding Methods Applied

11.3.3.1 Conventional Breeding

Fast screening analysis methods In breeding, selection for or against plant secondary compounds is costly and time consuming. Therefore, any method optimizing analyses in time and costs is advantageous for breeding and often part of a breeding project. Near-infrared spectroscopy (NIRS), for example, is a good technique to speed up analysis time and lower analysis costs. The essential oil content

and the major essential oil compounds of marjoram could be analysed simultaneously by NIRS with a measurement time of 1 minute only (Schulz et al. 1999). Essential oil content and composition is normally analysed by distillation (content) and subsequent gas chromatography (GC) (composition). Distillation is a time-, labour- and sample material-consuming process. Solvent extraction, however, can be fast, better parallelized and adapted to small amounts of plant material. By quantitatively analysing the essential oil compounds of solvent extracts, distillation can be circumvented (Novak et al. 2002a, b). Also analysis time of GC can be improved by using an approach called 'fast GC' by combining high pressures with narrow bore columns (Lamien-Meda et al. 2009).

Hybrid breeding In marjoram, a stable cytoplasmic sterility (CMS) was identified (Fig. 11.1). No restorer could be found (Novak et al. 1999). The CMS was used to develop a hybrid system. For the maintainer system, five breeding lines already selected for aroma and yield were used to cross with the CMS line for some generations in order to obtain good female lines with an already good overall performance and to establish a maintainer system for the valuable CMS. To establish the pollinator side, 49 accessions of marjoram were evaluated for sensorial parameters (aroma quality, essential oil content and composition and leaf colour). These parameters were combined with selection for high-yielding plants (plant height and diameter, herb/stem ratio, yield of herb). Since optimal harvest time for marjoram is at the beginning of flowering, days to flowering were recorded as well. Of these 49 accessions (one was a misnamed accession and

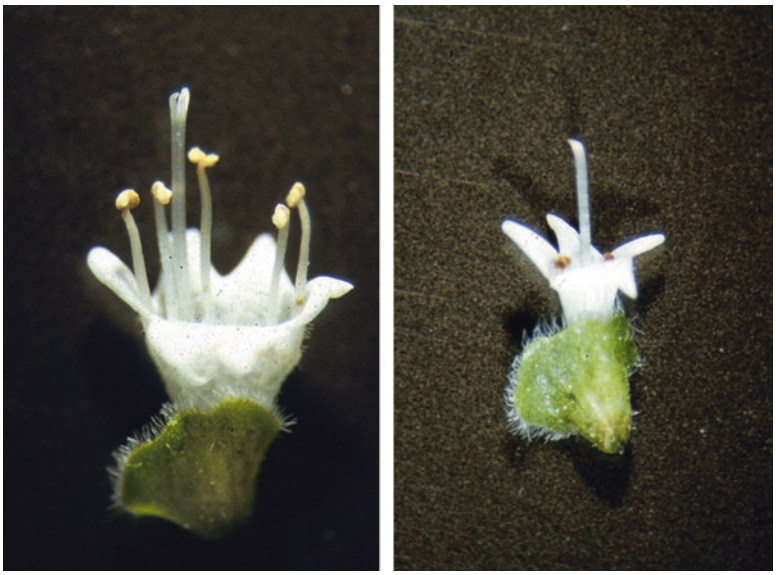

Fig. 11.1 Male-fertile (left) and male-sterile flower (right) of *O. majorana*. The anthers of the male-sterile flowers are stunted, but also the flower size is smaller

turned out to be *O. vulgare*), the best 20 accessions were selected as pollinator partners. The herb yield could be predicted by the maximum bush diameter and the days between planting and harvesting ($R^2 = 0.64$) (Langbehn et al. 2001). In herb yield, the best combination was 17% superior to the standard variety 'Marcelka'. The essential oil content of the best combination was more than doubled compared to the standard variety (2.6% compared to 1.24% in 'Marcelka') (Pank et al. 2002). The combinations were also sensorially evaluated (colour, smell (Table 11.1) and taste (Table 11.2)). From the sensorial perspective (Table 11.3), all replications of one hybrid combination were described as 'bitter', a clear off-flavour for marjoram. The parents of this combination, however, were not bitter. In another case, all hybrid combinations of a pollinator accession, described as bitter, were evaluated as bitter with the exception of one combination. In a 2 years' testing, smell and taste correlated well within a year ($r = 0.73$ and $r = 0.89$ in 1998 and 1999, respectively), but correlation between the years was low. However, taste correlated also well with the colour in the respective year ($r = 0.59$ and $r = 0.68$ in 1998 and 1999, respectively) (Novak et al. 2002c).

Table 11.1 List of actually or previously registered cultivars of marjoram

Country	Cultivar	Register type[a]	Variety status	Registration year
Belarus	*Malakhit*	NLI	Registered	2007
Germany	*Marietta*	PBR	Surrendered	1997
	Erfo	PBR	Surrendered	1997
	Tetrata (tetraploid)	PBR	Surrendered	1999
	Max	PBR	Registered	1997
Hungary	*Magyar*	NLI	Registered	2000
	Francia	NLI	Registered	1959
Netherlands	*Sapphire*	PBR	Expired	1993
	Max	COM	Registered	1999
	Petrofsky	PBR	Expired	1994
Poland	*Miraz*	NLI	Terminated	1982
European Union	*Lizbell*	PBR	Surrendered	2003
Russian Federation	*Kulinar*	NLI	Registered	2014
	Massandra	NLI	Registered	2015
	Skandi	NLI	Registered	2003
	PikantnyJ	NLI	Registered	2003
	Termos	NLI	Registered	2004
	Sadovnik	NLI	Registered	2018
	Bajkal	NLI	Registered	2004
	Lakomka	NLI	Registered	2002
	Tushinskij Semko	NLI	Registered	2000
	Prekrasnyj	NLI	Registered	2014
Slovak Republic	*Marcelka*	NLI	Registered	1967
	Kutnoshorska, krajova	NLI	Surrendered	1941
	Bzenecka, krajova	NLI	Surrendered	1941

[a]*COM* commercial registers, *NLI* national list, *PBR* plant breeders' rights

Table 11.2 Scores and their description for appearance of marjoram (based on TGL 37785/03∗)

Category	Description
0	Spoilt
1	Colour deviating, very high content of small stalks and stems, very high content of tiny parts
2	Greyish green to greyish brown round leaves, parts of leaves and flowers too high, high content of small stalks and stems, very high content of tiny parts
3	Green to brownish green round leaves, parts of leaves and flowers slightly augmented, small stalks augmented, stems in a less degree, high content of tiny parts
4	Green to greyish green round leaves and parts of leaves and flowers, small stalks only sporadic, low content of tiny parts
5	Green to greyish green round leaves and parts of flowers, small stalks only sporadic, very low content of tiny parts

∗Technical standards, quality specifications and technical delivery conditions of the German Democratic Republic (GDR)

Table 11.3 Scores and their description of smell and taste of marjoram (based on TGL 45611∗)

Category	Description
smell	
0	Spoilt
1	Not typical, strange
2	Hardly typical, impure, hardly aromatic, not spicy
3	Still typical, not completely pure, slightly aromatic, not so spicy
4	Typical, pure, aromatic, spicy
5	Typical, pure, fully aromatic, spicy
taste	
0	Spoilt
1	Not typical, strange
2	Hardly typical, impure, hardly aromatic, strong bitter note, not spicy, haylike
3	Still typical, not completely pure, slightly aromatic, bitter note somewhat dominating, not so spicy, slightly haylike
4	Typical, pure, aromatic, mild bitter note, spicy
5	Typical, pure, fully aromatic, mild bitter note, spicy

∗Technical standards, quality specifications and technical delivery conditions of the German Democratic Republic (GDR)

Genotype by environment interaction (G × E, 34 genotypes on three locations) was not significant, while for plant height, flowering time and essential oil content, G × E was significant. For leaf colour (L∗a∗b∗ system), G × E was only for the coordinate ∗a not significant (Novak et al. 2003).

11.3.3.2 Molecular Tools

Biosynthesis One enzyme, sabinene hydrate cyclase, is responsible for the formation of *trans*-sabinene hydrate, *cis*-sabinene hydrate and *cis*-sabinene hydrate acetate in marjoram (Hallahan and Croteau 1988, 1989). *trans*-Sabinene hydrate ('non-marjoramy') and the sum of *cis*-sabinene hydrate and *cis*-sabinene hydrate (both 'marjoramy') are always present in a fixed ratio and can therefore not be influenced by selection (Hallahan and Croteau 1988; Novak et al. 2002a).

DNA markers developed for *Origanum* (confer to the chapter oregano) will work in most cases well for marjoram. For example, all microsatellites developed from *O. vulgare* from Novak et al. (2008a) and Ince et al. (2014) cross amplified with marjoram. The SRAP and EST-SSR markers of Taşcıoğlu et al. (2018) were successfully applied for all different sections of the genus *Origanum*, including *O. syriaca*, which is very closely related to *O. majorana*.

11.3.4 Breeding Results Achieved/Economical Transfer (Registered Cultivars/Patents, Trial Results)

For a long time, two group cultivars were distinguished, 'bud marjoram' (or 'German marjoram') and 'leaf marjoram' (or 'French marjoram'). Just a few cultivars were registered of which many are not protected any more (Table 11.1).

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Chapter 12
Origanum vulgare L. and *Origanum onites* L. (Oregano)

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12.1 Botany (Taxonomy, Origin, Distribution, Cytology, Plant Description)

Within the Lamiaceae, the genus *Origanum* L. is in the large subfamily Nepetoideae, tribe Mentheae, with *Thymus* L., *Thymbra* L. and *Micromeria* Benth. as some of the closest relatives (Bräuchler et al. 2010; Ietswaart 1980; Li et al. 2016). *Origanum* has a complex taxonomy complicated by a considerable amount of gradually merging or intermediate morphological characters and hybridization. More than 300 scientific names have been given to the presently about 70 recognized *Origanum* taxa (Skoula and Harborne 2002) reflecting the difficulties of species delimitation. Many local floras are based on divergent taxonomic concepts. Among the different genus concepts used the taxonomic revision of Ietswaart (1980) is the most widely accepted. Based on the analysis of morphological characters (e.g. size and shape of bracts, calyx teeth and corollas) he recognized three groups, ten sections and 38 species. Since his monograph six new *Origanum* species were described (Carlström 1984; Danin 1990; Danin and Künne 1996; Duman et al. 1995; Dirmenci et al. 2018) raising the number of species to 44. Details of Ietswaart's taxonomic revision are still debated. Recent molecular analyses revealed high complexity also at the molecular level supporting the hypothesis that hybridization has been an important speciation mechanism in the genus (Lukas et al. 2013; Katsiotis et al. 2009; Taşcıoğlu et al. 2018).

Origanum species are usually subshrubs or woody perennial herbs that usually grow up to heights of about 80 cm and have ovate leaves with white or purple flowers. The aerial organs of the plant, especially stems, leaves and bracts, are often densely covered by glandular and non-glandular hairs. The glandular hairs secrete

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an essential oil with a very characteristic, well-known odour. Most of the *Origanum* species are locally distributed within the Mediterranean where they usually grow in mountainous areas (sea level up to 1800 (−4000 m)) on dry, rocky, often calcareous soils, often in coniferous or mixed woods in partial shade. About 70 percent of the *Origanum* species are endemic and occur on an island or mountain (group) only (Ietswaart 1980).

Origanum species are diploid with $2n = 30$ (32) chromosomes (Bakha et al. 2017; Ietswaart 1980; Yildiz and Gucel 2006; Bakha et al. 2017; Morone-Fortunato and Avato 2008). *Origanum* species have small genomes (e.g. 1.43 pg/2C in *O. vulgare* ssp. *virens* (Bakha et al. 2017)).

The term oregano usually summarizes those *Origanum* species or chemotypes that are especially rich in phenolic monoterpenoids carvacrol and/or thymol. Together with their precursors γ-terpinene and *p*-cymene they were denominated 'cymyl pathway' (Skoula and Harborne 2002). Today, most of the commercially traded oregano plant material derives from two species, *O. vulgare* and *O. onites*. With *O. syriacum* and *O. dubium* there are two more species of high local importance.

12.1.1 Origanum vulgare L. (Group C, Section Origanum*)*

Within the genus, *O. vulgare* possesses by far the largest distribution area and can be found wide over Europe and in parts of Asia and North Africa. *O. vulgare* is a woody perennial with heights up to 100 cm and pilose, hirsute or glabrous (sometimes glaucous) stems. The leaves (6–40 mm long, 5–30 mm wide) are petiolate to subsessile, ovate, oval or roundish with acute or obtuse tops and entire or serr(ul)ate margins. Leaves are usually hirsute or pilose to glabrous – sometimes glaucous with hardly visible to rather conspicuous sessile glands. Stems (partly), leaves, bracts and calyces are sometimes (deeply) purple coloured (Ietswaart 1980). As can be seen from Ietswaart's species description *O. vulgare* is rather variable in its morphological characters leading him to differentiate six subspecies. The three subspecies appearing in the more northern parts of the distribution area (ssp. *vulgare*, ssp. *virens* (Hoffmannsegg et Link) Ietswaart and ssp. *viride* (Boissier) Hayek) are usually poor sources of volatiles. The subspecies on the southernmost range of the distribution area (ssp. *glandulosum* (Desfontaines), ssp. *hirtum* (Link) Ietswaart and ssp. *gracile* (Koch) Ietswaart) are typically good sources of essential oils (Kokkini 1997; Lukas et al. 2015).

12.1.2 Origanum onites L. (Group B, Section Majorana*)*

Origanum onites is distributed in southern Greece, on Crete and other Greek islands and in western and southern Turkey. One isolated population is known from Sicily (Ietswaart 1980). The stems of *O. onites* are up to 100 cm long; the

younger parts are hirsute and glandular pilose. Leaves (3–22 mm long, 2–19 mm wide) are shortly petiolate (the lower ones), ovate or oval, with acute or acuminate tops and often remotely serr(ul)ate margins. The leaves are usually hirsute and glandular pilose. Compared with many other *Origanum* species the morphological characteristics of *O. onites* are rather homogeneous and the species can usually be easily discerned.

12.1.3 Origanum syriacum L. (Group B, Section Majorana)

Origanum syriacum has a large distribution area in the eastern Mediterranean and can be found in southern Turkey, on Cyprus, in Syria, Lebanon, Israel and Jordan and on the Sinai Peninsula (Ietswaart 1980). The stems of *O. syriacum* are up to 90 cm long; the younger parts of the stems are tomentose or hirsute and somewhat glandular pilose. The leaves (3–35 mm long, 2–23 mm wide) are clearly petiolate to (sub)sessile, ovate, oval or heart shaped, with obtuse to acuminate tops and entire, remotely crenulated or serrulate margins. Size, shape and indumentum of leaves are rather variable. The leaves are usually green or whitish, slightly hirsute-tomentose to densely tomentose and usually densely covered with sessile glands (Ietswaart 1980). According to the high morphological variability present within *O. syriacum*, three varieties, var. *syriacum*., var. *bevanii* Ietsw. and var. *sinaicum* Ietsw., were defined (Ietswaart 1985). *Origanum syriacum* shows a close morphological and genetic relationship to *O. majorana* (Ietswaart 1980; Lukas et al. 2013) but the two taxa differ significantly in their essential oil chemotype that is rich in cymyl compounds in *O. syriacum* and rich in sabinyl compounds in *O. majorana*.

12.1.4 **Origanum dubium** *Boiss. (Group B, Section Majorana)*

Origanum dubium is distributed on Cyprus (Paphos Forest) and the adjacent parts of Turkey (chiefly Amanus mountain range) (Ietswaart 1985). The stems of *O. dubium* are up to 100 cm long; the younger parts of the stems are glandular, densely pubescent with short, crispate hairs, sometimes intermixed with few longer hairs. The leaves (5–25 mm long and 3–20 mm wide) are shortly petiolate, generally subtending condensed, broadly and bluntly ovate or elliptic, with entire margins. The leaves are gland-dotted and canescent-furfurascent with short crispate hairs (Ietswaart 1985). *Origanum dubium* and *O. majorana* were long treated as one species (Ietswaart 1980, 1982), before extended morphological analysis (Ietswaart 1985) and molecular analysis (Lukas et al. 2013) supported the species status of *O. dubium*.

12.2 Economical Use (Plant Parts Used, Wild Collection/Cultivation, Domestication, Valuable/Undesired Plant Secondary Compounds, Main Production Areas, Economical Valuation/Parameters)

12.2.1 Bioactivity

Oregano has been used for many purposes in traditional medicine and in food because of its antimicrobial, antioxidant, expectorant, antispasmodic, carminative, antiviral, immunomodulatory and antiparasitic activities (Marrelli et al. 2018; Alagawany et al. 2018). The pronounced antimicrobial activity made oregano one of the most promising replacements for antibiotics in animal nutrition (Alagawany et al. 2018), but popularity as feed supplement goes way beyond this substitution (Giannenas et al. 2018).

12.2.2 Valuable Plant Secondary Compounds

Essential oil compounds the valuable plant secondary compounds of oregano, the phenolic monoterpenes carvacrol and thymol, are the main compounds in the essential oil of the cymyl chemotype. There is a huge variation in the relation of these compounds to other compounds, first in the relation to their precursors *p*-cymene and γ-terpinene, but also to other mono- and sesquiterpenes. The differences are due to genetic, seasonal influences and ontogenesis. So carvacrol/thymol is increasing during the summer months, while in winter, early spring *p*-cymene becomes higher (Johnson et al. 2004; Baranauskienė et al. 2013; Ozkan et al. 2010; Kokkini et al. 1997).

Rosmarinic acid is a caffeic acid ester with pronounced antioxidative and antiviral activities. In a collection of genebank accessions, Yan et al. (2016) found a variation between 7.2 mg/g DW and 41.3 mg/g DW. Shen et al. (2010) found a range between 14 and 64 mg/g in various *Origanum* species. The upper levels could make oregano to an interesting source for rosmarinic acid.

The anti-inflammatory active triterpenoids *oleanolic acid and ursolic acid* are present in many plant species. In oregano, they were found on average of 1.9 and 6.7 mg/g in 22 different species and varieties (Shen et al. 2010). *O. syriacum* was outstanding with 9.4 mg/g oleanolic acid and 24.1 mg/g ursolic acid on average of 7 accessions.

Thymoquinone is a compound with antioxidant, anti-inflammatory, analgesic and anticancer activity. In *O. syriacum* thymoquinone could be found in levels between 0.04% and 24% of the essential oil compounds (Lukas et al. 2009).

12.2.3 Undesired Compounds

Numerous chemotypes of the essential oil are known besides the cymyl type that includes carvacrol/thymol, which can be regarded as unwanted for an 'oregano-typical' use (de Martino et al. 2009; Morshedloo et al. 2018; Mastro et al. 2017).

Arbutin, a hydroquinone derivative, is used in cosmetics to lighten the skin and in medicine for its diuretic and urinary anti-infective properties. This compound was found in *O. dubium* at significant levels of 20.8 mg/g (Lukas et al. 2010). Due to the high activity, this compound is regarded as unwanted compound in food, although the levels in oregano are not alarming.

12.2.4 Uses and Production

The dried herb and the essential oil of the above-ground parts are the major traded goods from oregano. Until the turn of the millennium, almost all of the material in trade was wild collected. Since then, cultivation is increasing steadily, but wild collection still is the predominant supply of the market. Oregano became very popular after World War II when the Italian pizza became popular, first in America, then worldwide. Italy has a huge inland consumption. Therefore, the Italian production is consumed inland and no significant amounts are on the international market. First, Greece was the major supplier of the American market. Therefore, oregano originating from *Origanum* species is called 'Greek oregano' in trade, in contrast to 'Mexican oregano' originating from *Lippia graveolens*.

Besides Greece, Albania, Israel, Morocco, Lebanon and Argentina, Turkey is one of the main countries involved in production and export of oregano, where it is widely grown in the Aegean Region. Large-scale cultivation started with *O. onites* cv. Izmir in the province of Denizli in 1993 as alternative to tobacco and continued then in the provinces Manisa, Antalya, Isparta, Aydin, Mugla, Burdur and Balikesir (Koksal et al. 2010). Turkey exported about 10,000 tons in 2010 (Koksal et al. 2010) and about 15,000 tons of oregano (60 million $ worth) in 2014 (Sari and Altunkaya). In Turkey, only *O. onites* and *O. vulgare* are cultivated. Most of the Turkish oregano plant material collected from the wild and sold in markets derives from *O. minutiflorum*, *O. onites* and *O. vulgare* subsp. *vulgare* (Gürbüz et al. 2011).

Za'atar is a popular herbal mixture from the Near East consisting of dried and ground leaves of *O. syriacum* (za'atar), powdered seed coats of sumac (*Rhus coriaria*, Anacardiaceae) as acidulant and giving the mixture its typical red colour, salt, roasted sesame seeds and olive oil. Lebanon exported in 2008 dried oregano herbs, za'atar mix and oregano infusion herbs for a value of approx. USD 1.9 million. In 2011, the export market size increased to USD 2.4 million (20% in 3 years), of which 50% was exported as za'atar mix and 700 to 800 t as pure oregano herb. Oregano exports represent about 25% of all herb exports. Lebanon itself imports za'atar mix from Syria (70%) and Jordan (20%) (GEF-UNDP-LARI 2013).

Argentina produces about 600 tons per year on an area of 500 hectares, mainly in the provinces Mendoza, Cordoba and San Juan. Most cultivated species are here *O. vulgare* ssp. *viridulum* Nyman and *O.* x *majoricum* Cambessedes (a hybrid between *O. vulgare* ssp. *vulgare* and *O. majorana*). Exemplary names of varieties/ecotypes/clones are 'compacto', cordobés', 'criollo', 'mendocino', 'nativo', 'verde limón', 'negrito', 'peruano' and 'green Spanish' (Varela et al. 2014).

In the market of fresh herbs, oregano is one of the most important plants. Supply is here internationally from Israel via airfreight, but also inland production is of importance in the different countries.

12.3 Breeding

12.3.1 Flower and Pollination Biology

Flowers and flowering: In group C of the genus (section *Origanum*) the inflorescences are composed of spikes with (sub)sessile flowers consisting of a 2.5–4.5-mm-long hirtellous, pilosellous or glabrous calyx with five (sub)equal teeth and a 3–11-mm-long, pilosellous, purple, pink or white corolla. In group B of the genus (section *Majorana*) that includes besides *O. onites* also *O. syriacum*, *O. dubium* and *O. majorana* (marjoram), the spikes are arranged in a false corymb with flowers consisting of a one-lipped, 2–3-mm-long pilosellous calyx and a 3–7-mm-long 2-lipped, pilosellous, white corolla.

Oregano is a long-day plant. The Argentinian 'criollo ecotype' (*O. vulgare* ssp. *hirtum*) showed greater sensitivity to artificial lengthening of light hours than the 'compacto ecotype' (*O. vulgare* ssp. *vulgare*) and therefore earlier flowering (Davidenco et al. 2012).

12.3.1.1 Pollination

O. vulgare is known to be attractive to a wide range of flower-visiting insects. Its attractiveness is so high that the species was used as a baseline to compare the pollinator attractiveness of flowers of a range of ornamentals, where only a few percent of up to 69 observed species/varieties were more attractive than *O. vulgare* (Garbuzov et al. 2017).

12.3.1.2 Hybridizations

Hybridization in the genus *Origanum* is frequent and reported, e.g. between *O. libanoticum* x *syriacum* var. *bevanii* (*O.* x *adonidis* Mouterde), *O. majorana* x *vulgare* ssp. *vulgare* (*O.* x *applii* (Domin) Boros), *O. ehrenbergii* x *syriacum* var.

bevanii (*O.* x *barbarae* Bornmüller), *O. onites* x *vulgare* ssp. *hirtum* (*O.* x *intercedens* Rechinger), *O. onites* x *sipyleum* (*O.* x *intermedium* Davis), *O. scabrum* x *vulgare* ssp. *hirtum* (*O.* x *lirium* Heldreich ex Halácsy), *O. majorana* x *vulgare* ssp. *virens* (*O.* x *majoricum* Cambessedes), *O. microphyllum* x *vulgare* ssp. *hirtum* (*O.* x *minoanum* Davis), *O. bargyli* x *syriacum* var. *bevanii* (*O.* x *pabotii* Mouterde), *O. laevigatum* x *syriacum* var. *bevanii* (*O.* x *symeonis* Mouterde), *O. micranthum* x *vulgare* ssp. *hirtum* and *O. sipyleum* x *vulgare* ssp. *hirtum* (Ietswaart 1980).

Appl (1928, 1929) was the first to report in detail about the Mendelian inheritance of marjoram or oregano-typical traits in a hybrid between *O. majorana* and *O. vulgare*. Bariotakis et al. (2016) found in the hybrid *O.* x *intercedens* a reduced seed viability of 57% compared to 70% and 81% in the parent species *O. vulgare* ssp. *hirtum* and *O. onites*, respectively, indicating that – although at reduced levels – generative propagation of hybrids is possible.

Since the calyx shape is so different between the sections, it can be used to trace hybrids between the sections because of a multitude of intermediary forms. The calyx shape was analysed in a natural hybrid between *O. vulgare* and *O. majorana* and five different traits could be classified, each triggered by a single gene independent from each other. The five traits are (1) bell-shaped vs. tubular-shaped calyx, (2) angular vs. round teeth on the upper lip, (3) entire vs. denticulate upper lip, (4) fully developed vs. reduced lower lip and (5) entire vs. denticulate lower lip (Novak et al. 2002).

12.3.1.3 Gynodioecy, Male Sterility

Origanum is a gynodioecious genus, which often has female (male-sterile) and hermaphrodite individuals together in a population ranging in natural populations between 1% and 62% (Kheyr-Pour 1980). The evolutionary advantage of gynodioecy is the promotion of outbreeding that is depending on the ratio of male-sterile plants in a population. In plant breeding, male sterility can be used as an efficient tool in planned crossings. Two loci are responsible for gynodioecy in *Origanum*; a dominant factor *F* causes anther suppression and a recessive class *hh*. *HH* and *Hh* suppress factor F and lead to hermaphrodite flowers (Lewis and Crowe 1956). Kheyr-Pour (1980) found in progenies of three male-sterile individual plants one distinct cytoplasmic factor and for each individual plant another specific restorer gene. Male sterility of a fourth male-sterile plant was polygenic. Extending this study to more plants (Kheyr-Pour 1981), they found another four restorer genes and possibly also cytoplasmic differences. Interestingly, some plants with different genetic background for male sterility originated from the same natural population indicating a wide polymorphism in the genus.

12.3.2 Propagation Strategies

12.3.2.1 Vegetative Propagation

Propagation by cuttings Propagation of oregano by stem cuttings is not difficult. Rooting hormones like indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and Hormex (a rooting powder containing IBA) increased the number of roots per cutting and the rate of root formation (Kuris et al. 1980). After treatment with growth hormone, cuttings can be directly put into soil when kept subsequently under humid condition. A division of the rootstock, preferentially in spring, is also possible.

In vitro propagation The multiplication and growth of *O. vulgare* axillary buds after surface sterilization of the buds in a 20% sodium hypochlorite (4.9% active chlorine) solution for 20 min. was found to be best with 6 benzylaminopurine (BAP) concentrations between 0.5 and 1 mg/l. The culture medium consisted, besides BAP of the macronutrients according to Murashige and Skoog (1962), the micronutrients of Nitsch and Nitsch (Nitsch and Nitsch 1969), FeEDTA (25 mg/l), thiamine HCl (0.4 mg/l), myo-inositol (100 mg/l), sucrose (20 mg/l) and agar (7 g/l). Addition of auxin was not necessary because of early rooting, and the survival rate was 95% (Morone-Fortunato and Avato 2008). An in vitro co-cultivation of oregano (*O. vulgare*) with *Pseudomonas* spp. prevented physiological malformations associated with vitrification (Shetty et al. 1995) or hyperhydricity (Shetty et al. 1996).

12.3.2.2 Generative Propagation

Oregano has small seeds with a thousand seed weight of 0.1 mg (Gallagher 2014). Seed weight in *O. vulgare* is positively correlated to final germination percentage (Pérez-García et al. 2003).

An oregano plant produces a large numbers of seeds in autumn. There is some germination in autumn, but most of the emergence of seeds is in the following spring. Oregano produces large, persistent seed banks (Gallagher 2014). Oregano has a seed dormancy that can be overcome by chilling (4 °C for 8 weeks) or higher germination temperatures (22 °C and 12 °C day and night temperature, respectively) or a certain ratio between red and far-red light. So low temperatures and low-red to far-red light ratios in autumn prevent germination of oregano seeds in nature (van Tooren and Pons 1988). A temperature range between 15 and 20 °C with an optimal pH range of the aqueous medium between 6 and 7 was best for *O. elongatum* (Belmehdi et al. 2018), *O. compactum* (Laghmouchi et al. 2017) and *O. vulgare* ssp. *hirtum* (Thanos et al. 1995). High NaCl concentration negatively affects seed germination of *Origanum* (Belmehdi et al. 2018; Liopa-Tsakalidi et al. 2011; Laghmouchi et al. 2017). Oregano has an absolute light requirement for germination and germination can be promoted by green safelight or far-red light. Old seeds germinate better than fresh seeds (Thanos et al. 1995).

Seed production capacity was tested in Switzerland at 450 m a.s.l. and resulted in 10 kg/ha seeds in the first year and 300 kg/ha from the second year onwards with a germination capacity between 60% and 70%. Seed maturation took 40–50 days counted from the end of flowering (end of October) (Rey et al. 2002).

12.3.3 Breeding Methods Applied

12.3.3.1 Conventional Breeding

An electronic nose was used in selecting *O. vulgare* ssp. *hirtum* correlating well to the major compounds of the essential oil and therefore being a good instrument to be used in a breeding process (Bernáth et al. 2006).

Szabó et al. (2010) selected 20 elite plants of *O. vulgare* ssp. *hirtum* with a high essential oil content (7–8.6%) and a high carvacrol content in the oil (70–93%). Furthermore, they evaluated the correlation between the essential oil content and the density of the epidermal glandular hairs on the upper leaf side, the site of formation and storage of the essential oil in oregano. This correlation was not strong enough ($r \leq 0.5$) to be used as morphological marker selecting for high essential oil types.

O. dubium is very rich in essential oil (up to 8%) and therefore used to produce essential oil. Positive mass selection was applied by Turgut et al. (2017) in order to select high essential oil content clones for distillation. In a first step, they selected and propagated by stem cuttings 100 plants with an essential oil content higher than 4%. In these plants, they found a variation between 5 and 41 g dry biomass yield, 5–28 g dry leaf yield and 5–14% essential oil content with carvacrol rates in the oil between 72% and 88%. In a next step, they selected the 30 most interesting plants for further analysis (mean biomass 50 g, mean leaf yield 27 g, mean leaf/stem ratio 1.34, mean essential oil content 7.4% and a mean carvacrol content of 85%). Out of this intermediary step, they selected the best 10 plants for an exact field trial (randomized block design, 50 plants per plot in 3 replicates). Here, one clone combined a high leaf biomass yield (795 g/plot of 50 plants) with a high essential oil content of 11% and a carvacrol content in the oil of 85%.

In Greece, clones and native populations were evaluated and 34 individuals selected based on earliness in blooming, plant growth habit, density of foliage, branching density and type of inflorescence for further intensive breeding work (Goliaris et al. 2003). Sarrou et al. (2017) selected two types of families from *O. vulgare* ssp. *hirtum*, self-pollinated (SP) and open pollinated (OP) by developing half sib lines using a honeycomb breeding approach. At this selection stage, plants were selected based on essential oil content, carvacrol content in the essential oil, dry weight of leaves and flowers (biomass) and ratio of leaves/flower to stem. OP plants were superior in biomass production compared to SP plants in both experimental years. The most efficient, healthy and phenotypically superior 40 plants were followed in detail. Their essential oil content ranged between 6% and 11% and carvacrol from 52% to 87%.

12.3.3.2 Molecular Tools

Biosynthesis Seven terpene synthases for mono- and sesquiterpenes were identified in *O. vulgare* and their expression studied in two different cultivars. The amount of mono- and sesquiterpenes was predominantly regulated on the transcriptional level (Crocoll et al. 2010).

DNA markers such as random amplified polymorphic DNA (RAPD, (Katsiotis et al. 2009; Tonk et al. 2010), sequence characterized amplified regions (SCAR) (Marieschi et al. 2010) and amplified length polymorphisms (AFLP) (Ayanogç Lu et al. 2006; Bariotakis et al. 2016; Azizi et al. 2012), sequence related amplified polymorphism (SRAP) (Taşcıoğlu et al. 2018) and microsatellites (SSR) (Ince et al. 2014; Novak et al. 2008) were developed and applied in the genus *Origanum*.

12.3.4 Breeding Results Achieved/Economical Transfer (Registered Cultivars/Patents, Trial Results)

Simonnet et al. (2010) compared in 2 years on three locations three oregano varieties, namely, 'Carva', 'Origalia' and 'Bolier' (plant density of 70 cm × 30 cm) (Table 12.1):

- 'Carva' is a clone hybrid between *O. vulgare* ssp. *viridulum* and *O. vulgare* ssp. *hirtum* distributed by mediSeeds (Switzerland).
- 'Origalia' is a synthetic cultivar of clones, distributed by iteipmai, France.
- 'Bolier' is a population cultivar distributed by Hem Zaden, the Netherlands.

Cultivars for uses as medicinal and aromatic herbs:

- Argentina: *O. vulgare* 'Alpa Sumaj FCA-Inta', 'Aguanda FCA-INTA', 'Don Bastias'
- Australia: *O. vulgare* 'OREG04'
- Belarus: *O. vulgare* 'Malakhit', 'Greta'
- Bulgaria: *O. vulgare* 'Hebros'
- Germany: *O. vulgare* ssp. *hirtum* 'Vulkan'
- Israel: *O. syriacum* 'Tabor', 'Oren', 'Benotan', 'Carmeli'
- Russian Federation: *O. vulgare* 'Kudesnitsa', 'Karamel'ka', 'Mila', 'Slavnitsa', 'Feya', 'Nadezhda', 'Naryadnaya', 'Sibirskaya Melodiya', 'Raduga', 'Dushistyj Puchok', 'Zima', 'Organza', 'Severnoe Siyanie', 'Belaya', 'Medovyj Aromat', 'Arbatskaya Semko', 'Hutoryanka'
- Turkey: *O. onites* 'Taysi 2002', 'Ceylan 2002'
- USA: *O. vulgare* ssp. *hirtum* (essential oil rich), 'Greek Kaliteri', 'Hot and Spicy'

Table 12.1 Mean values from oregano variety trial in 2 years on three locations

Cultivar	Leaf/stem ratio	Biomass yield (leaves and flowers) [kg/ha]	Essential oil content [%]	Essential oil yield [l/ha]
Carva	52	2772	7.6	211
Origalia	45	2517	7.2	177
Bolier	50	2371	6.0	143

Ornamental cultivars have usually very low essential oil content and are of limited use for aromatic purposes or producing essential oil. Examples of ornamental cultivars are as follows:

- *O. vulgare* ssp. *vulgare*: 'Aureum', 'Aureum Crispum', Charm White', 'Max Royal', 'Compactum'
- *O. laevigatum*: 'Purple Charm', 'Herrenhausen', 'Hopleys', 'Rosenkuppel'
- *O. rotundifolium* Boiss.: 'Bellissimo', 'Kent Beauty', 'Juno', 'Lumino', 'Lulu', 'Sakore 001'

Fig. 12.1 Four different Origanum species in their natural habitat. O. onites (top left), O. syriacum (top right), O. majorana (bottom left), O. dubium (bottom right).

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Chapter 13
Petroselinum crispum (Mill.) Nyman (Parsley)

Frank Marthe

13.1 Botany (Taxonomy, Origin, Distribution, Cytology, Plant Description)

Parsley (*Petroselinum crispum*) belongs to the family Apiaceae (syn. Umbelliferae). Many species of this family are used for long time as medicinal and aromatic plants as well as for vegetables.

According to Hanelt (2001) the genus *Petroselinum* J. Hill, Brit. herb. (1756) 424, includes two species, *P. crispum* and *Petroselinum segetum* [L.] W.D.J. Koch (corn parsley). *P. crispum* occurs naturally in Western Europe from the British Isles to the Iberian Peninsula. Parsley comprises considerable variability according to its long and different use. von Linné (1753) described it as *Apium petroselinum*. Actually, parsley belongs to genus *Petroselinum* with the name *Petroselinum crispum* (Mill.) Nyman, Consp. fl. eur. 2 [1879] 309 (nomen in syn.) ex A.W. Hill, Hand-list herb. pl. Kew ed. 3 (1925) 122 Airy-Shaw in Kew Bull. (1938) 256.

The name of the genus *Petroselinum* was derived from the Greek word petra (Πέτρα) for rock or stone and Latin word selīnum for plants growing on stony soil. The name of the species *crispum* comes from the Latin crispus and means curled. Common names for parsley are *Arabian*, بقدونس (madanous); *Chinese*, 芫荽 (yánsuī); *French*, persil, turnip-rooted parsley (persil à grosse racine); *German*, Petersilie, turnip-rooted parsley (Wurzelpetersilie); *Italian*, prezzemolo; *Persian*, جعفری (jaaferi); *Portuguese*, salsa; *Russian*, петрушка (petruška); *Spanish*, perejil; *Turkish*, maydanoz; *Vietnamese*, Mùi tây.

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Common names combined with parsley exist meaning other species than *P. crispum* (e.g. Chinese or Japanese parsley for coriander (*Coriandrum sativum* L.)). Sometimes mitsuba or Japanese parsley (*Cryptotaenia canadensis* [L.] DC. ssp. *japonica* [Hassk.] Hand.-Mazz.) from Japan is confused with parsley. Beaked parsley, French parsley or gourmet parsley refers to chervil (*Anthriscus cerefolium* [L.] Hoffm.). Vietnamese parsley sometimes means Vietnamese coriander or rau răm (*Polygonum odoratum* Lour.) or water dropwort or cần nước (*Oenanthe javanica* [Blume] DC.) (Small 1997).

Two convarieties are differentiated in parsley. The convar. *radicosum* (Alef.) Danert in Mansf. Verzeichnis (1959) 322 with eatable swollen taproots and convar. *crispum* where the roots are not eaten. In all populations of convar. *radicosum* leaves are not crispy; they belong to var. *tuberosum* (Bernh.) Crov. Crispy leafed accessions of var. *erfurtense* Danert were not availible from gene banks. Parsley roots of convar. *radicosum* are used as vegetable. Among convar. *crispum* different leaf types exist: accessions with crispy leaves attributed to var. *crispum*, with non-crispy leaves to var. *vulgare* (Nois.) Danert and var. *neapolitanum* Danert also known as Neapolitan or Italian parsley with fleshy and longer petioles, respectively (Fig. 13.1). In the Mediterranean region, some cultivars are grown for these edible leaf stalks (Small 1997). Accessions of convar. *crispum* var. *silvestre* (Alef.) Danert, the wild type of parsley, were also not available from gene banks (Danert 1959).

The origin of parsley is doubtful, but comes probably from the eastern part of Mediterranean area or Western Asia, where natural variability is highest (Hanelt and IPK 2001). North Africa has also high variability (Ipor and Oyen 1999). The annual

Fig. 13.1 Parsley (*Petroselinum crispum*); (**a**) flat leaves of convar. *crispum* var. *vulgare*; (**b**) crisped leaves of convar. *crispum* var. *crispum*; (**c**) taproots of convar. *radicosum* var. *tuberosum*

accessions, which do not require vernalization, come from this region. Actually, parsley is grown at the subtropical and temperate zone around the world and escaped from cultivation and naturalized on many places around the world. It grows also naturally in the western part of the Mediterranean area and so it overlaps with the region of *P. segetum*. There is no information about natural or artificial bastards of both species.

The chromosome number of parsley is $2n = 2x = 22$ and of *P. segetum* 2n = 18 (Darlington and Wylie 1955). Own evaluation was done by flow cytometry for level of ploidy of 50 parsley accessions obtained from the federal ex situ collection of agricultural and horticultural plants at the Leibniz Institute of Plant Genetics and Crop Plant Research at Gatersleben, Germany (IPK). For all of them the diploid status ($2n = 2x = 22$) was detected. The DNA amount of diploid *Petroselinum crispum* was measured as 2C = 4.0 pg (Bennett and Leitch 1995).

Parsley is mostly a biennial species. It can grow also semi-perennial in greenhouse or warmer regions. Annual accessions also exist without requirement of vernalization. Biennial accessions form in the first vegetation period the rosette of leaves. The length of the tripinnate leaves with numerous leaflets varies in a wide range inside the species from around 8 to 25 cm. Leaves of var. *crispum* are shorter and the longest are found in var. *neapolitanum*.

In relation to other medicinal and aromatic plants, genetic resources of parsley are considerably represented in ex situ collections worldwide. The biggest parsley sets exist in the following countries: Spain, 351 accessions; Germany, Federal ex situ Gene Bank at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 242 accessions; United States, 249 accessions (USDA 2019); Poland, 229 accessions; Hungary, 163 accessions; Russian Federation, N.I. Vavilov All-Russian Research Institute of Plant Industry (VIR), 66 accessions; Ukraine, 163 accessions; Portugal, 113 accessions; Bulgaria, 82 accessions; United Kingdom, 67 accessions; Romania, 57 accessions; and Czech Republic, 40 accessions (Eurisco 2019). In the collections, many landraces exist from long traditions of use at geographically different places as well as old varieties.

13.2 Economical Use

Parsley has been used as a crop plant for more than 2500 years. The first mentioning was made by Theophrastus (322 BC), an ancient Greek botanist and physician (Small 1997). Hippocrates (ca 460–ca 370 BC) applied parsley as a diuretic. The ancient Greeks held parsley sacred, using it not only to adorn victors of athletic contests but also for decorating tombs. It was never brought to the dinner table. The ancient romans brought it to Central Europe and in the Carolingian Empire, Charlemagne (747/748–814) enacted the Capitulare de villis (ca 795) which included also parsley. Up to the end of Middle Ages, parsley was mostly used as a medical plant.

At present, it is grown in temperate and subtropical climate worldwide and predominantly used as aromatic plant for cooking and garnishing. In Germany parsley

is the most important spice plant, cultivated on more than 1800 ha (Schmitz and Pforte 2014). In the growing segment of potted spice plant production in Germany, parsley ranked second (19%) behind basil (*Ocimum basilicum* L., 47%).

13.2.1 Plant Parts Used

All parts of the plant including leaves, stems and taproots are usable. Products from harvested parsley leaves for seasoning of soups, sauces, dressings and meet dishes and for garnishing are marketed dehydrated, frozen or as fresh green bunches. Since some years pots with green parsley plants as pot herb are an increasing segment. Big amount of parsley is used dehydrated for industrial processed foods. It is important especially for soups and meat dishes.

Parsley roots are used fresh as vegetable and in soups. It is also often used for flavouring and to enhance the taste of meat in combination with celeriac, carrot and leek. Dehydrated pieces of parsley root are used alone or as mix with other root vegetables in a similar way as the fresh ones.

For the drying process horizontal belt dryers are used with temperatures starting between 90 °C and 120 °C. With decreasing moisture of the processed leaves, the drying temperature is reduced to 80 °C to 60 °C. The process finishes with 4–6% residual moisture content (Hoppe et al. 2013). The relation of fresh weight to dry weight for parsley leaves is about 7:1 and for parsley roots about 10:1. The drying process for roots occurs with 40 °C. The roots are sliced in small pieces before drying (Dachler and Pelzmann 1999).

The essential oil of parsley is applied in the food industry and as a fragrance in perfumes and for cosmetics (Feldheim 1999).

Parsley has carminative, tonic and aperient effects, but it is mainly used for its diuretic properties (Warncke 1994). It stimulates the appetite, has anti-inflammatory properties and has the ability to induce menstruation because it stimulates gentle contractions of the uterus.

Traditionally, it has been used for flatulent dyspepsia, colic, cystitis, dysuria, bronchitis cough in the elderly, dysmenorrhoea, functional amenorrhoea, myalgia and specifically for flatulent dyspepsia with intestinal colic.

The oral consumption of the essential oil (Petroselini aetheroleum) with high content of apiole can lead to intoxications. Parsley is safe in normal food quantities of green and dehydrated material (Hoppe et al. 2013).

The pharmaceutical use is not recommended by the European Medicines Agency (EMA) because of the side effects especially in the use of essential seed oil. The consumption of fresh or dehydrated leaves and root products is absolutely risk-free. A urological phytopharmaceutical in Switzerland is Asparagus-P, film-coated tablets (active ingredients, Asparagi radicis pulvis, Petroselini herbae pulvis; company, Phytaris Naturwissen GmbH). Parsley is used in homoeopathic pharmaceuticals and in folk medicine.

13.2.2 Cultivation

Parsley is a culture, which is grown on fields or greenhouses and plastic tunnels. It has to be sown at beginning of the vegetation period. For the temperate climate of Central Europe, this should be from beginning of March on. The plants form a rosette of leaves and the first harvest takes place 10–12 weeks after start of cultivation. According to the weather conditions every 21–30 days a harvest of the new grown leaves can occur. For harvest the plants of flat leaf type should reach a height of 20–25 cm. Varieties of the crispy leaf type are not so high. The cut should be 4–6 cm above the ground (Hoppe et al. 2013).

For growing parsley, sandy and sandy clay soils are preferable with a relatively high level of humus from 1 to 5% and a pH value from 6 to 7 (Dachler and Pelzmann 1999). Parsley is a cool season plant that grows optimally at temperatures between 7 and 16 °C, while growth slows considerably below 7 °C (Small 1997). The germination of parsley seeds is slow and can reach up to 26 days under low temperature. Germination is best at 12–15 °C.

For parsley seeds, a pretreatment by soaking the seeds for 18 hours in 20 ± 2 °C warm water and subsequent re-drying at 30 °C maximum temperature is useful. Seed dormancy can be broken by stratifying in a refrigerator with around 5 °C or briefly freezing.

Parsley leaves tolerate temperatures down to −6 to −8 °C. Roots will survive the winter with temperatures down to −10 °C. However, alternating frosts can lead to complete loss by rot. For seed production in the second year, it can be necessary to uproot the root ends of growing season and to replant them in spring.

A crop rotation system that includes parsley should guarantee a growing intermission of minimally 5 years, better up to 7 years between parsley but also to other Apiaceae. Suitable preceding crops are species depressing weed well.

13.2.3 Valuable/Undesired Plant Secondary Compounds

Parsley is rich in antioxidants (especially in vitamin C, (CAS 50–81-7), in the flavonoid luteolin (CAS 491–70-3), in vitamin A and in folic acid (vitamin B9, CAS 59–30-3). It contains essential oils in roots, leaves and fruits with clear differences in amount and composition according to the used plant tissue (Hoppe et al. 2013).

The essential oil content of parsley roots of the taprooted form (*P. crispum* convar. *radicosum*) is with 0.1–0.3% lowest in relation to leaves (0.02–0.9%) and fruits (1.0–6.0%). After steam distillation of taproots the main components of the essential oil are β-pinene (CAS 127-91-3, 21–40%), β-phellandrene (CAS 555-10-2, 7–14%), apiole (CAS 523-80-8, 15–42%) and myristicin (CAS 607-91-0, 5–15%). Extracts of uneatable roots of *P. crispum* convar. *crispum* contain terpinolene (CAS 586-62-9, 7–43%), β-pinene (4–30%), apiole (20–57%), myristicin (4–21%), small amounts of elemicin (CAS 487-11-6) and others (Franz and Glasl 1974; Gijbels

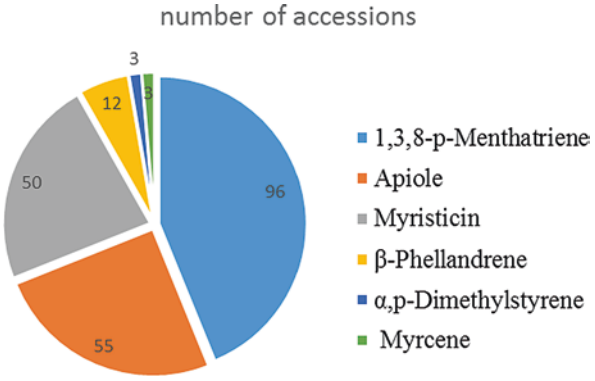

Fig. 13.2 Absolute numbers of main component types in 219 accessions of the essential leaf oil

et al. 1985; Warncke 1994). Parsley roots contain flavonoids (0.2–1.6%) with the main component apiin (CAS 26544-34-3), furanocoumarins (to 0.1%) with oxypeucedanin as main furanocoumarin (CAS 737-52-0), bergapten (CAS 484-20-8), imperatorin (CAS 482-44-0), isopimpinellin (CAS 482-27-9) and methoxsalen (CAS 298-81-7) (Baumann et al. 1988; Chaudhary et al. 1986), phthalides as ligustilide (CAS 4431-01-0), senkyunolide (CAS 62006-39-7), butylphthalide (CAS 6066-49-5) (Gijbels et al. 1985), polyenes as falcarinol (CAS 21852-80-2) and falcarindiol (CAS 55297-87-5) (Bohlmann 1967; Nitz et al. 1990).

The essential oil content of leaves has amounts of 0.02-0.9%. A set of 219 accessions which included the parsley set from Federal ex situ Gene Bank for Agricultural and Horticultural Crop Species at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) at Gatersleben, Germany, was characterized for composition of essential leaf oil (from the first cut) by hydrodistillation and gas chromatography. The main components were in 96 accessions 1,3,8-p-menthatriene (CAS 18368-95-1), apiole in 55 accessions, myristicin in 50 accessions, β-phellandrene in 12 accessions, α,p-dimethylstyrene (CAS 1195-32-0) in three accessions and myrcene (CAS 123-35-3) in another three accessions (Fig. 13.2). Further components are α-pinene (CAS 80-56-8) and β-pinene, α-phellandrene (CAS 99-83-2), *p*-cymene (CAS 535-77-3), terpinolene, (*E*)-β-farnesene (CAS 18794-84-8), germacrene D (CAS 37839-63-7), crispane (CAS 79803-28-4) and crispanone (CAS 11053-21-7) (Spraul et al. 1992; Hoppe et al. 2013).

Minor components with small or very small levels are methyl 2-methylbutanoate (CAS 868–57-5), 1-octen-3-one (CAS 4312-99-6), (*Z*)-1,5-octadien-3-one (CAS 65767-22-8), 2-(4-methylphenyl)propan-2-ol (CAS 1197-01-9), 2-isopropyl-3-methoxypyrazine (CAS 25773-40-4), 2-sec-butyl-3-methoxypyrazine (CAS 24168-70-5), p-mentha-1,3,8-triene (CAS 18368-95-1), (*Z*)-6-decenal (CAS 105683-99-6), (*E*,*E*)-2,4-decadienal (CAS 25152-84-5), linalool (CAS 78-70-6), citronellol (CAS 106-22-9) and β-ionone (CAS 14901-07-6) (PubChem 2019, TGSC 2019, Bauermann et al. 1993, Franz and Glasl 1974, Lawrence 1981, 1990, MacLeod et al. 1985, Pino et al. 1997, Porter 1989, Spraul et al. 1991, Warncke1994). All these volatile components are involved in flavour formation of parsley leaves.

Parsley leaves contain flavonoids (1.9–6.6%) with the main component apiin (apigenin-7-*O*-(6-*O*-apiosyl)glucoside (CAS 86546–87-4)). Further components are luteolin-7-apiosylglucoside, apigenin-7-glucoside, 6″-acetyl-apiin, isorhamnetin-3,7-diglucoside and chrysoeriol-7-apiosylglucoside (CAS 33579–63-4) (Grisebach and Bilhuber 1967; Nordstrom et al. 1953; Yoshikawa et al. 2000).

Furanocoumarins of parsley leaves reach up to 0.2%. The main components are oxypeucedanin and bergapten (5-methoxypsoralen, CAS 484–20-8). Further furanocoumarins in parsley are xanthotoxin (8-methoxypsoralen, CAS 298–81-7), psoralen (CAS 66–97-7), imperatorin (CAS 482–44-0) and isopimpinellin (CAS 482–27-9) (Ashraf et al. 1980; Baumann et al. 1988; Chaudhary et al. 1986; Teuscher and Lindequist 1994).

In fresh parsley leaves vitamin C (ascorbic acid, CAS 50–81-7) can reach 0.12–0.4% of fresh weight (Feldheim 1999; Manderfeld et al. 1997; Scheunert and Theile 1952).

The essential oil content of fruits (Petroselini fructus) is 1.0–6.0% with the main components apiole, myristicin and 1-allyl-2,3,4,5-tetramethoxybenzene (CAS 15361–99-6) (Hoppe et al. 2013). Because of the relatively high concentration of essential oil, only fruits were used for essential oil production.

13.2.4 Main Production Areas

Parsley belongs to the most significant medicinal and aromatic plants worldwide regarding the area under cultivation and harvest size. For international export parsley production of Egypt is the most important for dehydrated parsley as well as for green bunches and frozen spice. Uzbekistan is important for production and export to other Asian countries. However, parsley growing is also important in India, Malaysia, Indonesia, China and the Philippines. For America Canada is an important producer of dehydrated parsley flakes. The United States with production concentration in California, New Jersey, Florida and Texas and smaller volumes from Ohio, Massachusetts and other states is a significant producer and a significant market for fresh and dehydrated parsley (Small 1997).

For the member states of European Union, around 5.000 ha are estimated. Production of dehydrated parsley is important in Germany, France, the Netherlands and Poland. Production of green bunches and frozen spice food is important in France, Belgium, the Netherlands, Great Britain, Germany and Poland. Production of potted plants comes from the Netherlands, Germany, Belgium, France, Great Britain and Poland (Junghanns 2018). For turnip-rooted parsley the United Kingdom is an important production area. Countries of Central and South Eastern Europe Slovakia, Romania and Bulgaria have tradition in growing and using turnip-rooted parsley. Many accessions in gene bank come from this region.

13.2.5 Economical Valuation/Parameters

Under the conditions of Germany the yield of dry mass of first cut ranges from 800 to 900 kg/ha. For the second cut 1.0–1.1 t/ha are possible. All in all up to 5 cuts per year and up to 5.3 t/ha dry mass per year are possible. The importance of early sowing was demonstrated by Kienast (Hoppe et al. 2013). They reached 2012 6.1 t/ha and year dry mass yield by sowing in February, 12 kg/ha seed rate and distance between rows of 12–13 cm.

Essential oil produced from parsley is of lower value than fresh and dried products. Verlet (1993) estimated the annual world value of parsley essential oil from seed to be $600,000.00 (US), while that of "parsley herb" essential oil amounted to an additional $360,000.00 annually.

13.2.6 Pests and Diseases

The cultivation of parsley on fields or in greenhouses requires a monitoring of phytopathological situation of this crop.

For Central Europe behind pests especially fungi, viruses and bacteria are of significance. The requirement of products without phytopathological damages stands in conflict with the interest of consumers in goods produced nearly without or without chemical pesticides and the decreasing number of approved active substances. The best way to produce parsley without residuals is to use resistances to the pathogens. For breeding projects, information about the biology of the pathogens is necessary. Also evaluation data for sources of resistance are essential.

Economically important diseases are Septoria blight caused by *Septoria petroselini* (Lib.) Desm., downy mildew (*Plasmopara petroselini* Săvul. & O. Săvul.), powdery mildew (*Erysiphe heraclei* DC. ex Saint-Aman) and Alternaria leaf blight caused by *Alternaria radicina* Meier, Drechsler & Eddy.

Septoria blight (*S. petroselini*) is worldwide one of the most important pathogens for parsley. It is seed-borne (Tahvonen 1978). Pycnidia are fixed permanently within the pericarp of the schizocarpic fruit, containing pycniospores for mass infection (Ferri 1969).

Lesions of Septoria blight are round or angular and greyish brown, clearly bordered by a darker brown margin to the surrounding tissue, and have small or no chlorotic zones (Fig. 13.3). Spores containing black pycnidia appear within lesions (Meyer et al. 2010; Marthe and Scholze 1996; Cerkauskas and Uyenaka 1990).

Plasmopara petroselini was separated from *Plasmopara nivea* by Săvulescu and Săvulescu (1951).

Downy mildew on parsley was found several times in Germany at the end of the nineteenth and the first half of the twentieth century (Brandenburger and Hagedorn 2006). Together with Septoria blight, they are currently the most relevant pathogens.

The downy mildew causing fungus *P. petroselini* induces faint chlorotic spots on the upper surfaces of the leaves as first symptoms. On the corresponding lower surfaces, sporangiophores and white-to-greyish white mycelium develop (Fig. 13.4). Lesions grow fast and parts of the leaf or whole leaves rot (Amein et al. 2006;

Crepel and Inghelbrecht 2003). Spores are small, oval and hyaline. Downy mildew is an important pathogen in the production of potted parsley. Infection spreads very rapidly in greenhouse or plastic tunnel especially in the period of shorter days. The infected crop develops a typical smell.

Powdery mildew (*E. heraclei*) on parsley is a worldwide significant pathogen (Marthe et al. 2003; Scholze et al. 1996). The economic importance of *E. heraclei*

Fig. 13.3 Septoria blight on parsley; (**a**) lesions; (**b**) lesions with black pycnidia

Fig. 13.4 Downy mildew on curled leaf parsley; (**a**) plants with necrotic leaves; (**b**) lower leaf surface with necrosis and sporangiophores

for production under field conditions in Germany is lower than *S. petroselini* and *P. petroselini*. The rapidly increasing production of parsley pots around the year is much more affected by *E. heraclei*. Powdery mildew attacks also other members of Apiaceae family. This pathogen may occur early in the season and cause losses in quality of the market value of the product. *E. heraclei* on parsley has an increasing range worldwide. The fungus was first described in Brazil: Sao Paulo, *E. heraclei*, introduced through contaminated seeds (Rosa et al. 2008).

In the USA, the disease was several times observed and described (Raid et al. 2007; Glawe et al. 2005; Koike and Saenz 1994). In Europe, powdery mildew has been an important pathogen for a long time.

The identification is easy because of the mycelia growing on the leaf surface (Fig. 13.5). Haustoria of oval shape establish in epidermis cells for absorbing nutrients (Fig. 13.6). Infected leaves show chlorosis and crinkling (Marthe et al. 2003; Koike and Saenz 1994).

Alternaria radicina Meier, Drechsler & Eddy (syn. *Stemphylium radicinum* [Meier, Drechler & Eddy] Neerg.) causes *Alternaria leaf blight* and damping off of seedlings (Gärber et al. 2007; Marthe and Scholze 2006; Nawrocki 2005; Marthe et al. 2003; Nowicki 1997; Gärber and Ulbrich 1996).

Infestation by *A. radicina* causes important damages by leaf spots (Meyer et al. 2010) and loss of quality depending on annual weather conditions. *Alternaria* species are seed born on parsley. The conidia adhere together with mycelium external on the seeds and so they are good diagnosable.

Infestation starts from older leaves with brown-coloured small round lesions. At this stage the differentiation of lesions to early infestation by *S. petroselini* is nearly impossible. But with progression of the disease, characteristic lesions will develop. The Alternaria lesions expend rapidly usually from margin of leaf and are shaped irregularly. Inside the lesions, concentric zones can be visible with moderately graded colours. The transitions to non-necrotic area of leaf are fluid conditioned by a chlorotic zone (Fig. 13.7). Inside the lesions and successional on the whole dieback

Fig. 13.5 Powdery mildew on parsley; (**a**) plants with ecto-mycelium; (**b**) leaf with mycelium and mass production of asexual hyaline spores

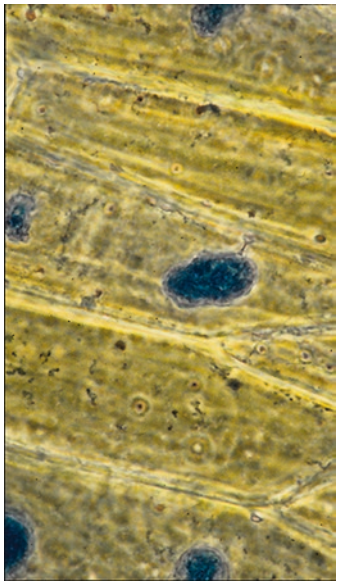

Fig. 13.6 Powdery mildew on parsley, haustoria formed in epidermis cells; coloured with Cotton Blue

Fig. 13.7 Alternaria leaf blight on parsley; (**a**) plants with necrotic leaves, (**b**) leaf with necrosis and chlorotic zones

leaves grey to black conidia are built terminally at the end of branched mycelia. This is the reason for the characteristic black colour of infested parts of the plants.

In nearly in all cases associated with specialized *A. radicina* the species *Alternaria alternata* [Fr.] Keissler (synonym *Alternaria tenuis* C.G. Nees.) followed as saprophyte. This species is not specialized to species or plant families and widespread through climatic zones. The conidiospores evolve as chains by constriction from mycelia. *A. alternata* is also able to set very small lesions on parsley leaves. For this a longer period with high air moisture and around 20 °C are necessary. However, the damages are not of economic importance.

The generalist *Sclerotinia sclerotiorum* (Lib.) de Bary causing *Sclerotinia rot* (Nowicki 1997; Scholze et al. 1996) are also relevant phytopathogen for parsley. It is easy to identify by the more or less big black sclerotia embedded in cotton-like mycelia. Attacked plants are destroyed by macerating of the stalk basis.

There are more pathogenic fungi damaging parsley as Fusarium wilt (*Fusarium oxysporum* Schltdl.) (Nawrocki 2005; Scholze et al. 1996), Pythium root rot (*Pythium* spp.) (Tsuchida et al. 2018; Petkowski et al. 2013; Gärber and Borchers 2000; McCracken 1984) and Rhizoctonia root rot (*Rhizoctonia solani* J.G. Kühn) (Nawrocki 2005; Monnet and Thibault 2002; Uematsu et al. 1993).

Viruses of economic impact on parsley are the widespread *Celery mosaic virus* (CeMV), the *Apium virus Y* (ApVY) and a virus complex probably from the *Carrot red leaf virus*, (CtRLV), the parsley-specific *Carrot mottle virus* (CMoV) and *Carrot mottle mimic virus* (CMoMV). In addition, the *Apium virus Y* and the *Carrot yellow leaf virus* (CYLV) are possibly involved in this virus complex (ICTV 2019; Hoppe et al. 2013; Meyer et al. 2010).

Bacteria can cause leaf spots and macerations, especially by *Pseudomonas* spp. (Reintke et al. 2016; Bull et al. 2011).

13.3 Breeding

Breeding Objectives

For optimization of parsley lines by using methods of plant breeding many requirements exist. For breeding success as well as for economic result the choice of breeding aims is of particular importance.

For many crops, the group of *yield parameters* is of big or biggest interest. That is also true for parsley. Breeding objectives of this group for leaf production are size of yield, amount of marketable yield, essential oil content, fast regrowth after cutting, colour of leaves, crimping of the leaf blade tissue between the secondary veins, relation of leaves to stalk, height and density of plants.

Breeding objectives for turnip-rooted parsley are yield of taproots, length and diameter of the taproot and a smooth taproot.

For many of these characters the International Union for the Protection of New Varieties of Plants (UPOV) has exact descriptors under UPOV code: PETRO_CRI (UPOV 2005, Table 13.1, Figs. 13.8 and 13.9).

Table 13.1 Characteristics and examples from varieties of parsley, including also turnip-rooted parsley

Number an footnotes	Characteristic
1. (∗)	Plant: height
2.	Plant: width
3. (∗)	Plant: density of foliage
4.	Plant: number of leaves
5.	Leaf: attitude
6. (∗)	Leaf blade: curling
7. (∗), (+)	Leaf blade: intensity of curling
8.	*Only varieties with leaf blade curling* Plant: appearance of surface of canopy
9.	Only varieties with leaf blade curling Leaf blade: upward reflexing of lobes
10. (∗)	Leaf blade: length
11. (∗), (+)	Leaf blade: width
12.	Leaf blade: ratio length/width
13. (∗)	Leaf blade: intensity of green colour
14. (+)	Leaflet: shape
15. (+)	Leaf blade: distance between first and second pair of leaflets
16.	Leaflet: undulation of margin
17. (+)	Petiole: length
18. (+)	Petiole: thickness
19. (+)	Petiole: anthocyanin coloration
20. (∗)	Root: thickening of main root
21. (∗)	Only root parsley varieties Root: length
22. (∗)	Only root parsley varieties Root: width
23. (∗)	*Only root parsley varieties* Root: ratio length/width
24.	Only root parsley varieties Root: branching

From UPOV (2005), simplified
(∗): Characteristics included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate
(+): See Fig. 13.9

A considerable number of breeding aims exist for *resistances to pathogens and for stress tolerance*. Usable resistances can be deciding for the qualification to be cultivated. This is evident for ecological cultivation but is also true for controlled cultivation. The availability of active components for plant protection is restricted. In the near future the number of such substances for pest management will decrease drastically.

In extensive tests for resistance to *Septoria blight* caused by *S. petroselini* no resistant variety could be found. For these tests it was necessary to develop a method for cultivating the fungus on artificial medium and a method for testing the resis-

Fig. 13.8 Classification of the curling intensity of the leaf blade: (**a**) weak, 3; (**b**) medium, (**c**) 5; strong, 7; (**d**) very strong, 9. (From UPOV 2005)

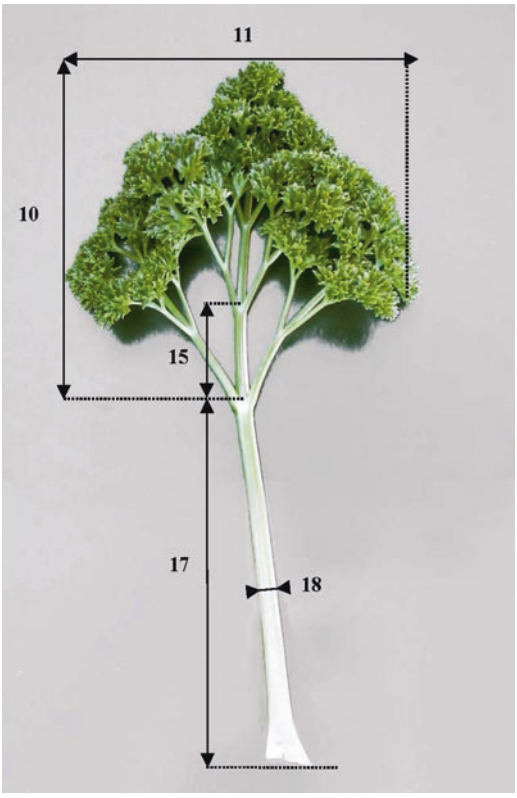

Fig. 13.9 Instruction for measuring of characters listed in Table 13.1: (10) leaf blade, length; (11) leaf blade, width; (15) leaf blade, distance between first and second pair of leaflets; (17) petiole, length; (18) petiole, thickness. (From UPOV 2005)

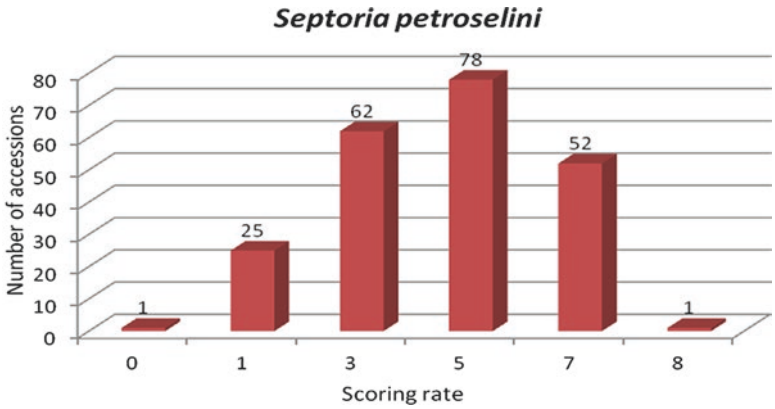

Fig. 13.10 Number of parsley accessions scored for lesions by *Septoria petroselini* under natural infection. Scoring rates 0, 1: free or nearly free of symptoms; 3, 5: moderately susceptible; 7, 8: highly susceptible. (From Marthe et al. 2013)

tance in climate chamber (Marthe and Scholze 1996). The pycniospores necessary for artificial inoculation were produced on artificial vegetable juice medium in petri dishes under sterile conditions. Concentration of spores in a water suspension was approximately 10^6/ml. Young plants were sprayed with 3 ml of the suspension per plant. After inoculation plants were incubated under controlled conditions at temperatures of 18–20 °C and a day/night light regime of 16/8 h. For good infection results it is necessary to provide more than 95% humidity for at least 4 days after inoculation. After an incubation time of 21 days, when susceptible leaves are showing lesions, disease assessment was carried out. No variety or gene bank accession was found free of symptoms for all single plants. However, clear differences in reaction to the pathogen have been found between the accessions of germplasm material tested in climate chamber. This type of resistance is characterized by populations including plants with low level of symptoms. In resistant populations, first symptoms appear later and infestation is lower in comparison to highly susceptible varieties and accessions. From resistant populations, plants free or nearly free of symptoms were selected and self-pollinated to increase homozygosity.

In a resistance test under natural infection pressure 220 accessions were scored. The rating scale consists of level 0 (without any lesion) to 9 (plants die by action of pathogen). Level 9 has not be observed in our sample set. Scoring rates 0 and 1 are classified as ‘free or nearly free of symptoms’, 3–5 are ‘moderately susceptible’ and 7 and 8 are ‘highly susceptible’. Only one accession was free of symptoms. The results generate a distribution similar to a Gaussian curve (Fig. 13.10). This characterizes the quantitative action of the resistance (Marthe et al. 2013). Crossing experiments for characterization of resistance inheritance indicate an oligogenic situation (Bruchmüller 2013).

Since the beginning of the twenty first century *downy mildew* caused by *P. petroselini* is as an economically important pathogen. Therefore, the availability

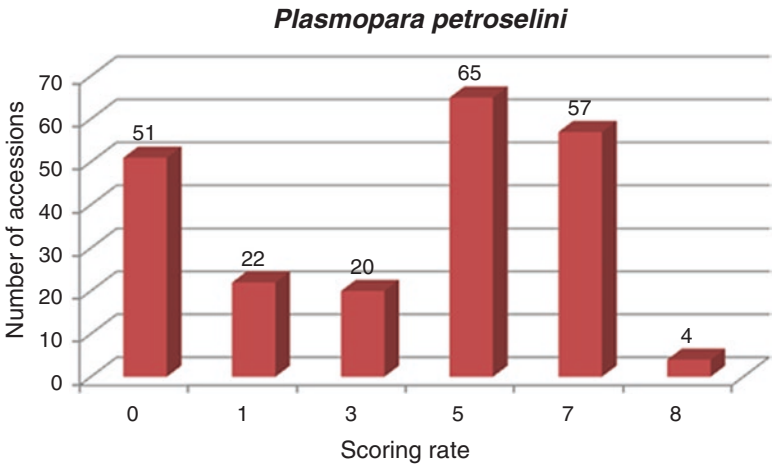

Fig. 13.11 Number of parsley accessions with scoring rates 0, 1: free or nearly free of symptoms; 3, 5: moderately susceptible; 7, 8: highly susceptible to natural infection by *Plasmopara petroselini.* (From Marthe et al. 2013)

of resistances is of high interest. Leinhos et al. (2012) developed a method for testing downy mildew resistance. For inoculation a spore suspension of 1 x 10^5 sporangia/ml in water was used. The temperature for infection is from 4 °C to 15 °C with an optimum of around 10 °C. The temperature is correlated with duration of leaf wetness. For a high infection rate 24 hours (4 °C) to 4 hours (15 °C) are necessary. The latency period was calculated with 8 days (14–24 °C day temperature, 3 °C night temperature and 77% relative air moisture). Twelve days after infection, the area of infested leaves and sporulation reached its maximum.

First results from tests with inoculation indicate that variety 'Felicia' was free of symptoms out of nine varieties tested (Krauthausen and Leinhos 2007).

In a test under natural infection Marthe et al. (2013) found 73 accessions free or nearly free of symptoms under 219 accessions and varieties. The results generate a bimodal distribution of accessions free of symptoms and susceptible accessions (Fig. 13.11). This is an indication for existence of qualitative resistance. The accessions free of symptoms of *P. petroselini* together with variety 'Felicia' tested free of symptoms are first candidates for intraspecific resistance in parsley. These candidates should be tested again for level of resistance. The accessions free of symptoms also open up the possibility to look for candidates for tests of race differences in *P. petroselini*. From many other downy mildew varieties a high number of different races are known (Marthe et al. 2013).

Powdery mildew caused by *E. heraclei* is also significant for parsley production, especially for production of potted plants and fresh green leaves. But also field cultures can be attacked by powdery mildew depending on weather conditions. Powdery mildew is an obligate biotrophic pathogen. The cultivation on artificial medium is an unsolved problem. The pathogen has to be cultivated on living plants

for mass production of spores and storage of isolates. For resistance test, Marx and Gärber (2014) developed a method for inoculation. The germination of the conidia correlates with temperature. At all tested temperatures from 6 °C to 30 °C conidia are germinable. The highest level of germination with 80% was from 15 °C to 20 °C. Lower and higher temperature reduced the level of conidia germination. The temperature influences also the length of incubation period. The shortest period was 7 days at the temperature of 25 °C. Lower temperatures (10 °C, 18 days) and higher temperatures (30 °C, 12 days) prolonged the incubation period. However, the level of air moisture (30, 50, 70% at 25 °C) has no influence on the length of incubation period.

For sporulation the level of air moisture has influence. The number of produced conidia increases with higher air moisture. The maximum sporulation was reached at 70% air moisture. At temperatures from 20 °C to 25 °C level of sporulation was higher than on 15 °C (Marx and Gärber 2012).

Although *E. heraclei* has been a significant pathogen in Europe for a long period, no results are available of extensive evaluation for resistant and susceptible accessions. Two evaluations of gene bank accessions were made under natural infection (Marthe et al. 2013; Marthe et al. 2003). From this, it was concluded that resistances exist in the tested set. Before final judgement of resistance, the candidates for resistance have to be tested under controlled conditions with inoculation.

Marx and Gärber (2012) tested 11 isolates of powdery mildew from parsley (10) and dill (*Anethum graveolens* L., 1) on two varieties of parsley. The scoring 15 days after inoculation generated nearly 100% of infected leaf surface on half of the isolates. The other half infected only 25% of leaf surface.

These results demonstrate the necessity for testing race specificity of different isolates of *E. heraclei* because for many other varieties of species of powdery mildew high numbers of different races are known. Variability in response to powdery mildew was evident offering possibilities to develop resistant cultivars.

Alternaria leaf blight causes leaf necrosis, which results immediately in a loss of quality. Under Central European conditions it can be found in nearly each year, but major damages depend on weather conditions and are not likely to occur frequently or regularly. For evaluation of collections including gene bank accessions for sources of resistance a technique was adapted for tests under controlled conditions in a climate chamber. *A. radicina* and *A. alternata* can be cultivated on artificial medium. On malt extract agar (25 ml/l, 23 g/l agar-agar, 0.25 mg/l chloramphenicol) monosporic isolates develop under sterile conditions at room temperature large amount of spores. Suspension of conidiospores in water (1×10^6 conidia/ml) were sprayed on six-week-old plants (3–4 ml/plant). Inoculated plants were cultivated in a climate chamber with saturated air moister, temperature of 19 °C and light of 16 hours. Disease assessment was carried out 7 days after inoculation and a second time 14 days after inoculation by estimating the lesions in relation to leaf surface (Marthe and Scholze 2006).

The results of different tests with a set of varieties and gene bank accessions indicate clearly resistances in the tested set. It was possible to differentiate two material groups that differ in the level of adaptation to temperate European growing

conditions. The number of resistant varieties from the European temperate zone was higher than accessions from other zones. There are resistances to *A. radicina* inside the European breeding material. However, the control of resistance in the breeding process is recommended (Marthe et al. 2003).

From the use as spice plant, significant *quality parameters* are of importance.

The most important is the *shelf life*. Because of relatively fast green colour loss of parsley bunches, the production of potted parsley plants gained in importance.

In the market consumers prefer minimally processed vegetables and a broad spectrum of fresh aromatic plants. For this the shelf life characteristics are of importance. Parsley has antibiotic acting secondary metabolites. Nevertheless, different microbes occur on and inside the leaves. Unhygienic contaminations in the production process by bacteria harmful to consumers can only be solved by hygienic standards (Santos et al. 2014). For this the post-harvest processing as fast drying starting with temperature of 90 °C and higher and also the pressurized CO_2 disinfestation process has proven its reliability.

The complexes of *smell and taste* are also important. The optimization of this breeding aims should be handled with respect to resistance or repellent action of some sensory effective substances, which are also included in resistance responses.

Parsley is known for both an outstanding and unique flavour and bioactive secondary metabolites (Cazzola et al. 2011). The sensory quality as well as the composition of volatile compounds of freshly harvested parsley, dried parsley and essential oil has been studied in the past (Masanetz and Grosch 1998a, 1998b; Jung et al. 1992; Whitfield and Last 1991; MacLeod et al. 1985). A total of around 80 volatiles have been identified, of which a smaller number of 17 odorants show a relatively high aroma impact. The flavour of freshly harvested and cut parsley leaves is caused mainly by p-mentha-1,3,8-triene, myrcene, 2-sec-butyl-3-methoxypyrazine, myristicin, linalool, (Z)-6-decenal and (Z)-3-hexenal (Ulrich et al. 2011).

Hoberg et al. (2007) developed a method for the evaluation of sensory features and their variability by a panel of testers. Every test person tastes the standard variety ‘Grüne Perle’ and collects descriptive, objective attributes for the categories. The resulting method includes 29 suitable sensory attributes for taste, odour, retronasal odour and mouth feeling. In addition, the estimated hedonic acceptability was noticed by each tester. The sensory method was adapted to breeding and does not focus on characterization of substances and their impact on sensorially overall impression. It enables evaluation of varieties and gene bank accessions as well as the monitoring over the breeding process. The sensorial characteristics and the content and composition of metabolites of most accessions conserved in gene banks are not characterized. In the set tested by Hoberg et al. (2007) it was found that the year of harvesting, the resistance against *Septoria petroselini* (Lib.) Desm. and above all the habitus have a significant effect on the majority of the sensorial parameters.

However, so far the interrelations between aroma compounds and their functionality in resistance mechanisms are widely unknown, not only in parsley. The results show that no simple and straight correlation exists between sensorial quality and resistance properties. The recognized variability of the sensory features and the described method for their objective evaluation offer good chances to breed well tasting parsley with resistances to pathogens in the future.

The group of breeding aims resulting from requirements of pharmacopoeia is for the factual scope of European Pharmacopoeia application not of special interest. A monography for parsley does not exist in Ph.Eur. 9 (2017) besides the general requirements of the monograph herbal drugs. The *German Homoeopathic Pharmacopoeia* (Homöopathische Arzneibuch HAB 2018) included *Petroselinum crispum* convar. *crispum* for use as fresh whole plant at stage of flowering begin with no requirements for metabolites.

13.3.1 Flower and Pollination Biology

Flowers and Flowering: Pollination

After the first winter, the stalk of biennial parsley will shoot immediately. The leaves of the flower stalk are smaller and pinnation is simpler. Flowering plants reach a height of 60–120 cm. At the end of the stalk umbels of different ranks are formed. Inside the umbels, a time gradient exists with the oldest partial inflorescences at the external circle and the younger unripen flowers in the internal circles. Such a gradient exists also inside the partial inflorescences, which group flowers arising from the same point. Each flower has five petals. The colour of petals varies between accessions from green to white (Fig. 13.12).

Protandry is common in the family Apiaceae and occurs in parsley as well. The five anthers start anthesis around 5 days before the two stigmata are receptive to pollen (Fig. 13.13). At the time of receptiveness for pollination, the receptacle nectaries produce a sugar-rich liquid for rewarding pollinating insects. At this time, the flower gets shiny and the stigmatic papillae reach the length of flower radius. In a flower two carpels are fused to a bicarpellate pistil from which the schizocarpic fruits develop (Fig. 13.14). The mature fruits can break into two mericarps of one single fruit (commonly called ‘seed’) each.

Male Sterility

Male sterility is not seldom in family Apiaceae. The model plant for this is carrot (*Daucus carota* L.) where cytoplasmic male sterility (cms) is used in practical

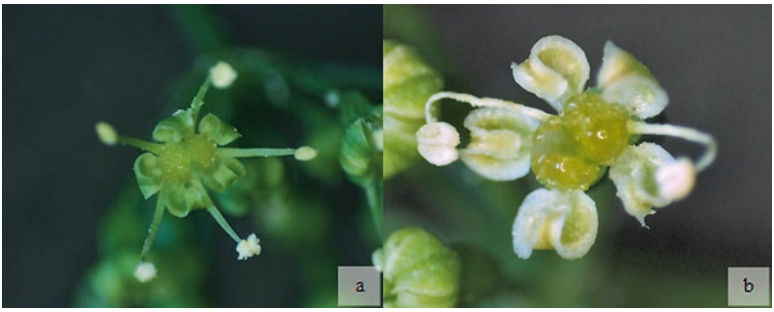

Fig. 13.12 Flowers of parsley; left, green petals; right, white petals

Fig. 13.13 Inflorescence of parsley; left, (double) umbel; right, flower with the two immature and non-receptive stigmata

Fig. 13.14 Inflorescence of parsley with almost ripe schizocarpic fruits

breeding. Two types of male sterility exist in carrot: the brown anther type and the petaloid type. The molecular mechanisms for cms in carrot are under investigation (Tan et al. 2018; Szklarczyk et al. 2014; Robison and Wolyn 2006). For celery (*Apium graveolens* L.) male sterility was found and characterized (Quiros et al. 1986). For fennel (*Foeniculum vulgare* MILL.) the existence and use of male sterility is documented (Palumbo et al. 2018; Pank et al. 2007). For parsley no source for male sterility or cytoplasmic male sterility was found. In own investigations on about 200 accessions and varieties no male sterile plants were detected.

Hybridizations

Crossing technique is a prerequisite to obtain genetic recombination at the beginning of the breeding process or genetic analyses. For hybridization, emasculation of an

umbel has to be performed. The anthers of the earliest third of the flowers should be fully developed. At this time, the flower is not ready for pollen reception. All older flowers with receptive papillae have to be removed manually by a tweezer. For this a head loupe generates a stereoscopic image. Furthermore, the younger flowers and buds have to be removed by a tweezer because their anthers would develop pollen. The remaining flowers with unripe papillae have to be flushed out by a strong water jet to remove all pollen. At the end, the emasculated umbel has to be controlled for remaining anthers and then isolated by a bag. The material of the bag can be a translucent plastic film with micropores. Three to five days later the pollination has to be performed by touching the papillae with flowering umbels from the male crossing partner. After pollination the emasculated umbel has to be isolated again by a plastic bag. About 1 week later the fruit growth should start and the bag can be removed.

13.3.2 Propagation Strategies

Generative Propagation
After winter the roots of the vegetative growing period are used for seed production. The harvest takes place when the first seeds are ripe. Selective harvest of oldest umbels decreases the loss of fully ripened seeds but is labour intensive.

Vegetative Propagation
There are no strategies for vegetative propagation in parsley.

13.3.3 Breeding Methods Applied

Conventional Breeding
The breeding of parsley started with selections from land races. An example is the curled parsley ‘Mooskrause’ from the nineteenth century, which was divided into ‘Mooskrause 1’, ‘Mooskrause 2’ and ‘Mooskrause 3’.

Different breeders started programs for maintenance of the variety. This resulted in differences between the selections ‘Mooskrause 2’ (Germany), ‘Moskrul 2’ (the Netherlands), ‘Moss Curled 2’ (Great Britain), ‘Nain Frisé mousse’ (France) or ‘Nano ricciuto 2’ (Italy). From this so-called umbrella variety many new varieties were developed by selection.

Other examples are the flat-leaf parsley ‘Amsderdamse Snij’, ‘Einfache Schnitt 3 Typ Hamburger Schnitt’ and the turnip-rooted parsley ‘Halblange - Berliner’.

Interspecific crosses between parsley and celery (*Apium graveolens* L.) has been successfully implemented since the beginning of the twentieth century (Becker-Dillingen 1926, Becker 1943/44, Skiebe cited from Becker 1962). These sexual crosses were successful only with celery as female. Later on, also reciprocal crosses were done (Madjarova et al. 1973; Madjarova and Bubarova 1978; Madjarova 1978; Honma and Lacy 1980; Lacy and Honma 1981). The aim of these crosses was to get

higher yielding bastards but primarily to transfer resistance against Septoria blight into the bastard. Celery is resistant to *Septoria petroselini* and parsley to *Septoria apiicola* Speg. Some of the produced bastards were more tolerant to the *S. apiicola* (Madjarova et al. 1973; Honma and Lacy 1980) but they report the resistance as polygenetic and recessive. The resistances were instable and had no practical impact on breeding resistant varieties (Ochoa and Quiros 1989). The variety 'Festival 68' from Bulgaria was developed from an interspecific bastard of parsley and celery (Madjarova and Bubarova 1978) but is susceptible to *S. petroselini*.

Hybrid Breeding

In the species *P. crispum* a tremendous variability is present. Also groups of different origin and consequently high phylogenetically distance exist. This is an important prerequisite for hybrid variety breeding. However, the lack of male sterility elements and no information on the intensity of hybrid effect prevented hybrid breeding.

Molecular Tools

The small budgets for molecular analyses in parsley as part of the medicinal and aromatic plants causes the lack of using molecular techniques in the past. With the development of new methods especially in next-generation sequencing and a price drop, it is possible to use this approach also in minor crops. From genome-based sequencing (GBS) AP2/ERF transcription factors from the transcriptome sequence under different abiotic stresses were detected (Li et al. 2014). Such data open the chance to use molecular methods for intensive parsley research.

Genome-wide association study (GWAS) resulted in not verified candidates for marker associations with curling of leaves, formation of turnip roots and lignification of the root (Bruchmüller 2013). The evaluation of different genotypes included also the analysis of sensorial characteristics in relation to the nontargeted volatile patterns (headspace-SPME-GC) and resistance to *Septoria petroselini*. The more resistant genotypes are characterized by several negative sensory characteristics. In contrast, the contents of some volatile compounds correlate highly and significantly either with resistance (e.g. hexanal and alpha-copaene) or with susceptibility (e.g. p-menthenol). Some of these compounds with very strong correlation to resistance are still unidentified and are presumed to act as resistance markers (Ulrich et al. 2011; Hoberg et al. 2007).

Phylogenetic distances have been calculated by using of RAPD and ISSR markers (Domblides et al. 2010). The analysis of 32 populations, mostly varieties, but also seven accessions of Russian gene bank VIR, St. Petersburg, describe considerable distances between accessions.

13.3.4 Breeding Results Achieved/Economical Transfer (Registered Cultivars/Patents, Trial Results)

There exist a high number of varieties. But the high number of local races or land races is of special interest. Table 13.2 contains the actual registered varieties for European Union.

Table 13.2 Plant variety catalogue for parsley (*Petroselinum crispum*, H-25) of plant variety database – European Commission (2019)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
36004 RZ	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)	(1)	Leaf[2]
36504 RZ	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)	(1)	Leaf[2]
A grosse racine gros hâtif	b FR x		Root[1]
Afrodite	b DK 57 (Hild Samen GmbH)		Leaf[1]
Alba	a CZ 1495 (Moravoseed CZ a.s.), a HU 102445 (Moravoseed s.r.o.)		Root[1]
Alto	a FR 8067 (Vilmorin)		Leaf[1]
Amsterdamse Snij	b NL x	Fijne Snij NL	Leaf[1]
Andrei	a RO 1031 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Bacău)		Root[2]
Arat	b NL 8 (Bejo Zaden BV)		Root[1]
Arctica	a NL 8 (Bejo Zaden BV)	H	Root[1]
Argon	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Aroma	a NL 8 (Bejo Zaden BV)		Root[1]
Arsem	a RO 1072 (S.C. Unisem S.A. Bucureşti)		Root[2]
Astra	a CZ 1495 (Moravoseed CZ a.s.)		Leaf[1]
Atika	a CZ 239 (SEMO a.s.)		Root[2]
Berliner		= Halblange	Root[1]
Berlinova	a DE 9989 (Satimex Quedlinburg GmbH)		Root[1]
Bravour	b NL x, b UK 176 (J.E. Ohlsens Enke A/S)		Leaf[1]
Catalogno		= Gigante di Napoli	Leaf[1]
Champion	b UK 189 (A.L. Tozer Ltd.)		Leaf[1]
Commun 2 Comun 2 Comune 2 Plain Leaved (Sheeps) 2 Comum 2	b FR x b ES x b IT 125 (SAIS Società agricola italiana sementi) b UK 38 (E.W. King & Co. Ltd.)	De hoja lisa 2 ES Plain or single 2 IT Simple 2 FR Toscano 2 IT	Leaf[1]
Comum 2		= Commun 2	Leaf[1]
Comun 2		= Commun 2	Leaf[1]
Comun 3 Gewone Snij 3	b ES 3051 (Royal Sluis) b NL 78 (SVS Holland BV)	De hoja lisa 3 ES Einfache Schnitt 3 NL	Leaf[1]
Comune 2		= Commun 2	Leaf[1]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Cukrowa	a PL 67 (Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o.o.)		Root[1]
Curlina			Leaf[1]
Danubiu	a RO 1072 (S.C. Unisem S.A. Bucureşti)		Leaf[2]
Darki	b DK 57 (Hild Samen GmbH), b UK 176 (J.E. Ohlsens Enke A/S)		Leaf[1]
Darklett	a DE 2549 (Hild Samen GmbH)		Leaf[1]
De hoja lisa 2		= Commun 2	Leaf[1]
De hoja lisa 3		= Comun 3	Leaf[1]
Doble rizado		= Frisé vert foncé	Leaf[1]
Dobra	a CZ 256 (Seva-Flora s.r.o.)		Root[2]
Domaći lišćar	b HR 177 (Podravka d.d.)		Leaf[2]
Efez	a CZ 239 (SEMO a.s.)		Root[2]
Einfache Schnitt 3		= Comun 3	Leaf[1]
Extra triple curled 2		= Mooskrause 2	Leaf[1]
Favorit	b NL 8 (Bejo Zaden BV)		Leaf[1]
Félhosszú		= Halblange	Root[1]
Fest	a CZ 1495 (Moravoseed CZ a.s.)		Leaf[1]
Festival 68	a CZ 1495 (Moravoseed CZ a.s.), a PL x		Leaf[1]
Fidelio	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Fijne Snij		= Amsterdamse Snij	Leaf[1]
Francesa Frisada		= Frisé vert foncé	Leaf[1]
Frisé vert foncé Gekrulde Donkergroene Doble rizado Francesa Frisada	b FR x, b NL 65 (Pieterpikzonen BV) b ES x	Rizado verde oscuro ES	Leaf[1]
Gala	a PL 356 (Hortag Seed Co.)		Leaf[2]
Gazela	a PL 854 (Vera-Agra sp. z.o.o.)		Root[2]
Gekrulde Donkergroene		= Frisé vert foncé	Leaf[1]
Gekrulde	b NL x		Leaf[1]
Gewone Snij 3		= Comun 3	Leaf[1]
Gigante catalogno		= Gigante di Napoli	Leaf[1]
Gigante d'Italia		= Gigante di Napoli	Leaf[1]
Gigante d'Italia		= Gigante di Napoli	Leaf[1]
Gigante di Chioggia		= Gigante di Napoli	Leaf[1]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Gigante di Napoli Gigante d'Italia	b IT x a DE 2549 (Hild Samen GmbH)	Catalogno IT Gigante catalogno IT Gigante d'Italia IT Gigante di Chioggia IT Verde scuro d'Italia IT	Leaf[1]
Grüne Perle	a DE 86 (Karl und Walter Hild)		Leaf[1]
Halblange Halvlang Berliner Halflange Félhosszú	a CZ x, a DE x, a PL x, a SK x b NL x a HU 151508 (ZKI Zöldségtermesztési Kutató Intézet Zrt.)		Root[1]
Halflange		= Halblange	Root[1]
Halvlang		= Halblange	Root[1]
Hamburska	a PL 356 (Hortag Seed Co.)		Root[2]
Hanácká	a CZ x, a SK 250 (Zelseed spol. s r.o.)		Root[1]
Ines	a DE 7092 (GHG Saaten GmbH)		Leaf[1]
Jadran	a CZ 239 (SEMO a.s.)		Root[1]
Jagienka	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Root[2]
Junák	a SK 432 (P.K. SEM spol. s.r.o.)		Leaf[2]
Kadeřavá	a CZ x		Leaf[1]
Kaśka	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Root[2]
Kinga	a PL 67 (Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o.o.)		Root[1]
Konika	a CZ 1495 (Moravoseed CZ a.s.)		Root[1]
Korai cukor	a HU 151508 (ZKI Zöldségtermesztési Kutató Intézet Zrt.)		Leaf[1]
Krista	a CZ 256 (Seva-Flora s.r.o.)		Leaf[1]
Kudrnka	a CZ 239 (SEMO a.s.)		Leaf[1]
Laica	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Lange Oberlaaer	a AT 72 (Austrosaat Österreichische Samenzucht- und Handels-AG)		Root[1]
Laura	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Lenka	a PL 1087 (Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa w Ożarowie Mazowieckim Spółka z o.o.)		Root[1]
Lisette	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Makói hosszú	b HU 191126 (Fekete János)		Root[2]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Marunka	a CZ 239 (SEMO a.s.)		Leaf[1]
Messis	a PL 938 (AdvanSeed ApS)		Leaf[1]
Mooskrause 2 Extra triple curled 2 Riccio muschiato 2 Riccio verde scuro 2 Moskrul 2 Moss Curled 2 Nain frisé mousse Nano ricciuto 2	b NL x b UK x b FR x		Leaf[1]
Moskrul 2		= Mooskrause 2	Leaf[1]
Moss Curled 2		= Mooskrause 2	Leaf[1]
Nain frisé mousse		= Mooskrause 2	Leaf[1]
Nano ricciuto 2		= Mooskrause 2	Leaf[1]
Natalka	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Leaf[1]
Nutka	a PL 1087 (Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa w Ożarowie Mazowieckim Spółka z o.o.)		Leaf[2]
Olomoucká dlouhá Ołomuńcka	a CZ x, a SK 250 (Zelseed spol. s r.o.) a PL 218 (Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa)		Root[1]
Oltis	a RO 1033 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Buzău)		Root[2]
Orbis	a CZ 239 (SEMO a.s.)		Root[1]
Orfeo	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Ory	a RO 1033 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Buzău)		Leaf[1]
Osborne	a CZ 1495 (Moravoseed CZ a.s.)		Root[1]
Ołomuńcka		= Olomoucká dlouhá	Root[1]
Peione	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Pesto	a PL 356 (Hortag Seed Co.)		Leaf[2]
Petronelia	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)		Leaf[1]
Petruschka	a DE 7092 (GHG Saaten GmbH)		Leaf[1]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Plain Leaved (Sheeps) 2		= Commun 2	Leaf[1]
Plain or single 2		= Commun 2	Leaf[1]
Polina	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)		Leaf[2]
Prairie	a NL 8 (Bejo Zaden BV)		Leaf[2]
Riccio muschiato 2		= Mooskrause 2	Leaf[1]
Riccio verde scuro 2		= Mooskrause 2	Leaf[1]
Rizado verde oscuro		= Frisé vert foncé	Leaf[1]
Roksana	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Root[2]
Rosette	b NL 136 (A.L. Tozer Ltd.)		Leaf[1]
Samba	a PL 187 (SPÓJNIA Hodowla i Nasiennictwo Ogrodnicze sp. z o.o.)		Root[1]
Simple 2		= Commun 2	Leaf[1]
Sonata	a PL 187 (SPÓJNIA Hodowla i Nasiennictwo Ogrodnicze sp. z o.o.)		Root[1]
Starke	b DK 56 (Weibulls Horto AB)		Leaf[1]
Starlett	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Thujade	b NL 65 (Pieterpikzonen BV)		Leaf[1]
Titan	b NL 8 (Bejo Zaden BV)		Leaf[1]
Toscano 2		= Commun 2	Leaf[1]
Troja	a CZ 239 (SEMO a.s.)		Root[2]
Verde scuro d'Italia		= Gigante di Napoli	Leaf[1]
Vistula	a PL 67 (Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o.o.)		Root[2]
Walser Petersilie	b AT 148 (Arche Noah)		Leaf[2]
Warta	a PL 187 (SPÓJNIA Hodowla i Nasiennictwo Ogrodnicze sp. z o.o.)		Root[1]
Wega	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Zaharat	a RO 1031 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Bacău)		Leaf[1]

(1): Variety denomination approved in the form of a code
a: Basic seed
b: Standard seed
Country code: AT – Austria, CZ – Czech Republic, DE – Germany, DK – Denmark, ES – Spain, FR – France, HR – Croatia, HU – Hungary, IT – Italy, NL – the Netherlands, PL – Poland, RO – Romania, SK – Slovakia, UK – United Kingdom
Used plant parts
1 (Kraus-Schierhorn 2019)
2 (Blüthner 2019)

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Chapter 14
Rosa x *damascena* Mill. (Rose)

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14.1 Introduction

Although the genus *Rosa* consists of more than 200 species, only a small number have been cultivated as essential oil crops including *R.* x *damascena* Mill. (Bulgaria, Turkey, Iran, India, Pakistan, Egypt, France, China, Russia), *R. alba* L. (Bulgaria), *R.* x *centifolia* L. (Morocco, France, India, Pakistan), *R. gallica* L. (Egypt), *R. rugosa* Thunb. (China) and *R. bourboniana* Desp. (India, Pakistan). The predominant rose species used worldwide for production of rose oil is *R.* x *damascena* also known as the Damask rose (Kovacheva et al. 2010a). Almost the whole production in Bulgaria and Turkey, which are the two biggest producers of rose oil in the world, supplying over 80–90% of this product to the market, is based on a single genotype of *R.* x *damascena* f. *trigintipetala* Dieck (the thirty-petalled rose), which has been vegetatively propagated for centuries (Ağaoğlu et al. 2000; Baydar et al. 2004; Rusanov et al. 2005b). The flowers of *R.* x *damascena* are traditionally used for production of rose oil, rose concrete, rose absolute and rose water, which are included in first-rate perfume products as well as in products of the pharmaceutical and food industries (Douglas 1993; Mirali et al. 2012). This chapter is focused on *R.* x *damascena* as the species with the highest impact on the rose essential oil industry.

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14.2 Botany

14.2.1 Taxonomy

Roses are classified in division Magnoliophyta, class Magnoliopsida, order Rosales, family Rosaceae, tribe Roseae, genus *Rosa* L., subgenus *Eurosa* Focke. *R.* x *damascena* Mill. belongs to section Gallicanae D.C. (Rehder, by Topalov and Irinchev 1967; Hrjanovskii 1958; Saakov 1965). According to the classification of the American Rose Society, roses are divided into three groups: species roses, wild roses; old garden roses, those that existed before 1867; and modern roses, those developed after 1867. *R.* x *damascena* belongs to the old garden roses (Shahbazi and Esmaeili 2012; Tobyn et al. 2011).

In Bulgaria, the cultivated *R.* x *damascena* has gained its well-known name "Kazanlak oil-bearing rose" (Kornova and Michailova 1994; Topalov and Irinchev 1967), because of the region where it is cultivated around the town of Kazanlak. Dieck 1889 (By Topalov 1978) defines the Kazanlak oil-bearing rose as *R. gallica* var. *damascena* Mill. f. *trigintipetala* Dieck, i.e. rose with 30 petals, and Crepin 1895 (by Topalov 1978) marks it as an ecotype of *R.* x *damascena* Mill., which is grown in Bulgaria. Hegi (1923), Stoyanov (1948) and Saakov (1965) accept Keller's definition of the Kazanlak rose as *R.* x *damascena* Mill. var. *trigintipetala* (Dieck) R. damascena Mill. var. trigintipetala (Dieck) R. Kell.

Hayek 1927, 1931 and Bonstedt 1931 (by Topalov and Irinchev 1967), note that var. *trigintipetala* differs from *R.* x *damascena* Mill. in that its flowers possess a stronger scent. The same author (Bonstedt 1931) points out that it is not var. *trigintipetala* Dieck. that is cultivated in Bulgaria for the production of rose oil but another variety called var. *kazanlik*. Based on morphological traits and agricultural characteristics, Topalov (1978) distinguishes the Kazanlak oil-bearing rose from the Damask rose and defines a new taxonomic unit called *R. kazanlika* V. T. sp. Nova, sect. Gallicanae D.C. Although this taxonomic definition has been used by some authors (Margina et al. 1997; Zlatev et al. 2001), it is not widely accepted. Due to the fame of the commonly used name "Bulgarian oil-bearing rose", some authors use the taxa *R. damascena* var. *Bulgaria* (Oka et al. 2014). According to the Germplasm Resources Information Network (GRIN) Taxonomy, the name of the species is *R.* x *damascena* Herrm., with synonyms *R. gallica* f. *trigintipetala* Dieck, *R.* x *damascena* var. *trigintipetala* (Dieck) Koehne and *R.* x *damascena* Mill. It is a cultivated hybrid and is therefore correctly written as *R.* x *damascena* Mill. (Tobyn et al. 2011).

14.2.1.1 Parental Origin

A number of speculations exist about the parental origin of *R.* x *damascena*. Some authors believe that the summer Damask roses, which bloom only in the summer, are a hybrid of *R. gallica* and *R. moschata* J. Herm, while autumn Damasks, which have a second bloom in the autumn, are a hybrid of *R. gallica* and *R. phoenicia*

Boiss. (Hurst 1941; Gudin 2000; Baydar et al. 2016). This model of parental origin, however, was not confirmed by the application of molecular markers, which showed that the summer Damask "Kazanlik" and the autumn Damask "Quatre Saisons" share identical microsatellite profiles (Rusanov et al. 2005b). The only study involving the use of molecular data on the parental origin of *R.* x *damascena* to date indicates that this hybrid species is probably of triparental origin, including several crosses between *R. gallica*, *R. moschata* and *R. fedtschenkoana* Reg. (Iwata et al. 2000).

14.2.2 Origin, Distribution and Domestication

14.2.2.1 Indications from the Antiquity for the Existence of Oil-Bearing Roses

Nazarenko et al. (1983) mentions that references to the oil-bearing rose as an ornamental plant are found in the literary sources referring to 2700 BC. In ancient Greece, according to Bunyard (1936) (by Topalov and Irinchev 1967), the Damask and the "hundred-leaved" (*R.* x *centifolia*) rose, which were brought from the Middle East, as well as the local species *R. sempervirens* L., *R. canina* L., *R. spinosissima* L. and possibly *R. gallica* became the base of the cultivated variety of roses. The first scientific information on roses and rose growing in ancient Greece are found in the works of Herodotus (fifth century BC) and especially Theophrastus (fourth century BC). Herodotus mentions the rose plantations around the rivers Tigris and Euphrates and the cultivation of a sixty-leaved rose with a much stronger scent in the gardens of king Midas, who lived near the Bermion mountain (Doxa) in Vodena, Greece. In Book VI of the *Enquiry into Plants*, Theophrastus, founder of the Old World Botany, who lived in the time when Alexander opens the doors of the East to enrich the Mediterranean flora with new plants, says that the roses grown differ in colour of the flowers, the sweetness of the scent and the number of petals. He noted the cultivation of roses, which have 5, 12, 20 and even 100 petals. According to Bunyard (by Topalov and Irinchev 1967), Theophrastus referred to *R.* x *damascena*, *R. alba*, *R.* x *centifolia* and possibly *R. gallica*. Theophrastus was the first to write about the influence of the soil conditions as well as the methods of rose propagation over their growth and development (Nazarenko et al. 1983; Evtuhova 2016). According to Aristotle (384–322 BC), the Greeks used the Egyptian methods of obtaining rose products and the cultivation of roses they adopted from the Phoenicians. The latter is also mentioned by Widrlechner (1981). Given the skills that people have had and the attitude towards beautiful plants, the probability one of the roses described by Herodotus (fifth century BC) and Theophrastus (fourth century BC) to be *R.* x *damascena* is significant.

In antiquity, wild roses have grown throughout the eastern Mediterranean. The flowers had 5–100 petals. It is hard to confirm how double flower forms originated. It may have occurred in the course of evolution as a result of a spontaneous mutation

process and then selected and propagated by experienced florists. Widrlechner (1981) also suggests that it was possible that *R.* x *damascena* had been the result of the interaction between conscious selection and the opportunity for the movement and recombination of many roses in the eastern Mediterranean region.

14.2.2.2 Origin and Domestication of the Oil-Bearing Rose

The country of origin of the oil rose, as most modern authors consider, is Persia (today Iran) (Nazarenko et al. 1983). From Iran, the oil rose has spread to Turkey, Egypt, India, Bulgaria, Spain, France, Russia and other countries. The Damask rose is the national flower of Iran (Haghighi et al. 2008), where it is known as Gole Mohammadi (Boskabady et al. 2011).

There is no single opinion in the world literature on the domestication of *R.* x *damascena*. Many authors point out that this species has not been collected in the wild. According to Widrlechner (Widrlechner 1981), Boissier (1872) was the first to note that *R.* x *damascena* had not been found spontaneously in Asia, which was later also stated by Hegi (1923), Komarov (1940) and Nilsson (1972). On the other hand, Pal (2013), quoting other authors, mentions that *R.* x *damascena* first grew wild, and it is still self-growing in Caucasus, Syria, Morocco and Andalusia. According to Topalov and Irinchev (1967), the cultivated variety of roses created in the Middle East countries – Assyria, Babylonia and Persia – was made up of the local wild species *R.* x *damascena* and *R.* x *centifolia* and in ancient China and India, the founders of the rose varieties became the beautiful, gentle and evergreen roses *R. indica* L., *R. thea* and others. Originally in the areas of Iran (Sumer and Akkadia) and on the eastern coast of the Mediterranean Sea, *R.* x *damascena* grew in natural conditions and in Assyria and Cilicia *R.* x *centifolia*, *R. gallica* and other species (Nazarenko et al. 1983). It is quite natural that the species so widely distributed in Iran and Syria could have been obtained as a result of selection and long-time open pollination between wild species and their natural hybrids. On the other hand, Shepherd (1954) (by Widrlechner 1981) stated that the first writing of *R.* x *damascena* was not until the 1500s.

14.2.2.3 Distribution of the Oil-Bearing Rose in Asia and Europe

A new stage in the development of rose growing in the world occurred during the seventh century when the Arabs, by conquering a number of countries, formed a large Islamic state bordering to the east with India and to the west with Spain. Using the experience and old traditions of Syrians, Persians and Egyptians, they created a new, flowering rose production related to the extraction of rose oil and rose water by distillation of rose flowers (Topalov 1978). Bliznakov (1934) notes that during the eighth and ninth centuries, rose water extracted by distillation was an important commercial product, which was traded from China to South India and from Persia to the Byzantine Empire. Most of the rose water was produced by Persia. During the

sixteenth and the seventeenth century, significant quantities of rose oil were sought for the developing perfumery industry in Europe. By the seventeenth century, rose cultivation had spread from Iran to India, northern Africa and Turkey (Baser 1992). During the seventeenth century, the oriental market was dominated by the Indian rose oil and later at the end of the seventeenth and the beginning of the eighteenth century Persian, Egyptian, Tunisian and Bulgarian rose oil (Bliznakov 1934).

Zarev (2008) points out the thirteenth century (during the Crusades) as the beginning of the oil-bearing rose introduction from the Middle East to Europe. According to a number of authors, rose growing in Bulgaria started during the sixteenth century (Topalov 1978; Astadzhov 1988b; Bliznakov 1934; Ganchev 1976; Georgiev 1932; Karaivanov 1966; Popova and Kozhuharova 1987; Topalov 1954; Michoff 1950) for production of rose water, used in the lifestyle of eastern peoples. Baser (1992) states that there is evidence of rose cultivation for the production of rose water in European Turkey dating back to the seventeenth century. Towards the end of that period, rose cultivation was introduced by a Turkish shepherd to Bulgaria, which at that time was a province of the Ottoman Empire.

14.2.3 Plant Description

14.2.3.1 Specifics in Growth and Development

R. x *damascena* passes through the following developmental phases: bursting of axillary buds, foliation, flower budding, flowering and second growth (Topalov 1962). The bursting of the buds is related to the onset of the juice movement and is related to extension of the buds up to 2 cm. The optimal temperature for this phase is about 10–12 °C. It lasts an average of 16 days – from the last 10 days of March to April 10.

The foliation begins with the appearance of the first leaf and continues until the flower buds appear (10–12th of April to the end of April). During this phase, five true leaves are sequentially differentiated from the vegetation tip of the buds. Following certain temperature impact, after the fifth leaf is formed, the first flower bud starts to differentiate from the vegetation tip. During the development of the flower organs, two new flower buds start to emerge at the base of the developing flower. Later on, the two new flower buds are set on their foundation, and so the inflorescence is formed.

Flower budding begins with the appearance of the first flower buds and ends with their blossoming (from the end of April to the end of May). At this phase, the accumulation of essential oil begins. Flowering begins from the end of May and lasts for 20–30 days. It is characterized by a full opening of the rose flower petals and continues until their fall.

Second growth After the end of the rose campaign, the flowering twig resumes its growth from the axillary bud of the last leaf, in the so-called second growth, which

lasts until late autumn. The more shoots develop during the second growth, the more flowers will be set in spring.

The oil-bearing rose is branching annually as its reproductive organs are laid on the branches developed the previous year. Branching increases the yields progressively, and this process lasts up to 7–8 years. After 8–10 years, the rose shrubs start aging and yields are reduced, resulting in the need of periodic rejuvenation.

14.2.3.2 Flowers

The flowers are located in a panicle. There are 3–9 and sometimes more (20–30) flowers on a twig, which bloom in succession. All flowers are actinomorphic, i.e. symmetrical. The Kazanlak rose blooms in late May and early June. The weight of a flower is 2–2.5 g on average. During flower development, the sepals are first to differentiate, followed by the petals. Carpels and stamens are formed at the latest. Each flower has five sepals, which, together with the flower peduncle, are covered with soft spines and glandular hairs. They are long in shape and sharp and possess several lateral branches. Inside, the sepals are smooth and whitish, and from the outside, they are pale green. The rose petals have a pink colour. According to Hegi (1923) and Bilov et al. (1962), the flowers of *R.* x *damascena* possess a large number of petals (50). The most common are 20–30 normal and 5–10 deformed petals, with a triangular shape and strongly rounded tops (Kovacheva 2007). The stamens are about 100–120. According to different authors, the number of stamens varies as follows: 78 ± 1 (Semenova et al. 2014), 88–116 (Zŭraw et al. 2015), 120 (Topalov and Irinchev 1967) and 71.00 ± 0.384 until 96.33 ± 0.333 (Farooq et al. 2011). The stamens consist of filamentous stalks and two anther sacs. According to Staykov (1965), 67% of the pollen grains are round in shape and 33% are oblong. Oblong grains are not viable. The outside of the anther sacs is covered with large cells filled with liquid. When the liquid evaporates, the cells shrink and the anther sacs open securing the release of pollen. The carpels in the Kazanlak *R.* x *damascena* are around 40–45 (Topalov and Irinchev 1967; Topalov 1978). Semenova et al. (2014) reported 29 ± 1 carpels in *R.* x *damascena* f. *trigintipetala* (Dieck) R. Keller and Zŭraw et al. (2015) reported 43 ± 3. The ovaries are superior, with a single ovule, and are attached to the base of the receptacle. The interior of the receptacle is soft, covered with plenty of long hairs. The styles have different lengths, depending on the location of the ovary in the receptacle. The stigmas are rounded and are located close to the anthers, but lower than they are.

14.2.3.3 Fruits

R. x *damascena* f. *trigintipetala* develops accessory fruits with oblong-ovate shape, orange-red to violet-red in colour. Most of the fruits fall off before ripening and the ones that remain produce 1–3 seeds. Because of the thick outer shell, the seeds need

stratification to germinate. The seeds are the real fruits of the rose. They are walnut shaped and are located on the inner walls of the false fruit or at its base.

14.2.3.4 Leaves

The leaves are odd-pinnate compound, composed of 5–7 leaflets on a common leaf petiole. The shape of the blade is oval to ovate, with a rounded base and a blunt or slightly pointed tip. Their periphery is jagged and their surface is covered with cuticle and hairs (more down the leaves), which limits excessive evaporation. The leaf veins are reticulate, bulging from the lower leaf surface and concave from the top surface. At its base, the leaf petiole possesses oblong, cohesive stipules. Along its entire length, the leaf petiole is covered with red-brown hairs and at the bottom with prickly spines curved back to the base of the petiole. The leaves fall with the onset of winter frosts.

14.2.3.5 Stem

The rose shrub is formed from 10–15 stems reaching up to 2–2.5 m height, as well as from the numerous side branches. The rose shrub branches into geometric progression, which leads to annual increase in yields. The young twigs have a grassy green colour and are covered with two types, relatively densely located prickles – short straight and long slightly curved downwards. In young shoots, they are reddish brown in colour and with aging they become grey-brown. After 8–10 years the shrubs start aging, which necessitates their rejuvenation. Branches of the shrub are cut deeply in order to provoke new growth of young shoots.

R. x *damascena* has two types of shoots – vegetative and flowering. The vegetative ones develop from the lower buds of the older branches. They grow vigorously at a height of up to 1 m and serve to rejuvenate old branches. Flowering twigs are shorter (up to 20–30 cm) and are much more numerous. They develop from the axillary buds on the tops of one-year-old branches, usually form five leaves, and then the flower buds sequentially differentiate from their vegetation peak. After overblowing of the flowers or their harvesting, the axillary bud of the last leaf starts to develop and further prolongs the flowering twig. The more the shoots of this second growth in the shrubs, the more flowers they will develop in the spring of the next year. The very differentiation of buds from the second growth occurs in the spring, after resumption of the juice movement and in the required influence for some time at high-temperature amplitudes from 4°°C to 16 °C.

14.2.3.6 Roots

Rosa x *damascena* is a perennial shrub. The root system consists of adventitious, highly developed ropelike skeletal branches formed by stem nodes and possesses a pronounced suction power. The skeletal roots have a well-defined geotropy. Their

branches reach 12th order and the length is over 3 m. They provide water and nutrients from the lower soil layers and therefore can successfully grow on permeable and sloping terrains. The root mass is placed superficially up to 40 cm deep. Geotropism and the high degree of branching make it possible to use soil moisture to the maximum extent and determine the cold-resistant nature of the rose shrubs. With aging of the root system, some of the skeleton branches successively die and in their place new ones are formed; thus the root system periodically rejuvenates.

14.2.4 Biodiversity

During the past two decades, a number of studies related to the analysis of genetic diversity among *R.* x *damascena* genetic resources available in the different countries of cultivation and natural growing of this species were performed. First, Ağaoğlu et al. (2000) analysed the genetic diversity among 14 *R.* x *damascena* f. *trigintipetala* accessions obtained from ten different locations in Isparta, Turkey, applying RAPD markers. The results showed identical profiles for all accessions. In a follow-up study Baydar et al. (2004) applied AFLP and SSR markers, analysing 15 accessions from 15 plantations from Turkey and again the results showed identical DNA profiles. Rusanov et al. (2005) analysed the genetic diversity among the *R.* x *damascena* genetic resources available in Bulgaria. Although in the past several studies pointed that different forms of *R.* x *damascena* have existed in Bulgaria, including forms with simple and double flowers and different colouration of the petals (Nikolov 1924; Georgiev 1927), currently only forma *trigintipetala* exists in collections and is industrially cultivated in the country. The study included accessions and cultivars used for industrial cultivation in Bulgaria as well as accessions from Turkey, Iran and India and several Damask rose accessions maintained in western European collections as garden roses (Rusanov et al. 2005b). The results showed that all accessions from Bulgaria and Turkey as well as the analysed accessions from Iran and India possess the same genotype, thus proving their origin from a common ancestor. Interestingly, the western European old garden Damask roses "Quatre Saisons" and "York and Lancaster" also possessed the same genotype as the one used in Bulgaria and Turkey for industrial production of essential oil. "Quatre Saisons" is an autumn Damask rose, characterized by a second bloom in the autumn in contrast to summer Damasks like the f. *trigintipetala* genotype used for cultivation in Bulgaria and Turkey, which blooms only once in summer. The obtained results rejected the hypothesis that these two types of Damasks have different parental origin as suggested earlier by Hurst (1941).

The geographic origin of *R.* x *damascena* is considered to be ancient Persia (nowadays Iran) from where it later spread to Turkey, Bulgaria, North Africa and West Europe. Indeed, several studies showed that in contrast to Bulgaria and Turkey, which are currently the two major exporters of rose oil in the world, (Kovacheva et al. 2010b), a number of different genotypes of *R.* x *damascena* were identified in Iran (Babaei et al. 2007; Farooq et al. 2013; Kiani et al. 2008, 2010; Pirseyedi et al.

2005; Tabaei-Aghdaei et al. 2006), Pakistan (Farooq et al. 2013), India (Kaur et al. 2007), Syria (Mirali et al. 2012) and Saudi Arabia (Amal et al. 2018).

As has already been shown, the Damask rose variety "York and Lancaster" possesses the same genotype as *R.* x *damascena* f. *trigintipetala* grown in Bulgaria and Turkey when comparing the profiles of SSR markers (Rusanov et al. 2005b). In 2000, Yokoya et al. (2000) determined that *R.* x *damascena* var. *versicolor* West. (var. "York and Lancaster") is a tetraploid species with a set of 4x = 28 chromosomes based on chromosome count and determination of DNA amounts. This result demonstrates that the major part of the rose oil world production is based on cultivation of a tetraploid genotype of *R.* x *damascena*. However, in Iran, in addition to the prevailing tetraploid genotype, also triploid and hexaploid genotypes were found based on flow cytometry analysis or microsatellite allele number (Babaei et al. 2007; Kiani et al. 2010).

The obtained results from biodiversity studies utilizing molecular markers demonstrated that almost the entire essential oil production industry is currently based on the industrial cultivation of a single genotype, which has been propagated vegetatively for centuries. Although a number of different *R.* x *damascena* genotypes were shown to exist in Iran and neighboring countries, still the industrial potential of these genotypes needs to be evaluated. Many of these "new" Damask roses have shown to be also different in terms of flower morphology, content and composition of the rose oil (Kumar et al. 2013; Mirali et al. 2012; Tabaei-Aghdaei et al. 2007). The existence of international standard for rose oil obtained from *R.* x *damascena* Mill. (ISO 9842:2003) has put some limits in the variation of chemical composition of the distilled essential oil from this species, and during the years the industry has shown to be quite conservative on demanding a product with constant quality, which is strongly related to the chemical composition and the used genotype. However, in the past few years a new product based on distillation of essential oil from the flowers of *R. alba* has emerged, which was well accepted by the market and which is different in composition from the traditional oil from *R.* x *damascena*. This is an indication that diversification of the essential oil products based on the utilization of different types of oil-bearing roses might be accepted well by the industry and the obtained products might find their own market even the composition of their essential oils is different from the traditional oil from the *R.* x *damascena* f. *trigintipetala* genotype cultivated in Bulgaria and Turkey on which ISO 9842:2003 is based. This could open opportunities for development of new cultivars and hybrids based on intra- and interspecific cross-pollination of *R.* x *damascena*.

14.2.5 Cultivation

As a perennial plant, the oil-bearing rose may last for more than 50 years and for industrial purposes 20–25 years. The duration of this period depends on the location, the soil type and the applied agrotechnology.

14.2.5.1 Propagation Strategies

Propagation of the oil-bearing rose by seeds (self-pollination, controlled crossing, free pollination) produces heterogeneous planting material that does not retain the properties of the parental forms (Nazarenko 1983). The resulting plants differ in plant habitus, petal colouration, number of petals, flower weight, number of prickles, resistance to diseases and pests and other traits (Nazarenko 1978, 1983; Mihailova and Kalaidzhieva 1984; Vlasova 1985; Staykov and Kalaidzhiev 1972, 1980; Baydar et al. 2016; Kovacheva 2014). For these reasons, as well as due to the breeders' concerns about the possibility of losing the quality of the traditional rose oil (Nikolov et al. 1977), for centuries, oil roses in Bulgaria and Turkey (Ağaoğlu et al. 2000; Baydar et al. 2016) have been propagated only by vegetative means, which preserves the characteristics of the parent plants.

Old Methods of Vegetative Propagation

The classic methods of vegetative propagation of the oil-bearing rose are "kesme", "improved kesme", "Chinese layering" and grafting on a dog rose (*R. canina*) rootstock. All these methods are old, are no longer applied and are not effective enough. Nowadays, a newer method is applied in Bulgaria – rooting of green cuttings in polyethylene greenhouses.

Kesme – original old Bulgarian propagation method with propagation coefficient 1:2 to 1:2.5. Rose shrubs cut from the surface of the ground are used as a propagation material. Cutting and planting is done in late autumn (November, December) and at the latest by February, before the beginning of vegetation. The shrub is cleaned from dry and damaged by agrilus branches and the branches are placed in pre-open furrows (35 cm deep and 40 cm wide) and covered with 10 cm soil and 5–6 cm organic mulch. In the spring, after the warming of the soil (April), shoots are developed from the buried branches. Disadvantages of the method are as follows: the propagation material is obtained from old roses, often strongly attacked by diseases and pests, and low propagation coefficient – 1 ha of old plantation creates 1–2 ha of new plantation.

Improved kesme – based on the above-described method. The distance between rows is 1–1.5 m. In the autumn of the same year or the next, after the end of the growing season, the young rooted roses are cut from the mother shrub and used to create new plantations. Higher yields (20–30%) are achieved compared to the original kesme method. Eight to ten hectares of new plantation are created from 1 ha old plantation.

Chinese layering – old plantations that can be irrigated are used to create nursery rows. The roses in the rows are bent and placed in a pre-dug ditch in the spacing next to the row followed by addition of soil and mulch. In autumn, the roses are taken out and new plantation is created with them. Advantages of the method: Higher reproduction coefficient from the above-mentioned methods 1:10 to 1:15. Disadvantage of the method is manual performance of most of the work.

Propagation by grafting on a dog rose rootstock – the method is based on grafting of a bud taken from the oil rose shoot and grafted on the root collar of a dog rose. The dog rose plant is 1 year old and obtained by seed propagation. Advantages of the method are as follows: high multiplication factor, increased flower yields by an average of 11% compared to non-grafted rose shrubs and the rootstock which affects the resistance of rose bushes to rust attacks and black leaf spots (Zlatev et al. 2001). Disadvantages of the method are as follows: lots of manual labour; shoots of the dog rose that start to appear in the following years, which need to be cut annually; and considerably raised costs for production of propagating material.

Currently Applied Methods of Vegetative Propagation

Production of Planting Material by Rooting of Green Cuttings

The rooting of mature and green cuttings is well known in the agricultural practice and is applied for the propagation of trees, shrubs and other plants. It is also applied in the propagation of oil-bearing roses in some countries. In India and other warm climates, mature cuttings are used for rooting of oil-bearing roses (Zlatev et al. 2001).

Since 1986, the Institute of Roses, Essential and Medical Cultures, Kazanlak, Bulgaria, has introduced new technique for the production of planting material of oil roses by rooting green cuttings in a cultivation facility (Nedkov et al. 2005). This method is based on the ability of parts of healthy leafy shoots placed under favourable conditions to form a root system and organs above ground. The implementation of this method of rooting requires the use of a nursery plantation for the production of shoots from which cuttings are prepared. Four to five-year-old plantation is used as a nursery plantation, which is cut out in February at a height of 5–10 cm from the base of the plants. This makes it possible to obtain a large number of shoots suitable for green rooting by the cutting period (June–August). Rooting takes place in a polythene greenhouse. Cuttings of 10–12 cm in length are prepared from the chopped part of the cut shoots. They are treated with biostimulators and are set for rooting in the greenhouses. Throughout the process, plant protection measures are carried out to combat rust, black leaf spots, aphids and mites. The prepared planting material is taken out in autumn (November). It has a well-developed root system, with 30–50 roots not less than 5 cm in length and root nerve thickness of not less than 3 mm. The above-ground part includes the stem, which is the remainder of the cutting, along with the new growth and the leaves. Its total length is not less than 11 cm. After taking it out, the propagation material is planted immediately or at the latest within 1–2 days at the site designated for the establishment of a new plantation. Advantages of the method are as follows: high reproduction coefficient 1:50–60; shortened production period (plants that are suitable for establishment of new plantations are developed for a period of 4 months); a flower yield of 1000–1500 kg/ha obtained 18 months after planting, unlike old methods, which take 3–4 years until the beginning of flowering; and obtaining a large number of propagating material on a small area (250 cuttings per 1 m^2). During the last 30 years, in Bulgaria all rose plantations have been created using the method of rooting green cuttings.

Tissue Culture Propagation (In Vitro)

In vitro propagation of roses has played an important role in the rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants. A number of reports exist for in vitro propagation of *R.* x *damascena* (Kornova et al. 2000, 2001; Kornova and Michailova 1994; Ginova et al. 2012; Ginova and Kondakova 2013) and *Rosa* sp. (Ara et al. 1997; Bhat 1992; Pati et al. 2006; Mirza et al. 2011; Canli and Kazaz 2009). Although micropropagation is accompanied by many difficulties (Ginova and Kondakova 2013; Iliev et al. 2010), this method is already in place in industrial production. During the past two years (2016–2017), development of oil rose plantations from in vitro propagated material at industrial scale has already begun in Bulgaria. This activity is carried out by the first private in vitro laboratory "Industrial Plants" (www.industrial-plants.net), which is located in the town of Kazanlak in the heart of the Rose Valley, Bulgaria.

14.2.5.2 Agrobiological Requirements to Climate Conditions

Moderate temperature and high atmospheric humidity, especially during the flowering period, are the most favourable climatic conditions for optimal flower and essential oil yields of the industrially cultivated *R.* x *damascena* f. *trigintipetala*. Low winter temperatures, when plants are in a relative rest, have a great impact on frosting of the roses. The rose shrub is very sensitive to sudden temperature fluctuations: sudden colds (up to −5, −10 °C) with strong winds in the winter are more dangerous than the gradual drop in temperature to −15 °C. Frosting of the shrubs is observed when the minimum temperature drops below −25 °C and during a windy weather.

The pace of warming during spring is of a great importance for the plant development. *R.* x *damascena* f. *trigintipetala* is sensitive to spring frost. As a plant without pronounced winter rest, during the warming in February and early March (above 4 °C), the rose shrub begins vegetation. Each frost after that causes damages usually expressed in frosting of individual parts of the branches, and in case of more cold, whole branches and even whole shrubs are frozen. A slow rise of the temperature in March and April results in slowing of the plant growth and diminishing of the risk of freezing during a period of re-cooling. A well-expressed 24-hour temperature amplitude during the prolonged spring is crucial for the development of reproductive organs, synthesis of essential oil and flowering duration.

The distribution of rainfall during the year is another important factor for rose growing. In the Rose Valley, Bulgaria, most rainfall occurs in May (average of 106 mm for a 30-year period) and in June (95 mm), when the flower buds grow and the flowering and rose harvest begin. During this period, the number of rainfall days is the highest, creating conditions for high atmospheric humidity, which is a prerequisite for obtaining high flower and essential oil yields (Topalov 1978; Topalov and Irinchev 1967; Zlatev et al. 2001; Nedkov et al. 2005).

14.2.5.3 Agrobiological Requirements to Soil Conditions

R. x *damascena* f. *trigintipetala* grows well and produces high yields on deep, aerated soils, relatively rich in humus substances, with a neutral or close to neutral soil reaction. The conditions of soil formation, geographic location and geomorphological conditions were studied by various authors (Atanasov 1965; Boneva 1969; Koinov et al. 1998; Kolarova et al. 1964). Penkov and Kovacheva (2013) defined the cinnamon forest soils leached as one of the most suitable for growing the oil-bearing rose (pH 6.7–6.9). The pseudopodzolic soils, surface overmoisturing, should be considered as unsuitable for growing this crop.

14.2.5.4 Agrotechnology of *R.* x *damascena* Mill f. *trigintipetala*

The preparation of the land to create new plantations includes weed control, excessive fertilization with organic and mineral fertilizers and deep treatment (plowing, dredging or trenching at a depth of 50–70 cm) depending on the soil type. Processing is carried out during the period May–August. Planting is performed manually or mechanically. When planting, the area should be kept free of weeds by several shallow treatments. The scheme applied in Bulgaria includes 2.80–3.00 m distance between rows, 0.70–0.90 m intra-row distance and around 4500–5000 plants per hectare. The most suitable planting time is November, when there is enough moisture in the soil and the days are still warm, ensuring good planting. Alternatively, planting can also be performed during the winter months and early in spring under appropriate agrometeorological conditions. In the first 2–3 years after planting, the care is limited to creating optimal water-air and nutritional regime consisting of several mechanized treatments and nourishing of the plants with mineral fertilizers. When soil is thickened and weed level is high, shallow plowing or milling can be applied. The last treatment is the autumn plowing at a depth of 18–20 cm.

14.2.5.5 Pruning

Cleaning

Pruning to clean from dried and agglomerated wood is done every year, during the winter months or early spring before the start of juice movement. Dry and agrilus sick branches are cut. When the plants become compressed in the row due to their growth, dying of the basal branches occurs. In this case, the basal branches should be cut to the base or higher – above a strong vegetative shoot.

Contour or Corrective Pruning

This type of pruning aims to regulate growth and yields. With the development of rose shrubs, flowering shoots grow to a height, with individual plants reaching more than 3 m. Because of limited lighting, the development of flower buds on the shrub is disproportional, which causes the flowers to be located on the surface of the shrub. The regulation of growth and yield through pruning, carried out immediately after the flowering at a height of 110–130 cm, helps to reduce the height of the bush and increase the yield by 46.4%. Pruning has a significant impact on the recovery of plant vigour, extension of the utilization term of the shrub, increase in the average flower weight and daily yield on harvesting by 35.7%. It is recommended that pruning be carried out every 3 years. Various attempts have been carried out in Bulgaria to determine the optimal moment and way of corrective and contour pruning (Paskalev 1986; Kovacheva and Nedkov 2009; Astadzhov 1980; Astadzhov 1988b). The regulation of flowering and yields of oil-bearing rose by pruning were studied by other authors (Paul et al. 1995; Porwal et al. 2002; Singh et al. 2002). Much of the research includes treatment methods and the time of the applied pruning (Singh and Ram 1987; Safari et al. 2004), the annual pruning of the tops of the shoots (Astadzhov 1980, Astadzhov 1988b), pruning under the subtropical climate of India (Singh et al. 2002) and pruning of ornamental roses with study of the effect of application on the flower yield (Zarina et al. 2004) and essential oil (Hassanein 2010).

In contour pruning, the yield is relatively high during the whole period of its application, but this does not solve the basic problem – the decay of the basal twigs, the thickening on the circumference of the shrub and the location of the flowers at a favourable height. In this case, shrubs need to be rejuvenated periodically. After 8–10 years of living, rose shrubs lose their active growth and gradually reduce the flower yields (Topalov and Irinchev 1967). In order to stop this aging, periodic rejuvenation of the rose shrubs needs to be carried out.

Contour pruning is also necessary when the surface part of the shrubs is damaged by frost, hail damage and other causes. Studies on the basic (rejuvenating) pruning of oil-bearing roses have been performed related to the degree of frost on the shrubs (Tshachev and Paskalev 1982), rejuvenating pruning under the mulch surface (Paskalev and Tshachev 1982), rejuvenating pruning in relation to development of a cutting apparatus (Todorov et al. 1989) and the effect of pruning (rejuvenation) in individual varieties of oil-bearing roses (Kovacheva and Nedkov 2012).

Rejuvenation takes place in spring before the start of the sap flow in February–March, but shrubs bloom during the next year. The bushes are cut at a height of 5–10 cm from the base. The new shoots that develop from the adventitious buds provide high yields already the next year after rejuvenation. Recently, this treatment has begun to be carried out right after the rose harvesting. With good care, a satisfactory yield is achieved in the following year. However, when a slight growth is observed during summer and extremely cold temperatures in the winter, plants are endangered by frost.

14.2.5.6 Fertilization

R. x *damascena* f. *trigintipetala* grows better on aerated soils that have the potential to promote a deep root system. Two periods of intensive absorption of nutrients have been established. The first period is the budding phase until the end of the rose harvesting. During this period the shoots grow rapidly, forming the flower buds and the roses massively bloom. Shrubs absorb about 58% of nitrogen, 64% of phosphorus and 54% of potassium (Zlatev et al. 2001). The second period is that of secondary growth, characterized by rapid growth of shoots after flowering. Plants absorb about 32% of nitrogen, 26% of phosphorus and 33% of potassium (Zlatev et al. 2001).

Excessive fertilization with phosphorus and potassium for a period of 3 years is applied to the lands assigned for new rose plantations. Flowering rose plantations can also be fertilized with phosphorus in periods of 3 years or annually. Amounts are determined based on the soil stocks.

Rose plantations are fertilized annually with nitrogen fertilizer, which is introduced with the first spring treatment of the plantation before the growing season. The nitrogen fertilizer is introduced twice – before the vegetation ½ of the norm and after the flowering the remaining amount. During the first and the second year after establishment of a new plantation, reduced doses of nitrogen fertilizer are recommended.

The microelements are extremely important for the development of roses and the obtaining of high yields. In the absence of Fe, Mg and Ca a varying degree of chlorosis occurs. Lambev (2011) reports an increase in yields of 7–11% when treated with a liquid bio-fertilizer containing a large set of macro- and microelements, phytohormones and other BAS including: humic acids (17 species), fulvic acids, amino acids as well as soil microorganisms.

14.2.5.7 Irrigation

Although the oil-bearing rose is known to be a deep-rooted and dry-resistant crop, droughts very often cause a severe reduction in flower yield. The yields are reduced both over the years with a dry winter-spring season when the stocks of moisture in the deep soil layers are small and in the case of droughts during the vegetation period, which has a bad effect on the growth. The rose needs large amounts of water in the soil from the beginning of the budding until the end of the vegetation and from the air humidity – from the beginning to the end of the flowering period.

In years when there is not enough winter humidity, it is necessary to make early spring water irrigations, which also have a positive effect in restraining the plants from growing and protecting them from damages caused by late spring frosts. In dry summer years, soil moisturizing and refreshment irrigations are required to fill the daily consumption and to maintain high air humidity in order to maintain high flower and essential oil yield during the flowering period. After the flowering period and during the hottest summer months, two or three waterings should be given depending on the degree of drought.

14.2.5.8 Flower Harvesting

The duration of the flowering period depends largely on the weather conditions. In warm and dry weather, flowering passes quickly – for 12–15 days. In cool and wet weather, it lasts for 25–30 days.

The flowering duration is a result of the biological feature of the oil-bearing rose to form a number of flower buds sequentially on a flowering twig during their differentiation period. The same sequence occurs during the flower opening. Thus, due to the non-simultaneous formation of the flower buds, as well as due to the non-simultaneous development of the individual flowering branches, the flowering is prolonged.

The flower is picked manually, with a slight twist, which separates the whole flower from its peduncle. Typically, an experienced rose picker can gain 20–30 kg of flowers. The picked up flower is placed in polyethylene sacks without tapping and crushing. Bags are tied up and settled under a shade until their transportation to the processing facilities. The highest content of essential oil is in the newly opened flowers, when they have the shape of a cup and the anthers of the stamens are light yellow. This is the so-called stage 5 of flower development. The earliest stage of flower development in which it can be picked is the third stage, i.e. when only the first outermost petal of the bud has opened.

The best time for picking starts in the morning around 5 a.m. Later start of the rose picking may result in large amounts of the rose flowers to be not harvested until 10.30–11 a.m. When picking up after this time, and in hot weather, the rose oil content drops sharply. In cloudy and cool weather, harvesting can continue until 12–1 p.m., and in cloudy and rainy weather, it can continue during the whole day.

14.2.5.9 Disease and Pest Control

Mechanical combat consists of (a) choosing a suitable predecessor (roses should not be planted 3–4 years after roses and rose hips), (b) timely weed control, (c) use of resistant and tolerant varieties and (d) winter-spring cleaning of the dry branches and their burning.

Chemical combat. During the vegetation period according to the prognosis and the instructions of the specialists, 2–3 pre-blooming sprays with fungicides against rust and black leaf spots, combined with insecticides against aphids, agrilus and other pests, are carried out at intervals of 10–14 days and 1–2 post-blooming sprays against diseases and pests.

Major Diseases

Rust Rust is caused by the fungus *Phragmidium mucronatum* Pers. It attacks the leaves but develops on all green overground parts of the rose shrubs. Strong attack causes leaves to fall in summer, exhaustion of the shrubs, reduction of yields and

growth of the plants as well as the shrubs easily frost. The weakened rose plantations are prone to attack by agrilus.

Black leaf spots Black leaf spots are caused by the fungus *Diplocarpon rosae* Wolf (*Marssonina rosae* Trail), which develops on the leaves and causes yellowing and fading.

Grey mould The cause is *Botrytis cinerea* Er. Most often, it affects the flower buds and the tips of the young shoots, which become brown and dry.

Major Pests

Agrilus cuprescens Men. (*A. mokrzeskii* Obebn.) is the most dangerous pest of the oil-bearing rose. Damage can reduce yields by 20 to 40%.

Rose curculio (*Homalorhynchites hungaricus* Fussly). The adult and the larvae cause harm. At a density of 1.5 pests on a rose shrub, an average of 2250 flower buds per hectare are destroyed. A similar damage is caused by the strawberry weevil (*Anthonomus rubi* Herbst.). Serious damage is also caused by *Syrista parreyssi* Spin., *Arge ochropus* Gmel. and other *Arge* species.

14.3 Economic Use

14.3.1 Plant Parts Used and Products Developed

The usable part of the *R.* x *damascena* shrub is the flower, which is processed to produce different flower products – rose oil, rose water, rose absolute and rose concrete, which are applied in the high perfumery, food and pharmaceutical industries.

14.3.1.1 Rose Oil

Rose oil is obtained by hydrodistillation of rose flowers. The oil content of *R.* x *damascena* is 0.03–0.05%, the maximum being at the 4th– 5th stage of flower development (Staykov et al. 1975). This is the stage of half-open to fully open bud with yellow stamens. At this point the most important terpene alcohols, citronellol, geraniol and nerol, quickly grow up to 60%. Industrial yields vary over the years, but on average, 1 kg of oil is obtained from 3000 kg of rose flowers. The classical technology of rose oil production includes double distillation – once from fresh flowers and second time from the distilling water. Proper process management, starting with flower picking, transportation, storage, hydromodule, speed, temperature and duration of the distillation, is critical to the yield and quality of rose oil.

Rose oil obtained from *R.* x *damascena* f. *trigintipetala* is a complex mixture of over 300 components. This composition makes it indispensable in the top perfumery industry and determines its potential in human and veterinary medicine. The chemical composition of the essential oil consists of terpenic, phenyl derivatives and hydrocarbon compounds (Balinova-Tsvetkova et al. 1977; Ponomaryova et al. 1979; Kovats 1987; Pellati et al. 2013). A small number of sulfur-containing components have also been identified (Omata et al. 1991). The amounts of eight of its constituents including citronellol, geraniol, nerol, ethanol, β-phenylethanol, heptadecane, nonadecane and heneicosane are under international standard regulation (ISO 9842:2003).

The name "Българско розово масло" (Bulgarsko rozovo maslo) has been included in the European Register of Protected Designations of Origin (PDO) and Protected Geographical Indications (PGI) by the Commission Implementing Regulation (EU) No 1020/2014 of 25 September 2014.

14.3.1.2 Rose Water

In Bulgaria and Turkey, rose water is considered a by-product in the distillation of rose oil. It can also be obtained by stacking less raw flower material. It contains a minimum amount of oil (about 0.02–0.09%), the composition of which differs from regular oil with high content of phenyl ethyl alcohol (Agarwal et al. 2005; Ulusoy et al. 2009; Verma et al. 2011). However, in some countries like Iran, rose water is considered the main product of interest during the distillation, where it finds application as a pleasant odorous substance during religious ceremonies.

14.3.1.3 Rose Concrete

Rose concrete is a red-orange mass with a characteristic scent of roses, which is obtained by extraction of fresh rose flowers with a hydrophobic solvent (hexane). It serves mainly as a source for obtaining rose absolute. The flowers are soaked in the solvent several times until the flavouring substances are completely extracted. In industrial conditions, stationary or rotor extractors are used. The yields are around 0.25%; i.e. 1 kg of concrete is produced from 400 kg of rose flowers. Applying ultrasound increases efficiency and reduces extraction time without negative quality changes (Elenkov et al. 1976).

An alternative to the conventional rose concrete production is the use of liquefied gases. Extraction with supercritical CO_2 at different pressure, temperature and processing time results in a product with specific properties (Baser et al. 2003; Da Porto et al. 2015; Moates and Reynolds 1991). When using 8 MPa/40 °C, the obtained product contains about 29% citronellol, 49% phenylethyl alcohol and 3% stearoptene (Baser et al. 2003). Citronellol 0.49%, phenylethyl alcohol 3.94%, farnesol 6.73% and heptadecane 13% (Da Porto et al. 2015) were reported at 5 MPa/15 °C. The yield is unstable and the parameters of the technology have not yet been established.

Subcritical extraction of rose flowers with 1,1,1,2-tetrafluoroethane produces an interesting product with specific qualities (Wilde 1996). At 0.5 MPa/60 min the yield is about 0.14%, and the composition includes phenylethyl alcohol 59% and citronellol 12.3% (Nenov et al. 2016). With the use of 10% dimethyl ether as a co-solvent the best composition was achieved with 69.6% phenylethyl alcohol, citronellol 3.8% and nonadecane 6.1% (Baser et al. 2003).

14.3.1.4 Rose Absolute

Rose absolute is obtained by extraction of rose concrete with ethyl alcohol. After filtration at cold conditions and separation of the solid paraffin fraction, a reddish liquid remains with a typical rose scent. The high quality concrete contains over 60% absolute. It contains essential oil components, with phenylethyl alcohol (over 70%) being essential. The extraction conditions are soft (room temperature) and the product has a scent that reflects the composition of the rose flower itself. Some perfumers prefer absolute for use in perfume compositions.

In recent years, attempts have been made to obtain absolute by extraction of the concrete with liquefied gases – carbon dioxide or tetrafluoroethane. The technology has greater flexibility with easy pressure variation and lack of residual solvent in the product. When using 80 bar/40 ° C, the product contains 50% phenylethyl alcohol (Reverchon and Della Porta 1996; Reverchon et al. 1997). The application of 1,1,1,2-tetrafluotethane also gives promising results, with the best quality of absolute being achieved by a 10-minute extraction with a high alcohol content of 62.4% phenylethanol and 6.9% citronellol and minimum content of hydrocarbons (Kürkçüolu and Baser 2003).

14.3.2 Economic Parameters of Rose Growing and Essential Oil Production in Bulgaria and Turkey

Presently, the main producers of rose essential oil, which supply 80–90% of the total rose oil produced to the world market are Bulgaria and Turkey (Kovacheva et al. 2010b).

Bulgaria has long-standing traditions in the cultivation and processing of oil-bearing roses. The export orientation of the sector has made it emblematic for the country. The area of oil rose cultivation has increased steadily over the last 10 years. The growing investor interest is determined by the steady growth in prices of the rose oil and the rose flower material. The total area of rose plantations in 2017 was nearly 4070 ha, which was 14% more than in 2010. The annual production of rose flower material in Bulgaria during the last few years was between 7500 and 8500 tonnes. The harvest of rose flowers in 2017 reached a peak of nearly 13,000 tonnes. Most of the big companies dealing with oil rose flower processing and traders in Bulgaria

invest in their own plantations. According to estimates (Inteliagro 2017), nearly 30% of the plantations are operated by 15 major market players in the country. Other distilleries work with long-term contracts with farmers, providing them with planting material, know-how and even financial support for investments in new plantation areas. The third, smallest segment of the market are producers who sell spontaneously, according to the proposed highest purchase price. Over 30 companies and more than 50 distilleries are producing and trade with rose oil in Bulgaria. According to the indicative data during 2017, the production of rose oil was above 3 tonnes in this year, compared to 2.53 tonnes during 2016 (Inteliagro 2017). Theoretically, the production potential is twice as large, given the available areas, a possible (realistic, not optimal) yield of 5 t/ha of rose flowers and an average ratio of oil/flower = 1/3300.

Oil-bearing rose plantations in Turkey until 1999 were 2758 ha with annual flower production of 9940 tonnes; 80% of rose oil production was from Isparta and 20% from Burdur and Afyonkarahisar districts (Ağaoğlu 2000). Baser et al. (2003) reported that the annual production of rose oil from 1998 to 2000 was 1800 kg, 1600 kg and 1400 kg and that of rose concrete was 2500 kg, 2200 kg and 2300 kg, and the processed flower material was 9200, 8600 and 6200 tonnes. According to Kart et al. (2012), although there were some fluctuations between 1980 and 2009, rose production has seen double increment with the increase of production from 4870 tonnes to 10,000 tonnes. A mature rose field normally yields 5 tonnes of fresh roses per hectare. The yield from more carefully nurtured field may go up to 7–8 tonnes per hectare (Baser 1992). According to Giray and Kart et al. (2012), 8510 tonnes of rose flowers, 1310 kg of rose oil, 1899 kg rose absolute, 8050 kg rose concrete and 100 tonnes of rose water were produced in 2009 in Isparta only.

14.3.3 Main Production Areas

Rose oil yield and composition is largely affected by a number of factors including the geographic area and respective soil and climatic conditions of rose growing. This is the reason why the main oil-bearing rose plantations in Bulgaria and Turkey are located in areas with favourable climatic conditions with moderate temperatures and high humidity during the flowering period. In Bulgaria this is the region between the mountains of Stara Planina and Sredna Gora, where the famous Rose Valley of Bulgaria is located. The districts of Plovdiv and Stara Zagora concentrate 90% of the total planted area with oil-bearing roses in the country and 80% of the number of farms. The main production centres are the municipalities of Karlovo, Pavel Banya and Kazanlak.

In Turkey, the cultivation of oil-bearing roses for distillation of rose oil dates back to the late nineteenth century when Turkish emigrants transferred and planted oil-bearing rose from Bulgaria in the region of West Anatolia (Topalov and Irinchev 1967). Baydar (2006) and Baser (1992) confirm that the oil-bearing rose was introduced to the Isparta province of Turkey in 1880. Today the main rose-growing regions in Turkey are Isparta (Giray and Kart 2012), Burdur, Denizli and Afyonkarahisar.

R. x *damascena* is the national flower of Iran and Iranians have been calling this plant the flower of Prophet "Mohammad" (Mahboubi 2016). The main product produced from the oil-bearing rose in Iran is rose water used during religious ceremonies, while rose oil is just a by-product. The major production areas in Iran are Kashan, Fars, Kerman and Azerbaijan. The most famous rose production region is Kashan where the highest quality rose water in the country is produced (Haghighi et al. 2008). Kerman Province with 2297 hectares of rose gardens and 6198 tonnes of flower production is one of the important rose production regions (Baradaran et al. 2012).

In India, the distribution of *R.* x *damascena* is limited to the mountains. Approximately 2500–3000 hectares (ha) of Indian land are dedicated to rose cultivation, including Himachal Pradesh, Kashmir, Pushkar in Rajasthan, Hasayan, Ettah, Kannauj, Ghazipur, Lucknow in Uttar Pradesh, Bihar and some areas in Punjab. (Kumar et al. 2013). The highest acreage of Damask rose lies in the Western Himalayan region of the northern plains where the land is ideal for its cultivation (Shawl and Adams 2009).

In Morocco, oil-bearing rose has been an industrial culture for centuries. Rose plantations are concentrated in the southern part of the country and occupy a small area of the Dade Valley. Nowadays, rose oil is produced from *R.* x *damascena*, grown in El Kelaa M'Gouna (https://www.biolandes.com). According to information from the International Trade Centre (www.intracen.org), Morocco has 30 hectares of *R.* x *damascena* plantations.

In Pakistan, the main rose cultivation regions are in Punjab and Sindh Provinces (Farooq et al. 2011). In Syria, the Damask rose can be found in Damascus, many of its suburbs and in Aleppo (Mirali et al. 2012). In Saudi Arabia *R.* x *damascena* Mill f. *trigintipetala* is grown in the Taif region (Amer et al. 2016).

In Russia, oil-bearing roses are currently grown in the southern regions, using high yield cultivars developed by hybridization between *R.* x *damascena*, *R. gallica* and *R. alba* (Egorova and Stavtseva 2016).

R. x *damascena* has also been naturally growing or industrially cultivated in other countries including Tunisia, Ukraine, Georgia, Moldova, Kyrgyzstan and Azerbaijan (Nazarenko et al. 1983).

14.4 Breeding Strategies and Achievements

14.4.1 Desired Traits

The main targets for improvement of *R.* x *damascena* f. *trigintipetala* are related to:

- Increased flower and essential oil yields
- Improved resistance to fungal diseases and pests
- Decrease of the amounts of undesired compounds in the essential oil like methyleugenol, which has been shown to cause cancer in rodents (Rietjens et al. 2005)

Another direction for breeding, which also directly correlates with yield, is the possibility for introduction of recurrent blooming. In contrast to Chinese roses, which bloom continuously from March to October, *R.* x *damascena* f. *trigintipetala* is a once blooming rose as the whole blooming process happens in May and June for a relatively short period of around 3 weeks.

The strategies for improving traits in *R.* x *damascena* include the application of standard breeding methods like clonal selection, mutagenesis and cross-pollinated breeding and the possible application of modern biotechnological approaches.

14.4.2 Clonal Selection

Several varieties in Bulgaria have been created based on clonal selection of best performing plants among the population of *R.* x *damascena* f. *trigintipetala* in the country.

Variety "Iskra" is characterized by increased in number and length branches (Astadzhov 1978). It reaches a height of 140–150 cm during the third year of flowering. The number of flowers on one flowering twig is 5–8 and on one shrub ranges from 850 to 970. The number of petals in the flowers is between 30 and 36, with a mean weight of 1 flower being 2.39 g. In the flowering plantations, the yields of fresh flower are in the range of 6400–7000 kg/ha, while the average oil content is 0.047%. The average damage caused by winter frost measured for a period of several years with diverse climatic conditions is 52%, percentage of rust attack is 20% and black leaf spot attack is 15% (Nedkov et al. 2005; Kovacheva 2010).

Variety "Svezhen" has been developed by the method of mass selection from the population of *R.* x *damascena* f. *trigintipetala* in Bulgaria, followed by cloning and pooling of similar clones (Staykov and Astadzhov 1975). The shrubs are vibrant, well branched, fast growing and reaching a height of 160–200 cm. Typical for "Svezhen" is the stacking of a larger number of flower buds per one flowering twig. The total number of flower buds per 1 shrub is 877, the average number of petals is 32, the average weight of 1 flower is 2.37 g, the yield of fresh flowers is 7320 kg/ha and the essential oil content is 0.054%. Frost under natural conditions is 56%, rust attack is 18% and attack by black leaf spots is 16% (Staykov and Astadzhov 1975; Zlatev et al. 2001; Nedkov et al. 2005).

Along with the development of new varieties, many *R.* x *damascena* f. *trigintipetala* clones, representing about 95–97% of all areas of this crop in Bulgaria, have been studied (Astadzhov 1975, 1988a, b, 1989). Plants with a higher number of flowers, larger flower sizes, high oil content, disease resistance and lower pest and frost attacks have been selected. Based on this selection, an "improved population", consisting of several clones of oil-bearing roses, has been developed. This "improved population" is high yielding 7200–7660 kg/ha of fresh flower, a rose oil content of 0.051%, rust attack of 13.9% and attack of black leaf spots of 20.4% and possesses excellent grade for essential oil quality (Kovacheva 2010).

Although the varieties "Svezhen" and "Iskra" have been shown to have a number of positive agronomic characteristics, they have not been used for industrial cultivation. Instead, the developed "improved population" has been massively propagated and used in production since the 1980s to present days.

14.4.3 Mutagenesis

Tsvetkov developed two new cultivars based on *R.* x *damascena* f. *trigintipetala* in Bulgaria through radiation and chemical mutagenesis, coupled with clonal selection (Tsvetkov 1984).

Cv. "Elejna" is a high yield cultivar with an average yield of 6670 kg/ha, reaching 11,860 kg/ha and essential oil content of 0.052%. It is characterized by high resistance to critical negative temperatures and increased resistance to attack by economically important diseases — rust and black leaf spots.

Cv. "Yanina" is also a high flower yield cultivar with an average yield of 5130 kg/ha, reaching 10,070 kg/ha. Essential oil content is 0.045%. Yanina is also characterized by good cold resistance and increased resistance to economically important diseases.

Until now, these two cultivars – developed through mutagenesis – have not been used for industrial cultivation.

14.4.4 Cross-Breeding

According to data from breeders, diseases and pests can cause flower yield reduction in some years in the range of 20–40% making improved disease resistance a major breeding trait. At the same time, other oil-bearing rose species like *R. gallica* and *R. alba* show much higher resistance to fungal diseases compared to *R.* x *damascena* f. *trigintipetala* making them a desired parent in interspecific breeding strategies.

Due to the breeders' concerns for losing the quality of the rose oil (Nikolov et al. 1977), the oil-bearing rose in Bulgaria has been propagated by vegetative means. For the same reason the use of hybrids has not been tolerated. Looking for opportunities to introduce the desired traits (high yield of fresh flowers, high content of essential oil, cold resistance and high resistance to diseases), during the period 1973–1978, breeding based on cross-pollination has been initiated (Popova and Kozhuharova 1987; Staykov and Kalaidzhiev 1972, 1980; Mihailova and Kalaidzhieva 1984). The breeders managed to develop hybrids and lines with good economic qualities, highly resistant to diseases, but with characteristics of the essential oil different from the international standard. A new oil rose cultivar called "Prolet" was created (*R.* x *damascena* x *R. gallica* subsp. Eryostila Kell var.

Austriaca Br.), but it was not accepted by the industry because of the desire not to change quality of the Bulgarian rose oil.

A number of new cultivars have been created in Russia and Ukraine using cross-pollination (Demidov 1983; Nazarenko 1983) where *R.* x *damascena* f. *trigintipetala* participated in the series of crosses as one of the parents. For example, the cultivars Novinka, Kooperatorka, Bukuria, Julskaja, Michurinka (Kluka 1964), Ukraina, Djalita, Ulskaia and Vilena (Nazarenko et al. 1983) are hybrids developed by crossing *R.* x *damascena* (the Kazanlak rose) x *R. gallica* and cultivar "Pionerka" was developed by crossing *R. gallica* x *R.* x *damascena* (Kluka 1964; Demidov 1983). The cultivars "Lanj" (*R. alba* x cv. Michurinka) and cv. "Raduga" (cv. "Prolet" x cv. "Crimean Red" (*R. gallica*)) were created by the method of remote hybridization (Nazarenko 1983; Nazarenko et al. 1983; Nazarenko et al. 1984; Nazarenko and Grishchenko 1990). Variety "Tavrida" (Nazarenko et al. 1983) was developed based on individual selection of plant derived from free pollination of *R.* x *damascena*.

In more recent years, new hybrids based on crosses between *R.* x *damascena*. f. *trigintipetala* and *R. gallica* cv. "Crimean red" showing increased resistance to diseases and good quality of the rose oil were also created (Kovacheva 2010).

In spite of the good characteristics of the developed varieties and cultivars obtained through cross-pollination strategies, none of them has managed to become widely cultivated for industrial purposes. The main concerns from industry are the undesired changes in the essential oil composition and therefore in quality compared to the traditional essential oil obtained from the original *R.* x *damascena* f. *trigintipetala* genotype traditionally grown in Bulgaria and Turkey.

14.4.5 Undesired Plant Secondary Compounds

A number of studies showed that methyleugenol, a compound found in a wide range of natural products, is carcinogenic and can cause cancer in rodents (Rietjens et al. 2005). Methyleugenol is present in the oil of *R.* x *damascena* in quantities up to 3.5% (Jirovets et al. 2002; Kovats 1987). In the United States, it is listed as a safe substance according to 21 CFR §172.515 (FDA 2004), but has been provisionally unauthorized since 2015 (FDA 2018). In Europe, EU Regulation 1334/2008 prohibits its addition to food and limits its concentration in food or cosmetic flavours (European Commission 2008; European Commission 2000). Although the content of methyleugenol in rose oil is quite low, the researchers have been looking for ways to decrease its amount in rose oil and related products. In 2011, while studying the changes in volatile composition during different stages of *R.* x *damascena* flower development, Rusanov et al. discovered that methyleugenol was not detected in extracts of *R.* x *damascena* flowers until stage 6 (Rusanov et al. 2011). In a follow-up study the same authors compared the composition of *R.* x *damascena* essential oils distilled from several types of flower material including full-blown rose flowers (stages 6–7 of flower development), rose buds up to stage 5 of flower development,

rose petals only and petal-less flowers (Rusanov et al. 2012). The obtained results showed that methyleugenol was reduced around two times in rose oil distilled from rose petals but more than 5 times in essential oil distilled from rose buds up to stage 5. The amounts of the analysed compounds, which are part of the international standard for rose oil (ISO 9842:2003) showed no significant deviation from the rose oil traditionally distilled from full-blown flowers with the exception of heptadecane with a slight increase of around 1%. The yield of rose oil from kg of flower material was not significantly different between rose buds up to stage 5 and full-blown flowers. Moreover, the yield of rose flower material increased more than 2 times when harvesting rose buds compared to full-blown flowers from the same planting area. The obtained results allowed the authors to suggest a change in the traditional harvesting practices from harvesting of full-blown flowers (stages 6–8) towards picking up buds and flowers up to stage 5. However, these results still need to be verified on an industrial scale.

Decrease of the amounts of methyleugenol could be also achieved by cross-breeding with other rose species possessing lower amounts of this compound. For example, the quantity of methyleugenol in the essential oil of *R. alba* is only in traceable amounts or not identified at all (Dobreva 2010). The composition of the essential oil from *R. alba* although different from the oil of *R.* x *damascena* in the relative amounts of the individual compounds is as a whole quite similar as a set of compounds. This makes *R. alba* a suitable candidate for breeding based on cross-pollination with *R.* x *damascena* and for evaluation of the quality of the rose oil of the obtained hybrids and lines.

14.4.6 Molecular Breeding Perspectives

In 1999 the first genetic linkage map of roses was constructed by Debener and Mattiesch (Debener and Mattiesch 1999) and since then the knowledge about the rose genome and the way it works has been steadily growing. A number of studies related to mapping of QTLs and monogenic traits led to the development of a unified genetic map of diploid roses (Spiller et al. 2011) where agronomically important traits like flower colour (Debener and Mattiesch 1999), petal number (Debener and Mattiesch 1999; Hibrand-Saint Oyant et al. 2008; Koning-Boucoiran et al. 2012; Roman et al. 2015), date of flowering (Hibrand-Saint Oyant et al. 2008; Remay et al. 2009; Roman et al. 2015), black spot resistance (von Malek and Debener 1998; Yan et al. 2005), powdery mildew resistance (Linde et al. 2004, 2006; Moghaddam et al. 2012; Yan et al. 2005), recurrent blooming (Crespel et al. 2002; Hibrand-Saint Oyant et al. 2008; Remay et al. 2009) as well as flower- and scent-related genes and QTLs (Remay et al. 2009; Spiller et al. 2010) were mapped and their location linked to particular DNA markers.

Most of the research related to genetic mapping in roses has been carried out in diploid species. However, modern roses as well as *R.* x *damascena* are tetraploid with mode of inheritance disomic or tetrasomic depending on the particular locus

(Koning-Boucoiran et al. 2012; Rusanov et al. 2005a). Recently, Bourke et al. (2017) presented an ultra-high density linkage map of a tetraploid *R. hybrida* where also the synteny with the sequenced genome of *Fragaria vesca* L., which is the closest related sequenced species, was analysed. The sequence of the rose genome has just recently been revealed and published in 2018 (Raymond et al. 2018). The availability of the rose genome sequence will soon allow linking particular loci in the rose genetic map with candidate genes. This will make the availability of the rose genome sequence an invaluable source of information allowing accelerated breeding in roses with the help of molecular markers linked to genes, which determine traits of interest.

Recently, a project on discovering the genetic background of flower architecture in *R.* x *damascena* f. *trigintipetala* was initiated at the AgroBioInstitute, Bulgaria, based on analysis of a population of plants obtained after self-pollination of *R.* x *damascena* f. *trigintipetala*. The plants from the population showed wide variation in flower characteristics including number of petals and number of stamens (Rusanov et al. unpublished data) as around 30% of the plants possessed simple flowers while the rest developed semi-double and double flowers. Analysis of the expression of several flower homeotic genes showed that only the expression of *R.* x *damascena* AGAMOUS was changed in plants with simple flowers (around 3–4 times upregulated) compared to plants with double flowers (Rusanov et al. unpublished data). The segregation of the four alleles of *R.* x *damascena* AGAMOUS in the population did not correlate with the number of flower petals for the plants in the segregating population. However, analysis of several markers in the vicinity of the *Blfo* locus, which was previously shown to control the double corolla phenotype in diploid roses (Debener and Mattiesch 1999), allowed the identification of a dominant allele of the SSR marker Rh58, which was linked in 92% of the cases with the double corolla trait. Using this or more closely linked markers will allow early selection of hybrids with double corolla in breeding programs based on cross-pollination including *R.* x *damascena* as a parent. The double corolla trait is especially important in *R.* x *damascena* breeding since the majority of rose oil is located in the epidermal cells of the flower petals and plants with double corolla (higher number of petals) will have clear advantage in terms of essential oil yield compared to simple flower plants. The obtained results show that information from diploid roses can be effectively transferred to breeding of the tetraploid *R.* x *damascena*.

14.4.7 Biotechnology Perspectives

14.4.7.1 Improvement Through Genetic Engineering

Development of genetically modified plants is already a mature technology, which has been applied to numerous plant species including roses (Borissova et al. 2005). The function of a number of genes, which are known to be directly related to important flower- and scent-related characteristics in roses, is also known and can be

utilized for improvement of *R.* x *damascena*. However, the current public opinion in the EU and Bulgaria, which is one of the biggest producers of rose oil in the world, has been strongly against the development and application of genetically modified plants. This has resulted in a very restrictive legislation in Bulgaria, which prohibits the release of genetically modified plants in the environment of several crops including *R.* x *damascena*. All this makes the application of gene transfer technology on oil-bearing roses in Bulgaria groundless. To the best of our knowledge, GMO roses based on *R.* x *damascena* have not been developed in the rest of the world as well.

14.4.7.2 New Plant Breeding Techniques

During the last decade a new field has emerged based on the application of the so-called new plant breeding techniques (NPBTs). Several NPBTs like zinc finger nucleases, TALENs and Crisp/Cas9 are related to the development of double-strand breaks in the host genomic DNA, which can result in either the induction of precisely targeted mutations in the genome or to the integration of foreign DNA when a copy of such is present in the cell. As such NPBTs are a bordering techniques between classical mutagenesis and genetic engineering, which are still not regulated by the law in the EU and to a great extent in the rest of the world. The future development of the regulatory framework in this respect will determine the opportunities for application of these techniques in the breeding of new oil-bearing rose cultivars. In the case of *R.* x *damascena*, NPBTs may allow the development of new cultivars based on precise mutagenesis in genes, which are known to be related to important flower-, scent- and disease-related traits. NPBTs have a number of advantages over classical breeding techniques, allowing the introduction of new traits based on targeted mutagenesis and at the same time the preservation of the rest of the genome context. Unlike traditional mutagenesis, where the introduced mutations are random, unpredictable and with unknown location in the genome, NPBTs are precisely targeting genes of interest. The time necessary to develop new rose cultivars with NPBTs will also be dramatically reduced avoiding the necessity to pass through "tough" crossings in roses, for example, between the tetraploid *R.* x *damascena* and diploid rose species leading to the development of often sterile triploid hybrids, which hampers further necessary backcrosses.

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Chapter 15
Rosmarinus officinalis L.: Rosemary

Merita Hammer and Wolfram Junghanns

15.1 Botany (Taxonomy, Origin, Distribution, Cytology, Plant Description)

Rosemary (*Rosmarinus officinalis* L.), as other well-known herbs like basil, thyme, oregano, mint or its closest relative sage, is taxonomically classified under Mentheae, the largest and economically most important tribe of the subfamily Nepetoideae within Lamiaceae family. The genus *Rosmarinus* has been classified differently by different authors. The old classifications (Bentham 1876; Briquet 1987) include the genus *Rosmarinus* into the subfamily of Ajugoideae. Recent studies on phylogeny of Mentheae (Walker et al. 2004; Moon et al. 2010), using analysis of characters like morphology of leaves, fruits and pollen, confirmed the tribal relationships, but did not corroborate the subfamily affiliation suggested by the old classification (Bentham 1876). Wunderlich (1967), based on a survey of pollen, ovule embryo sac and seed morphology, included *Rosmarinus* into the subfamily Nepetoideae.

Taxonomic reviews based on morphological and molecular data (Harley et al. 2004; Moon et al. 2008; Wagstaff et al. 1995; Walker and Sytsma 2007) demonstrated a close relationship between *Rosmarinus*, *Perovskia* Kar., *Meriandra* Benth., *Salvia* L., *Zhumeria* Rech. F. & Wendelbo, *Lepechinia* Willd. and *Dorystaechas* Boiss. & Heldr. Considering the high similarity with *Salvia*, also Drew et al. (2017) suggested to group the genus *Rosmarinus* together with *Salvia*, *Dorystaechas*, *Meriandra*, *Perovskia* and *Zhumeria*. Two fertile stamens (as opposed to four, the usual number of mint family members), bireticulate sexine ornamentation of pollen (as opposed to perforate; Moon et al. 2008), and the presence of "large crystals in the innermost cell layer of the mesocarp" (Ryding 2010) are distinctive common characters of *Salvia*, *Rosmarinus* and these genera. Based on such similar

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morphology, *Salvia* and *Rosmarinus* have been considered by previous taxonomic classifications as closely related and are placed as one genus of the subtribe Salviinae in the recent taxonomic review of Lamiaceae (Harley et al. 2004).

Rosemary is supposed to be of Plio-Quaternary origin (Segarra-Moragués et al. 2016; Hewitt 1996, 1999). Mateu-Andrés et al. (2013) supposed that the distribution of rosemary in its present geographical areal has followed three different routes: a northern route expanding along the northern side of the Mediterranean and two southern routes, one from the west to east through North Africa and reaching Cyrenaica and a second one to the south-west of the Iberian Peninsula, from where it came back to the south-central areas.

In contrast to the high diversity of other genera of mint family such as *Salvia*, *Origanum* or *Thymus*, the genus *Rosmarinus* L. includes only three species (Greuter et al. 1986):

1. Rosmarinus officinalis L. synonyms: R. lavandulaceus De Noé; R. laxiflorus De Noé.
2. *Rosmarinus eriocalyx* Jord. & Fourr. syn. *R. tournefortii* (Murb.) Joh. & Maire.
3. *Rosmarinus tomentosus* Hub. – Mor. & Maire.

All three species are diploid chamaephytes with 2n = 24 chromosomes (Rosúa 1986).

Rosmarinus officinalis L.

Among three species of the genus, *R. officinalis* is the most widely spread, present as native in Portugal, Spain, France, Italy, Greece, Albania, Bosnia and Herzegovina, Croatia, Montenegro, Morocco, Algeria, Libya, Tunisia, Egypt, Cyprus and Turkey and naturalized in Bulgaria, Crimea, Azores, Canary Islands, Cape Verde, Bermuda, Texas and Central Mexico. It can be found from sea level to 1600 m a.s.l., usually growing in arid, semi-arid and calcareous habitats (Morales 2010). High genetic variability and adaptability of *Rosmarinus officinalis* L. populations in the western part of the Mediterranean, as well as the coexistence of this species with the other two species of the genus in North Africa and Andalucia, suggest that the diversity centre of the species is located in this territory (Mateu-Andrés et al. 2013).

The huge variability of different morphological characters (Turrill 1920) considered by different authors to be influenced by environmental factors (Morales 2010; Segarra-Moragués et al. 2016; Valverde et al. 2016) makes the taxonomic classification of the species difficult. Several attempts have been made to describe different infraspecific taxa like *R. officinalis* subsp. *palaui* O. Bolós & Molinier (Bolòs et al. 2005) or recently *Rosmarinus officinalis* L. subsp. *valentinus* P.P. Ferrer, A. Guillén & Gómez Nav., (Ferrer-Gallego et al. 2014), which have not been accepted by taxonomical reviews (Rosúa 1986; Greuter et al. 1986; Rosselló et al. 2006; Segarra-Moragués and Gleiser 2009; Morales 2010).

Rosmarinus officinalis L. is an evergreen perennial, occasionally shrub-like, up to 3 m high, usually erect, with no rooting, woody highly branched stems (Morales 2010). The branches are erect, climbing or decumbent and young branches hairy grey-green. The leaves, numerous on twigs, 15–40 mm by 1.2–3.5 mm are sessile,

leathery (due to numerous trichomes), opposite, strongly recurved and entire-margined, glabrous and dark green on the upper surface and greyish-green and densely tomentose underneath, due to the presence of typical hairs, rich in glands containing the volatile oil, of which the minimum content specified is 1.2% (European Pharmacopoeia 2004). The flowers, blooming from March to October, grow on tomentose inflorescences in the leaf axils of the upper part of the branches. The fruits are tetracheni with free achenes, oblong, smooth, brownish.

R. officinalis subsp. palaui (O. Bolós & Molinier) Malag. is an endemic from the Balearic Islands. It is characterized by a decumbent habit, up to 80 cm high, small purple corolla, very leafy branches, short leaves, succulent, is dark green and bright, flat lateral lobes of the lower lip.

Rosmarinus officinalis subsp. valentinus P.P. Ferrer, A. Guillén & Gómez Nav from the eastern part of the Iberian Peninsula is distinct by prostrate habit up to 20 cm high, small calyx and white corolla with clear purplish sparkles, slightly leafy branches, narrow fairly thick leaves, lush green colours.

Rosmarinus officinalis subsp. *valentinus* frequently hybridizes with subsp. *officinalis*.

Rosmarinus eriocalyx Jord. & Fourr. is a narrow endemic, native to northwest Africa and southern Spain, where only few scattered populations exist (Rosúa 1981, 1986; Morales 2010). Small leaves (5–15 mm long and less than 2 mm broad) as well as the presence of long pluricellular hairs on the calyx surface are distinctive characters of this species. With a prostrate growth habit, it can reach up to 1 m of height. The plant grows widely in southern Iberian Peninsula (Almeria Province) and on rocky ground and pastures in the mountainous areas of Algeria, Libya and Morocco (Arnold et al. 1997), but it is also cultivated in these regions.

Rosmarinus tomentosus Huber-Morath & Maire so-called Romero bianco, is a southern Iberian endemic, native to Andalucia (Granada and Malaga), where it can be found on coastal and inland cliffs at altitudes between 0 and 250 m a.s.l. (Rosselló and Sáez 2000). This species has a similar calyx indumentum and also bears a distinctive V- to Y-shaped marking on the entire and distinctly concave lower corolla lobe like *R. eriocalyx*. But it is quite distinct from the latter one by its dense compact habit and silver-grey tomentose leaves which are borne in dense clusters. It is a chasmophytic shrub with abundant branching; stems and small leaves are covered with thick, greyish-white tomentum (Rosúa 1981). This species is predominantly outcrossing, as are most species of Labiatae (Owens and Ubera-Jiménez 1992), with entomophilous pollination mainly by Hymenoptera, such as bees and bumblebees.

Hernández Bermejo and Clemente Muñoz (1994) reported about a very few populations still existing, well adapted to extreme sun exposure, scarce precipitation, strong winds and saline environment. Due to reproductive limitations of this species, difficulties in rooting of cuttings and scant flowering, low seed productivity, very low germination capacity and prolonged latency periods, only very few young

plants are found in the wild; therefore, the species is categorized as "endangered". *Rosmarinus tomentosus* was firstly recognized as a separate species in 1941. Recent research has indicated that *R. tomentosus* forms a monophyletic group, which is included within paraphyletic group of *R. eriocalyx* (Rosselló et al. 2006).

There are very few molecular data of the population genetics of the species. Zaouali and Boussard (2008) reported a high correlation between allozyme variability and structure and composition of essential oils. Studies of Segarra-Moragués and Gleiser (2009) indicated high genetic variability by using nuclear microsatellites.

15.2 Economical Use (Plant Parts Used, Wild Collection/Cultivation, Domestication, Valuable/Undesired Plant Secondary Compounds, Main Production Areas, Economical Valuation/Parameters)

Among all *Rosmarinus* species, only *R. officinalis* had gained medicinal, pharmaceutical and industrial importance.

15.2.1 Bioactivity

Rosemary is not only a culinary herb but also a highly appreciated medicinal plant of great pharmacological potential, considering its properties like antioxidant, antimicrobial, anticancer, antidepressant, neuroprotective, cholinergic, anti-inflammatory, analgetic, antispasmodic, diuretic, expectorant and carminative (Andrade et al. 2018; Amaral et al. 2018; Pintore et al. 2002; Del Pilar Sánchez-Camargo and Herrero 2017). As one of the most abundant sources of antioxidant compounds, rosemary is becoming increasingly important not only in food conservation and stability but also in preventive medicine, in the control of pathogen-caused human diseases and recently in plant protection and veterinary medicine. The greatest interest is concentrated on the possible development of new drugs on rosemary basis.

15.2.2 Valuable Plant Secondary Compounds

The phenolic compounds such as carnosic acid and carnosol are primarily responsible for the distinctive antioxidant activity of rosemary. They give rosemary a mild bitter taste and affect the generation of off-flavours. Their distinctive antioxidant properties have made rosemary a very promising plant material for pharmacy and cosmetics and valuable for the food industry.

Carnosic acid and carnosol content in rosemary vary widely. Carnosic acid content is found to range from 3 mg/g DW to 50 mg/g DW and carnosol content from 0.2 to 0.4% DW (Birtić et al. 2015; Hidalgo-Fernández et al. 1998a; Richheimer et al. 1996; Schwarz and Ternes 1992). The factors influencing this variation are genetic (variety), development stage of plants (ontogenesis) and environmental conditions like physical and chemical properties of the soil or hydric stress.

The highest level can be reached at the beginning of growth phase. Luis and Johnson (2005) found that high temperatures combined with low precipitation rates decrease the level of carnosic acid. Investigations made by other authors confirm the influence of water stress and light intensity on the concentration level of diterpenes in rosemary plants (Munné-Bosch et al. 2000).

Rosmarinic acid is another diterpene with antioxidative and antiviral activity supposed to be effective against neurodegenerative diseases (Andrade et al. 2018). A minimum of 3% of rosmarinic acid is specified for Rosmarini folium drug (European Pharmacopoeia, fifth Edition).

To the antioxidant, antimicrobial and anti-inflammatory compounds of rosemary belong also triterpenoids like *oleanolic and ursolic acids*. The later one is considered a trypanocidal constituent in rosemary (Abe et al. 2002).

15.2.3 Undesired Compounds

According to WHO Monograph, eugenol, which can be metabolized to a quinone methide, is considered to be toxic, but its content in rosemary leaves is low, so that there is no risk for human health. Johnson et al. (2001) reported that certain extraction methods, like methanol extraction of rosemary, contribute to a higher concentration of eugenol, thus increasing the risk of toxicity.

15.2.4 Uses and Production

Rosemary is considered to be one of the most traded herbs in the international market (Ravikumar 2000). Even if cultivation is increasing steadily, wild collection is still the major supplier of trade (Zeineb 2012; ProGuiRosemary 2012).

Leading regions of rosemary production are Europe, Northern Africa, Mexico and the United States. Svoboda and Deans (1992) reported Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal, Tunisia and Morocco as the main producers of rosemary.

Rosemary is widely used for flavouring food in Mediterranean cuisine and as a highly appreciated medicinal plant in folk medicine as well as pharmacy, cosmetics and phytocosmetics.

A range of rosemary products can be found on the market, but the most important ones, beside the fresh areal parts or pot plants, are dried leaves usually harvested before flowering, essential oil and different extracts as powder, infusion or dry extracts.

The extracts obtained from rosemary with main compounds like rosmarinic acid, carnosic acid and carnosol (Samuelsson and Bohlin 2001; Anon 2009; Begum et al. 2013) are widely used in cosmetic applications and since about 20 years as natural antioxidants in food industry, replacing the synthetic antioxidants for food preservation. Since 2008 rosemary extract has been approved by EU as natural food additive under the product name E392 (EFSA 2008). Rosemary extracts are legalized as effective natural antioxidants for food preservation in Japan and China as well (Birtić et al. 2015).

The identification of carnosic acid as key contributor to the antioxidant activity has led to an increased usage of rosemary extracts (Birtić et al. 2015).

Aerial parts of rosemary plants are used for production of essential oil, obtained by steam distillation. The main constituents of the rosemary essential oil, noted for their antimicrobial and anti-fungal effect, are 1,8-cineole (20–50%), α-pinene (15–26%), camphor (10–25%), bornyl acetate (1–5%), borneol (1–6%), camphene (5–10%) and α-terpineol (12–24%). Limonene, β-pinene, β-caryophyllene and myrcene present in the oil give it a special flavour (ESCOP 2003).

Rosselló et al. (2006) found no significant differences in volatile compounds among rosemary species, but recent studies on *R. eriocalyx* consider the essential oil of this species to be different from that of *R. officinalis*. The typical phenolic diterpenes like rosmadial have not been found in the essential oil of *R. eriocalyx* (Santana-Meridas et al. 2014); however, this species, besides *R. officinalis*, can be considered as a good source of bioactive compounds, especially phenolic acids and flavonoids.

There are three main oil chemotypes described, of which the chemical properties are significant for the market (Pintore et al. 2002; Satyal et al. 2017):

- Camphor-borneol oils with approximately equal ratios (20–30%) of 1,8-cineole, α- pinene and camphor (Spain, France, Italy, Bulgaria, Greece).
- Cineol oils with over 40% of 1,8-cineole (Morocco, Tunisia, Turkey, Yugoslavia, Greece, Italy, France).
- Verbenone oils containing up to 15% verbenone (France, Egypt).

One other chemical composition could be defined according to the comparatively higher amount of *myrcene* in oils from Argentina, Portugal and Spain (Pintore et al. 2002).

A number of studies have reported the influence of variety and bioclimatic conditions on composition of essential oil of rosemary (Gouyon et al. 1986; Zaouali et al. 2005; Figueiredo et al. 2008; Zaouali and Boussard 2008; Segarra-Moragués et al. 2016).

Cineol and verbenone chemotypes are supposed to have impact on future markets, considering the increases of their market demand (ProGuiRosemary 2012; Satyal et al. 2017).

Spain has traditionally been the largest supplier of the oil, followed by Morocco and Tunisia (Farooqi and Sreeramu 2001). The world consumption of rosemary essential oil is increasing gradually over the years, from 400–500 tons during the year 1980–1984 to 5000 tons at the beginning of millennium, out of which about 2000 tons were produced in Spain (Gopal et al. 2000). The major market in the world for essential oils is the United States, followed by Japan with about 10% of the world demand and Europe. However, production continues to be concentrated in Europe, with seven of the world's largest essential oil processing firms. The major users of essential oil are soft drink companies in the United States and producers of flavours and fragrances in France, Great Britain and India or pharmaceutical industry in Switzerland and Germany.

Essential oil is used widely for food preservation, as an additive in pre-packaged meat products, in aroma therapy and in cosmetics as an ingredient in soaps, creams, candles, deodorants, hair tonics and shampoos. It is also used in many household cleaners and air fresheners and recently as a major constituent of some organic pesticides.

15.3 Breeding

15.3.1 Flower and Pollination Biology

The flowers of rosemary are sessile, gathered in clusters. They grow in the leaf axils of the upper part of the branches. The bell-shaped green or reddish calyx is initially tomentose, becoming later glabrous. The upper lip of corolla is two-lobed and the lower three-lobed with a colour variation from pale to bright blue, pink or white and a size variation from 8.5 to 13.5 mm (Fig. 15.1). Herrera (2005) found that plants, growing at higher elevations, produce larger corollas in comparison to plants growing in lowlands and supposed this variation to be a response of the plant to the environmental conditions.

Rosmarinus officinalis L. has hermaphrodite as well as androsterile flowers. Floral traits and pollen grain characteristics, both likely evolved in relationship to pollinators (Wilson et al. 1996; Fenster et al. 2004; Lamborn et al. 2005), are specific for each variety. Rosemary flowers like *Salvia*, in contrast to the other members of mint family with only two stamens, which bear a fertile locus.

15.3.1.1 Pollination

Rosemary plants are self-compatible, but their protandry, typical for Lamiaceae in combination with nonsimultaneous maturation of anthers and stigma, prevents self-pollination, making necessary an intermediator for pollination, thus promoting cross-pollination. Nectar and scent produced by a huge number of rosemary flowers

Fig. 15.1 Flowers of different rosemary varieties (*Rosmarinus officinalis*). Flowering rosemary plants, grown in greenhouse, IPK, Gatersleben, Germany

attract various insects, among them honeybees (Herrera 2005; Segarra-Moragués et al. 2016). A variation of pollen size and fertility has been observed on different rosemary populations, supposing genetic factors (Herrera 1986, 1987; Torres 2000; Lamborn et al. 2005; Beaulieu et al. 2008; Knight et al. 2010).

15.3.1.2 Hybridizations

As above mentioned, the huge morphological variability of rosemary causes problems in species delimitation. Valverde et al. (2016) have supposed that genetic variability reflects the effects of environmental heterogeneity. Segarra-Moragués et al. (2016) have recently shown significant genetic differentiation between rosemary plants (*Rosmarinus officinalis*) growing in siliceous vs. calcareous soils. However, morphological markers allowed the identification of plants with intermediate morphological characters, suggesting possible introgression or hybridization between different rosemary taxa.

Rosúa (1981, 1986) and Morales (2010) have described two interspecific hybrids, including *R. officinalis*, *R. eriocalyx* and *R. tomentosus*:

Rosmarinus x *noeanus* Maire ex Rosúa

= R. eriocalyx Jordán & Fourr x R. officinalis L.
= R. tournefortii x R. officinalis (Maire 1932), a semi-prostrate shrub, with narrow leaves and mid blue flowers

The existence of such hybrids with introgression character is explainable for species growing in the same geographical region like the three species of rosemary (Rosúa 1981, 1986), but the hybrid between *R. eriocalyx* and *R. officinalis* has been also found in North Africa (Libya, Algeria, Tunisia), which is not the distributional area of *R. eriocalyx* (Greuter et al. 1986).

Rosmarinus x mendizabalii Sagredo ex Rosúa

= R. tomentosus Huber -Morath & Maire x R. officinalis L.

Populations of the hybrid, *R. x mendizabalii* Sagredo ex Rosúa (Rosúa 1981, 1986), have been found in the Guadalfeo valley and very rarely at the eastern and western coast of Granada, where *R. tomentosus* is widely distributed.

15.3.1.3 Gynodioecy and Male Sterility

Gynodioecy is a common phenomenon in the Lamiaceae family. Ubera-Jiménez and Hidalgo-Fernández (1992) described three types of plants, in rosemary wild populations, plants bearing hermaphrodite (or male and female fertile) flowers, female (or male sterile) flowers and intermediate flowers, that can be distinguished morphologically from each other by corolla and filament sizes, colours of filaments and types of pollen. The hermaphrodite flowers have a large corolla and viable pollen grains at anthesis. MS (male sterile) flowers are distinguished by small corollas, very short staminal filaments and brown aborted anthers. The intermediate flowers (INT) have like hermaphrodite flowers a large corolla, but indehiscent whitish anthers that contain nonviable reduced pollen grains.

Different degrees of gynodioecy (proportion of female versus hermaphrodite flowers) have been observed in rosemary individuals during the flowering season (Ubera-Jiménez and Hidalgo-Fernández 1992). After Hidalgo-Fernández et al. (1998b, 1999), MS and INT flowers show special characteristics during different stages of microsporogenesis: in MS, flowers were observed necrotic areas in the anther tissues before meiosis and in INT flowers vacuolization of tapetum in later stages of microsporogenesis compared to anthers of MS flowers. Promotion of outcrossing by gynodioecy is considered to be an evolutionary advantage of gynodioecious plants (Horovitz 1980). In *R. officinalis* gynodioecy is controlled by cytoplasmic (mitochondrial) genes (Hidalgo-Fernández et al. 1998b).

15.3.2 Propagation Strategies

Rosemary is propagated generatively by seeds or vegetatively by cuttings, layering or division of roots. In vitro propagation is used to get higher multiplication rates from selected plant material.

15.3.2.1 Vegetative Propagation

Propagation by cuttings is the easiest and widely applied method. Usually soft and semi-hardwood cuttings of 10–15 cm length are used to propagate new plants efficiently. Among the root-inducing hormones which are being extensively used com-

mercially, auxins like IBA (indole butyric acid), NAA (naphthalene acetic acid), IAA (indole acetic acid) or Hormex have been proven to have a good effect on root formation (Kumar and Armugam 1980). After treatment cuttings can be inserted in a proper growing medium half to two thirds of the length. Hennig et al. (2002) reported a rooting rate of 88% by propagation in multipot pallets (Fig. 15.2) in low fertilized organic substrate covered by a punched film.

Layering can be applied during the summer by pegging some of the lower branches under sandy soil. After rooting the plants can be separated from the parent plant. Although laying propagation has high survival rate (Dong et al. 2012), it is not effective for a large-scale multiplication as normally needed for cultivation.

In vitro propagation For plants like rosemary with poor rooting ability, in vitro propagation is an effective method to obtain in a short time large amounts of plant material with high uniformity for large-scale cultivation. Usually plant apex, leaves, seeds or seedling explants are used for in vitro multiplication. For rapid clonal multiplication, cultivation of in vitro axillary buds of *Rosmarinus officinalis* on MS basal medium supplemented with 6-benzylaminopurine (BAP) concentration between 0.5 and 0.8 mg/l, indolepropionic acid (IAA) 0.25–0.5 mg/l and sucrose (50 mg/l) has shown the best results with a survival rate of 88% (Zhang et al. 2006; Leelavathi et al. 2013). Tests with sterilizing reagents have shown that a solution containing 75% ethanol would result in mass explant deaths and the sterilization with 0.2% $HgCl_2$ (w/v, 5 min) was found to be the best one (Dong et al. 2012). To reduce teratogenic effect on the tube seedlings by plant hormone accumulation, it is recommended to reduce or renounce the addition of growth regulators like cytokinins and auxin.

15.3.2.2 Generative Propagation

Propagation by seed is not effective considering cross-pollination and typically very slowly seed germination of rosemary. Rosemary has small, oblong, brownish seeds with a thousand seed weight of about 0.98–1.2 g and average germination capacity of 30–50% (APAT 2003).

Heat pretreatment of seed for germination stimulation is not necessary although rosemary seeds can resist comparatively high temperatures (+40°/+60 °C for 24 hours) not having a negative effect on germination ability. It has been proved that cold stratification of seeds for 30–60 days favours the speed and uniformity of germination, therefore spring-sowing, using seeds that may have undergone cold stratification for a short period, is recommended.

Temperatures around +20 °C and absence of light are the optimal conditions for a rapid emergence. The seedlings are rather delicate and must be protected from intense sun during the first stages of their development.

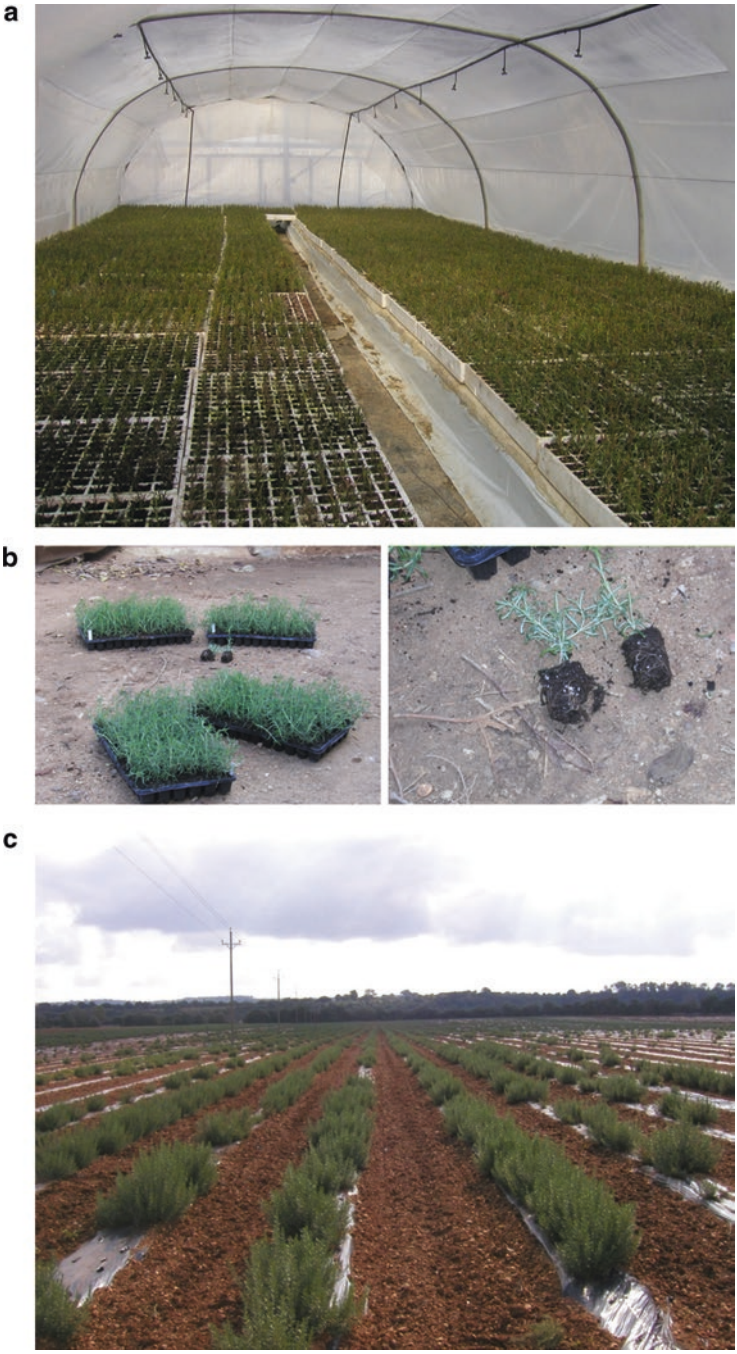

Fig. 15.2 (**a**–**c**) Rosemary cultivation, from cutting propagation to large field cultivation. (**a**) Propagation by cutting in multipot pallets. (**b**) Rosemary plantlets. (**c**) Open field cultivation

R. officinalis' strategy of adaptation to the Mediterranean climate envisages a flowering and a subsequent dispersal over a very lengthy period.

This leads to the formation of large soil seed banks, thus allowing the species to survive.

Propagation by seed is not usual under natural conditions. In rosemary populations, less than one third of the open-pollinated flowers produce seed, and there is evidence that seed set is sensitive to inbreeding depression (Hidalgo-Fernández and Ubera-Jiménez 2001). Protandrous flower of rosemary favours cross-pollination. Although there are no data available on the effect of inbreeding depression on the quality of seeds, Madeiras et al. (2009) found a correlation between low germination rates and high rates of empty seeds. Insect-mediated geitonogamy causing high rates of self-fertilization combined with a strong post-zygotic purging of inbred embryos that produce many apparently healthy but empty seeds seems to be present in rosemary plants (Garcia-Fayos et al. 2018).

Seed set by MS plants indicates that gynodioecy provides additional cross-pollination in wild populations. Whereas seeds corresponding to cross-pollination are more abundant and heavier, seed set by self-pollination shows strong inbreeding depression (Hidalgo-Fernández and Ubera-Jiménez 2001).

Collection and processing of the seed are quite simple. Cool, dry environments are used for seed storage (+3°/+5 °C).

15.3.3 Breeding Methods Applied

15.3.3.1 Conventional Breeding

Rosmarinus officinalis is available in a broad range of varieties, forms, races and ecotypes with an abundance of colours and sizes of leaves, flowers (blue, violet, pink, white) (Fig. 15.3), scents (pine to lemon) and growth habits (upright, prostrate, twisting, creeping) (Fig. 15.4). Exploitation, characterization and evaluation of natural diversity are still a focal point of rosemary breeding programmes.

Six cultivars of rosemary resulted from a prebreeding programme on characterization of spontaneous Sardinian populations of rosemary. Applying mass selection, 20 plants have been selected, cutting propagated and transplanted in open field for characterization. After analysis of morphological, phenological and chemical data (Table 15.1), different cultivars like 'Gerrei', 'Costa Paradiso', 'Sette Fratelli', 'Sant Antioco', 'Cala Gonone' and 'Vignola' were selected (Mulas and Mulas 2005).

Recently research work is concentrated on identification and chemotaxonomical evaluation of genetic resources of rosemary.

Individual selection based on the above-mentioned genetic diversity has been applied in many cases with high efficiency.

The majority of rosemary plant material under cultivation is a result of clone and line selection, using wild populations as a starting material.

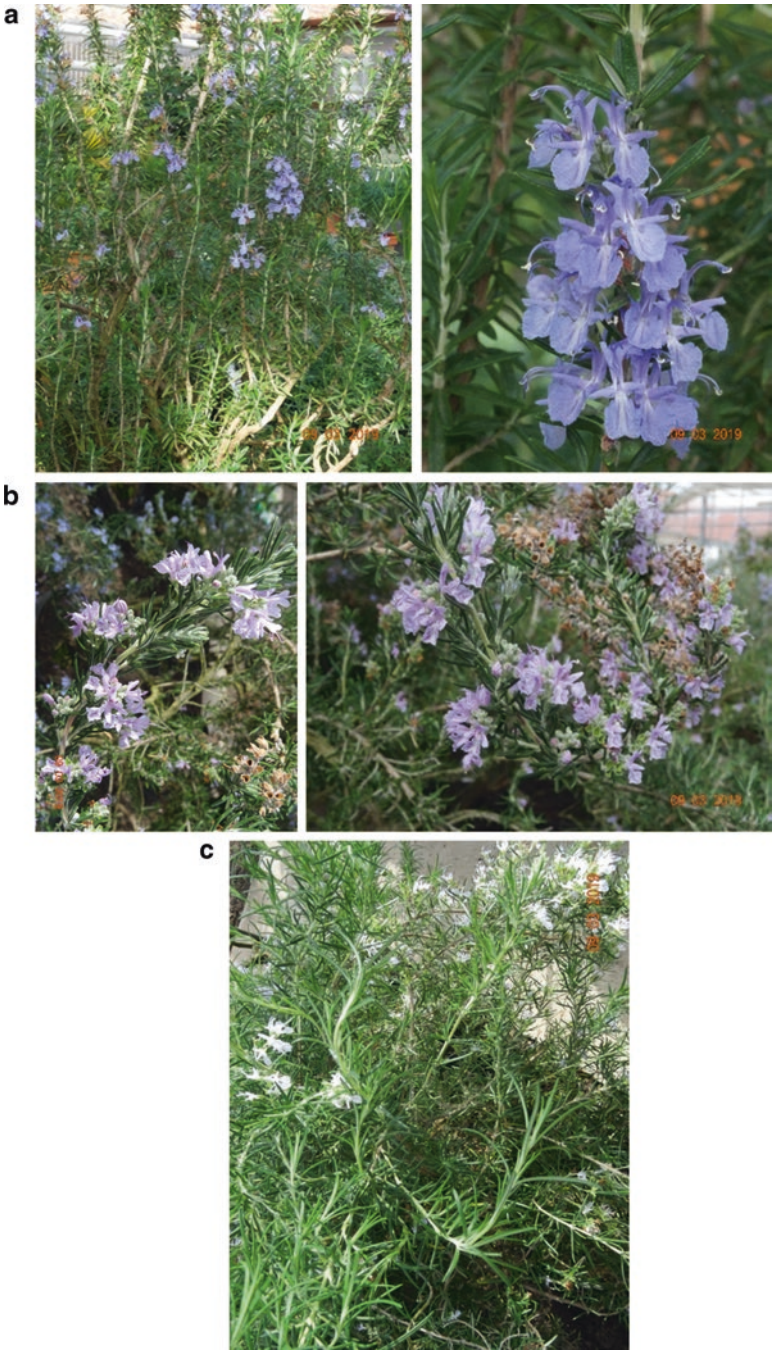

Fig. 15.3 Rosemary varieties distinguished among other characters by corolla colour and size (Greenhouse, IPK, Gatersleben, Germany). (**a**) 'Tuscan Blue'. (**b**) 'Majorca Pink'. (**c**) White Flower Rosemary

Fig. 15.4 In relation to growth habit, various rosemary types from upright to prostrate and twisting or creeping can be found grown naturally. (**a**) 'Pyramidalis' (Greenhouse, IPK, Gatersleben, Germany) (**b**) Creeping rosemary

Fig. 15.4 (continued)

Table 15.1 Some characteristics of selected cultivars

Cultivar	Growth habit	Chemotype
'Gerrei'	Erect, high	Verbenone
'Costa Paradiso'	Compact, medium	Verbenone
'Sette Fratelli'	Prostrate, low, above	12% verbenone
'Sant Antioco'	Vigour plants, the highest biomass	Rich in camphor, 21.4%, and 1,8-cineole, 17.6%
'Cala Gonone'	Erect, medium	Borneol type
'Vignola'	Erect	43.8% α-pinene

Special rosemary cultivars with high content of essential oil (above 5%) and highly abundant in carnosic acid (4–10%) on weight basis of air-dried leaves, including 'VAU3', 'Daregal', 'Farinole', '4 English', 'Severn Sea', 'Miss Jessop's Upright', '1 English' and 'Lighthorne culinary' (Wellwood and Cole 2004), are developed by clonal selection. Clones of rosemary with a high content of essential oil ranging from 4 to 7% and carnosic acid up to 17% have been selected in Germany (Junghanns and Hammer 2017).

Plant tissue culture-based techniques have also been used to develop high rosmarinic acid-producing clones. Clonal lines, originating from a single heterozygous seed, were inoculated with *Pseudomonas* sp. to stimulate synthesis of total phenolics and rosmarinic acid (Yang et al. 2009).

Selection under environmental pressure has been and still is a challenge in rosemary breeding. Cold resistance of rosemary is a very important breeding aim especially for northern countries.

Selection of frost-tolerant cultivar of rosemary the 'Harmat' (Domokos et al. 1997) makes the cultivation of rosemary possible in Hungary (Stefanovits-Bányai1 2003).

15.3.3.2 Molecular Tools

PCR-based RFLP techniques were used to characterize and identify different varieties of rosemary at the molecular level, considering 18S, 5,8S and 26S RNA genes with two internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) sequence for rosemary identification (Rosselló et al. 2006; Ibrahim et al. 2011). Analyses of nuclear microsatellites have also been used to confirm certain genetic variability in the species (Segarra-Moragués and Gleiser 2009). Variable polymorphic markers (codominant SNP marker) are used to understand the diversity patterns and relationship between different accessions of rosemary (Lohwasser et al. 2018).

15.3.4 Breeding Results Achieved/Economical Transfer (Registered Cultivars/Patents, Trial Results)

Besides the 6 varieties mentioned above (Table 15.1), more than 20 varieties of rosemary (Begum et al. 2013) are selected for garden and culinary use (Table 15.2). They are grouped according to similar characteristics of growth habit, cold resistance or flavour in **Upright rosemary**, **Creeping rosemary**, **Pine-Scented rosemary**, **Arp rosemary**, **Madalene Hill rosemary**, **Pink rosemary**, **White rosemary**, **Dancing waters rosemary** (shorter mounding varieties with dark blue flowers), **Golden Rain rosemary** (weeping foliage), **Spice islands rosemary** (thick juicy leaves and very upright growth) and **Blue boy rosemary** (very small varieties, small leaves and little light blue flowers).

Table 15.2 Rosemary varieties selected for culinary and ornamental purposes as garden plant

Variety	Growth habit	Flower colour	Special characters
Albus	Upright	White flowers	Lemon-scented, cold-resistant
Arp	Upright	Pale blue	Green-grey leaves Speckled yellow
Benenden blue	Upright	Deep blue	Leaves narrow, dark green
Blue boy	Twisted	Light blue	Dwarf, small leaves, the smallest of all the rosemary varieties
Blue rain		Pink	
Golden rain	Upright	Blue	Leaves green, variegated, with yellow streaks
Gold dust	Upright	Blue	Dark green leaves, with Golden streaks but stronger than 'Golden rain'
Gorizia	Upright	Blue	Densely packed branches of dark green leaves, fragrant
Hill hardy	Upright	Blue	Needlelike foliage, fragrant
Lockwood de forest	Procumbent	Lavender blue	Selection from 'Tuscan blue', dark green leaves
Ken Taylor			
Majorca pink	Upright	Pink	Very needlelike leaves
Miss Jessop's upright	Upright	Blue	Distinctive tall fastigiate form, with wider leaves bred especially for flowering
Kenneth's prostrate (Prostratus)	Creeper	Blue	Lower, groundcover fast grower, flowering late summer
Pine-scented	Upright	Blue	Feathery needlelike leaves grown as miniature Christmas tree
Pyramidalis	Upright		Fastigiate form, pale blue flowers
Remembrance (or 'Gallipoli')	Upright		From the Gallipoli peninsula
Roseus	Upright	Pink flowers	
Salem	Upright	Pale blue	Cold-resistant similar to 'Arp'
Severn Sea	Upright	Deep violet	Arching branches
Sudbury blue	Upright	Blue	
Tuscan blue	Robust upright	Blue	Flowering mid-spring to late summer, extremely fragrant, bred especially for dense flowering

After Begum et al. (2013)

Cold-resistant varieties (from −10 °C to −15 °C) like *Rosmarinus officinalis* 'Arp' and *R. officinalis* 'Madalene Hill' (syn. 'Hill Hardy') are the most commonly grown varieties, followed by *R. officinalis* 'Albus', *R. officinalis* 'Benenden Blue', *R. officinalis* 'Goodwin Creek', *R. officinalis* 'Herb Cottage', *R. officinalis* 'Logee's Light Blue', *R. officinalis* 'Miss Jessop's Upright' and *R. officinalis* 'Salem'.

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Chapter 16
Salvia officinalis L. and *Salvia fruticosa* Mill.: Dalmatian and Three-Lobed Sage

Corinna Schmiderer and Johannes Novak

Dalmatian sage (*Salvia officinalis* L.), Spanish sage (*Salvia officinalis* subsp. *officinalis* (Vahl) Gams, syn. *S. lavandulifolia* Vahl) and Greek, Turkish or three-lobed sage (*S. fruticosa* Mill., syn. *S. libanotica* Boiss. & Gaill., *S. triloba* L.) are in commercial terms the most important *Salvia* species besides Chinese sage (*S. miltiorrhiza* Bunge), clary sage (*S. sclarea* L.) and chia (*S. hispanica* L.). Since *S. officinalis* and *S. fruticosa* are closely related, they will be discussed together in one chapter.

16.1 Botany (Taxonomy, Origin, Distribution, Cytology, Plant Description)

Salvia is the largest genus of the Lamiaceae and contains between 900 and 1000 species distributed in Europe, Asia, Africa and America. Traditionally all species within the tribe Mentheae with only two stamens, and each stamen expressing an elongated connective, are included in the genus *Salvia*. Recent molecular phylogenetic data showed that this genus boundary leads to a paraphyletic origin of the genus, because five small genera (including *Rosmarinus*) are embedded into *Salvia* clades (Walker et al. 2004; Walker and Sytsma 2006; Will and Claßen-Bockhoff 2014). In the Mediterranean and the Irano-Turanic regions, 30–40 species are found, categorized within the genus as the type section *Salvia* (Hedge 1972).

Salvia officinalis, the type species of the genus, is originally native to North and Central Spain, to South France and to the Western part of the Balkan Peninsula

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(Fig. 16.1). It is widely cultivated as an herb and naturalized in parts of South and Central Europe to Asia Minor. The species is hardy to −18 °C or less, but several cultivars are less hardy and need protection from freezing and thawing. Depending on the climate, this species grows as a shrub or well-branched sub-shrub up to 60 cm height. The grey-green leaves are simple, oblong and more or less narrowed at the base. The surface is rugose, white-pubescent beneath, greenish above and densely pubescent when young. The inflorescences are composed of verticillasters with 5–10 flowers, which have a 10–14-mm-long calyx and an up to 35-mm-long, violet-blue, pink or white corolla.

Salvia lavandulifolia Vahl, which is also of commercial interest, is now accepted as *S. officinalis* subsp. *lavandulifolia* (Vahl) Gams (Bolòs O. de and Vigo 1983; Govaerts et al.). This shrub of up to 50 cm is native to Central, South and East Spain, just extending into Southern France. The leaves are up to 50 mm long, simple, petiolate, narrowly oblong or oblong-linear, crenulated and tomentose. The verticillasters possess 6–8 flowers with an 8–12-mm-long often reddish-purple, pubescent, glandular-punctate calyx. The corolla is 20–25 mm, blue or violet (Hedge 1972; Clebsch and Barner 2008).

Salvia fruticosa Miller (syn. *S. libanotica* Boiss. & Gaill., *S. triloba* L.) is native to the Central and Eastern Mediterranean region, from Southern Italy to Israel (Fig. 16.1). It is hardy to −7 °C and is a drought-tolerant shrub up to 60–120 cm. The stems and lower surfaces of the leaves are white-tomentose. The laminas are simple or pinnate with one or two pairs of ovate lateral segments and a large oblong-elliptical terminal segment. The verticillasters bear 2–6 flowers, with a 5–8-mm-long calyx and a 16–25-mm-long lilac, pink or rarely white corolla (Hedge 1972; Clebsch and Barner 2008).

All sage species discussed in this chapter are diploid with $2n = 14$ chromosomes (Maksimović et al. 2007; Esra et al. 2011). The genome size of *S. officinalis* is 0.95 pg/2C with a base composition of 38.52 GC% (Maksimović et al. 2007). The shortest chromosome length of *S. fruticosa* is 1.02 µm and the longest 2.70 µm. The total haploid chromosome length is 14.59 µm (Esra et al. 2011).

Fig. 16.1 *S. officinalis* (left) and *S. fruticosa* (right) in the wild (Albania)

16.2 Economical Uses (Plant Parts Used, Wild Collection/Cultivation, Domestication, Valuable/Undesired Plant Secondary Compounds, Main Production Areas, Economical Valuation/Parameters)

The leaves of both species are used as food or medicine, dried or as essential oil, oleoresin or other forms of extracts. The essential oil is traded as Dalmatian sage oil (*S. officinalis*), Spanish sage oil (*S. officinalis* subsp. *lavandulifolia*) or Greek sage oil (*S. fruticosa*).

16.2.1 Food Use

16.2.1.1 Leaves

The flavour of *S. officinalis* leaves can be described as warm with a spicy flavour. They are used for flavouring baked goods, condiments, relishes, fats, oils, gravies, meat products, processed vegetables and soups. In contrast to *S. officinalis*, Spanish sage (*S. officinalis* subsp. *lavandulifolia*) leaves are characterized by a camphoraceous, fresh odour with a cineole top note. The odour of *S. fruticosa* leaves is described as harsh, rosemary- and camphor-like (Burdock and Fenaroli 2010).

Rosmarinic acid extracts and extracts containing diterpenes like carnosic acid, carnosol, etc. are used for food preservation due to their strong antioxidant activity (Berdahl and McKeague 2015).

16.2.1.2 Essential Oils

- Sage oil (*S. officinalis*): Sage essential oils are produced from the leaves (0.5–2.1%). The considerable variation of the essential oil composition found may be due to the quality of the plant material (genetic influence, influence of harvest time, different chemical types, use of fertilizers, etc.) as well as to the methods used for distillation. The oil is characterized by a typical thujone odour, mobile and almost colourless to pale-yellow (Burdock and Fenaroli 2010). The main compounds in sage essential oils are 1,8-cineole (7–17%), α-thujone (1–37%), β-thujone (2–14%) and camphor (3–12%) (Lamien-Meda et al. 2010b). The essential oil composition – especially of *S. officinalis* – is polymorphic beyond environmental influences, and numerous chemotypes are described in literature (Tucker and Maciarello 1990; Perry et al. 1999; Novak et al. 2006; Schmiderer et al. 2013; Lamien-Meda et al. 2010b).
- Spanish sage oil (*S. officinalis* subsp. *lavandulifolia*): It is a slightly yellow liquid and consists mainly of 1,8-cineole, linalool, linalyl acetate, linalyl isovalerate and d-camphor (Burdock and Fenaroli 2010).

- Greek sage oil (*S. fruticosa*): It is characterized by a very high content of 1,8-cineole, accompanied by camphor, α- and β-pinene and myrcene (Karousou et al. 1998).

In Europe, the maximum limit for the sum of α- and β-thujone (two major monoterpenes in *S. officinalis*; see below) in alcoholic beverages except those produced from *Artemisia* species is 10 mg/kg (Regulation (EC) No 1334/2008). Since α- or β-thujone used in food must come from natural sources, *S. officinalis* is – besides *Artemisia* species – an important source for thujone-containing alcoholic beverages.

16.2.2 Biological Activities and Medicinal Uses

Both species have anti-inflammatory, antibacterial, antiviral and spasmolytic activity (Todorov et al. 1984). *S. officinalis* is used in folk medicine for its secretion-promoting and antilactagogue effects (Wichtl 2004). Clinically proven are its antihidrotic effect, beneficial effects on cognitive performance and mood (Eidi et al. 2006; Scholey et al. 2008), effect against menopausal hot flushes (Bommer et al. 2011), its positive effect in treating hyperlipidaemia (Kianbakht and Dabaghian 2013) and symptomatic relief of inflammations of the mouth and throat (Hubbert et al. 2006). *S. fruticosa* shows antihypertensive (Todorov et al. 1984) and CNS effects (Imanshahidi and Hosseinzadeh 2006).

The two sage species are represented in the European Pharmacopoeia with four monographs:

(1) Salviae officinalis folium, Ph.Eur. 9.0, 1370: the minimal content of essential oil is 12 mg/kg and 10 mg/kg for *Salvia officinalis* for whole and cut leaves, respectively.
(2) Salviae trilobae folium, Ph.Eur. 9.0, 1561: 18 mg/kg and 12 mg/kg for *Salvia fruticosa* whole or cut leaves, respectively.
(3) Salviae lavandulifoliae aetheroleum, Ph.Eur. 9.0, 1849: an essential oil profile within a defined range must be fulfilled (purity test).
(4) Salviae tinctura, Ph.Eur. 9.0, 1889: produced from *S. officinalis* leaves with a minimum content of 0.1% (m/m) essential oil.

16.2.3 Other Uses

Both species have also ornamental value (Clebsch and Barner 2008; Sotti 1999). Because of winterhardiness and natural leaf variations, mostly *S. officinalis* is used for ornamental purposes.

Furthermore, *S. officinalis* is the source for the monofloral sage honey, characteristic for the Croatian coast and islands (Bilandžić et al. 2017; Tuberoso et al. 2012; Kenjerić et al. 2008; Jerković et al. 2006).

16.2.4 Production

Albania – Europe's leading sage herb producer for both species – exports over 1500 tonnes annually; Bosnia-Herzegovina exporting 352 tonnes, mostly originating from wild collection (Kathe et al. 2003a), is shifting now into cultivation with 400 farmers in 2012 cultivating sage in the Albanian province of Shkoder. Seventy percent of sage imports into the USA are from Albania (Paul 2015). Albania and Croatia are the biggest producers of *S. officinalis* essential oil ('Dalmatian sage oil') with about 40 tonnes/year, whereas the production of Spanish sage oil (*S. officinalis* subsp. *lavandulifolia*, yield 0.8%) on the Iberian peninsula is about 5 tonnes per year (Kalemba and Wajs 2012).

16.3 Breeding

16.3.1 Breeding Targets

Both species are perennial plants and cultivated for about 5 years. Therefore, *winterhardiness* is an issue for cultivating *S. officinalis* in regions with cold winters. However, winterhardiness is a breeding target followed in those regions 'unconsciously' by pragmatically using genotypes surviving winter.

Primary actual breeding targets are:

1. *Yield of leaves*, where a high *ratio of leaves to stem* contributes positively to leaf yield and post-harvest processing.
2. *Content of the essential oil* that should be as high as achievable.
3. *Composition of the essential oil*, only to assure a certain profile/flavour. The only exception of introducing a new flavour profile was the cultivar 'Newe Ya'ar No. 4' (Newe Ya'ar Research Center, Israel) a hybrid between *S. officinalis* and *S. fruticosa* with an essential oil profile intermediary between its parents (Dudai et al. 1999).

However, due to new challenges in cultivation and new commercial applications, possible new breeding targets will be:

1. *Resistance to fungal diseases*. So far, *S. officinalis* was regarded as a robust plant in cultivation. However, fungal diseases are increasingly threatening sage cultivation.
2. *Breeding for antioxidant activity* with the two sub-targets that are independent from each other:
   (a) Breeding for a high content of water-soluble antioxidant rosmarinic acid
   (b) Breeding for a high content of lipid-soluble antioxidant diterpenes (carnosic acid, etc.)

In the moment, lemon balm (*Melissa officinalis*) is the candidate amongst the rosmarinic acid-rich Lamiaceae for producing this antioxidant compound (Kittler et al. 2018), while rosemary (*Rosmarinus officinalis*) is the candidate for the antioxidant diterpenes (Ban et al. 2016). However, the two sage species are so variable in antioxidant compounds that the plant could compete with the species mentioned above after selecting for these compounds (Lamien-Meda et al. 2010a; Sarrou et al. 2017; Sarrou et al. 2016).

16.3.2 Flower and Pollination Biology

The hermaphroditic flowers of both sage species are protandric. Sage is preferentially cross-pollinated but self-pollination is possible. The flower is ready for pollination when the style of the pistil becomes longer than the upper lip (Fig. 16.2). The fruit is composed of a maximum of four nutlets, corresponding to the 4-partite ovary (Fig. 16.3). The dark brown nutlets are almost globular, up to 2.5 mm in diameter, glabrous and smooth with a mass of 1000 seeds of 7.6–7.8 g (Sváb 1992).

S. officinalis is not flowering without flower induction by cold temperatures (Novak et al. 2005). The minimum duration of cooler temperatures for flower induction is 6 weeks with an upper limit temperature of 12 °C day and approximately 10 °C night. Flower induction is accompanied by an obvious change in leaf colour from greyish to light green and a stronger leaf development immediately after the cold period (Fig. 16.4). Critical temperature and its duration are not dependent from the genotype. However, the duration until flowering, observed in three genotypes, was variable within 15 and 26 days. Very young plants did not flower at all, so a certain plant age must be reached before *S. officinalis* is able to flower. This minimum plant age is between 6 and 12 months. The minimum time between two cycles of flower induction of one plant is 20 weeks. Therefore, a minimum cycle flower to flower is about 32 weeks, seed maturation not included.

Fig. 16.2 Artificial pollination of *S. officinalis* with a cotton pad. The cotton pad was coloured with ink to make the pollen better visible

Fig. 16.3 Optimum pollination resulting in four nutlets per flower

Fig. 16.4 *S. officinalis* leaves before (leaf on the bottom) and after (leaf on top) flower induction

Sage species have flowers with a sophisticated pollen transfer mechanism and offer nectaries at the base of the flower (Claβen-Bockhoff et al. 2003). So honey bees (*Apis mellifera* L.) and buff-tailed bumblebees (*Bombus terrestris* L.) are amongst the most important pollinators for *S. officinalis* (Koch et al. 2017). Bumblebees and solitary bees (*Synhalonia grandis*, *Anthophora dispar*, *A. biciliata*, *A. dufourii*, *A. nigriceps*, *A. plumipes*, *Ceratina cucurbitina*, *Eucera clypeata*, *E. dalmatica*, *E. nigrifacies* and *Habropoda tarsata*) were important pollinators of wild growing *S. pomifera*. Other genera of small to medium solitary bees (*Ceratina*, *Halictus*, *Lasioglossum*, *Melecta*, *Nomada*, *Osmia*) seem to collect pollen only without pollinating the flowers ('pollen thieves') (Ne'eman and Dafni 1999). The average nectar volume in *S. fruticosa* is 0.15 µl with a concentration of 30% equivalents of sucrose, and the average fruit set was 20 fruits per plant (Ne'eman and Dafni 1999).

Hybridization is possible between the subspecies of *S. officinalis* (Gómez et al. 1995) as well as between *S. officinalis* and *S. fruticosa* (Putievsky et al. 1990). Artificial crossings *S. officinalis* x *S. fruticosa* resulted in a crossability of 36%

(reciprocal cross: 34%), compared to 85% and 92% crossability within the two parents, respectively. The viability of the F1 was 38% and 35% for the species crossings, compared to 93% and 90% of the parents. Pollen fertility was low in the hybrids, which was no more than 16%, compared to 90% in the parents, and seed set in open pollination was also low with 9% compared to 65% in *S. officinalis* and 37% in *S. fruticosa* (Putievsky et al. 1990). In nature, hybridizations between the two species are also rare because *S. fruticosa* flowers earlier than *S. officinalis* with just a small overlapping period. That may be the reason why Schmiderer et al. (2013) did not find any indication of hybridizations in essential oil profiles in an overlapping region of Albania.

16.3.3 Fungal Diseases

So far, *S. officinalis* was regarded as a robust plant in cultivation without hardly any disease problems. However, this changed recently and especially fungi are more and more threatening cultivation. Important fungal diseases of *S. officinalis* are downy mildew (*Peronospora salviae-officinalis* Y.J. Choi, Thines et H.D. Shin) and a fungus causing root rot (*Boeremia exigua* (Desm.) Aveskamp, Gruyter et Verkley). Recently, *Boeremia exigua* was identified on autumn sage (*S. greggii*) (Garibaldi et al. 2016) and seems to infect several sage species. *Phomopsis sclareae* Sarwar. causes specific symptoms on sage stem in the form of necrotic spots, peeling off and bark breaking, while *Alternaria alternata* (Fr.) Keissler is responsible for leaf necrosis and defoliation (Zimowska 2008). *Phytophthora cryptogea* Pethybr. & Laff. causes root necrosis (Koike et al. 1997; Çakir et al. 2017). In the moment, no information is available about possible resistances in the two sage species.

16.3.4 Propagation Strategies

16.3.4.1 Seed Germination

The optimum germination temperature for *S. officinalis* is 25 °C (constant) or 30 °C/20 °C (day/night). At temperatures below 10 °C and above 35 °C, growth is retarded and inhibited. Germination is ambivalently influenced by light: below 20 °C germination is inhibited by light, while above 20 °C light stimulates germination (Oberczian and Bernáth 1988). Hydropriming (soaking seeds in water, drying of seeds) for 12 h at 30 °C can improve final germination rate by 25% (Dastanpoor et al. 2013). Gibberellic acid at 500 and 1000 mg/L, magnetic field at 15 mT (radicle length only) and laser irradiation at 650 nm (200 mW) increased germination and growth of *S. officinalis* seeds (Abdani Nasiri et al. 2018).

16.3.4.2 Vegetative Propagation by Cuttings

Cuttings of *S. officinalis* normally root in 2–3 weeks at 18–20 °C. A treatment of the cuttings with auxins (IBA or NAA) in a concentration of 5 ppm has shown stimulating effects on rooting of *S. officinalis* (Gudeva et al. 2017). Although *S. officinalis* roots readily without further stimulation, the length and number of roots could be increased by commercial rooting products (Nicola et al. 2005).

16.3.4.3 In Vitro Propagation

Nodular meristematic callus was induced on the basal cut surface of apical shoot explants of *S. officinalis*, grown on Murashige and Skoog (MS) medium supplemented with 4.5–13.5 μM thidiazuron (TDZ) (Tawfik and Mohamed 2007). The callus can be maintained on medium with 4.5 μM thidiazuron and 0.45 mM ascorbic acid. Shoot differentiation is possible with a medium containing 4.4–8. μM 6-benzyladenine supplemented with 0.45 mM ascorbic acid. The shoots developed roots on MS medium with 4.9 μM indole-3-butyric acid. In another protocol (Santos-Gomes and Fernandes-Ferreira 2003), 6.6 μM 6-benzyladenine in combination with 0.23 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 μM kinetin gave the highest number of shoots per explant. Rooting was induced by transfer of shoots to MS devoid of any growth regulator.

Shoot proliferation of *S. fruticosa* is successful on MS medium with 0.75 μM 6-benzyladenine. A medium containing 2.7 μM indole-3-butyric acid is favourable for rooting (Arikat et al. 2004).

16.3.5 Genetic Resources and Cultivars

16.3.5.1 In Situ Genetic Resources

Natural populations of *S. fruticosa* from Crete showed a distinct geographical gradient in their essential oil profile as well as in their genetic constitution from East to West (Skoula et al. 1999). Rešetnik et al. (2016) analysed the genetic diversity of wild and cultivated/naturalized populations of *S. officinalis* including a wide range of natural populations from Slovenia to Greece in their study. Interestingly, all cultivated/naturalized populations (sampled in Kosovo, Serbia, Romania and Moldavia) were very distinct from natural populations and originated from only one geographical area. Most of the genetic diversity was based on differences among individuals within populations. However, still some spatial genetic differences between populations were identified as well, clustering geographically the natural populations into three sub-clusters. The highest genetic diversity was found in the central part of the eastern Adriatic coast, while genetic diversity decreased towards the edges of its distribution area.

16.3.5.2 Ex Situ Genetic Resources

Noteworthy collections of sage species exist in Croatia with 116 accessions of *S. officinalis* and 28 accessions of *S. fruticosa*, in the Czech Republic with 24 accessions of *S. officinalis*, in Germany with 21 accessions of *S. officinalis* and 1 hybrid of *S. officinalis* x *S. fruticosa* and in the USA with 11 accessions of *S. officinalis*. A part of the German accessions were characterized phytochemically and genetically (Lamien-Meda et al. 2010a, b; Mader et al. 2010). Seven populations of *S. officinalis* cultivated in Greece and originating from the region of Kozani, West Macedonia (Greece), were comprehensively analysed by Sarrou et al. (2017).

16.3.5.3 Cultivars

- Medium to good yields and essential oil contents between 1.7% and 2.1% are reported from the cultivars 'De Resniresti' (Romania), 'Dessislava' (Bulgaria) and 'Freitaler Herkunft'.
- 'Extrakta' and 'Gute Selektionsherkunft PHASA', a re-selection from 'Extrakta' (Pharmasaat Artern, Germany), are described as vigorous and rich in leaves.
- 'Bona' (Institute of Natural Fibres and Medicinal Plants, Poznan, Poland) is a high growing, good tillering cultivar with long elliptical big leaves (Seidler-Łożykowska et al. 2015).
- 'Krajova' is a cultivar from the Czech Republic.
- The cultivar 'Newe Ya'ar No. 4' (Newe Ya'ar Research Center, Israel) is a highly productive hybrid between *S. officinalis* and *S. fruticosa* (Dudai et al. 1999). 'Moran', 'Nazareth', 'Silverline' and 'Caspit' are further sage cultivars from Israel.
- 'Regula' (Agroscope RAC Changins, Switzerland), a hybrid between a male sterile and a male fertile clone, is characterized by a favourable leaf/stem ratio (leaf: 70%) and a high essential oil content of 2% (Carron et al. 2005).

16.3.5.4 Cultivars for Ornamental Use

Optical variations range from very broad leaves (cv. 'Maxima', cv. 'Berggarten') over purple stems (cv. 'Purpurascens') to variegated leaves (cv. 'Icterina'). A genotype combining purple stems and variegated leaves is called cv. 'Tricolor' (Sotti 1999).

16.3.5.5 Biosynthesis and Molecular Tools

The biosynthesis of the main essential oil compounds of *S. officinalis* was elucidated, and genes for the first step (1,8-cineole synthase, bornyl diphosphate synthase and sabinene synthase) were identified (Wise et al. 1998). Two of the three

genes were under transcriptional control (Grausgruber-Gröger et al. 2012; Schmiderer et al. 2010). Part of the biosynthesis of antioxidant active diterpene carnosic acid was elucidated in *S. pomifera* and *Rosmarinus officinalis*, two species closely related to the species discussed here (Ignea et al. 2016; Božić et al. 2015; Trikka et al. 2015). Usefulness and problems of transforming *S. officinalis* with *Agrobacterium tumefaciens* were discussed by Luwańska et al. (2017).

Microsatellite markers were developed for *S. officinalis* with good cross-amplification in related species (amongst others *S. fruticosa*) (Radosavljević et al. 2011; Radosavljević et al. 2012). EST sequences exist from a trichome-specific cDNA library of *S. fruticosa* (Chatzopoulou et al. 2010). From these EST sequences, SNP and SSR markers were developed for *S. officinalis* (Mader et al. 2010). SRAP markers were used to study the genetic relationship of five *Salvia* species, including *S. officinalis* (Aghaei et al. 2017).

16.4 Summary and Outlook

The two species *S. officinalis* and *S. fruticosa* are used for many purposes in food and medicine. So far, monoterpenes were the most important active ingredients, making them – besides their use as herb – large volume essential oil crops. Now other plant secondary compounds of the two species are coming more and more into focus like the antioxidative compounds rosmarinic acid and carnosic acid. The main breeding targets in the past were essential oil content and biomass. Since fungal diseases in sage cultivation are increasing, resistance breeding will possibly become an important part of plant protection strategy for these sage species. So far, there is no optimization for antioxidative plant compounds like rosmarinic and carnosic acid, although the variability present in genetic resources makes increases in productivity and uniform quality by breeding and interesting approach.

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Chapter 17
Salvia sclarea L.: Clary Sage

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17.1 Introduction

Clary sage, *Salvia sclarea* L., is a biennial or perennial herbaceous plant of the Lamiaceae family native to Central Asia and Southern and Central Europe. It has been grown for ornamentals and medicinal uses in various areas where it is spontaneously sown.

Historically, clary sage has been used for numerous applications such as the flavouring of wine and beers, perfume additive and to a lesser extent for medicinal purposes (Lawrence 1994). Today, the main uses are on the one hand the essential oil obtained by steam distillation of the plant and on the other hand a "concrete" obtained by solvent extraction of the straw leftover after distillation (Elnir et al. 1991; Farkas et al. 2005). This last fraction (so-called concrete) has a high level of sclareol (up to 50%), a diterpene precursor used to produce Ambrox and Ambroxan after hemi-synthesis (Decorzant et al. 1987; Barrero et al. 1993). This molecule is characterized by a noble amber, woody, dry odor and a great olfactory and diffusive power, even at very low dilutions. Since the end of the 1990s, it has replaced ambergris formerly extracted from the whale and incorporated into fine perfumes.

Producing almost 10 tonnes of essential oil a year, France was the leading producer in the middle of the twentieth century. Countries such as the USA, Bulgaria, China, Hungary and India are reported to produce several tonnes a year. For sclareol, France is currently the largest European producer with an annual production of 10–20 tonnes.

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The global market for sclareol today is estimated at several hundred tonnes of pure sclareol. The economic importance of sclareol has led to numerous studies aiming to pinpoint the organs of the plant in which the secondary metabolites accumulate. By far, the calyces were shown to be the organs of the plant richest in sclareol (Schmiderer et al. 2008).

17.2 Biology

Reaching heights of 90–120 cm under field conditions, the stems are erect, robust and covered with glandular hairs and are typically highly ramified. The leaves are broadly ovate and rough, and the high density of glandular hairs causes them to appear grey.

The flowering period usually occurs between May and September in the second year of growth. The flowers range from white to light purple and are arranged in tight verticils carried by thick stems. The calyx is 5 mm long, pubescent, with 5 spiny teeth distinctly separated in an upper block of 3 and a lower block of 2. The corolla is 15-25 mm long, two lips featured, the curved upper lip hiding the stamen and most part of the style, the apex of the lower lip being 3-lobed and generally less colored than the upper. The nectar-producing flowers ultimately produce fruits in the form of an achene. Plants are hermaphrodites and pollinated by insects such as the carpenter bee (*Xylocopa* sp.). In the wild, this species is often found growing along dry rocky slopes (iteipmai technical dossier 2008).

To go from bud formation to the production of mature seeds, plants require approximately 60 days. This interval will vary depending on the climatic conditions. Once the flower has bloomed, it persists for a maximum of 96 hours; if not fertilized, flowers fade 12 hours after pollination (Haque and Ghoshal 1981; Lawrence 1994). Flowering occurs in a specific order progressing along the spike from the bottom to the top. The two flowers in the central position in the whorl bloom first. This is followed by the flowering of the whorls held by the secondary spikes which occurs successively with approximately 2–5 days between each consecutive spike. The full flowering period often occurs between May and September and can last up to 20 days for main and first-order lateral spikes.

The two stamens of clary sage are hidden under an “upper lip” and are articulated at their base by a “pedal” referred to as the staminal lever that allows the stamens protrude from the upper lip when mechanic pressure is applied (Claßen-Bockhoff et al. 2004; Reith et al. 2006). As soon as the ovary becomes receptive, the two branches of the stigma move away and out of the upper lip. There is no difference in maturity between the male and female parts of the flower. There is no self-incompatibility reported; however, spontaneous self-fertilization hardly occurs under natural conditions. Thus, cross-fertilization and self-fertilization are possible, and both have good results (Şenol et al. 2008).

The species is practically entirely propagated through seed propagation.

17.3 Applied Farming Methods

17.3.1 Planting

Clary sage is adapted to well-exposed, rocky soils that warm quickly, preferably with a pH between 6 and 7. The species is sensitive to low temperatures even though it is grown up to 800 m above sea level. Hydromorphic soils or zones with excessive rainfall will not allow the plant to express its full genetic potential. Aside from the added value of the different compounds that are extracted from clary sage, this crop is also interesting from an agronomic point of view as it is a good starter crop in rotations. Often implanted through direct drilling, the plant can remain in place for 3 or 4 years. As a biennial species, the plant will bloom in the second year of vegetation, but it can be maintained for a third or fourth year.

Although it is possible in some areas to sow at the end of the summer in mild and rainy conditions, in most countries, the direct drilling occurs between April and May, as soon as the soil temperature approaches 8–10 °C. The emergence will often occur within the next 15 days (Giannouli and Kintzios 2000; Saharkhiz et al. 2009).

17.3.2 Harvesting

The harvest is often organized for the end of July in the first year that the plant produces flowers. The state will vary depending on climatic conditions. Various harvesting methods exist. In traditional methods, the plants are left in the field for a short period post cutting in order to reduce their water content prior to their distillation, or alternatively bales of the plant are prepared for distillation. However, the most common method currently used is the silage method which makes use of a corn cutter bar. This method renders the harvest significantly less labour-intensive and has been shown to allow for improved content for the secondary metabolites of interest. This technique gives rise to yields that are between 25 and 30 kg of essential oil/ha in the case of population varieties and up to 50 kg/ha with selected varieties.

17.3.3 Optimizing yields and oil content

Numerous studies have been carried out to optimize different aspects of the crop management strategy in the aim of improving yield and oil content. For example, seed density has been shown to significantly impact sclareol productivity due to high levels of competition among plants in denser sown plots. Tests were also carried out with the aim of reducing losses of sclareol during the harvest, which is mainly affected by the way the plant is treated, and by the machine-related ventilation. Harvest losses are estimated at around 30%. By suitable harvesting means, the

losses can be reduced to a minimum of 10% (Taarit et al. 2011; Verzar-Petri et al. 1985).

In theory, to obtain maximum oil levels, harvest should take place at a time when the majority of flowers have reached full maturity. This goal, however, is in practice difficult to reach due to the fact that the main flowering spikes reach maturity prior to the lateral spikes forcing the grower to make a compromise.

17.3.4 Diseases and Pests

Clary sage is a plant that is hardly not often impacted by disease. At the level of pests, a beetle (*Arima marginata*) can cause damage on young shoots as soon as the vegetation starts in April to May. Chemical control of weeds, diseases and pests is possible, and despite it being a speciality crop, often at least a few plant protection products are registered in the different producing countries.

17.4 Breeding Clary Sage

17.4.1 Breeding Works Carried out Around the World

The first way to improve clary sage cultivars was to exploit the rich diversity observed (morphological and chemotypic) to select superior individuals from wild populations (Bernáth 2002). This selection has resulted in varieties with significantly improved agronomic characteristics compared to wild populations (Kintzios 2000). Subsequently, in the 1990s, new ways of improvement were exploited. For example, Ukrainian studies described the use of inter-species crosses to improve resistance of clary sage to biotic and abiotic stresses, by introgressing resistance from the neighboring species, *S. grandiflora* and *S. aethiopis* (Kintzios 2000). Moreover, it has been demonstrated that some clary sage accession well tolerates inbreeding. Self-crossing experiments gave rise to the development of elite lines, which sometimes revealed the expression of new characters that were non-expressed in heterozygous plants (Goncearіuc 2009). The ability to create sage lines also allowed to explore the hybrid pathway and to maximize the effect of heterosis through the use of homozygous parents. The success of the hybrid varieties was also due to the fact that their large-scale development has been made possible by the discovery of male sterility in some Bulgarian and Moldovan accessions (Kaul 1988; Goncearіuc 2009, 2014). Genetic improvement through mutations and modification of ploidy level have also been investigated. A success story for the development of a mutant line has been described in the work of Mekhraz (Mekhraz et al. 1988). The authors produced mutants from the cultivar “Trakiia” and obtained a “remarkable” line (named afterwards cultivar “Boyana”), whose yield in essential oil was higher than that of the starting variety.

A third step in clary sage improvement can be noted in the early 2000s. The availability of molecular biology tools has allowed to finely consider the genomic information to help in improving breeding schemes. For *Salvia sclarea*, these novel tools have been used to decipher the metabolic pathway of the biosynthesis of essential oil and sclareol compounds, including the genes regulating these pathways.

Therefore, in one study released in 2008, Karaca and colleagues (Karaca et al. 2008) developed RFLP molecular markers (restriction fragment length polymorphism) and "minisatellite" markers for the clary sage. Likewise, the analysis of clary sage transcriptome deciphered various proteins involved in secondary metabolite biosynthesis pathways and their regulation (Legrand et al. 2010) and in the synthesis of sclareol (Caniard et al. 2012). All these studies, by providing information on the genes involved in the secondary metabolite biosynthesis pathways in clary sage, contributed to explore the of biotechnology engineering approaches (Georgiev and Pavlov 2017).

17.4.2 Breeding Work Achieved at iteipmai

At iteipmai, the choice was made to breed synthetic varieties to maximize the heterosis effect while focussing on reduced number of parents chosen for their ability to transmit their traits (i.e. yield, content in essential oil and titration in sclareol) to the progeny.

The breeding work has started in 1998 collecting cultivars gathered from various origins.

Figure 17.1 summarizes the different steps leading to the selection of a synthetic variety.

The first step consists in characterizing each population gathered and each outperforming individual (clones) within populations. Plants were harvested individually to estimate the weight of fresh and dried flowers. Essential oil was extracted and characterized using GC-FID. This primary screening allows the identification of the best performing clones selected for their intrinsic agronomic and biochemical values. Once identified, these clones were multiplied through cuttings and transplanted into two types of assays: (1) a so-called poly-cross where cross pollination is facilitated adding bees of pollinating insects and (2) line evaluation of selected clones. From the poly-cross, the seed lots are harvested plant by plant and sown by line. Lines are evaluated on the same traits as previously described plus some additional agronomic traits such as flowering pattern and resistance to biotic and abiotic stresses. The dataset obtained is statistically analysed to identify the clones (also called mother lines) that transmit their traits to their off-springs; this is named general combining ability (GCA). We kept only the mother lines that have a good GCA; the remaining ones were eliminated. A second row of poly-cross was made using the only good GCA mother clones multiplied by cutting; the harvested seeds are named *synthetic* 0 also named stock seeds. To maximize the mix of desired alleles, it is recommended to have to subsequent cycles of open pollination. So, the seeds synthetic 2 are the commercial seeds (See Fig. 17.1).

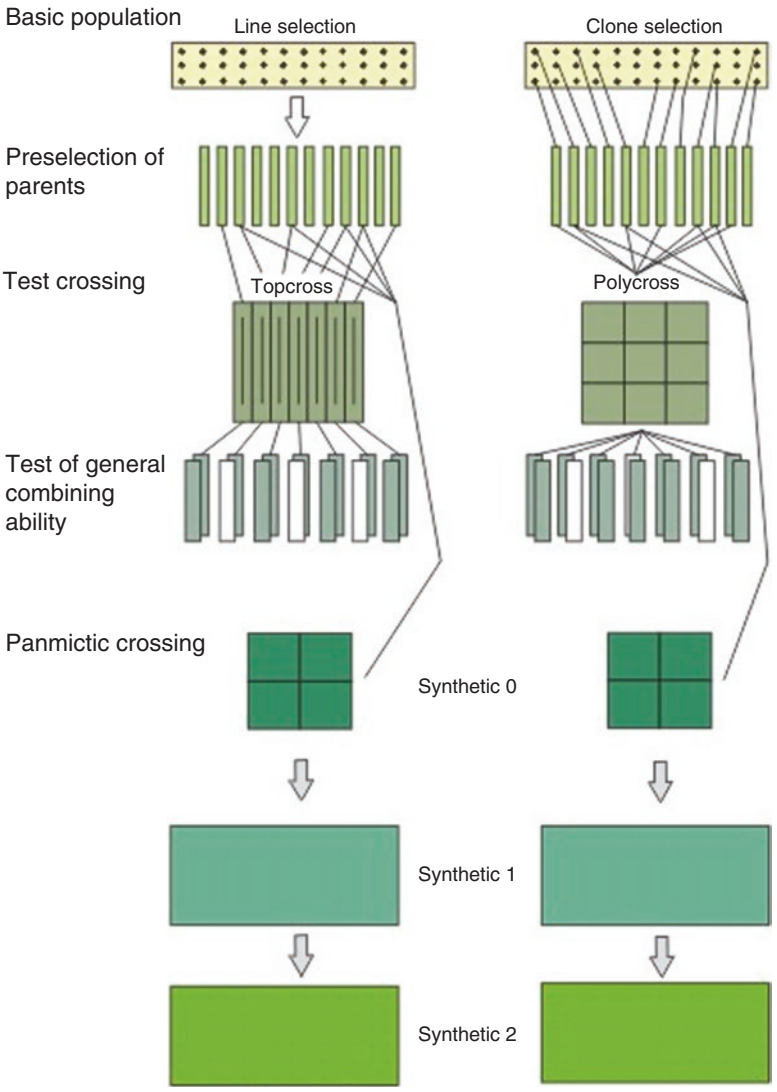

Fig. 17.1 Breeding schemes for synthetic varieties using inbred lines (left) or clones (right)

Finally, three commercial varieties, namely, "VS1", "Scalia" and "Toscalia", were bred at iteipmai. These varieties performed significantly better than previously evaluated varieties: increased production of essential oil ranging from +50 to +100% vs. control and improved titration in sclareol reaching +30 and +40%.

Fresh flower weight and yield in essential oil are traits highly influenced by growing conditions. With the context of climate change, it becomes more and more important to select varieties for their earliness (flowering period occurring before the hottest moment of the summer). This work has started with the selection of "Toscalia" that flowers around 8 days earlier than "Scalia" (can vary depending on climatic conditions).

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Chapter 18
Thymus vulgaris L.: Thyme

José F. Vouillamoz and Bastien Christ

18.1 Botany

18.1.1 Taxonomy

The genus *Thymus* is part of the Lamiaceae family and is composed of 215 accepted species, of which *Thymus vulgaris* L., the garden thyme, is by far the most commercially used species (Morales Valverde 2002). The etymology of 'Thymus' possibly comes from the Greek word *thuô* meaning fumigation, or from *thio* meaning perfume, while 'vulgaris' is the Latin for 'common' (Amouretti and Comet 1993).

The first official description of the species *T. vulgaris* was done in 1753 by Linné in the first edition of his Species Plantarum; however, earlier descriptions by Dodonaeus in 1616 and by Bauhin in 1623 can be found (Morales Valverde 2002). Based on morphological characters, botanists have long debated about the number of sections in the genus *Thymus*. *T. vulgaris* was initially placed in the *Serpyllum* section by several authors, and later in the *Thymus* section (Jalas 1971). In addition, Morales recognized two subspecies (Morales Valverde 2002): *T. vulgaris* subsp. *vulgaris* and *T. vulgaris* subsp. *aestivus* (Willk.) A.Bolòs & O.Bolòs from western Balearic Islands and eastern Spain with different chromosome numbers (see below), to which we must add a third possible subspecies that has also been described in Catalonia, *T. vulgaris* subsp. *palearensis* (O.Bolòs & Vigo) O.Bolòs & Vigo.

The frequent presence of hybrids adds another layer of taxonomic complexity in the *Thymus* group (Morales Valverde 2002). Using cpDNA and mtDNA barcoding, Belhassen et al. (1993) and Federici et al. (2013) observed high intraspecific and low interspecific genetic variation among *Thymus* species, accompanied by low gene flow among populations, and could not suggest species-specific barcodes.

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Similarly, at the species level and on a regional scale, Tarayre et al. (1997) observed high levels of cpDNA diversity within and among *T. vulgaris* populations in southern France.

18.1.2 Origin

T. vulgaris is native to the Western Mediterranean basin, from Spain and the Balearic Islands extending to south-eastern Italy. Thyme was already used by ancient Greeks, and also supposedly by Sumerians (3'500 BC) and ancient Egyptians, although the species was more likely to be *T. capitatus*. It was propagated towards northern Europe by Romans and much later (during the sixteenth century) to North America by European settlers (Amouretti and Comet 1993).

18.1.3 Distribution

Natural populations of *T. vulgaris* are found on the northern part of Western Mediterranean region, namely, Spain, France and Italy (Hegi 1927). *T. vulgaris* can grow up to 1600 m in its northernmost populations in the Aosta Valley of the Italian Alps (Rey 1989), and it has been reported in Spain at altitudes of up to 2000 m (Blanca et al. 2009). It was also mentioned in Portugal and Greece by Hegi, but species identification remains questionable (Hegi 1927). Similarly, further research is required to confirm the records from North Africa (Morales Valverde 2002).

18.1.4 Cytology

According to the Index to Plant Chromosome Numbers (IPCN) and the Chromosome Counts Database (Rice et al. 2015) (both accessed Feb 15th 2018), the sporophytic chromosome number for *T. vulgaris* is $2n = 30$, based on plant material from Spain, France or Italy.

However, chromosome numbers of $2n = 28$, 56, 58 or 60 have been reported for *T. vulgaris*:

- $2n = 28$ was found for *T. vulgaris* L. subsp. *vulgaris* in Alicante, Madrid and Valencia regions of Spain (Morales Valverde 1986), but it is possible that it is the result of a misidentification with *Thymus zygis* ($2n = 28$) (Elena-Roselló 1980; Morales Valverde 1980).
- $2n = 56$ was found for *T. vulgaris* in France (Bonnet 1966). This number was also found for *T. froelichianus* Opiz and *T. longicaulis* K. Presl (Morales Valverde 1986), but a misidentification is unlikely since these species are not present in

France. A tetraploidization of *T. zygis* is not plausible either, since this species is not found in France.

$2n = 58$ and $2n = 60$ were found for *T. vulgaris* subsp. *aestivus* (Reut.) A. Bolòs & O. Bolòs in Alicante and Valencia regions of Spain under the names *T. aestivus* (Reut. ex Willk.) or *T. vulgaris* var. *aestivus* (Reut.) F. Quer. The subspecies *aestivus* possibly corresponds to aneuploid populations of tetraploidized *T. vulgaris* (Mewes et al. 2008).

18.1.5 Plant Description

T. vulgaris is a perennial semi-evergreen shrub growing 10–50 cm tall by 40–50 cm wide, with erect to semi-patent woody branches. The stem is quadrangular and becomes woody with age. The leaves are linear to elliptical, 3–8 mm long and 0.5–2.5 mm wide, with a variable number of glandular hairs. The bracts are similar to the leaves but wider, sometimes with a flat margin. The inflorescence is a capitulum. The calyx is 3–4 mm long with short hairs and the tube is campanulate. The corolla is whitish to pale purple. *T. vulgaris* is highly variable in shape of leaves, bracts and inflorescence, either in wild populations or in cultivated forms (Tutin et al. 1976; Morales Valverde 2002).

18.2 Economical Use

T. vulgaris has been used since ancient times for aromatic and medicinal purposes. As an aromatic plant, it is used today to flavor food (e.g. cheese and meat), liqueurs and herb blends. As a medicinal plant, the antimicrobial and antioxidative properties of its essential oil are used to treat alopecia, dental plaque, dermatophyte infections, bronchitis, cough, inflammatory skin disorders and gastrointestinal distress (Mewes et al. 2008; Hosseinzadeh et al. 2015; Satyal et al. 2016). In cosmetics, thyme extract is used in shampoos, toothpastes, hair conditioners, colognes, soaps, detergents and creams (Lawrence and Tucker 2002). According to the European Pharmacopoeia, the essential oil content of *T. vulgaris* must be higher than 1.2% (v/w, obtained by hydrodistillation) with thymol and carvacrol representing together at least 40%.

18.2.1 Plant Parts Used

The industry uses mostly leaves and flowers of *T. vulgaris*. Compared to stems, leaves and flowers have higher concentrations in bioactive metabolites such as terpene hydrocarbons, oxygen terpene derivatives, sesquiterpene hydrocarbons,

oxygen sesquiterpene derivatives, saturated aliphatic hydrocarbons and phytosterol derivatives (Guillén and Manzanos 1998).

18.2.2 Wild Collection/Cultivation

T. vulgaris was originally wild harvested in Mediterranean countries such as France, Italy and Spain, where the species is considered 'Least Concern' on the IUCN Red List due to its widespread distribution, stable populations and the absence of major threats (Khela 2014). Today, *T. vulgaris* is mostly cultivated in many countries including Morocco, France, Italy, Spain, Switzerland and the USA, but there is no worldwide data on its cultivation because most countries combine data from spices and herbs for statistics (Lawrence and Tucker 2002).

18.2.3 Domestication

Like many other aromatic plants, wild *T. vulgaris* has been taken into cultivation rather than domesticated, as evidenced by the important morphological and phytochemical similarities between wild and cultivated individuals.

18.2.4 Valuable Plant Secondary Compounds

The most important constituents of commercial essential oil from *T. vulgaris* are thymol (23–60%), α-terpineol (18–50%), *p*-cymene (8–44%), carvacrol (2–8%) and linalool (3–4%) (Satyal et al. 2016). Numerous chemotypes (CT) have been described for *T. vulgaris* (Kaloustian et al. 2005; Keefover-Ring et al. 2009; Satyal et al. 2016), which makes it one of the herb species with the highest essential oil diversity. By profiling terpenes and phenolics, a study identified 20 distinct CT in 85 essential oils (Satyal et al. 2016).

The most important chemotypes in natural populations are thymol CT, carvacrol CT and *p*-cymene CT (Kaloustian et al. 2005; Satyal et al. 2016). It was shown that the major monoterpenes are controlled by epistatic relationships between loci, each having a dominant and a recessive allele: geraniol > α-terpineol > thuyanol > linalool > carvacrol > thymol (Vernet et al. 1986).

The phytochemical profile of *T. vulgaris*, especially monoterpenes, does not only vary based on the genotype but also according to abiotic factors such as location, elevation, cultural practices, harvest time, drying methods and storage conditions (Venskutonis 2002; Kaloustian et al. 2005; Keefover-Ring et al. 2009; Satyal et al. 2016). As a consequence, properties of the essential oil of plants harvested in spring differ from plants harvested in fall (Kaloustian et al. 2005). In addition, enantiomeric

distributions of chiral compounds can also vary, resulting in distinct medicinal and biological activities, flavors and aromas: for example, L-linalool has anticonvulsant activity in a mouse model, whereas D-linalool is inactive (de Sousa et al. 2010).

In addition to volatile compounds, *T. vulgaris* accumulates various specialized metabolites such as flavonoids and other polyphenols (Pereira and Cardoso 2013) and triterpenoids (Jäger et al. 2009).

18.2.5 Main Production Areas

T. vulgaris is cultivated and collected wild in most European countries, with Spain producing around 90% of the world production of thyme oil (*T. vulgaris* and *T. zygis* combined). *T. vulgaris* is also produced in France, Germany, Switzerland, Portugal, Italy, Bulgaria, Hungary, Turkey and Greece. Productions also exist in Russia, North America, North Africa (Algeria and Morocco), China, Japan, India, South Africa, Chile (naturalized), Brazil, New Zealand (naturalized), Iran, Saudi Arabia and Cameroon (Lawrence and Tucker 2002; Stahl-Biskup and Saez 2003).

18.2.6 Economical Valuation

There is currently no available statistics on the global market for the production of *T. vulgaris* worldwide, but the annual essential oil production is estimated at 4 tons, and the annual global trade of thyme (*Thymus* sp.) is about 15,000 tons.

18.3 Breeding

18.3.1 Flower and Pollination Biology

Gynodioecy was already described for *Thymus serpyllum* and *T. vulgaris* by Charles Darwin in 1877 (Darwin 1877). Gynodioecy is a dimorphic sexual system characterized by the presence, within in the same population, of individuals with normal hermaphroditic flowers (male fertile, MF) and others with significantly smaller flowers with absent or non-functional anthers (male sterile, MS). In the MF, which are proterandric, anthers open 2 or 3 days before the gynoecium is receptive and then dry out (Assouad and Valdeyron 1975). Cross-pollination is ensured by bees, but seed set is poor (0.2–0.5 per flower on average). This might be the result of inbreeding depression, since pollination can occur between flowers of the same plants, as shown by the constant rate of self-fertilization in a population, while it is highly variable from one plant to another (Brabant et al. 1980). The seed set is more

than doubled in the MS flowers (Assouad and Valdeyron 1975). The variation of the proportion of MS between 5 and 95% (mean around 63%) between *T. vulgaris* populations is probably the result of different selection pressure: MS prevail in high vegetation cover, whereas larger proportions of MF are found in rocky sites (Dommée et al. 1978).

18.3.2 Propagation Strategies

Propagation can be done by seeds for clone hybrids and population varieties and by vegetative or in vitro multiplication for clonal varieties.

18.3.3 Seed Propagation

Propagation by seeds is more affordable than vegetative propagation and usually provides a better rooting of the plantlet. When propagated by seeds, no significant difference in yields or quality was observed in Switzerland between direct sowing and planting for *T. vulgaris* (Carron et al. 2010).

18.3.4 Vegetative Propagation

Clones can be multiplied by plant division or cuttings, but these methods are more expensive and induce more superficial rooting than propagation by seeds. In vitro propagation is even more costly than plant division or cuttings, but this method is well suited to maintain mother plants of clonal varieties. In vitro propagation of *T. vulgaris* was first successfully carried out in 1992 using apical and axillary buds and multiplying nodal segments on semi-solid Nitsch and Nitsch medium containing kinetin and indole-3-butyric acid or 1-naphthaleneacetic acid (Furmanowa and Olszowska 1992). More recently, Ozudogru et al. (2011) were able to obtain 380 genetically stable plantlets of *T. vulgaris* from one single explant using shoot tips on semi-solid MS medium supplemented with kinetin and gibberellic acid.

18.3.5 Breeding Methods Applied

For the generation of clone hybrids, breeders took advantage of the natural gynodioecy of *T. vulgaris*. In Switzerland, almost 100 hybrids were obtained by open pollination of MS clones with MF clones, thus avoiding the castration process and possible self-fertilization, from which three thymol CT varieties were selected:

'Varico 1', 'Varico 2' and 'Varico 3' (Rey et al. 2004; Carlen et al. 2010). A similar program was carried out at ITEIPMAI in France and resulted in the breeding of three club varieties with distinct chemotypes: 'Carvalia' (carvacrol CT), 'Linalia' (linalool CT) and 'Thymlia' (thymol CT).

18.3.6 Breeding Targets, Important Traits and Biotic and Abiotic Stresses

Multiple cultivars of *T. vulgaris* have been bred to meet the requirement of the industry regarding homogeneity, productivity and phytochemical content. This section describes several traits of *T. vulgaris* that have been and/or could be improved by breeders.

18.3.6.1 Yield (Plant Material and Essential Oil)

Yields of plant material and essential oil have been major targets of *T. vulgaris* breeding programs. Currently, major cultivars yield around 2–3 tons/hectare (dry matter) in the first year of cultivation and around 4–5 tons/hectare (dry matter) in the second and following years (Aeschlimann et al. 2017). The essential oil content of *T. vulgaris* cultivars typically represents 1.5–6.5% of the dry weight of leaves (Teuscher et al. 2005).

18.3.6.2 Resistance (Abiotic/Biotic)

Most common pests feeding on *T. vulgaris* are leafhoppers (Cicadellidae), various Lepidoptera and leaf beetles (Chrysomelidae). These insects cause damages early in the season on young plants and during warm conditions. However, damages induces by invertebrate pests on *T. vulgaris* cultures are often not important. It can be postulated that the increase of the essential oil content during breeding programs has indirectly improved the resistance of cultivars to some of these pests. In Switzerland, where most of the production of *T. vulgaris* is certified organic, insect pests are controlled with biopesticides such as spinosad when damages are becoming too important.

Soil diseases caused by fungi, i.e. *Fusarium oxysporum* and *Pythium* spp., have been reported to cause the death of whole plants and are a concern in some regions of France (ITEPMAI 2009). Soil diseases are often transmitted by vegetative propagation techniques such as plant division. In addition to cultivar resistance, it is important to perform crop rotation and use cultural practices that favor soil health.

18.3.6.3 Adaptation to Cultivation Processes (Pre-/Postharvest Processes)

Homogeneity within populations has been improved by *T. vulgaris* breeding programs to facilitate harvesting and improve plant material quality. The harvesting date, time of the day and weather conditions influence the quality of the final product (Venskutonis 2002). The first cutting is usually performed at flowering, when *T. vulgaris* is most aromatic, and the second 2 or 3 months later. Cutting height, which influences yield, is optimal being between 10 and 15 cm from the ground. During the day, harvest should be done after morning dew has disappeared and preferably on sunny days. Hand harvesting is the rule for plants collected in the wild and machines (harvester-loaders) for cultivated fields.

Only a small amount of *T. vulgaris* is consumed fresh (Venskutonis 2002). Fresh plant material can be stored a few days at 0 °C and 2–3 weeks after treatment with gibberellic acid. Fresh *T. vulgaris* can also be stored frozen for up to 1 year. Although these processes retain more flavors, most of the thyme is consumed dehydrated. *T. vulgaris* is considered dried when the final amount of water is around 8–12%. Drying is mostly performed using heated air (solar dryers or hot flow oven-dryer), and the control of drying temperature and duration is crucial for maintaining essential oil content and quality. For *T. vulgaris*, the optimal temperature is between 35 and 40 °C (Aeschlimann et al. 2017). Cleaning is an important process before and after drying. Several cleaning methods exist and can be used in combination to remove biological, metallic or mineral contaminants: magnets, sifters, air tables, destoners, air separators, indent separators and spiral separators. Grinding of the dehydrated parts can then be achieved with various equipment that can produce different particle sizes.

18.3.7 Breeding Results

Breeding programs have been mostly carried out for *T. vulgaris* in Germany, Switzerland and France. The classification of cultivars is very complex, the difference often being related to the essential oil composition or content. In addition, numerous hybrids make it difficult to assign cultivars to the correct species (Morales Valverde 2002). Table 18.1 lists the major cultivars that are used for commercial cultivation. Several other cultivars, such as ‘Château Queribus’, ‘Compactus’, ‘Fragrantissimus’, ‘Haute Vallée de l’Aude’, ‘Lemon Variegated’, are primarily cultivated as ornamental plants (Naktuinbouw 2005).

Note that English winter thyme, German winter thyme (Deutscher Winter), French summer thyme, French winter thyme (Narrow-Leaf French) and Greek thyme are not cultivars but genetically diverse population varieties from various origins. These populations show higher average coefficient of variation of most traits, and their essential oil content is often lower than in clones or clone hybrids.

Table 18.1 List of major cultivars of *T. vulgaris* used for commercial production (ITEPMAI 2009; Dachler and Pelzmann 2017)

Name	Description
'Carvalia'	Hybrid of clones released by ITEIPMAI (France), rich in carvacrol
'De Dolj'	From Romania
'Krajovy'	From Czech Republic
'Linalia'	Hybrid of clones released by ITEIPMAI (France), rich in linalool
'RH-1'	From Germany, winter-resistant
'RH-2'	From Mainz (Germany)
'Sloneczko'	From Poland
'Thymlia'	Hybrid of clones released by ITEIPMAI (France), rich in thymol
'Varico 1'	Hybrid of clones released in 1993 by Agroscope (Switzerland), winter hardy, rich in essential oil (Rey et al. 2004)
'Varico 2'	Hybrid of clones released in 2004 by Agroscope (Switzerland), winter hardy, rich in essential oil (Rey et al. 2004)
'Varico 3'	Hybrid of clones released in 2011 by Agroscope (Switzerland), winter hardy, very rich in essential oil. Thymol chemotype with high homogeneity and high yield (Carlen et al. 2010)

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Chapter 19
Valeriana officinalis L. s.l.: Valerian

Michael Penzkofer and Heidi Heuberger

19.1 Botany

Valeriana officinalis L., the common valerian, has a long application history in the European folk medicine. This is also illustrated by the multitude of commonly used names, e.g. all heal, Capons's tail, cat's love, and garden heliotrope (Dweck 1997). The origin of the botanical name can be traced back to the Roman province Valeria in Pannonia, the region west of the Balaton (Hungary). The etymological description of the Latin *valere* (be healthy, be strong) was introduced later (Mayer 2003).

19.1.1 Taxonomy

Valeriana is the eponymous genus of the family Valerianaceae and comprises about 150–350 species (Dweck 1997; Bell and Donoghue 2005). Recent studies discuss the classification of *Valeriana* within the Caprifoliaceae family (Chase 2009). The systematic classification down to the genus category is clear and shown in Table 19.1; however the taxonomy within the *Valeriana officinalis* aggregate (agg.) is not yet clarified (Buttler et al. 2008). Concerning the difficult taxonomic order, the taxa are classified in the different studies as species, subspecies or varieties. A frequently used classification is the basic taxonomic order of the *Valeriana officinalis* agg. resulting from the investigations of Skalinska (1947), Walther (1949), Titz (1969, 1984), Titz and Titz (1979, 1981, 1982), and Titz et al. (1983).

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Table 19.1 Classification of the *Valeriana officinalis* agg. according to Kirschner and von Raab-Straube (2017)

Category	Classification
Regnum	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superordo	Asteranae
Ordo	Dipsacales
Familia	Valerianaceae Batsch
Genus	*Valeriana* L.

For valerian in Central Europe, Walther (1949) classified the five species *V. exaltata*, *V. collina, V. pratensis*, *V. procurrens* and *V. sambucifolia* by including morphological, chorological and chromosomal aspects. The main morphological characteristics for differentiation are leaf morphological traits and runner formation. In principle, Titz (1984) uses the same classification for the valerian in Germany/Bavaria but defines them as 'types' instead of species. Keller (1973) describes for Switzerland the three subspecies *V. officinalis* L. ssp. *exaltata*, *V. officinalis* L. ssp. *collina* and *V. officinalis* L. ssp. *procurrens*.

For valerian in the Czech Republic, Holub and Kirschner (1997) differentiated between the species *V. officinalis L.*, *V. stolonifera* CZERN. and *V. excelsa POIR.* and allocated further subtypes to each of them. Buttler et al. (2008) applied this concept onto the German flora and substituted *V. stolonifera* CZERN. by *V. pratensis* DIERBACH. A recently published study shows that in Southern Germany three ecologically and morphologically distinct groups of *V. pratensis* can be differentiated (Gregor et al. 2016). Thereby, the informally recognized 'Frankonia type' of Titz (1984) was now formally described and was indicated as *V. pratensis* subsp. *franconica* Meierott & T.Gregor. Table 19.2 shows the current accepted taxonomy of the *Valeriana officinalis* agg.

The taxonomy is complex, and the currently existing results are difficult to interpret. It is not really clear to which species or subspecies the medically used valerian belongs to, therefore the appendix sensu lato (s.l.) is often used the appendix sensu lato (s.l.). In 'the broad sense', the whole *Valeriana officinalis* agg. is addressed in this case.

19.1.2 Origin and Distribution

The species complex of *Valeriana officinalis* L. s.l. is characterized by an enormous variability and can be divided into numerous smaller species, with partly limited distribution areas. Overlaps of the species exist. The natural occurrence of the species complex is located in the temperate and boreal zone of Europe and Asia (Fig. 19.1a). The natural distribution areas are predominantly located between the latitudes of 30° and 70° (Meusel and Jäger 1965, 1978, 1992). In Germany, valerian

Table 19.2 Genus *Valeriana* L. included taxa of the *Valeriana officinalis* agg. according to Kirschner and von Raab-Straube (2017)

Species	Subspecies
Valeriana armena P. A. Smirn.	
	Valeriana armena P. Smirn. subsp. *armena*
	Valeriana armena subsp. *grossheimii* (Vorosch.) Vorosch.
Valeriana colchica Utkin	
Valeriana excelsa Poir.	
	Valeriana excelsa Poir. subsp. *excelsa*
	Valeriana excelsa subsp. *salina* (Pleijel) Hiitonen
	Valeriana excelsa subsp. *sambucifolia* (Pohl) Holub
	Valeriana excelsa subsp. *versifolia* (Brügger) Buttler & al.
Valeriana hispidula Boiss.	
Valeriana officinalis L.	
	Valeriana officinalis L. subsp. *officinalis*
	Valeriana officinalis subsp. *nemorensis* (B. Turk) F. Martini & Soldano
Valeriana pratensis Dierb.	
	Valeriana pratensis Dierb. subsp. *pratensis*
	Valeriana pratensis subsp. *franconica* Meierott & T. Gregor
Valeriana rossica P. A. Smirn.	
Valeriana stolonifera Czern.	
	Valeriana stolonifera Czern. subsp. *stolonifera*
	Valeriana stolonifera subsp. *angustifolia* Soó
Valeriana wolgensis Kazak.	

is naturally common mainly in the southern and eastern regions (Fig. 19.1b). *Valeriana officinalis* L. s.l. is also common in the East and West of North America (Meyer 1951; USDA 2017). *Valeriana officinalis* L. s.l. grows mainly on fresh to moist habitats, e.g. moist and spars deciduous forests, ditches, banks, shrubberies and meadows. Due to the frost tolerance, valerian can still be found in mountain regions up to altitudes of 1.800 m MSL (Heuberger et al. 2012a).

19.1.3 Cytology and Aspects of Molecular Biology

19.1.3.1 Chromosome Number

Valeriana officinalis L. s.l. presents a basic chromosome number of $1n = 1\times = 7$. The number of chromosomes is one important characteristic to identify the different taxa. Early taxonomic investigations often base on it. The ploidy levels are compatible with the taxonomic classification of Walther (1949). The mentioned

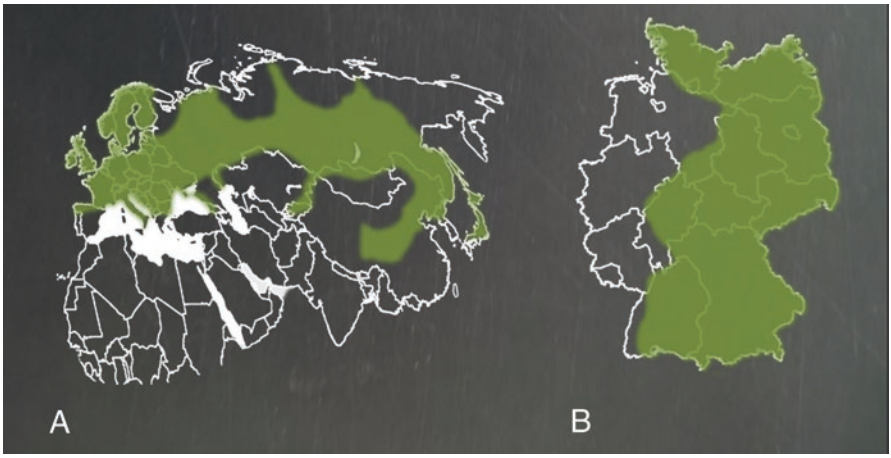

Fig. 19.1 The natural and predominant occurrence of the species complex of *Valeriana officinalis* L. s.l. in Europe and Asia (**a**) and specifically in Germany (**b**). (Data source: Meusel and Jäger (1965, 1978, 1992) and BfN (2017))

Valeriana exaltata is diploid, *V. collina* and *V. pratensis* are tetraploid, and *V. procurrens* and *V. sambucifolia* are octoploid. The same applies to the classification of Titz (1969). The taxonomical characterization proposed by Holub and Kirschner (1997) also conforms to the ploidy levels: *V. officinalis L., V. stolonifera CZERN.* and *V. excelsa* POIR. are di-, tetra- and octoploid, respectively.

In nature, mainly di-, tetra- and octoploid valerians are found, but Hidalgo and Vallès (2012) and Heuberger et al. (2012b) also reported on natural hexaploid cytotypes. Forced hybridizations between different species and cytotypes of the *Valeriana officinalis* aggregate are possible, and tri- and hexaploid cytotypes could be generated, but not each cytotype-crossing combination was similarly successful (Konon and Korneva 1980; Penzkofer et al. 2014a). A natural hybridization of species with geographically overlying habitats cannot be excluded (Titz 1969).

Up to the present time, it is still unclear how the cytotypes originated. Recently it was tried to investigate and clarify the origin of the polyploid complex by using genome analysis. Two independent research groups genotyped di-, tetra- and octoploid plants by an amplified length polymorphism analysis (AFLP). Heuberger et al. (2012b) analysed 106 varieties and accessions, collected and described in 1985–1993 and known as BLBP1-BLBP86 (Stahn and Bomme 1998), as well as varieties and accessions of commercial trade seeds and from several gene banks of Europe and the USA. Fischer (2012) collected 2009 seven valerian populations growing wild in western Austria and adjacent regions of Bavaria (Germany).

In both studies, the two-dimensional main component analyses showed that each of the three ploidy levels built a separate cluster. The genetic similarity was higher within than between the cytotypes, but single overlaps were observed. Heuberger et al. (2012b) showed that within the tetraploid cluster some accessions formed subclusters, but the accessions could be genetically curtailed, because of breeding

processes in the past. The high degree of consensus indicates a long-term development with limited gene flow between the cytotypes.

To verify the origin of the cytotypes, other methods are more suitable. The DNA sequence of the nuclear internal transcribed spacer region (ITS region) is a non-functional, tandem-like repeated DNA sequences, located between nuclear, ribosomal DNA (rDNA). ITS sequences have a high degree of polymorphism and are often used for genealogical studies and for identifying the genetic variability. If a mutation occurs among the tandem-like sequences in the ITS region, by a molecular process called 'concerted evolution', all other tandem sequences will be replaced through this mutated sequence, or the mutated sequence gets lost again (Liao 1999). In autopolyploid organisms, this process can affect all homologous chromosomes (multivalents). In the case of strictly allopolyploids, 'concerted evolution' occurs mostly within bivalents, and the two variants of a sequence can remain permanently.

Heuberger et al. (2012b) analysed the ITS regions (627 bp – ITS1-5.8S-ITS2) of several single plants of different cytotypes of *V. officinalis* L. s.l. and compared available valerian sequences from NCBI-GenBank (Benson et al. 2005). In both ITS regions of some tetra- and octoploid plants, two sequence variants were found. The positions and the coexistence of the two different ITS sequences indicate that hybridizations occurred and, hence, an allopolyploid genesis. The results of the genotyping and ITS region analysis can be understood as an indication of a possible allopolyploid origin, at least for the step from the diploid to the tetraploid level. However, an autopolyploidization followed by a longer, independent evolution of the cytotypes with an introgression by other taxa would also be possible. In order to clarify the formation of the polyploidy complex, further investigations are necessary.

19.1.3.2 Genome Size

Data on genome size are available for an increasing number of plant species (Bennett and Leitch 2012). The genome size is variable and is caused in principal based on polyploidy, aneuploidy and change in DNA content of monoploid genomes or chromosomes (Bressler et al. 2017). Polyploidy is the main variation factor for the absolute genome size (2C) in *V. officinalis* L. s.l. Due to the lower number of chromosome sets, diploids show a smaller genome size than tetra- and octoploids. A correlation of genome size and ploidy level is to be presumed and was shown by Hidalgo and Vallès (2012) (Table 19.3). The absolute genome sizes are varying in literature, because of physically varying genome sizes of the different analysed plant material and the use of different internal genome size standards.

Despite of the correlation of polyploidy level and genome size, the genome size of polyploid valerian is not linearly proportional to the ploidy level. The investigations of Geyer (2013) and Bressler et al. (2017) showed different monoploid genome sizes (1C in pg) for the different cytotypes. The monoploid genome sizes of tetra- and octoploids are about 13% and 26%, respectively, smaller than the monoploid genome of diploids in both studies. The differences between the monoploid genome

Table 19.3 Overview of absolute genome sizes ± standard deviation (2C, in pg) of valerian at different ploidy levels (2*n* = 2× to 8×) collected from different references

Ploidy levels					
Diploid (2*n* = 2×)	Triploid (2*n* = 3×)	Tetraploid (2*n* = 4×)	Hexaploid (2*n* = 6×)	Octoploid (2*n* = 8×)	Reference
2.97 ± 0.04		4.62 ± 0.08	6.51 ± 0–11	8.15 ± 0.25	Hidalgo et al. (2010) Hidalgo and Vallès (2012)
3.16 ± 0.03				8.05 ± 0.08	
3.37 ± 0.05	4.70 ± n.a.	5.85 ± 0.14	8.20 ± n.a.	10.21 ± 0.13	Geyer (2013)
3.39 ± 0.06		5.84 ± n.a.	8.32 ± n.a.	9.91 ± 0.17	
				9.97 ± 0.20	
				10.00 ± 0.26	
2.97 ± 0.03		5.10 ± 0.38		8.78 ± 0.46	Bressler et al. (2017)

Values of single plants, lacking standard deviation, are marked with n.a. (not available)

size and that of tri- and hexaploids were about 7% and 18%, respectively (Hidalgo et al. 2010; Geyer 2013).

19.1.4 Plant Description

19.1.4.1 Life Cycle (Ontogeny)

Valeriana officinalis L. s.l. is an herbaceous perennial plant, but in the early life period it behaves like a winter annual. The first year is distinct by predominantly vegetative growth. In the following, the plants hibernate with their subterranean plant parts (rhizome) and with their young, vegetative shoot buds. The first generative phase follows after vernalization (Fig. 19.2a) in which the shoot buds are induced (Fig. 19.4a). In the following spring, the basal leaves (rosette) appear from February, and, by internode stretching, the rosettes form a leafy shoot with a terminal inflorescence. After flowering and seed ripening, the inflorescence and the basal leaves undergo senescence, followed by the formation of new basal leaves and underground shoot buds until senescence starts for hibernation. This enables valerian to renew itself repeatedly for several years.

19.1.4.2 Morphology of Above Ground Plant Parts (Texture and Habitus)

As complex as the taxonomy, as variably depicted is also the morphology. The plants of the *Valeriana officinalis* aggregate form imparipinnate leaves, with 7–23, lanceolate to linear and weakly to strongly serrated leaflets (Fig. 19.2c). From the cotyledon stage to young plant, the leaf shape changes with each new leaf (Fig. 19.2b). During the growing period, new leaves are continuously generated.

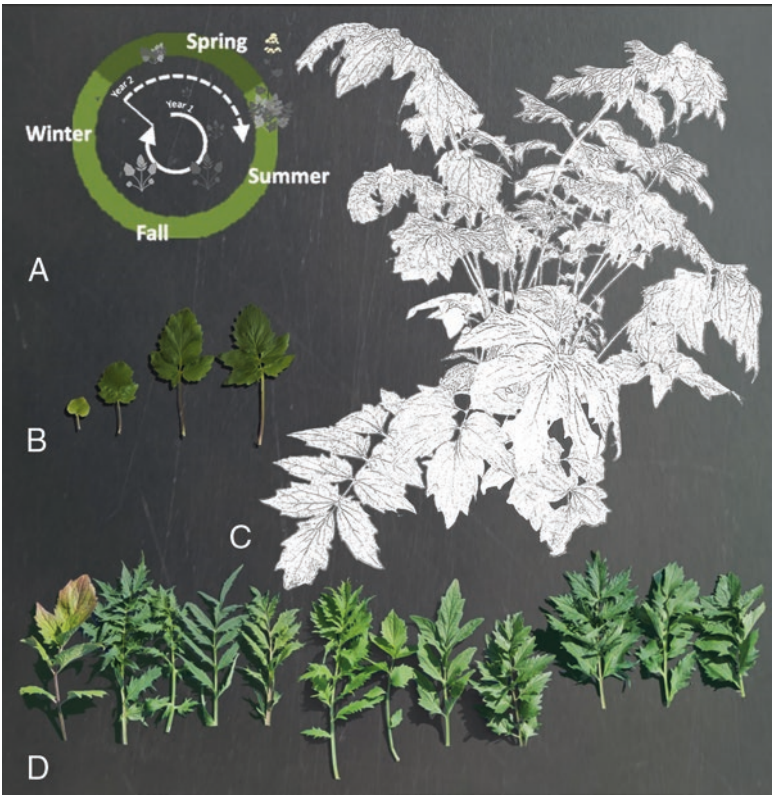

Fig. 19.2 Morphology in the vegetative phase (first year) of *Valeriana officinalis* L. s.l. (**a**) Stages of development (ontogeny). (**b**) Different leaf shapes during the young plant development. (**c**) In the first year, valerian usually forms a leaf tufts. (**d**) Different leaf morphologies and leaf textures of tetraploid valerian

Therefore, the leaf color can vary within one plant and during the season. The leaf color ranges from deep green to light green and can show blue-grey and reddish tints. The leaflet surfaces, the petiole and the midrib can be more or less hirsute. In the first year, the plant reaches heights of about 30–60 cm (Fig. 19.2a) (Heeger 1956; Jäger and Werner 2002; Gregor et al. 2016).

19.1.4.3 Morphology of the Inflorescence and the Seeds

Depending on genetic background and plant vitality, plants of the *Valeriana officinalis* aggregate form one to several shoots with a terminal inflorescence and reach heights of up to 2 m. In addition to the terminal inflorescence, further inflorescences can develop out of the leaf axilla of the upper third to the upper half of the shoot (Reichling et al. 1994). The inflorescence shoots can be hirsute or glabrous and

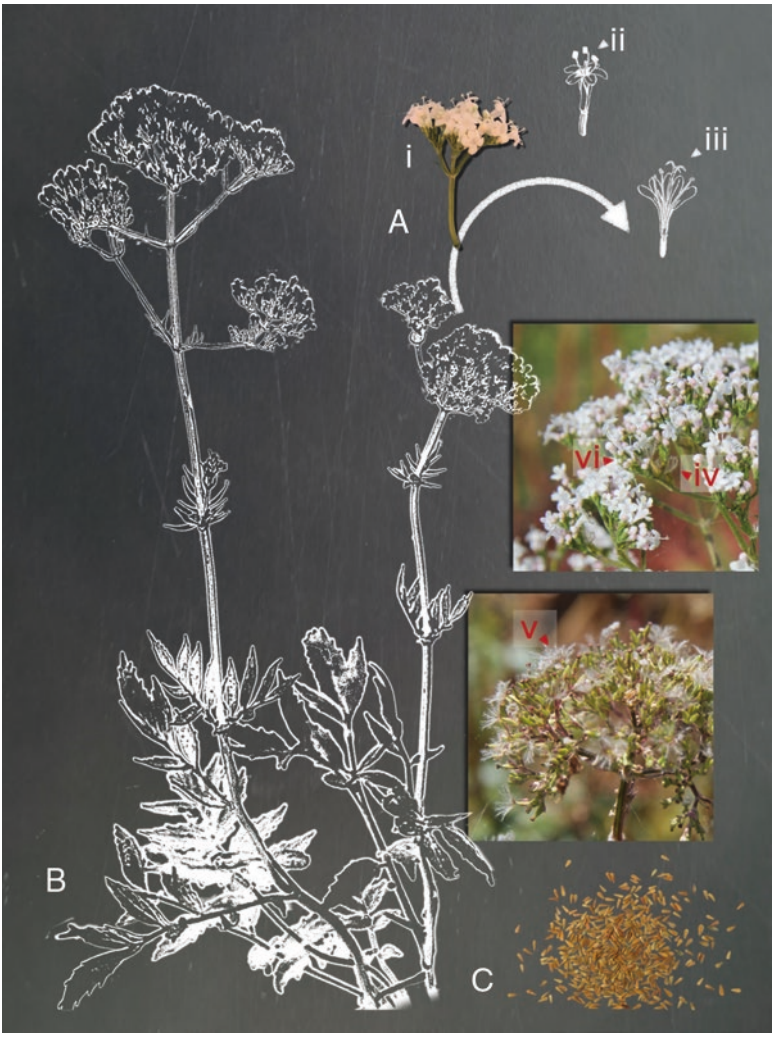

Fig. 19.3 Development of inflorescences and seeds in the generative phase of *Valeriana officinalis* L. s.l. (**a**) Type of inflorescence and flower development stages. (i) Part of the inflorescence, showing the panicle like cyme. (ii) Single protandric flower with mature stamina and immature carpels. (iii) Single flower with three stigma segments open after pollen maturity. (iv–vi) Inflorescence exhibiting different maturity stages of flowers and seeds at the same time. iv + v Young seeds with pappus, vi Different bud stages. (**b**) Illustration of a valerian inflorescence. (**c**) Valerian seeds

show often a more or less reddish coloration. The blooming period lasts from May to August. The inflorescence is forming a paniculate and umbrella-shaped cyme (Fig. 19.3). The flower color is white to pale rose. The corolla consists of five petals, is spurred on and is asymmetric. Valerian flowers exhibit three stamina and three adnate carpels.

Fig. 19.4 Dried root system, including rhizome and roots, of *Valeriana officinalis* L. s.l.

The seeds of *Valeriana officinalis* L. s.l. are about 3 mm long and form a 1000 seed mass between 0.4 and 1.1 g. The seed surface exhibits longitudinal ribs and a light to dark brown color and is more or less hairy (Heeger 1956; Bomme 2001). At the time of seed ripening, the seeds bear a pappus (Fig. 19.3a, v).

19.1.4.4 Morphology of the Root System

Valeriana officinalis L. s.l. forms a very fine structured root system (Fig. 19.4). The more or less thick adventitious roots that derive from the rhizome and the thinner lateral roots interweave to a carpet-like matted network (Fig. 19.5b). The rhizome is short and cylindrically shaped. The formation of long upper and subterranean runners is often described for wild valerians (especially octoploids) at the origin habit (e.g. Titz and Titz 1982) but is rarely found in cultivated accessions and breeding material (Heuberger et al. 2010). Possibly, this ergonomically hindering characteristic has already been eliminated unknowingly in earlier selection works. Sometimes, new vegetative shoots form own roots; thus, the rhizome size is increasing (Fig. 19.5c, i).

19.2 Economical Use

19.2.1 Cultivation, Harvesting and Processing

All commercially used valerian raw material is derived from cultivation. The methods of cultivation vary and depend on local conditions and on the individual operating structure. The following sections are to be understood as an overview of the basic principles in the cultivation of valerian for the dried root production.

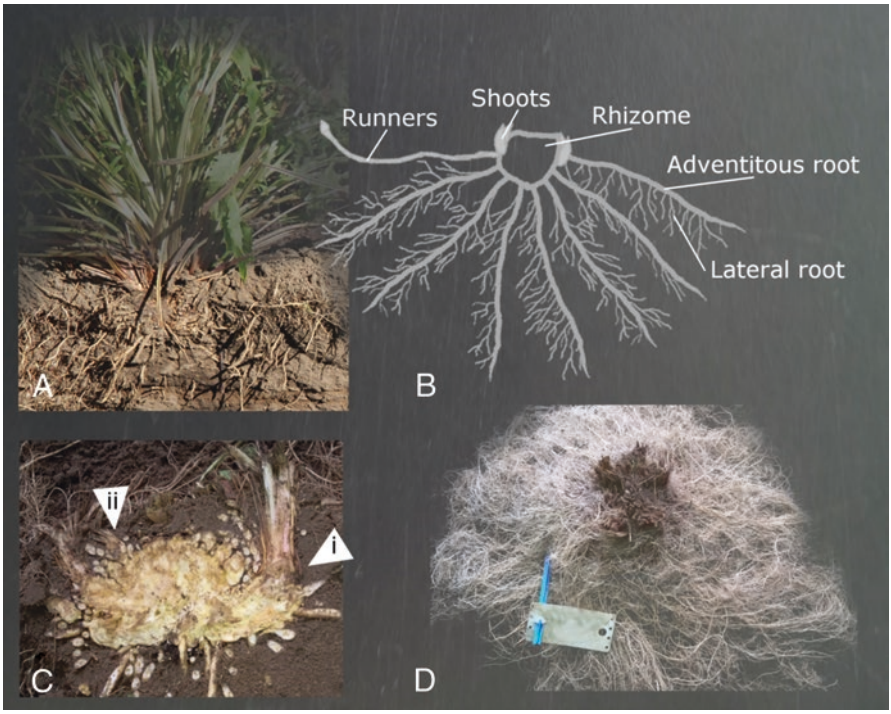

Fig. 19.5 The root system of *Valeriana officinalis* L. s.l. (**a**) In situ cross section. (**b**) Schematic illustration of the root system, which includes the adventitious and lateral roots, the rhizome, the shoots and, if formed, the runners. (**c**) Vertical cross section through a rhizome at vegetative stage before hibernation. (i) Base of a new vegetative and unstretched inflorescence shoot. (ii) Small vegetative bud. (**d**) Plan view to a fine structured, carpet-like and matted root system

19.2.1.1 Location

Valerian is a robust crop, hardy against spring frost and can be cultivated in rough areas, if a sufficient precipitation or irrigation is ensured of about 600–700 mm/year. Valerian has no high requirements on the soil but grows well in argillaceous peat soils with high humus contents. From a technical point of view, these soils should be avoided, because of the difficulties arising from the adhesion of soil particles in the root system in harvest and postharvest processing. For cultivation, porous soils are selected such as sand or sandy loam, with low incidence of stones or gravel and low weed infestation, especially of root spreading weeds (Bernath 1997; Bomme 2001; ESCOP 2009).

19.2.1.2 Crop Rotation

Valerian shows no special requirement to the preceding agricultural crop, except that it should not form slowly decomposing root stocks, perennial roots or other overwintering plant parts, which could spread out again (e.g. peppermint, horse radish and

liquorice). Legumes and cereals are suitable preceding crops. Cereals may follow valerian, because possible spreading of overwintering parts of the valerian root system can be controlled and prevented easily by herbicides. Else, valerian has positive effects in the crop rotation. To prevent the development of diseases and pests, Bomme (2001) recommends a 4- to 5-year break of cultivation of valerian and lamb's lettuce.

19.2.1.3 Cultivation

The standard procedure for valerian field cultivation is the planting of young plants in spring (Heuberger et al. 2012a). The planting in autumn is also possible if it is planted not longer than 1–2 months before the first frost (Bernath 1997). The plants should not exceed a certain developmental stage before hibernation to prevent vernalization (see more in Sect. 19.3.1). The same aspect is to be considered for the possible direct sowing in autumn, but then a second condition accrues that for a successful overwintering the plants must have developed three to five leaves (Bernath 1997). A sowing in spring should be avoided, because of the clearly lower root yield (Neumaier and Fröhlich 2013). The cultivation on ridges offers advantages for weed control and harvest. A yield difference to a usual flat ground culture does not exist (Bomme 1984).

The young plants for the spring planting are cultivated under protected conditions. The sowing starts at mid-February. The seeds will be placed as single grain or tuff (3–5 seeds) in multipot plates. Broadcast sowing and the pricking out (transplanting) of single plants into pots are also possible and often done during breeding. At a germination temperature of 20 °C, the duration of the germination phase is about 1–2 weeks. Thereafter, a gradual temperature lowering to 16 °C and a final cool and ventilated phase for hardening for at least 3 days are recommended. The well hardened young plants can be planted with a common planter at the end of March to early April. Usual stand densities are 40,000–100,000 plants or tuffs per ha at row distances of 30–50 cm and distances between the plants of 20–30 cm (Heuberger et al. 2012a).

19.2.1.4 Cultivation Measures

During field cultivation, weed control, irrigation and fertilization are the important maintenance works. Weed control occurs predominantly by using mechanical equipment and is supplemented by manual weeding. Chemical weed control is possible where authorized herbicides are available. Valerian grows naturally on fresh to moist habitats and is classified as crop with high water requirements. Heeger (1956) estimates 650 mm as adequate precipitation. Vetter et al. (1991) provided guideline values of 15–130 mm additional water demand from the end of June to the end of August based on long-term observations in central Germany. Bomme et al. (2002) included practical experiences and assumed up to five water charges

summing up to 90–150 mm. However, more frequent application of smaller doses can be advantageous on light soils. The fertilization is usually carried out before and during cultivation, depending on the availability of nutrients, the fertilizer used, the cultivation system, the development stage of the plants and weather conditions. According to Bomme and Nast (1998), the average nutrient uptake is 100 kg N/ha, 48 kg P_2O_5/ha, 162 K_2O/ha, 21 kg MgO/ha and 90 kg CaO/ha based on 15 t/ha fresh root mass and 20 t/ha fresh leaf mass. To calculate the fertilizer application rates, mineral N in 0–30 cm soil depth in spring and the mineralization potential of the soil have to be considered.

In general, diseases of valerian are relatively rare. In practice so far, no economically relevant attack by powdery mildew (*Erysiphe valerianae* [Jacz.] Blumer) has been observed in the field (Heuberger et al. 2012a). Other fungal parasites are described, which also have little relevance for root production and are rarely imperiling (Hoppe 2007). Kinked stems and wilt phenomena can occur on plants used for seed production caused mainly by *Phoma exigua* Sacc.

Animal pests like insects and arachnids are not important in root and seed production in the field. Mammals such as hares, deer, rodents, etc. occasionally cause damage to seed propagation stocks. Nematodes such as *Meloidogyne* spp., *Pratylenchus* spp., *Paratylenchus* spp. or *Paralongidorus maximus* (Heuberger and Penzkofer 2017) can bring the cultivation to a standstill on infested areas, especially after close crop rotations (Fig. 19.6). The damage patterns of the soil-borne nematodes are extremely diverse and range from leaf deformations and growth disturbances to the death of the plants. Typical are the deformations and constrictions on the roots or the gall-like root structures. The infections mainly occur in restricted field areas (Bomme 2001; Hoppe 2007). Occasionally, viruses and phytoplasms occur, which can lead to leaf spotting, growth depression and deformation (Fig. 19.7) (Hoppe 2007; Heuberger et al. 2012a).

Fig. 19.6 Valerian roots with gall-like deformations (black arrow) caused by nematodes

Under greenhouse conditions, powdery mildew and some insects (e.g. aphids and whiteflies) can cause a serious damage at the plants, which can even lead to total loss.

19.2.1.5 Harvest and Post-Harvest Treatment

The start of the harvesting phase is determined by several parameters. These can be the operational structures and processes, the cultivated varieties and the climatic conditions. Bernath (1997) sees the optimum time for the root harvest from Mid-October to Mid-November, whereas Bomme (2001) already would start at the end of September. In practice, however, a later harvest is mostly carried out based on the operating procedures and yield advantages and may last until February before the new leaves appear (Heuberger et al. 2012a).

The dried root drug may contain a maximum of 5% of residual leaf stems (Ph. Eur.9.1 2017). Therefore, before the root harvesting, the leaves must be cut off as close to the soil surface as possible that is commonly done by using mower or beater. The root harvest can be carried out with adapted sugar beet or potato harvesters (Bomme 2001; Neumaier 2017) or harvesters for tree nurseries.

Due to the very fine structured root system, the freshly harvested root system can contain up to 75% soil mass (Heuberger et al. 2012a). Following to the harvest, several cleaning steps are needed to remove the soil and to drop the content of HCl insoluble ash to the required maximum content of 5% and the percentage of other impurities to 2% (Ph.Eur.9.1 2017). Depending on the individual procedure, the root stocks are comminuted, freed from bulk soil and stones and washed with water. Different combinations of choppers, rippers and sieves are used. The washing is carried out often with drum washing machines using high water rates. The washing

Fig. 19.7 Deformations of shoots and inflorescences, caused by unspecified phytoplasms

process line is often combined with an air bubble pool and a sedimentation tank. The last step of washing is performed with fresh potable water.

The washed roots are dried at 40–45 °C and with high air volumes in flat dryers or drying hurdles, performed as intermittent drying allowing the water in the rhizomes to migrate to the tissue surface. Belt dryers are less suitable because of the long drying time and the poor flowing properties of the root material. The maximum allowable residual moisture content of 12% is reached after 20–40 hours of net drying time (Heuberger et al. 2012a; Ph.Eur.9.1 2017; USP41 2017).

19.2.2 Valuable/Undesired Plant Secondary Compounds

The valerian root drug contains a large number of other secondary compounds, which have been isolated and characterized from the root and the rhizome (Trauner 2009; Wichtl 2009): sesquiterpenes (Stoll and Seebeck 1956), iridoids (valepotriate) (Thies and Funke 1966), lignans (Bodesheim and Hölzl 1997; Schumacher et al. 2002), flavonoids (Fernández et al. 2004; Marder et al. 2003), alkaloids (Torsell and Wahlberg 1966; Gross et al. 1971), amino acids, phenolic carboxylic acids, sterols, carbohydrates and others. A detailed compilation and overview of the diverse substance groups and ingredients are also given by Heuberger et al. (2012a). The characteristic smell of valerian develops during the drying process; it is mainly caused by the liberation of isovaleric acid (Sticher et al. 2015).

The components described in this publication are contained in larger quantities in the root drug and in essential oil, respectively, or are important for the medical application and the determination of the root drug quality. The contents and the composition can be quite diverse and are influenced by the plant genetic properties, the origin of the plant material and the method of extraction (Bos 1997; Houghton 1997).

The valerian root drug contains 0.3–1.0%, in some cases up to over 2.0%, essential oil (Houghton 1997; Wichtl 2009; Heuberger et al. 2012a). The essential oil content is determined by water distillation (Ph.Eur.9.1 2017; USP41 2017). The quality standards for the minimum essential oil content is defined by the European Pharmacopoeia as 4 ml essential oil/kg for uncut root (*Valerianae radix*) and 3 ml essential oil/kg for cut root (*Valerianae radix minutata*) (Ph.Eur.9.1 2017). The US Pharmacopeia defined minimum essential oil content for valerian root as 0.5% and for powdered valerian root as 0.3% (USP41 2017). In the Japanese Pharmacopoeia, *Valeriana officinalis* L. s.l. is not listed (JP XVII 2017). The essential oil is composed of a mixture of monoterpenes and sesquiterpenes; until now, approximately 150 components have been identified. The most frequent major constituents of essential oil of *Valeriana officinalis* L. are the monoterpenes borneol and its esters bornyl acetate and bornyl isovalerate. Further monoterpenes of the essential oil are camphor, camphene, 1,8-cineole, α-pinene and myrcene (Stoll et al. 1957; Reichling et al. 1994; Houghton 1997; Bos 1997). The most frequent sesquiterpenes are valerenal, valerenol, valerenyl acetate, valerenyl isovalerate, kessan, kessanyl acetate,

α-kessyl acetate, faurinone, patchouli alcohol, α-curcumene, β-bisabolene and others (Houghton 1997; Reichling et al. 1994; Bos 1997). The valerenic acid, hydroxyvalerenic acid, acetoxyvalerenic acid and 3β,4β-epoxyvalerenic acid are cyclopentane-sesquiterpenes and form a subgroup of the sesquiterpenes (Stoll and Seebeck 1956; Reichling et al. 1994; Houghton 1997; Dharmaratne et al. 2002).

These low-volatile sesquiterpene acids are typical for *Valeriana officinalis* L. s.l. and can be used as marker substances in the quality control of valerian preparations (Hänsel and Schulz 1982; Navarette et al. 2006; Ph.Eur.9.1 2017). However, sesquiterpene acids are also found in other *Valeriana* species, e.g. *Valeriana angustifolia* Tausch and *Valeriana montana* L. (Bos et al. 1997). Hydroxyvalerenic acid may be an artefact, which is probably formed from acetoxyvaleric acid by unfavorable storage conditions, an indication for a possible careless treatment and processing (Bos 1997). The quality standards for the minimum content of sesquiterpene acids (valerenic acid and acetoxyvalerenic acid), calculated as valerenic acids, are defined by the current European Pharmacopoeia as 0.17% sesquiterpene acids for the un-cut root (*Valerianae radix*) and 0.10% sesquiterpene acids for the cut roots (*Valerianae radix minutata*) (Ph.Eur.9.1 2017). The US Pharmacopeia demands also a minimum sum of 0.17% sesquiterpene acids, where hydroxyvalerenic acid is also included in the sum. The content of valerenic acid, within the sum, must be at minimum 0.05% (USP41 2017).

A further group of compounds that appear in valerian are the valepotriates. These bicyclic monoterpenes are instable compounds and will be decomposed quickly under acidic or alkaline conditions or by influence of heat (Bos 1997; Wichtl 2009). Extracts from the roots of *Valeriana officinalis* L. contain up to 2% of a mixture of valepotriates. In contrast, the root extracts from *Valeriana edulis* Nutt. ex Torr. & Gray (synonym: *V. mexicana* DC) and *Valeriana wallichii* DC. (synonym, *V. jatamansi* Jones) contain 3–6% and 4–8 (12)% of valepotriates, respectively (Hölzl 1996; Bos 1997; Sticher et al. 2015). These two last valerian species were originally used to isolate valepotriates, which were used to influence psychovegetative and psychosomatic disorders, restlessness, anxiety disorder as well as against concentration weakness (Sticher et al. 2015). Nowadays, the understanding of the medically active substances has changed through intensive research. Valepotriates act mutagenic after metabolic activation. For this reason, the importance of valepotriate-rich preparations has been lost (Sticher et al. 2015).

The effectiveness was attributed so far to different groups of elements. Sticher et al. (2015) classified the lipophilic group (e.g. the sesquiterpenes, the sesquiterpene acids and the borneol) and hydrophilic group (e.g. lignans and flavonoids) as active substance classes. The mechanism of action is probably caused by the linkage of extractives or single ingredients to the adenosine receptors, the subtypes of the serotonine receptors and the GABA receptor complex, respectively (Sticher et al. 2015). The latest investigations revealed that the valerenic acids stimulate subtypes of the $GABA_A$ receptors and thus a soporific effect is initiated (Khom et al. 2007; Benke et al. 2009; Becker et al. 2014). Murphy et al. (2010) identified valerenic acid as the substance with the highest anxiolytic effect in valerian. Acetoxyvalerenic acid also links to the GABA receptors, but without anxiolytic effect, so that it inhibits the potential of the valerenic acid (Felgentreff et al. 2012).

19.2.3 Major Production Areas

The growth conditions, required by valerian and the traditional use of valerian roots in (folk) medicine, are reflected in the production areas. Nowadays, *Valeriana officinalis* L. s.l. is cultivated in many countries of Eastern Europe and Western Asia, namely, Poland, Bulgaria, Romania, Ukraine, Russia, and in some countries of Central Europe, such as the Netherlands, France and Germany (Bernath 1997). The production in Eastern Europe and Western Asia is often characterized by large-scale cultivation if the technical requirements do exist for it, while in Central Europe and partially in Eastern Europe and North America, medium- and small-sized farms are predominant (less than 100 ha).

19.2.4 Economical Valuation/Parameters

Valeriana officinalis L. s.l. is a classic medicinal plant in the western medicine. The dried root system is used as raw material mainly for extraction, in smaller volumes for essential oil distillation and for herbal tea production. Fresh roots are also used for the extraction of pressed juice (Schoenenberger 2014). A use of aboveground plant components such as flower extracts as a plant additive to promote plant health, flower formation, etc. has only a small importance, and its efficacy is scientifically rarely well documented.

In Germany, the dried root of valerian is a component of about 86 phytopharmaceuticals and homeopathics[1], creating a quantified demand for dried valerian roots only in Germany at approximately 1000 tons and a market size of about 4 Mio. € (FNR 2013, 2014). In North America (the USA, Canada and Mexico) due to other admission procedures, more than 1000 products with valerian root are obtainable (LNHPD 2018; COFEPRIS 2018; NIH 2018).

Two business markets are served with valerian root drug: on the one hand, the main market, which is looking for a pharmacopoeia-compliant product, and, on the other hand, a smaller, highly specific market which requires, for example, high or specific content levels of secondary compounds or a particular secondary compound composition. Both markets are dominated by contracted cultivation with its advantages of full product tracking, further customer specifications, etc. Organic valerian root drug is available and has a small market share where the root drug is used for food supplements or dietary products (food market sector).

[1] The number mentioned corresponds to the number of listed preparations. According to the approval procedure in Germany, several approvals are possible on the market which differ in dosage and dosage form. Accordingly, the number of approvals is equal or greater than the number of preparations.

For growers, the economic success is a result of the root drug yield per area, production costs, market price and the adherence to pharmacopoeias or contractually recorded quality specifications. The root drug yield ranges mainly between 2 and 4.5 t/ha and generates a benefit between 3000 and 20,000 €/ha. Reduced by the variable costs, the contribution margin calculates for −3400 to 5600 €/ha under German conditions (Bomme et al. 2002; Heuberger 2014).

The production of dried valerian roots is highly labor- and cost-intensive, due to the necessity to crop establishment by planting as well as the time- and energy-consuming harvest and drying of the roots and rhizomes (Heuberger et al. 2012a). About 60% of the production costs are caused by the drying, the processing and the personnel (sum of labor hours: 215–379). Thirty percent must be calculated for the planting material (transplants) (Bomme et al. 2002; Heuberger 2014). For seeds, used in agricultural cultivation, the catalog seed prices range from 270 to 650 €/kg, depending on variety and seed quality (Jelitto 2015; NLC 2016; PS 2017; RH 2017).

19.3 Breeding

19.3.1 Flower and Pollination Biology

Valerian flowers exhibit each three stamina and carpels. Dichogamy (protandry) is occurring, in which the pollen-covered anthers protrude from the flowers about 1–2 days before the stigma is opening the three flaps. This protects the single flower from self-pollination (autogamy), but not from pollination within the inflorescence (Fig. 19.3a, i–iii). Thereby, *Valeriana officinalis* L. s.l. is generally regarded as cross-pollinated species, which can certainly be suspected by the enormous variability within populations (Heeger 1956; Heuberger et al. 2012a). Penzkofer et al. (2018b) determined a natural cross-pollination (allogamy) between 77 and 97%. The inflorescence exhibits different maturity stages of flowers and seeds at the same time, because of continuously appearing new flowers during the blooming period (Fig. 19.3a, iv–vi).

Valerian is a winter annual plant and requires a vernalization for flowering. The requirement and the intensity of vernalization are depending on the genetic background, age and cultivation history of the plant material as well as on the duration and temperature of vernalization. To artificially induce flowering during cultivation, potted plants can be kept in cooling chambers for a minimum of 8 weeks at 4 °C and 8 hours light per day (Honermeier et al. 2011; Heuberger et al. 2012c). An additional application of phytohormones (e.g. gibberellic acid (GA3)) is helpful to shorten the vernalization period but is not obligatory. More important seems to be the plant age. The number of leaves is a good indicator for the maturity of the plant apex to translate low temperatures into the initiation of the generative plant organs. Seedlings should have a minimum number of 6–10 leaves. The reaction onto cool

temperatures of young plants, originated from in vitro propagation (cloning), can deviate. Presumably, no flowers are formed because of the imbalance of phytohormones. After flowering and seed ripening, the flowering shoot undergoes senescence, and new vegetative shoot buds are formed. Without further vernalization, this newly formed shoot buds would not turn flowering.

19.3.2 Propagation Strategies

Valerian can be propagated generatively as well as vegetatively; however, vegetative propagation is used for the field production only in special cases. Vegetative propagation is predominantly used in research and seed production where genetically uniform plant material is needed or special genotypes should be conserved. The simplest vegetative propagation is the division of the rhizome into several parts with regenerative buds (Heeger 1956). Eisenhuth (1966) reported vegetative propagation by runners of the variety 'Merkator', bred in the former German Democratic Republic. Nowadays, vegetative mass propagation is commonly performed by using plant tissue cultures. *Valeriana wallichii* DC can regenerate from cell suspension cultures and can be well propagated in vitro (Mathur et al. 1988, 1989; Viola and Fritz 1991; Mathur 1992). Enciso-Rodríguez (1997) described a method for micropropagation of *Valeriana edulis* ssp. *procera* by using germinated seeds for in vitro establishment. Seeds from *Valeriana officinalis* L. s.l. were used as starting material for the investigation to rapid in vitro shoot regeneration by Tansaz et al. (2014). Penzkofer et al. (2018a) described the micropropagation by using inflorescences at an early bud stage as starting tissue and side shoots as propagation parts. This method is useful, if of an existing adult plant larger numbers of genetically identical plants are needed.

The commonly used method for propagation is the production of seeds. Seed production needs special care and technology, because of the continuous ripening and dropping of seeds within the inflorescence (Fig. 19.3iv–vi). Therefore, large-scale seed production in the field will be usually performed by specialized seed producers (Bernath 1997). The seeds are harvested as soon as the first seeds turn brown by cutting the flower stalks on the base. Self-propelled harvesting machines that are suitable to harvest the entire large shoots are used. Subsequently, a post-ripening can be carried out under cool and ventilated conditions (ESCOP 2009). For a good seed quality and the protection of losing the seed quality from seed harvest to sowing, a good drying and dry storing of the seeds are the most effective and indispensable measure. The most important aspect during the post-ripening and drying period is ventilation. On the one hand, this ensures cooling and protects the seed from overheating. Secondly, by using of dry air, it ensures a reduction in the moisture content in the seeds (Humpisch 2008). After cleaning the seeds and removing small and poorly filled seeds, the seeds should be stored under dry, dark and cool conditions. A storing temperature of 2 °C extends the storage period for valerian from one to more than 4 years (Hoppe 2009, p. 655).

19.3.3 Applied Breeding Methods and Techniques

Compared to other agricultural crops, breeding of valerian – as well as breeding of many other medicinal and spice plants – is at an early stage of development (Hoppe 2009). Innovations in breeding and cultivation techniques are closely interrelated and can influence each other.

Bernath (1997) summarized the development of valerian breeding into four periods, reflecting to the chronological development (Fig. 19.8). In the first period, dated back to the early 1930s, mass selection and simple selective breeding were done in native wild populations. The attention laid on the morphological and production characteristics, especially their root productivity. The only chemical character which was taken into consideration was the essential oil. The second period (1950s) is characterized as the time where the mass selection was expanded and systematical analyses of populations were realized (Eisenhuth 1956). The aim of the third period (as of the 1970s) was the stabilization of root yield and quality, mainly of the ingredient contents and the ingredient composition. The still existing high variability of the plant material was a risk for serving the demand of the pharmaceutical industry for constant raw material. At the late 1980s, the fourth period is characterized by the application of the new genetic knowledge and the use of traditional and new methods of plant breeding.

In contrast to other medicinal plants, for valerian, an unusually large number of varieties have been bred. However, a lot of them are not available anymore in the seed trade (Heuberger et al. 2012a). In addition, numerous accessions exist for individual varieties as they were not under variety protection. About the development of

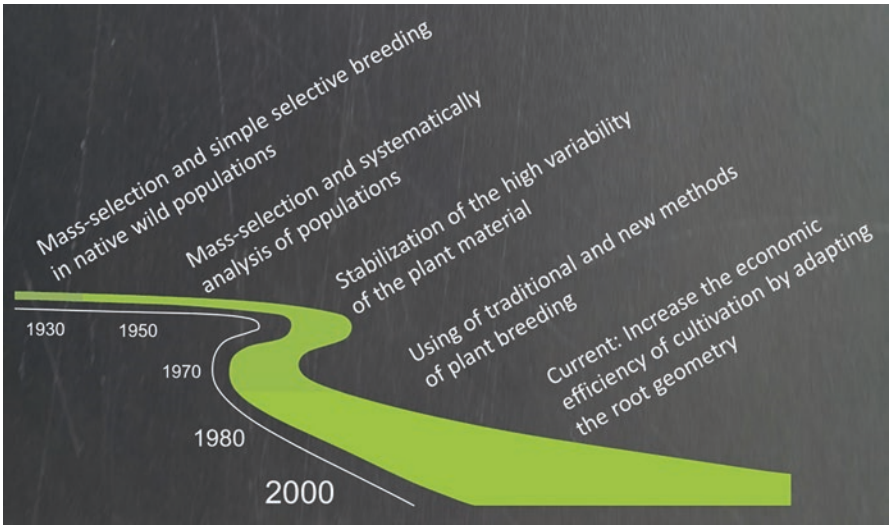

Fig. 19.8 Timeline of valerian breeding periods from the early twentieth century to now

these varieties, no or just little information are documented. Therefore, the authors report in the following on their own findings and experiences in the breeding of valerian and indicate references if it is possible.

19.3.3.1 Breeding Techniques

The creation of new variability and selection are two basic process steps that are used in varying ways in almost any variety development. For this purpose, different techniques and methods have been developed. In this context, the technique is to be understood as a treatment or procedure used in breeding, and the method is the application of different techniques during the breeding process. A universal scheme or procedure does not exist for valerian. However, a multitude and often also a mixture of different breeding techniques and methods are applicable, depending on the breeding target and the facilities of the breeder.

Creation of New Variability

The fundamental step during breeding is the creation of new variability. Pollination control is the one most often used as well as the simplest technique and can be done in two directions. Either the combination of geno- or phenotypes or the exclusion of not wanted geno- or phenotypes. The use and manipulation of the ploidy levels are mostly more complex and are predominantly used in primary stages of the breeding or in research.

Control of Pollen Transfer

The *Valeriana officinalis* L. s.l. complex exhibits such variability so that crossings within the complex are sufficient for the creation of new variability. Since valerian is used exclusively medically, the breeding result must be in compliance with the identity requirements of the pharmacopoeia. Therefore, interspecific and bridge crossings with other valerian species are of minor relevance.

An essential aspect of breeding is the control of pollination (Acquaah 2012). The targets of these pollination control techniques are ideally to maximize the proportion of hybrid or inbred seeds in the offspring.

Support of Pollen Transfer Between Different Geno- and Phenotypes

Pollen transfer between different geno- and phenotypes leads to hybridization and can be controlled or maximized by application of mechanical/technical or chemical treatments, or by using genetical characteristics of the parent plants, which influence the sexual biology (Brown and Caligari 2008). A mechanical/technical pollination control is often based on the removal of anthers from the bisexual flowers of valerian. Kempf (1986) elaborated some basic information about emasculation (cas-

Fig. 19.9 Emasculation of valerian inflorescences. Left: The emasculation must be done by hand for each single flower in young inflorescences. Right: Inflorescence with emasculated flowers. Unneeded flowers/buds were removed

tration) techniques. The optimal floral stage is if the flower buds are already swollen but the corolla is not yet opened. Due to the different maturity stages of flowers that arise sequentially within the inflorescence of *Valeriana officinalis* L. s.l., emasculation must be carried out continuously. Flowers that are not needed must be removed, preferably at early bud stages to hinder pollination with own pollen (Fig. 19.9). A manual emasculation is in principle possible, but in most cases too time-consuming and too expensive for the low seed yield generated (Acquaah 2012). Therefore, it is mainly used for scientific investigations (Penzkofer et al. 2014a; Heuberger and Penzkofer 2017).

In order to generate a higher castration rate, the hot water emasculation technique was developed in grain and rice breeding and successfully applied in further crops (Mukasa et al. 2007; Tong and Yoshida 2008; Otsuka et al. 2010; Hussain et al. 2012; Stetter et al. 2016). Valerian shows no or only a slight difference in the temperature sensitivity between male and female flower parts, so that the hot water emasculation could not be successfully initiated. The treated flower shoots died (>40–45 °C), or fertile pollen was developed (<35–40 °C) (Kempf 1986). Kempf (1986) described also a chemical castration with flurenol (9-hydroxy-9-fluorocarboxylic acid), a morphactin, which inhibits or modifies the growth and formation of higher plants already at very low concentrations. Thereby, among other things, the formation of the carpel and stamen will be affected. However, flurenol reduced the germination rate of the received seeds. It should be noted that the use of gametocides is not allowed in every country and also not in the context of organic cultivation.

Male sterility can support cross-pollination. Shugaeva (1979) reported from valerian inflorescences with completely reduced anthers and total pollen sterility. Furthermore, plants were described that contain normally developed androgynous flowers as well as flowers with male sterility at the same time. The ratio of the two

types of flowers varied from plant to plant and changes during the process of flowering (Shugaeva 1979). However, the simultaneous appearance of these two types of flowers seems to be an exception. Inheritance studies of Shugaeva (1979), where the progeny of male sterile plants were studied after free cross-pollination with fertile plants, show that the male sterility was maintained in the offspring. In the first generation, on average 44% of the descendants were male sterile and in the second generation still 35%. The reduction of the anthers was based on the degeneration of the tapetal and sporogenic cells. Like Shugaeva (1979) observed, the degeneration occurred in early stages of the premeiotic and meiotic period. The female organs of male sterile plants were not concerned and developed without deviation so that female fertility was preserved. In valerian, male sterility is presumably based on a combination of heritable sterility, shown in the preservation of male sterility in following generations and the influence of low temperatures in the flower bud stage (Shugaeva 1979). In order to use male sterility during breeding and in seed production, it is necessary that the inducing principle for male sterility (genomic or cytoplasmic) is known and is not manipulated by non-controllable external influences.

Prohibition or Restriction of Pollen Transfer Between Different Geno- and Phenotypes

The prohibition or restriction of pollen transfer between different geno- and phenotypes is the second aspect of pollination control. Isolation means that the transfer of pollen between different plants is prevented. Heeger's (1956) descriptions lead to the assumption that valerian is predominantly pollinated by insects. Entomological studies showed that honey bees (*Apis* sp. L.), mining bees (*Andrena* sp. Fabr.) and syrphid flies (*Eristalis* sp. Latr.) are often found in valerian inflorescences. Therefore, the main task of isolation is to lock out the vectors, because pollination takes place in the flight radius of the vectors and is additionally promoted by wind.

The classic proceeding is the isolation of one valerian plant or inflorescence. This is done predominantly for developing homogeneous descendants (inbred lines) (Vömel and Hölzl 1979; Konon and Novikova 1981). Of course, also more plants with the same (clones) or similar (populations) genetical configuration can be isolated together. The appropriate isolation technique for single plants or shoots is the isolation of inflorescences shortly before flowering with glassine bags. The valerian pollen grain sizes lie between 26 and 80 µm and depend on the degree of polyploidy (Weberling and Bittrich 2016; Halbritter 2016; Wetzler 2010). Other materials, like textile bags or perforated plastic (Crispac-) bags, are not sufficiently pollen-proof compared to glassine bags (Heuberger and Penzkofer 2017). Many isolations can be performed in a greenhouse at the same time (Fig. 19.10). The insertion of blowflies supports the pollination and rises up the amount of seeds.

Depending on the purpose of isolation, also for more than one plant, and for plants with different genetic configuration, isolation techniques can be applied. The isolation of two inflorescences of different plants promotes the intended crossing since a high outcrossing rate can be assumed, like Penzkofer et al. (2018b) reported. The procedure is similar to the isolation of one inflorescence, only that now two

Fig. 19.10 Inflorescences of valerian individually isolated with glassine bags in a greenhouse

inflorescences are taken together in one isolation bag. It is important that both combining partners show an equal flowering stage to prevent an imbalance of foreign flowers and pollen. If both combining partners are similarly fertile, the seeds formed are a mixture of seeds originated from reciprocal crossings and of inbreeding. Ideally, the inbred seeds do not exist or are quantitatively negligible. The determination of a maternal effect is difficult to realize, because the inflorescences quite tangle with each other and the seeds can usually not be attributed to the respective combining partner. Otherwise, an exact knowledge of different seed parameters (e.g. weight, Size and shape) of the combining partners must be on hand to develop seed separation techniques.

If it is desired to differentiate the combining partners, the use of isolation cabins allows the access and the processing of single plants. The isolation cabins are with gauze-covered frames and can be placed in greenhouses or, as seen in Fig. 19.11, outdoor.

For a good plant health, the gauze should be highest possible transparent, breathable and permeable to moisture. Therefore, the mesh size should not be smaller than the valerian pollen grain which varies between 26 and 80 µm depending on the degree of polyploidy (Weberling and Bittrich 2016; Halbritter 2016; Wetzler 2010). As gauze does not provide a pollen proof isolation, additional measures are necessary. One option is the use of overpressure, in which pollen-free air is pressed from the outside into the cabin. Another option is to rise up the concentration of the pollen inside the cabin by inserting blowflies, because their movement promotes pollen shedding from plants. A large distance between the cabins is also conducive. There are no systematic studies on how far the valerian pollen is transported. From practical breeding experiences, 100–150 m should be sufficient, if the cabins are not

Fig. 19.11 Blooming valerian in an isolation cabin in the field

placed in main wind direction. If the distance increases further (over 300 m) and a structural hurdle (house, hedge) is placed in between, the cabins can be dispensed. Such isolation sites are often used for commercial seed production.

Manipulation of the Ploidy Level

The naturally present different cytotypes in valerian (see Sect. 19.1.3) provide possibilities of creation of new variability and new plant traits. Plant breeders typically try to cross tetraploid and diploid plants to create triploid plants (Otto et al. 2015). Triploidy often leads to non-viable gametes and sterility by distorting effects on meiosis (Otto and Whitton 2000). Furthermore, the development of a polyploid series (e.g. from 1× to 8×) can provide information on whether the performance of valerian can be improved with increasing numbers of genomes. Due to the fact that the valerian accessions with different ploidy levels also differ in their genetical composition (Heuberger et al. 2012b), a polyploid series of valerian must base on the same haploid (monoploid) plant.

In principle, two procedures are necessary to implement the creation of triploids and the polyploid series: crossings between different ploidy levels and reduction and doubling of the ploidy level/development of haploids (monoploids) and double haploids. Presently, none of the mentioned procedures are really established. However, attempts have been made to this, which will be briefly described below.

Crossings Between Different Ploidy Levels

Penzkofer et al. (2014a) reported from reciprocal crosses between di-, tetra- and octoploid origins of valerian. Cross-pollination was carried out by emasculation (castration) and manual pollen transfer. The inflorescences were protected against foreign pollen by isolation. The ploidy level of the descendants was determined using flow cytometry and microscopic chromosome counting.

Not all descendants showed the cytotype, which would be expected due to the ploidy of the crossing parents. In addition, within some crossing offspring, different ploidy levels were detected. Varying ploidy levels within the mother plants could be excluded. The observed results may be caused by distorting effects during meiosis or through incomplete emasculation, which enabled self-pollination. As long as these two points cannot be effectively controlled, crossings between different ploidy levels are only conditionally applicable.

Reduction and Doubling of the Ploidy Level

Reduction and doubling (polyploidization) of the chromosome sets, also called double-haploid technique, are biotechnological procedures that are also applied in breeding of medicinal plants (Ferrie 2009). A reduction to a monoploid (usually a haploid) with a subsequent doubling leads to homozygous plant material. This way to create homozygous plant material is alternative to the continuous inbreeding and is often preplaced by hybrid breeding. For tetraploid valerians, two reduction steps and two doubling steps would be necessary each, to create full homozygosity.

In gametes, the ploidy level is naturally reduced by half. Therefore, from early gamete stages in the androecium and gynoecium, plants with reduced ploidy level can be generated. Using microspores, unripe anthers and embryo rescue are common techniques. Several times, it was tried to develop viable haploid valerian plant material. Microspores were extracted and plantlets could be regenerated in some cases, but they were not haploids (Bal and Touraev 2009; Nietsch 2010; Göttl 2011). Rather, somatic cells of the mother plant developed to the new plantlets, or, less likely, a spontaneous doubling of chromosomes occurred. Moreover, an embryo rescue after pollination with pollen of corn salad (*Valerianella locusta* L.) and elderberry (*Sambucus nigra* L.) was also less successful (Heuberger et al. 2012c).

Presuming a successful reduction, the original ploidy level will be usually restored. Moreover, doubling the chromosome sets may be performed from higher ploidy levels. The doubling of the chromosome sets is possible by using colchicine. Heuberger et al. (2012c) determined a colchicine concentration of 0.1% for young plants and 0.4% for seeds, which is in minimum necessary for chromosome doubling. Increasing the colchicine concentration up to 0.6%, the portion of plants with a doubled chromosome set was increased. However, it must be ensured that all cells, which are able to divide, are being treated and further; it must be also controlled that, in the next generation, the generated plants bear a doubled set of chromosomes.

Selection

Selection presents the most important tool during breeding. The response to selection depends usually on the selection intensity, the variability of the parental population and the heritability (genotype-environment interaction).

The selection intensity depends on the possibilities of the breeding work and the existing (amount) plant material. A high genetic variability exists among populations and can also be observed within most valerian populations. The finding of plant material with wanted characteristics should not be problematic. However, valerian shows a genotype-environment interaction for some important characteristics (Heuberger et al. 2012c). This study and further own observations of the authors show an interaction of the secondary compounds and the morphological root structure, with the year of cultivation and the local condition. The local condition is probably mostly defined by the soil conditions affecting the soil structure and water availability. Therefore, it is absolutely necessary to select under target environmental conditions and to review new varieties in different locations before commercial launch.

A challenge during each selection process is to recognize the appropriate plant material as soon as possible and to receive the plant material nondestructively and without loss. If the performance of the valerian should be determined in the usual field cultivation system, the root system being the most important plant part is harvested in autumn of the first cultivation year. Visual evaluation of the root system, yield measurements and chemical analyses will follow. Hence, just one selection step can be conducted in 1 year, and the entire selection process will be inevitably stretched.

Genotypic Selection

In general, selection can be based on genotypic and/or phenotypic properties. However, genome-based selection does not yet play a role in valerian breeding. The knowledge of the structure, the function and the evolution of genomes has developed further in the past, but up to now, the techniques of genome analysis, especially marker-assisted selection, have not yet been applied for valerian breeding.

Phenotypic Selection

Selection for Morphological and Agro-economic Criteria

The possibilities of phenotypical selection in valerian range from morphological characteristics of the root system (e.g. root structure), the aboveground plant parts (texture and habitus) and the inflorescence (e.g. flower time and range). The most important agro-economic parameters are root and seed yield.

Usually, the above-shown characteristics will be examined during or after field cultivation. Usually, cultivation takes 1 year. To abbreviate a selection step, correla-

tions of simple and early detecting morphological characteristics with breeding targets would be helpful (indirect selection) (Bernath 1997). However, no significant evidence for clear correlations between morphological or early observable characteristics exist for valerian (data from Noller (1989), described by Bernath (1997)).

Many properties could be also viewed from potted plants in the greenhouse, but due to the different cultivation conditions, a transfer of the results to other environments requires detailed correlation studies.

Selection for Analytical Criteria

The analytical selection applies predominantly to the secondary compounds indicated in the pharmacopoeias (Ph.Eur.9.1 2017; USP41 2017). The analytical methods and procedures are described there (e.g. water distillation, thin-layer chromatography and liquid chromatography (HPLC)).

The analysis of the secondary compounds can be done for single plants or for a group of plants based on a mixed sample. If the plant material is needed for further breeding steps, the root system can be divided into halves (through the rhizome). One section will be analyzed; the other section will be cultivated and used for further breeding steps. The secondary compounds are not equally distributed in the entire root system (Penzkofer et al. 2014b) (Fig. 19.12); therefore, the mass proportion of the analyzed components (rhizome, adventitious roots, lateral roots) should be the same in the two sections.

The detection of secondary compounds in young plants could be useful to accelerate the breeding process. Bohr et al. (1996) investigated and compared the secondary compound contents of in vitro cultivated valerian seedlings and of their generated clone lines in the field. However, a reliable conclusion for this work was not possible.

The contents of secondary compounds are very variable traits, which are influenced by the genetic constitution, as well as by the environmental conditions, the stage of plant development (ontogenesis) and the processing of the plant organ until analysis. The different secondary compounds of valerian do not behave in similar ways. According to Hörner (1989) and Bos et al. (1998), the content of essential oil increases until the end of the vegetative phase and decreases with the development of the generative plant parts. The content of valerenic acid reaches the highest values in or just before of the flowering period (Noller 1989; Bos et al. 1998). Bernath (1997) discusses the influence of growth factors (light, temperature, water requirement, soil conditions, nutrition) on the secondary compound production and describes quite different results. In studies of the authors, no genotype by location interaction was found when cultivation and processing techniques were the same at all locations. In contrast, a genotype by year interaction was detected for the sesquiterpene content.

Therefore, cultivation, harvest and processing conditions during breeding should be quite similar to the common cultivation conditions.

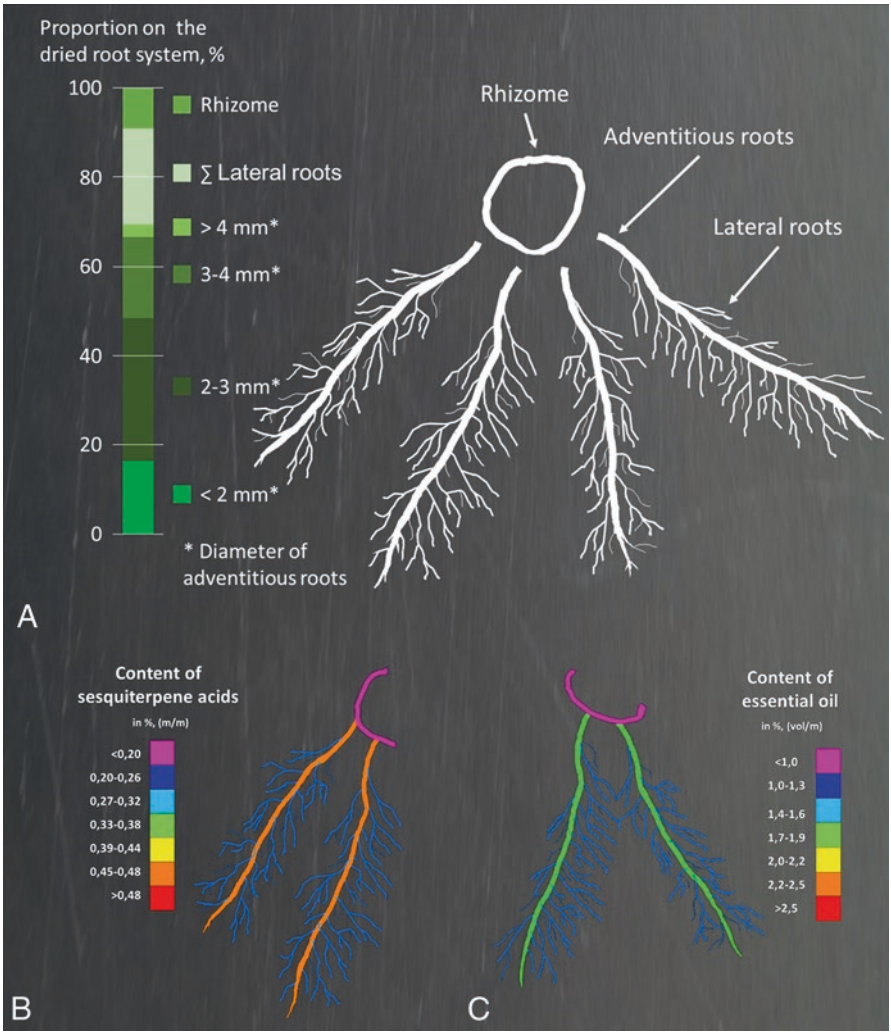

Fig. 19.12 (**a**) Components of the valerian root system and the mass proportion of the different parts within the root system. Distribution of (**b**) sesquiterpene acid contents and (**c**) essential oil contents within the root system. (Data source: Penzkofer et al. (2014b))

19.3.3.2 Breeding Methods

Method Using Self-Pollination Strategy (Inbreeding)

Inbreeding means the recurrent pollination with own pollen (self-pollination). The principle techniques are shown in Sect. "Creation of New Variability". The main aim of inbreeding is to create higher homozygosity in plant material. The fully inbred plant material shows no longer allele variation, and all or nearly all loci are homozygous. The gene combinations are fixed and can be identically reproduced.

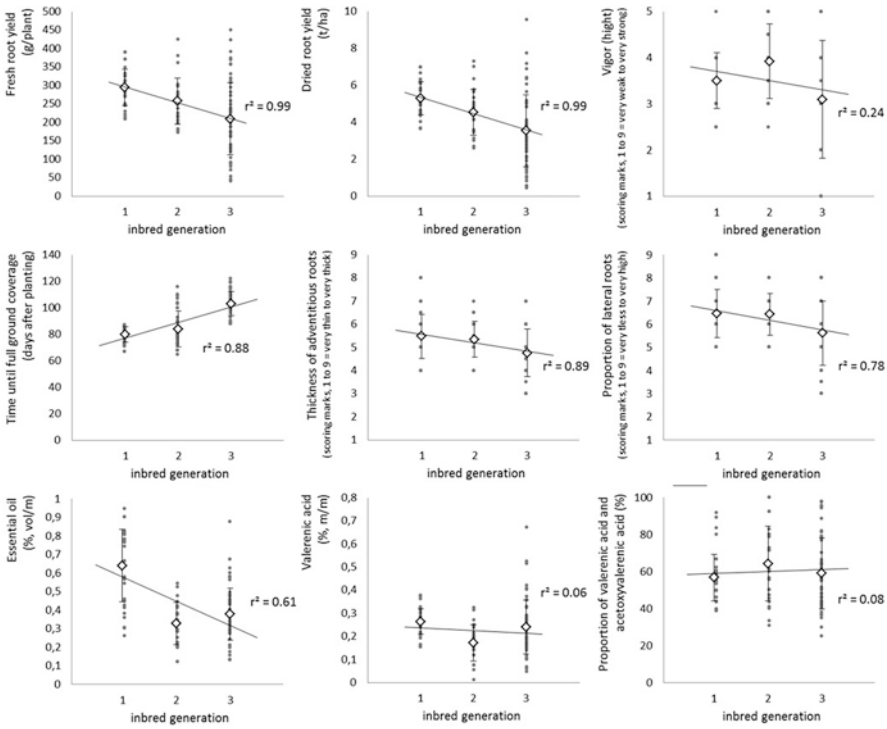

Fig. 19.13 Development of the performance of the fresh root yield, dried root yield, vigor (top, left to right), time until full ground coverage is achieved, thickness of the adventitious roots, proportion of the lateral roots (middle, left to right), content of essential oil and valerenic acid, as well as the proportion of valerenic acid and acetoxyvalerenic acid (bottom, left to right) from the first to the third inbred generation of valerian. Each data point is shown (grey dots) that was used for calculation the mean value (white rhombus). The vertical lines represent the standard deviation. The diagonal line represents the regression line and r^2 the coefficient of determination of the mean values

Already from the first inbred generation, inbreeding depression occurs in valerian and is enforced with increasing inbreeding levels (Fig. 19.13). The inbreeding effect on vitality-indicating characteristics, like the longer time until full ground coverage, the reduced vigor (plant height) or the lower seed yield (not shown), illustrates that more than three inbreeding steps are difficult to realise and often lead to non-viable plant material.

The shown data originated from a breeding program (Penzkofer and Heuberger 2018). This means that only the best inbred lines, with good prospects to reach the breeding targets, have been transferred into the next inbred generation. Therefore, the visible inbred depression is certainly less strongly developed compared to inbreeding without any selection.

The majority of cultivated valerian is tetraploid. Compared to diploids, it is more difficult to identify homozygous plants and to determine the frequency of dominant alleles due to five possible allele combinations, from nulliplex (aaaa) to simplex (Aaaa) to quadruplex (AAAA) (Schmalz 1989; Stoskopf et al. 1993). In addition, it takes longer time to reach full homozygosity by classic inbreeding steps. In diploids, the theoretical portion of 97% of homozygous dominant alleles would be reached in the sixth inbred generation. In contrast, in the case of tetraploids like valerian, such a level of homozygosity is statistically reached after the 21st generation starting from a duplex genotype or after the 19th generation starting from a triplex genotype. Considering the high inbreeding depression, shown from the third inbred generation, reaching full homozygosity by classic inbreeding is not possible for valerian.

Despite all challenges, different generations of inbred lines of valerian could be developed (Konon and Novikova 1981; Kempf 1986). Penzkofer and Heuberger (2018) describe two selection strategies, which can be applied during inbred line development:

The selective selection strategy: Each inbred generation will be tested in the field, and single plants (elites), which correspond with the breeding target, will be selected only from the best inbred lines. The elites are the mother plants, on which the next inbred generation will be generated by selfing. The performance of these inbred lines and elites is well characterized, but each inbreeding generation lasts for 2 years as the selective selection strategy goes along with the natural life cycle.

The rapid selection strategy: In this selection strategy, after harvesting the inbred seeds, they are immediately sown and the plant material is cultivated in a greenhouse; the generative phase will be induced by an artificial vernalization in cooling chambers, and the plants are returned for blooming and selfing into the greenhouse. This concept has the advantage that one inbred generation per year can be generated. However, the inbred lines and especially the mother plants on which the next inbred generation are generated were not tested in field and their performance is not known except for general vitality.

For practical breeding, to save time and to avoid an exponentially rising number of lines, a mixture of both selection strategies is feasible. At one time, the field performance of the inbred lines should be tested in the field. This can be done between two inbred generations or parallel to the development of the next generation. Otherwise, there is the risk that inbred lines will be developed and continued, which do not show the desired breeding targets.

Methods Using Cross-pollination Strategies (Population Breeding, Crossbreeding, Hybrids and Synthetics)

Due to the high tendency for natural cross-pollination (xenogamy), methods of population breeding with open pollination are applied for valerian. The simplest method for valerian seems to be mass selection, followed by seed production by open pol-

lination. For Germany, this is documented since the end of the eighteenth century (Heeger 1942).

Selection of individuals is still an appropriate method for starting new breeding programs of valerian (Heuberger et al. 2012c). The selection can be done before flowering or after flowering, with different emphasis of the criterions (Table 19.4). Criterions, assessable before flowering time, are most important for growers, whereas after- or during-flowering criterions are important for seed producers and the breeder. Some more details concerning the criterions are given in the following chapters (Sect. 19.3.4). A selection before flowering is useful to reduce the genotypic frequency of recessive-negative alleles (characteristics). The pollen of such individuals is no longer available for pollination.

A two-step selection is feasible. At first, individuals are selected before flowering according to the agronomic and root quality criterions. The half-root technique described in Sect. "Selection for Analytical Criteria" allows chemical component analysis and further breeding with the identical plant. Secondly, during or after flowering, the performance of these selected individuals in the generative phase can be considered.

Using a simpler method without analyzing chemical constituents on a single plant base, some growers could develop their own varieties by selecting for agronomic traits during the cultivation. Such selection steps were done recurrently until the desired agronomic characteristic became genetically fixed. The maintenance breeding of such varieties is very important to hinder a performance drop in the next generation. At the same time, the content of essential oil and of valerenic acids must be controlled at population level.

Targeted crossbreeding was used for valerian breeding to combine different characteristics, which are missing in one parent each (Heuberger et al. 2012c). The used crossing techniques are described in Sect. "Control of Pollen Transfer". Usually, the newly created plant material represents the base for further breeding steps (e.g. F_1–F_2 or selection of single plants for new crosses or inbreeding).

According to undocumented evidence from the practical breeding, through crossing between siblings of the same inbred line, with similar and breeding-aim-conform characteristics, a re-vitalization of the viability and the performance of

Table 19.4 Selection criteria for valerian and their time of selection with respect to flowering. The consequences of the selection during a special period are shown in the text

Criterions (examples) which could be assessed:	Before flowering	After/during flowering
Content or special compilation of secondary compounds	•	
Root yield	•	
Special root morphology aspects	•	
Homogeneity (as defined by the plant varieties protection law)	•	•
Disease and pest tolerance	•	•
Flowering and pollination behavior		•
Seed formation and seed yield		•

depressed inbred lines can be realized. This may be explained by the fact that classic inbreeding practically never leads to homozygosity and a crossing can attenuate the inbred depression. A full recombination cannot occur, because the genetic background of the crossing partners is the same, especially if inbreeding and crossing steps have been repeated several times. An example of possible re-vitalization is given in Table 19.5 showing the combination of plants within the inbred line C. However, not every inbred line and every characteristic behaves in this way.

Ideally, each individual combining partner and the resulting combination (offspring) show a good performance. For valerian, comprehensive hybrid performance and combining ability tests were not yet executed. Penzkofer and Heuberger (2018) investigated the offspring (F_1) of four crossed inbred lines (I_3) (Table 19.5). The crossings were performed on the base of single plants. Inbred line A and inbred line B originate from the same I_2 inbred line.

The examined progenies showed different values of negative and positive mid-parent heterosis (MPH), both between the inbred lines and between the characteristics (Table 19.5). In sum, the crossings between different inbred lines exhibit a higher MPH than crossings of the same inbred lines. This reflects the low degree of homozygosity that can be achieved after three cycles of inbreeding of the tetraploid valerian.

The combining ability of the inbred lines (general combining ability, GCA, Table 19.6) or of the single combinations, where single plants acted as combining partners (specific combining ability, SCA, Table 19.7), applies as benchmark for a successful development of a hybrid variety. The inbred lines A and B show in sum a negative and the inbred lines C and D a positive GCA. This is less amazing, because of the same origin of the inbred lines A and B.

The breeding lines of Penzkofer and Heuberger (2018) did not exhibit SCA or GCA for the plant trait “thickness of adventitous roots”. This was not expected as thick roots were the main breeding aim of their project. Caused by the consequent selection towards thick-rooted inbred lines, the root thickness rating of the F_1-offspring was maybe too imprecise, because of the resemblance of the offspring, or the root thickness cannot be further increased by crossing.

Manual crosses at valerian are not really practical to produce a sufficient amount of crossing seeds, and the use of gametocides seems not to be acceptable for medicinal plants. Male sterility systems were observed, but not sufficiently developed for breeding application. Therefore, other concepts were developed (Penzkofer et al. 2016). Due to the high tendency for natural cross-pollination (xenogamy), high inbred parental hybrid components should produce hybrid seeds under open pollination conditions. Certainly, the harvested seed lot will contain a fraction of inbred seeds. This fraction must be kept at a tolerable amount. Inbred seeds usually show lower vitality and a reduced growth rate (Bernath 1997; Penzkofer and Heuberger 2018) (Fig. 19.13) and will be either suppressed or compensated by the hybrid plants in the established field crop. In the introduced hybrid breeding concept, or in case of more than two crossing components (synthetic variety), the main aim is the

Table 19.5 Mid-parent heterosis (MPH) in % of nine important breeding characteristics from crossings of four inbred lines (I_3, A–D) of valerian

Combining partner (inbred line)		Thickness of adventitious roots	Proportion of lateral roots	Dried root yield	Time until full ground coverage	Valerenic acid	Essential oil	Proportion of valerenic acid and acetoxyvalerenic acid	∑
No. 1	No.2								
A	A	21.4%	−27.3%	−20.8%	−2.8%	−1.9%	−18.2%	−10.3%	−59.8
A	B	3.0%	−15.6%	−5.4%	0.7%	44.1%	8.4%	−17.5%	17.7
A	C	−5.7%	8.3%	29.0%	10.2%	7.3%	7.8%	8.6%	65.6
A	D	9.1%	9.5%	49.0%	6.2%	26.4%	−6.0%	−3.5%	90.8
B	B	5.3%	−8.7%	−10.8%	−6.2%	38.2%	−5.4%	−19.2%	−6.9
B	C	−1.3%	16.2%	70.6%	18.9%	12.1%	17.6%	−12.6%	121.6
B	D	−6.1%	−2.3%	38.4%	5.2%	4.8%	−3.6%	−16.2%	20.1
C	C	−23.8%	50.0%	17.0%	8.9%	−19.0%	14.4%	0.5%	48.1
C	D	−7.5%	23.5%	29.7%	12.8%	−34.1%	−20.6%	−17.5%	−13.6
D	D	−10.5%	−20.0%	−27.5%	−21.4%	6.8%	21.3%	−20.0%	−71.3

Table 19.6 General combining ability (GCA) of nine important breeding characteristics of four inbred lines (I_3, A–D)

Combining partner (inbred line)	Thickness of adventitious roots	Proportion of lateral roots	Dried root yield	Time until full ground coverage	Valerenic acid	Essential oil	Proportion of valerenic acid and acetoxyvalerenic acid	$\sum$
A	−0.219	−0.4	−3.538	−1.875	−0.019	−0.007	−0.004	−2.3
B	0.386	0.267	−7.926	0.908	−0.029	−0.047	−0.071	−8.3
C	−0.136	0.3	2.773	1.142	0.001	0.032	0.167	2.0
D	−0.031	−0.167	8.692	0.175	0.047	0.023	−0.092	8.6
$\sum$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
p-value	0.159	0.025*	<0.001***	0.189	<0.001***	0.027*	<0.001***	

GCA and *p*-values determined according to Griffing (1956)
Signif. codes: < 0.001 '***', < 0.01 '**', < 0.05 '*'

Table 19.7 Specific combining ability (SCA) of nine important breeding characteristics from crossings of four inbred lines (I_3, A–D)

Combining partner (inbred line)		Thickness of adventitious roots	Proportion of lateral roots	Dried root yield	Time until full ground coverage	Valerenic acid	Essential oil	Proportion of valerenic acid and acetoxyvalerenic acid	∑
No. 1	No.2								
A	A	0.186	−0.5	−12.295	−2.425	−0.021	−0.035	−0.049	−10.3
A	B	−0.419	−0.167	−5.717	−0.542	0.014	0.059	−0.024	−5.7
A	C	−0.064	−0.033	4.401	0.725	0.022	0.033	0.058	3.7
A	D	0.331	1.6	29.445	6.542	0.025	−0.015	0.068	24.9
B	B	−0.025	−0.167	−10.779	−5.825	0.001	−0.015	0.04	−5.1
B	C	0.414	−0.033	18.83	6.192	0.025	0.043	−0.043	13.0
B	D	−0.331	0.267	16.372	5.092	−0.013	−0.024	0.057	11.2
C	C	−0.314	−0.233	−14.609	−6.458	0.005	0.01	−0.047	−8.7
C	D	0.414	0.233	3.213	4.858	−0.059	−0.129	−0.088	−1.3
D	D	−0.192	−0.967	−28.861	−8.158	0	0.072	0.028	−21.8
	∑	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	p-value	0.489	0.003**	<0.001***	<0.001***	<0.001***	0.015*	0.037*	

SCA and *p*-values determined according to Griffing (1956)
Signif. codes: < 0.001 '***', < 0.01 '**', < 0.05 '*'

genetically identical reproducibility of the variety seeds by using vegetative propagated (cloned) and preserved crossing components. However, information of the development of synthetic varieties of *Valeriana officinalis* L. s.l. does not exist.

19.3.4 Breeding Targets

The breeding targets for valerian have changed several times in the past years. The breeding targets are always to be considered in connection with the state of the existing breeding and cultivation techniques and the re-evaluation of the secondary compounds.

19.3.4.1 Yield (Roots)

The root yield is an important aspect for the economic success of the valerian root production and one of the most important factors for the grower. Therefore, during the breeding process, it must always be paid attention to root yield, even if the root yield is not the prior selection aim. Bernath (1997) reported that root productivity was a subject in the beginning of breeding in the early 1930s.

19.3.4.2 Yield (Secondary Compounds)

Like other medicinal plants, also valerian acts as supplier for pharmacologically usable secondary compounds. If valerian will be used medicinally, the breeding/selection aim must be in accordance with the specifications of the commonly used pharmacopoeia (Ph.Eur.9.1 2017; USP41 2017). Especially the contents of essential oil and valerenic acids, including their derivatives and their ratios, must be considered. Further specifications concerning the secondary compounds can be agreed on by the breeder, grower and purchaser.

After the importance of valepotriates has reversed due to their mutagenic activity, the breeding of valepotriate-rich varieties was discontinued.

From a breeding point of view, the high phenotypical and genetic variabilities, which is observed in the different valerian accessions, provide a good base for selection and fixing higher contents of secondary compounds during the development of new varieties (Bernath 1997).

19.3.4.3 Breeding for Optimizing Certain Plant Secondary Compounds

Through intensive research, the understanding regarding the pharmacological activity of substances, appearing in valerian, has changed repeatedly, or new findings were added. Beside valerenic acid, also acetoxyvalerenic acid binds at the GABA

receptors but without anxiolytic effect (Felgentreff et al. 2012). Thus, the potential of valerenic acid is inhibited by acetoxyvalerenic acid.

Within valerian populations (accessions and varieties), single plants or groups of plants can be found with a proportion of more than 90% valerenic acid with respect to the sum of valerenic and acetoxyvalerenic acid (Stahn and Bomme 1998; Heuberger et al. 2012c). Through a combination of single plant selection and the creation of inbred lines, this high proportion of valerenic acid can be fixed in plant material (Junghanns et al. 2017; Penzkofer and Heuberger 2018). It is helpful for breeding that the characteristic proportion of valerenic acid shows a lower sensitivity to inbreeding depression.

19.3.4.4 Resistance

Valerian is considered as a very healthy and robust crop so that an intense resistance breeding was less relevant so far. During the breeding process, plants prone to diseases (e.g. against powdery mildew) can be suspended from further breeding through simple negative selection. Penzkofer and Heuberger (2018) observed strong powdery mildew occurence in each generation of a sensitive inbred line. This suggests that the powdery mildew sensitivity is a heritable characteristic.

19.3.4.5 Adaptation to Cultivation Processes

In contrast to other medicinal crops, the cultivation of valerian is rather uncomplicated, predominantly well investigated and well established. Breeding works for plant characteristics relevant during early cultivation, and early processing steps (seed production, crop establishment and field cultivation) were done, if at all, just as secondary aspects.

More attention should be given to the root harvest, cleaning and drying, because these are cost-intensive processes, and quality losses mostly occur there. The very finely structured and very strong interweaved carpet-like matted network of the root system is deep-seated in the ground and must be harvested and processed with considerable expenditure. A high mechanical strain on the root system, in which roots can break, can lead to a decrease of root yield. Furthermore, adherent soil must be washed out intensively resulting in losses of secondary compounds. Valerian varieties with thicker and less branched root systems, in combination with a high root yield and high content of secondary compounds, would counteract (Fig. 19.14) (Heuberger et al. 2012c; Heuberger and Penzkofer 2017; Penzkofer and Heuberger 2018). In addition, varieties with smaller rhizomes would be beneficial for faster and less energy-consuming drying.

Fig. 19.14 Two different valerian root system structures and the difference in soil mass that is still adherent after mechanical harvesting. Left: Fine structured and very strong interweaved carpet-like matted network root system: a larger amount of soil is hold in the root system. Right: Thicker and less branched root system: a lot of soil mass has been discharged

19.3.4.6 Breeding Results Achieved/Economical Transfer (Registered Cultivars/Patents, Trial Results)

For valerian, a large number of varieties (e.g. 'Anthos' (later 'Anton'), 'Lubelski', 'Polka' and 'Trazalyt') have been bred and developed, especially in Central and Eastern Germany and in Eastern Europe (Heuberger et al. 2012a). Also known are the many valerian accessions, collected and tested by the Bavarian State Research Center for Agriculture and described with 'BLBP' numbers (Bomme et al. 1999; Heuberger et al. 2012b). However, most of these varieties are not readily available in the seed trade. In addition, the original characteristics of, for example, 'Anthos' and 'Polka', are not known anymore. Within the BLBP collection for valerian, there are nine accessions of 'Anthos' and 16 accessions of 'Polka' with highly varying properties within one varietal type. Just few varieties, 'Vival', 'Valeripharm' and 'Valerimed', have got the official plant variety protection and are registered in Germany (BSA 2017). These varieties of Swiss and German origin, respectively, are not available for the public, and no information is available on their development and breeding history. The restricted availability of breeding history information and of seeds is also given for the Dutch and French varieties.

Compared to other agricultural crops, valerian breeding is done just by a few companies or public research stations. Extensive breeding projects are rare. Much information exists from the German breeding project 'Breeding of valerian to improve production profitability and quality of the raw material within the scope of the joint research project to improve the international competitiveness of the German medicinal plants production', financed by the Federal Ministry of Food and Agriculture and a business consortium and realized by the Bavarian State Research Center for Agriculture (Heuberger et al. 2012c; Penzkofer and Heuberger 2018; Heuberger and Penzkofer 2017). Through selection and crossbreeding, a valerian variety with a coarser root system (Sect. 19.3.4.5) and reliable ingredient contents

has been developed. It will be promoted as Weila®riana (trademarked). In this breeding project, several basic breeding and research themes were investigated. Some results have been incorporated into this book chapter.

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Chapter 20
Zingiber officinale Roscoe: Ginger

Meenakshi Kumari, Manoj Kumar, and S. S. Solankey

The aromatic substances of vegetable origin used in food as preservatives and flavours are known as spices. Various plant parts such as fruits, seeds, flowers and bark have economic importance in human diet due to their peculiar flavouring properties based on their content of essential oils. Medicinal plants are generally known as "Chemical Goldmines" as they contain a multitude of natural chemicals, which exert beneficial bioactivity in humans and animals. Ginger is one of the most important spices, which is scientifically known as *Zingiber officinale.*

It is valued for its light yellow liquid (curcuma oil) with aromatic and persistent odour obtained from rhizomes. It is widely used in Ayurveda, Siddha, Chinese, Arabian, African, Caribbean and many other medicinal systems to cure a variety of diseases like pain, nausea, vomiting, asthma, cough, inflammation, dyspepsia, loss of appetite, palpitation, constipation and indigestion. The essential oil and oleoresin contributing to these properties are well known (Weiss 2002), and these ingredients are often extracted and exported.

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20.1 Taxonomy

Ginger (*Zingiber officinale* Rosc., Zingiberaceae) is one of the most important spices as well as medicinal crops of India, mainly cultivated in subtropical areas since many centuries and used at larger scale around the globe. The plant belongs to genus *Zingiber*, family Zingiberaceae, in which four other genera of economic interest can be found (*Alpinia*, *Amomum*, *Curcuma* and *Elettaria*).

The English botanist William Roscoe named the plant *Zingiber officinale* in 1807. The genus name is from the Greek word 'zingiberis', which is derived from the Sanskrit word 'shringavera', aptly meaning 'shaped like a deer's antlers', while 'officinale' pertains to the medicinal properties of the rhizomes (Elzebroek and Wind 2008). The genus *Zingiber* includes 80–90 (or even 150) species (Holttum 1951; Wolff et al. 1999; Ravindran et al. 2005).

Based on plant stature and yield ginger plants are classified into three groups viz., : (1) plants with small size with many tillers along with a small rhizome, (2) plants having medium size with an intermediate number of tillers and a medium-sized rhizome and (3) plants with large size and fewer tillers and which produce larger rhizomes (Ravindran et al. 2005).

20.2 Origin and Distribution

Ginger represents the long history of its cultivation in India and China and is supposed to be originating from Southeast Asia from where it was introduced to other parts of the world (Ravindran and Babu 2005). The exact information about the plant's origin is unavailable due to its long history of cultivation in these regions.

The species is found in its cultivated state and is not known in a wild state (Purseglove 1981a). A few other researchers explored it from Eastern Asia, Indo-Malayan region, Africa, America and Northern Australia where it is now distributed widely and used as spice for over 2000 years (Bartley and Jacobs 2000). Gagnepain (1908) described the Indochinese region, Myanmar, Cambodia, Laos and Vietnam among the least known hotspots of the family Zingiberaceae the latest comprehensive study being over a century old.

20.3 Cytology

Several workers reported the somatic chromosome number of ginger as n = 11 (Federov 1969; Ramachandran 1961; Omanakumari and Mathew 1985) The diploid chromosome number $2n = 22$ was displayed by all the cultivars. Application of colchicine at sprouting bud of ginger rhizome helped in development of autotetraploid ginger, $2n = 44$ (Ramachandran 1982; Ramachandran and Nair (1992a, b). The length of the diploid chromosomes ranged from 1.6 μm to 4.3 μm and had

median and submedian centromeres. Ramachandran and Nair (1992a, b) reported one or two associations of four chromosomes at the first metaphase in the case of diploid ginger, while a high frequency of quadrivalents at the first metaphase was observed in tetraploid ginger. At the first anaphase, both types represented bridge-fragment configuration. Diploid ginger showed a pollen fertility of 13%, whereas in the case of tetraploid, ginger fertility was 85%. The high sterility in diploid ginger is mostly due to heterozygosity for gross structural changes of chromosomes (Ramachandran and Nair 1992a, b). Structural hybridity involving interchanges and inversions was reported in *Zingiber officinale* (Ramachandran 1961).

20.4 Plant Description

Zingiber officinale is a monocotyledonous herb of the wet tropical region. It is a 2–4-foot-tall perennial herb with grass-like leaves up to a foot in length. It has an underground rhizome that is used for culinary and medicinal purposes (Kemper 1999). The underground parts contain several small, solid rhizomes, more often branched like a palm, but the shape of rhizomes mainly depends on soil texture of the site of cultivation. Rhizome grown in loose soil are more valuable due to their straight and undeformed nature as loose and friable soil provides less mechanical resistance in their development. The rhizomes are surrounded by small scales and bear several fines, fibrous roots, which branch frequently in the surface soil. Ginger plants having slender aerial stem, which raises up to 1 m height and is closely wrapped by sheathing leaf bases. The light green leaves arranged in an alternate manner are oblong with strongly pointed end, having around 15 cm length and 2 cm width with a pronounced mid-rib, and tend to be rolled upwards. Normally, the inflorescence is leafless but sometimes leafy which is a reproductive shoot about 30 cm long and appears directly from rhizomes. The emergence of flower in ginger plants depends upon growing conditions of that place. In some parts of the world, it produces flowers very rarely, while in other parts it flowers at a regular basis. When flowering, seeds are produced only occasionally. The inflorescence known as spike is about 6 cm long and has solitary flowers in the axils of greenish-yellow bracts. Pale yellow flowers have a short calyx tube and longer corolla tube (1.5–2.5 cm) which bursts at the mouth into three unequal, pointed lobes, the upper one bowed down as a hood over the anther. Ginger flowers have only a single functional stamen with a short filament, two distinct pollen sacs and a broad connective prolonged into a spur. A slender style passes between these two pollen sacs and is detained by them. The lower lip of the flower is known as labellum shaped by a large purple and yellow mottled staminode, which is merged to the corolla tube. It is thought that it is derived from three non-functional stamens. The inferior ovary is composed of three cells and consists of numerous ovules in axile placentation, but hardly develops as a fruit. If it develops into a fruit, the fruit is thin walled with three valved structures known as capsule, comprising numerous small, black, angled seeds.

20.5 Economical Uses

Ginger is safely used in medicine, pharmaceutical and food industries. The underground stem (rhizome) is the highly demanded trade product. The stimulating aroma and the pungent taste are the key features of ginger to make it an essential ingredient of most world cuisine and of the food processing industry. In western countries, ginger is used in gingerbread, biscuits, cakes, puddings, soups, pickles, beer and wine. The unique flavour property of ginger is basically the combination of pungency and aromatic essential oil. The aroma of ginger is due to 1 to 3% of volatile oils with the main compounds bisabolene, zingiberene and zingiberol, while pungency is due to the non-volatile gingerols, shogaols, paradols and zingerone (Dhanik et al. 2017).

The importance of ginger is considered in traditional Chinese, Indian and Japanese medicine for over 25 centuries (Castleman 2001). Ginger has several diverse medicinal uses and important to promote digestion and as an antiflatulent or carminative to reduce gas and bloating (Lewis and Elvin-Lewis 2003; Chevallier 2000). It also acts as an anti-inflammatory against rheumatic pain and arthritis (Altman and Marcussen 2001; Bliddal et al. 2000) but also against inflammation caused by gamma radiation (Abd El-Salam and Hassan 2017). Ginger is also reported to have possible antitumorigenic effects (McCann 2003). It has antiemetic properties used during pregnancy (Apariman et al. 2006). Ginger is recognized as a plant with a high content of antioxidative compounds by several researchers (Shobna and Naidu 2000; Halvorsen et al. 2002; Eleazu et al. 2012). The powder of ginger is as effective as ibuprofen in the management of postsurgical sequelae (Rayati et al. 2017).

20.6 Domestication

The plant was domesticated for the first time in Asia or India where it was cultivated in wet tropics of southern India or Asia since ancient time, having high rainfall and fairly high temperature, but commonly some shady places. Other than these, it is also cultivated in different regions of West Africa and in West Indies, Jamaica, producing the top-grade ginger of the world (Cobley and Steele 1995).

The precise and reliable history of ginger was briefly explained by Elzebroek and Wind (2008). The earliest recording of Chinese herbals gives an idea about ginger and confirms the use of ginger in culinary and medicinal practices of natives of Asian countries. The Greeks were also familiar with the ginger plant as it was cited by the Ancient Greek physician, botanist and apothecary Dioscorides (40–90 AD) in his works. Plinius Secundus (23–79 AD 79), a Roman writer, naturalist and philosopher, also explained the medicinal uses of ginger in his work, *Naturalis Historia*. During the ninth century, it was well recognized as a spice in

Germany and France. In the thirteenth century, Arabian traders brought ginger from India to East Africa. In the sixteenth century, Portuguese brought ginger to West Africa and started its cultivation. Later on, its cultivation was initiated in Mexico by a Spaniard, Francesco de Mendoza. The long period of domestication might have played a major role in the evolution of this crops' sterility, propagated solely vegetatively (Ravindran et al. 2005).

20.7 Valuable/Undesired Plant Secondary Compounds

The major valuable ingredient responsible for pungency is gingerol ([6]-gingerol) present in higher concentration, whereas other gingerols, such as [4]-, [8]-, [10]- and [12]-gingerol, are present in lower concentrations. At high temperature, these compounds are thermally unstable hence converted into shogaols, which impart a pungent and spicy-sweet fragrance (Wohlmuth et al. 2005). In dried ginger, gingerols are also transformed to the corresponding shogaols with faster rate, of which [6]-shogaol is the most common dehydration product (Ok and Jeong 2012).

The gingerols usually occur in oil cells of the rhizome as pungent yellow oil but can also form a crystalline solid with low melting point (Butt and Sultan 2011). The amount of active compounds is not uniform, and it may vary according to growing condition and cultivar (Gruenwald 2004). Sesquiterpene compounds such as bisabolene, zingiberene, zingiberol, sesquiphellandrene and curcurmene also occur in moderate quantity and contribute to the flavour of ginger (Kemper 1999).

20.8 Main Production Area

Indigenous to warm tropical climates, ginger is widely grown in Asia, Africa, India, Jamaica, Nigeria, Indonesia, Bangladesh, Thailand, the Philippines, Mexico and Hawaii (Evans 1989). It is also cultivated in Fiji, Brazil, Sierra Leone, Japan and Australia. Nigeria is identified as a top-ranking country for area of cultivation with a share of 56% of the total global area followed by India (24%), China (4.5%), Indonesia (3.4%) and Bangladesh (2.3%). Among the top producing countries, India ranked first with total biomass production of 33% followed by China (21%), Nigeria (13%) and Bangladesh (11%) in the world. It is grown in most of the Indian states. However, 65 per cent of country's total production is contributed by 15 states, namely, Andhra Pradesh, Andaman and Nicobar Islands, Assam, Karnataka, Odisha, Meghalaya, Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Bihar, Chhattisgarh, Madhya Pradesh, Maharashtra and Gujarat (Rajeev and Thomas 2015).

20.9 Breeding

Sakai et al. (1999) reported the broad range of pollination and breeding systems in ginger.

20.9.1 Flower and Pollination Biology

The corollas of ginger flowers are white in colour and each – normally bisexual – flower is enclosed by a bract (a leaf-like structure) (Purseglove et al. 1981b). But sometimes ginger also shows monoecious unisexual flowers (Peter et al. 2007). Three calyx types are present in the ginger flower; among these one is larger than the others and light yellow transparent, so that when the flowers begin to bloom, they seem to with a tinge of red, which, in fact, is the colour of the labellum protected by calyx. The labellum is pale yellow inside and dark red inside and mixed with yellow spots. When the flowers bloom, pistil stalk shaped curved edges touching the labellum. Bracts are arranged in a spiral manner and the inflorescence is known as spike which is a conical shape structure where flowers occur in clusters. The presence of a labellum (two or three fused stamens) which is joined with a pair of petal-like sterile stamen shows the close relationship of Zingiberaceae flower with orchids. The slender flower tubes are the source of nectar in ginger. The blooming of a bright flower occurs only for a few hours in the afternoon until late afternoon (01:00–05:00 pm), and this is pollination period for the single flower by insects (Melati-Palupi and Bermawie 2015). Endress (1994) also reported the mode of pollination in Zingiberaceae family by animals. Bees, hawk moths and birds were found to be the main pollinators (Ippolito and Armstrong 1993). The flower of large white ginger is less valuable for the production as it is mainly propagated by rhizome and because these flowers are not used for ornamental purpose. On the other side, under normal climatic condition, these plants flower very rarely, and even if they produce flowers, seed setting is very rare. The quantity of pollen grains germinated on stigma or self-incompatibility reaction decides the ratio of fertile pollen and non-fertile pollens (Ramachandran 1982). Large variations were observed in fertility of red ginger pollen (*Zingiber officinale* var. *rubrum*) which varies from 6 to 45 per cent (Rachman 1998). Peter et al. (2007) stated that the failure in fruit formation and seed setting may be influenced by several factors such as failure of pollination due to an insufficient number of pollinators. Melati-Palupi and Bermawie (2015) reported that flowering in ginger starts from 4 to 7 months after planting, depending upon climatic conditions. High temperature leads to early and more flowering, but normal temperature and humidity lead to flowering for a longer time.

20.9.2 Propagation Strategies

The vegetative part, the rhizome, is the main propagating material in ginger. The environmental as well as photoperiodic factors lead to failure of flower and seed set, as well as the high sterility among *Zingiber* flowers (Ravindran et al. 2005). For propagation purposes, healthy and larger-sized rhizomes should be harvested from disease-free plants. Disease-free and healthy clumps are identified in the field, when the crop is 6–8 months old and still green. The rhizomes for propagation should possess one or two good buds and weigh about 20–25 g each. They are cut into small pieces of 2.5–5.0 cm length. The propagation rhizomes should be stored carefully. In India, local practices such as spreading layers of leaves of *Glycosmis pentaphylla* are followed by farmers. Storing the propagation rhizomes in pits under shade results in higher germination. A convenient size pit can be made under shed to protect the rhizome from sun and rain. Cow dung is commonly used for pasting the pits' wall. Then, these pits can be used for storage of rhizome along with well-dried sand/saw dust (i.e. put one layer of seed rhizomes and then put 2-cm-thick layer of sand/saw dust). Proper aeration should be given to propagation rhizomes by leaving enough space at the top of the pits. Timely inspection, i.e. 20 days once inspection, is necessary to remove the shrivelled and disease-infected rhizomes (Rajeev and Thomas 2015). In planting, the weight of reproductive organs necessary depends on the area of cultivation and the method of cultivation adopted. In southern India, the weight varies from 1500 to 1800 kg/ha in plains, whereas, at higher altitude, it goes up to 2000 to 2500 kg/ha.

20.9.3 Breeding Methods Applied

In clonally propagated crops like ginger, the two important components of biodiversity are species diversity and varietal diversity which permits selection forces to act on it (Sasikumar et al. 1999). The evolution of this crop experienced a lot of changes in its physiological and anatomical structure due to the long history of domestication of gingers into diverse geographical niches. This variability was not so dominant in cultivars grown in the same region compared to the ones growing in different geographically distant locations (Ravindran et al. 2005). The presence of this genetic variability gives a chance for utilization in crop improvement and sustainable development.

The main purpose of crop improvement is to develop high-yielding varieties with wide adaptation, high-quality parameters (oil, oleoresins) and low fibre, besides resistance to major pest and diseases such as rhizome rot and shoot borer. Several methods such as introduction, selection, mutation, polyploidy breeding and also biotechnological approaches are being used for improving the ginger cultivars in India as well as other parts of world. Hybridization is not practicable in ginger due to sterility.

20.9.4 Selection

During the initial years of crop improvement, foremost importance was given for the collection of a large number of germplasm from different localities, their comparative yield evaluation and selection of superior types based on yield and quality traits. The yield potential and unique feature of rhizome vary according to existing factors in the particular region. The high yield potential and quality of the exotic cultivar 'Rio-de-Janeiro' were proven by various researchers (Kannan and Nair 1965; Thomas 1966; Muralidharan and Kamalam 1973). The yields of the cultivars 'Himachal', 'Kuruppampadi', 'China' and 'Maran' are analogous to 'Rio-de-Janeiro' (Jogi et al. 1978; Nybe et al. 1980; Mohanty et al. 1981; Thangaraj et al. 1983). The most popular varieties among farmers are 'Rio-de-Janeiro', 'Himachal Pradesh', 'Kuruppampadi', 'Maran', 'Nadia' and 'Burdwan'. The highest yield of variety 'Thingapuri' was observed in Odisha (Panigrahi and Patro 1985). The best quality character, i.e. the highest oleoresin content, was observed in 'Rio-de-Janeiro' and 'Maran', while the highest essential oil content was found in 'Karakkal'. The cultivars 'China' and 'Nadia' were the lowest in crude fibre content, whereas a higher fibre content was observed in 'Kuruppampadi', 'Maran', 'Jugijan', 'Ernad Manjeri', 'Nadia', 'Poona', 'Himachal Pradesh', 'Tura' and 'Arippa' (Jogi et al. 1978; Nybe et al.1980).

20.9.5 Mutation Breeding

In sterile vegetatively propagated plants, variability can be created by exposing the plant organs to physical and chemical mutagens. Once the induced variability is fixed, it can be maintained by vegetative propagation. Rattan (1994) reported that the use of ethyl methanesulfonate (EMS) as a chemical mutagen leads to reduced growth and increased cytological irregularities. Gamma rays also showed a similar effect as chemical mutagens (Rattan 1994). Jayachandran and Mohanachandran (1992) observed that most of the induced changes appearing in the R_1 generation were in chimeric form and expressed a stunted or semi-dwarfing effect and were inhibitory on production of rhizomes.

20.9.6 Polyploidy Breeding

Successful stable tetraploids in ginger with $2n = 44$ were developed by Ramachandran (1982) and Ramachandran and Nair (1992a, b) by treating the sprouts with 0.25 per cent aqueous colchicine. The polyploids have the features like vigour growth, larger rhizome size and early flowering than the diploid. However, in polyploid rhizomes, lower oil content was observed compared to diploid rhizomes.

20.10 Biotechnological Approaches

Limited variability in ginger genotypes is mainly due to absence of seed setting which hinders the crop improvement programmes. The diseases such as rhizome rot caused by *Pythium aphanidermatum* and bacterial wilt caused by *Ralstonia solanacearum* are the major production constraints in ginger cultivation. Use of biotechnological tool can be considered as boon for ginger improvement due to its wider application.

20.10.1 Micropropagation

Many workers reported the clonal multiplication of ginger from vegetative buds (Hosoki and Sagawa 1977; Nadgauda et al. 1980; Babu et al. 1997; Sharma and Singh 1997; Rout et al. 2001). Infected rhizomes are the main source of disease inoculum in ginger. Production of pathogen-free planting material of elite cultivar is possible by using tissue culture technique. Tissue-cultured plants required a minimum of two crop seasons for development of rhizomes with normal size that can be used as propagation rhizomes for commercial cultivation. Rout et al. (1998) reported genetic uniformity in micropropagated plants by molecular characterization. However, some percentage of polymorphism was reported by Babu et al. (2003).

20.10.2 In Vitro Pollination

There is no natural fruit or seed set in ginger due to sterile pollen grains and self-incompatibility reaction. Nevertheless, artificial supplementation of required chemicals and nutrient to young flowers along with in vitro pollination helps in the development of fruit and later on the plants that can be generated from these fruits (Babu et al. 1992b; Valsala et al. 1997). The successful application of in vitro pollination to overcome the pre-fertilization barriers for getting successful fruit set was reported by Nazeem et al. (1996).

Plant regeneration and somaclonal variation:

The use of leaf, vegetative bud, ovary and anther as explants for regeneration of plantlets through a callus phase has been reported by several workers (Babu et al. 1992a, 1996; Babu 1997; Kacker et al. 1993, Rout et al. (1998); Samsudeen 1996; Samsudeen et al. 2001). Somaclonal variations can be created by using this system which is not possible by conventional breeding due to failure of seed set. The variability in somaclones was reported for various agronomic characters and other yield traits, during field trial of these clones (Samsudeen 1996; Babu et al. 1996; Babu 1997). RAPD characterization of these somaclones also specified the profile variations representing genetic differences (Babu et al. 2003). Isolation of *Pythium-*

tolerant ginger by using culture filtrate as the selecting agent was described by Kulkarni et al. (1984).

20.10.3 Anther Culture

Anther callus obtained from diploid and tetraploid ginger can be used for plant regeneration (Samsudeen et al. 2001; Babu 1997). Ramachandran and Nair (1992a, b) observed the formation of callus and development of roots and rhizome-like structures from excised ginger anthers which were cultured on MS medium containing 2,4-D and coconut milk.

20.10.4 Microrhizomes

Many researchers reported the in vitro induction of microrhizomes in ginger (Bhat et al. 1994; Sharma and Singh 1995; Babu 1997; Sunitibala et al. 2001; Shirgurkar et al. 2001; Babu et al. 2003; Peter et al. 2002; Ravindran et al. 2004). The plants developed from microrhizome have lesser plant height but more tillers. The genetic stability occurs in the plants obtained from in vitro cultured rhizomes as compared to micropropagated plants and is considered as an important source of disease-free planting material ideally suited for germplasm exchange, transportation and conservation (Babu et al. 2003).

20.10.5 Protoplast Culture

Leaf tissues and cell suspension cultures of ginger could be used for isolation of protoplast. 2.5×10^5 protoplast per gram leaf sample can be obtained by digesting leaf tissue in an enzyme solution containing macerozyme R10 (0.5%), hemicellulase (3%) and cellulose Onozuka R10 (5%), when incubated for 10 hours at 15 °C followed by 6 hours at 30 °C. Out of this, 72 per cent of the protoplast were viable with a size of 0.39 mm. These viable protoplasts could be successfully plated on culture media and made to develop up to microcalli stage (Babu 1997; Geetha et al. 2000).

20.10.6 Genetic Transformation

The plasmid vector sp. AHC 25 and promoter Ubi-1 (maize ubiquitin) were used through bombardment, and the transient expression of GUS was effectively induced in ginger embryogenic callus tissue (Babu 1997).

20.11 Molecular Characterization

Sasikumar et al. (2004) analysed 96 accessions using RAPD profiling and interrelation ship studies. Moderate to low polymorphism was detected in ginger due to the vegetative mode of reproduction.

20.11.1 Candidate Genes

Candidate gene approach is more appropriate for ginger improvement due to the absence of sexual reproduction. A candidate gene for *Pythium* resistance was isolated by using primers designed from conserved motifs of similar resistance genes as well as a ddRT PCR approach. Cloning, sequencing and comparison of differently expressed fragments were done with the known sequences (BLAST searches) and putative resistance gene fragments identified. Cloning and characterization of a mannose-binding lectin from ginger rhizomes were reported by Chen et al. (2005).

20.12 Processing of Ginger

Production of dry ginger is done by adopting two peeling steps of ginger rhizomes to remove the outer skin and sun drying to a safe moisture level during its processing.

20.12.1 Peeling

Peeling is the process of removing scaly epidermis which helps in early drying. For peeling, bamboo splits with pointed end are used, and the outer skin of fully matured rhizomes is removed by scraping which leads to enhanced drying. Oil-bearing cells, which are present just below the outer skin of rhizomes, may be damaged during deep scraping with knife. Excessive peeling should be avoided, as it results in reduced essential oil recovery from dried product. Proper washing of peeled rhizome is necessary to avoid a deterioration of quality. A better quality of dry ginger is obtained from clean-peeled (smooth-textured) ginger which is also known as Jamaican ginger, whereas Indian ginger (especially that one grown in Kerala and most of the southern states) is roughly peeled (unbleached ginger) (Rajeev and Thomas 2015).

20.12.2 Drying

At the time of harvest, ginger contains a moisture content of about 80–82%, which is brought down up to 10% for its safe storage. Sun drying is more common in most of the developing countries which takes about 8–10 days for complete drying. Sun-dried ginger can be identified by brown colour appearance and irregular wrinkled surface. Type of cultivar and climatic conditions decide about dry ginger yield recovery which ranged from 19 to 25% (Rajeev and Thomas 2015).

20.12.3 Polishing, Cleaning and Grading

The removal of dry skin and wrinkles from the surface of ginger can be possible by polishing. It is the process of rubbing the peeled and dried rhizome against a hard surface. Manual cleaning of dried ginger is more common to remove the extraneous matter and the light pieces. After cleaning of dried ginger, grading is done based on size of the rhizome, its colour and shape and the extent of residual lime (in the case of bleached ginger) (Rajeev and Thomas 2015).

20.12.4 Storage

Insects like *Lasioderma serricorne* (cigarette beetle) are the major problems during storage of dry ginger in gunny bags. So airtight high-density polyethylene or similar packaging materials can be used for storage of dry ginger. Reduction in aroma, flavour and pungency of dried ginger occurs when it is stored for more than 2 years (Rajeev and Thomas 2015).

20.12.5 Bleached Ginger

Production of bleached ginger is done by plunging scrapped fresh ginger in a slurry of slaked lime, Ca $(OH)_2$ (1 kg of slaked lime/120 kg of water), followed by sun drying. After drying of adhering water from rhizome, it is again dipped in slurry. The same process is repeated until the rhizomes become uniformly white in colour. Bleaching of dry ginger is also done in a similar manner. Application of lime is essential for ginger as it gives better appearance and less susceptibility to the attack of insect pests during storage and shipping.

20.13 Important Varieties Evolved

20.13.1 IISR: Rejatha

This variety was released during the year 2001 from IISR Calicut by following the pedigree selection method. Plants have the height of 68 cm with green colour aerial shoots. Plumpy, round and bold rhizomes with three layered compact clumps are the main features of this variety. Rhizomes are brown in colour with a low fibre content and are rich in oil and oleoresin. It has an essential oil content of about 2.4% with dry recovery of 23% and 4% fibre content. Plants mature in 200 days and yield about 22.4 t/ha.

20.13.2 IISR: Mahima

This variety was released by adopting pedigree selection method during the year 2001 from plants that have green-coloured aerial shoots with a height of 65 cm. Rhizomes are brown in colour and plumpy and bold with low fibre content. The essential oil content yield is 1.7% with total dry recovery of 19% and fibre content of 3.3%. Plants mature in 200 days and have a yield capacity of 23.2 t/ha. This variety is resistant to root knot nematode.

20.13.3 ISR: Varada

This variety was released in the year 1996 by following pedigree selection method from IISR, Calicut. Plants have green-coloured aerial shoots with a plant height of 72 cm. Rhizomes have a bluish yellow core with reddish brown colour scale colour. It has good quality and a high yield with plumpy rhizomes having flattened fingers with low fibre content. Dry ginger is less prone to storage insect damage. Farmers are of opinion that Varada is tolerant to diseases. Plants are ready for harvest in 200 days and yield about 22.6 t/ha.

20.13.4 Suprabha

It is a clonal selection from Kunduli. Rhizomes are plump having a low fibre content (4.4%) with wide adaptability and suitable for both early and late sowing. Plants mature in 229 days and yield about 16.6 t/ha. This variety gives dry recovery of 21% with 8.9% oleoresin and 1.9% of essential oil content (AICRP on Spice, Calicut).

20.13.5 Suruchi

This is also a clonal selection from Kunduli. Plants have profuse tillers with a bold rhizome and early maturity. It is suitable for both rainfed and irrigated condition. Rhizomes are ready for harvest in 218 days with a yield of 11.6 t/ha. It gives total dry recovery of 24% with an oleoresin content of 10.9% and an oil content of 2.0% (AICRP on Spice, Calicut).

20.13.6 Suravi

It is an induced mutant of Rudrapur. Rhizomes are plumpy with dark-skinned yellow flesh, suitable for both irrigated and rainfed conditions. Plants mature in 225 days and yield about 17.5 t/ha. This variety gives the dry recovery of 24% with oleoresin content of 10.2%. It has a crude fibre of 4% and an oil recovery of 2.1% (AICRP on Spice, Calicut).

20.13.7 Himgiri

It is clonal selection from Himachal collection. It is best suitable for green ginger and less susceptible to rhizome rot disease. It is suitable for rainfed condition. Plants are ready for harvest in 230 days with a yield of 13.5 t/ha. It gives dry recovery of 20.2% with an oleoresin content of 4.29%. It has a low fibre content of 1.6% with a high oil recovery about 6% (AICRP on Spice, Calicut).

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Chapter 21
DNA Content (C-Values), Chromosome Numbers, and Mating System of Medicinal, Aromatic, and Stimulant Plants

Wolf-Dieter Blüthner

Medicinal, aromatic, and stimulant plants are common in nearly all plant families. The historical knowledge is based more on long experience of folk medicine than on scientific research. In a number of species (not in terms of quantity), the major parts of medicinal, aromatic, or stimulant plants are still collected from the wild; cultivation is rather the exception. However, the number of species domesticated increases fast with breeding as one of the most important processes driving the economic efficiency of cultivation. Absolute prerequisite for a systematic improvement through breeding is the knowledge of scientific biological basics like mating system, ploidy level, and DNA content. This is especially true where a molecular method gives us better insights and is planned to speed up the breeding process.

The following compilation summarizes the results of DNA content (C-values), chromosome numbers, and mating system from 2255 species. Although this number seems to be high, it is still below 10% of all plants used by man as medicine (Willis 2017). For this compilation, some very helpful databases need to be highlighted, like the "Angiosperm DNA C-Value Database" (Bennett and Leitch 2012) and the "Index to Plant Chromosome Numbers" (Goldblatt and Johnson 1979). Furthermore, *The Families and Genera of Vascular Plants* (Kubitzki 1990–2016), *Dictionary of Cultivated Plants and Their Centres of Diversity* (Zeven and Zhukovsky 1975), *The Encyclopedia of Herbs and Spices* (Ravindran 2017), and *Culinary Herbs* (Small 1997) were important reference points facilitating this compilation.

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Correction to: *Petroselinum crispum* (Mill.) Nyman (Parsley)

Frank Marthe

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This book was inadvertently published with missing part figures of Figures 13.3a, 13.5a, 13.7b and few missing details from Table 13.2 which has now been updated and corrected.

The updated online version of this chapter can be found at
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Fig. 13.3 Septoria blight on parsley; (**a**) lesions; (**b**) lesions with black pycnidia

Fig. 13.5 Powdery mildew on parsley; (**a**) plants with ecto-mycelium; (**b**) leaf with mycelium and mass production of asexual hyaline spores

Fig. 13.7 Alternaria leaf blight on parsley; (**a**) plants with necrotic leaves, (**b**) leaf with necrosis and chlorotic zones

Table 13.2 Plant variety catalogue for parsley (*Petroselinum crispum*, H-25) of plant variety database – European Commission (2019)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
36004 RZ	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)	(1)	Leaf[2]
36504 RZ	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)	(1)	Leaf[2]
A grosse racine gros hâtif	b FR x		Root[1]
Afrodite	b DK 57 (Hild Samen GmbH)		Leaf[1]
Alba	a CZ 1495 (Moravoseed CZ a.s.), a HU 102445 (Moravoseed s.r.o.)		Root[1]
Alto	a FR 8067 (Vilmorin)		Leaf[1]
Amsterdamse Snij	b NL x	Fijne Snij NL	Leaf[1]
Andrei	a RO 1031 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Bacău)		Root[2]
Arat	b NL 8 (Bejo Zaden BV)		Root[1]
Arctica	a NL 8 (Bejo Zaden BV)	H	Root[1]
Argon	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Aroma	a NL 8 (Bejo Zaden BV)		Root[1]
Arsem	a RO 1072 (S.C. Unisem S.A. Bucureşti)		Root[2]
Astra	a CZ 1495 (Moravoseed CZ a.s.)		Leaf[1]
Atika	a CZ 239 (SEMO a.s.)		Root[2]
Berliner		= Halblange	Root[1]
Berlinova	a DE 9989 (Satimex Quedlinburg GmbH)		Root[1]
Bravour	b NL x, b UK 176 (J.E. Ohlsens Enke A/S)		Leaf[1]
Catalogno		= Gigante di Napoli	Leaf[1]
Champion	b UK 189 (A.L. Tozer Ltd.)		Leaf[1]
Commun 2 Comun 2 Comune 2 Plain Leaved (Sheeps) 2 Comum 2	b FR x b ES x b IT 125 (SAIS Società agricola italiana sementi) b UK 38 (E.W. King & Co. Ltd.)	De hoja lisa 2 ES Plain or single 2 IT Simple 2 FR Toscano 2 IT	Leaf[1]
Comum 2		= Commun 2	Leaf[1]
Comun 2		= Commun 2	Leaf[1]
Comun 3 Gewone Snij 3	b ES 3051 (Royal Sluis) b NL 78 (SVS Holland BV)	De hoja lisa 3 ES Einfache Schnitt 3 NL	Leaf[1]
Comune 2		= Commun 2	Leaf[1]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Cukrowa	a PL 67 (Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o.o.)		Root[1]
Curlina			Leaf[1]
Danubiu	a RO 1072 (S.C. Unisem S.A. Bucureşti)		Leaf[2]
Darki	b DK 57 (Hild Samen GmbH), b UK 176 (J.E. Ohlsens Enke A/S)		Leaf[1]
Darklett	a DE 2549 (Hild Samen GmbH)		Leaf[1]
De hoja lisa 2		= Commun 2	Leaf[1]
De hoja lisa 3		= Comun 3	Leaf[1]
Doble rizado		= Frisé vert foncé	Leaf[1]
Dobra	a CZ 256 (Seva-Flora s.r.o.)		Root[2]
Domaći lišćar	b HR 177 (Podravka d.d.)		Leaf[2]
Efez	a CZ 239 (SEMO a.s.)		Root[2]
Einfache Schnitt 3		= Comun 3	Leaf[1]
Extra triple curled 2		= Mooskrause 2	Leaf[1]
Favorit	b NL 8 (Bejo Zaden BV)		Leaf[1]
Félhosszú		= Halblange	Root[1]
Fest	a CZ 1495 (Moravoseed CZ a.s.)		Leaf[1]
Festival 68	a CZ 1495 (Moravoseed CZ a.s.), a PL x		Leaf[1]
Fidelio	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Fijne Snij		= Amsterdamse Snij	Leaf[1]
Francesa Frisada		= Frisé vert foncé	Leaf[1]
Frisé vert foncé Gekrulde Donkergroene Doble rizado Francesa Frisada	b FR x, b NL 65 (Pieterpikzonen BV) b ES x	Rizado verde oscuro ES	Leaf[1]
Gala	a PL 356 (Hortag Seed Co.)		Leaf[2]
Gazela	a PL 854 (Vera-Agra sp. z.o.o.)		Root[2]
Gekrulde Donkergroene		= Frisé vert foncé	Leaf[1]
Gekrulde	b NL x		Leaf[1]
Gewone Snij 3		= Comun 3	Leaf[1]
Gigante catalogno		= Gigante di Napoli	Leaf[1]
Gigante d'Italia		= Gigante di Napoli	Leaf[1]
Gigante d'Italia		= Gigante di Napoli	Leaf[1]
Gigante di Chioggia		= Gigante di Napoli	Leaf[1]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Gigante di Napoli Gigante d'Italia	b IT x a DE 2549 (Hild Samen GmbH)	Catalogno IT Gigante catalogno IT Gigante d'Italia IT Gigante di Chioggia IT Verde scuro d'Italia IT	Leaf[1]
Grüne Perle	a DE 86 (Karl und Walter Hild)		Leaf[1]
Halblange Halvlang Berliner Halflange Félhosszú	a CZ x, a DE x, a PL x, a SK x b NL x a HU 151508 (ZKI Zöldségtermesztési Kutató Intézet Zrt.)		Root[1]
Halflange		= Halblange	Root[1]
Halvlang		= Halblange	Root[1]
Hamburska	a PL 356 (Hortag Seed Co.)		Root[2]
Hanácká	a CZ x, a SK 250 (Zelseed spol. s r.o.)		Root[1]
Ines	a DE 7092 (GHG Saaten GmbH)		Leaf[1]
Jadran	a CZ 239 (SEMO a.s.)		Root[1]
Jagienka	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Root[2]
Junák	a SK 432 (P.K. SEM spol. s.r.o.)		Leaf[2]
Kadeřavá	a CZ x		Leaf[1]
Kaśka	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Root[2]
Kinga	a PL 67 (Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o.o.)		Root[1]
Konika	a CZ 1495 (Moravoseed CZ a.s.)		Root[1]
Korai cukor	a HU 151508 (ZKI Zöldségtermesztési Kutató Intézet Zrt.)		Leaf[1]
Krista	a CZ 256 (Seva-Flora s.r.o.)		Leaf[1]
Kudrnka	a CZ 239 (SEMO a.s.)		Leaf[1]
Laica	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Lange Oberlaaer	a AT 72 (Austrosaat Österreichische Samenzucht- und Handels-AG)		Root[1]
Laura	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Lenka	a PL 1087 (Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa w Ożarowie Mazowieckim Spółka z o.o.)		Root[1]
Lisette	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Makói hosszú	b HU 191126 (Fekete János)		Root[2]
Marunka	a CZ 239 (SEMO a.s.)		Leaf[1]
Messis	a PL 938 (AdvanSeed ApS)		Leaf[1]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Mooskrause 2 Extra triple curled 2 Riccio muschiato 2 Riccio verde scuro 2 Moskrul 2 Moss Curled 2 Nain frisé mousse Nano ricciuto 2	b NL x b UK x b FR x		Leaf[1]
Moskrul 2		= Mooskrause 2	Leaf[1]
Moss Curled 2		= Mooskrause 2	Leaf[1]
Nain frisé mousse		= Mooskrause 2	Leaf[1]
Nano ricciuto 2		= Mooskrause 2	Leaf[1]
Natalka	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Leaf[1]
Nutka	a PL 1087 (Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa w Ożarowie Mazowieckim Spółka z o.o.)		Leaf[2]
Olomoucká dlouhá Ołomuńcka	a CZ x, a SK 250 (Zelseed spol. s r.o.) a PL 218 (Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa)		Root[1]
Oltis	a RO 1033 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Buzău)		Root[2]
Orbis	a CZ 239 (SEMO a.s.)		Root[1]
Orfeo	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Ory	a RO 1033 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Buzău)		Leaf[1]
Osborne	a CZ 1495 (Moravoseed CZ a.s.)		Root[1]
Ołomuńcka		= Olomoucká dlouhá	Root[1]
Peione	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Pesto	a PL 356 (Hortag Seed Co.)		Leaf[2]
Petronelia	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)		Leaf[1]
Petruschka	a DE 7092 (GHG Saaten GmbH)		Leaf[1]
Plain Leaved (Sheeps) 2		= Commun 2	Leaf[1]
Plain or single 2		= Commun 2	Leaf[1]

(continued)

Table 13.2 (continued)

Variety name	Country maintainers	Synonym	Used plant part (1, 2)
Polina	a NL 108 (Rijk Zwaan Zaadteelt en Zaadhandel BV)		Leaf[2]
Prairie	a NL 8 (Bejo Zaden BV)		Leaf[2]
Riccio muschiato 2		= Mooskrause 2	Leaf[1]
Riccio verde scuro 2		= Mooskrause 2	Leaf[1]
Rizado verde oscuro		= Frisé vert foncé	Leaf[1]
Roksana	a PL 92 (PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o.)		Root[2]
Rosette	b NL 136 (A.L. Tozer Ltd.)		Leaf[1]
Samba	a PL 187 (SPÓJNIA Hodowla i Nasiennictwo Ogrodnicze sp. z o.o.)		Root[1]
Simple 2		= Commun 2	Leaf[1]
Sonata	a PL 187 (SPÓJNIA Hodowla i Nasiennictwo Ogrodnicze sp. z o.o.)		Root[1]
Starke	b DK 56 (Weibulls Horto AB)		Leaf[1]
Starlett	a DE 2549 (Hild Samen GmbH)		Leaf[1]
Thujade	b NL 65 (Pieterpikzonen BV)		Leaf[1]
Titan	b NL 8 (Bejo Zaden BV)		Leaf[1]
Toscano 2		= Commun 2	Leaf[1]
Troja	a CZ 239 (SEMO a.s.)		Root[2]
Verde scuro d'Italia		= Gigante di Napoli	Leaf[1]
Vistula	a PL 67 (Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o.o.)		Root[2]
Walser Petersilie	b AT 148 (Arche Noah)		Leaf[2]
Warta	a PL 187 (SPÓJNIA Hodowla i Nasiennictwo Ogrodnicze sp. z o.o.)		Root[1]
Wega	a NL 26 (Enza Zaden Seed Operations BV)		Leaf[1]
Zaharat	a RO 1031 (Staţiunea de Cercetare-Dezvoltare pentru Legumicultură Bacău)		Leaf[1]

(1): Variety denomination approved in the form of a code
a: Basic seed
b: Standard seed
Country code: AT – Austria, CZ – Czech Republic, DE – Germany, DK – Denmark, ES – Spain, FR – France, HR – Croatia, HU – Hungary, IT – Italy, NL – the Netherlands, PL – Poland, RO – Romania, SK – Slovakia, UK – United Kingdom
Used plant parts
1 (Kraus-Schierhorn 2019)
2 (Blüthner 2019)

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