Sustainable Development and Biodiversity 8

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S. Mohan Jain *Editors*

Genetic Diversity and Erosion in Plants

Case Histories

Volume 2

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Sustainable Development and Biodiversity

Volume 8

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Preface

The term erosion implies gradual loss of something important that will eventually undermine the health or stability of dependent individuals or communities. As applied to genetic diversity, erosion is the loss of genetic diversity within a species. It can happen fairly quickly, as with a catastrophic event, or change in land use that removes large numbers of individuals and their habitat. But it can also occur more gradually and go unnoticed for a long time. Genetic erosion represents the loss of entire populations genetically differentiated from others, or the loss or change in frequency of specific alleles within a population, or the species as a whole, or the loss of allelic combinations in plants, trees, and animals.

Until the 1940s, the centers of origin of crop species and woody plants were considered limitless sources of genetic variability. After World War II, agriculture in developing countries suffered great changes. The expanded use of improved varieties resulted in the reduction of traditional varieties, a process called genetic erosion. The expansion of the agricultural frontiers also contributed to the risk of loss of the wild relatives of crop species. Some 10,000 different plant species have been used by humans for food and fodder production since the dawn of agriculture 10,000 years ago.

Yet today just 150 crops feed most human beings on the planet, and just 12 crops provide 80 % of food energy, while wheat, rice, maize, and potato alone provide 60 % of stable food. Reduction of agricultural biodiversity means fewer options for ensuring more diverse nutrition, enhancing food production, raising incomes, coping with environmental constraints, and sustainably managing ecosystems. Recognizing, safeguarding, and using the potential and diversity of nature are critical for food security and sustainable agriculture. Biodiversity conservation targets three interdependent levels: ecosystems, species, and genes. Genetic erosion can represent the loss of entire populations genetically differentiated from others, the loss or change in frequency of specific alleles (i.e., different forms of a gene) within populations or over the species as a whole, or the loss of allelic combinations. Genetically eroded populations may be less competitive with new introduced invasive species. Genetic diversity is important to a species' fitness, long-term viability, and ability to adapt to changing environmental

conditions. Genetic erosion can be addressed at several levels in the spectrum of management activities. This book deals with a broad spectrum of topics on genetic erosion and biodiversity in crop plants and trees.

We believe that this book will be useful to botanists, geneticists, molecular biologists, environmentalists, policy makers, conservationists, and NGOs working for the protection and conservation of species in a changing environment.

M.R. Ahuja
S. Mohan Jain

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Chapter 1
Genetic Diversity, Erosion, and Conservation in Oil Palm (*Elaeis guineensis* Jacq.)

Claude Bakoumé

Abstract African oil palm (*Elaeis guineensis* Jacq.) is a perennial crop that offers a variety of products for food, non-food, and medical uses worldwide. Sustainable oil palm development is expected from the species with high genetic diversity within ex situ and in situ populations. From the Guinea golf in Africa, oil palm adapted to the humid tropics of Africa, Southeast Asia, and Latin America, thanks to this high genetic diversity. Indicators of the species' genetic variability include (i) multiple fruit shell forms, (ii) diverse fruit exocarp color types, and (iii) wide variation of morphological and agronomic characters. The high genetic variability within oil palm materials has been confirmed by molecular marker techniques. As for many other plants, pests and diseases, breeding, and human activities in natural oil palm groves are responsible for genetic erosion or loss of alleles or genes resulting from the death of oil palms, i.e., decreasing population size. In fact, molecular markers have revealed low genetic diversity in breeding populations which are usually smaller than natural collections. Efforts have been taken to preserving oil palm germplasms and to collecting and conserving new materials from the natural oil palm belt in Africa for improved oil palm profitability and for posterity. Constraints in oil palm conservation are the requirements of large space (at 148 palms per hectare) and regular maintenance incurring high upkeep costs. Furthermore, the long-term in vitro conservation techniques have not yet been established and seeds cannot tolerate storage at a low temperature. Fortunately, locals in the African oil palm belt are concerned with the preservation of the species' natural groves. They select oil palms to fell for palm wine production, maintain existing palm trees, create suitable conditions for the growth of seedlings, and do not cut seedlings during bush clearing or weeding of farms.

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1.1 Introduction

1.1.1 The Oil Palm Elaeis Guineensis

Oil palm (*Elaeis guineensis* Jacq.), a perennial species, is a diploid (2n = 32) monocotyledon. It belongs to the family *Arecaceae* (also known as *Palmae*), to the subfamily *Aracoideae*, and is grouped with *Cocos* (the coconuts) in the tribe *Cocoineae* (or *Cocoeae*). *Elaeis guineensis* is a member of the subtribe *Elaeideae* and of the genus *Elaeis* (Uhl and Dransfield 1987). *E. guineensis* (Fig. 1.1) forms with the American *E. oleifera* (Fig. 1.2) the two cross-fertile and commercially important species of the genus.

Its root system is composed of primary, secondary, tertiary, and quaternary roots. The greatest quantity of roots is found in the first 45 cm of the topsoil (Taillez 1971). However, primary roots of palms around 13 years old can absorb water from water table at a depth of 5 m (Dufrene 1989). The early growth of the oil palm is transversal, i.e., formation of a wide stem without internodal elongation. The vertical growth varies in the range of 20–75 cm per year, depending on the provenance and the agro-ecological environment. Harvesting becomes increasingly difficult for tall palms, which can reach a height of 15–18 m when they are more than 25 years old. The stem is cylindrical (45–60 cm in diameter), not ramified and terminated by a unique crown of 40–45 pinnate leaves. The oil palm is a monoecious plant characterized by alternating series of male and female inflorescences. An inflorescence is initiated at the axil of every leaf, which will abort or develop into male or female inflorescence (Beirnaert 1935). The oil palm is naturally allogamous and mostly insect pollinated. The bunch completes its maturation

Fig. 1.1 African *Elaeis guineensis* (*source* Madi Galdima)

Fig. 1.2 American *Elaeis oleifera* with their characteristic leaning (then crawling) stem

in 4.5–6 months after pollination. As the palm ages the bunch weight increases while the number of bunches produced per palm declines. Well-set bunches carry 500–4000 fruits. It is believed that the life span of the oil palm may be up to 200 years (Purseglove 1972).

1.1.2 Importance of Oil Palm

The oil palm offers the highest oil yield (4–5 tons) per hectare compared to other oil crops. The importance of oil palm (*Elaeis guineensis* Jacq.) and its products have kept increasing, making the oil palm industry a multipurpose industry. Oil palm is both food and cash crop. The crude oils extracted from the mesocarp (also called pulp) and the endosperm (also known as kernel) are used as cooking oils, for animal nutrition, and for industries (Ngoko et al. 2004). Palm oil (mostly crude palm oil) contains tocopherols (vitamin E), tocotrienols, carotenoids, polyphenols, and all extracts that possess cardiovascular health benefits (Carbonneau 2013; Monde et al. 2013). Tocotrienols (TT) and carotenoids combat vitamin A deficiency. Worldwide clinical trials are investigating TT's effectiveness in stroke and pancreatic cancer prevention (Khosla 2013). Transdermal application of TT contributes to the prevention of breast cancer (Wahid 2006).

Since palm oil is semi-solid at ambient temperature, melting at about 35 °C, unlike liquid vegetable oils which become semi-solid only with partial hydrogenation, a chemical process which leads to parallel production of the unnatural trans-fatty acids responsible for cardiovascular diseases, a more desirable solid fat content can be obtained by blending liquid vegetable oils with palm oil. This property of palm oil has recently increased its consumption in the United States to over one million tons (Global Oil and Fats Business 2013).

Not only is palm oil a raw material for the oleochemical industry, it is also in demand for biodiesel production. There are technologies to produce oil palm products, for example, pulp and paper, molded particle board, and plywood (Wahid 2006).

Fig. 1.3 Electric power generator running on crude palm oil

The possibility of extracting ethanol from oil palm fronds cut during harvesting or pruning, which were previously left to rot, has raised hope in the palm oil-producing countries. There are two suitable and commercially proven technologies for use of palm oil for electric power generation; one is to run on palm kernel shell or wood chips and another is to run on crude palm oil (Fig. 1.3), which has already been implemented in Liberia (Walden 2014). Other oil palm products of paramount importance in Africa include palm wine, palm cabbage, leaflets for thatching, the petioles and rachis for fencing or for protecting top of mud walls, and other materials for making baskets, nets, ropes, and band rooms (Bakoume 2006).

In the developing countries an increase of 83 % in the consumption of grains and oil seeds is predicted for 2013–2022 along with a 92 % increase in the world imports of grains and seeds (Basiron 2013). Palm oil production costs are low due to low input requirements, and for production of the same amount of oil, oil palm requires one-tenth the land area required by soya. Moreover, palm oil is the cheapest oil in the world despite the fact that production is relatively less mechanized and therefore labor-intensive (Fig. 1.4).

In 2012, palm and kernel oils represented 32 % of the world's oil and fat production at 185 million tons and 60 % of the 72.34 million tons exported (Global Oil and Fats Business 2013). Oil palm is a crop that amplifies the success of

Fig. 1.4 Traditional extraction of palm oil in Nekom, Central Region of Cameroon

economic and social development. It is a reliable and sustainable growth catalyst and a useful model to be adopted by developing countries. Oil palm development contributes to advancing poor African economies such that they can move the continent towards sufficiency for edible oil, provide employment, and improve the quality of life (Bakoumé 2013a, b). Ascertaining the genetic diversity which ensures the sustainability of the species, assessing the eventual genetic erosion, and evaluating conservation initiatives are justified for a crop with the merit to be called "God's gift to mankind" by some and "the golden crop" by others.

1.1.3 Genetic Diversity in Oil Palm

Genetic diversity is defined by the total number of genetic characteristics in the genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary. In fact, both genetic diversity and genetic variability are found in oil palm. Genetic diversity in oil palm will be viewed with reference to its distribution area, fruit types and fruit forms, and agronomic and morphological characters as well as to results of assessments done using molecular markers.

1.1.3.1 Genetic Diversity Supported by Wide Geographic Distribution

There are physical, historical, and linguistic reasons to support the African origin of the oil palm (*E. guineensis*). One of the physical evidence is the fossil pollen found in Miocene sediments in the delta of the river Niger (Zeven 1964). A report from Ca' da Mosto, a Portuguese explorer of the Guinea coast from 1453 to 1460, provided the historical evidence of the existence of oil palms in Africa a long time

ago. It gave first mention of a palm that strongly suggested the oil palm (Crone 1937). Linguistic agreement has been founded on the fact that all the West African vernacular names of oil palm are short and directly translated to mean oil palm, while Negro names for the oil palm in Surinam are a corruption of its African Yoruba, Fanti-twi, and Kikongo names. Even the Brazilian name *dende* may be derived from the Kimbundu word *ndende* of Angola (Hartley 1988).

The center of distribution of oil palm as supported by studies using isozyme and DNA-based marker techniques is a zone covering Nigeria, Cameroon, and Angola (Ghesquiere 1985; Hayati et al. 2004; Kularatne 2000). From its center of distribution, oil palm seeds spread through the agency of gravity and water, of animals, or of man (Hartley 1988). Humans are clearly by far the foremost contributing factor in seed dispersal, deliberately or by accident. Oil palm arrived in Madagascar when African elements entered the island, as early as the ninth century (Purseglove 1972) and in Southeast Asia through Amsterdam Botanic Gardens and Mauritius in 1848 (Hardon and Thomas 1968). In the seventeenth century, *E. guineensis* was introduced into South America from Africa with the slave trade; a semi-wild grove covering about 20,000 hectares exists in Brazil (Barcelos 1998).

Today the oil palm exists in wild, semi-wild, and cultivated states in the equatorial tropics of Africa, Southeast Asia, and America (Hartley 1988) between latitude 16°N and latitude 21°S where it has survived to a wide range of environmental conditions (Fig. 1.5). According to Jacquemard (1995), although the soil and climate features of the main areas of high bunch production are a rainfall of at least 1800 mm evenly distributed throughout the year, a mean temperature ranging from 29 to 33 °C and a minimum temperature above 18 °C, a total of 1800 h per annum of sunshine, and an altitude varying between 0 and 300 m above sea level, still spontaneous or sub-spontaneous oil palms are found a few kilometers south of

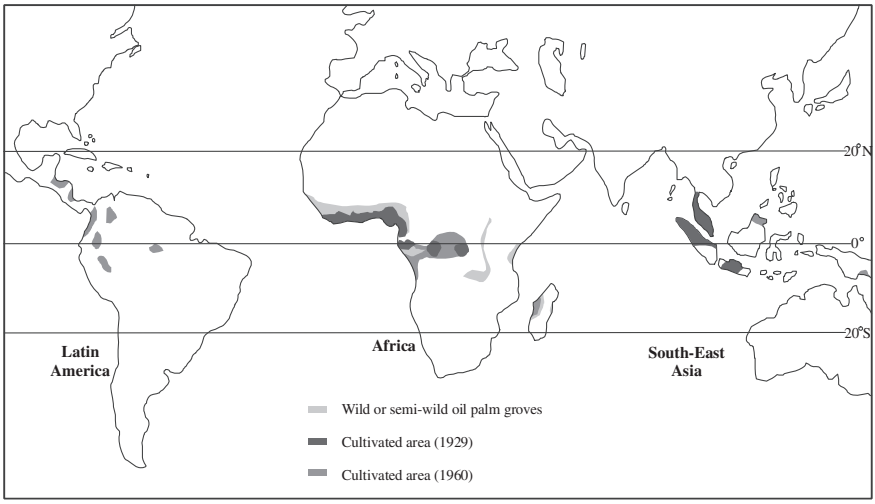

Fig. 1.5 Oil palm (*E. guineensis*) growing areas (reproduced from Jacquemard 1995)

Saint-Louis in Senegal at latitude 16°N where rainfall is only 200–400 mm and also at up to 3000 m above sea level at Kikango in Cameroon (Rajanaidu 1994).

Nature and subsequently humans have placed oil palm in a wide range of habitats, climates, and spaces. Under such circumstances, any species must have a pool of genetic diversity if it has to survive environmental pressures exceeding the limits of developmental plasticity (Yeh 2000). If this is not the case, failure to extension would become inevitable. We believe that genetic diversity contributes to oil palm's adaptation to different environments. With greater variation, it is more likely that some oil palms will possess allele variants that are suited for marginal environments and therefore will allow them to survive and produce offspring. The oil palm population will continue for more generations because of the success of these individuals. Bakoumé (2006a, b, c) suggested that the high genetic diversity found in natural oil palm collections from Africa maintained by the Malaysian Palm Oil Board (MPOB) can explain the species' plasticity in its adaptation to various environments in its actual large distribution area. In Madagascar, oil palm develops different flower and fruit characteristics as a reaction to long-lasting drought episodes. In Bamenda, in the northern region of Cameroon situated at more than 1700 m above sea level where low temperatures prevail, local natural oil palms grow normally and start producing fresh fruit bunches in 3–4 years after planting like the improved *tenera* in the lowland area. Oil palm grows tall and thin under shade in the forest as it competes for light with other forest tree species. Its growth rate is reduced under dry spell conditions. Thanks to its high genetic diversity, the species demonstrates phenotypic plasticity when it is exposed to different environments. In short, genetic diversity plays an important role in the survival and adaptability of oil palm to its wide and diverse distribution area.

1.1.3.2 Diversity in Fruit Form

The oil palm produces bunches bearing fruits numbering from a few hundred up to 4000. The fruit is a sessile drupe varying in shape from nearly spherical to ovoid or elongated and bulging somewhat at the top. The fruit length varies from 2 cm to more than 5 cm, and its weight ranges from 3 g to over 30 g. The fruit is covered by an exocarp or skin. A cross section shows a mesocarp (pulp containing palm oil) and an endocarp (a shell) surrounding an endosperm or kernel, from which palm kernel oil is extracted (Fig. 1.6).

Oil palm is cultivated for its fruits that contain oils in both the mesocarp (palm oil) and the kernel (palm kernel oil). The fruit deserves special consideration for its characteristics in the classification of the species. In fact, the three varieties of oil palm are based on the form of the fruit, namely the presence or absence of a shell, a monofactorial trait (*sh* from "shell"). The two homozygotes are the *dura* (sh^+sh^+) with a thick shell and the *pisifera* (sh^-sh^-), shell-less, usually female sterile due to premature rotting of fruits. The hybrid *tenera* (sh^+sh^-), the product of a cross between a *dura* and a *pisifera* (P), has a thin shell surrounded by a fiber ring (Fig. 1.7).

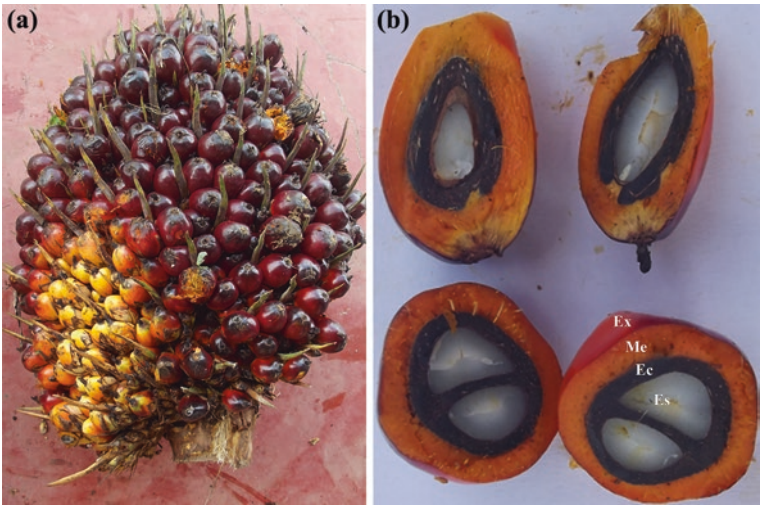

Fig. 1.6 **a** Oil palm bunch, **b** Oil palm fruit components: *Ex*—exocarp, *Me*—mesocarp, *Ec*—endocarp, *Es*—endosperm (*source* Madi Galdima)

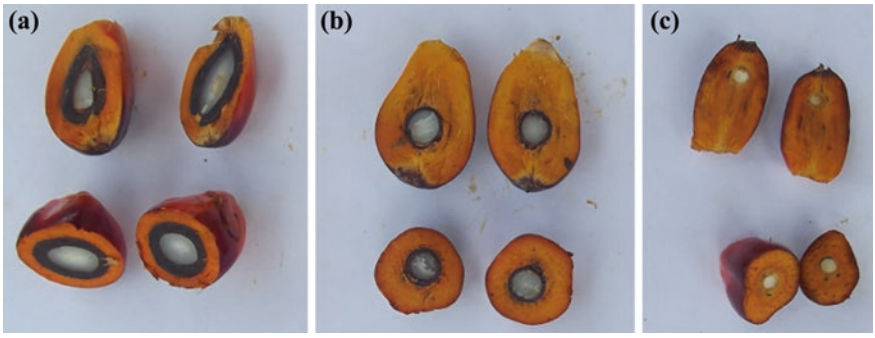

Fig. 1.7 **a** *Dura*, **b** *Tenera*, **c** *Pisifera* (*source* Madi Galdima)

Although *dura*, *tenera*, and *pisifera* are mainly determined on the basis of the endocarp (shell) in the fruit, that is not all that distinguishes them. They are also distinct from each other for some few morphological, physiological, and bunch production characteristics (Table 1.1).

Genetic diversity in terms of fruit form is of importance to oil palm development because traits of interest to the oil palm industry are associated with variety. New genotypes for new traits for increased profitability of oil palm business are accessed through the collection of desired phenotypes from African natural oil palm groves. There is a need to look at the varietal diversity in the natural oil palm groves from Africa. The varietal composition of accessions from recent oil palm collections in the continent should provide a picture of the actual diversity of fruit

Table 1.1 Distinctive characteristics of *dura*, *pisifera*, and *tenera* palms

Characteristics	Type of oil palm		
	Dura	*Pisifera*	*Tenera*
Female inflorescence	Average to large	Small	Average to very large
Bunch number	Low	High	Average to high
Bunch size	Large	Small	Medium to large
Fruit	Sessile drupe, spherical or ovoid	Rare, parthenocarpic, elongated, oblong	Ovoid, oblong, elongated
• Genotype at the shell locus (*sh*)	Homozygote for the presence of a shell (sh^+ sh^+)	Homozygote for the absence of a shell (sh^- sh^-)	Heterozygote for the presence of shell (sh^+ sh^-)
• Mesocarp/fruit	Up to 80 %	>95 %	Up to 96 %
• Weight	3–50 g	<10 g	3–50 g
Number of leaves[a]	Low	High	Average
Cycle	Completed after 3–6 years	Uncompleted and usually limited to production of abortive bunches	Completed after 3–6 years
Commercial interest as regards to oil yield	Limited	None	High[b]
Common uses	Female or male parent in breeding Female parent in seed production	Male parent in breeding and commercial seed production	Male or female parent in breeding Hybrid for commercial plantations
Potential palm oil yield after selection	10 t/ha	Almost not productive	18.5 t/ha
Transmission of tolerance to *Fusarium* wilt	Good	Mediocre	Mediocre

[a]Broekmans (1957)
[b]Since 1960, all commercial oil palm planting materials have been *tenera* (also known as D × P), thereby increasing the oil-to-bunch ratio by 30 %, that is, from 16–18 % in *dura* to 22–26 % in *tenera*

forms that exists in the natural oil palm groves. Natural oil palm groves are composed of close to 98 % *dura* and 2 % *tenera* with traces of *pisifera*. Four joint oil palm collections have recently been carried out, two of them in Angola, one by Angolan National Coffee Institute (ANCI) and Centro de Investigación en Palma de Aceite (Centre of Oil Palm Research) (Cenipalma) (Rey et al. 2004), then another by Indonesian Palm Oil Board (IPOB), MPOB, and ANCI and the other two in Cameroon, one by Cenipalma and the Institute of Agricultural Research for Development of Cameroon (IRAD) and another by IPOB and IRAD. Despite the emphasis on *tenera* fruit form during the collection, actually the greatest proportion of palms in the natural groves was *dura* (83.2 % vs. 16.6 %). Only one *pisifera* was found, and it was in the Cameroon natural oil palm groves (Table 1.2). Indeed, *pisifera* is considered rare by local communities. It is easy for villagers to

Table 1.2 Fruit forms in natural oil palm collections from Angola and Cameroon

Year	Country of oil palm collection	Collecting organizations	Total number of accessions	Composition in fruit form		
				dura	*tenera*	*pisifera*
2002	Angola	Cenipalma/ANCI	137	123	14	0
2007	Cameroon	Cenipalma/IRAD	74	53	20	1
2008	Cameroon	IPOB/IRAD	103	89	14	0
2010	Angola	IPOB/MPOB/ANCI	127	102	25	0
Mean (%)			100	83.2	16.6	0.2

locate all the *tenera* because they are only a few. Furthermore, *tenera* are given a distinctive name translated into "small shell," "abundant mesocarp" in all the areas explored in Cameroon while *dura* are called by a generic name meaning simply "oil palm."

It is most likely that the frequencies of alleles sh^+ and sh^- and those of the genotypes *dura*, *tenera*, and *pisifera* have been maintained in the wild oil palm groves where poor *dura* with regards to mesocarp-to-fruit ratio and rare *pisifera* are preferably felled for the production of palm wine, a beverage very appreciated by locals in Africa. The diversity of form is essential for breeding and seed production programs. *Dura* and *pisifera* are crossed to produce *tenera* (hybrid) for supply to oil palm growers. *Tenera* are crossed with their *pisifera* descendants to increase the number of pollen donors (*pisifera*). By so doing, breeders and seed producers have augmented the frequencies of the allele sh^- to levels that could hardly be achieved if nature were to act alone. In conclusion, the diversity of fruit forms has been maintained in the natural oil palm groves as well as ex situ in field genebanks by research and seed production centers. No one variety has been lost over time despite high human interference.

1.1.3.3 Diversity in Fruit Type

The exocarp of the fruit displays a diversity of colors controlled by a monogenic inheritance (Latiff 2000). Three variants are commonly known (Fig. 1.8):

- *Nigrescens*: unripe fruits are deep violet to black at the apex and ivory colored towards the base, which turns to reddish or orange as it ripens. It has the highest content of carotenoids.
- *Virescens*: unripe fruits are green and turn to light reddish-orange at ripeness with a small greenish apex. The distinction between unripe and ripe bunches is clear.
- *Albescens*: unripe fruits are deep green and ripen to a pale yellow. It has little or no carotene.

In natural oil palm groves, oil palms with *nigrescens* fruit predominate over *virescens* with only traces of *albescens*. The collections referred to in Sect. 1.1.3.2

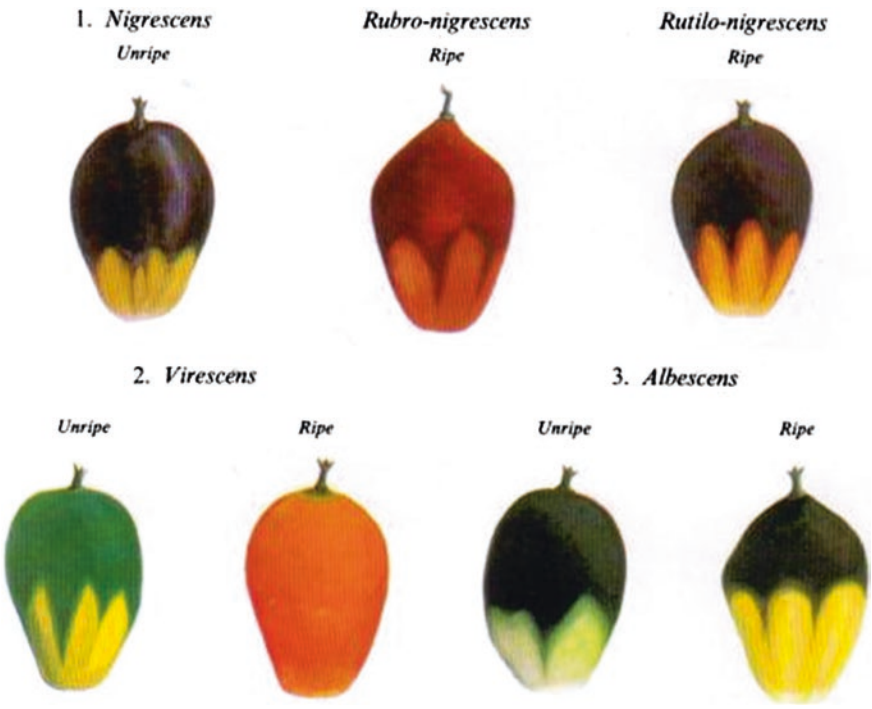

Fig. 1.8 *1 Nigrescens*, *2 Virescens*, *3 Albescens* (reproduced from Hartley 1988)

confirm this natural distribution. In fact, on average, 90 % of oil palms collected in Angola and Cameroon were *nigrescens* (Table 1.3) despite the importance given to *virescens* fruit type because of the building interest of oil palm growers in bunches that are easily identifiable when ripe. The IPOB/IRAD collection of natural oil palms in Cameroon seems to have enriched the number of fruit types with one new type, an intermediate between *virescens* and *nigrescens*. It was found in extreme eastern region of Cameroon at the border with Republic of Congo. Oil palms producing *virescens* fruits and *albescens* fruits are given names that reflect the perception that they are distinct. For example, the generic name of oil palm is *nipil* and *virescens itondopil* by the Banen people of the central region of Cameroon.

Diversity of fruit types is maintained in the African natural oil palm groves. With the recent interest in *virescens* fruits, a few *virescens* accessions from early 1900s crop expeditions in Africa were acquired as a curiosity have drawn new interest. The dominance of the *virescens* gene over the *nigrescens* gene contributes to increase its allelic and genotypic frequencies. The *ablescens* fruit type is generally less represented (0.30 % on average) and unknown to locals in Africa who are, however, interested in preserving all the different fruit types represented in the natural oil palm groves. Therefore, the *albescens* fruit type does not suffer human interference, a situation which constitutes an asset for its continued existence.

Table 1.3 Composition in fruit type of natural oil palms collected in Angola and Cameroon

Year	Country of oil palm collection	Collecting organizations	Total number of accessions	Composition in fruit type (%)			
				Nig.	*Vir.*	*Alb.*	Intermediate (*Nig./Vir.*)
2007	Cameroon	Cenipalma/IRAD	74	89	11	0	0
2008	Cameroon	IPOB/IRAD	103	93	5	1	1
2010	Angola	IPOB/MPOB/ANCI	127	87	12	0	0
Mean			304	90.0	9.3	0.3	0.3

Nig. Nigrescens, *Vir. Virescens*, *Alb. Albescens*

1.1.3.4 Variation of Morphological and Agronomic Traits

In this section, morphology and agronomic traits refer, in the sense of Weaver et al. (1969), to the outward appearance (shape, structure, color, and pattern) as well as the form and structure of the internal parts of oil palm or of oil palm fruits which present an established concern for the oil palm industry. For example, short oil palms are easier to harvest than tall ones, increasing labor productivity and reducing harvesting cost. Recently, Isa et al. (2013) confirmed the positive correlation ($r = 0.50$) between the mesocarp-to-fruit ratio and the oil-to-bunch ratio. Although Maxted et al. (1997) recognized that the use of morphological characters is an indirect method for the measurement of genetic diversity, even after the advent of direct measurement using protein- and DNA-based markers, morphological traits are still successfully used to assess the genetic diversity in crops, including oil palm. The coefficients of variation of morphological traits are still used as a measure of the degree of genetic diversity within and among oil palm populations.

Variation in Natural Oil Palms Collected in the Late 1900s

Natural oil palm populations collected in Nigeria in 1973 and maintained by MOPB showed considerable genetic variation (Table 1.4), which offered breeders opportunities to select individual palms with desired agronomic traits. The coefficients of variation varied from 23.1 % in the rachis weight to 92.5 % for fruit-to-bunch ratio within populations and from 7.5 % for fruit-to-bunch ratio to 77.0 % in the rachis length.

Evaluation of the natural oil palm germplasm materials collected in Cameroon and the Democratic Republic of Congo in 1984 and maintained by MPOB also revealed high coefficients of variation for bunch yields and bunch characteristics (Table 1.5), indicating the high genetic diversity existing in the species in natural oil palm groves in Africa. The evaluation took into account the variety of oil palm (*dura* and *tenera*). It was noted in Sect. 1.1.3.2 that the two varieties differ not only in the endocarp (shell) but also in many other bunch and vegetative characters. The low coefficients of variation for fruit-to-bunch ratio, irrespective of the variety, in natural oil palm collections from Nigeria, Cameroon and Democratic Republic of Congo are congruent with the low heritability of the character ($h^2 = 0.16$; Rajanaidu et al. 2000).

Table 1.4 Variation for morphological characters in Nigerian oil palm collections (reproduced from Rajanaidu and Rao 1986)

Characteristic	Coefficient of variation (%)	
	Within populations	Between populations
Bunch characters		
Average bunch weight	57.3	42.7
Fruit-to-bunch ratio	92.5	7.5
Endosperm-to-fruit ratio	53.8	46.2
Endocarp-to-fruit ratio	78.3	21.7
Mean fruit weight	74.3	25.7
Mesocarp-to-fruit ratio	59.2	40.8
Vegetative characters		
Frond production	52.7	47.3
Rachis length	23.1	77.0
Leaflet number	69.2	30.8
Height increment	47.8	52.2
Leaf area	40.4	59.7

Table 1.5 Means and coefficients of variation for bunch production and bunch characteristics in Cameroon and DRC oil palm materials (reproduced from Kushairi et al. 2003)

Characteristic	Cameroon				DRC			
	dura		*tenera*		*dura*		*tenera*	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
FFB (kg/palm)	91.3	34.2	101.6	33.0	84.1	35.8	93.1	34.2
Bunch number	13.5	35.2	14.8	34.1	10.7	36.7	11.6	36.0
ABWT (g)	6.9	23.0	7.0	22.5	8.1	25.8	8.2	22.7
MFW (%)	8.4	27.7	7.1	23.0	10.1	29.8	7.1	16.5
MNW (%)	4.9	27.7	2.3	27.6	5.6	31.7	2.0	30.0
Fruit/bunch (%)	65.3	9.1	63.0	8.6	67.3	8.5	65.1	7.2
Oil/bunch (%)	11.9	25.5	18.5	19.7	13.6	23.2	20.1	7.9
Kernel/bunch (%)	8.3	22.0	8.6	26.4	8.3	21.6	9.1	20.4
Oil/palm/yr (kg)	11.5	39.5	19.6	36.0	12.0	39.7	27.1	21.0
Kernel/palm/yr (kg)	8.0	38.8	9.0	41.1	7.3	38.4	12.2	26.1
TEP (kg/palm)	16.2	35.7	25.0	33.1	15.6	35.9	34.5	20.5

DRC Democratic Republic of Congo, *FFB* fresh fruit bunch, *ABWT* average bunch weight, *MFW* mean fruit weight, *MNW* mean nut weight, *TEP* total economic product (oil+kernel)

Variation in the Most Recent Natural Oil Palm Collections

The most recent natural oil palm collections were the prospection carried out in the central region of Ghana (Okyere-Boateng et al. 2012), an IPOB/ANCI expedition in Angola, a Cenipalma/IRAD exploration in Cameroon, and an IPOB/IRAD collection in Cameroon (Table 1.6). In the absence of fruit-to-bunch data, the

Table 1.6 Variation for bunch and vegetative characters in recent natural oil palm collections

Country	Year of collection	Fruit form	Estimated parameter	Bunch weight (kg)	Peduncle length (cm)	Rachis weight (kg)	Weight of 10 fruits (g)	Weight of 10 nuts (g)	Mesocarp to fruit (%)
Ghana	2011	Dura	Mean	9.0	–	0.8	57.5	35.0	38.7
			CV (%)	11.9	–	11.4	5.3	5.3	3.7
		Tenera	Mean	9.0	–	1.0	63.6	35.0	63.8
			CV (%)	31.1	–	41.6	17.3	5.3 7	4.2
Angola	2010	Dura	Mean	23.4	–	–	141.2	72.4	48.2
			CV (%)	45.1	–	–	28.9	30.3	18.3
		Tenera	Mean	21.8	–	–	107.8	33.0	68.1
			CV (%)	39.3	–	–	31.3	32.5	13.8
Cameroon	2007	Dura	Mean	12.0	18.2	1.1	127.0	74.6	40.6
			CV (%)	63.1	38.2	75.6	28.7	30.4	13.1
		Tenera	Mean	8.5	17.2	0.8	94.4	24.0	71.6
			CV (%)	54.5	44.5	82.5	46.8	42.4	17.3
Cameroon	2008	Dura	Mean	21.1	–	3.7	115.1	67.1	41.2
			CV (%)	47.3	–	45.2	28.1	28.4	18.8
		Tenera	Mean	19.6	–	2.7	84.5	31.0	62.3
			CV (%)	33.7	–	16.9	26.9	21.3	12.7

(continued)

Table 1.6 (continued)

Country	Year of collection	Fruit form	Estimated parameter	Weight of 10 kernels (g)	Leaf 17 length (cm)	Petiole length (cm)	Number of leaflets	Leaflet length (cm)	Leaflet width (cm)
Ghana	2011	Dura	Mean	8.4	–	–	–	–	–
			CV (%)	29.3	–	–	–	–	–
		Tenera	Mean	9.0	–	–	–	–	–
			CV (%)	23.8	–	–	–	–	–
Angola	2010	Dura	Mean	–	511.6	124.4	337.0	97.1	5.0
			CV (%)	–	15.2	19.6	8.8	15.6	13.9
		Tenera	Mean	–	541.7	128.5	351.8	99.6	5.0
			CV (%)	–	18.0	18.4	9.1	13.2	14.2
Cameroon	2007	Dura	Mean	12.8	496.0	133.0	297	84.5	4.3
			CV (%)	27.4	23.8	20.5	12.0	16.3	21.6
		Tenera	Mean	7.5	511.6	135.2	296	82.8	4.2
			CV (%)	49.0	17.1	17.6	13.9	9.6	17.4
Cameroon	2008	Dura	Mean	–	485.6	122.3	328	93.1	5.0
			CV (%)	–	12.6	14.8	6.0	11.6	28.3
		Tenera	Mean	–	476.3	111.3	325	96.6	4.5
			CV (%)	–	16.6	11.6	8.7	8.8	27.2

smallest genetic variation, in absolute terms, was observed for the number of leaflets (6.0–13.9 %). When the variety of palm was taken into consideration, the coefficients of variation were relatively low in *dura* accessions (8.9 %) compared with *tenera* ones (10.6 %) (Table 1.6).

There was considerable variation for the rest of the characters on which breeders can capitalize. The large variation of morphological and agronomic traits in the African oil palm groves was indicative of the genetic variation that exists in Ghana, Angola, and Cameroon natural oil palm populations.

1.1.3.5 Genetic Diversity Revealed by Genetic Markers

A genetic marker is a sequence of DNA that is usually recognizable by a restriction enzyme that is diagnostic for a given chromosome (Hoelzel and Dover 1991). A molecular marker or DNA-based marker is a fragment of DNA sequence that is associated with a part of the genome. It can be described as an observable variation, arising due to mutation or alteration in the genomic loci. Molecular markers allow us to look at variation in the DNA itself. They are generally neutral and free of environmental variation (Caligari 2003). They allow understanding the extent and distribution of genetic variation, the analysis of specific genes, and understanding gene action (Pons and Chouache 1995). Genetic diversity is an important determinant of population viability and adaptability (Smith and Wayne 1996).

Molecular markers are sophisticated and more reliable tools than simple observation of morphological characteristics in the assessment of the genetic diversity of both the breeding populations and natural oil palm germplasm materials. The genetic markers used include the (i) isozymes, (ii) restriction fragment length polymorphisms (RFLPs), (iii) random amplified polymorphic DNA (RAPD), (iv) amplified fragment length polymorphism (AFLP), (v) inter-simple sequence repeat (iSSR), and (vi) simple sequence repeat (SSR). The measures of genetic diversity considered are (i) percentage of polymorphic loci, (ii) mean number of alleles (A_o), (iii) mean effective number of alleles (A_e), (iv) observed heterozygosity (H_o), and (v) expected heterozygosity (H_e). High genetic diversity has been found in both the natural oil palm collections and breeding materials irrespective of both the country of origin and the genetic marker technique used (Table 1.7). Hayati et al. (2004) mentioned that the expected heterozygosity ($H_e = 0.184$) observed in oil palm was higher than that reported for other palm species. The number of alleles per loci is high in both the natural oil palm and breeding stocks. According to Ghesquiere (1985), the number of alleles per loci is a good guide for assessing genetic diversity of materials from a prospection or material undergoing selection.

Bakoumé et al. (2014) has assessed the genetic diversity of African oil palm collections maintained at MPOB as well as Deli, La Me (Côte d'Ivoire), and Bahia (Brazil) breeding materials. Deli materials were derived from the four palms introduced in 1848 in Indonesia. The Bahia material was derived from African oil palm introduced in Brazil in the seventeenth century. Materials from the La Me

Table 1.7 Genetic diversity of oil palm breeding and germplasm materials revealed by genetic markers

Oil palm materials	Genetic markers	Genetic diversity					References
		P	A_o	A_e	H_o	H_e	
Natural oil palm collections from 10 African countries maintained at MPOB (Malaysia) and three breeding materials	SSR	93.1	5.0	3.3	0.458	0.644	Bakoumé et al. (2014)
Natural oil palm collections from Angola	SSR	100	6.5	–	0.54	0.656	Arias et al. (2013)
Natural oil palm collections from Cameroon	SSR	100	6.361	3.648	0.579	0.649	Arias et al. (2012)
Natural oil palm collections from Cameroon	SSR	96.4	4.71	2.30	0.65	0.75	Ajambang et al. (2012)
Breeding and germplasm materials maintained at La Me and Dabou (Côte d'Ivoire), Pobè (Benin), Manaus (Brazil)	SSR	100	4.942	–	0.609	0.604	Cochard et al. (2009)
Breeding materials from four populations maintained at IOPRI (Indonesia)	Isozymes	36.4	1.813	–	0.332	0.300	Purba et al. (2009)
	AFLP	61.0	19.2	–	–	–	
Natural oil palm collections from 11 African countries maintained at MPOB (Malaysia)	Isozymes	71.4	1.80	1.35	0.186	0.184	Hayati et al. (2004)
Breeding materials from six provenances in West Africa and one in Southeast Asia	Isozymes	89.2	2.558	–	0.299	–	Ghesquiere (1985)

MPOB Malaysian Palm Oil Board, *IOPRI* Indonesian Oil Palm Research Institute

source consisted of 21 *tenera* collected around 1922 from wild oil palm groves of Bingerville in Côte d'Ivoire. They found that the average number of alleles and effective numbers of alleles per locus in the Deli *dura* maintained at Dabou (Côte d'Ivoire) ($A_o = 3.5$, $A_e = 2.5$) and *tenera* ($A_o = 3.6$, $A_e = 2.7$) breeding materials maintained at La Me were considerable and comparable to values shown by natural oil palm accessions of certain populations from Senegal and from Cameroon. The Deli *dura* (MPOB and Dabou) materials which had undergone several generations of selection had lower H_e value (0.549), in absolute terms. The genetic diversity in

La Me ($H_e = 0.618$) and Bahia (Brazil) ($H_e = 0.668$) materials which have undergone fewer generations of selection was average and higher than that detected in Deli *dura* materials. However, Duncan's multiple range test of comparison of mean values of A_o, A_e, and H_e of the populations and the breeding materials did not permit clear separation, indicating that A_o, A_e, and H_e values were comparable from one natural oil palm population or breeding material to another. However, it should be remembered that Deli, La Me, and Bahia oil palms were represented by small numbers of samples in the study. In fact, Deli *dura* (Dabou and MPOB) were represented by two oil palms per progeny, La Me *tenera* by three oil palms per progeny, and all the Bahia materials by four palms against ten oil palms per progeny of natural oil palm accession. The high genetic diversity observed might have resulted from the out-crossing behavior of oil palm (Bakoumé 2006a, b, c), the large sizes of the natural oil palm populations from where they were sampled, and the presence of rare alleles (frequency $p < 0.05$) and alleles at low frequency ($0.25 > p \geq 0.01$). According to Namkoong et al. (2000), in large populations, disfavoured alleles, deleterious alleles, and even neutral alleles with respect to fitness are present at low frequencies. The presence of alleles at rare and intermediate frequencies determines the polymorphism of a locus (Ghesquiere 1985). The high genetic diversity found in oil palm can explain its plasticity with regards to its adaptation to changing environments (Savolainen and Kuittinen 2000) and to its actual large distribution area. To sum up, the existing genetic diversity in oil palm materials allows the species to survive bouts of intense selection and environmental pressure.

1.1.4 Genetic Erosion

Genetic erosion is described as the loss of particular alleles or genes, as well as of varieties or even whole species. Genetic erosion occurs because each individual organism has unique genes which are lost when it dies without having a chance to breed. It is also compounded and accelerated by habitat fragmentation. Loss of genetic diversity is thought to reduce the ability of a population to adapt to changing environment (Beardmore 1983). Low genetic diversity can cause reduced biological fitness and increase the chance of extinction of that species or population. For Friis-Hansen (1999), genetic erosion is not just due to the reduction in the number of plants of a species or in a geographic range of a species, but, more importantly, to the loss of genetic variation among plants or, more precisely, the loss of some of the diverse forms of genes (i.e., gene variants or alleles) that are responsible for the phenotypic variation and variation in the life cycles of the species. It is understood that all factors leading to the death of oil palm, to preferring some individuals at the expense of others, which are therefore neglected and condemned to disappearance, contribute to genetic erosion.

1.1.4.1 Factors Contributing to Genetic Erosion in Oil Palm

Pests and Diseases

Pests and diseases attack oil palm at all stages of development of the plant, i.e., germinated seed, seedling, immature oil palm, and mature oil palm. Surprisingly, they are found in all wild or semi-wild and cultivated areas of the humid tropics of Africa, Southeast Asia, and South America.

Pests

Damage done by insects and small mammals leads to the death of oil palm. Some of them are continent-specific and others are not (Table 1.8).

Table 1.8 Oil palm-killing pests

Common name	Scientific name	Oil palm developmental stage	Damages	Continent of prevalence
Black weevil	*Temnoschoita quadripustulata*	Pre-nursery and nursery seedling	Larvae attack the bulb base of the leaves, resulting nearly always in the death of the affected seedling	Africa
Recilia	*Recilia mica*	Nursery seedling	• The insect is a vector of blast • The base of the spear decays and can be removed easily, giving a strong odor of rot • The roots rot, leading usually to the death of the seedling	Africa
Brown-black beetle	*Rhynchophorus phoenicis*	Immature and mature oil palm	• The weevil is attracted by exudates of wounded tissues • A wound provides entry for the insect and a suitable cavity where up to 400 eggs are deposited • Larvae bore closely through the growing points, terminal buds, trunks and crown of oil palm, leading to the death of the palm • An early symptom of *R. phoenicis* infestation is yellowing of the fronds (Fig. 1.9)	Africa
	R. palmarum	Immature and mature oil palm	Similar to those of *R. phoenicis* (Fig. 1.9)	America

(continued)

Table 1.8 (continued)

Common name	Scientific name	Oil palm developmental stage	Damages	Continent of prevalence
Groundhog or cutting-grass	*Thryonomys swinderianus*	Nursery seedling and field immature oil palm	• Eats into the base of leaves surrounding the heart of the young oil palm • Devours bud tissue of the collar on the ground (Fig. 1.10)	Africa
African brush-tailed porcupine or porcupine	*Atherurus africanus*	Field immature oil palm	Similar to those of *T. swinderianus*	Africa

Fig. 1.9 **a** Oil palm infested by *R. phoenicis*, **b** Larva and adult of *R. phoenicis* (*source* Claude Bakoume), **c** Larva and adult of *R. palmarum* (reproduced from Drenth et al. 2012)

Fig. 1.10 **a** *Thryonomys swinderianus* (cutting-grass), **b** Damages on young oil palm (*source* Claude Bakoume)

Diseases

All oil palm distribution areas suffer from numerous diseases. The three major endemic diseases which seriously limit oil palm cultivation include (i) vascular wilt in Africa caused by *Fusarium oxysporum* f.sp. *elaeidis*, a soil-borne fungal pathogen, (ii) basal stem rot or *Ganoderma* in Southeast Asia and in Africa caused by *Ganoderma boninense*, another soil-borne fungal pathogen, and (iii) bud rot disease in Latin America caused by *Phytophthora palmivora* a fungus present in the soil and in the oil palm roots.

Vascular *wilt*, also known as fusariose, is present or latent in the entire African oil palm belt. The disease either kills infected palms or enfeebles the plant. When a commercial plantation is infected with wilt, more than 50 % of the palms can be lost. Renard (1979) has reported two forms of symptoms of vascular wilted palms (Fig. 1.11):

- The typical or acute symptoms through which the palm dies about 6 months after the appearance of fusariose.
- The chronic symptoms in which the palm survives, but weakened and is unproductive.

A cross section of a diseased tree bole or stem shows brown, pink, or black discoloration of the vascular bundles in the tissues (Fig. 1.11).

Lower leaves of basal stem rot diseased oil palm collapse and hang vertically downwards along the trunk complete with their petioles, some dry and others green. Multiple unopened spears appear in the crown. Lesions and fruiting bodies also known as sporophores grow at the base of the diseased and/or dead palms (Fig. 1.12). Losses of palms due to this disease can reach up to 80 %.

Bud rot disease in Latin America is a complex disease strongly suspected to be caused by *P. palmivora*, which can destroy plantations of several thousand hectares (Durand-Gasselin 2012). The spear leaf of the affected oil palm collapse, the production of new spear leaf stops, and meristemic tissues and young leaves primodia are discolored (Fig. 1.13). Drenth et al. (2012) observed that disease expression is a function of the environment. In wet coastal areas, the wilt of the spear leaf is followed by the death of palm unlike in savannah areas where the

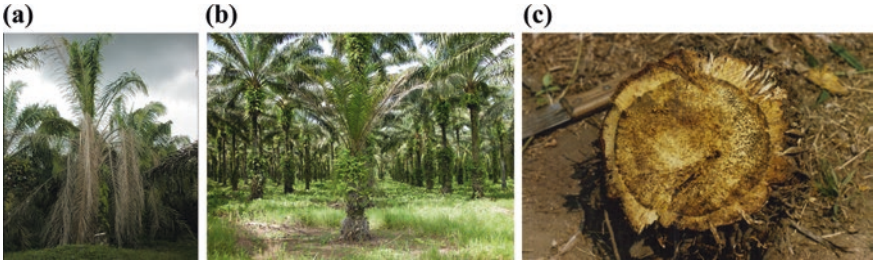

Fig. 1.11 **a** Acute symptoms, **b** Chronic symptoms, **c** Black and brown fibers in the bole (reproduced from Sékou et al. 2013)

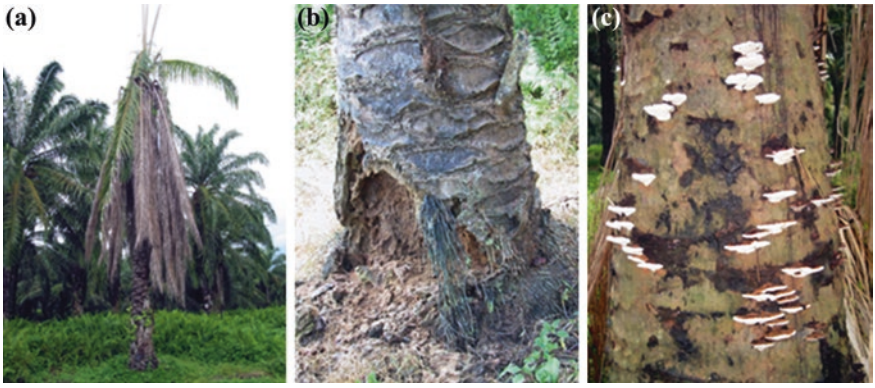

Fig. 1.12 **a** Leaf symptoms, **b** Lesion of the base of the stem, **c** Sporophores (reproduced from Durand-Gasselin 2012)

Fig. 1.13 **a** Collapse of spear leaf, **b** No emission of new spear leaf, **c** Discoloration of meristem and young leaves tissues

wilt of spear leaf is followed by an apparent remission during dry season. The authors also noticed that bud rot-affected palms attract boring insects, notably *Rhynchophorus palmarum*, which probably speeds their death.

Human Activities in Natural Oil Palm Groves

Humans are clearly by far the most important factor in oil palm dispersal within its current African belt and in the American and Asian continents. They develop agriculture and build cities with educational and health facilities and construct roads to meet human needs for food, habitat, and mobility from one area to another. However, the oil palm does not grow in primary forest or the savannah (Corley and Tinker 2003). Instead it flourishes there when humans start felling the forest or settle. Forest and bush clearings for implementation of monoculture-based

industrial plantation or in the development or expansion of human settlements and cities lead to destruction of natural oil palm groves with their considerable stock of genes and alleles. In 2007, the Cenipalma/IRAD joint expedition found at Widikum in the southwest region of Cameroon that sub-division offices were located where, in 1984, MPOB had collected accessions with interesting agronomic traits. Indeed, the desired oil palms had ceased to exist.

Development of cities and roads results in the fragmentation of natural oil palm populations. Fragmentation of populations is responsible for the genetic erosion in all plants, including oil palm. Andrew et al. (2000) observed that fragmentation reduces the overall number of individuals and the mean population size and also causes the spatial isolation of remaining populations. The authors opined that the most obvious genetic effects of population fragmentation are the loss of genetic diversity at the population and species level, change in interpopulation structure, and an increase of inbreeding.

Oil Palm Breeding

Breeding is considered a genetic adjustment of plants to social, cultural, economic, and technological aspects of the environment (Frankel 1968, cited by Chaudhary 1984). It is also the management of genetic variability to develop superior varieties (Chaudhary 1984). In both cases, breeding aims are to the passing on of desirable traits of given crops while omitting the undesirable ones. The Dumpy E260, Deli-type material, developed at Elmina estate in Kuala Lumpur, well known for its palms with large girth and slow height increase (Jagoe 1952) was widely distributed among oil palm research centers but abandoned and even felled later for the variability of its fruit-to-bunch ratio and some abnormalities of fruit set. Chaudhary (1984) also noticed that in India with the spread of high-yielding varieties old ones were going out of cultivation and were in danger of being lost.

Abandonment of oil palms with undesirable traits leads to reduction of the size of base populations. Such small populations experience genetic drift because alleles at very low frequencies are likely to be lost (Bakoumé et al. 2014), resulting in a loss of genetic diversity (Namkoong et al. 2000). Small populations may not bear enough variability to respond to changing environmental conditions (Outi and Helmi 2000) or to new consumer demands. Bakoumé et al. (2006) found alleles at high frequencies ($p \geq 0.75$) in Deli MPOB, Deli Dabou, La Mé, and Bahia breeding materials were found only at low to intermediate frequencies in the natural oil palm populations. They suggested that several generations of selection may have favored these alleles in these materials. On the other hand, alleles that were rare in Deli MPOB breeding materials were common in natural oil palm populations, suggesting their reduction after many years of selection. Deli materials which had already undergone several generations of selection exhibited the lowest expected heterozygosity in comparison with natural oil palm collections from seven African countries ($H_e = 0.501$ vs. 0.716; Cochard et al. 2009) and with MPOB's natural oil palm collections ($H_e = 0.554$ vs. 0.691; Bakoumé et al. 2014).

The smaller the population on a relative scale, the more magnified the effect of genetic erosion because non-selected oil palms are not given the chance to pass individual alleles and combinations of alleles to any descendants.

The oil palm industry is interested in planting materials with high kernel-to-fruit ratio, reduced palm height, tolerance to *Ganoderma*, high iodine value, high oleic acid, carotene and lipase content, vitamin E, long stalk, and non-abscission of fruits for higher productivity. Unfortunately, the base populations of current breeding programs can only poorly respond to these requirements due to their narrow genetic diversity. In Côte d'Ivoire, only five original palms chosen from a survey made in the Bingerville region including Bingerville Botanic Gardens and also from the wild palm grove are still represented in the La Mé breeding population (Cochard et al. 2000). The Institut National pour l'Étude Agronomique du Congo Belge (INEAC) in the Democratic Republic of Congo (formerly Zaire) planted ten open-pollinated *tenera* bunches: one from the famous Djongo (meaning 'the best') and nine from groves at Yawenda planted at Palmeraie de la Rive at Yangambi in 1921; but in the end, the selected palms were all descended from the Djongo palm (Hartley 1988). At the N'dian (Cameroon) oil palm research station, out of a total of 35,000 palms recorded for yield and bunch characteristics, crosses and selfs of only 19 *tenera*, six *dura*, and two fertile *pisifera* were selected for the next generation of selection thus leading to a reduction of the original genetic diversity.

1.1.5 Conservation of Oil Palm

1.1.5.1 Conservation of Breeding Oil Palm Materials

Oil palm breeding populations that have been developed from few original palms have therefore been termed "breeding populations of restricted origin (BPRO)" by Rosenquist (1986). The main interest of both public and private breeders, including those of oil palm plantations in any country, is in germplasm which can be utilized for attaining specific goals. In fact, very few original *dura* female parents and a handful of *tenera*/*pisifera* male parents constitute the pedestal on which rest commercial planting materials worldwide. The source of females (Deli *dura*) was four palms planted in Bogor Botanic Gardens, Java, in 1848 (Wood 1981). The ten *tenera*/*pisifera* male parents originated mostly from Djongo (SP540), Binga, and Yangambi (Democratic Republic of Congo), Bamenda, Ekona, N'dian (syn. Lobé), and Widikum (Cameroon), NIFOR (Nigeria), La Mé (Cote d'Ivoire), Pobè (Benin), and Sibiti (Republic of Congo). Descendants of these original *dura* and *tenera*/*pisifera* are kept by the oil palm research institutions of all countries. Conservation of a whole breeding population is almost not achievable. In practice, only selected oil palms are given particular attention for conservation; they are selfed or crossed with each other for seed production and next generations of selection.

At Marihat Baris (Indonesia) estate, 15 out of 2000 palms planted in 1915 were selected and self-pollinated (Hartley 1988). High priority is given to at La Me

Fig. 1.14 **a** Visiting seed producer only interested to LM2T, **b** Kissing LM2T a pride for a visiting breeder to La Me (Côte d'Ivoire) (*source* Claude Bakoume)

(Côte d'Ivoire) and La Dibamba (Cameroon) to conserving descendants from DA128D (128th *dura* selected at Dabou in Côte d'Ivoire) for their tolerance to heart rot disease in Latin America. In Côte d'Ivoire, six original oil palms (LM2T, LM5T, LM7T, LM9T, LM10T, LM13T) from the prospection carried out at Bingerville (Côte d'Ivoire) planted at La Me oil palm research station in 1926 and their descendants from self-pollinations or crosses constituted the sole sources of pollen for commercial seeds till 2003 (Bakoumé et al. 2006). LM2T or second genitor of *tenera* fruit form selected at La Me (Côte d'Ivoire), which represents the source of more than 95 % of Deli x La Me planting materials and the unique source of pollens tolerant to *Fusarium* wilt, is the most protected oil palm in the plot among the six. Its trunk is supported by numerous cables from the base to the top of the trunk (Fig. 1.14). Palms LM7T and LM9T were killed by *Fusarium* wilt without apparently constituting a great loss for La Me's breeding and seed production programs although Namkoong et al. (2000) have stressed that large population sizes are key for the conservation of oil palm populations for future adaptability.

1.1.5.2 Conservation of Natural Oil Palm Collections

Domesticated plant species such as oil palm depend upon the broad genetic base found in their wild relatives. For example, in 1970 the maize crop of the United States with very restricted genetic base was severely threatened by corn blight. However, improvement of tolerance of cultivated varieties was possible and thanks to the introduction of genes for tolerance to corn blight from Mexican wild and less inbred varieties of maize (Maxted et al. 1997). The earliest recorded plant expedition in the world is likely the one organized by Queen Hatshepsut of Egypt in 1500 B.C. that sought incense trees in East Africa (Chaudhary 1984). In oil palm, the main interest of both public and private breeders is in (oil palm) germplasm

collections consisting of different types of variability for yield, yield components, plant height, maturity, resistance to disease, pests and other stress conditions, and quality (Gill 1989). Oil palm research stations, centers or departments in most countries have acquired natural oil palm collection materials through collaboration, exchanges, and expeditions carried out in the African oil palm belt.

A total of about 2790 accessions have been collected from the natural oil palm groves of 11 African countries between 1973 and 2011 (Table 1.9). Expeditions have mostly been initiated by oil palm research institutes from Malaysia, Indonesia, and Colombia. However, Nigeria, Ghana, and Côte d'Ivoire have also organized supplementary collections. Prior to its own expedition, Côte d'Ivoire received some of the natural oil palms jointly collected in 1973 by MPOB and NIFOR.

To date, collection from natural oil palm groves spans a long band from Senegal in the extreme west to Tanzania in the east as far as Madagascar. Countries of the African natural oil palm belt not yet explored include Liberia, Togo, Equatorial Guinea, Gabon, Republic of Congo, and Central African Republic. The planting of the 2790 accessions at the rate of 50 oil palms (or seedlings) minimum per accession at 148 oil palms per hectare requires a total of 1886 ha, i.e., 943 ha in each of the

Table 1.9 Accessions collected from natural oil palm groves in Africa

Exploring organization (country)	Country explored	Year	Number of accessions
MPOB (Malaysia)	Nigeria	1973	919
	Cameroon	1984	95
	Zaire	1984	369
	Tanzania	1986	60
	Madagascar	1986	17
	Angola	1991	54
	Senegal	1993	104
	Gambia	1993	45
	Sierra Leone	1994	56
	Guinea	1994	61
	Ghana	1996	58
CENIPALMA (Colombia)	Angola	2002	137
	Cameroon	2007	74
IPOB (Indonesia)	Cameroon	2008	103
	Angola	2010	127
CSIR (Ghana)	Ghana	2003–2011	347
NIFOR (Nigeria)	Nigeria	1991, 2004	95
CNRA (Côte d'Ivoire)	Côte d'Ivoire	1969, 2011	69
Total			2790

MPOB Malaysian Palm Oil Board, Cenipalma Centro de investigación en palma de aceite (Centre for Oil Palm Research) (Colombia), *IPOB* Indonesian Palm Oil Board, *CSIR* Council for Scientific and Industrial Research (Ghana), *NIFOR* Nigeria Institute for Oil Palm Research, *CNRA* Centre National de Recherche Agronomique (National Centre for Agronomic Research) (Côte d'Ivoire)

group of exploring countries and group of explored countries. (Note that an accession corresponds to a palm from which a ripe bunch bearing numerous fruits has been collected.) Vegetative parameters, bunch production, and bunch characteristics must be recorded in the framework of the evaluation of the accessions. This work will last at least 10–12 years after planting. In this process, putative interesting genotypes are identified and taken care of, ensuring that one main objective of the explorations of natural oil palm groves in Africa is met: to ensure conservation of a wide range of oil palm genetic resources for posterity.

1.1.5.3 Constraints to Oil Palm Conservation ex situ

Problems inherent to conservation of oil palm ex situ include (i) the limited storage period of the seeds, (ii) the large space required; only 148 palms are planted per hectare, and (iii) the upkeep costs. The height of oil palm is also a limiting factor to its exploitation when it is more than 25 years. In 2003, I had to use three aluminum poles connected end to end (3 × 5.6 m) to collect leaflets for DNA extraction from 28-year-old oil palms selected from Nigeria natural oil palm materials planted at Kluang (Malaysia) in 1975. The long-term in vitro conservation of zygotic and somatic embryos in liquid nitrogen at −196 °C (cryopreservation) tested experimentally by MPOB and the French IRD/CIRAD (Institut de recherche pour le développement/Centre de coopération internationale en recherche agronomique pour le développement) is not yet conclusive or of routine use. Storage of seeds rarely exceeds 2 years as germination rate diminishes with time. Hence, beyond 2 years, seeds are sold by seed producers in the form of germinated seeds only (Bakoumé et al. 2008). Ex situ field planting is by far the most popular oil palm conservation method currently used. Once evaluation of natural oil palm collections is completed, selected oil palms are utilized for broadening the genetic diversity of breeding populations and initiating new breeding programs (Rajanaidu et al. 2000). Non-selected oil palms are simply abandoned and felled when space is needed for planting of new materials. On rare occasions, non-selected oil palms are harvested if the yield and height justify it.

To ensure adequate space as well as cost-effective conservation of natural oil palm resources ex situ, Bakoumé (2006a, b, c) proposed an assessment of the genetic diversity and genetic structure of new collections at the pre-nursery level when seedlings are 3–4 months old. He recommended limiting the number of populations to plant for the field genebank to those with high allelic diversity, rare alleles, and high heterozygosity, ensuring a good representation of the total genetic variation and desired agronomic traits existing in the oil palm collections. According to the author, high genetic diversity implies a high level of additive genetic variance, upon which progress in oil palm breeding depends. This diversity is also a source of novel genes for oil palm improvement, The assessment of the genetic diversity and genetic structure of MPOB oil palm germplasm materials using microsatellite markers showed that out of 213 accessions from natural groves from ten African countries sampled for the study, 25 (12 %)

were representative of the entire sample with negligible loss of allelic diversity and genetic diversity. Indeed, there are considerable efforts towards the development of molecular tools (molecular markers, proteomics, lipodomics, and metabolomics), which would ease the very precise identification of desired candidate palms for introgression in the breeding program.

1.1.5.4 Conservation of Natural Oil Palm Groves in Situ

At the village level, a palm growing naturally on farmland, whether actual or abandoned, and near a dwelling belongs to the owner of that farmland or dwelling. Furthermore, oil palm is understood to belong to the person who first removed the leaf bases from the rough stem to facilitate the climbing, pruning or harvesting of its fruit bunches (Bakoumé 2006a, b, c).

Locals in Africa have already become concerned with the conservation and sustainability of natural oil palms in view of the income generated and the variety of products offered to them. Although no or very few plantings were carried out, locals generally have taken measures to ensure the existence and exploitation of natural oil palm groves for present and future generations. These measures have included maintenance of existing palm trees, creation of suitable conditions for the development of seedlings, interdiction against cutting seedlings during bush clearing or weeding of farms, and selection of mature palms to be felled for palm wine production. Bakoumé (2006a, b, c) reported that one peculiarity in Africa where crude palm oil is generally consumed by households is that red crude palm oil produced from natural oil palms is preferred to that obtained from selected *tenera*. "Man red palm oil," produced from natural palm groves of the Man region in Côte d'Ivoire, has gained popularity in local dishes. In Liberia, crude palm oil extracted from improved *tenera* is believed to be suitable only for the manufacture of soaps. These considerations add to the paramount importance to Africans in the continent's oil palm belt of conservation in situ of natural oil palm groves (Fig. 1.15)

Fig. 1.15 Dense spontaneous African oil palms in an *Elaeis guineensis* grove (*source* Claude Bakoume)

and their associated genetic diversity. Most of the oil palms from which bunches were collected during organized expeditions have been preserved by locals wherever they participated to the collection.

1.2 Conclusion

Genetic diversity is still on the high side in oil palm breeding materials and natural groves. There has been no loss yet of a fruit form or of a fruit type. Variation of morphological and agronomic traits seems to have been conserved in breeding materials and, over time, in natural groves, as indicated by assessments of oil palm collections of 1900s and 2000s. Genetic erosion is furthered by pests and diseases common or specific to current areas of distribution of cultivated and natural oil palms. Road construction, development of cities, and the monoculture practice typical of modern agriculture lead to the destruction of oil palm natural habitats in Africa. Selection carried out in the germplasm oil palm materials results in the abandonment of non-selected genotypes with inevitable loss of alleles and genes. Emphasis must be put on long-term preservation of maximum genetic diversity within the field genebank composed of breeding materials and natural oil palm collections. Given the large space needed and maintenance costs related to ex situ conservation of oil palm germplasm, molecular marker techniques, and agronomic evaluation will be very useful in determining and reducing the number of populations to conserve while maintaining maximum genetic, morphologic, and agronomic variability.

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Chapter 2
Genetic Diversity, Genetic Erosion, and Conservation of the Two Cultivated Rice Species (*Oryza sativa* and *Oryza glaberrima*) and Their Close Wild Relatives

Ahmadi Nourollah

Abstract Rice cultivated gene pool includes two species. Asian rice, *Oryza sativa,* displays a very large phenotypic diversity resulting from a long history of domestication driven by human demographic expansion and sympatry with its wild relatives. African rice, *Oryza glaberrima,* represents a typical case of domestication bottleneck. Recent sympatry of the two species in Africa has given birth to new diversity. Current rice in situ genetic diversity results from the succession of a number of long-standing evolutionary events and the contemporary reversal of the trend of increasing diversity, referred to as genetic erosion. Since the early twentieth century, human demographic growth, agricultural modernisation and the advent of formal breeding systems, have affected the in situ diversity of cultivated rice species and their wild relatives. The evolutionary processes had produced a very large number of Landraces (LV) of which some 500,000 are conserved ex situ. The contemporary changes have resulted in the replacement of a large proportion of LV by a small number of Modern varieties (MV) in more than 70 % of rice-growing areas in Asia and Latin America, 38 % in Africa. The most important feature of rice in situ diversity emerging from our case studies in China, South and Southeast Asian countries, West Africa and Madagascar, is the diversity of situations. Aggregated data suggest massive absolute genetic erosion and sharp reduction of diversity indexes, particularly in irrigated ecosystems. Detailed surveys indicate smoother genetic erosion in rainfed ecosystems. However, the perspectives of rice in situ genetic diversity are gloomy even in rainfed ecosystems. The most realistic and promising option for the future is a dynamic management in the framework of the emerging concept of *ecological intensification.*

Keywords Rice · *Oryza sativa* · *Oryza glaberrima* · Genetic erosion · In situ diversity

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2.1 Introduction

Several types of events in the history of a crop lead to the diversity levels found at the present time. Such long-standing events include the domestication bottleneck (as only a subset of the diversity in the progenitor found its way into the domesticated species), directional selection (that affects key domestication genes, such as those regulating fruit size), dispersal bottlenecks (in which a small founder population experiences intense selection for agronomically desirable characteristics) and gradual increase of genetic diversity as a consequence of gene flow between the domesticated species and its wild relatives, or as a consequence of de novo generation of diversity through mutations and recombinations (van de Wouw et al. 2009).

A more contemporary event that has dramatically affected crop diversity found at the present time in farmers' fields, or in situ diversity, is the modernization of agriculture. It started in the middle of the nineteenth century in Europe and North America leading to the replacement of the large number of local varieties or landraces (LV) of major crops by a small number of modern varieties (MV). At present, in North America and northwestern Europe, LVs have become almost absent (Evenson 2003). In Asia and other developing countries, the phenomenon started in the beginning of the twentieth century and gained momentum in the 60s with the advent of the Green Revolution.

Taking place in the centers of genetic diversity of major food crops, the Green Revolution raised concerns about the survival of the genetic resources of those species (Harlan 1975). The perception of this threat gave birth to the concept of genetic erosion describing the process of loss of genetic diversity in agriculture (Pistorius 1997). It also gave impetus to national and international initiatives for collection and ex situ conservation of genetic resources on the one hand, for the in situ conservation of the LVs by farmers, on the other hand. Analyzing data from 27 crop species from five continents, to determine overall trends in crop varietal diversity on farm, Jarvis et al. (2008) found that for all crops, LVs dominated the planting area (from 80 to 100 % of the total crop area). The exception was rice, for which the range was from 7 to 100 % across the six sites.

About half of the world's population relies on rice as their staple food and rice cultivation provides livelihood for millions of people. Thanks to the extremely large morphological and physiological diversity of its ecotypes, rice is cultivated in a very broad range of environmental conditions in tropical, subtropical, and temperate regions around the world, in more than 100 countries and on every continent except Antarctica (Maclean et al. 2002). Rice cropping areas stretch from the latitude of 40° south, in Argentina, to 53° north in China; from the seaside to almost 3000 m of altitude in Nepal; from deepwater swamps (5–6 m deep) to strictly aerobic soils of steep slope in mountainous tropical areas; and from very acidic soils to the brackish waters of mangrove zones (Fig. 2.1). Likewise, its large diversity allows meeting a very broad range of grain quality requirements.

Being a major food crop, rice genetic diversity has undergone all of the abovementioned impoverishment and enrichment events. Its large phenotypic diversity

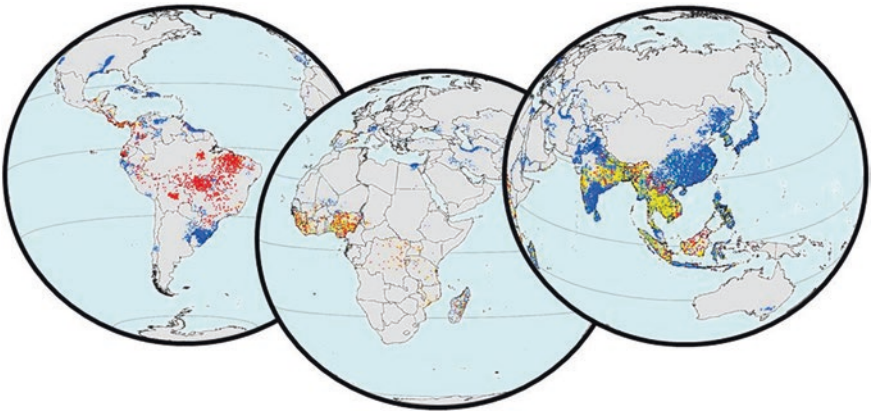

Fig. 2.1 Major rice growing areas and cropping ecosystems. Each *dot* represents 5000 ha of rice. *Blue* irrigated ecosystem; *Yellow* rainfed lowland ecosystem; *Red* rainfed upland ecosystem (After Rice Almanac; 2013)

results from a long history of domestication, driven by human demographic expansion, and by sympatry of the cultivated rice ecotypes with their wild relatives. Moreover, there are two species of cultivated rice, *Oryza sativa* L. or Asian rice, and *Oryza glaberrima* Steud or African rice, with distinct domestication histories. Last but not least, sympatry of these two species during the last five centuries in Africa has also given birth to a new original diversity.

This paper will provide a reminder of rice genetic diversity and of its early evolution, and present the post-Green Revolution evolution of rice in situ genetic diversity through a number of case studies. The extent of the effects of the modern breeding efforts on rice in situ diversity will be discussed. As wild species of *Oryza* have proved to be an important source of genes that add value to the cultivated rice genome, the gene pool considered includes the two cultivated rices and their close wild relatives.

2.2 Rice Gene Pool, Genetic Diversity, and Ex Situ Conservation

The genus *Oryza* is divided into four species complexes: the *O. sativa*, *O. officialis*, *O. ridelyi,* and *O. granulata* species complexes (Table 2.1). All members of the *Oryza* genus have a multiple of $n = 12$ chromosomes. While fertile offspring can be obtained rather easily from interspecific crosses within each complex, this is much more difficult in across-complexes crosses (Vaughan et al. 2003). The two cultivated rice species *O. sativa* and *O. glaberrima* belong to the *O. sativa* complex together with six closely related diploid wild species of the AA genome group: *O. nivara* and *O. rufipogon* present throughout Asia and Oceania; *O. barthii*

Table 2.1 Classification and distribution of species in the genus *Oryza*

Taxa	Genome	Distribution
Sativa species complex		
O. sativa	AA	Worldwide
O. glaberrima	AA	West Africa
O. nivara	AA	Tropical Asia
O. rufipogon	AA	Tropical Asia
O. meridionalis	AA	Tropical Asia to Northern Australia
O. barthii	AA	Africa
O. longistaminata	AA	Africa
O. glumaepatula	AA	South America
Officinalis species complex		
O. minuta	BBCC	Philippines, Papua New Guinea
O. officinalis	CC	Tropical Asia—Papua New Guinea
O. rhizomatis	CC	Sri Lanka
O. malampuzhaensis	CCDD	India
O. punctata	BB	Africa
O. schweinfurthiana	BBCC	Africa
O. eichingeri	CC	Africa, Sri Lanka
O. alta	CCDD	Central and South America
O. grandiglumis	CCDD	South America
O. latifolia	CCDD	Central and South America
O. australiensis	EE	Australia (*Australiensis* section)
O. brachyantha	FF	Africa (*Brachyantha* section)
O. schlechteri	HHKK	Indonesia and Papua New Guinea
O. coarctata	KKLL	South Asia to Myanmar (*Padia* section)
Ridleyi species complex		
O. longiglumis	HHJJ	Indonesia and Papua New Guinea
O. ridleyi	HHJJ	Southeast Asia—Papua New Guinea
Meyeriana species complex (*granulata species complex*)		
O. granulata	GG	South and Southeast Asia
O. meyeriana	GG	South and Southeast Asia
O. neocaledonica	GG	New Caledonia

endemic in West Africa; *O. longistaminata* found throughout Africa; *O. meridionalis* native to Australia and *O. glumaepatula* endemic in Central and South America. Divergence between *O. glaberrima* and *O. sativa* goes back to 0.6–0.7 million years (Zhu and Ge 2005; Ammiraju et al. 2008). The *O. officinalis* complex comprises five diploids BB, CC, and EE genomes, and six tetraploids with BBCC or CCDD genomes. The remaining species are more distantly related to the cultivated species, with genomes FF, GG, HHJJ, and HHKK.

The centers of species diversity and genomic diversity are the islands from Southeast Asia to the Pacific Ocean. Nine of the 24 wild relatives of rice occur in

Indonesia, and 7 of the 10 genome types are found in the Asian–Pacific islands. In addition, distinctive sets of species assemblages are associated with South Asia, Africa, and the Americas: each continent has its own set of wild species, and only one wild species (*O. eichingeri*) is found in more than one continent.

The geographic distribution of each cultivated species coincides with the ones of an annual autogamous and a perennial allogamous wild species of *O. sativa* complex: respectively, *O. nivara* and *O. rufipogon* for *O. sativa*; *O. barthii* and *O. longistaminata* for *O. glaberrima*. These wild species constitute the two ancestral pools that were directly subject to domestication and gave birth to the two cultivated species, though there is still debate over the relative contribution of the annual and perennial ancestors to domestication, especially in the case of *O. sativa*.

2.2.1 Asian Rice Gene Pool and Genetic Diversity

In Asia, *O. rufipogon* grows in perennial swamps across a broad geographic range spanning eastern India, Indochina, and portions of southern China. Men originally harvested it by continuous rationing. The domestication process started some 10,000 years ago by planting rice seeds outside those permanent wetlands, in seasonally wet terrain where selection for the annual growth habit that characterizes *O. sativa* took place. Out-planting away from wild stands would also have allowed selection toward non-shattering to be retained more easily with each successive monsoonal planting season (Allaby et al. 2008). Thus, the very process of radiation and migration on the part of humans was an essential part of the domestication process for rice right from the start.

The early spread of the Asian rice is tightly associated with the outflow of Neolithic lifestyles in the eastern Asian region, in a "spread, pause, adapt, spread, pause again" mode, in relation with environmental barriers and constraints (Bellwood 2011). The major steps are (i) 8000–6000 BC: pre-domestication of *japonica* rice in China, the Yangzi, Han, Huai, and lower Yellow River basins, (ii) 6000–3500 BC: gradual spread of Neolithic lifestyles through southern China, accompanied by an increasing predominance, especially after 4000 BC of fully domesticated (non-shattering) rice, (iii) 3500 BC: Neolithic settlement of Taiwan, presumably following developments in Fujian and/or Guangdong, (iv) 3000–2000 BC: Neolithic settlement of mainland Southeast Asia from Guangdong and Guangxi into northern Vietnam, and possibly down the Mekong river into southern Vietnam and Thailand, (v) 2000–1500 BC: Neolithic settlement of the Philippines and central Indonesia, via Taiwan, (vi) 500 BC establishment of wet rice cultivation in regions of high population growth such as Java and Bali.

Although domestication of the *indica* subspecies from annual forebears within a vast region south of the Himalayas Mountains (likely eastern India, Myanmar, and Thailand) takes place as early as 7000–4000 BC, it does not make an appearance in Southeast Asia until about 2000 years ago, contemporary with early contacts with India.

The domestication bottleneck was probably not very severe due (i) to large effective population sizes during the domestication process, as large quantities of grain were needed for subsistence and (ii) very likely multiple domestications. Directional selection then played a relatively important role. For instance, in glutinous rice the *waxy* locus shows a reduced nucleotide variation compared to other unlinked genes in the rice genome (Olsen and Purugganan 2002). Foundation effects are clearly visible when local or country level rice diversity is compared to global rice genetic diversity (Barry et al. 2007a; Radanielina et al. 2013a). Gene flow between *O. sativa* and its wild relatives have certainly played an important role in shaping the diversity of the cultivated rice LVs. Analyzing the DNA sequence variation in *O. sativa* and *O. rufipogon*, across 111 randomly chosen gene fragments, Caicedo et al. (2007) observed a genome-wide excess of high-frequency derived single nucleotide polymorphisms (SNPs) in *O. sativa* varieties. They concluded that the simple bottleneck model could not explain the derived SNP frequency spectrum in rice. Instead, a bottleneck model that incorporates selective sweeps, or a more complex demographic model that includes subdivision and gene flow, offers more plausible explanations for patterns of variation in domesticated rice varieties.

Later on, as *O. sativa* spread around the world, it has, especially the *indica* form, differentiated into a great number and diversity of LVs. *O. sativa* reached Madagascar (through India) and Europe (through Greece and Italy, and subsequently Spain) over 2000 years ago, and subsequently spread to other parts of Africa through Mozambique and to other countries of southern Europe. Secondary centers of distinctive *indica*-like diversity are particularly notable in Madagascar and Sri Lanka. More recent distinctive secondary centers of diversity are also apparent in Africa and the Americas. One of the most recent introductions, slightly over 100 years ago, is to Australia.

O. sativa displays a very large phenotypic diversity. This phenotypic diversity is associated with differentiation into two major genotypic groups, the *indica* and *japonica* types (Oka 1983). Given the low level of fertility of *indica* × *japonica* hybrids, they are also referred to as subspecies. Surveying polymorphism at 15 isozyme loci in a sample of 1688 LV, Glaszmann (1987) distinguished, besides the two subspecies, some other minor groups such as *aus-boro* and *aromatic* (Fig. 2.2). The origins of these minor groups are still a matter of research and debate. The *indica–japonica* differentiation is also associated with ecological specialization (Khush 1997).

- The *indica* group, particularly diverse, is widespread across the tropical lowlands.
- The *japonica* group, less diverse, comprises two subgroups: the tropical subgroup cultivated in the upland ecosystems of tropical regions; and the temperate subgroup cultivated in the lowland ecosystem of countries such as Japan, Europe, and the USA.
- The *aus-boro* rice of the Bangladesh region.
- The *aromatic* rice from the Iran–Afghanistan–Pakistan–Nepal–North India region.

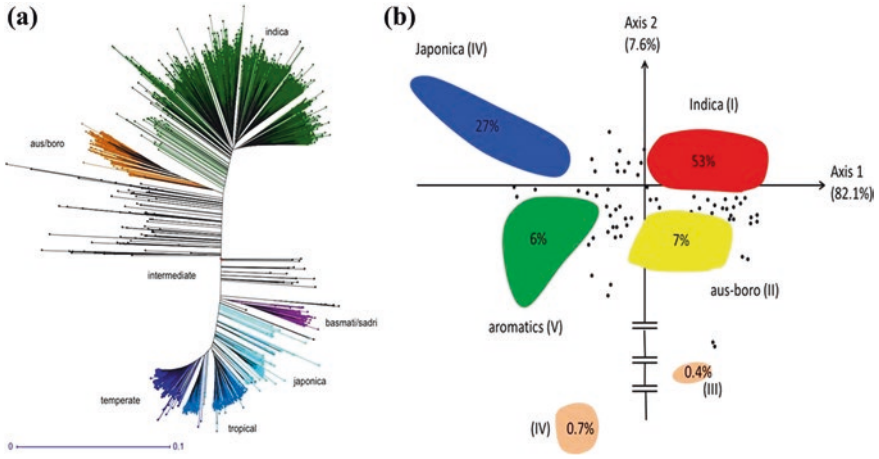

Fig. 2.2 Structuration of *O. sativa* genetic diversity. **a** Classification of 3000 rice accessions into five distinct varietal groups based on 5 sets of 200,000 random sets of SNP from the 18.9 million discovered SNP variants discovered (After 3000 rice genomes project, 2014). **b** Projection of 1688 Asian rice accessions on the first plane of a factor analysis of correspondences of isozyme variation at 15 loci. Sizes of the groups are indicated. *Isolated dots* represent 90 varieties with intermediate positions or unstable classification (After Glaszmann1987)

Using genotypic data at 169 SSR loci in a sample of 232 accessions, Garris et al. (2005) confirmed the above-mentioned classification and showed that 37.5 % of the variation was due to differences among groups with the remaining 62.5 % due to differences within groups. Differentiation between groups estimated with pairwise F_{ST} statistics was high between groups with values ranging from 0.20 to 0.42. The lowest F_{ST} were observed between *temperate and tropical japonica* (0.20) and between *aus* and *indica* (0.25). The five groups are not endowed with the same amount of intragroup diversity (Table 2.2). The *indica* group has the highest intragroup diversity, followed by the tropical *japonica* and *aus-boro* Garris et al. (2005). Recently, Zhao et al. (2011) tagging the amount of intragroup genomic variation, by an array of 44,000 SNPs across 413 diverse accessions of *O. sativa* collected from 82 countries and measuring it by the pairwise SNP linkage disequilibrium (LD) among these SNPs, confirmed the particularly high diversity of the *indica* group (Table 2.2). On average, LD drops to almost background levels around 500 kb–1 Mb, reaching half of its initial value at about 100 kb in *indica*, 200 kb in *aus-boro* and tropical *japonica*, and 300 kb in temperate *japonica* (Zhao et al. 2011). The structuring of *O. sativa* in 5 groups (*indica*, tropical *japonica*, temperate *japonica*, *aus-boro,* and *aromatic*) was confirmed by the most recent and massive genotypic data produced in the framework of the 3000 rice genomes project (Fig. 2.2). The geographical distribution of the different genetic groups in major rice-growing areas of Asia is presented in Fig. 2.3.

This structuring of rice diversity into several groups with unequal intragroup diversity results from its autogamous reproduction system, its domestication

Table 2.2 Diversity parameters of the 5 major genetic groups within *O. sativa*

Study	Diversity parameters	*Aus*	*Indica*	*Aromatic*	*Japonica*		Adm
					Trop	Temp	
1	Sample size	57	87	14	96	96	62
	Private SNPs	822	1851	77	398	376	
	Polymorphic SNPs	23,270	30,449	12,059	24,813	14,688	
	MAF ≥ 0.05	18,012	20,259	12,039	13,051	7775	
2	Sample size	21	79	19	41	48	24
	No. of alleles/locus	5.1	7.3	3.4	4.9	6.1	
	Gene diversity	0.54	0.55	0.39	0.39	0.47	
	Average PIC value	0.52	0.52	0.38	0.37	0.46	
3	Sample size	48	124	52	43	43	63
	Gene diversity	0.15	0.19	0.11	0.16	0.12	

1: Zhao et al. (2011); 2: Garris et al. (2005); 3: Ahmadi et al. (2013). *Adm* admixes. Private SNPs are unique to one specific group; Polymorphic SNPs are considered to be those that segregated in one specific group, irrespective of whether they also segregate in another group; *MAF* minor allele frequency

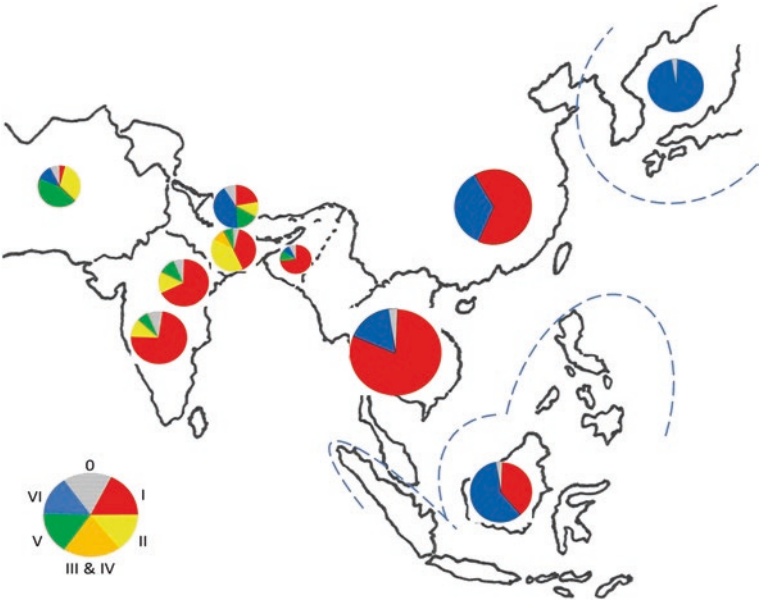

Fig. 2.3 Geographical distribution of the six varietal groups in major rice growing areas of Asia based on isozyme variation at 15 loci, in 1688 Asian landraces. I: *indica*; II: *aus*; III and IV: deepwater; V: *aromatic*; VI: *japonica*; 0: unclassified varieties (After Glaszmann 1987)

history, and the independent population histories of different groups. For example, the source of high level intragroup variation in the *indica* group relative to the others, with no evidence of a genetic bottleneck, could include mitigation of the domestication bottleneck by gene flow due to sympatric wild relatives or

a historically larger effective population size due to overland dispersal routes. Likewise the high level of intragroup diversity of *aus-boro* despite its small area of distribution argues for independent domestication.

While the existence of these groups is not contested, debate about their origin(s) continues. Indeed, while the domestication of *O. sativa* goes back to an estimated 10,000 years (Higham and Lu 1998; Sweeney and McCouch 2007), the seniority of the *indica-japonica* differentiation within its wild ancestor *O. rufipogon* has been estimated at more than 100,000 years (Wang et al. 1992). Based on this early differentiation, it was often concluded that *O. sativa* had undergone two independent domestications from the divergent pools of *O. rufipogon* (Second 1982; Cheng et al. 2003). However, considering the domestication not as an event but as an evolutionary process promoted by interactions between plant and man, Oka (1988) suggested multiple and diffuse domestications of *O. sativa*, in a large area stretching from the Himalayan footsteps of India to China. The latest in-depth analysis of the domestication sweeps and genome-wide patterns based on genome sequences from 446 geographically diverse accessions of *O. rufipogon* and from 1083 cultivated *indica* and *japonica* varieties reveals that the *japonica* group was first domesticated from a specific population of *O. rufipogon* around the middle area of the Pearl River in southern China, and the *indica* group arose subsequently from crosses between *japonica* rice and local wild rice as the initial cultivars spread into South East and South Asia (Huang et al. 2012).

Whatever the early history of domestication of *O. sativa*, by the mid-twentieth century, selective pressure exerted by man and the new environments he colonized had given birth to hundreds of thousands of LVs, each adapted to the specific environmental conditions and cropping requirements of a small agricultural area. No direct statistics regarding the number of such *O. sativa* rice LVs are available neither at the level of individual countries nor at the global level. A rough estimate of the minimum number is provided by the number of accessions of *O. sativa* genetic resources being conserved in international and national genebanks around the world: over 500,000 in 2007 (IRRI 2010).

2.2.2 African Rice Gene Pool and Genetic Diversity

In Africa, *O. glaberrima* was domesticated independently from *O. barthii* (syn. *O. breviligulata*) in the inland delta of the upper Niger River, in what is today Mali, some 2000 or 3000 years ago. The species then spread to two secondary centers of diversification, one on the coast of Gambia, Casamance and Guinea Bissau, the other in the Guinean forest between Sierra Leone and western Ivory Coast (Portères 1970; Second 1982).

O. glaberrima represents a typical case of reduction of genetic diversity observed in crops compared to their wild progenitors because of dual bottlenecks imposed by domestication and breeding (Buckler et al. 2001; Zeder et al. 2006). Indeed, based on isozyme, RFLP, SSR and MITE markers, all previous studies

have found dramatic reduction in genetic diversity associated with the domestication of African rice and have revealed substantially lower genetic diversity in African than in Asian rice (Second 1982; Wang et al. 1992; Ishii et al. 2001).

The most recent analysis of diversity comparing SNP variation in of independent genes between *O. glaberrima* and *O. barthii* showed that both cultivated and wild African rice maintained extremely low levels of nucleotide diversity. Moreover, genetic loss in African rice is much more severe after domestication, with 76 % less diversity in the domesticated species than in its wild progenitor (Li et al. 2011). An obvious explanation for the low genetic diversity of *O. glaberrima* would be a severe genetic bottleneck during its domestication from small initial populations of *O. barthii*. The ecogeographical diversity seems so low that clustering analysis is unable to refine the domestication place and dispersion of *O. glaberrima* (Li et al. 2011). Therefore, the hypothesis developed by Portères (1970) remains today the most probable considering that African rice was first domesticated in the inland delta of the upper Niger River and subsequently spread in two secondary centers along Sahelian rivers and their tributaries.

Genome-wide LD investigated in 198 accessions of *O. glaberrima* using 93 SSR markers (Mande et al. 2005) detected very high levels of LD among distantly located loci, separated by more than 100 cM (~25,000 kb). Free recombination among loci at the population genetic level was shown (i) by a lack of decay in LD among markers on the same chromosome and (ii) by a strictly increasing composite likelihood function for the recombination parameter. This suggested that the elevation in LD was due not to physical linkage but to other factors, such as population structure. Structure analysis using the Bayesian clustering analysis approach confirmed this hypothesis, indicating that the sample of *O. glaberrima* in this study was subdivided into at least five cryptic subpopulations. Two of these subpopulations clustered with control samples of *O. sativa*, subspecies *indica* and *japonica*, indicating that some *O. glaberrima* accessions represent admixtures. The remaining three *O. glaberrima* subpopulations were significantly associated with specific combinations of phenotypic traits—possibly reflecting ecological adaptation to different growing environments and plant type described by Portères (1970): the floating, non-floating, and upland types (Mande et al. 2005).

Until the mid-fifteenth and early sixteenth centuries, *O. glaberrima* was the only rice species grown in West Africa. Some ethnical groups such as the Jola of south Senegal were growing wet rice and using intensive techniques, such as diking to retain rainwater and transplanting, at the time they first encountered Europeans (Linares 2002). Although it is not known with certainty when and where the first varieties of *O. sativa* were introduced into West Africa, the general consensus is that, beginning in the sixteenth century, the new species spread and was adopted by peoples living on the Upper Guinea Coast who had previous experience growing the local African species. Since then, *O. glaberrima* has undergone an extinction process. Given its lower productivity, its high degree of shattering and its red caryopses, not praised by the European merchants and colonizers, *O. glaberrima* was soon relegated to the rank of secondary species cultivated in marginal rice-growing ecosystems (deepwater, depleted upland areas) and/or for specific purposes, such as traditional ceremonies (Linares 2002).

2.2.3 Ex Situ Conservation of Rice Genetic Resources

According to a survey implemented by International Rice Research Institute (IRRI) in 2007, over 500,000 accessions of rice genetic resources are conserved in international and national gene banks around the world (IRRI 2010). The majority are only kept in a small number of gene banks (Fig. 2.4). The largest six gene banks are all in Asia, and together conserve around 70 % of total world holdings. In order of number of accessions, they are: IRRI, the National Bureau of Plant Genetic Resources (NBPGR) in India, the Institute of Crop Germplasm Resources (CAAS) in China, the China National Rice Research Institute (CNRRI), the National Institute of Agrobiological Sciences (NIAS) in Japan, and the Rural Development Administration (RDA) gene bank in the Republic of Korea. These gene banks hold well-organized long-term seed storage facilities.

The three largest collections outside Asia are: Africa Rice, the National Center for Genetic Resources Preservation (NCGRP) in the USA, and Brazil; but these collections are considerably smaller than the large Asian collections, and together they hold only 10 % of the global holdings. The remaining 20 % of global

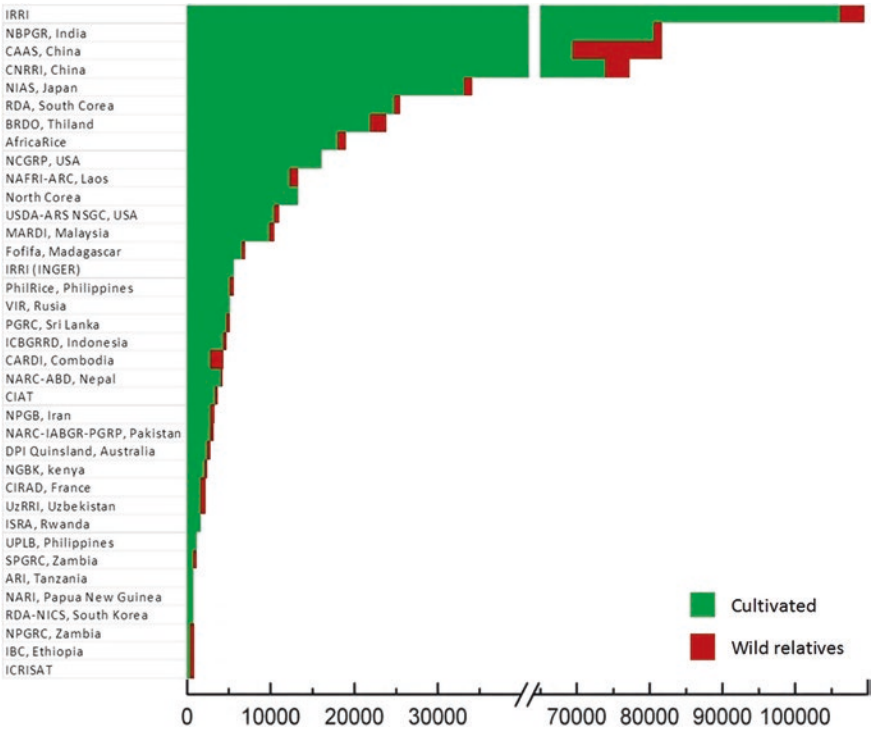

Fig. 2.4 Ex situ conservation of the rice gene pool. Numbers of cultivated or wild rice accessions held at the institutes indicated (as in May 2007). Total number of accessions: 575,029 (After IRRI 2010)

holdings are distributed across a large number of small national collections widely distributed through rice-growing regions of the world.

Accessions of wild rice are conserved ex situ in fewer gene banks, presumably because of the difficulties of their conservation and use. The largest collections of wild rice are at CAAS and CNRRI in China and at IRRI, with other significant collections at the Indonesian Center for Rice Research (ICRR), the Biotechnology Research and Development Office (BRDO) in Thailand, NBPGR India, and NIAS Japan.

In the early 2000s, IRRI took the initiative of developing a Global Conservation Strategy for Rice. After a large array of consultations, the strategy document was completed and distributed for review in April 2010 (IRRI 2010). The initiative was motivated by the need for rice scientists to have free access to the entire rice gene pool, including that of wild rice species, so that they could incorporate desirable genes into varieties that are evermore productive and/or tolerant to abiotic and biotic stresses. The objective is to establish an overarching strategy that will ensure the efficient and effective conservation of rice genetic resources globally and that will identify priority collections for support, upgrading, and/or capacity building. The strategy is intended to be an evolving program of assessment, prioritization, and action with respect to global rice genetic resources.

Key strategic targets are ensuring the global gene pool is securely conserved, ensuring the global gene pool can be effectively used and sharing and cross-referencing information to support joint actions and decisions. Recognizing that gene banks differ in mandate, targets and resources, sharing responsibilities emerges as a potentially important tool to improve efficiency and effectiveness (IRRI 2010).

2.3 In Situ Diversity and Genetic Erosion of Wild Rice Gene Pool

Agricultural modernization, the Green Revolution, and more generally human demographic and economic growth, have not only affected the in situ diversity of the two cultivated rice species but also that of their wild relatives. The habitats of wild species of *Oryza* have been increasingly suffering from the growth of human activities. No data are available about the recent evolution of the habitats of *O. glaberrima* wild relatives in Africa. We report here only on the case of *O. sativa* wild relatives.

Molecular surveys and screening of germplasm from different *Oryza* species has shown broad interpopulation diversity (Oka 1988; Second 1985). However, detailed inter- and intrapopulation genetic studies of *Oryza* species, other than those closely related to the cultivated rices, are lacking primarily because they are less common, populations are small and widely scattered and formerly were not useful to rice breeders. Here we will focus on the *O. rufipogon* case.

O. rufipogon is comprised, like *O. sativa*, of genetically identifiable subpopulations that show strong geographical and ecological differentiation (Banaticla-Hilario et al. 2013). It has a higher genetic diversity at the molecular level than *O. sativa*.

This contrasts with the variation pattern observed at the phenotypic level, in which *O. sativa* is more diverse than *O. rufipogon* (Morishima 2001). Comparison of the genetic diversity of *O. rufipogon* from nine countries in Asia (China, India, Thailand, Burma, Bangladesh, Cambodia, Indonesia, Malaysia, and Philippines), using RFLP markers (Sun et al. 2000), indicated that China's *O. rufipogon* had the largest genetic diversity which was followed by India. The average gene diversity of South Asian wild rice was higher than the one of Southeast Asia. Chinese *O. rufipogon* also had the highest degree of heterozygoty. *O. rufipogon* had 25 % more polymorphic loci and 40 % more alleles per loci than *O. sativa*.

Natural hybridization between wild and cultivated rice occurs frequently and hybrid derivatives are found abundantly as weed types. Gene flow mainly comes from predominantly inbreeding cultivated races to *O. rufipogon* which exhibit an outcrossing rate ranging from 10 to 60 % (Oka 1988). Nowadays, truly wild populations without introgression of genes from cultivated rice in tropical rice-growing areas are very rarely found.

Following the worldwide effort of collection and ex situ conservation of LVs of *O. sativa*, undertaken after the spread of the improved varieties of the Green Revolution, a large number of accessions of wild relatives of the two cultivated rice species were collected during the 70s and 80s. Unfortunately, the momentum for protection of wild relatives of *O. sativa* did not last very long.

During the last 50 years, the habitats of wild species of *Oryza* have undergone rapid destruction due to the extension of rice-growing areas and/or urban development, resulting in local extinction of the wild species. For instance, in Taiwan, *O. rufipogon* was declared extinct in the wild due to urban development as early as the mid-70s (Kiang et al. 1979). In Thailand, a well-studied heterogeneous population of *O. rufipogon* in the northeast regions, showing genic divergence from other *O. rufipogon* populations of the country, was destroyed by the development of an army camp (Morishima 1986). The great flood of 1988 in Bangladesh destroyed many deepwater rice fields. This resulted in a shortage of rice straw for cattle. Populations of wild rice were consequently decimated as a shortage of forage intensified during the dry season. Along the cattle trading route from Tamil Nadu to Kerala in South India, grazing is extremely heavy; only the underwater parts of wild rices remain in many ponds (Vaughan and Chang 1992). In China, despite its abundant genetic diversity and relatively wide distribution, wild rice populations have declined so rapidly that they were listed as "*rare and endangered plants*" in the Chinese Red Data Book of Plant Species (Fu and Jin 1992).

While local extinctions due to destruction of habitats are easily recorded, a more insidious threat to rice wild relatives is the slower environmental changes and population fragmentation, leading to changes in populations' genetic structure.

In Thailand, a diachronic (1985–1994) analysis of the wild rice population in the central plain of the country, using isozyme variability at 17 loci, revealed severe decrease in gene diversity. Fragmentation of the population during the study period of 10 years has not only caused loss of genetic variability but has also forced the habitually outbreeding plants to inbreeding, thus accelerating genetic drift. Likewise, signs of introgression of the wild rice by cultivated rice, blurring

the intrinsic nature of wild rice, were detected. This acknowledgment of rapid genetic erosion has led the authors to call for action in the area of in situ conservation (Akimoto et al. 1999).

In China, gene flow from cultivated rice is considered as one of the most important threats that may alter the genetic structure of natural populations of *O. rufipogon* and eventually lead to its genetic erosion. Effective isolation measures are recommended in the regions where in situ conservation projects are carried out. And reintroduction is proposed as a complementary option to in situ conservation of remaining natural populations (Song et al. 2005). More recently, an extensive field investigation, of 201 natural populations or habitats of *O. rufipogon*, suggests that (i) the majority of the natural populations have been extinct, which has led to serious fragmentation of the population system as a whole; (ii) the surviving populations have become small in size and thus fragmented within the population as a result of the loss of subpopulations and (iii) extinction of wild rice germplasm seems closely related to the biodiversity related education of the rural population (Gao et al. 2012). The authors propose a strategy for in situ conservation that includes rules for selecting conservation sites, enhanced biodiversity education, creation of a positive incentive system for local communities and the involvement of local governments and academic institutions.

In India, the Indo-Gangetic plains are endowed with a great diversity of wild rices, still growing in their natural habitats. It is also one of the most intensively farmed zones of the world and is crucial for food security (Thakur and Pandey 2009). Analysis of diversity of 35 wild rice populations collected in 2011 from natural habitats of eastern Uttar Pradesh and Buxar district of Bihar, using 25 SSR markers and 14 phenotypic traits, clearly demarcated the wild rice accessions into two main groups representing *O. rufipogon* and *O. nivara* (Singh et al. 2013a, b). The widespread distribution of the two species in this region indicates that these species are still secure in the wild but there is great pressure on this habitat due to the developmental needs of the growing human population; risks for the loss of these populations include competition with weeds and land clearing for agriculture and developmental activities. Singh et al. (2013a, b) concluded that urgent action is needed for conservation of this gene pool; more extensive exploration, collection, and careful molecular analysis should be undertaken to ensure that this diverse resource remains available to support rice improvement.

In the Mekong Delta, because of new irrigation schemes dedicated to double or triple rice cropping with modern short duration varieties, the area for deepwater and floating rice has declined drastically. This has also affected *O. rufipogon*, which has been a weed in deepwater rice fields, and the potential for gene flow among the two species (Bui Chi Buu, pers. comm.).

Little information is available about the in situ maintenance of wild relatives of *O. sativa*, in other Asian countries. Vaughan and Chang (1992) advocated in situ conservation of wild *Oryza* species and suggested that priority should be given to *O. longistaminata* in Africa and to O. *rufipogon* in Asia as these species are genetically heterogeneous and difficult to conserve ex situ. They proposed a list of high priority sites in different Asian countries where several different *Oryza* species

occur together, or where unusually large stands of *O. rufipogon* are present, and ought to be protected. Likewise, noting that some wild rices were prized as healthy and/or auspicious food, they suggested a further enhancement of productivity of natural stands of wild rice by elimination of competition from other plant species, protection from grazing cattle, or the use of more effective harvesting techniques. A new global survey is needed to update information about the current in situ maintenance of rice wild relatives.

A positive fact somewhat balancing these negative trends is the introduction of genes and alleles from the wild relatives of *O. sativa* into improved rice varieties and the broad dissemination of these varieties. For instance, the IR64 variety cultivated in Asia over millions of hectares and extensively used in breeding programs worldwide bear an *O. rufipogon* introgression fragment representing approximately half of the short arm of rice chromosome 8 (Ballini et al. 2007).

2.4 In Situ Diversity and Genetic Erosion in Cultivated Rice

Crop genetic erosion is referring to a reversal of the trend of increasing diversity after the domestication and dispersal bottlenecks, as a consequence of scientific and formal breeding systems and modern agriculture where a relatively small number of breeders has replaced the multitude of farmers involved in the generation and maintenance of diversity.

Genetic erosion has been given at least three definitions: (i) absolute loss of a crop, variety, or allele (Peroni and Hanazaki 2002), (ii) reduction in richness of the total number of crops, varieties, or alleles (Hammer and Laghetti 2005) and (iii) reduction in evenness of the frequencies of varieties or alleles in a given place (Khlestkina et al. 2004), as it is evaluated by Shannon's index (Maughan et al. 1996) or Nei's gene diversity index (Nei 1973).

The first definition is really incomplete as it does not take into account what replaced the lost diversity. The evaluation of reduction in richness is a better indicator for genetic erosion, as it does recognize the dynamics in the system. However, richness might only poorly reflect increased levels of uniformity in agriculture and the level of richness found depends on the intensity of the investigation. The evenness parameter provides information on the risks of losing alleles or varieties due to skewed distributions of each diversity unit.

Wheareas the concept of genetic erosion emerged in the 60s, as early as the beginning of the eleventh century, in some places, the need to intensify rice production in the face of population growth has resulted in the centralized selection of rice varieties to grow and the abandonment of existing varieties. For instance, in 1012, faced with an influx of migrants from the north and a real shortage of arable land, Zhao Heng, the Emperor of China ordered two annual rice crops using a short duration variety imported from Annam (Jeanguyot and Ahmadi 2002). Likewise, in Japan, increasing the application of commercial fertilizers (fishmeal, soybean cakes)

in the late 1800s and chemical fertilizers in the early 1900s led to an early interest in the development of varieties with shorter stems. One of the first such varieties was selected in 1877 and soon replaced several local varieties (Matsuo 1955).

The contemporary trend of replacement of a large number of rice LVs by a small number of MVs goes back to the early years of the twentieth century when national rice research organizations were established in major Asian rice-growing countries, such as China and India. It had its first acceleration in the 1950s with the FAO Asian rice breeding program; some of the products of this program, such as the Mahshuri variety, are still cultivated over millions of hectares. The replacement process reached its momentum with the spread of semidwarf inbred lines developed by the IRRI and of hybrid rice varieties developed in China.

The percentage of the rice-growing area covered by MVs in 1998 was estimated to almost 70 % in Asia and in Latin America. At the same time, the share of improved rice varieties was only 38 % in Africa (Evenson and Gollin 2003). This evolution has contributed to the reduction of diversity in two ways: a foundation bottleneck due to the utilization of a limited number of LVs for the development of new varieties, and a reduced diversity due to directional selection for genes involved in dwarfing and/or response to fertilizers.

While several authors have analyzed the impact of the Green Revolution on rice in terms of areas covered by MVs, yield increase, etc. (e.g., Pinstrup-Andersen and Hazell 1985), no direct quantitative assessment of its impact is available in term of genetic erosion at the global level. Furthermore survey methodologies and diversity indicators used in the few studies undertaken at the individual country or at subcountry levels are too heterogeneous for any form of formal meta-analysis. Therefore, we will rely on a small number of case studies to draw a general picture of rice genetic erosion during the last 40 years and the current state of in situ diversity.

2.4.1 Rice In Situ Genetic Diversity in China

China used to possess a remarkably rich biodiversity of *O. sativa.* This richness is illustrated by the large number of rice LVs collected and preserved in the national rice genebanks: since the beginning of the twentieth century, the majority of Chinese rice germplasm has been collected through several survey and collection campaigns. Some 76,646 accessions have been catalogued and 67,444 preserved, including 48,420 LVs, 4335 improved inbred lines and 5584 wild rice accessions (Ying 2000). About 93 % of rice LVs have been collected in a vast region in the south of the Qinling Mountains and Huaihe River, while about 6 % come from areas north of the Huaihe River in eastern China and less than 1 % from northern China (Cao et al. 1995). The LVs included both *indica* and *japonica* accessions and were classified into 50 “varieties” and 962 forms (Yu 1996).

China was also the country which first developed and popularized semidwarf inbred rice varieties in the 1960s and later hybrid rice varieties in the 1970s. In 2008, hybrid rice occupied about 63.2 % of the total rice production area, or 18.6 out of 29.4 Mha. In 2003 and 2004, in the most intensive rice-growing areas, such as the southern provinces of Hunan, Jiangxi and Sichuan, the adoption rates of hybrid rice, reached 75–91 % of the total rice acreage (Li et al. 2009). Moreover, the majority of hybrid combinations are derived from a small number of male-sterile and restorer lines and some of the hybrids have been grown on very large areas for a very long time. In 1984, only 42 hybrid varieties were available. Between 1984 and 2003, 14 hybrid combinations and four inbred lines were each planted on an area larger than 650,000 ha. In 1990, the most popular hybrid, Shanyou 63, was planted on more than 6.2 Mha (Cheng et al. 2007).

However, the average planting area and the share of planting area of large-scale extended varieties has decreased from 9.3 Mha (41.4 %) in 1986–1990 to 7.53 Mha (34.3 %) in 1991–1995, to 3.99 Mha (16.9 %) in 1996–2000 to 1.23 Mha (5.7 %) in 2001–2003. Likewise, the number of varieties with growing areas larger than 75,000 ha increased from 296 in 1986–1990 to 485 in 2001–2003. The main factor contributing to the increase of the number of varieties was hybrid rice. The number of hybrid varieties increased remarkably from 42 to 233 in 18 years, while the number of inbred varieties remained almost unchanged (Yang et al. 2006). Whatever this recent evolution, it is allowed to speculate that the spread of the improved rice varieties (inbred or hybrid) in southern China, home to 93 % of the registered LVs, has certainly provoked major genetic erosion, in terms of absolute diversity, and also of richness and evenness of in situ diversity.

The Yunnan province is hosting a significant share of Chinese rice diversity with more than 6000 LVs registered. This abundant genetic diversity of LVs originated from a combination of socioeconomic (large number of ethnic groups), environmental (altitudes of 400–2406 m) and cropping system (upland and irrigated lowland) diversity. Surveying four villages in two regions of the Yunnan province with the most genetically diverse rice LVs, Sun et al. (2012) did not detect major absolute genetic erosion between 1980 and 2007. Conversely, Zhu et al. (2003) surveying a total of 44 townships, in the framework of developing a conservation strategy through crop diversity management, had noticed the almost disappearance of LVs among the varieties cultivated by farmers. This contradictory information coming out of the two case studies in the same province is very likely due to differences in target ecosystems and cropping systems, upland rice in the first case and lowland rice in the second case.

Thus the available data lead to the conclusion that while rice in situ diversity has been maintained in some marginal areas and cropping systems, the mainstream rice-growing areas and cropping systems have undergone a very drastic reduction of diversity. In order to have a more precise idea of the change in in situ diversity a comparison of genetic diversity of a representative panel of MVs currently grown with one in the representative panel of 48,000 Chinese rice LVs is needed (Gao 2003).

2.5 Rice In Situ *Genetic* Diversity in South Asia (Bangladesh, India and Nepal)

2.5.1 Bangladesh

Bangladesh is famous for extensive rice biodiversity, allowing rice cultivation over three different cropping seasons (*aus*, *aman,* and *boro*) as well as in specific agro-ecological conditions. It is reported that the IRRI genebank contains more than 8000 traditional rice varieties collected from Bangladesh. Formal rice research there dates back to 1935. Cultivation of MVs really started in the 1950s under an FAO project, and then intensified at the beginning of the 1970s with IR8 developed by IRRI. In 1981, the area grown with MVs rice was about 22 % of the total rice-growing area. Between 1973 and 2005, the Bangladesh Rice Research Institute (BRRI) has released 57 improved rice varieties, while a few additional ones had been released by other institutions (Hossain et al. 2013).

The total number of LVs as well as the area planted with LVs in Bangladesh is declining over time (Hossain et al. 2012). However, several LVs are still popular among farmers/consumers due to their special traits. They are maintained in small areas as special purpose rice (such as *kalizira* for *polao*), for superior grain quality that fetches a high price in the market (such as *Katari bhog*) or for tolerance to extreme environmental stresses (such as *Mota dhan* in the coastal areas). Hossain and Jaim (2009) reported that farmers in Bangladesh still cultivate more than 1000 LVs.

In order to provide precise information on the diffusion of MVs and the disappearance of LVs, a nationwide farm survey was undertaken in 2005, using a multistage random sampling for selection of villages and a purposive sampling of the households. A total of 14,400 farmers distributed in 1800 villages from 600 blocks from the six regions of the country, representative for diversity in farm size, farmer's age, education, etc., were interviewed (Hossain et al. 2013).

The survey found that 515 rice varieties were cultivated during the *aman* (monsoon) season of 2004, 261 varieties in *boro* (dry season) season of 2005 and 295 in the *aus* (pre-monsoon) season of 2005. The top ten varieties for the *aman* season all belonged to improved types, covered 1.1–26.5 % of the total *aman* crop area and accounted for over two-thirds of this area. In *boro* season, the top ten varieties were again improved ones and the top two together covered about 60 % of the rice-growing total area. In *aus* season, eight of the ten top varieties were improved types but their individual share of the total cropping area was much smaller, varying from 2.0 to 9.0 %. Regarding regional variability, in favorable areas with irrigation facilities and homogeneous terrain, only a few varieties occupy the landscape. In diverse ecosystems with large variations in microecology, farmers are growing a much larger number of varieties. The survey also revealed that 572 LVs for the *aman* season and 426 LVs for the *boro* season were extinct (not cultivated any more) or on their way toward extinction (not cultivated over a significant area). The main reason given for dropping these formerly popular

varieties is their low yield, as reported by more than 70 % of the farmers. The other reasons are a longer growing duration, lodging because of heights and weak stems, pest incidence, etc. (Hossain et al. 2013).

Thus, in Bangladesh, rice in situ diversity has undergone both absolute genetic erosion and change in the evenness of utilization of existing diversity to the benefit of the MVs. The fact that almost all MVs are progenies of crosses involving Bangladeshi LVs somewhat balances this skewed evenness. However, an improved offspring contains only a small share of the diversity of its parent landrace as, similar to other places, each Bangladeshi landrace holds a high level of genetic variation, whereas the MVs are monomorphic (Choudhury et al. 2013).

2.5.2 India

India is home to some of the most singular compartments of *O. sativa* rice genetic diversity such as the *aus* and *Basmati* groups, and almost all major rice-growing ecosystems: irrigated (50 % of the rice-growing area and 65 % of production), rainfed lowland (LLE) (32 and 27 %), rainfed upland (ULE) (13.5 and 6 %), and deepwater rice (4.5 and 2 %). The Indian National Bureau of Plant Genetic Resources is maintaining some 80,000 rice accessions (IRRI 2010).

India was also one of the countries that hosted the earliest (1950–54) international effort in developing improved rice varieties. Launched simultaneously in India and Southeast Asian countries by FAO, and based on inter-subspecies crosses (*indica* × *japonica* and the reverse) the project led to varieties such as Mahshuri and ADT-27, which are still grown on millions of hectares. Since then, a total of 946 rice MVs were officially released all over India and as soon as 1980, the area cultivated with MVs was estimated to 18.5 Mha, 47 % of the country rice-growing area (Pinstrup-Andersen and Hazell 1985).

No data about the current state of rice in situ diversity countrywide or even about the current share of MVs countrywide are currently available. However, recent surveys undertaken in northeastern and eastern India (Assam, Orissa, Jharkhand, and West Bengal) on the adoption of MVs, also provide some insight into the state of current in situ diversity in the country.

2.5.2.1 In Orissa

The survey was conducted, in 2008, in 6529 households representing all 30 districts of the state (Hossain et al. 2012). It revealed that farmers were cultivating a large number (723) of varieties, most of them LVs, under rainfed conditions in the rainy season. Comparatively, the number (29) of varieties grown under the irrigated conditions of the dry season was much smaller, and all were MVs. However, even during the wet season, four of the five top varieties, covering about 54 % of the rice-growing area, were of improved type. Varietal diversity was also important

at the household level. The largest number of rice varieties grown by a single farmer was 14. However, about 30 % of the farmers cultivated only one variety while 33 % cultivated two varieties, 21 % cultivated three varieties and 16 % more than three varieties. The existence of a large number of LVs is directly related to the diversity of agroecological conditions in the Orissa state where rice is grown in the pre-monsoon season (mostly direct-seeded in the uplands), in the monsoon season (transplanted in LLEs) and in the summer dry season (mostly in the irrigated lowlands). These three seasons account for 17, 76 and 7 %, respectively, of the total rice area (Hossain et al. 2012).

2.5.2.2 In Jharkhand

The survey was conducted in 2007 in 3219 households representing 20 out of 24 districts of the state (Hossain et al. 2012). It showed wide variations in diversity and concentration of rice varieties grown in the highland, medium land, and lowland ecosystems. A total of 145 varieties were identified and the highest number was found in medium land (71), followed by lowland (55) and highland (19). Among these varieties, 70, 84 and 75 %, respectively, were LVs. In the highlands, a landrace grown by 65 % of farmers covered about 69 % of the rice-growing area. In the medium land, four MVs covered 87 % of the rice-growing areas with almost equal shares, and three LVs covered an additional 8 % of the medium-land areas. Finally, in the lowlands, an improved variety grown by 58 % of farmers covered 55 % of the total rice-growing area. It was followed by three LVs with shares of 3–5 %.

2.5.2.3 In Assam

The survey was carried out in 2008, in 200 households from four villages, representative of the two districts with a high prevalence of submergence stress and a high proportion of rainfed rice area (Pandey et al. 2012). In 2008, the state produced 5.39 Mt of rice on an area of 2.46 Mha, 70 % of the total cultivated areas (www.indiastat.com). It is grown in three seasons: a wet season (65 % of the total rice area) in July to December and in the two dry seasons (12 %) from January to May and November to May (23 %).

The extent of adoption of MVs in terms both of the proportion of households (97 %) and of area (61 %) was fairly high. However, almost all those households were also growing LVs and the average proportion of area dedicated to LVs was 39 %. The highest proportions of MVs were observed in LLEs which are the least prone to drought or flood stress. The total number of MVs was about 15. The number of LVs was not inventoried. Among the MVs, the share of area was 45 and 55 % for old MVs (released before 1990) and new MVs, respectively. One old MV and one new MV covered each 30 and 37 % of the MV grown areas, respectively.

2.5.2.4 In West Bengal

The survey was carried out in 2008 in 300 households from two villages in each of the four representative rainfed districts of the state (Pandey et al. 2012). West Bengal rice production accounted for 16 % of India's 131 Mt production in 2009. Rice-growing areas include irrigated lowlands as well as drought and salinity prone LLEs and uplands. On average, rice is grown on at least 84 % of the cultivated areas during the wet season and on 64 % during the dry season. In 2009, the average yield was 3.9 t/ha, among the highest in the country.

MVs dominate regardless of season: 92 and 100 % of farmers were growing only MVs during the wet and dry seasons respectively. None were growing LVs during the dry season, 7 % were growing both MVs and LVs in the wet season. Strong correlations were observed between the rice cropping season and the generation of MVs grown. Old-generation MVs (released before 1990) are grown mainly during the wet season (77 % of the total MV grown areas), while new-generation MVs dominate during the dry season (99 % of the total MV grown areas). Whereas ICAR statistics have reported the release of some 47 rice varieties for West Bengal since 1960, the survey showed that around 20 MVs were grown during the wet season and only nine MVs during the dry season. Among the MVs, a rather new variety, released in 1994, occupies 43 % of the total MV area of the sample farmers, and an old MV, released before 1990, was grown on 39 % of the area.

These very valuable surveys show highly contrasted situations of rice in situ diversity in India. While in Orissa and Jharkhand, LVs still have an important share of the rice-growing area, it is not anymore the case in Assam and in West Bengal. The data also show a large variability among varieties as regards the share of farmers who are growing them and the area they cover. This uneven utilization of varieties is not directly related to the spread of MVs. Some LVs are also very popular and are grown by a large share of farmers, while some others have very specific usages and are grown by a much smaller number of farmers.

2.5.3 Nepal

In Nepal, rice is grown on 1.5 Mha, from low elevation areas (50 m) to high mountain valleys and mountain slopes (2830 m), the highest altitude of rice-growing locations in the world. Rice is mainly cultivated during the wet monsoon season in LLE and upland ecosystems. It is also grown during the spring season as an irrigated crop. According to national statistics (Singh 2009), from 1961 to 2008, rice production grew at 1.7 % per annum, but yield increased only by 20 kg/year. While in 1981, the area grown with MVs was about 26 % of the total rice-growing area (Pinstrup-Andersen and Hazell 1985), in 2008 this share was of 88 %.

A survey conducted in 2008 in 300 households in the districts with a high prevalence of drought prone LLEs across different regions of the country

(Pandey et al. 2012) revealed that on average, MVs covered 86 % of the total rice area. Important variations existed among sites in terms of both proportion of area covered by MVs, number of farmers growing them, and season: an average of 99 % of farmers and 98 % of the area in flat low elevation sites, 47 % of farmers and 71 % of the area in hill sites. MVs are predominant in lowlands, more limited in unbounded uplands. In all cases, the proportion of farmers growing MVs (91 %) is higher than the proportion of area under MVs (86 %) indicating that farmers also grow some LVs on some portions of their farms. Most of the MVs belonged to older generations (released in the early 1970s and 1980s). It accounted for approximately 60 % of the total MVs area.

The average percentage of farmers growing only LVs was 9 % (1–23 % according to sites) and the average percentage of the area was 14 % (2–29 %). The average percentage of farmers growing MVs and LVs was 26 % (6–43 % according to sites).

These data suggest major evolutions of rice in situ diversity in the country. But it does not necessarily imply absolute genetic erosion. Indeed, a survey of the uptake of three modern rice varieties by farmers in high-altitude villages in the Kaski district of Nepal (Steele et al. 2009) found that although seven LVs had been dropped in favor of the MVs, the allelic diversity of the remaining LVs cultivated over up to 40 % of the rice area, compensates the loss. Using a model, the authors found that the partial replacement of LVs increased genetic diversity if the MVs were adopted on up to 65 % of the area. Only above these levels did overall diversity decline.

2.6 Rice In Situ Genetic Diversity in Southeast Asia (Cambodia, Laos, Thailand, and Vietnam)

In some Southeast Asian countries, farmers are living in relatively homogeneous rice-growing environments, where controlled irrigation and good access to fertilizer inputs are the norm. There, they have realized tremendous production gains, thanks to the introduction of high-yielding rice MVs developed by national and international rice breeding programs. As soon as 1980, the area grown with MVs reached 60 % of the total rice-growing area in Indonesia, 55 % in Malaysia, and 78 % in the Philippines. This is not the case for some other countries. Irrigation covers only 12 % of Cambodia's rice land, 23 % in Laos, and less than 30 % of Thailand. In 1980, the area share grown with MVs in Thailand only reached 9 %.

2.6.1 Cambodia

In Cambodia, rice is grown over more than 2.5 Mha, mainly in LLE ecosystems (84 %). In the mid-70s, while the country was achieving its greatest ever rice crop,

under the effect of the Green Revolution, the Red Revolution dramatically disturbed rice production and the maintenance of the national rice genetic resources with more than 500 accessions. A new rice collect campaign was undertaken in the 20 provinces of the country from 1989 to 1996. It harvested more than 6000 samples. From these samples a total of 2557 distinct LVs accessions were identified and stored in genebanks in Cambodia for short- and medium-term storage and a duplicated set at the IRRI genebank for long-term storage (Smolders 2002). The largest share (88 %) of those LVs was collected in LLE ecosystems, followed by the ULE (10.6 %) and deepwater/floating (1.2 %) ecosystems. Only 0.2 % of samples came from the irrigated/recession ecosystem. A very large phenotypic diversity was observed among those LVs for spikelet and caryopsis color, for aroma, amylose contents—including 8 % glutinous varieties—duration and photoperiod sensitivity.

Thus, rice in situ diversity was still high in Cambodia at the end of the 1990s. According to more recent reports (Ouk and Sakhan 2010), despite the country's ambition to become a "rice basket" and a major exporter of milled rice, almost 80 % of the rice-growing area is still cultivated with LVs. An unusual threat to rice in situ diversity is the emergence of invasive weedy rice, especially in the area with a high prevalence of the wild species *O. rufipogon*.

2.6.2 Laos

In Laos, rice is the single most important crop accounting for more than 80 % of the cropped area. In 1998–1999, the rice harvested area was estimated at 717,000 ha, the LLEs accounting for 67 % of the area and 71 % of the production, and the ULEs for 21 % of the area and 12 % of the production. Dry season irrigated rice only accounted for 12 % of the area and 17 % of the production.

A 5-year systematic collect program of rice varieties implemented between 1995 and 2000 led to the collection of 13,192 accessions of cultivated rice, and 237 accessions of six wild rice species. The number of accessions collected from the northern region mainly cultivating upland varieties was much higher (44.8 % of the total) than in the central (35.1 %) and southern (20.1 %) provinces. Unfortunately, this strikingly high density of Lao rice in situ diversity, with an average of one accession every 54 ha, conceals a rapidly changing environment. MVs are rapidly replacing the LVs, particularly in the favorable LLE environment. While in 1993, less than 10 % of the whole LLE area was grown with improved cultivars, in 2002, they covered more than 50 % of the rainfed area in the central provinces of Khammouane, Saravane, Borikhamxay, Champassak, and Savannakhet and Vientiane. Some farmers no longer grow LVs. In Savannakhet, the area planted to MVs is as high as 80 %. Only MVs are grown in the dry season irrigated environment throughout the country (Appa Rao et al. 2002).

2.6.3 Thailand

Thailand is one of the ten top rice producing countries with 11 Mha and it is one of the three top rice exporters. More than 80 % of the rice-growing area is under rainfed conditions. Less than 20 % of the area can be irrigated for rice cropping in the dry season. Thailand is home to one of the most famous aromatic rices, Kaw Dawk Mali. The ex situ collection of rice germplasm at the National Rice Genebank, which began in 1937, holds 24,000 entries (Rerkasem and Rerkasem 2002).

While in 1980, the share of MVs grown rice area was of 9 %, it had reached more than 75 % in 1996. However, important regional variability existed on the one hand, and some of the MVs, especially the aromatic ones, are in fact, pure lines extracted from LVs. LVs were still grown over some 20 % of the country's cultivated rice land in 1997 (OAE 1998).

A survey of rice germplasm grown in Thailand's main rice ecosystems (upland, irrigated, deepwater, and acid sulfate soil) was conducted, in 2002, through interviews of 75–80 % of the farmers in villages representative of these ecosystems (Rerkasem and Schaal 2002). There were 14–18 named varieties found to be grown in each village. The average number of varieties grown by a farmer ranged from 1.5 in irrigated lowland villages to three in shifting cultivation upland rice villages. Each village reported the loss of 10–14 named varieties during the last 15–20 years. In villages representative of the irrigated lowland ecosystem, the two top varieties were of MV type. The most popular one was cultivated by 75 % of the farmers and covered 55 % of the land. The second important variety (aromatic glutinous MVs) was grown by 34 % of the farmers on 18 % of the land. The remaining 27 % of the land were cultivated with the 13 other varieties, each grown by only a few farmers. The villages representative of the LLE and upland ecosystems were much more conservative with their rice germplasm. But similar to the irrigated ecosystem, all the LVs were not evenly used.

A common feature of the rice germplasm is the variation within seed lots and the practice of seed selection by individual farmers giving rise to differences between seed lots within the same varieties. Analysis of the genetic structure of 33 subpopulations of the same local rice variety collected from 33 farms in 13 villages in Chiang Mai and Mae Hong Son provinces in northern Thailand, using Microsatellite markers, revealed a high level of intra subpopulations variation despite predominant inbreeding in the crop. It also showed slight but significant genetic differentiation among villages. The data suggested that rice LVs are a dynamic genetic system that responds to evolutionary forces, imposed both by nature and by humans (Pusadeea et al. 2009).

2.6.4 Vietnam

Vietnam is also one of the ten top rice producing countries, with 7.3 Mha of rice-growing area and 35.6 Mt of rice production in 2007, and one of the three top rice exporters. There are three major areas: the southern Mekong River delta (60 % of

the total rice area), with a warm and humid climate throughout the year; the northern Red River delta (32 %) with tropical monsoons and cold winters; and the highlands in the North hosting the upland rice cropping systems (8 %). No precise data are available about the number of Vietnamese LVs rice accessions conserved ex situ, as several independent collections exist: the National Plant Resource Centre maintains over 3000 rice accessions in the National Genebank; the Cuu Long Rice Research Institute has a collection of over 1200 LVs; the Vietnam Agricultural Science Institute maintains some 6500 rice accessions (VAAS 2006). These numbers probably do not reflect the Vietnamese overall wealth of rice genetic diversity.

The utilization of improved rice varieties in the country goes back to 1950 at least, with the development of short duration varieties allowing double rice cropping in the mountain areas of North Vietnam. By the mid-1970s the share of MVs had already reached 35 %. Starting with the release of IR8 both in the northern and southern delta rice-growing areas, a total of 89 IRRI breeding lines have been released as varieties in Vietnam. IRRI varieties now cover 70 % of the rice-growing areas in Vietnam (http://irri.org/our-work/locations/vietnam, Jun 28, 2014).

Data about the current state of rice in situ diversity in the country are somewhat contradictory. A survey carried out in 2001, in the Red River delta and in the Mekong River delta showed that the number of LVs, though still remaining considerably high, was much lower than that of MVs. The area planted to rice LVs was also very small, accounting for less than 4 % in irrigated rice lands, up to 21 % in the marginal rainfed environments such as upland, coastal sandy and flood-prone areas, which represent some 2 Mha. According to another study looking at the structural change of rice varieties cultivated in three sites of the Mekong River delta (Nguyen 2005), the general trend showed an increase in the total number of varieties at the community level, mainly due to the increase in the number of MVs. The number of LVs decreased in the areas where it was higher before 1980. The reduction of the number of LVs and the lower evenness of the share in total rice area both contributed to the decrease in the diversity index. It was also noted that most LVs were grown by few households and over small areas. However, although the MVs were used with increased frequency, some LVs were maintained as common varieties with a large distribution. The study also indicated that 100 % of LV seed used by farmers was supplied by informal systems. Finally, a recent effort in collecting rice LVs yielded around 1000 accessions in the Mekong River delta only. After elimination of the duplicates, 812 have been conserved and evaluated by Can Tho University, among which 517 were reintroduced for cultivation and evaluation by farmers under various growth conditions (VAAS 2006).

2.7 Rice In Situ Diversity in West Africa and Madagascar

West Africa is not only home to the African species of cultivated rice *O. glaberrima,* but has also hosted the Asian species of rice *O. sativa* for more than five centuries; as a consequence, the sympatry of the two species has also given birth to a new original diversity.

In the 1970s, fears of the negative effects of the Green Revolution led national and international institutions to undertake collect campaigns in different African countries and in Madagascar. Several thousands of accessions, including wild species were thus collected. Today, the Africa Rice genebank is maintaining some 16,000 accessions including 1500 *O. glaberrima*.

During the last 50 years (1960–2010), a total of 708 rice MVs have been officially released in Africa, among which some 260 have been widely cultivated in one or more countries (Sanni et al. 2013). However, the Green Revolution has not affected rice cropping systems anywhere as intensely as in Asia. In 1998, MVs covered only 38 % of the rice-growing area in Africa (Evenson and Gollin 2003). More recent analyses consider that, to some degree, a rice revolution has already begun in Africa and practices that have proved successful in Asia could also be applied in Africa. But for many reasons, Africa's rice revolution has been, and will continue to be, characterized by a mosaic of successes, situated where the conditions are right for new technologies to take hold (Larson et al. 2010).

Here we will provide insight into rice in situ diversity in Africa through two country case studies and a special focus on *O. glaberrima*.

2.7.1 Rice In Situ Genetic Diversity in Guinea

Guinea is a center of diversification of the cultivated species *O. glaberrima* (Portères 1950), and an important reservoir of rice genetic diversity in West Africa. It has been proposed as an area for the in situ conservation of African rice varieties (Bezançon 1995). Currently, rice is cultivated on an area of 800,000 ha. Slash-and-burn cropping of upland rice accounts for 65 % of the rice-growing area, LLE for 19 % and 'mangrove' rice cultivation for 16 %. For several decades, efforts to disseminate improved rice varieties have been undertaken in the country (Dalton and Guei 2003). This was particularly the case for the NERICA varieties during the last 15 years (Jones et al. 1997).

The organization of rice genetic diversity in the country was analyzed using SSR markers genotyping of 170 accessions collected in farmers' fields in the Maritime Guinea region (Barry et al. 2007a). Similarly to what has been observed in Asia, the organization was tightly linked with the rice-growing ecosystems, with *indica* varieties grown in the lowlands and tropical *japonica* in the uplands. The two major ecotypes of *O. glaberrima* ("floating" and "upright") were also present. Moreover, an original genetic compartment was detected, highlighting the occurrence of *glaberrima* × *sativa* hybridization. Allelic diversity was found to be comparable to that noted worldwide for *indica* and *japonica* groups of *O. sativa*, but not as large for *O. glaberrima*.

Recent changes in rice in situ diversity was analyzed on the basis of a survey of 1679 farms located in 79 villages of the four regions of the country in 2001 (Barry et al. 2009). Varietal diversity was high, especially in forest Guinea and lower Guinea with 33 known varieties and 21 cultivated varieties per village, on

average. LVs accounted for 72–88 % of the total number of varieties present in each village. The number of varieties per village had increased during the last 5 years (1996–2001) by 10–30 %, according to the region, for the LVs and by 40–80 % for the MVs, particularly the newly promoted NERICA MVs. The evenness index calculated at the village level was generally much lower than one, indicating a high variability in the number of farmers cultivating each of the varieties present in the villages. A very small share of varieties present in the village could be considered as major varieties, used by more than 50 % of farms. There were also considerable differences between and within regions in the number of major varieties. Most of the major varieties belonged to the LV category. Depending on the region, MVs accounted for only 10–30 % of the major varieties in the villages, while NERICA represented only 5 % of the major varieties.

The temporal evolution of rice genetic diversity was further monitored through a diachronic comparison approach using samples collected in six villages in 1982 and in 2003. The names and number of varieties inventoried and the polymorphism of microsatellite markers were used as diversity indicators (Barry et al. 2008). The number of varieties appeared not to be comparable between the two dates, due to differences in the collection methods. The varietal composition had evolved very substantially between the two collection dates. Many long duration varieties present in 1982 had been abandoned and several MVs had been introduced. The mean number of alleles per locus and per accession was significantly higher in accessions collected in 2003. Pairwise comparisons of the mean number of alleles per locus in 1982–2003 homonymous accession pairs indicated higher intra-accession diversity for the 2003 collections (Fig. 2.5). Genetic differentiation, measured with the F_{ST} values, was very high and significant for more than 80 % of these pairs of accessions. The overall genetic differentiation between accessions from the two collection dates was also significant. Furthermore significant changes were observed for allelic composition. However, alleles specific of each collection date had a much lower frequency, compared to alleles common to the two collection dates.

Finally, partitioning rice genetic diversity between farms, varieties, and within-variety diversity were analyzed using the genotype at 10 SSR loci of 1200 individual plants belonging to 45 accessions collected in eight farms (Barry et al. 2007b). It revealed an even share of molecular variance between and within accessions, while the farm effect was almost nil. Local varieties had a multiline genetic structure. The number of multilocus genotypes was proportional to the utilization rate of the variety in the village. The F_{ST} values between different accessions of each variety were significant which indicated low genetic consistency in the variety names. This varietal structure could mainly be explained by the migration phenomenon and the high varietal turnover. Compared to allelic diversity, multilocus genotypic diversity seemed to be the most suitable indicator of the quantitative distribution of diversity at different management scales (accession, farm, and village). The within- and between-farm F_{ST} values were in the same order of magnitude. The within-farm diversity was not farm-specific but quantitatively high, i.e., up to 50 % of the total genotypic diversity of a given village. Given the relative

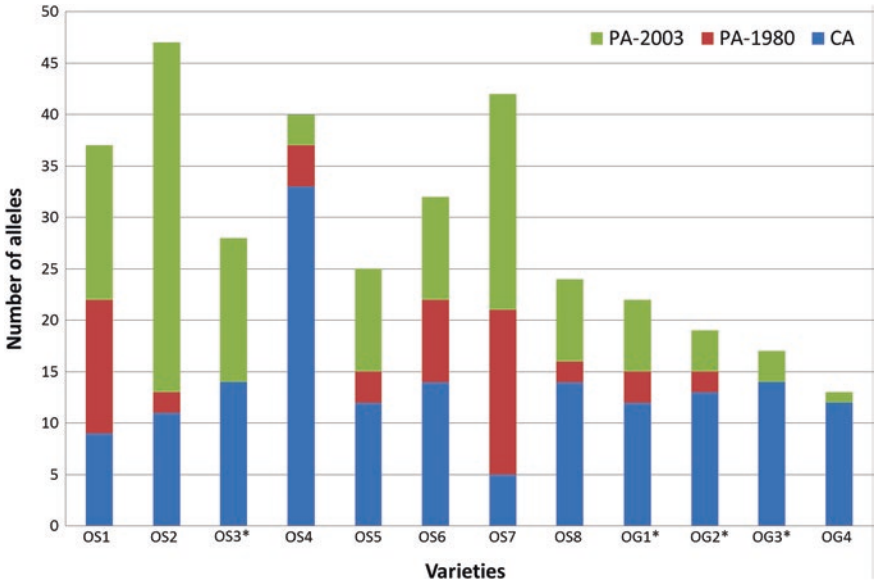

Fig. 2.5 Allelic composition over 10 SSR loci of homonymous rice varieties collected in 1979 and in 2003 in six villages of Maritime Guinea. *OS O. sativa* variety; *OG O. glaberrima* variety. Variety number followed by an *asterisk* indicate nonsignificant F_{ST} between the two collections dates. *CA* Allele common to 1979 and 2003; *PA* private allele. The PA had a frequency of at most 20 %, whereas more than 80 % of CA had a high frequency of over 20 % (After Barry et al. 2008)

importance of the within-variety diversity, the in situ approach stands out as the most effective solution. As farms do not host specific diversity the in situ approach could be implemented by working with a small number of farms.

It was concluded that the rice in situ diversity pattern was typical of the subsistence farming system with a high share of LVs. Over the last 30 years, genetic diversity had been maintained or even enhanced. The recent dissemination of NERICA varieties had not caused any form of genetic erosion. These short-duration varieties were mainly used as a complement to the long-duration LVs and thus enhanced varietal diversity. Given the relative importance of the within-variety diversity, Barry et al. (2007b) advocated for the in situ conservation of rice genetic resources in Guinea.

2.7.2 Rice In Situ Genetic Diversity in the Island of Madagascar

Madagascar is producing some 3.6 Mt of rice over 1.3 Mha of irrigated lowlands (20 %), LLEs (59 %), ULE (10 %) and swidden uplands (SUE). The national genebank maintains more than 5000 rice LVs. All major rice genetic

groups are present in the country including the aromatic groups. The highlands of Madagascar have been identified as a key site of rice genetic diversity. Using morphophysiological and isozymic data, Ahmadi et al. (1991) have identified, in addition to the well-known *indica* and *japonica* subspecies of *O. sativa*, an atypical group specific to Madagascar and preferentially present in the central highlands of the country. Comparing diversity patterns in 1105 SNP loci—including LD, introgression patterns and haplotypes—between a panel of 147 Malagasy rice varieties, and a reference panel of 370 Asian rice, we recently confirmed the existence of the Malagasy-specific group (*Gm*). Pattern of diversity of *Gm* group positioned it halfway from *indica* and *aus* groups (Fig. 2.6).

The *Gm* group most probably arose from founder effect coming from intermediary forms of rice originating from either India or Sri Lanka that did not belong to the five major *O. sativa* groups. It then underwent human selection for cold tolerance. Madagascar also hosted cold tolerant tropical *japonica* varieties, with very long grain. Migration bottleneck has resulted in 30–40 % reduction of diversity among the *indica* and *japonica* groups in Madagascar. The Malagasy panel also showed much fewer *indica* × *japonica* recombinations compared to the Asian panel, suggesting that the two groups had undergone much less recombinations when migration to the Island occurred (Ahmadi et al. 2013).

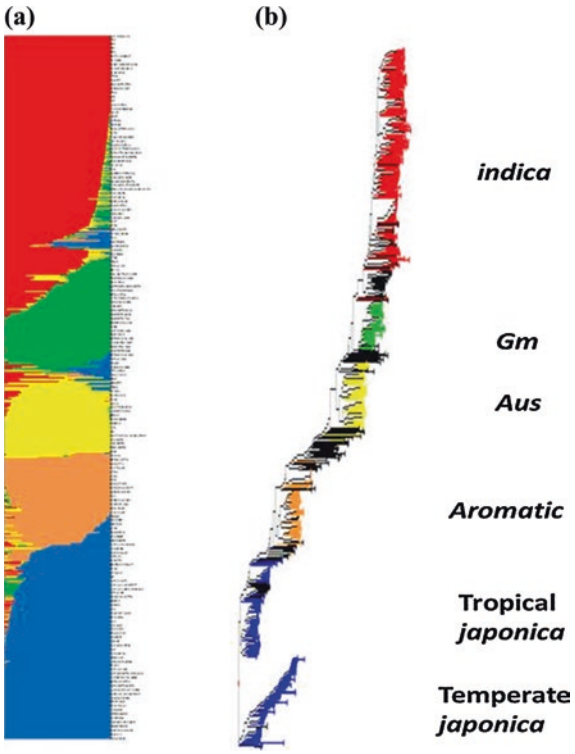

Fig. 2.6 Structuring of rice genetic diversity in Madagascar. **a** Population structure in the Asian (373 accessions) and Malagasy (147 accessions) panels estimated from 1105 genome-wide SNPs. **b** Distance-based neighbor-joining tree of the same panels. *Gm* group specific to Madagascar

To define conservation strategies, a multidisciplinary analysis was performed focusing on rice genetic diversity and factors shaping its distribution in the Vakinankaratra region of Madagascar. Individual and collective surveys, and collection and characterization of samples of cultivated rice varieties in 1050 farms located in 32 villages were realized (Radanielina et al. 2013a). A total of 349 rice accessions were collected, 306 grown in the lowland ecosystem and 43 in the upland ecosystem. Among these accessions, several collected in different villages had the same name. The 306 lowland accessions comprised 149 distinct names and the 43 upland accessions, 19 distinct names. Among the lowland accessions, 77 % were of LV type, while all upland accessions were of MV type. The proportion of MVs was higher in low altitude villages. The proportion of farmers using MVs was 48 % in villages below 1250 m altitude, 18 in villages between 1250 and 1500 m, 11 % between 1500–1750 m, and only 6 % above 1750 m.

The average numbers of rice varieties used per village (10.9) and per farm (2.2) were comparable with other traditional agrosystems, nevertheless great regional variability was observed. The determinants of this variability were the altitude, the village production system, the type of rice cultivation system, and the farm economic wealth. An important disparity in the frequency of the use of varieties was observed with large proportions of "minor" varieties used by less than 10 % of farms (Fig. 2.7).

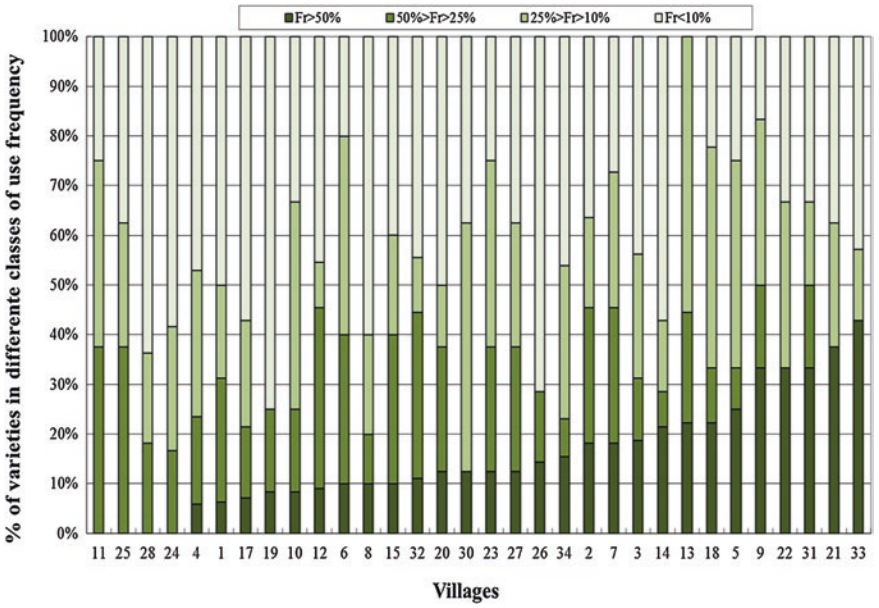

Fig. 2.7 Relative importance of four categories of varieties according to the proportion of farms (Fr) that use them, in 33 villages of Vakinankaratra region of Madagascar. The number of varieties per village ranged from 6 to 19 with an average of 10.9 (After Radanielina et al. 2013b)

Partitioning of the molecular variance between intervals of altitude, villages and farms revealed that, within-village variance represented more than 75 % of the total, and within-farm variance, 70 % of within-village variance. Genetic diversity at the individual field level, or within-variety diversity, was also high in fields cropped with LVs. However, the within-variety diversity at the level of an individual farm represented a rather small fraction of the total diversity of the variety estimated by sampling several villages (Radanielina 2013a).

It was concluded that, given the hierarchical distribution of molecular variance, a small number of samples per scale (altitude interval, village and farm) could allow to capture most of the genetic diversity observed. However, within-variety diversity was also important making ex situ conservation strategies impractical and costly.

2.7.3 *In Situ Diversity of the African Cultivated Rice Species O. Glaberrima*

O. glaberrima was the only cultivated rice in West Africa until the fifteenth century. No direct statistics regarding the current share of rice-growing areas or the number of *O. glaberrima* varieties still cultivated are available. A rough estimate of the minimum number of such varieties in the mid-1970s is provided by the number of *O. glaberrima* accessions conserved in international genebanks: some 2000, probably with several duplicates.

Recent germplasm collection campaigns conducted in different West African countries inventoried the presence of significant numbers of *O. glaberrima* accessions. In Niger, the germplasm collection conducted in 2008 in 51 villages covering the diversity of rice-growing ecosystems of the country yielded 241 non-redundant accessions, among which 25 % belonged to *O. glaberrima* species (Sow 2011). In Burkina Faso, a survey covering almost all rice-growing districts in the country led to the identification of 320 LVs accessions, among which 15 % belonged to *O. glaberrima* species (Kam 2011). In Guinea, our germplasm collection conducted in 2011, in 35 villages distributed over three natural regions of the country out of four, yielded 496 accessions, among which 19 % belonged to *O. glaberrima.* A similar collection campaign conducted in the same villages of Guinea in 1980 had yielded 250 accessions, among which 23 % belonged to *O. glaberrima* (our unpublished data).

However, our recent (2012 and 2013) detailed, village-level analyses of *O. glaberrima* in situ diversity in three West African countries (Burkina Faso, Guinea and Senegal), based on a diachronic approach, did not confirm the reassuring pictures drawn by those large-scale collection campaigns.

In the Cascades region of Burkina Faso, a detailed survey at the level of three villages, cropping rice mainly in the LLE ecosystem, led to an inventory of more than 20 known *O. glaberrima* varieties, only three of which were still cultivated. In each village, less than 10 % of the farmers were growing *O. glaberrima* varieties on less than 50 % of their rice-growing area.

In Guinea, the situation was more contrasted. The survey was conducted in three villages representative of the LLE and ULE and SUE rice cultivation system of Maritime Guinea. A total of 22, 19 and 24 varieties were inventoried in the LLE, ULE and SUE village, respectively. The LLE village had only *O. sativa* varieties while the ULE village had five *O. glaberrima* varieties and the SUE village 10 *O. glaberrima* varieties. Compared to the inventory of rice varieties collected in the same village between 1979 and 1982, the LLE village had lost its unique *O. glaberrima* varieties but had gained 13 *O. sativa* varieties. The ULE village had lost five *O. glaberrima* varieties but had gained eight *O. sativa* varieties. In the SUE village, while the *O. glaberrima* varieties had been present for generations, all *O. sativa* varieties had been introduced progressively since 1980. The LLE village had two major varieties (cultivated by more than 50 % of the farmers), both *O. sativa* LVs. The ULE and SUE village had only one major variety, respectively, an *O. sativa* LV and an *O. glaberrima* LV. In general, *O. glaberrima* was perceived as the most "*economical rice*" because of its higher yield in the most unfertile soils and its excellent swelling after cooking. It was also considered as the rice of the poorest, because of its poor grain appearance.

In Senegal, a free-listing survey conducted in 12 villages of the Casamance region led to an inventory of 281 known rice varieties among which 205 were still cultivated. And among these last accessions, only four belonged to the *O. glaberrima* species. The number of varieties present in each village varied from 12 to 44 and the number of varieties per farm from three to six. Comparatively, a collection campaign conducted in the same village in 1975 had yielded some 60 *O. glaberrima* accessions.

The contrasted pictures drawn by the large-scale collection campaigns and the more detailed survey at the individual village level is probably due to the fact that the collection campaigns were specifically oriented toward *O. glaberrima* varieties and did not evaluate other parameters like the proportion of farmers growing *O. glaberrima* or the area grown with *O. glaberrima* varieties. Our village-level survey suggests that even in the most remote and marginal areas the use of *O. glaberrima* varieties is declining sharply. The most optimistic estimates of the current share of area cultivated with *O. glaberrima* varieties in Africa would be less than 0.1 %.

2.8 Conclusions

The report on the process of "development of the global conservation strategy for rice" (IRRI 2010) indicates the existence of more than 500,000 accessions of rice genetic resources conserved in international and national genebanks around the world. A very large proportion of these accessions were collected during the 1970s in order to protect local LVs against one of the drawbacks of the Green Revolution leading to the rapid replacement of the multitude of LVs by a small number of MVs, particularly in the favorable irrigated rice ecosystems of Asia.

Whereas reports on the impact of the Green Revolution, inventorying areas cropped with MVs, indirectly provide some insight into the evolution of rice in situ genetic diversity, no direct quantitative estimate of the global genetic erosion is available, whatever the term of genetic erosion is considered.

The most important feature of rice in situ diversity emerging from our case studies is the diversity of situations. Almost all aggregated data suggest massive absolute genetic erosion and a reduced evenness in the utilization of the existing genetic diversity, particularly in irrigated ecosystems. In this relatively homogeneous rice-growing environment, the absence of major abiotic stresses, the controlled irrigation and the good access to fertilizer inputs has facilitated the deployment of a small number of MVs with large adaptability, nicknamed mega-varieties, over several million hectares each. Detailed surveys indicate that in Asia the pace of replacement of LVs by MVs was smoother in rainfed rice ecosystems compared to the irrigated ecosystem. This lesser penetration of MVs in the rainfed lowland and upland ecosystems is due to their much poorer performances under the adverse and spatiotemporally variable abiotic constraints (drought, submergence, salinity, iron toxicity, …) and the often limited access farmers have to fertilizer and other resources in these ecosystems. In Africa, while the extinction of *O. glaberrima* is accelerating, no significant genetic erosion is observed among the *O. sativa* LV in the rainfed ecosystems. So far, in both Asia and Africa, the spread of MVs in the rainfed ecosystems is not synonymous to absolute genetic erosion as they do not completely replace the LVs. The partial replacement of LVs by MVs may even lead to increased genetic diversity.

However, the relative sparseness of rice in situ diversity in the rainfed ecosystems may not last very long. The prospects are gloomy. Indeed, yield increase through the utilization of improved varieties in all rice-growing ecosystems constitutes the central pillar in the strategy of the international rice research community for taking up the challenge of the increasing demand for rice while the rice growing area is not extensible. The strategy relies on the development of a new generation of MVs endowed with multiple tolerances to abiotic stresses encountered in the rainfed ecosystems. The new submergence tolerant varieties endowed with the submergence tolerance gene *Sub1* provide a flavor of this evolution. A small number (4–5) of these varieties are spreading at an unprecedented pace in India, Bangladesh and Nepal, and are expected to cover more than 5 Mha by 2014 in these three countries (Singh et al. 2013a, b). Similar breakthroughs are expected for salinity and drought tolerance in the near future, in both Asia and Africa. As the rainfed lowland ecosystems coincide with major centers of rice genetic diversity, especially in west and south Asia, where genetic diversity is much higher and the genetic structure is more complex (all genetic groups are encountered there, together with many unclassifiable varieties), the spread of the new generation of MVs will almost certainly lead to sharp genetic erosion, similar to the one observed in the irrigated ecosystem during the Green Revolution.

Since the preoccupation of increasing rice productivity will almost certainly prevail over the maintenance of rice in situ diversity, new options need to be considered. One of these options is the establishment of protected areas in a small

number of sites where representatives of different rice genetic groups are sympatric of their wild relatives. It assumes incentive mechanisms for rice farmers in the area. It also assumes the revival of the momentum for preservation of in situ genetic diversity among the scientific community which has declined sharply during the last decade. Another complementary and promising direction is the valorization of genetic diversity in the framework of the emerging concept of *ecological intensification* (Griffon 2007). It assumes a more detailed understanding of biological interactions involved in improving the performances (primary production and its stability) of deployment of genetic diversity at the landscape level (varietal mosaic) and/or at the individual plot level [varietal mixture or monospecific stands endowed with a functional diversity (Kiær et al. 2009)] to help select the best possible complementary components. Which traits are to be diversified, and how to go about it without adversely impacting the homogeneity desirable for other traits? Similarly, the concept of *evolutionary plant breeding* has emerged recently. It envisages the deployment of varietal stands that are capable of adapting to changes in environmental conditions (Döring et al. 2011). Given the rapid development of precision and high throughput breeding methods, on the one hand, and the spreading of information sharing tools, on the other hand, it should be possible to move in a near future from the Green Revolution model of widely deploying a small number of improved varieties toward the new model of *ecological intensification* or *evergreen revolution* with a larger (and more diverse) number of improved varieties and a more precisely targeted deployment.

Genetic erosion is affecting not only the cultivated rice species *O. sativa* and *O. glaberrima*, but also their wild relatives. In situ conservation of these species is all the more necessary as ex situ conservation is very difficult. This indispensable conservation requires more awareness about their importance and the establishment of protected areas.

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Chapter 3
Genetic Diversity and Erosion in Berries

Samir C. Debnath

Abstract *Fragaria* (strawberry; Rosaceae), *Rubus* (brambles: raspberry and blackberry; Rosaceae), and *Vaccinium* (blueberry, cranberry and lingonberry; Ericaceae) are among the most important berry crop genera worldwide. Berry fruits are rich in vitamin C, cellulose, and pectin, and produce anthocyanins which have important therapeutic values, including antitumor, antiulcer, antioxidant, and antiinflammatory activities. As in other crops, biodiversity of berry crops decreased dramatically at the species and intraspecific levels, leading to genetic erosion due to complex process involving both human and environmental drivers. Genetic erosion occurs when old varieties in farmers' fields are replaced by newer ones. Conservation of genetic resources is of prime importance to prevent genetic erosion. Efforts are being made to conserve the biodiversity of berries across Europe and North America. Utilization of diverse, locally adapted germplasm is required for the future viability of berry production. There is a pressing need to develop reliable methods for conserving, maintaining, and identifying berry germplasm and for assessing genetic biodiversity for practical breeding purposes and proprietary-rights protection. The introduction of molecular biology techniques, such as DNA-based markers, allows direct comparison of different genetic material independent of environmental influences. This paper presents the progress in-depth of various aspects of molecular diversity analyses in wild berry species and cultivars collected from Canada, Europe, and USA. Different molecular markers detected a sufficient degree of polymorphism to differentiate among wild clones and cultivars, making these technologies valuable for cultivar identification and for the more efficient choice of parents in berry breeding programs. The chapter describes in-depth the progress of various aspects of berry crop diversity, erosion,

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and their characterization at molecular and chemical levels, and on the employment of molecular markers for the assessment of genetic diversity, relatedness and population structure in berry crop germplasm.

Keywords Antioxidants · Berry crops · Biodiversity · Molecular markers · Pedigree analysis

3.1 Introduction

Berry crops are dicot angiosperms, bear small to moderate-sized fruits on herbs, vines or shrubs; and are usually vegetatively propagated to maintain true-to-type (Debnath 2003a). The most important berry crops worldwide include strawberry (*Fragaria* × *ananassa* Duch., Rosaceae), blueberry (*Vaccinium corymbosum* L., *Vaccinium angustifolium* Ait., *Vaccinium ashei* Reade; Ericaceae), cranberry (*Vaccinium macrocarpon* Ait.), black currant (*Ribes nigrum* L., Grossulariaceae), table and wine grapes (*Vitis* spp., Vitaceae), raspberry (*Rubus idaeus* L., Rosaceae), and blackberry (*Rubus* spp.). Other major berry crops having large production areas are the hybrid berries ('Logan' and 'Boysen', *Rubus* spp.), black raspberry (*Rubus occidentalis* L.), lingonberry (*Vaccinium vitis-idaea* L.), gooseberry (*Ribes. uva-crispa* L.), and red currant (*Rubus. rubrum* L.). Bilberry (*Vaccinium myrtillus* L.), cloudberry (*Rubus chamaemorus* L.), edible honeysuckle/Haskap (*Lonicera caerulea* L., Caprifoliaceae), elderberry (*Sambucus canadensis* L., Caprifoliaceae), Juneberry/saskatoon (*Amelanchier* sp., Rosaceae), and sea buckthorn (*Hippophae rhamnoides* L., Elaeagnaceae) are some of the regionally important minor berries (Finn 1999). Although grapes have been an integral part of the human society for thousands of years and are the single most important crop grown in the world, the production of blueberries, cranberries, strawberries, and raspberries is also a profitable agricultural enterprise that began in the early nineteenth century.

The importance of berry fruits in horticulture lies in their dual role as in the landscape and of food. The fruits themselves are highly prized for their varying shapes, textures, flavors, and colors. The berry fruit group is quite diverse and comprises simple (e.g. blueberry, cranberry) and composite fruits derived from single or multiple fused fertilized ovaries (e.g. strawberry, mulberry, raspberry, blackberry) (Vicente and Sozzi 2007). Main challenges in the distribution of premium-quality berries include postharvest losses due to over-ripening, excessive softening, and pathogen attack (Cantín et al. 2012; Terry 2011).

Berry fruits are highly nutritious and contain relatively high levels of vitamin C, cellulose and pectin, and produce anthocyanins which are believed to have important therapeutic values, including antitumor (Koide et al. 1996), antiulcer (Cristoni and Magistretti 1987), antioxidant, and antiinflammatory activities (Wang et al. 1999). They are good sources of natural antioxidants including carotenoids, vitamins, phenols, flavonoids, dietary glutathionine and endogenous metabolites, and exhibit a high level of antioxidant capacity against free radical

species: superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen (Wang and Jiao 2000). A large and growing body of studies has convincingly established the anticancer potential of singly purified constituents found in berry fruits (Seeram 2006). These phytochemicals include phenolics such as anthocyanins (pigments that impart the attractive colors to berry fruits), quercetin (a ubiquitous flavonol also found in apple skins), proanthocyanidins (flavonol polymers common to grape skin and seeds, blueberries, cranberries), hydrolyzable tannins (particularly ellagitannins, found in strawberries, black raspberries, red raspberries, blackberries, muscadine grapes), and other flavonoid-related molecules. The benefits of these high antioxidant activity fruit include reduction of carcinogens in humans (Chung et al. 2002), protection against tumor development (Kresty et al. 2001), and reversal of age-related effects on memory (Bickford et al. 2000). *Vaccinium* anthocyanidins neutralize free radical damage to the collagen matrix of cells and tissues that can lead to cataracts, glaucoma, varicose veins, hemorrhoids, peptic ulcers, heart disease, and cancer. Anthocyanins are associated with increased neuronal signaling in brain centers, mediating memory function as well as improved glucose disposal, benefits that would be expected to mitigate neurodegeneration. They improve the integrity of support structures in the veins and entire vascular system, enhance the effects of vitamin C, improve capillary integrity, and stabilize the collagen matrix (the ground substance of all body tissues). The proanthocyanidins (condensed tannins) produced by cranberries have been shown to help prevent urinary tract infections through reduced adhesion of uropathogenic *Escherichia coli* (Howell et al. 2005). Lingonberry fruits and leaves are used medicinally to lower cholesterol levels and treat stomach disorders and rheumatic diseases as well as an agent to treat bladder and kidney infections (Novelli 2003).

Analysis of genetic diversity in berry crop germplasm can facilitate reliable classification of genotypes and identification of subsets of core accessions with possible utility for specific breeding purposes. Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including genetic variability analysis in a germplasm (Cox et al. 1986), identification of diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell 1998) and introgression of desirable genes from diverse germplasm into the available genetic base (Thompson et al. 1998).

3.2 Diversity and Erosion

Wide genetic diversity within a species is required for its survival and adaptation to changing environments. As in other crops, domestication in berry crops has limited the genetic diversity to useful genotypes, adapted to the needs of humans as well as local growing conditions. Genetic erosion is defined as the loss of variability from crop populations in diversity centers, i.e., areas of domestication and

secondary diversification (Brush 1999). It implies the disappearance of genetic variability in a population so that the net change in diversity is negative causing a reduction in the total number of crops, varieties or alleles. Ecological theories regarding extinction are built primarily for wild populations (Tunstall et al. 2001). Along with the industrialization in the nineteenth century, the diversity of domestic crops decreased dramatically at the species and intraspecific levels, leading to genetic erosion or the loss of cultivated and wild species, subspecies, landraces, former varieties and single genes. Crop genetic erosion is due to complex process involving both human and environmental drivers. Modern plant breeding efforts, climate change and environmental degradation, the effects of urbanization and modern agricultural practices and natural disasters or human conflicts resulting in a large-scale displacement of farmers, are the major causes of genetic erosion (Richards and Ruivenkamp 1997). Globally, this process has led to a decrease in crop diversity in many farming systems, frequently because traditional varieties are being replaced by modern varieties (FAO 1997). Berry species that cannot meet changing demands by farmers and consumers become neglected and farmers are more interested to cultivate promising berry cultivars that have high market value. Although diversity is lost once the crops are grown on farm, the need for genetic resources in order to develop new and improved varieties started to increase. Novel genetic variation can be created by sexual hybridization within and across berry species. However, as landraces are often well adapted to specific environments, they do have a clear advantage in marginal areas. Besides their direct use, these genetic resources are very valuable in breeding programs (IPGRI 2004). Although berry production is increasing and becoming more economically important on the global scene, some countries have insufficiently invested in conservation of genetic resources due to insufficient facilities and inadequate support for berry preservation and distribution (Hummer 2007). Special attention to conserve wild and threatened berry clones and to maintain their diversity should be given. Improvement programs in minor berry crops need to be encouraged, so that these berries can keep their place in farming systems and in food chain, while modernization in horticulture is continued.

3.2.1 Blueberry

A decrease in genetic diversity among cultivated blueberries as opposed to wild blueberries was evident from a study with wild and cultivated blueberries where the average number of alleles, the average Shannon's index per locus (Shannon and Weaver 1949) and the number of unique alleles were greater in wild accessions than in domesticated accessions (Boches et al. 2006). Brevis et al. (2008) reported that the southern highbush blueberries are less genetically diverse than previously thought. The heterozygosity of the cultivated highbush blueberry has continued declining as a consequence of selective breeding (Brevis et al. 2008).

3.2.2 Raspberry

The wild red raspberry populations are genetically distinct from cultivated raspberries (Graham et al. 2003). Over a 10-year period of study, a decline in population size was observed in Scottish wild red raspberry plants (Graham et al. 2009) which might have resulted mainly from habitat degradation leading to erosion of genetic diversity (Young et al. 1996). Graham et al. (2009) reported that only 18 of the 80 alleles present in the wild were found in cultivated raspberries. They found that the unique diversity was eroded primarily due to human impact. In the long term, this might affect the viability of populations to changing selection pressures due to climate change (Young et al. 1996).

3.2.3 Strawberry

The strawberry has centers of diversity in Eurasia and North and South America and the primary cultivated gene pool is octoploid (Hancock et al. 1996). Landraces are being lost though human encroachment, natural disasters and displacement by commercial cultivars. Although cultivars are being developed for more than 300 years, the primary cultivated gene pool has a narrow germplasm base (Hancock et al. 1996; Debnath et al. 2008). To prevent genetic erosion, conservation of *Fragaria* species is of prime importance. For proper conservation, it was suggested that diploids and tetraploids *Fragaria* genetic resources need to be obtained from Asia, native octoploids from northwestern Alaska, *F. virginiana* from Canada and the United States, *F. moschata* and *F. viridis* from Eastern Europe, and heirloom varieties that possess unique genetics from available sources (Hummer 2007).

As in with other crop species, there is an urgent need to accept, and deal with, the requirements of protecting berry crop plants, their habitats and ecosystems. A global strawberry conservation strategy was initiated through the Global Crop Diversity Trust (Trust). This was a joint initiative of the Food and Agriculture Organization of the United Nations (FAO) and the International Plant Genetic Resource Institute (IPGRI) on behalf of the centers of the Consultative Group on International Agricultural Research (CGIAR) (Hummer 2007). The Trust is a public–private partnership that was established under law in October 2004 and aimed to protect the availability and accessibility of the wild and cultivated gene pools (Hummer 2007).

3.3 Conservation of Genetic Resources

Plant germplasm is the living tissue from which new plants can be grown. It can be a seed or a plant part (a stem, a leaf, a pollen, or even a few cells) that can be cultured into a whole plant (http://pgrc3.agr.gc.ca/about-propos_e.html). It contains

the genetic information for the plant's hereditary makeup. Berry germplasm needs to be maintained for preserving the genetic diversity of berry plants, their wild relatives, and plants present and unique in the biodiversity. The US National Plant Germplasm System was established to collect, maintain, distribute, evaluate, and document germplasm of strawberry, currant, gooseberry, blackberry, raspberry, blueberry, and cranberry and their wild relatives. A collection of 200 genotypes of cranberries are preserved as seed or maintained as clones in the Genebank at the National Clonal Germplasm Repository in Oregon (Zdepski et al. 2011). In Canada, the system plays a significant part of Agriculture and Agri-Food Canada's commitment to the Canadian Biodiversity Strategy in response to the Convention on Biological Diversity. The Canadian Clonal Genebank was designated in 1989 as the primary germplasm repository for fruit tree and small fruit crops. Originally located near Trenton, Ontario it moved in 1996 to Harrow, Ontario. The Canadian Clonal Genebank located in Harrow, Ontario is responsible for conserving, characterizing, virus indexing, and distributing tree and small fruit; for example, apple and strawberry (http://pgrc3.agr.gc.ca/about-propos_e.html).

Natural stands of cranberries, lowbush blueberries, lingonberries and cloudberries are harvested in the Eastern and Atlantic Canadian provinces. Due to the increased demand for nutritious, natural fruit-based drinks and other products that use processed the berries, local demand now exceeds production. An increasing demand for high-quality berries in Canada has intensified the need to select superior wild-growing plants for cultivation. To this end, a program to develop improved berry cultivars at the Atlantic Cool Climate Crop Research Centre of Agriculture and Agri-Food Canada in Newfoundland and Labrador was established in 1999 where more than 1000 wild cranberry, lowbush blueberry, lingonberry and cloudberry clones have been collected and are being used in berry improvement program (Debnath 2000). The wild clones and cultivars sampled in this study and the accompanying assessment of genetic diversity represent essential tools for germplasm management and plant breeding efforts.

Berry crop biodiversity are being conserved in Europe (Bartha-Pichler 2006) and an interest in the conservation of genetic resources has led to studies on wild raspberry populations in various countries. In Scotland, wild raspberries were found to be genetically and physiologically different from each other and from cultivars (Marshall et al. 2001; Graham et al. 1997, 2003). Similar studies using phenotypic characteristics have been carried out on 12 wild raspberry populations in Russia (Ryabova 2007) where wild populations were examined for characteristics which may be useful in cultivated raspberries. Finn et al. (2003) evaluated black raspberry (*R. leucodermis*) populations for traits of importance for use in red and black raspberry breeding and suggested that the conservation of genetic resources may prove to be invaluable in securing germplasm for future breeding programs.

Berry germplasm can be maintained through in situ or ex situ conservation. While the former method preserves the living plants in a natural state in the habitat where they naturally occur, the ex situ approach is the conservation outside of the natural environment. In in situ conservation, large ecosystems can be left

intact as protected reserve areas with minimal intrusion or alteration by humans. In ex situ conservation, preservation and propagation are accomplished outside the natural habitat where they occur. Plants can be conserved in botanical gardens, greenhouses. The preservation of seeds or other plant materials can also be done in germplasm banks under appropriate conditions for long-term storage.

Although conventionally seeds are used to store the germplasm in many plant species, berry plants are genetically heterozygous and they are propagated vegetatively to ensure that desired genetic characteristics are preserved.

3.3.1 In Vitro Conservation

In vitro or tissue culture methodologies for berry crop conservation represent an important tool for ex situ conservation. The prerequisite for in vitro germplasm conservation is the establishment of efficient micropropagation systems.

Being genetically heterozygous, berry crops are vegetatively propagated. Micropropagation is a multibillion dollar industry being practiced for clonal mass propagation of specific genotype and of parental stocks for hybrid seed production, maintenance of pathogen-free (indexed) germplasm, and use as the initial step in a nuclear stock crop production system and year-round production of plants. It is based on enhanced axillary bud proliferation and on the ability of differentiated, often mature plant cells, to redifferentiate, and develop new meristematic centers that are capable of regenerating fully normal plants. Regeneration is potentiated through two morphogenic pathways: (1) organogenesis—the formation of unipolar organs and (2) somatic embryogenesis—the production of bipolar structures and somatic embryos with a root and a shoot meristem (Steward et al. 1970). Although somatic embryogenesis is amenable to mechanization, making possible the use of bioreactors for large-scale production of somatic embryos and their delivery through encapsulation into artificial seeds, it often exhibits abnormalities with respect to morphology (Halperin 1966) as well as physiology. Although most of the micropropagation protocols used semi-solid gelled medium, significant opportunities exist for the application of bioreactor propagation in berry crops (Debnath 2011a). Automation, using a bioreactor, is one of the most effective ways to reduce the costs of micropropagation (Paek et al. 2001). The system improves the efficiency of in vitro propagation of berry crops; shoot proliferation is about three times more than on gelled medium (Debnath 2009a). Less cytokinin concentration (1–2 μM zeatin) is required in liquid culture (Debnath 2009a) compared with gelled medium (2–4 μM) for maximum shoot proliferation of *Vaccinium* species (Debnath 2004). Protocols for berry crop micropropagation have been reviewed elsewhere (Debnath 2003a, 2007a, 2011a; Graham 2005; Skirvin et al. 2005; Debnath et al. 2012b). Different explant types through distinct morphogenic pathways, aiming at conservation, multiplication, and germplasm improvement of berry crops, have been used (Table 3.1).

Table 3.1 Examples of micropropagation in *Fragaria*, *Rubus* and *Vaccinium* species

Species	Micropropagation pathway	Explant used	References
F. × ananassa	Shoot regeneration	Leaf	Passey et al. (2003), Hanhineva et al. (2005), Debnath (2005a, 2006)
F. × ananassa	Shoot regeneration	Petiole	Passey et al. (2003), Debnath (2005a, 2006)
F. × ananassa	Shoot regeneration	Sepal	Debnath (2005a, 2006)
F. × ananassa	Shoot regeneration	Stem	Graham et al. (1995)
F. × ananassa	Shoot regeneration	Root	Passey et al. (2003)
F. × ananassa	Shoot regeneration	Runner	Liu and Sanford (1988)
F. × ananassa	Shoot regeneration	Stipule	Passey et al. (2003)
F. vesca	Somatic embryogenesis	Leaf	Zhang et al. (2014)
R. chamaemorus	Shoot proliferation	Node	Debnath (2007b)
R. idaeus	Shoot regeneration	Leaf	Debnath (2010, 2014a)
V. angustifolium	Shoot proliferation	Node	Debnath (2004)
V. angustifolium	Shoot regeneration	Leaf	Debnath (2011b)
V. corymbosum	Shoot poliferation	Hardwood and softwood cuttings	Gonzalez et al. (2000)
V. corymbosum	Shoot regeneration	Leaf	Cao et al. (2002)
V. macrocarpon	Shoot proliferation	Node	Debnath and McRae (2001a, 2005)
V. macrocarpon	Shoot regeneration	Leaf	Qu et al. (2000)
V. vitis-idaea	Shoot proliferation	Node	Debnath (2005b), Debnath and McRae (2001b)
V. vitis-idaea	Shoot regeneration	Leaf	Debnath (2005c)
V. vitis-idaea	Shoot regeneration	Hypocotyl	Debnath (2003b)
V. deliciosum	Shoot regeneration	Leaf, stem	Debnath and Barney (2012)
V. membranaceum	Shoot regeneration	Leaf, stem	Debnath and Barney (2012)
V. ovalifolium	Shoot regeneration	Leaf, stem	Debnath and Barney (2012)

Berry crop genetic resources can be conserved in vitro by slow growth or by cryopreservation for medium and long-term storage, respectively. In vitro preservation requires less space and, consequently, of labor costs for the maintenance of collections. The techniques are in use worldwide for storage of elite genotypes, wild species, modern and local cultivars, and genetically transformed material (Engelmann 2004).

For medium-term storage, slow growth of strawberry and raspberry shoots in vitro can be stored at 2–4 °C for 24 months (Reed 1991; Lisek and Orlikowska 2001a, b) or longer (Mullin and Schlegel 1976) on media without growth regulators, although multiplication after storage is significantly lowered (Lisek and

Orlikowska 2001b). Lisek and Orlikowska (2004) reported that in vitro storage of strawberry and raspberry shoot tips in alginate beads containing a medium without growth regulators results in 90–100 % survival after 3 months in storage at 4 °C, although it decreases multiplication of raspberry.

Sakai and Nishiyama (1978) were the first to successfully cryopreserve in vivo winter buds of raspberry by a method including slow-freezing at −5 °C and prefreezing to −45 °C prior to immersion in liquid nitrogen. Reed and Lagerstedt (1987) treated in vitro-grown raspberry shoot tips with a cryoprotectant composed of polyethylene glycol, glucose, and dimethylsulfoxide, after which the material was prefrozen at −40 °C and then immersed in liquid nitrogen. An efficient cryopreservation procedure for in vitro-grown raspberry shoot tips was developed based on encapsulation–vitrification and encapsulation–dehydration by Wang et al. (2005). Encapsulation–dehydration technique was also successfully applied for cryopreservation of shoot tips in *Ribes* (Reed et al. 2005). Kartha et al. (1980) reported cryopreservation of strawberry shoot tips using the slow-freezing method. It was possible to cryopreserve strawberry meristems in liquid nitrogen for 28 years without a decrease in viability (Karen and Kartha 2009). Five *F.* × *ananassa* cultivars and two wild species (*F. chiloensis* and *F. virginiana*) have been screened using the encapsulation–dehydration method and/or a protocol which compromises vitrification and encapsulation–dehydration by Clavero-Ramírez et al. (2005). Apices were encapsulated in an alginate gel, precultured on media containing high levels of sucrose (0.8 M) or a combination of 0.4 M sucrose and 2 M glycerol. They reported that the recovery rates varied from 23 to 63 % among genotypes. Cryopreservation using an aluminum cryoplate was successfully applied to in vitro-grown strawberry shoot tips where the shoots were cold-hardened at 5 °C for 3 weeks and precultured at the same temperature for 2 days on Murashige and Skoog (1962) medium containing 2 M glycerol and 0.3 M sucrose (Yamamoto et al. 2012). The shoots were then placed on the aluminum cryoplate containing alginate gel. After storage in liquid nitrogen, shoot tips attached to the cryo-plate were directly immersed into 2 ml of a 1 M sucrose solution for regeneration. Following this procedure, Yamamoto et al. (2012) reported that the average regrowth level of vitrified shoot tips of 15 strawberry cultivars reached 81 %. Kami et al. (2008) performed cryopreservation in cranberry using shoot apices and plant vitrification solution with dilution.

3.4 Genetic Markers

Markers have a key role in the study of genetic variability and diversity. Different marker systems have been established at morphological, physiological, and DNA levels for the assessment of diversity in berry plant populations. Each of these markers used to characterize similarities and differences between individuals, has its specific strengths and constraints, e.g. regarding the number of available markers, the polymorphism per marker, the mode of inheritance or the genomic

location of the markers, etc. To obtain unbiased estimates of the genetic diversity within a population, attention has to be paid to the choice of the marker system utilized as well as of the statistical methods applied once an appropriate dataset is obtained. In strawberry, the genetic loci regulating runnering or nonrunnering habits (*r*), precocious flowering (semperflorens; *s*), and yellow fruit color (*c*) were defined by Brown and Wareing (1965a, b). During the last three decades, classical strategies for the evaluation of genetic variability, such as comparative anatomy, morphology, embryology, and physiology, have increasingly been complemented by biochemical and molecular techniques. These include the analysis of chemical constituents (metabolomics), but most importantly relate to the development of molecular markers. Determining plant metabolome is the profiling of the small molecules (chemical compounds) found throughout the plant in response to a variety of growth conditions. Plant metabolites may act as markers of quality traits, potentially expediting the appraisal of experimental lines during breeding. Analysis of secondary metabolites is, however, restricted to those plants that produce a suitable range of metabolites which can be easily analyzed and which can distinguish between varieties. These metabolites which are being used as markers should be ideally neutral to environmental effects or management practices.

Although traditional cultivar identification is based on morphological traits of leaf, flower, and fruit (Dale 1996), its usefulness is limited to closely related cultivars that often cannot be distinguished by morphological indices alone. Furthermore, expression of morphological features is often affected by the environment, including the method of cultivation. Examples of morphological diversity in some berry crops are presented in Table 3.2. Karyotype analysis cannot reveal alteration in specific genes or in small chromosome arrangements.

Application of molecular markers for plant germplasm improvement was initiated in the early 1980s with the use of isozyme markers to speed up the introgression of monogenic traits from exotic germplasm into a cultivar background (Tanksley and Rick 1980). Later, Beckmann and Soller (1986) described the first use of restriction fragment length polymorphism (RFLP) (Botstein et al. 1980) markers in crop improvement including theoretical issues related to marker-assisted backcrossing for improvement of qualitative traits. Lande and Thompson (1990) pioneered the theoretical studies of marker-assisted selection (MAS) for quantitative traits. With the development of molecular marker technologies, many new alternative methods have become available for generating large number of genetic markers.

3.4.1 Protein-Based Markers

Molecular markers are designed to characterize variation at the level of DNA, RNA (theoretically), and protein. Protein (enzyme- and non-enzyme) molecular markers provide indirect information about plant genome structure. Isoenzymes,

Table 3.2 Examples of diversity analysis for morphological characters in *Fragaria*, *Rubus* and *Vaccinium* species

Species	Results obtained	Reference
Fragaria spp.	A study with 37 North American octoploid strawberry populations for 44 morphological traits, grouped the genotypes into five clusters: east of the Missouri River (*Fragaria virginiana* ssp. *virginiana*), the Black Hills (*F. virginiana* ssp. *virginiana* and ssp. *glauca*), from the eastern Cascades to the eastern Rocky Mountains (*F. virginiana* ssp. *glauca*), the western Cascades and Olympic Peninsula (*F. virginiana* ssp. *platypetala*) and the Pacific coast (*F. chiloensis*)	Harrison et al. (1997)
Fragaria spp.	Petiole color, leaf mass/area ratio, leaflet length and width, flower and receptacle diameter, petal width, flowers/inflorescence) were significantly different between the *F. chiloensis-platypetala* and *F. virginiana-glauca* species complexes. Variation among populations within provenances was low while a much larger amount of variability was observed among plants within populations	Harrison et al. (2000)
R. idaeus	While wild raspberries had a longer and broader compound leaf with the longer length of the petiole, the terminal and lower leaflets, cultivated raspberries had a broader lower leaflets, and the shape of the terminal leaflet is more often of broad obovate form	Ryabova (2007)
	Phenetic analyses with genotypes of *Vaccinium* section *Myrtillus* scored for 13 characters generated five robust clusters. *Vaccinium parvifolium* is the most distinct cluster, followed by the "*myrtiIlus-scoparium*" complex, then *V. membranaceum*, *V. caespitosum* and the "*ovalifolium-deliciosum*" complex. Biosystematic studies suggest that the five clusters comprise seven taxa	Vander Kloet and Dickinson (1999)
V. oxycoccos	Great phenological variation was observed among a collection of European cranberry (*V. oxycoccos*) clones. Leaf size, berry shape, berry size and fruit color at full maturity were found most clearly distinguished	Česonienė et al. (2013)

the "electrophoretically separable variants of one enzyme system" (Bergmann et al. 1989), are coded by genes at one or often several loci. Variants that are coded by alleles at the same locus are called allozymes.

Multilocus analysis considers the results for various loci belonging to one or, more commonly, a large number of enzyme systems. Protein variants in isozyme analysis are distinguished by gel electrophoresis and visualized by an enzyme-specific staining mixture, where substrate, co-factor and an oxidized salt are included. Isozymes are not necessarily products of the same gene, and they can be active at different life stages or in different cell compartments. Allozymes are codominant markers and can discrimination between homozygous and heterozygous genotypes which is important for precise estimations of population genetics parameters, especially in cross-pollinated species like berry crops. However, they provide fewer markers compared to DNA-based methods. Other disadvantages of isoenzymes include: limited number of enzyme loci for which staining protocols are available, occurrence of developmental and seasonally dependent enzyme expression, highly toxic stain constituents, and the banding pattern of dimeric loci (enzymes with two subunits) which is difficult to interpret without segregating populations (Tanksley and Orton 1983; Meerow 2005).

3.4.2 DNA Markers

The shortcomings of biochemical markers resulted in the development of markers based on DNA polymorphisms (Kan and Dozy 1978) that detect nucleotide sequence variation at a particular location in the genome. Unlike comparative anatomy, morphology, embryology, and physiology, DNA markers generate DNA 'fingerprints,' which are distinctive patterns of DNA fragments resolved by electrophoresis in agarose or acrylamide gels and detected by staining or labelling. The separation of these fragments is accomplished by exploiting the mobility with which different-sized molecules are able to pass through the gel. The DNA fragments of different lengths are visualized using a fluorescent dye specific for DNA, such as ethidium bromide. The gel shows bands corresponding to different nucleic acid molecules populations with different molecular weight. Fragment size determination is typically done by comparison to commercially available DNA markers containing linear DNA fragments of known length (Westermeier 2005).

DNA-based markers used in diversity analysis includes RFLP, random amplified polymorphic DNA (RAPD) (Williams et al. 1990), simple (short) sequence repeat (SSR) (Tautz and Renz 1984), sequence characterized amplified region (SCAR) (Paran and Michelmore 1993), sequence-tagged sites (STS) (Olson et al. 1989), amplified fragment length polymorphism (AFLP) (Vos et al. 1995), inter simple sequence repeat (ISSR) (Gupta et al. 1994; Zietkiewicz et al. 1994), and single nucleotide polymorphisms (SNPs) (Brookes 1999; Primmer et al. 2002). Reviews of these techniques are available (Nybom 2004; Varshney et al. 2005a). The polymerase chain reaction (PCR) (Saiki et al. 1985) has the ability to overcome many of the shortfalls in the Southern blotting RFLP technique. PCR-based DNA marker systems can use either primers designed from arbitrary or non-specific sequences such as RAPD and AFLP, or primers designed from known

sequence to target a single specific locus such as SSRs and STSs. Nevertheless, it is possible to identify important criteria by which to assess the value of any particular marker system to a chosen application. For accurate and unbiased estimates, criteria like (i) sampling strategies, (ii) utilization of various datasets on the basis of the understanding of their strengths and constraints, (iii) choice of genetic similarity estimates or distance measures, (iv) clustering procedures and other multivariate methods in analyses of data, and (v) the objective to determine genetic relationships need to be considered (Mohammadi and Prasanna 2003). Choosing the most appropriate technique may be difficult and often a combination of techniques is needed to obtain the information on diversity. To date, several types of markers including RFLP, RAPD, AFLP, ISSR, SSR, expressed sequence tag (EST)-PCR, and cleaved amplified polymorphic sequences (CAPS) derived from EST-PCR markers have been used for diversity analysis of berry crops (Debnath 2008; Debnath et al. 2012a).

In RFLP, the DNA sample is digested by restriction enzymes and the resulting fragments are separated according to their lengths by gel electrophoresis. Although RFLPs are codominant markers and unlimited, they require elaborate laboratory techniques including development of specific probe libraries, use of radioisotopes, southern blot hybridization procedures and autoradiography that make them labor intensive, time consuming, and costly (Kesseli et al. 1994).

RAPDs markers consist of short 10-base primers of arbitrary nucleotide sequences (>50 % guanine-cytosine) for amplification of multiple fragments of genomic DNA. Each product is derived from a region of the genome that contains two short segments in inverted orientation, on opposite strands that are complementary to the primer and sufficiently close together for the amplification to work. The amplification products are separated on agarose gels in the presence of ethidium bromide and visualized under ultraviolet light. The RAPD analysis is very simple, rapid, and it requires a small quantity of DNA and generates numerous polymorphisms. Being dominant markers, they cannot distinguish between homozygotes and heterozygotes and thus are less informative in some type of genetic analyses than codominant markers like isozyme and RFLP markers. The reproducibility of RAPD markers has been questioned in the literature (Hedrick 1992) which can largely be overcome by improvements in laboratory techniques and band scoring procedures (Nybom and Bartish 2000). Maintenance of consistent reaction conditions is of prime importance to obtain reproducible band profiles on the gels.

In AFLP, total genomic DNA is digested using two restriction enzymes. Double-stranded nucleotide adapters are ligated to the DNA fragments to serve as primer binding sites for PCR amplification. A subset of the restriction fragments is selected to be amplified. This selection is achieved by using primers complementary to the adaptor sequence, the restriction site sequence and a few nucleotides inside the restriction site fragments. The amplified fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies (Vos et al. 1995). Partial DNA digestion causes artifacts in AFLP analysis (Arnau et al. 2002). A major drawback for AFLP is that like RAPD, the

investigated loci are biallelic (dominant marker) and heterozygotes cannot be distinguished from homozygotes (Nybom 2004).

ISSR primers target microsatellites, repeating sequences of 2–6 base pairs of DNA, which are abundant throughout the plant genome (Wang et al. 1994). The primers used in ISSR analyses can be based on any of the SSR motifs (di-, tri-, tetra-, penta- ,or mixed-nucleotides) found at microsatellite loci, giving a wide array of possible amplification products which can be anchored to genomic sequences flanking either side of the targeted SSR (Gupta et al. 1994; Zietkiewicz et al. 1994). ISSRs cost less and are easier to use than AFLPs and do not require prior knowledge of flanking sequences, like SSRs (Reddy et al. 2002). However, like RAPD and AFLP, ISSR markers are also dominant markers. Compared to RAPD and AFLP, ISSR overemphasizes differences between closely related populations and attributes less variation to differences over large geographical distances (Qian et al. 2001).

SSRs or microsatellites are tandem repeats of a very short nucleotide motif (1–5 base pairs) that can be amplified with PCR primers specific to the flanking regions of these repeats. They are robust, highly reproducible, and are easy and fast to assay. SSRs have become an important tool in crop germplasm management and diversity analysis.

SCARs are PCR-based enhanced arbitrary amplified DNA markers produced by cloning, sequencing, and PCR amplification of arbitrary sites in the genome (Paran and Michelmore 1993). SCAR sequence analysis provides an estimate of the extent of duplex formation between arbitrary amplified DNA product termini. These markers can be developed from RDPD (Bautista et al. 2003), AFLP (Schmidt et al. 2003), or ISSR fingerprints (Albani et al. 2004). SCARs are usually dominant markers; however, some of them can be converted into codominant markers by digesting them with tetra cutting restriction enzymes. The most significant drawback of SCAR markers is development time and expense.

CAPS, originally named PCR-RFLP (Maeda et al. 1990), is a combination of the PCR and RFLP that involves amplification of a target DNA through PCR, followed by digesting with restriction enzymes (Michaels and Amasino 1998). Hence, CAPS markers rely on differences in restriction enzyme digestion patterns of PCR fragments caused by nucleotide polymorphism between samples. Although CAPS markers are inherited mainly in a co-dominant manner (Matsumoto and Tsumura 2004), the ability of CAPS to detect DNA polymorphism is not as high as SSRs and AFLPs because nucleotide changes affecting restriction sites are essential for the detection of DNA polymorphism by CAPS. Furthermore, the development of CAPS markers is only possible where mutations disrupt or create a restriction enzyme recognition site. An alternative marker called derived-CAPS (dCAPS) that eliminate the problems related with CAPS markers by generating mismatches in a PCR primer have been developed which are used to create a polymorphism based on the target mutation (Michaels and Amasino 1998).

SNPs are a single-base substitution/indel in the genome of an individual (Primmer et al. 2002) and are the most abundant form of genetic variation in most

organisms (Cho et al. 1999). They are used for detecting smallest unit of genetic variation among individuals within a species and are usually biallelic variations between individuals that occur in genes (promoter, exons or introns) or between genes (intergenic) (Rafalski 2002).

ESTs are short DNA molecules (300–500 bp) reverse-transcribed from a cellular mRNA population (MacIntosh et al. 2001). EST-specific primers are followed by digestion with restriction enzymes to generate CAPS markers to detect polymorphism. ESTs represent the expression of genes in a tissue or a cell at a certain time or condition, and permit rapid identification of the expressed genes.

3.4.3 Comparison Among DNA Markers

Molecular markers can be (i) hybridization-based (e.g., RFLP), (ii) PCR-based (e.g., AP-PCR, CAPS, STS, RAPD, SCAR, AFLP, SSAP, SSR, ISSR, EST), and (iii) DNA chip and sequencing based (SNP). They may be dominant or codominant. PCR-based protocols are usually fast, require only a small amount of DNA, and easier to use than RFLPs. While RFLPs, RAPDs, and AFLPs are all highly polymorphic marker systems, SSRs offer the greatest amount of polymorphism. Of the four techniques, RFLPs and SSRs are codominant and AFLPs, RAPDs, and ISSRs are only dominant. Although RFLPs, AFLPs, and SSRs are more expensive, laborious and time consuming, they are much more robust than RAPDs (Powell et al. 1996). ESTs and SNPs have potential to lower costs even further, especially if automated facilities are available (Gupta et al. 1999). Unlike with RAPDs and AFLPs, where Hardy–Weinberg equilibrium must be assumed to calculate heterozygosity and allele frequencies, microsatellites allow a direct test of Hardy–Weinberg equilibrium, for estimation of allele frequency and heterozygosity (Meerow 2005). Maroof et al. (1994) reported that the chromosomal segments marked by microsatellite loci are under selective pressure, and may therefore provide useful markers for distinguishing genotypes with desired phenotypic characters (Meerow 2005).

PCR based methodologies including RAPD markers are relatively easy to use, and inexpensive as compared to other methods. However, RAPD markers have some limitations including seamless interlaboratory transferability and susceptibility to certain types of error, although in *Vaccinium mytrillus*, it has been reported that RAPD markers are as effective as AFLP markers for identifying clonal diversity (Albert et al. 2003). Estimates of genetic relatedness deduced from employment of the RAPD and SCAR methods were compared among 27 randomly chosen cranberry germplasm accessions (Polashock and Vorsa 2002). Although both methods produced comparable results above 0.90 coefficient of similarity, SCAR marker reactions provided more polymorphic markers on a per reaction basis than RAPD marker reactions and as such more readily separated closely related progeny. Jones et al. (1997) compared the reproducibility of RAPD, AFLP, and SSR techniques in European laboratories and reported that RAPDs were

difficult to reproduce. For AFLPs, a single-band difference was observed in one track, while SSR alleles were amplified by all laboratories, but small differences in their sizing were obtained.

3.5 Blueberry, Cranberry, and Lingonberry

The genus *Vaccinium* L. (family: Ericaceae; tribe: Vacciniae; subfamily: Vaccinoiodae) contains about 400 species, and one or more species are native to all continents except Antarctica and Australia (Vander Kloet 1988; Ballington 2001). It is typically characterized as having fleshy, more-or-less edible fruits with very high levels of vitamin C, cellulose, pectin, and anthocyanins possessing antitumor, antiulcer, antioxidant, and antiinflammatory activities (Wang et al. 1999) and includes bilberry, blueberry, cranberry, huckleberry, lingonberry, and whortleberry. Flora of Europe is quite poor in *Vaccinium* (Tutin et al. 1972); it comprises 8 species including the subgenera *Oxycoccus* and *Vaccinium*. In Himalayas, 9 species of *Vaccinium* are found in the regions: Sikkim and Bhutan (Grierson and Long 1983–1991). Native stands of several species have been managed for fruit production for at least 1000 years in USA and Canada (Hall et al. 1979). Cranberry (*V. macrocarpon* Ait., $2n = 2x = 24$), blueberry (*Vaccinium* spp., $2n = 2x = 24$, $2n = 4x = 48$, $2n = 6x = 72$), and lingonberry (*V. vitis-idaea* L., $2n = 2x = 24$) are three commercially cultivated *Vaccinium* fruit crops of economic importance which have been domesticated in the twentieth century. The recent introduction of cultivars of the tetraploid small cranberry (*V. oxycoccus* L.) will add some additional diversity to the cranberry group (Pliszka 1993). All cultivated blueberries belong in the section Cyanococcus of the genus *Vaccinium*. Species within this section are often called the "true" or cluster-fruited blueberries. Wild representatives of Cyanococcus are found solely in North America. The most recent classification of the Cyanococcus species includes a total of seven diploid species (*V. boreale* Hall and Aald., *V. corymbosum*, *V. darrowi* Camp, *V. elliottii* Chapm., *V. myrtilloides* Michx., *V. pallidum* Ait., and *V. tenellum* Ait.), six tetraploid species (*V. angustifolium* Ait., *V. corymbosum*, *V. hirsutum* Buckley, *V. myrsinites* Lam., *V. pallidum* and *V. simulatum* Small), and two hexaploid species (*V. ashei* Reade and *V. constablaei* Gray), with *V. corymbosum* and *V. pallidum* occurring at diploid and tetraploid levels.

Although the majority of cultivated blueberry hectarage is in the United States and in Canada, they are also grown commercially in Europe, Asia, Africa, Australia, New Zealand, and South America (Lehnert 2008). While the leading countries in cranberry production are the United States, Canada, Latvia, and Poland, its culture has also shown promise in Austria, Germany, and Russia (http://aesop.rutgers.edu/~bluecran/cranberrypage.htm). Commercial lingonberry production primarily involves harvesting of berries from wild populations in northern Europe, Asia and North America, with cultivated production still in its infancy compared with cranberry and blueberry (Ballington 2001).

Blueberry (*Vaccinium* section Cyanococcus L.) includes diploid, tetraploid, and hexaploid species native to North America (Camp 1945; Vander Kloet 1988). The primary gene pool of blueberry consists of three species, highbush [*V. corymbosum* L. ($2n = 4x = 48$)], lowbush [*V. angustifolium* Ait. ($2n = 4x = 48$)] and rabbiteye blueberries [*V. virgatum* Ait. (syn. *V. ashei* Reade; $2n = 6x = 72$)], whereas the remaining noncultivated Cyanococcus species constitute the secondary gene pool (Lyrene and Ballington 1986). There are five major groups of blueberry species which are commercially-grown: (1) lowbush (*V. angustifolium*, *V. myrtilloides* Michx., *V. boreale* Hall and Aald.; Fig. 3.1b), (2) highbush, (3) half-high (Fig. 3.1a), which are hybrid or backcross derivatives of highbush-lowbush hybridizations; (4) southern highbush, which were developed from hybridization of *V. corymbosum* with one or more species (mainly *V. darrowi* Camp and *V. ashei*), and (5) rabbiteye. There may also be pentaploid and aneuploidy blueberries resulting from interploidal hybridization (Bian et al. 2014). Pentaploid blueberry genotypes ($2n = 5x = 60$) may occur when hexaploid species are crossed with tetraploid species. Two female fertile pentaploid cultivars 'Pearl River' (Spiers et al. 1997) and 'Robeson' (Ballington and Rooks 2009) have been released. The fertile *Vaccinium* aneuploids

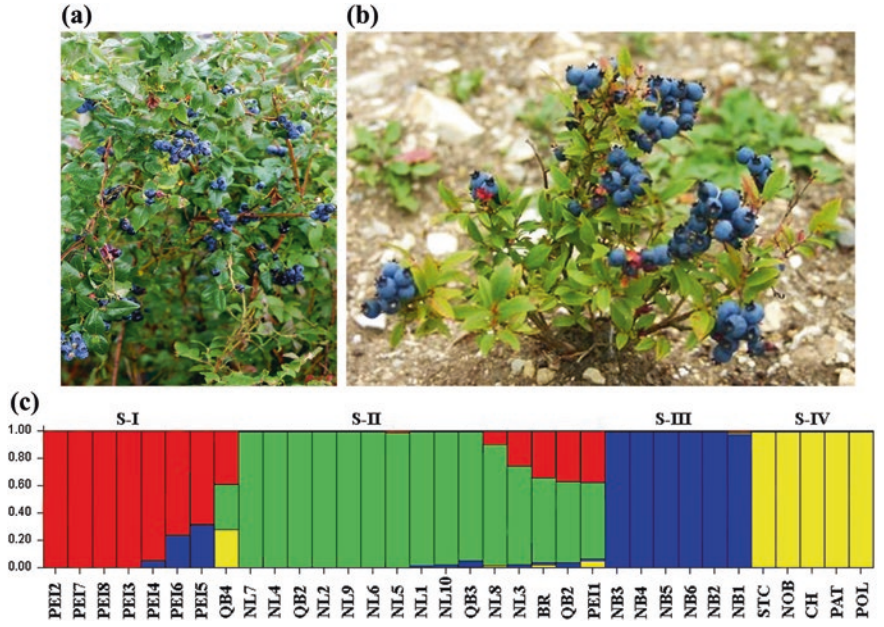

Fig. 3.1 Biodiversity in blueberry. **a** Half-high blueberry plants. **b** Lowbush blueberry plant. **c** Distribution of wild lowbush blueberry clones collected from four Canadian provinces: Prince Edward Island (*PE*), Quebec (*QC*), Newfoundland and Labrador (*NL*), and New Brunswick (*NB*); lowbush blueberry cultivar 'Brunswick' (*BR*); the half-high blueberry cultivars 'Chippewa' (*CH*), 'St. Cloud' (*STC*), 'Patriot' (*PAT*), and 'Northblue' (*NOB*) and the highbush blueberry cultivar, 'Polaris' (*POL*), in groups (SI, SII, SIII, SIV) according to EST-PCR and EST-SSR-based STRUCTURE analysis

were generated from the $4x \times 5x$ (and reciprocal) backcross progenies (Vorsa et al. 1986, 1987). During 100 years of blueberry improvement, homoploid and heteroploid interspecific hybridizations have been used extensively to combine useful traits found in the primary gene pool and to incorporate novel traits from wild germplasm. Southern highbush blueberries are low-chill (less than 600 h below 7 °C) interspecific hybrids derived from highbush blueberry, lowbush blueberry, rabbiteye blueberry, and wild diploid species native to the southeastern United States (Lyrene and Ballington 1986). The use of wild germplasm resulted in the incorporation of traits such as low chilling, tolerance to heat, drought, and mineral soils disease resistance and fruit quality traits such as picking scar, firmness, color, and flavor in southern highbush blueberries (Draper 1997).

Cranberries (Fig. 3.2a) belong to section Oxycoccus of genus *Vaccinium* which contains two species: *V. macrocarpon* Ait. and *V. oxycoccus* L. (Vander Kloet 1983). *V. maccocarpon*, the American or large-fruited cranberry, is an exclusively diploid vine that is native to North America with its natural distribution extending from Newfoundland, Canada west throughout the Great Lakes Region to Minnesota and south through the Appalachian Mountains to North Carolina and Tennessee in U.S.A. In contrast, *V. oxycoccus*, or little-leaved cranberry, is a polyploid species which has tetraploid ($2n - 4x = 48$) and hexaploid ($2n = 6x = 72$) individuals. *V. oxycoccus* is circumboreal. In Europe, it is present in the northwestern part of the continent, from Ireland, the British Isles and Scandinavia, throughout Central and Eastern Europe, the Balkan countries, Bulgaria and in Siberia (Asia) and Japan. It also occurs in Greenland and the northern part of North America (Jacquemart 1997).

Lingonberry is an important berry crop in many northern latitude countries (Gustavsson and Stanys 2000) and is used as both a medicinal plant (Novelli 2003) and an ornamental ground cover (Dierking and Dierking 1993). Various uses of lingonberry include juice, preserves, candy, jelly, syrup, ice cream, pickles, wine and liqueur (Gustavsson 1997). Native to north-temperate regions in Eurasia and North America (Pliszka and Scibisz 1989), this circumboreal woody, dwarf to low-growing, rhizomatous, evergreen shrub (Vander Kloet 1988) grows wild in diverse habitats, ranging from lowland to upland and mountain areas, in largely acid soils to pure peat bogs (Gustavsson 1997). The species is represented by two subspecies: ssp. minus (Lodd.) Hulten (1949) (Fig. 3.3a) and ssp. *vitis-idaea* L. (Fig. 3.3b) and the plant size constitutes the main difference between the two subspecies. Plants of *V. vitis-idaea* ssp. *vitis-idaea* are 25–30 cm in height, whereas plants of ssp. minus are approximately 20 cm high (Fernald 1950).

Haghighi and Hancock (1992) carried out an RFLP analysis of chloroplast and mitochondrial DNA of northern highbush blueberry cultivars and one representative of *V. ashei* using 23 restriction enzymes to identify variation and clarify mode of organelle inheritance. All species and genotypes displayed identical chloroplast DNA fragment patterns, but high degrees of polymorphism were observed in the mitochondrial genomes. ‘Bluecrop’ and ‘Jersey’ did not appear to have ‘Rubel’ cytoplasm as was previously believed. All hybrids contained maternal-type mitochondrial DNA.

RAPD has been used for estimation of relatedness and diversity in blueberry (Aruna et al. 1995; Burger et al. 2002; Albert et al. 2004, 2005; Debnath 2005d), cranberry (Novy et al. 1996; Stewart and Excoffier 1996; Polashock and Vorsa 2002; Debnath 2005a, b, c, d, 2007c; Fig. 3.2b) and lingonberry (Persson and Gustavsson 2001; Garkava-Gustavsson et al. 2005) wild accessions, clones, cultivars, and populations. Working with five cranberry cultivars and 43 wild clones collected from four Canadian provinces, Debnath (2007c) reported a high

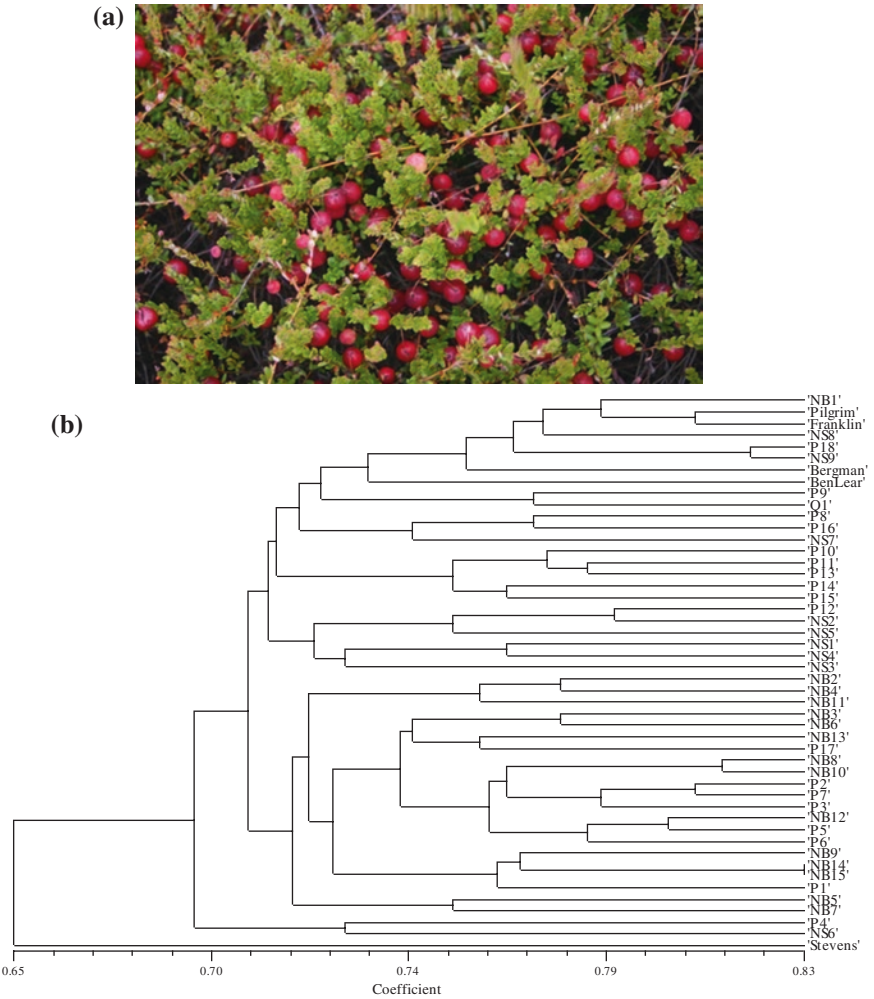

Fig. 3.2 Biodiversity in American cranberry. **a** American cranberry. **b** Unweighted pair-group method with arithmetic averages dendrogram estimating the genetic distance among five cranberry cultivars ('Stevens', 'BenLear', 'Bergman', 'Franklin', and 'Pilgrim') and 43 Canadian wild clones collected from Canadian provinces: Quebec (*Q*), Nova Scotia (*NS*), Prince Edward Island (*P*), and New Brunswick (*NB*), based on RAPD based Simple Matching similarity matrix

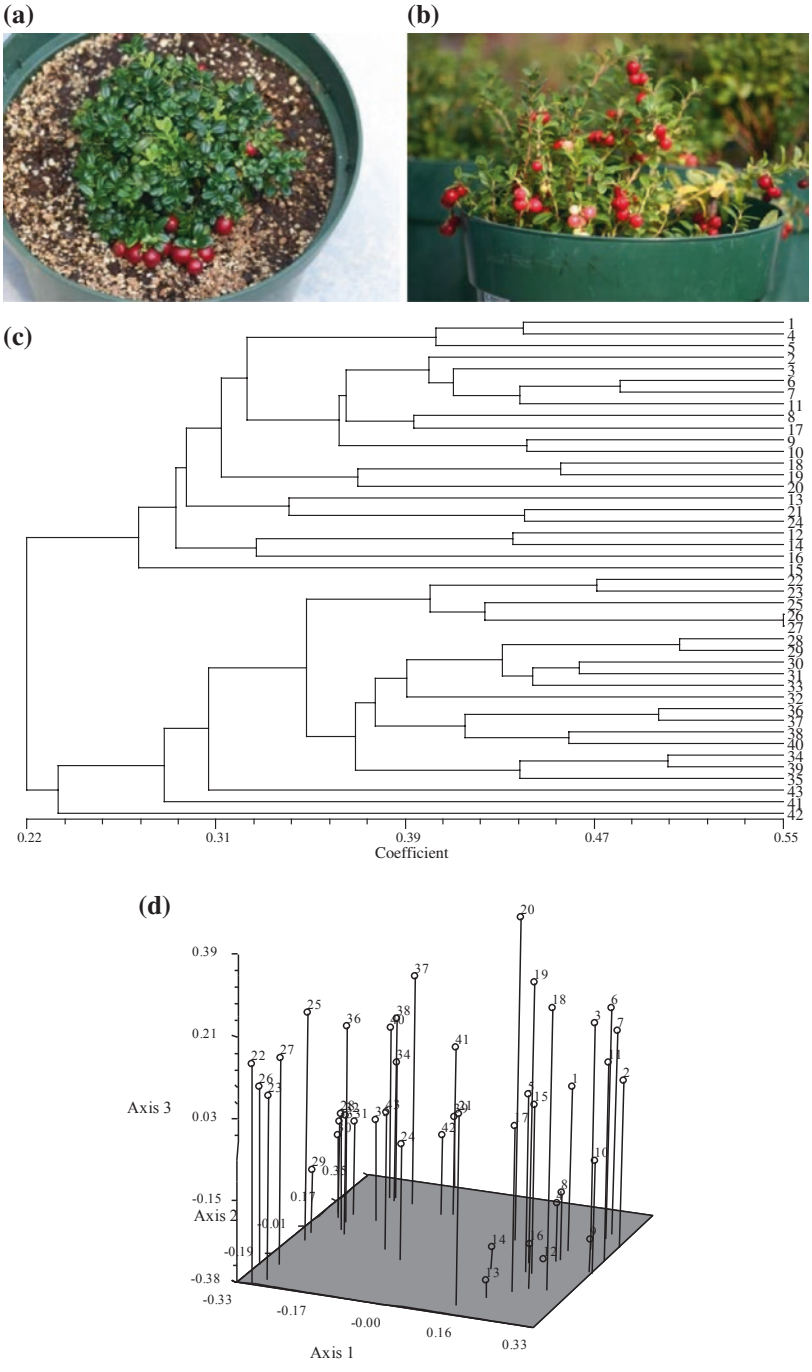
(a)
(b)
(c)
0.22
0.31
0.39
0.47
0.55
Coefficient
(d)
Axis 3
Axis 2
Axis 1
0.39
0.21
0.03
-0.15
-0.38
-0.33
-0.17
-0.00
0.16
0.33

Fig. 3.3 Biodiversity in lingonberry. **a** *Vaccinium vitis-idaea* ssp. *minus*. **b** *Vaccinium vitis-idaea* ssp. *vitis-idaea.* **c** Unweighted pair-group method with arithmetic averages dendrogram estimating the genetic distance among 43 wild lingonberry (*V. vitis-idaea* ssp. *minus)* clones (1–43) based on ISSR—Dice's similarity matrix. **d** Three-dimensional plot of the principal coordinate analysis of distance among 43 wild lingonberry (*V. vitis-idaea* ssp. *minus)* clones (1–43) ISSR—Dice's similarity matrix

proportion of genetic variation (90 %) as revealed by the analysis of molecular variance (AMOVA). A very high proportion of genetic variation (RAPD-based AMOVA) that can be invaluable in a breeding program (Barrett and Kidwell 1998; Thompson et al. 1998), was also observed within *V. uliginosum* L. (96 %, Albert et al. 2005), *V. mytrillus* L. (86 %, Albert et al. 2004) and *V. vitis-idaea* L. [89 % (Persson and Gustavsson 2001); 69–79 % (Garkava-Gustavsson et al. 2005)] populations. Debnath (2007c) identified that 10 % of total variation was due to geographical distribution in cranberry wild clones. Reasons for the lack of a geographical differentiation might be due to the result of a glacial bottleneck and rapid colonization coupled with autogamous breeding habit of cranberries (Stewart and Nilsen 1995). This low level of differentiation could also reflect high rates of regional gene flow resulting from both human migration and agricultural trade (Aldrich and Doebley 1992). Based on the RAPD and morphological data, a new taxon, *V. hiepii* Vander Kloet, sp. nov., was described. Cluster analyses clearly grouped *V. hiepii* with *V. chunii* Merr. ex Sleum., placing the new taxon within *V.§ Galeopetalum* (Vander Kloet and Paterson 2000). RAPD was also used to survey the genetic diversity in a population of *V. stamineum*, a clonal shrub that forms discrete patches (Kreher et al. 2000). High levels of genetic variation within some patches might be explained by somatic mutation; however, seedling recruitment was a more likely explanation (Kreher et al. 2000).

Albert et al. (2003) used AFLP to analyze 112 samples from a bilberry population and identified 32 clones and their spatial distribution. In the animal-dispersed *V. membranaceum* (Douglas ex Torr.), growing on volcanic deposits of Mount St. Helens (Washington, USA), genetic diversity in the newly founded population 24 years after the eruption was higher than in most of the source regions, suggesting a lack of a strong founder effect (Yang et al. 2008). High gene flow among sources and long-distance dispersal were inferred to be important processes shaping the genetic diversity in the young *V. membranaceum* population.

ISSR markers have been used successfully for diversity analysis of *Vaccinium* species (Fig. 3.3c, d). Levi and Rowland (1997) differentiated 15 highbush (or highbush hybrid) blueberry cultivars (*V. corymbosum* L.), two rabbiteye blueberry cultivars (*V. ashei*) and one southern lowbush (*V. darrowi*) selection from the wild using three ISSR markers. Although the *V. ashei* cultivars and *V. darrowi* selection grouped out separately from the *V. corymbosum* cultivars as expected, estimates of relative genetic similarity between genotypes within the *V. corymbosum* group did not agree well with known pedigree data. Forty-three wild lingonberry (*V. vitis-idaea* ssp. *minus* Lodd.) clones collected from four Canadian provinces were assessed for genetic variability (Debnath 2007d). Fifteen primers generated

356 polymorphic ISSR-PCR bands. A substantial degree of genetic diversity was found among the wild collections as was observed by cluster analysis. AMOVA indicated that geographical distribution explained 10 % of total variation. Four ISSR primers generated 113 polymorphic bands in 34 clones and eight cultivars of lingonberry and detected a sufficient degree of polymorphism to differentiate among lingonberries, making this technology valuable for germplasm management and more efficient choices of parents in lingonberry breeding programs (Debnath and Sion 2009). By assaying 36 ISSR polymorphisms in 21 populations of *V. stamineum* L. (deerberry) in eastern North America, from the range center to its northern limit where it has 'threatened' status, Yakimowski and Eckert (2008) reported that increased population isolation, rather than reduced population size, can account for the limited increase in genetic differentiation at range margins. Using six ISSR markers, Garriga et al. (2013) reported high levels of polymorphism among 10 highbush blueberry and three rabbiteye blueberry cultivars (80 % of polymorphic loci).

Polashock and Vorsa (2002) developed SCAR method for cranberry germplasm analysis. Nine primer sets were designed from RAPD-identified polymorphic markers for use in two multiplex PCR reactions. These primer sets generated 38 markers across a cranberry germplasm collection. Although SCAR markers can be employed for identifying closely related genotypes, the inferences of more distant genetic relationships are less certain. SCAR marker reactions provided polymorphic markers and as such readily separated closely related progeny. When SCAR primers were fluorescent dye labelled for computerized detection and data collection, reduced marker intensity relative to unlabeled reactions was one problem encountered.

Rowland et al. (2003b) used EST-PCR and EST-PCR-derived CAPSs markers to differentiate and evaluate genetic relationships among 15 highbush or highbush hybrid cultivars, two rabbiteye cultivars and two wild selections (one *V. darrowi* and one diploid *V. corymbosium*). Thirty PCR primer pairs were designed from the ends of the best quality sequences that were generated and were tested in amplification reactions with genomic DNA. Fifteen of the 30 primer pairs resulted in amplification of polymorphic fragments that were detectable directly after ethidium bromide staining of agarose gels. Several of the monomorphic amplification products were digested with the restriction enzyme AluI and approximately half resulted in polymorphic-sized fragments (CAPS markers). The polymorphic EST-PCR and CAPS markers developed in the study distinguished all the genotypes. A fair correlation between similarity coefficients calculated from marker data and coefficients of coancestry was found. It was also found that the EST-PCR primers designed from the blueberry EST databases of *Vaccinium* plant species are becoming a valuable source of PCR-based gene-specific markers for DNA fingerprinting and gene mapping. It was also found that the EST-PCR primers designed from highbush blueberries can be used to amplify genomic DNA from cranberries (Rowland et al. 2003a) and from lowbush (Bell et al. 2008) and rabbiteye blueberries (Rowland et al. 2010).

Genetic structure (Fig. 3.1c) is essential in association genetics, because a structured sampling set often results in false-positive marker trait associations (Lander and Schork 1994). Debnath (2014b) investigated the genetic structure and diversity of 28 wild lowbush blueberry clones collected from eight communities of four Canadian provinces: one lowbush, one highbush, and four half-high blueberry cultivars and two blueberry selections. Summary statistics, structure estimation, and clustering by neighbor-joining (NJ), principal coordinate analysis (PCoA), and by the AMOVA, using 10 EST-PCR and two EST-SSR primer pairs, were performed to characterize and discriminate the genotypes. A total of 213 markers were detected. Wide genetic diversity was evident from high values of expected heterozygosities, Shannon's index and polymorphism information content and from AMOVA. Structure analysis subdivided the lowbush blueberries into three distinct groups leaving the half-high and highbush blueberries into one cluster which was in agreement with the NJ clustering and PCoA. In a previous study of EST-PCR, variation among 25 genotypes of lowbush blueberries from four commercial fields in Maine, USA, Bell et al. (2008) detected a total of 81 polymorphic bands using 17 primers with 4.8 bands per primer in diploid and tetraploid Vaccinium species. Debnath (2014b) reported a wider range of blueberry genotypes including wild collections and selections, which would be expected given the broader geographic sampling. Working with highbush blueberries, Boches et al. (2006) reported that 9 out of 28 blueberry SSR loci were able to distinguish each unique accession. In *V. aungastifolium*, the average Shannon's index (1.93) (Debnath 2014b) was higher than those recorded for *V. uliginosum* (0.65) (Albert et al. 2005), *V.* myrtillus (0.55) (Albert et al. 2003), and for *Vaccinium* vitis-idaea (0.57) (Persson and Gustavsson 2001); but less than *V. corymbosum* (9.77) (Boches et al. 2006). EST-SSR loci are extremely variable in blueberries. Seventeen alleles per locus and very high levels of expected heterozygosity (He = 0.86) were observed among the blueberry genotypes by Debnath (2014b). Debnath (2014b) reported some inbreeding among the blueberry genotypes. Using STRUCTURE analysis, Debnath (2014b) observed the presence of admixtures in the wild blueberry germplasm that could be due to the result of a glacial bottleneck and rapid colonization of lowbush blueberries. This could also reflect high rates of regional gene flow resulting from both human migration and agricultural trade (Aldrich and Doeble 1992). A significant proportion of genetic variation between wild lowbush blueberry clones was evident from AMOVA analysis (39 %). Geographical variation contributed significant variation among localities within provinces as was evident from STRUCTURE, NJ, PCoA and AMOVA (Debnath 2014b). Bell et al. (2009) used EST-PCR markers to infer spatial genetic structure of four lowbush blueberry fields. Besides the clonal fidelity part of the study where they sampled at five different points within five clones, they also sampled across seven clones from four fields within 65 km of each other. They found within field variation to account for 91.6 % of the total genetic variation and among field variation to account for 8.4 %. A very high proportion of genetic variation was also observed within *V. uliginosum* (96 %) (Albert et al. 2005), *V. myrtillus* (86 %) (Albert et al. 2004) and *V. vitis-idaea* populations (89 %; Persson and Gustavsson 2001) through

RAPD-based AMOVA analysis, and within lingonberry (90 %) (Debnath 2007d) and lowbush blueberry clones (73 %) (Debnath 2009b) through ISSR-based AMOVA analysis.

Population structure using SSR markers has also been studied by Bian et al. (2014). For analysis of genetic diversity and population structure, 42 genomic SSR and EST–SSR markers with an average of 14.2 alleles and 56.0 allele phenotypes per locus were used to genotype a diverse blueberry population of 150 accessions. Cluster analysis grouped the accessions in a manner consistent with known information regarding species, ploidy levels, and pedigree. The analysis of population structure among blueberry accessions revealed inter- and intraspecific levels of stratification. Rabbiteye blueberry (*V. virgatum*) represents a genetically distinct subgroup within Cyanococcus. Three additional subpopulations were detected among highbush varieties that are largely attributable to distinctions between northern and southern highbush and founder effects of a single cultivar 'Weymouth' (Bian et al. 2014).

Genomic tools can hasten germplasm improvement for climatic adaptation and fruit and nutritional quality and for conservation of *Vaccinium* species. ESTs have been generated in blueberries from nonacclimated and cold acclimated flower bud libraries (Dhanaraj et al. 2004, 2007). The SSR and EST-PCR markers derived from highbush blueberry ESTs appear to be useful for cranberries (Bassil et al. 2009). Mapping populations and initial genetic linkage maps have been developed in *Vaccinium* species (Brevis et al. 2007). The microarray experiments with blueberries identified many transcripts whose abundances increase with cold acclimation and identified interesting differences in expression between acclimation under cold room and field conditions, and between cold-tolerant and cold-sensitive genotypes (Dhanaraj et al. 2007; Rowland et al. 2008). Georgi et al. (2013) constructed a cranberry genetic map based on four mapping populations segregating for field fruit-rot resistance that contains 136 distinct loci. It comprises 14 linkage groups totaling 879.9 cM with an estimated coverage of 82.2 %. Positioned on this map are quantitative traits loci for field fruit-rot resistance, fruit weight, titratable acidity, and sound fruit yield (Georgi et al. 2013).

Rowland et al. (2014) constructed a genetic linkage map of diploid blueberry from an interspecific diploid population that was comprised of 12 linkage groups and totals 1740 cM. The map included 265 markers based on SSR, EST-PCR, SNP, and RAPDs. While the estimated map coverage was 89.9 %, the average distance between markers was 7.2 cM. They identified one quantitative trait locus for cold hardiness on LG 4 (Rowland et al. 2014).

3.6 Rubus Species

The genus *Rubus* (Tourn.) L. contains approximately 750 highly variable and heterogeneous species (Robertson 1974; Thompson 1995) within 12–15 subgenera (Jennings 1988) with ploidy levels ranging from diploid to 14-ploid (Nybom 1985).

The members of this genus are called brambles that occur in all parts of the world except the desert regions. Plants are mostly perennial shrubs varying in habit from erect to trailing. Canes are usually biennial although a few species produce perennial or annual canes. The domesticated subgenera contain the raspberries, blackberries, arctic fruits and flowering raspberries. The most commercially important of the domesticated subgenera is Ideobatus (raspberries) which contains some 200 species showing considerable differentiation.

Commercially, the most important raspberries are the European red raspberry, *R. idaeus* L. subsp. *idaeus*, the North American red raspberry *R. idaeus* subsp. *strigosus* Michx, and the black raspberry (*R. occidentalis* L.). Red raspberry (*R. idaeus* L.; $2n = 2x = 14$) is an economically important berry crop with a high free radical scavenging capacity and it contains numerous bioactive compounds with potential health benefits (De Ancos et al. 2000). Raspberries are a temperate berry fruit crop but can also grow in areas with no chilling, where summers are very hot and soils are alkaline (Oliveira et al. 2002). Red raspberries are widely distributed in all temperate regions of Europe, Asia, and North America with the greatest diversity in China.

Enriching cultivated gene pool through incorporation of unique genes from wild germplasm is highly desirable in raspberry improvement. Cultivated raspberries are very different from their wild relatives. The former produces more but shorter and thinner canes than those produced by cultivated ones. While berries produced by cultivated raspberries are larger, the wild berries are small, soft, and crumbly with fewer but larger drupelets (Jennings 1988).

Molecular markers used for detecting genetic diversity in *Rubus* species have been demonstrated for allozymes (Cousineau and Donnelly 1992), RFLPs (Waugh et al. 1990), RAPDs (Graham and McNicol 1995; Graham et al. 1997; Weber 2003; Patamsytė et al. 2004; Badjakov et al. 2006), AFLPs (Kollmann et al. 2000; Ipek et al. 2009), minisatellites (Nybom 1995), ISSRs (Debnath 2007e, f), and SSRs (Graham et al. 2009; Dossett et al. 2012). Chloroplast DNA RFLP was used by Moore (1990) to investigate 21 red and black raspberry clones to determine cytoplasmic diversity. An M13 bacteriophage probe has been used to examine different *Rubus* spp. and a number of red raspberries (Nybom et al. 1990). A minisatellite probe was used by Kraft et al. (1996) to demonstrate that fingerprints of outcrossing species vary considerably compared to vegetative and apomictic clones. Chloroplast DNA sequence probes were used by Howarth et al. (1997) to examine genotypic and taxonomic relatedness in raspberry. Ribosomal DNA internal transcribed spacer region has been used to construct a phylogenetic tree with representatives from 20 species (Alice and Campbell 1999). Nybom and Schaal (1990) used RFLP markers to document genetic diversity in a wild black raspberry population in Missouri. They found 15 unique genotypes among 20 plants sampled along a 600 m stretch of roadside and suggested that the main mode of plant recruitment in this population was through sexually produced seed leading to intrapopulation diversity.

Weber (2003) examined genetic diversity in 14 cultivars black raspberry and two wild selections from New York using RAPD markers. Genetic diversity was

quite low; on average, there was 81 % similarity among polymorphic markers. However, more than half of this variability was accounted for by 'Black Hawk,' 'Cumberland,' 'John Robertson,' and the two wild selections. The remaining 11 genotypes had a collective marker similarity of 92 %. Weber (2003) asserted that many cultivars that originated as chance seedlings were probably from open pollination of other cultivars. Using RAPD markers, Graham et al. (2003) assayed a wider range of wild *R. idaeus* from 12 sites across a greater area of the United Kingdom and compared the accessions to European red raspberry cultivar 'Glen Moy'. Little gene flow was observed between wild populations and commercial cultivars. Forty *Rubus* species were analyzed using RAPD markers and found that molecular classification of species agreed with the traditional classification of *Rubus* in most cases, except for three species in the subgenus Malachobatus that clustered with the raspberry types in subgenus Idaeobatus (Pamfil et al. 2000). However, their RAPD-based taxonomy could not explain differential success of interspecific hybridization within each subgenus.

Raspberry domestication started around 500 years ago (Hedrick 1925), resulting in a reduction of both morphological and genetic diversity in red raspberry (Jennings 1988) with modern cultivars being genetically similar (Dale et al. 1993; Graham and McNicol 1995). Similar work using RAPD markers raised same concerns and the need for more incorporation of more diverse germplasm into black raspberry improvement (Weber 2003). Relatedness in blackberries has also been examined using pedigree analysis with similar findings recommending the diversification of the gene pool (Stafne and Clark 2004). This restricted genetic diversity is of serious concern for the future of *Rubus* breeding. Working with raspberry cultivars released between 1960 and 1993, Dale et al. (1993) reported that the genetic base from which the improvements were made was very narrow. The cultivars Lloyd George and Pyne's Royal, derived from *R. idaeus*, and Preussen, Cuthbert, and Newburgh that are derived from *R. idaeus* and *R. strigosus* dominated the ancestry of red raspberry. *R. idaeus* wild accessions from a Lithuanian germplasm collection were examined for genetic diversity using RAPD loci (Patamsytė et al. 2004). An environmental effect on diversity within populations was evident. DNA probes from two variable number tandem repeat (VNTR) loci were utilized to examine diversity in Philippine populations of *R. moluccanus* L. (Busemeyer et al. 1997). Similar results were also reported by Graham et al. (1997, 2003) who observed greater similarity within populations at each location than between locations. Additionally, apomictic reproduction was ruled out in these populations because no identical VNTR patterns were identified. Badjakov et al. (2006) analyzed 28 raspberry genotypes from the Bulgarian germplasm collection including 18 Bulgarian cultivars and breeding lines, eight accessions from outside Bulgaria and two wild species accessions, *R. occidentalis* and *R. adiene* using RAPD markers. They created a genetic similarity tree with two clusters, which corresponded to two pedigree groups among the Bulgarian genotypes. The genetic similarity within specific populations and the distinctness between them has been determined by RAPD markers and it had been suggested that there are strong limitations to gene flow through pollen and seed movement in red raspberry

(Graham et al. 1997, 2003). Research on natural populations of arctic raspberry has shown genetic diversity at levels near 50 % for among and within population estimates (Lindqvist- Kreuze et al. 2003).

AFLP has been used by Amsellam et al. (2000) to investigate the weedy raspberry species *R. alceifolius*. Considerably more genetic diversity was detected in its native range with diversity in nonnative ranges dependent on distance from the origin. Lindqvist-Kreuze et al. (2003) characterized diversity in six populations of wild arctic raspberry (*R. arcticus*) and 10 raspberry cultivars of Finland. AFLPs were highly effective in distinguishing 78 genotypes from 122 samples. Genetic variation was found to be high within populations, indicating a high degree of sexual reproduction, but interpopulation gene flow was low as measured by overall diversity among locations (Lindqvist-Kreuze et al. 2003).

Genetic diversity has been examined in natural populations of black raspberry (*R. coreanus*) in Korea using ISSR markers (Hong et al. 2003), and overall genetic relationships among populations were associated with geographic location. Cloudberry (*R. chamaemorus* L.; Rosaceae) (Fig. 3.4a), an important fruit crop in Scandinavia and northern Russia (Korpelainen et al. 1999), is a dioecious octoploid ($2n = 8x = 56$) perennial herb of boreal circumpolar distribution (Thiem 2003). It reproduces primarily through clonal growth (Makinen and Oikarinen 1974) and although sexual reproduction occurs rarely, this is obviously important for colonizing new habitats. Genetic studies on Finnish cloudberry populations indicate that the levels of genetic diversity within populations were quite low, comprising 2–4 clonal genotypes per population (Korpelainen et al. 1999). This has implications for domestication and breeding programs because the morphological variability observed may be largely influenced by environmental conditions. Therefore, plants for breeding programs should be selected from populations located some significant distance apart (Korpelainen et al. 1999). Wild cloudberries have a diverse variation (Fig. 3.4a, b). Debnath (2007f) studied 48 wild cloudberry clones, collected from four Canadian Provinces, for genetic variability using ISSR-PCR methods. Nine primers generated 138 polymorphic ISSR bands and a substantial degree of genetic diversity was found among the wild collection indicating a possibility of use of wild germplasm for cloudberry improvement.

Black raspberry (*R. leucodermis*) populations have also been evaluated for traits of importance for use in red and black raspberry breeding (Finn et al. 2003). Romanian (Rusu et al. 2006) and Bulgarian red raspberries (Badjakov et al. 2006) have been studied to determine their similarity with European and American germplasm using SSR markers. Badjakov et al. (2006) analyzed the 28 accessions with four SSR loci and demonstrated high levels of diversity within the collection Bulgarian red raspberries. Castillo et al. (2010) reported that of 13 SSRs evaluated in 48 genotypes of raspberries and 48 accessions of blackberries, three loci were highly polymorphic in each crop type, easy to score, and were mapped to single loci. They concluded that SSR-based analysis cannot be used to infer phylogenetic relationships. Working with Scottish wild red raspberry plants at 12 sites, Graham et al. (2009) screened 10 SSR loci and detected that only 18 of the 80 alleles present in the wild were found in cultivated raspberries. This highlighted

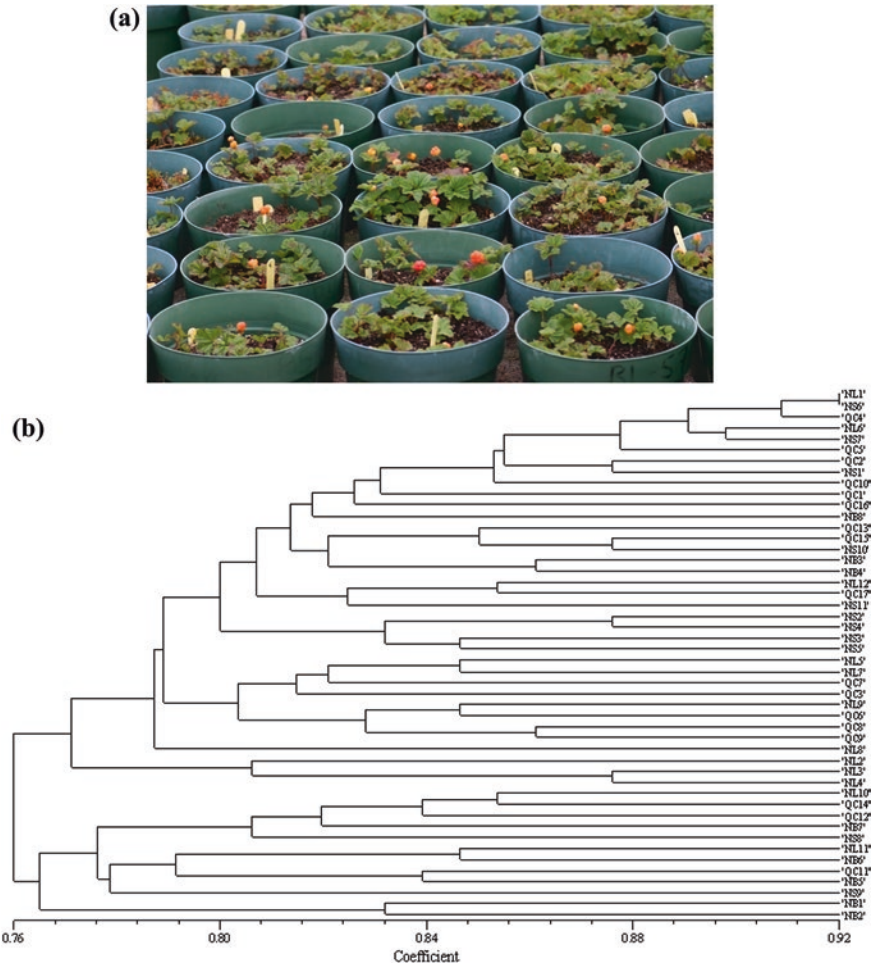

Fig. 3.4 Biodiversity in cloudberry. **a** Wild cloudberry germplasm. **b** Unweighted pair-group method with arithmetic averages dendrogram estimating the genetic distances among 48 wild cloudberry clones collected from Canadian provinces: Newfoundland and Labrador (*NL*), Quebec (*QC*), Nova Scotia (*NS*), and New Brunswick (*NB*), based on ISSR-Simple Matching similarity matrix

the genetic diversity available in wild population for future breeding. The plants in these 12 populations have been studied over a 10-year period during which the plant numbers in most of the populations have declined dramatically. The reason for the decline in plant numbers at most populations was largely due to human intervention and included activities such as land excavations and sheep grazing. This finding makes the decline in population number observed a concern, as this unique diversity is being eroded, primarily due to human impact. An additional

17 unique alleles were identified in the cultivars that were not present in the wild individuals studied. Diversity in this crop's wild relative and the population differentiation observed may have use in the future for breeding aimed at addressing climate change scenarios and consideration should be given to means of conserving the diversity revealed by these studies. Further loss of germplasm will have serious implications for long-term commercial red raspberry production. Most plant losses were readily explained by human impact and, consequently, turnover of populations may take place over a relatively short cycle (Graham et al. 2009). Similar results were also reported by Dossett et al. (2010) using SSR markers who found 12 black raspberry cultivars to be more closely related to each other than to any of the four wild accessions examined. These results, along with those of Weber (2003) and Nybom and Schaal (1990), suggest that wild populations have more genetic diversity than do current cultivars. However, Dossett et al. (2012) examined genetic diversity in 148 wild and cultivated black raspberry accessions using 21 polymorphic SSR markers and observed that black raspberry cultivars clustered tightly and showed higher than expected heterozygosity while that of wild accessions was low. This indicated that wild black raspberry germplasm is a relatively untapped resource available for future breeding (Dossett et al. 2012).

In raspberry, Graham et al. (2004) constructed a 789 cM genetic linkage map from a cross between the phenotypically diverse European red raspberry cultivar 'Glen Moy' and the North American cultivar 'Latham'. SSR and AFLP markers were utilized to create the linkage map. Bushakra et al. (2012) contracted linkage map in black and red raspberries consisting of DNA sequence based markers. They used an F_1 population developed from a cross between an advanced breeding selection of *R. occidentalis* (96395S1) and *R. idaeus* 'Latham'. While the 96395S1 genetic map consisted of six linkage groups and covered 309 cM with an average of 10 cM between markers, the 'Latham' genetic map had seven linkage groups and covered 561 cM with an average of 5 cM between markers. The alignment of the orthologous markers designed in the study suggested that the genomes of *Rubus* and *Fragaria* had a high degree of synteny and that synteny decreased with phylogenetic distance (Bushakra et al. 2012). Genotyping by sequencing was used to produce highly saturated maps for a *R. idaeus* pseudo-testcross progeny by Ward et al. (2013). The two resulting parental maps contained 4521 and 2391 molecular markers spanning 462.7 and 376.6 cM, respectively, over seven linkage groups.

3.7 Strawberry

The genus *Fragaria* is monophyletic and contains approximately 24 wild species, including 13 diploids ($2n = 2x = 14$), 5 tetraploids ($2n = 4x = 28$), 1 hexaploid ($2n = 6x = 42$), 4 octoploids ($2n = 8x = 56$) (Staudt 2008) and 1 decaploid ($2n = 10x = 70$) (Hummer et al. 2009) and naturally occurring hybrid species, including *F.* × *ananassa* ssp. *cuneifolia* Staudt ($2n = 8x = 56$), *F.* × *bringhurstii*

Staudt ($2n = 5x = 35$, $2n = 6x = 42$, $2n = 9x = 63$), and *F.* × *bifera* Duchesne ($2n = 2x = 14$, $2n = 3x = 21$) (Hummer et al. 2009). Many diploid and tetraploid species are endemic to Asia (Staudt 2005). Areas around the Sea of Japan and the Sino-Himalayan region are centers of diversity for *Fragaria* where most Fragaria diploids, tetraploids, and the decaploid are confined (Staudt 2005). The cultivated strawberry (*F.* × *ananassa* Duchesne ex Rozier) (Fig. 3.5a) is one of the youngest domesticated plants, developed in France in the 1700s by chance hybridization between the Scarlet or Virginia strawberry (*F. virginiana* Duch.) and the pistillate South American *F. chiloensis* (L.) Duch. (Hancock 1999). It is a dicotyledonous, perennial low-growing herb grown in most arable regions of the World. The crop is enjoyed by millions of people in all kinds of climates including temperate, mediterranean, subtropical, and taiga zones (Hancock et al. 1991).

Asia is the center of diversity for *Fragaria*. Several species from around the Sea of Japan include *F. iturupensis* Staudt (decaploid) found on Iturup Island, *F. mandshurica* (diploid) found on the continental Russian Far East to north Korea, *F. orientalis* (tetraploid) found along the Amur Valley and into China, *F. iinumae* Makino (diploid) in Honshu and Hokkaido, Japan and *F. nipponica* Makino (diploid) found in Honshu and Hokkaido Japan, Sakhalin, Russia and Kurils (Staudt 2005; Staudt and Olbricht 2008).

Strawberry development efforts began in the mid-eighteenth century (Hancock et al. 1996) and significant progress has been made in the past 50 years. Today, more than 500 commercial cultivars are grown worldwide (Hancock 1999). However, the commercial strawberry has a narrow germplasm base (Fig. 3.5b), even though its progenitor species have an extensive geographical range (Sjulin and Dale 1987; Hancock et al. 2002).

Isozyme markers were used by Arulsekar et al. (1981) to investigate diploidized nature of the octoploid strawberry using phosphoglucoisomerase (PGI) and leucine amino peptidase (LAP). Distinction between genotypes of the cultivated strawberry has been performed using PGI, LAP, and phosphoglucomutase (PGM) (Bell and Simpson 1994). Similar studies using isoenzymes have also been conducted on the Chilean strawberries, *F. chiloensis* (Gambardella et al. 2005).

DNA-based markers have been used by Kunihisa et al. (2006) who developed 24 PCR-RFLP markers and distinguished 65 Japanese strawberry cultivars and 96 progeny of a selfed 'Sachinoka' strawberry line. The results were highly reproducible across DNA extraction methods, organs and researchers. In addition, they investigated the inheritance of these markers using selfed lines of 'Sachinoka,' T'ochihime,' 'Nyoho,' and 'Cesena,' and checked whether it was consistent with Mendelian law. Strawberries have been extensively analyzed for relationship and diversity analysis using RAPD (Hancock et al. 1994; Graham et al. 1996; Harrison et al. 2000; Degani et al. 2001; Sugimoto et al. 2005), AFLP (Degani et al. 2001; Tyrka et al. 2002), ISSR (Arnau et al. 2002; Debnath et al. 2008) and SSR markers (Ashley et al. 2003; Cipriani and Testolin 2004; Lewers et al. 2005; Monfort et al. 2006; Cho et al. 2007). Degani et al. (1998) identified 10 RAPD markers and used to distinguish 41 strawberry cultivars grown in the United States and Canada. Hancock et al. (1994) has also examined the genetic diversity among eight related

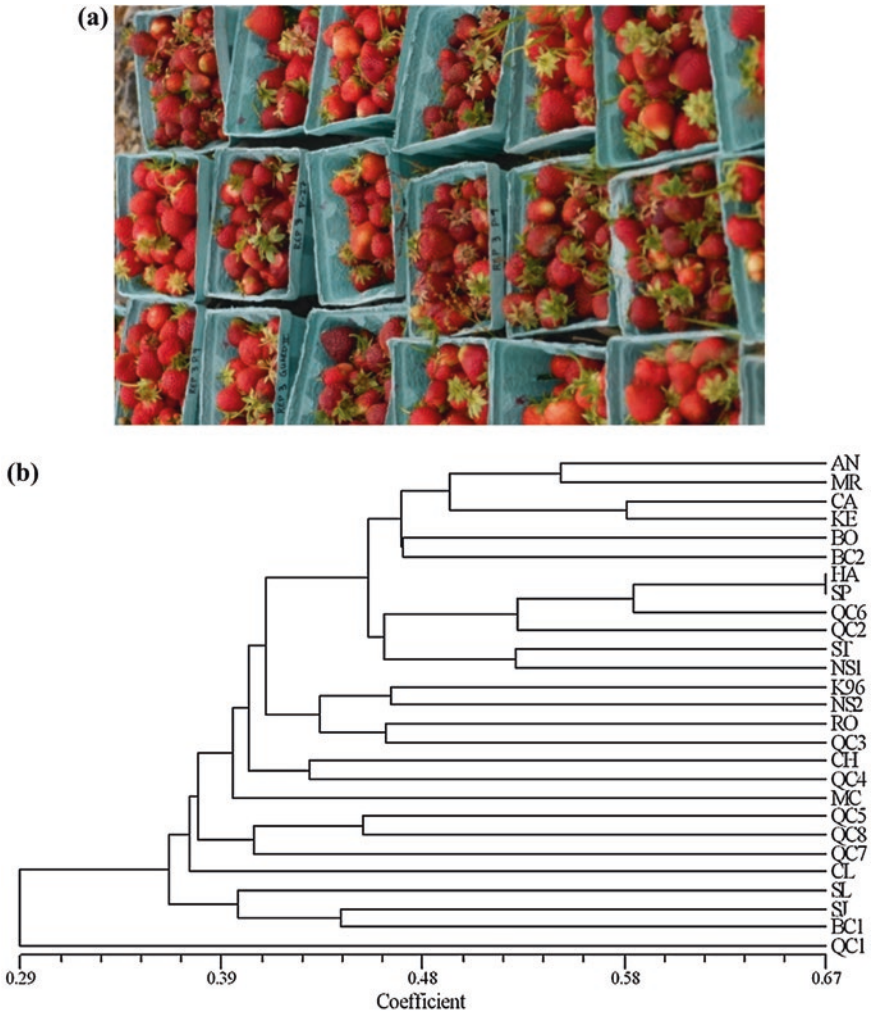

Fig. 3.5 Biodiversity in strawberry. **a** Variation in berry size, shape, and color at ripe. **b** Unweighted pair-group method with arithmetic averages dendrogram estimating the genetic distance among Canadian strawberry cultivars and breeding lines, based on ISSR coefficient-derived Jaccard's similarity matrix. Cultivars: *AN* ('Annapolis'), *BO* ('Bounty'), *CA* (Cavendish), *CH* ('Chambly'), *CL* ('Clé des champs'), *HA* ('Harmonie), *JO* ('Joliette'), *KE* ('Kent'), *MC* ('Micmac'), *MR* ('Mira'), *RO* ('Rosalyne'), *SJ* ('St-Jean d'Orléans'), *SL* ('St-Laurent d'Orléans'), *SP* ('Saint-Pierre'), *ST* ('Stolo'), and *WE* ('Wendy'). Thr rest of the genotypes are advanced breeding lines developed at Agriculture and Agri-Food Canada Research Centre at Kentiville, Nova Scotia (*NS*); Saint-Jean-sur-Richelieu, Quebec (*QC*), and Agassiz, British Columbia (*BC*) (Debnath et al. 2008)

strawberry cultivars and advanced breeding selections. However, RAPDs were unable to discriminate among the four subspecies of *F. virginiana* L. (Harrison et al. 2000). AFLP was used by Degani et al. (2001) to study genetic relationships

among 19 strawberry cultivars from United States and Canada. Genetic studies on *F. chiloensis* from Chile reported a genetic diversity for AFLP markers with just 29 % of AFLP polymorphisms in 61 accessions (Becerra et al. 2001). Tyrka et al. (2002) distinguished six strawberry cultivars and 13 salinity tolerant clones using a simplified AFLP assay based on a single cutting enzyme PstI—PstIAFLP.

ISSR markers were used to assess the genetic diversity in 216 accessions of *F. chiloensis*, which represented the two botanical forms present in Chile (*F. chiloensis* ssp. *chiloensis* f. *chiloensis* and *F. chiloensis* ssp. *chiloensis* f. *patagonica* (L.) Duch.) (Carrasco et al. 2007). High genetic diversity at the species level (polymorphic ISSR loci [P] = 89.6 %, gene diversity [h] = 0.24 ± 0.17, Shannon's index [S] = 0.37 ± 0.24) and a lower genetic diversity in f. *chiloensis* than f. *patagonica* were observed. The AMOVA showed a moderate genetic differentiation among accessions (fst = 14.9 %). No geographic patterns for ISSR diversity were observed. AMOVA, structure and discriminant analysis indicated that accessions tend to group by botanical form (Carrasco et al. 2007). Arnau et al. (2002) used five ISSR markers to characterize 30 strawberry varieties. With ISSR markers, Carrasco et al. (2007) reported a high genetic diversity at the species level and a lower genetic diversity in *F. chiloensis* than *F. patagonica*. Using 17 ISSR primers, Debnath et al. (2008) reported a narrow genetic base among 16 strawberry cultivars and 11 breeding lines developed in Canada, ranging from 63 to 77 %. Seventeen ISSR primers generated 225 polymorphic ISSR-PCR bands. Geographical distribution for the place of breeding program explained only 1.4 % of total variation as revealed by AMOVA (Debnath et al. 2008). Debnath and Ricard (2009) also reported a high degree of genetic similarity among 10 strawberry cultivars and nine breeding lines ranging from 45 to 73 %.

Ge et al. (2013) conducted cluster analysis of 16 strawberry cultivars using the 116 SNPs. The cultivars 'Meiho,' 'Sachinoka,' and 'Hokowase' showed a close genetic variation, which was consistent with the fact that they are from the same area. 'Chandler' and 'Honeye' also showed a close genetic variation. Although the 16 cultivars belong to different areas, they were grouped into three small subclusters. This revealed that the genetic variation between the cultivars was not much as expected (Ge et al. 2013).

Although microsatellite or SSR markers are very expensive (Gupta and Varshney 2000), their transferability was reported to be high within the genus *Fragaria* (Bassil et al. 2006). SSR has been studied by Ashley et al. (2003) and Hadonou et al. (2004) for variability and diversity analyses in strawberry. Using *Fragaria* species-derived SSRs, Njuguna et al. (2011) assessed genetic diversity of populations of the diploids, *F. iinumae* Makino and *F. nipponica* Makino, and examined intra- and interspecies relationships in overlapping populations.

Njuguna et al. (2011) reported that 20 of 82 *Fragaria*-derived SSRs were polymorphic among and within the two Japanese diploid strawberry species, *F. iinumae* Makino and *F. nipponica*Makino. Genetic diversity, based on the proportion of shared alleles between the two species, in *F. nipponica* (0.4542) and *F. iinumae* (0.1808) was significantly different. A high genetic diversity was reported in the outcrossing diploid species, *F. nipponica* compared to self-pollinating *F. iinumae* as seen from

the heterozygosity values (Ho) (0.4071 vs. 0.1336, respectively) and the number of alleles/locus (10.6 vs. 7.3, respectively) Njuguna et al. (2011).

Using 18 SSR markers, Yoon et al. (2012) identified 101 alleles with an average of 5.6 per locus and 21 specific alleles in 59 accessions of cultivated strawberries from Korea, Germany, United States, United Kingdom, and Japan. A model-based structure analysis revealed the presence of two populations. The accessions that were clearly assigned to a single population in which >70 % of their inferred ancestry was derived from one of the model-based populations. However, two accessions (3.4 %) in the sample were categorized as having admixed ancestry. Although, strawberries have, complicated ploidy levels and different parentages, most of the alleles were shared among the cultivated strawberries. Horvath et al. (2011) studied the genetic structure in strawberry cultivars using 23 SSR markers. Eight SSR markers were diploid, useful for cultivar discrimination with polymorphic information content (PIC) values between 0.29 and 0.74. Bayesian analyses of genetic structure identified four subpopulations. Three of them, American and modern northern European cultivars, American and modern southern European cultivars and old European cultivars, reflected the European breeding history of the cultivated octoploid strawberry. The fourth subpopulation, 'Intermediate' group cultivars comprised various origins that were introgressed with wild species such as *F. chiloensis* or *F. moschata*.

The markers derived from EST-based SSRs seem to be more transferable than those designed from SSR-enriched genomic libraries (Bouck and Vision 2007). As ESTs represent cDNA copies of expressed sequences (Adams et al. 1991), EST-SSRs are tightly linked to functional coding genes. They display higher cross-species transferability than non-EST-derived SSRs (Varshney et al. 2005b). Gil-Ariza et al. (2006) described EST-derived microsatellites from cultivated strawberry and their potential use for varietal identification and diversity study. Gil-Ariza et al. (2009) studied the similarity relationships and structure of 92 selected strawberry cultivars with widely diverse origins using EST-SSR markers. As was reported by Debnath et al. (2008) with ISSR analysis, a limited differentiation of modern cultivars, most probably as a consequence of the methodology of strawberry breeding, was noticed.

In strawberry, a number of cytological genome models including AABBBBCC (Federova 1946), AAA′A′BBBB (Senanayake and Bringhurst 1967), and AAA′A′BBB′B′ (Bringhurst 1990) have been proposed and the last one (AAA′A′BBB′B′) is most accepted with an allopolyploid genome composition in *F.* × *ananassa* (Hirakawa et al. 2014). Williamson et al. (1995) reported a measurable genetic linkage (1.1 cM) between an SKDH isozyme variant and the yellow fruit (c) locus and Yu and Davis (1995) observed a cosegregation of the Pgi-2 and non-runnering (r) locus in diploid strawberry. A number of linkage maps for cultivated strawberry have been developed using various molecular markers that characterized the genome of cultivated strawberry (Lerceteau-Kohler et al. 2003; Rousseau-Gueutin et al. 2008; Weebadde et al. 2008; Sargent et al. 2009; Zorrilla-Fontanesi et al. 2011). Linkage maps have also been developed for *F. virginiana*, a wild progenitor of the cultivated strawberry (Spigler et al. 2008). Sargent et al. (2006)

developed an enhanced linkage map of diploid *Fragaria* which was composed of 182 molecular markers (175 microsatellites, six gene specific markers and one sequence-characterized amplified region) and spans 424 cM over seven linkage groups. The average marker spacing was 2.3 cM/marker and the map contained eight gaps longer than 10 cM. A set of eight representative progeny allowed efficient assignment to linkage maps using a bin mapping strategy (Sargent et al. 2008). Hirakawa et al. (2014) conducted a study aiming at dissecting strawberry octoploid genome through comparison with its wild relatives, *F. iinumae*, *F. nipponica*, *F. nubicola* and *F. orientalis* by de novo whole-genome sequencing on an Illumina and Roche 454 platforms. The total length of the assembled Illumina genome sequences obtained was 698 Mb for *F.* × *ananassa* and ~200 Mb each for the four wild species.

3.8 Berry Crop Diversity for Antioxidant Activity

Berries are extremely high in antioxidants. The phytochemicals in plant tissues responsible for antioxidant capacity can largely be attributed to the phenolics, anthocyanins, carotenoids, and other flavonoid compounds. Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules. They inhibit the propagation or initiation of oxidizing chain reactions (Velioglu et al. 1998). The antioxidant activity of phenolic compounds are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxide (Osawa 1994). Berry fruits contain many of these components and are a good source of natural antioxidant substances such as flavonoids and phenolic acids. They have high antioxidant enzymes and oxygen radical scavenging activities. Berry fruits possess up to 4 times more antioxidant capacity than nonberry fruits, 10 times more than vegetables and 40 times more than cereals (Halvorsen et al. 2002). They are very rich in antioxidant vitamins A, C, and E and of phenolic compounds. Phenolics can account for 90 % or more of the overall antioxidant capacity found in berry fruit (Deighton et al. 2000). Phenolic acids present in berries are glycosides of hydroxylated derivatives of benzoic acids and cinnamic acid (Macheix et al. 1990). Main berry flavonoids are anthocyanins, proanthocyanins, flavonols, and catechins. Berries represent a significant dietary source of anthocyanins. Only 24 out of 100 common foods contain anthocyanins and nonberry anthocyanin containing foods typically contain less than 100 mg 100 g FW $-$ 1 (Wu et al. 2006). A linear relationship exists between total phenolic or anthocyanin content and oxygen radical absorbance capacity (ORAC) in various berry crops indicating that the berry antioxidant activity was mainly derived from the contribution of phenolic and anthocyanin compounds in berry fruits (Wang and Lin 2000).

Berry genotypes showed a wide range of variability although multiple factors can affect the antioxidant capacity (Table 3.3). A wide range of genetic diversity has been reported for blueberry genotypes (1.8-fold, Kalt et al. 2001; 2.9–3.5-fold,

Table 3.3 Examples of variation for total anthocyanin content (ACY), total phenolics (TPH) and antioxidant activity as determined by oxygen radical absorbing capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) or ED_{50} value used to express the concentration of an antioxidant required to quench 50 % of the initial 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH [ED_{50}]; a smaller ED50 value corresponds to a greater DPPH radical scavenging activity) in *Vaccinium*, *Rubus* and *Fragaria* species

Species	ACY mg 100 g^{-1}	TPH mg 100 g^{-1}	ORAC/ TEAC µmol TE g^{-1}	FRAP µmol TE g^{-1}	DPPH (ED_{50})	Reference
Fragaria × *ananassa*	10–30	173–313	11–19	7–14		Tulipani et al. (2008)
Fragaria × *ananassa*	6–44	–	–	–	9–40	Debnath and Ricard (2009)
Rubus spp.	52–627	126–1079	13–146	20–206	–	Moyer et al. (2002)
Vaccinium spp.	131–370	282–598		42–114	–	Yuan et al. (2011)
Vaccinium spp.	62–300	181–525	14–46	–	–	Prior et al. (1998)
Vaccinium spp.	34–515	171–961	19–131	19–161	–	Moyer et al. (2002)
Vaccinium spp.	131–370	282–598	–	42–114	–	Yaun et al. (2011)

Howard et al. 2003; 6.8-fold, Ehlenfeldt and Prior 2001). Duy (1999) reported that both total phenolic and anthocyanin content varied over about a 3.5-fold range while ORAC spanned an about 5-fold range in 135 wild blueberry clones. Moyer et al. (2002) reported a wide variation for total anthocyanin and phenolic contents and antioxidant capacities in 107 genotypes of nine *Vaccinium*, seven *Rubus* and five *Ribes* species. ORAC values ranged from 19 to 131 µM Trolox equivalents (TE) g-1 in *Vaccinium*, 13–146 in *Rubus* and from 17 to 116 in *Ribes* genotypes. Wild selections of rabbiteye blueberry from Florida and Georgia had the highest ORAC values (131, 129 and 122 ímol TE g-1).

Kalt et al. (2001) reported that lowbush blueberries were consistently higher in anthocyanins, total phenolics and antioxidant capacity, compared to highbush blueberries. Thirty-four wild lingonberry clones representing *V. vitis-idaea* ssp. *minus* and eight cultivars representing *V. vitis-idaea* ssp. *vitis-idaea* showed a wide variation among them for antioxidant activities (Debnath and Sion 2009). Antioxidant activity and anthocyanin content were higher in the berries of wild clones than those of the cultivars (Debnath and Sion 2009). Foley and Debnath (2007) found that lingonberry cutivar 'Erntedank' out-yielded 'Splendor' for berry production and produced more, but smaller berries with a higher antioxidant activity than those of 'Splendor'.

A wide natural variation was observed among 18 cranberry and 21 lingonberry wild clones collected from four Canadian Atlantic provinces and in seven European lingonberry cultivars for total phenolics and antioxidant activity (Petkau et al. 2010). Total phenolics and ORAC values were higher in wild lingonberry clones than those of the cranberry clones and lingonberry cultivars (Debnath et al. 2012a). A positive linear correlation was noticed between the total phenolics and ORAC values for these genotypes ($R^2 = 0.968$). Collecting the data on total

phenolics, ORAC values, and ultra-performance liquid chromatography profiles, the cranberry clones were grouped into two but the lingonberries into four groups. This study also identified valuable wild genetic resources for future use in berry crop improvement program (Debnath et al. 2012a).

A range of 11–19 TE g-1 antioxidant capacity among nine Italian strawberry genotypes was reported by Tulipani et al. (2008). Debnath and Ricard (2009) analysed diversity for antioxidant activity and anthocyanin content in 10 strawberry cultivars and nine breeding lines developed in Canada. Wide natural variation was observed among the strawberry genotypes for anthocyanin contents and antioxidant activities as was revealed by UPGMA cluster and Euclidean distance. Two major clusters were resolved leaving one selection as an outlier. The cultivars were intermixed with selections in both anthocyanin content and antioxidant activity-based dendrogram (Debnath and Ricard 2009).

3.9 Molecular Markers and Pedigree Analysis

Levi and Rowland (1997) examined highbush (or highbush hybrid) and rabbiteye blueberry cultivars and one southern lowbush selection from the wild using seventeen 10-base RAPD and seven 15- to 18-base SSR-anchored primers (primers comprised of SSR motifs) in PCRs. Fifteen RAPD and three SSR markers resulting from these reactions were chosen to construct a DNA fingerprinting table to distinguish among the genotypes. Similarity values were calculated based on 132 RAPD and 51 SSR bands, and a dendrogram was constructed based on the similarity matrix. The *V. ashei* cultivars and *V. darrowi* selection grouped out separately from the *V. corymbosum* cultivars as expected. However, estimates of relative genetic similarity between genotypes within the *V. corymbosum* group did not agree well with known pedigree data and, thus, indicated that RAPD and SSR data did not accurately assess the genetic relationships of cultivars within this species.

Debnath et al. (2008) reported that clustering based on ISSR data was different from that based on the coefficient of coancestry although both analyses showed narrow genetic diversity among the Canadian strawberry genotypes. Similar results were also reported by Degani et al. (2001) who observed a poor correlation between the AFLP-derived genetic similarity values with those of the coefficients of coancestry but a better correlation between the RAPD-derived values and the coefficients of coancestry in 19 strawberry cultivars. Hancock et al. (1994) and Graham et al. (1996) observed good agreements between genetic similarity values based on RAPD markers and pedigree information for eight strawberry genotypes. Arnau et al. (2002) reported consistent associations between strawberry varieties revealed by ISSR analysis with pedigree data although they did not perform pedigree analysis. Poor correlations between genetic similarities values and coefficients of coancestry might be due to inaccuracy in estimates. The assumptions underlying the coefficient of coancestry calculations may introduce inaccuracies in these estimates (Messmer et al. 1993). The pedigree data can be subjective, and

does not account for the effects of selection, mutation, and inadequate simplification in the underlying model that assumes equal parental contributions (Cox and Murphy 1990). These estimates may be biased due to selection pressure, unequal parental contribution and the relatedness of ancestors without a known pedigree. DNA markers have the advantage of directly detecting sequence variation among genotypes and therefore the ability to bypass the assumptions that are inherent to pedigree analysis. DNA markers may be affected by the number of markers analysed, their distribution over the genome and the accuracy in scoring the marker (Schut et al. 1997). Finally, incongruities can result from the clustering process whenever clusters are nonoverlapping due to which a genotype that is related to two other genotypes from separate clusters will only be grouped with the one to which it is most closely related.

3.10 Comparison of Diversity Analysis with Molecular Markers with Those from Data on Antioxidant Activity

Little is known on linkage between molecular markers and berry antioxidants. Multiple genetic and environmental factors affect production and accumulation of bioactive compounds. There are only two reports on comparison between diversity analysis based on molecular markers and on antioxidant activity in berry crops. Chemical diversity based on antioxidant activity and anthocyanin content and on molecular diversity based on ISSR markers in lingonberry did not show any correspondence, and the grouping obtained from molecular analysis did not match with grouping obtained from chemical traits (Debnath and Sion 2009). Similar results were also reported in strawberry (Debnath and Ricard 2009). Working with 19 Canadian strawberry genotypes, they reported that 14 were grouped together in one cluster by ISSR-UPGMA analysis while 16 were in one group when UPGMA analysis was done on data of antioxidant activity and anthocyanin contents. Clustering based on ISSR data was different from that based on the antioxidant activity and anthocyanin content data. ISSR markers are distributed throughout the genome and in the majority of cases most regions of the genome are not expressed at the phenotypic level (Dahlberg 2000). The noncoding regions (unexpressed) of genome are not accessible to phenotypic expression and might have resulted in disagreement between the chemical and molecular diversity. The weak correspondence between genetic distances from chemical and ISSR data most probably implies that these markers differ in their degree of genomic coverage. Antioxidant activity and anthocyanin contents in berries are affected by genetic differences (genotypes), the degree of maturity at harvest, preharvest environmental conditions, and postharvest treatment and storage conditions (Wang 2006). DNA markers have the advantage of directly detecting sequence variation among genotypes and therefore the ability to bypass the factors that affect antioxidant activity and anthocyanin contents. DNA markers may be affected by the number

of markers analyzed, their distribution over the genome and the accuracy in scoring the marker (Schut et al. 1997). Finally, incongruities can result from the clustering process whenever clusters are nonoverlapping due to which a genotype that is related to two other genotypes from separate clusters will only be grouped with the one to which it is most closely related. García et al. (2002) failed to correlate morphological and RAPD characterization in strawberry. It has been proposed that data on antioxidant activities and anthocyanin contents together with ISSR data could be used for germplasm management and more efficient choices of parents in berry breeding programs (Debnath and Ricard 2009).

3.11 Conclusions and Prospects

High population pressure has adversely affected genetic diversity worldwide. Traditional cultivars and their wild populations have been lost due to a number of reasons including alteration of cropping systems, monoculture of high yielding cultivars, alteration of arable systems, nonsustainable land use, deforestation, and/or agro ecosystem deterioration. This led to loss of genetic diversity within berry cultivars and their wild populations which is known as "genetic erosion." The loss of biodiversity is one of the most serious human impacts on the global environment and extinction of local crop cultivars might be a great threat to agricultural sustainability (Pei et al. 1993). The breeding practices during last 200 years resulted in the reduction of 600 strawberry genetic varieties. Initial diversity increased due to introgression of wild strawberry germplasm or using unrelated progenitors but these introgressions did not compensate for the loss of diversity observed in modern strawberry cultivars (Gil-Ariza et al. 2009).

Assessment of genetic diversity and germplasm characterization are important in berry crop improvement through selection. These studies were tremendously benefited from the development of various molecular marker techniques. Each marker system has its own strengths and limitations, making the choice of marker an important decision that has to be a compromise between reliability and ease of analysis, statistical power, and confidence of revealing polymorphisms evolutionary genetics. For an efficient diversity assessment, molecular markers ideally need to be selectively neutral, highly polymorphic, codominant, and well-dispersed throughout the genome and cost- and labor-efficient (Bretting and Widerlechner 1995). Genetic markers complying with these requirements are protein and DNA markers. Isozyme (allozyme; protein marker) analysis detects variation in proteins and provides fewer markers compared to DNA-based methods. As in other crop improvement programs, a combinatorial approach of accelerated gene discovery through genomics, proteomics, and other associated branches of biotechnology, as an applied approach, will speed up the berry improvement programs. DNA markers provide large number of polymorphisms and make direct inferences on genetic diversity and interrelationships among organisms without the confounding effects of the environment and/or faulty pedigree records (Weising et al. 1995).

RFLP markers are relatively highly polymorphic, codominantly inherited, and highly reproducible but have not been widely used in berry crops because the system is time consuming, involves expensive and radioactive/toxic reagents, and requires large quantity of high-quality genomic DNA. Because RFLP and SSR markers require prior knowledge of DNA sequences, a number of universal, dominant molecular marker types such as RAPD, AFLPs, and ISSR have also been employed in berry crop diversity studies. Some of these problems, like reproducibility, appear to be smaller for AFLP and ISSR than for RAPD (Zietkiewicz et al. 1994; Vos et al. 1995), presumably because they employ longer primers and higher annealing temperatures. Partial DNA digestion causes artifacts in AFLP analysis (Arnau et al. 2002) but these are difficult to detect unless plant tissue is sampled for DNA isolation at different periods during the growing season and from various organs. A major drawback for all the three methods is the fact that the investigated loci are biallelic and that attempts to distinguish heterozygotes from homozygotes on band intensity have not proven feasible (Nybom 2004). Different indices exist for the measurement of diversity, partitioning of diversity within and between crop populations, and the genetic distance between them (i.e., differentiation). It seems noteworthy that comparing data achieved with different molecular marker types and even measured at different marker loci of the same type is ambiguous as diversity measures are relative rather than absolute.

The development and application of molecular tools for berry crops would increase the speed and precision of the improvement and conservation process, particularly for traits that are difficult to characterize phenotypically, such as resistances to biotic and abiotic factors and for quality characters. Understanding the genetic control of commercially and nutritionally important traits and the linkage of these characteristics to molecular markers on chromosomes is of prime importance for crop improvement. The speed and precision of berry breeding can be improved by genetic linkage maps, thus facilitating the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes (Graham et al. 2004). In raspberry, cane pubescence (fine hairs) is controlled by the dominant gene H (either homozygous or heterozygous) and the recessive allele (h) in homozygous condition gives glabrous canes. Gene H is rarely homozygous because it is linked with a lethal recessive gene (Jennings 1988). Pubescent canes are more resistant to cane botrytis (Botrytis cinerea), cane blight (Leptosphaeria coniothyrium), and spur blight (Didymella applanata) than unpubescent ones (Knight and Keep 1958; Jennings and Brydon 1989) but more susceptible to cane spot (Elsinoe veneta), powdery mildew (Sphaerotheca macularis), and yellow rust (Phragmidium rubi-idaei) (Keep 1968, 1976; Anthony et al. 1986; Jennings and McGregor 1988; Williamson and Jennings 1992). This might be due to a linkage of this gene with the gene complexes that contribute to the resistance or susceptibilities of these diseases or the H gene might have pleiotropic effects on each of the resistances (Williamson and Jennings 1992). In raspberry, cane spininess and the root sucker traits of density and spread were found to be located on linkage group 2 for spines and group 8 for density and diameter (Graham et al. 2004). In the cultivated strawberry, linkage between major genes

and quantitative trait loci for disease resistance (Lerceteau-Kohler et al. 2005), flowering habit (Sugimoto et al. 2005; Weebadde et al. 2008), and traits associated with fruit quality (Zorrilla-Fontanesi et al. 2011) were observed. The 'pale-green' leafy phenotype is the most recent addition to linkage group IV in strawberry (Sargent et al. 2004).

Although berries can have remarkable variation in antioxidant content for different growing seasons and conditions, ripening stages, and storage conditions (Wang 2006), wide natural variation is available in wild clones/accessions. Wild berries are valuable genetic resources for high antioxidant activity and could be a valuable resource for including them into a berry breeding program. Berry genotype selection based on molecular analysis combined with antioxidant activity will play an important role in berry breeding and improvement program. Based on diversity analysis and biochemical data, crossing between wild lowbush blueberry clones and half-high blueberry cultivars was made in author's laboratory in order to develop high antioxidant containing and high yielding hybrid blueberries suitable for cultivation in cool climates of Canada (Debnath 2011c).

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Chapter 4
Genetic Erosion of *Phoenix dactylifera* L.: Perceptible, Probable, or Possible

Abdullah A. Jaradat

Abstract Genetic diversity of date palm (*Phoenix dactylifera* L.) encompasses genetic differences among and within species, subspecies, populations, cultivars, and individual clones in traditional oases and plantations. Components of this diversity can be estimated, throughout the tree's ontogeny, at the phenotypic, physiological, biochemical, and molecular levels using quantitative, qualitative, and ecological parameters. Due to recent extensive expansion of its cultivation in plantations, *P. dactylifera,* as a species, may not be immediately threatened by genetic erosion in spite of documented isolated cases where some oasis agroecosystems passed ecological thresholds, leading to irreversible changes in the ecosystem and the loss of valuable genetic resources and associated ecosystem services. However, threats to genetic diversity increased during the last ~30 years partly due to the introduction of improved mass propagation methods of a limited number of elite date palm cultivars to the exclusion of many others; this widespread practice may have led to genetic vulnerability of the species to biotic (e.g., red palm weevil [(*Rhynchophorus ferrugineus* (Olivier)] and Bayoud, caused by *Fusarium oxysporum* f. sp. *albedinis*) and abiotic (e.g., drought, heat, sand encroachment, desertification, aquifer depletion, and salinity) stresses, especially in view of climate change. Selecting a small number of resistant cultivars to biotic stresses is a further threat to the diversity of the species if the resistance to a particular disease or insect proved to be short lived because of changing climatic conditions or through a change in the virulence of the pest. Traditional propagation using offshoots of elite cultivars having desirable fruit quality traits may lead to the confinement of these cultivars to certain oases; its impact on genetic diversity will, most certainly,

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be influenced by plant quarantine regulations and the ease with which date palm propagules can be moved between oases, plantations, countries, and regions of the world; whereas the current massive propagation of a few elite date palm cultivars using tissue culture and other mass propagation methods, at the expense of less popular but genetically valuable adapted cultivars, may exacerbate the problem of genetic erosion. In order to combat potential genetic erosion, in-depth understanding of genetic diversity and genetic structure of the species' gene pool complex, which have been shaped and greatly altered by human and natural selection, clonal propagation, and spatiotemporal exchange of germplasm, is indispensable. This chapter will present a comprehensive assessment of the factors with tangible or potential impact on date palm genetic diversity; appropriate research methodologies to quantify and partition genetic diversity; and strategies that can be implemented at the oasis, plantation, regional, and global levels, to enhance sustainable use and conservation of maximum genetic diversity and combat genetic erosion in the date palm.

Keywords Abiotic stress · Agrobiodiversity · Biotic stress · Genetic diversity · Genetic erosion · Indigenous knowledge · Vulnerability

4.1 Introduction

Genetic diversity studies and discrimination among closely related date palm cultivars and clones to assess vulnerability to biotic and abiotic stresses are often extremely difficult. In addition, the history of the domestication of date palm remains poorly understood; the natural center of its origin is unclear, as no wild population is identified with certainty, whereas the time and place marking the beginning of its domestication is not known with precision (Zohary and Hopf 2000; Gros-Balthazard 2013; Pintaud et al. 2013). This fact makes it all the more necessary to characterize and evaluate the known wild species as components of agrobiodiversity, and as potential genetic resources for its improvement (Pintaud 2010). Spontaneous interspecific hybrids may have contributed to the development of well-known cultivars; however, these remain marginal as the major date production oases are located in areas where the date palm is not in contact with other species and where well-defined cultivars are produced with vegetative propagation (González-Pérez et al. 2004a, b; Gros-Balthazard 2013). Nevertheless, in-depth assessment of the genetic vulnerability of date palm to many threats (e.g., climate change, desertification, and salinity stress) requires a broad knowledge of the extent and distribution of its genetic diversity, both of which depend on the species evolution and its unique breeding system; past genetic bottlenecks; and ecological, geographical, and anthropogenic factors (Shabani et al. 2012; Pauls et al. 2013). Therefore, agrobiodiversity-based models for sustainable agriculture in oasis agroecosystems may provide the most cost-effective and durable solution for the problems associated with or emanating from such factors.

Fig. 4.1 Oases agroecosystems based on traditional three vertical stories of annual crops (e.g., alfalfa), fruit trees, and date palms with flood irrigation along the Nile valley, Egypt (**a**), multi-cultivars of mostly seedling- or offshoot-derived date palms, traditional management and canal irrigation at Al Ain oasis, United Arab Emirates (**b**), and plantation based on single elite cultivar produced by tissue culture that may lead to vulnerability to biotic and abiotic stresses and genetic erosion, and drip irrigation, that may lead to salinity problems, in the Jordan Valley (**c**) (Google Earth Images)

Oases in the Middle East and North Africa region are undergoing rapid physical and socioeconomic changes which, in the past, led to the collapse of a few oases, such as Sijilmasa in southern Morocco, and Timbuktu in Mali (Lightfoot and Miller 1996). Such changes can occur suddenly, although they often represent the cumulative result of a slow decline in agrobiodiversity and reduced ecological resilience of the oasis (Battesti 2012; Huang et al. 2013). Apart from a few documented cases, where date palm cultivation has diminished or even vanished, the worldwide date production has increased over time (Jaradat 2014a). However, threats to genetic diversity increased during the last ~30 years partly due to the introduction of improved mass propagation methods, such as tissue culture, of a limited number of date palm cultivars to the exclusion of many others (Racchi et al. 2013). Ultimately, mass propagation of pure and limited number of date palm genotypes predictably would lead to genetic vulnerability (Diaz et al. 2003; Fig. 4.1). The loss of agrobiodiversity has serious implications for species, ecosystem services, and people who depend on environmental and natural resources for their livelihood (Aboragab 2010; Sudheresan et al. 2013).

Recent reports suggested that the productivity and quality of date palm trees has been declining in its traditional growing areas . As much as 30 % of production can potentially be lost as a result of poor management as well as due to pest and disease attacks. In the Gulf countries and Egypt, the red palm weevil [(*Rhynchophorus ferrugineus* (Olivier)] has recently become one of the major date palm pests, while Bayoud disease caused by a parasitic fungus (*F. oxysporum* f. sp. *albedinis*) is a common threat to date palm in North Africa (Djerbi 1990). Another fungus disease which causes inflorescence rot in Iraqi date palm is *Fusarium proliferatum* with devastating effects on yield potential and yield quality (Hameed 2012). Several interacting technical and socioeconomic factors contributed to date palm degradation (Nixon 1951; King and Salem 2013). Currently, however, poor management is causing some oasis agroecosystems to pass ecological thresholds, leading to irreversible changes in these ecosystems and the loss of their ecosystem services. The impact of modern irrigation techniques

for human settlement in hyper-arid regions, for example, is demonstrated by the large quantitative and qualitative changes in vegetation cover that have occurred in several Middle Eastern and North African oases over the past 50 years. The impact of this technology on natural vegetation is demonstrated by the disappearance of date palm orchards and associated agrobiodiversity due to the depletion of desert aquifers (e.g., Wadi Alajal in Libya), abandonment and declining oasis (e.g., Um Elma'a, southwestern Libya; Elbarasi and Saeed 2013), or to seawater intrusion (e.g., coastal regions in the United Arab Emirates; Jaradat 2011).

For most *Phoenix* spp., the status of genetic vulnerability is not well known or documented. Although many of the species are cultivated as ornamentals, there are probably few "pure" *Phoenix* spp. in ornamental plantings due to its readiness to hybridize with many genetically compatible species. However, due to its extensive cultivation in oases and, most recently, in modern plantations, the date palm as a species may not be considered threatened, whereas wild *Phoenix* spp. germplasm (*P. acaulis*, *P. andamanensis*, *P. atlantica*, *P. caespitosa*, *P. canariensis*, *P. dactylifera*, *P. loureiroi* var. *loureiroi*, *P. loureiroi* var. *pedunculata*, *P. paludosa*, *P. pusilla*, *P. reclinata*, *P. roebelenii*, *P. rupicola*, *P. sylvestris*, and *P. theophrasti*) (Dransfield et al. 2008; Kruger 2011) may become extinct due to habitat destruction and climate change. Nevertheless, existing genetic diversity in cultivated date palm can be lost due to these factors if they result in the loss of local cultivars having specific genetic structures (Bodian et al. 2012). Species adaptive capacity, a highly variable attribute among species as a function of genetic and reproductive controls, fluctuates through phenotypic plasticity. Some elements of adaptive capacity are latent but can be released by changing climate conditions (Kittel 2012). A species' adaptive capacity depends on flexibility in its ecological relationships, which is primarily associated with the magnitude of its physiological response to climate change including interactions with hydrothermal, light, nutrients, salinity, and other climate-linked environmental variables (Kremer et al. 2014).

The genetic basis of adaptive capacity of *Phoenix* spp. may vary over its geographical range as a consequence of variation in biotic and abiotic environmental gradients affecting selective pressure, and climatic and evolutionary history (Chao and Krueger 2007; Chen et al. 2013). The structure and function of genetic diversity, through eco-regional diversity, contribute to the maintenance of species diversity and are themselves important conservation objectives; within this context, date palm diversity and agrobiodiversity are vulnerable to changing climates across wide areas of its distribution in complex and highly interactive ways (FAO/IPGRI 2002; Kittel 2012); the conservation of genetic diversity in date palm is important not only to sustain its genetic resources, but also to maintain agrobiodiversity at the oasis level (Kurup et al. 2009).

Genetic diversity and agrobiodiversity of *Phoenix* spp. are vulnerable to the climate acting either directly through ecological networks on the species, thus generating vulnerabilities at the population, oasis, and ecosystem levels, or indirectly through climatic alteration of the physical environment (e.g., eco-hydrology, salinity, and drought), and may act synergistically with other management and biotic

and abiotic stressors (de Jong et al. 2005; Ben Salem et al. 2011). Genetic stability assessment, as opposed to vulnerability, using time-series analyses should be undertaken wherever benchmark data are available for an oasis if we are ever to fully comprehend the degree of date palm genetic erosion. It will be most insightful when taken to the level of a date palm unique cultivar or an ethnotaxon (Nabhan 2007). Vulnerability, which is context dependent, of material and energy flows of an oasis and its ecosystem may emerge from the plant community composition as it relates to diversity of the functional types of species, or could be attributed to the complexity and strength of interactions, especially the extent to which trophic network structure is determined by bottom–up and/or top–down interactions within the oasis.

4.2 Is *Phoenix* spp. Threatened by Genetic Erosion?

Genetic erosion was first used in the early 1960s to describe the process of the loss of genetic diversity in agriculture (van de Wouw et al. 2009) and was applied at the crop, cultivars within a crop, and alleles within a cultivar. For most *Phoenix* spp., the status of genetic vulnerability to biotic and abiotic stresses and whether they are threatened by genetic erosion is not well known or documented. The overall partitioning of genetic diversity, based on results of phenotypic, biochemical and molecular markers, and fruit quality traits (Alfarsi and Lee 2008), suggests that date palm cultivars represent a complex gene pool within which historical movement of germplasm, recent introductions, and human selection are shaping its genetic structure (Elshibli and Korpelainen 2009; Karim et al. 2010; Krueger 2011).

The documentation of genetic erosion and loss of agrobiodiversity in date palm was more difficult than initially expected because time-series data are not generally available, especially in its centers of origin and diversity (Pintaud et al. 2013; Jaradat 2011, 2014b, c). Decline in population diversity of date palm (e.g., in Oman) can be attributed to abiotic (increased salinity in major date palm-growing regions and desertification) and biotic (insects and diseases), and urbanization of rural areas (Alyahyai and Alkhanjari 2008). For example, at Nakhila oasis (200 km west of Aswan in Upper Egypt), about 30 trees of date palm are remaining (Abdulghani and Fahmi 1994).

Although many species, other than *P. dactylifera*, are cultivated as ornamentals, there are probably few pure *Phoenix* spp. in ornamental plantings due to its readiness to hybridize with many genetically compatible species. However, the date palm as a species may not be considered threatened by genetic erosion due to its extensive cultivation in oases and most recently in modern plantations (RFAO/IPGRI 2002). On the other hand, wild species may become extinct due to habitat destruction and climate change. Though, existing genetic diversity in cultivated date palm can be lost due to these factors if they result in the loss of local cultivars having specific rare alleles or genetic structures (Abdallah et al. 2013; Elbarasi and Saaed 2013).

Ecological and socioeconomic factors are affecting the delicate equilibrium of oasis agroecosystems and may lead to genetic erosion; these include land degradation, inappropriate agronomic practices, frequent droughts, aquifer depletion, salinity, desertification, sand encroachment, and the introduction of exotic plant species into remote oases. In addition, date palm agrobiodiversity and production potential are threatened by a number of biotic and abiotic stresses (see Sects. 4.6.1 and 4.6.2). Date palm orchards in North Africa are aging; almost one-third of the productive trees in Algeria are beyond the limits of their productive years, almost half of the Tunisian productive date palms are more than 50 years old, whereas millions of Medjool trees, an elite date palm cultivar, were lost to the Bayoud disease (Djerbi 1990; Azeqour et al. 2002; Hamza et al. 2012; Allam and Cheloufi 2013).

One of the most commonly occurring hybrids is between *P. canariensis* and the introduced *P. dactylifera*. Hybridization between widespread and rare taxa may contribute to the extinction of endangered species (Francisco-Ortega et al. 2000). Gene flow between different species could prevent local differentiation or adaptation, leading to diminished fitness through outbreeding depression. This process will usually occur when the source population of the more common native or invasive species is genetically depauperate (i.e., poorly developed) and there is substantial immigration into the small target populations as was the case in the Canary Islands (Santana and Toledo 1999; Francisco-Ortega et al. 2000; Meekijjaroenroj and Anstett 2003). Such hybridization capabilities pose clear threat to the survival of the endemic *P. canariensis* and some individuals that had been morphologically identified as pure *P. canariensis* proved to be of a hybrid nature at the DNA level.

Assessment of interspecific hybridization and introgression between species and subspecies is important for the implementation of appropriate genetic conservation strategies. The remaining unique populations can be identified and properly conserved, while the extent to which they are endangered by the introduction of alien species should be evaluated in order to prevent eventual outbreeding depression. Therefore, the lack of knowledge about the nature, origin, and purity of the *P. canariensis* populations has stood in the way of their conservation efforts as an endemic species (Fransisco-Ortega et al. 2000).

4.2.1 Biology and Genetic Diversity of Phoenix spp.

Palms (Arecales: Arecaceae) are a plant group of 183 genera and over 2300 species in tropical and subtropical climatic regions of the world; the genus Phoenix has 14 formally described species. *Phoenix* spp. is morphologically and phylogenetically highly divergent from the other genera in the Arecaceae; it constitutes the monogeneric tribe Phoeniceae within the subfamily Coryphoideae. The position of *Phoenix* within the subfamily Coryphoideae has been confirmed by a generic-level phylogenetic analysis of the entire Arecaceae that included plastid and nuclear DNA sequences, cpDNA RFLPs, and morphological markers (Cornique and Mercier 1994; Ballardini et al. 2013). A phylogenetic analysis, based on 22

conserved genes of 15 representative plant mitochondria, showed that *P. dactylifera* is positioned at the root of all sequenced monocot mitochondrial genomes (Fang et al. 2012).

The date palm, throughout its long history as a domesticated multi-functional fruit tree, represented a powerful example of integrating sustainable use of limited soil and water resources, as well as renewable and recycled material resources, renewable inputs, and numerous outputs (de Grenade and Nabhan 2013b; Bai et al. 2014). Agrobiodiversity is a prerequisite for the proper functioning of the oasis agroecosystem, which is a complex system characterized by horticultural, agronomic, ecological, economic, social, and cultural dimensions; it represents the climax of rigorous management of scarce water and land resources in association with the date palm; the latter is the dominant component upon which the sustainable biophysical and socioeconomic structures of the oasis agroecosystem are based (Ilahiane 1996; Ghazouani et al. 2011; Kraiem et al. 2012).

Intraspecific genetic variation provides the basis for any evolutionary changes; therefore, it comprises the most important component of agrobiodiversity (Pauls et al. 2013). Biological diversity (or agrobiodiversity) includes diversity within species, between species and within ecosystems (Alyahyai and Alkhanjari 2008; Allam and Cheloufi 2013). Agrobiodiversity of the date palm consists of genetic, organismal (i.e., tree) and ecological diversities, whereas genetic diversity represents heritable variation within and among wild or domesticated populations of the species. Phenotypic diversity represents the interaction between genetic diversity and the environment, and it is an apparent indicator of date palm diversity. The latter represents the basis for selection and conservation, as well as for date palm improvement and sustainable utilization (Diulgheroff 2006; de Grenad and Nabhan 2013b). A relatively small part of the total genetic diversity in palms, including that of *P. dactylifera*, has been characterized, evaluated, and used for breeding and improvement purposes (Elhumaizi et al. 2002; Elshibli and Korpelainen 2009). One of the difficulties in estimating "functional" genetic diversity in palms is that their sexual expression is spatially separated at five discrete levels, and they are within flowers (in-between floral organs), within flower clusters (in-between flowers), within inflorescences (in-between partial inflorescences), within palms (in-between inflorescences), and in-between palms. The complexity of sexual expression in palms and its impact on genetic diversity only becomes clear when the spatiotemporal separation of male and female functions is considered as in the date palm (Younis et al. 2008; Masmoudi-Allouchi et al. 2009).

It is expected that oases in the center of origin and diversity of *P. dactylifera* contains cultivars with a wide range of phenotypic diversity. Numerous factors have impacted the continued survival of this genetic diversity, including biological and anthropogenic factors (Lovich and Bainbridge 1999; Gepts and Papa 2003). However, the strong human selection and clonal propagation of the date palm greatly altered its original genetic structure. Although the date palm may not be immediately threatened by genetic erosion, however, several reports directly or indirectly indicated that the level of genetic diversity as to the number of cultivars

in oases is declining due to interacting anthropogenic, biotic, and abiotic stresses, including desertification, salinity, diseases, and insects. Genetic diversity, which may be expressed as genetic differences between species, subspecies, cultivars, populations, or individual clones, can be quantified at the morphological, physiological, biochemical, or molecular levels (Elhoumaizi et al. 2002; Adawy et al. 2004; Ezebilo et al. 2013). Among the measures available to quantify genetic diversity within plant populations, the mostly used are the amount of polymorphism within populations, the allelic richness (i.e., the total number of alleles in the population), the gene diversity or probability that two random copies of the gene will have dissimilar alleles, and heterozygosity (i.e., the percentage of heterozygous genotypes in a population) (see Sect. 4.10; Ledig 1986; Honnay and Jacquemyn 2008).

Indicators derived from reproductive biology studies on *Phoenix* spp. in the Canary Islands suggest that many of the endemic species are outcrossers. The high total diversity within species, the relatively high level of population differentiation, and the outcrossing breeding systems have implications for the species conservation. Decreased population size in outcrossing species would certainly promote biparental inbreeding and increase inbreeding depression (Francisco-Ortega et al. 2000). Many interspecific hybrids have been recognized or suspected among the largely interfertile *Phoenix* spp. The spread of 'domesticated' *P. dactylifera* resulted in situations of sympatric distribution with wild species, thus promoting interspecific gene flow, particularly with the endemic *P. canariensis* in the Canary Islands and possibly with *P. theophrasti* in Turkey, *P. atlantica* in the Cape Verde Islands, and *P. sylvestris* in NW India. In addition, spontaneous and directed hybridization between species is an important aspect of *Phoenix* ornamental cultivation (Henderson et al. 2003, 2006).

In the Canary Islands, molecular evidence of hybridization between the endemic *P. canariensis* and the widespread *P. dactylifera* L. was detected using random amplified polymorphic DNA (RAPD) markers. Such interspecific hybridization between an endemic species (*P. canariensis*) and a common one (*P. dactylifera*) may result in putting the endemic species at risk if hybrid progeny and progeny from advanced hybridization are vigorous and fertile, or the common species may become at risk if the hybrid progenies are sterile or have reduced vigor (González-Pérez et al. 2004a). On the other hand, the relatively recent introduction of date palm to the oases of the Baja California peninsula may have impacted the endemic fan palm (*Washingtonia filifera* and *W. robusta*) (de Grenade and Nabhan 2013a). Similar to situations in Morocco (e.g., *P. atlantica*) and the Indian subcontinent (e.g., *P. sylvestris*), the introduction of *P. dactylifera* may have posed serious threats to the genetic integrity and conservation of the endemic species; the latter may become at risk from genetic assimilation or from cross-breeding depression. In addition, extreme cases of invasiveness are increasingly becoming an issue of great global concern, especially in light of the ever-increasing scale of human movement and trade globalization of date palm (Fiaboe et al. 2012).

Genetic diversity of date palm represents heritable variation within and between wild and domesticated populations grown in oases or modern plantations

(Zehdi et al. 2004a, b; Szabo 2013), whereas phenotypic diversity represents the interaction effect between the genetic diversity and the environment, and it is an apparent indicator of date palm diversity. The latter represents the basis for selection and conservation, as well as for its improvement for sustainable utilization (Rivera et al. 2008; Rhouma-Chatti et al. 2011).

4.2.2 Which Diversity Is Vulnerable?

The genus *Phoenix*, which is composed of 14 species naturally distributed in the Old World, comprises *P. dactylifera* L., cultivated for its fruits, while other species are grown for a wide range of ecosystem services (Gros-Balthazard 2013). Presumably, the *Phoenix* species were spread out of their natural distribution area for these and other reasons. It is therefore common to find species not naturally sympatric, growing together in cultivation or in the wild. *Phoenix* species are interfertile; interspecific hybridization of distinct species usually leads to fertile hybrid offspring. In anthropogenic contexts, such spontaneous gene flows are possible, if not common, under cultivation as well as in the wild (Gros-Balthazard. 2013). Allelic richness is important for breeders as a basis for the continuous improvement and adaptation of crops. Recent developments in molecular techniques have made it possible to study genetic erosion at the allelic level (van de Wouw et al. 2009). Allelic richness is important for the survival of a species as a significant loss of alleles can affect the evolutionary potential of even common species. The drawbacks of studying genetic erosion at the level of varieties or using pedigree information are overcome by looking into more detail at the genetic makeup of the genotypes.

Intraspecific genetic diversity is the most fundamental level of agrobiodiversity as it provides the basis for any evolutionary changes (Rhouma et al. 2008; Shapcott et al. 2009; Pauls et al. 2013), whereas agrobiodiversity of date palm is a prerequisite for the proper functioning of the multidimensional complex oasis agroecosystem characterized by horticultural, ecological, economic, social, and cultural dimensions (Nabhan 2007; Potcher et al. 2008; Mekki et al. 2013). The genetic diversity which has been recognized as an important factor for maintaining ecosystem services (Mace et al. 2012; Pauls et al. 2013; Gill et al. 2014), and consequently, the genetic structure of the gene pool complex of date palm, including wild, feral, and domesticated species, have been shaped and significantly modified by natural and human selection, clonal propagation and spatiotemporal exchange, and movement of its germplasm (Krueger 2011). Several key historical, geographical, ecological, and anthropogenic factors determine the magnitude and distribution of genetic diversity in *P. dactylifera*. In addition, this genetic diversity was shaped by the composition of date palm populations as genetically discrete clones representing highly heterozygous cultivars without the benefits of a dynamic mutation–recombination breeding system (Alrugaishi et al. 2007; Ataga et al. 2012; Bodian et al. 2012). The long-term and strong natural and human selection,

and clonal propagation of this unique clonally propagated perennial fruit tree in oasis agroecosystems greatly altered its original genetic structure. Selection, within and among date palm cultivars, is the primary force that shaped the levels and patterns of genetic diversity within and between its populations; this happens when certain selected individual female trees in the population are more likely to survive to maturity and produce more offspring (either seedlings or offshoots) than other trees (Elassar et al. 2005; Brown et al. 2013; Chen et al. 2014).

Several key historical, geographical, ecological, and anthropogenic factors determine the spatiotemporal distribution of date palm's genetic diversity. The impact of these factors and their interaction is reflected on the level of population differentiation and especially on fruit quality traits. Therefore, this genetic diversity is not randomly or uniformly distributed in space or time; it differs between oases and populations, or between regions and localities (Gaitto et al. 2003; Elshibli and Korpelainen 2008, 2009).

Traditional oases continue to play an important role in maintaining and enriching the genetic resources of date palm, and their genetic diversity through multiple processes and dynamic conservation practices, though a better understanding of the intraspecific genetic variation of the date palm and its distribution in oases ecosystems will be imperative for the proper conservation and sustainable use of its genetic diversity. Estimates of genetic diversity based on phenotypic, biochemical, and molecular markers, and fruit quality traits were utilized in assessing the population differentiation of date palm populations throughout its center of diversity. Vegetative phenotypic traits (e.g., percent spined midrib, apical divergence angle, maximal pinnae width at leaf top, percent solitary spines, and spine length at the middle and maximal spine angle) (Elhoumaizi et al. 2002; Ouarda et al. 2012; Forsman 2014), as well as reproductive and qualitative fruit traits at the fruiting stage (Glilcan 1997; Alobeed 2010; Hamza et al. 2012), provide a reasonably accurate method to analyzing phenotypic diversity in date palm and related species.

Variation in a small number of phenotypic descriptors fully discriminated between groups of date palm cultivars (Elkichaoui et al. 2013). However, precise identification of, and discrimination between, cultivars require the use of a large number of morphological markers and an assortment of complementary biochemical (Bendiab et al. 1993; Saker et al. 2000; Azeqour et al. 2002; González-Pérez et al. 2004a, b) and molecular markers (Khanam et al. 2012; Zhao et al. 2013; Mirbahar et al. 2014). However, some phenotypic traits may not exhibit variation in response to environmental or management factors and can be used as stable descriptors of date palm cultivars and for cultivar identification (Elhoumaizi et al. 2002; Schlichting and Wund 2013). Nevertheless, accurate estimates of genetic diversity in morphometric traits of the trunk, crown, fruiting, and fruit quality attributes, and their partitioning within and among the gene pool of date palm in its center of origin and diversity, are important considerations for a successful date palm industry. This is particularly significant for the long-term survival of date palm plantations due to the long-life expectancy of each generation and, in particular, due to the high maintenance cost of mature female trees.

The majority of analyzed studies based on isozyme and microsatellite markers reported larger within-population than among-population genetic diversity levels (Reviewed in Jaradat 2014c); they are better candidates than other markers in achieving more accurate genetic diversity analyses and in precisely identifying phylogenetic relationships (Trifi et al. 2000; Rhouma et al. 2008; Rhouma-Chatti et al. 2014). Most variation estimated for fruit quality traits was found among populations; however, substantial differences in genetic diversity components were found among and within populations. The empirical evidence derived from this assessment suggested that the genetic structure of date palm populations is controlled by the environment, isolation by distance, and the biological characteristics of female trees.

Understanding past human–climate–environment interactions is essential for assessing the vulnerability of the date palm and the oasis agroecosystem to future climate change (McGregor et al. 2009). This is particularly important in areas where anthropogenic stress coupled with sensitivity to climate variability and climate change are already impacting oases in southern Morocco (Ilahiane 1996; Sedra 2013), Egypt (Aboragab 2010; Battesti 2013), southern Libya (Elbarasi and Saaed 2013), Tunisia, and other countries (Trifi et al. 2000; Battesti 2012).

The spontaneous hybridization events that have been documented between the introduced *P. dactylifera* and the endemic *P. canariensis* in the Canary Islands pose a clear threat to the survival and conservation of the endemic species and may result in outbreeding depression or genetic assimilation (Gros-Balthazard 2013). A similar situation may develop in the Cape Verde Islands, where the endemic *P. atlantica* is threatened by the recently introduced *P. dactylifera* (Henderson et al. 2003, 2006). Therefore, it is suggested that a 'vulnerability approach' be adopted for conservation planning and should focus on enhancing adaptive capacity of *P. dactylifera* and that of the oasis, and employing strategies to lower risks from uncertainties of biotic and abiotic stresses. Although it is essential, however, incorporating vulnerability into conservation planning of *P. dactylifera* and other species is challenging because inherent limitations in predicting outcomes of vulnerability's complex dynamics, coupled with uncertainties in future climate forcing, make it difficult at best to anticipate specific consequences (Kittel 2012).

4.2.3 Historical and Current Genetic Bottlenecks

Climatic, environmental, and anthropogenic factors may have contributed to changes leading to the collapse of a few oases, such as Sijilmasa in southern Morocco, and Timbuktu in Mali (Lightfoot and Miller 1996; Jaradat 2011); these changes can occur suddenly, although they often represent the cumulative result of a slow decline in agrobiodiversity and reduced ecological resilience of the oasis. More recently, however, anthropogenic factors, including poor management, are causing some oasis agroecosystems to pass ecological thresholds, leading to

irreversible changes in the ecosystem and the loss of its services including the loss of valuable adapted cultivars (Bai et al. 2014; Mamat et al. 2014).

Besides a presumed 'domestication' bottleneck of the date palm (Zohary and Hopf 2000; Zohary 2004; Gros-Balthazard et al. 2013), a bottleneck caused by the dispersal of this and other fruit trees may have occurred as well. A secondary bottleneck can be even more severe than the domestication one if only a few individuals become disseminated by offshoots around a few oases (van de Wouw et al. 2009). Currently, however, farmers select date palm cultivars particularly on the bases of fruit quality traits and local adaptation (Alfarsi and Lee 2008). Therefore, depending on selection intensity, only a small part of the species genome that encompasses genes encoding for these traits is affected by this process and may have resulted in narrowing the genetic base among selected genotypes (Zehdi et al. 2004a, b).

The status of genetic diversity in North African countries is aggravated by the threat of destructive diseases such as the vascular fusariosis disease commonly known as Bayoud (Bendiab et al. 1993; Elhassni et al. 2007; Elmodafar 2010; Sedra 2013) and by the red palm weevil (*R. ferrugineus* (Olivier)) in the Middle East (Faleiro 2006; Elshafie et al. 2011; Fiaboe et al. 2012; Hazir and Buyukozturk 2013) and beyond (Ju and Ajlan 2011; Avalos et al. 2014). The lack of natural and effective resistance to these pests in addition to inefficient control measures and the absence of strictly applied national and regional quarantine measures are alarming signs of a potential genetic bottleneck. This is particularly significant for the long-term survival of date palm oases and plantations due to the long-life expectancy of each generation and, in particular, due to the high maintenance cost of mature female (and male) trees. In addition, threats to genetic diversity increased during the last ~30 years partly due to the introduction of improved mass propagation methods, such as tissue culture, of a limited number of date palm cultivars to the exclusion of many others (Saker et al. 2006; Jain 2011). Eventually, mass propagation of pure and a limited number of date palm genotypes would inevitably lead to a genetic bottleneck, genetic vulnerability, and may have serious implications for species survival, ecosystem services, and people who depend on environmental and natural resources of oases or plantations for their livelihood (FAO/IPGRI 2002; Shapcott et al. 2009; Kittel 2012).

4.3 The Oases Through the Lens of Complex Systems Science

Traditional oases represent the climax of age-old rigorous management, by many generations of farmers, of scarce water, and land resources in association with the date palm. Historically, sustainability depended on numerous human interactions that shaped oasis agroecosystems and enabled them to provide multiple ecological and socioeconomic services to meet the needs of local populations (Nabhan et al. 2010; Parrott et al. 2014). Depending on the nature of the oasis and its environment, social development in oases of the Old World supported sedentary,

hierarchical, agricultural societies, and nomadic pastoralists, as was described by Ibn Khaldun in the fourteenth century (de Grenade and Nabhan 2013a, b). These management practices, which can be encountered today in old and stable and transient oases (Marx 1999), ranged from settled communities with firm control over land and water resources, to communities who cultivated their orchards during some seasons and practiced nomadic pastoralism during others, to the purely nomadic communities.

Physiographically, oases are unique landscapes that exist within deserts in arid zones where human disturbances happen at the regional scale, while, demographically, oases are the most concentrated areas of human activities and they provide habitats for a wide range of plant and animal life (Wang and Li 2012; Mamat et al. 2014). However, over the past 50 years, combinations of anthropogenic and environmental factors have caused desert expansion and soil salinization and subsequent degradation of some oasis (Saaroni et al. 2004; Mamat et al. 2014).

Old World oases, due to their importance for the provision of local food security, high levels of agrobiodiversity and associated biological diversity, indigenous knowledge, and ingenuity of management systems, constitute a significant part of the Globally Important Agricultural Heritage Systems (GIAHS) network of FAO (Koohafkan and Altieri 2011). The outstanding landscapes in the GIAHs have been developed using local biophysical, economic, and sociocultural resources which evolved under specific ecological and sociocultural constraints. Under the provision of the network, oases can assist in promoting "dynamic conservation and adaptive management" of crop diversity as climatic, ecological, cultural, and economic conditions shift over time. This model differs from the "freezing of the genetic landscape" approach which promotes a purely conservationist approach of biological resources in a way which ignores cultural and economic factors (Wahba et al. 2007; Sawut et al. 2013).

Oases harboring complex species assemblages provide unique sites for formal and informal in situ crop and traditional knowledge conservation (Kendoucia et al. 2013; Misra 2013; Fernald et al. 2014). The oases agroecosystem integrates native, wild, and introduced species in highly interactive, but not strictly mutualistic relationships. In Baha California, for example, introduced date palms along with native California fan palms grow in the riparian ecosystems providing food resources to temporary and permanent resident species, ecosystem structure for nesting and habitat sites, shade for understory species, and organic matter that alters soil moisture and composition (Rouston 2012; de Grenade and Nabhan 2013a, b; Parrott et al. 2014). Similarly, in Old World oases, a number of fruit tree species (e.g., grapevines, pomegranates, and fig trees) form an understory, while introduced species provide food and habitat resources as well as contribute to the agroecosystem structure and function. For example, richness of the unique and typically small mountain oases in northern Oman is symbolized by 107 different crop species (including 33 fruit trees and 24 vegetable species) belonging to 39 plant families (Gebauer et al. 2009). On the other hand, endemic and introduced wild and cultivated plants that grow in Gafsa oasis in Tunisia (Boerma and Koohafkan 2008), for example, have high resilience to adverse climatic and

edaphic conditions. Varieties of cultivated species, including date palm, have been carefully introduced and selected for adaptation over many centuries by generations of farmers.

Elements of adaptive capacity, such as physiological, life history, and genetically based traits, may differ substantially for date palm populations at oases in the core of the species distribution (Middle East and North Africa) versus those at its peripheries (e.g., Elche in Spain, Sind in the Indian subcontinent, and parts of sub-Saharan Africa) (Rivera et al. 2008). Such populations may have different responses to similar climatic changes; therefore, the species' vulnerability will diverge geographically not only with varying availability of suitable habitats, but also with its location-specific genetically determined capacity to adjust to local niches (Pochter et al. 2008; Baer and Risbey 2009; Bai et al. 2014; Kremer et al. 2014).

This oasis diversity and its associated indigenous knowledge are fundamental resources for the inhabitants of the oases and constitute a deliberate assembly of livelihood options. Unfortunately, the indigenous knowledge associated with both the oasis agroecosystems and date palm agrobiodiversity, and its management, is being gradually lost and need to be maintained to ensure a sustainable way of life in the oases (Boerma and Koohafkan 2008; Elmodafar 2010; Misra 2013). A single oasis is insufficient to capture and maintain all historic varieties for the future (Nabhan et al. 2010); therefore, the oases system should be considered worthy of conservation as an aggregate.

4.3.1 The Oases: Legacy for the Future

The date palm comprised a vital element in developing traditional oases for early settlements in deserts of the Old World; the complex geometry of the old palm groves with the numerous and overlapping beds of cultivation required technical knowledge, and idiosyncratic characteristic of oasis inhabitants (Battesti 2013). Water shaped the design of oases and, to some extent, the composition of date palm groves; however, without the tree, the vertical structure providing needed shade for the growth of other, less hardy but nutritionally important species including grain crops, forage crops, vegetables, and fruit trees, would be lacking. Traditional oases have been developed, concisely or unconcisely, to support complex agroecosystems with higher levels of native agrobiodiversity than surrounding environments or small household gardens (Tengberg 2012; de Grenade and Nabhan 2013a, b). A few traditionally cultivated annual species or landraces have been lost over time, especially in isolated oases; however, new and exotic crop species have been occasionally adopted with the advent of modern transportation (Nabhan 2007). On the other hand, agrobiodiversity of perennial crops remained relatively stabile over time; this stability may relate to the need for crop ecotypes adapted to the challenging edaphic and climatic conditions in the oasis and may stem from the pride in, and adherence to traditional food systems in these oases.

As concentrations of cultural development, and inter- and trans-continental trade routes, oases in the Old World have served as nodes for exchange of knowledge and material in desert environments and resulted in the development of some of the most complex social and agroecological systems developed by humans (Marx 1999; de Grenade and Nabhan 2013a, b). The agrobiodiversities within these oases, including crop and livestock species, crop wild relatives, as well as indigenous knowledge, are resources for the future of humanity, especially in the face of environmental degradation, climate variability, and extreme events, and crop pest outbreaks.

For millennia, the mainstay of oasis livelihood was based on mixed farming of date palm, annual crops, forage crops, perennial fruit trees, and livestock. Recently, however, other economic activities, such as tourism and remittances from community emigrants, are increasingly supporting the livelihoods of oases communities (Koohafkan and Altieri 2011), while the overall trend has been a gradual move from mixed and random oasis date palm cultivation to a more intensive plantation system for date production of one or a few elite cultivars (Carr 2012; Jaradat 2011). The integrity of most oases' agroecosystems, and for thousands of years, reflected their ability to sustain services to humans; the identification of those services emerged from multi-sector partnerships within the oasis in which all stakeholders pursued agreements on the uses to which an ecosystem will be put, and linkages with other ecosystems were recognized (Misra 2013; Winfree 2013). The long-term objective was to enhance the capability of supporting and maintaining a balanced, integrated, adaptive, and mostly unique community of flora and fauna having species composition, diversity, and functional organization distinctive of the natural habitat in the immediate vicinity (Boerma and Koohafkan 2008; Boyd et al. 2013; Gill et al. 2014). Longitudinal studies, either of the old (e.g., Siwa in Egypt; Aboragab 2010; Battesti 2013) or relatively new (e.g., Baja California peninsula in Mexico; Nabhan 2007; Nabhan et al. 2010) oases, can help determine whether and how such isolated agroecosystems will function as a legacy for the future.

4.3.2 Management of Natural Resources in the Oases

Traditional land and water resources management systems and practices are highly dynamic, drawing on local knowledge, extensive experience, and experimentation (de Jong et al. 2005; Ashkenazi et al. 2012; Fernald et al. 2014). Unique examples of traditional water and land management techniques (Luedeling and Buerkert, 2008), in relation to sustainable food production and date palm genetic resources conservation, are exemplified in northern Oman where approximately 2600 mountain oases scattered in Alhajar mountain range, most of them are squeezed between cliffs of rugged mountain escarpments. Farmers in this part of the country have developed techniques for gathering and directing spring water in concrete channels, called aflaj (traditional water works for irrigation), and in some cases,

they carved tunnels into the rocks to tap subsurface aquifers (Lightfoot 2000). Approximately, 4000 aflaj exist in Oman supplying about one-third of the country's water demand. Oasis settlements typically consist of agro-pastoral communities cultivating a number of small and sometimes terraced areas (Marx 1999; Bodian et al. 2012; Battesti 2013; Bai et al. 2014). However, land degradation, gradual loss of agrobiodiversity, poor irrigation and drainage management practices, depletion of aquifers, frequent droughts, and the introduction of alien species, as well as cultural erosion of traditional and indigenous knowledge (Boerma and Koohafkan 2008; Eljuhani 2010; Kraiem et al. 2013; Zhou and Li 2013) are contributing, directly and indirectly, to genetic erosion of date palm diversity. In particular, poor irrigation and drainage practices call into question the sustainability of water resources for continued date palm irrigation, production, and eventually longevity and survival of traditional oases and plantations (de Fraiture and Wichelns 2008; Battesti 2012; Carr 2012; Alnaeem 2013). Due to lack of, or improper, drainage, shallow water tables are causing waterlogging and topsoil salinity problems in some oases (Masoud and Koike 2006; Ghazouani et al. 2011). This problem is compounded by the lack of salinity records that could enable the establishment of long-term salinity trends (Alhammadi and Edwards 2009; Trpler et al. 2011; Sperling et al. 2014); to date, there is no knowledge base available for identifying areas at potential risk to salinity (Jaradat 2011).

Poor or mismanagement of limited land and water resources has caused some oasis agroecosystems to pass ecological thresholds, leading, in a few well-documented cases (e.g., Sijilmasa in the oasis of Tafilalet, southern Morocco, and Timbuktu in Mali) to irreversible changes in the ecosystem and the loss of its ecosystem services, as well as the genetic erosion of local genetic diversity of date palm and associated crops (FAO/IPGRI 2002; Diulgheroff 2006; Dansi et al. 2013).

Fruit production plays an important role in the local economy and ecological survival of traditional oases. Historically, it accounted for more than 60 % of household income in some oases in North Africa; livestock is another important economic factor (Ilahian 1996; Marx 1999; Aboragab 2010; Li et al. 2013a, b). When compared with other products (e.g., other fruit trees and annual crops, which are progressively disappearing as components of the oases agroecosystem), date production has the highest added value, especially in the export sector of North African oases (Ben Salem et al. 2011; Omrani and Quessar 2011; Battesti 2012; Allam and Chloufi 2013). Land degradation, in the form of water- and wind-driven soil erosion, modifies soil structure and can have a negative impact on the cycling of soluble or mobile nutrients, with far-reaching impact on agrobiodiversity in general and genetic diversity, in particular, with a concomitant impact on date production as a provisioning service of the oasis. In all likelihood, climate change will increase the length and intensity of droughts in mid-latitudes where most date palm is currently grown; therefore, better land management and drought monitoring systems are needed (Luedeling and Buerkert 2008; Li et al. 2013a, b; McBratney et al. 2014).

Traditional social water management institutions in several Middle Eastern and North African countries have been largely replaced by new administrative methods such as the association of irrigation, the cooperative of agricultural services, town chiefs responsible for the smallest administrative unit, the agricultural engineering services, and local or regional farmers union (de Jong et al. 2005; de Fraiture and Wichelns 2008; Ben Salem et al. 2011; Battesti 2012). The absence of integrated traditional collaborative community approaches toward water management access to main water resources, and dispute resolution between water users, exacerbate the problem of increasing water shortages, and may lead to unsustainable land and water use (Li et al. 2013b; Mekki et al. 2013).

Shortages in groundwater resulted in conflicting water resource management options and led to salinization, water logging, and eventually deterioration in yield and quality of dates and other ecosystem services (Alnaeem 2013; Huang et al. 2013; Kendoucia et al. 2013; Mekki et al. 2013). Therefore, approaches to water management at several hierarchical levels within an oasis that integrate the provisioning of food, energy, and other ecosystem services are necessary in order to balance the multiple demands on this increasingly scarce resource (de Fraiture and Wichelns 2008). These approaches require full involvement of farmers, breaking disciplinary boundaries and encouraging greater cooperation among stakeholders from planning to implementation. Although governmental water policies endeavor to maintain a balance between the conflicting developmental needs of oases and those of neighboring urban centers, several technical and anthropogenic constraints are facing the increasingly diminishing water resources in the oases agroecosystems of the Middle East (Alnaeem 2013; King and salem 2013; EF), North Africa (de Jong et al. 2005; Heidecke and Heckelei 2010; Battesti 2012), and elsewhere (Huang et al. 2013; Li et al. 2013b).

4.3.3 Desertification versus Oasification

Temporal and spatial stabilities of oasis agroecosystems are affected by a balance between desertification and oasification forces. The balance between evolution and development of a more sustainable and productive oasis and desertification is largely dependent on abundance or shortage of water resources in the oasis (Omrani and Ouessar 2011; Mekki et al. 2013; King and Salem 2013). Changes in spatiotemporal water resources in the oasis are key determinants of the process of oasis evolution, whereas the effect of human activities on oasis evolution is manifested through their effect on water resources (Popenoe 1913; Battesti 2013), all of which impact agrobiodiversity and genetic diversity of date palm. As water becomes increasingly scarce, diversity is lost, cropping systems change, and social institutions are weakened; therefore, the need for documenting indigenous knowledge and values associated with life in the oases has become urgent (Jaradat 2011, 2014b, c). The process should include systematic and comprehensive documentation of local and traditional knowledge on date palm agrobiodiversity and proper

functioning of the oasis agroecosystems, cultivar identification, water and soil resources, and land-use options.

Land-use changes and options impact oases agroecosystem services and, in theory and practice, are essential for their sustainable development and stability (Bai et al. 2014; Fernald et al. 2014; Lu et al. 2014). Desertification is threatening the livelihoods of over one billion people with almost one million living in oases of the Old World (Alibekov and Alibekova 2007; Aboragab 2010; Ashkenazi et al. 2012; Chen et al. 2013), thus contributing to degradation and reduced productivity and resilience of oases agroecosystems, and to increased poverty and risk to human security. Present and future quantitative assessment of desertification and its impact on agricultural production and net revenue indicated that farmers in traditional Egyptian oases would lose approximately 20–25 % of their annual income due to degraded date palms and other fruit trees, such as olives (Aboragab 2010). Arbitrary exploitation of closed aquifers was implicated in the desertification process (Alnaeem 2013; Kraiem et al. 2012, 2013), while remedial actions will require reliable information to help better set priorities and to choose the types of actions that are most appropriate for combating the desertification problem.

Global circulation models suggest that dry and hyper-arid regions of the world could become hotter and drier (IPCC 2007; Hubener and Kerschgens 2007; Baer and Risbey 2009). If climate change increases the frequency and intensity of droughts, it would lead to more desertification and the loss of genetic diversity, genetic resources, sustainability, and productivity of vulnerable oases. As a result, some areas that are climatically suitable for date palm will become unsuitable in 50–70 years (Shabani et al. 2013a, b) including areas in the center of origin and diversity of the species (i.e. parts of the Arabian Peninsula, southern Iraq, western Iran, and across North Africa). Other parts of the world may become more suitable for date palm, including parts of North and South America and Australia.

Simulation studies suggested that temperature and drought stresses will play important roles in date palm adaptation and distribution in the twenty-first century, and the future distribution of date palms will most probably be impacted by climate change (Shabani et al. 2012). Recent examples from the Sahara Desert highlight the impact of climate change on fragile oasis agroecosystems, sand dune encroachments, and desertification. Ten oases in southern Morocco have lost ~40 % of their vegetation due to the combined effects of drought, depletion of groundwater, high temperature, and sand encroachment (Bodian et al. 2012), whereas date palm orchards, along with their genetic diversity, disappeared altogether from wadi Alajal in Libya due to the depletion of desert aquifers (Elbarasi and Saaed 2013; Racchi et al. 2013). Oases rehabilitation (i.e., oasification), as part of a national water policy where water resources, regional climate and socioeconomic conditions, and competition over limited water resources between urban and rural sectors in Tunisia were taken into consideration, was instituted in response to a dramatic threefold increase in the irrigated area during 35 years, mostly in the drier (i.e., desert) parts of the country (Omrani and Ouessar 2011).

4.3.4 Date Palm Agrobiodiversity and Ecosystem Services

Agrobiodiversity of the date palm is geographically highly structured due to long time isolation between oases, date palm groves, and gardens (Pintaud 2010; Tengberg 2012). Date palm cultivars have been selected to fit local edaphic and ecological conditions (Bendiab et al. 1993; Hamza et al. 2009; Bodian et al. 2012; Mirbahar et al. 2014), and quality traits have been selected to meet consumer demand and preferences (Jaradat and Zaid 2004; Alobeed 2010; Allam and Cheoufi 2013). In addition, seeds, but not vegetative material, have been transported due to logistical reasons, if not for the risk of spreading pests and diseases, and these seeds contributed to local cultivar selection and development (Johnson et al. 2013). The warning by the Convention on Biological Diversity that agrobiodiversity is facing eminent threat highlights the importance of the global initiative to safeguard global agrobiodiversity, especially in marginal and threatened habitats such as the oases. Species have been disappearing at up to 1000 times the natural rate, and this is predicted to rise dramatically (Adetola and Adepoju 2013). Natural agrobiodiversity is being damaged, often beyond repair and occasionally with little or no knowledge of the characteristics of the plant genotypes being lost (Dixon 2012). Oasis agrobiodiversity at the species level has functional consequences because the number and kinds of species, besides *P. dactylifera*, determine the traits that influence a large number of processes within and services provided by the oasis agroecosystem (Boerma and Koohafkan 2008; de Grenad and Nabhan 2013b; Rouston 2012; Gill et al. 2014). Changes in agrobiodiversity have been caused by a combination of internal and external factors modifying the functions of oases agroecosystems; historically, these changes resulted in economic impacts through the provisioning of less goods and services to local communities (Alyahyai and Alkhanjari 2008; Gebauer et al. 2009; Battesti 2013).

Theoretically, large genetic diversity within the oasis agroecosystem is expected to result in ecosystem stability (Boyd et al. 2013; Gill et al. 2014); however, genetic diversity itself was not always the driver of ecosystem stability; rather, the latter depends on the ability of the oasis to contain different species, or functional groups (e.g., different cultivars of date palm, different species and cultivars of fruit trees, forage crops, annual grain crops, vegetable crops, semi-domesticated crops, weedy crop relatives, etc.) that are capable of differential responses to biotic and abiotic stresses and to different management practices (Kittel 2012). For example, Algerian oases, typical of North African oases, historically harbored diversified landraces of date palms, other fruit species, and cultivars. However, this diversity is increasingly being subjected to genetic erosion due to anthropogenic and natural causes. A first step to preserve this heritage and reverse its genetic erosion was to establish an inventory of species and cultivars in the region of Touggourt in southeastern Algeria (Allam and Cheloufi 2013), whereas the agrobiodiversity of the ancient Sewa oasis in Egypt was described as being stable since the early nineteenth century (Battesti 2013); however, establishing a list of different local cultivars of date palm in that oasis proved to be far more difficult, even

though only ~15 named cultivars, mostly landraces, coexisted with other fruit trees and field crops (Nabhan 2007).

Ecosystem services within and across oases are not distributed evenly at different spatiotemporal scales, and their value will also often differ according to stakeholders' perception; the latter may influence the long-term status of agrobiodiversity in general, and genetic diversity of date palm and other crops in particular. Provisioning services (e.g., date production of elite cultivars) are the most common and important direct driving force leading to land-use change in oases (Sawut et al. 2013; Mamat et al. 2014) and plantations (Battesti 2012; Kraiem et al. 2013) which often either deteriorate the resource base or brings about negative externalities such as ground water depletion (Alnaeem 2013; Huang et al. 2013; Kendoucia et al. 2013) or salinization (Masoud and Koike 2006; Kraiem et al. 2012). The regulation services of oases ecosystem can be impaired by devegetation and vegetation degradation (McGregor et al. 2009), deterioration of soil structure due to lack (Lu et al. 2014) or loss of soil organic matter and organisms (Luedeling and Buerkert 2008), and soil contamination (Wang and Li 2012). Water vapor flux, due to changes in land surface reflection, leads to changes in the regional and global temperature and precipitation regime, and may contribute to micro-climate change with subsequent effects on date palm phenology and yield quality (e.g., new plantations in the Natroune valley of Egypt King; and Salem 2013, personal observations).

Supporting services in oases are essential for the supplying of all other services, but less directly useable by oases dwellers. However, land degradation impacts all other ecosystem services through its impact on these supporting services. Compared with the influences on other services, the impacts of land degradation on supporting services can be measured more directly as many supporting services are closely associated with the intrinsic biophysical properties of oases ecosystems.

Multiple cultural services, which are often valued and/or accessed in the context of local knowledge systems, have been developed over thousands of years and are quite often locally specific to each oasis or groups of oases (Ghazouani et al. 2011; Bodian et al. 2012; Battesti 2013; de Grenade and Nabhan 2013b). Oases dwellers developed and handed down a wealth of indigenous knowledge and skills in using the natural diversity of the environment for cultivating date palms, other fruit trees, perennial forage and annual crops, managing the soil, water and vegetation, and maintaining their livelihoods under difficult conditions. In these systems, genetic diversity, particularly of date palm, and agrobiodiversity are better protected, land degradation is better controlled, and food security and livelihoods are enhanced (Aboragab 2010).

4.3.5 Oasis Isolation and Integration: Impact on Genetic Diversity and Vulnerability

A set of environmental, political, socioeconomic, and cultural processes differentially shaped farmers attitudes and practices, as well as structure and functions of oases, orchards, and gardens in the Old (Nabhan 2007; Tengberg 2012; Battesti 2013) and New Worlds (de Grenada and Nabhan 2013a, b). Whether isolated by desert expanses (Ghazouani et al. 2011) or desert and saltwater (de Grenade and Nabhan 2013a, b), most oases are increasingly being linked through family or business connections, migrant workers, and transportation and communication networks; therefore, oases can be considered as components of an interconnected system of heritage sites (Nabhan et al. 2010; Koohafkan and Altieri 2011). Oasis diversity at the biological, cultural, and agricultural levels appears to have developed through dynamic periods of isolation and connection over hundreds, if not thousands of years, and across large desert distances using slow transportation means (Zohary and Hopf 2000; de Grenade and Nabhan 2013a, b).

The unique geography of a particular oasis, its cultural history, and agricultural evolution have led to the emergence of unique and high diversity being supported and managed in the oases, along with rich native, resident and migratory flora and fauna, complex agroecosystems, and equally intricate and innovative anthropogenic systems, cultures, and practices. Some oases harbor agrobiodiversity as integrated semi-wild and culturally managed communities in dynamic interactions and relationships across space and time. Processes of isolation, diffusion, and hybridization among oases may have led to the production of novel assemblages of heritage crops. When newly introduced plant species are limited to a single orchard or garden (e.g., in the Baha California oases; Rouston 2012; de Grenade and Nabhan 2013a), they may become more vulnerable to social and environmental stochasticity than well-established species. In order to conserve the total perennial crop diversity and associated management and indigenous knowledge systems, these oases should be considered as interdependent and interconnected sites, where some oases may serve as "source" areas for the "sink" areas that are more vulnerable to species loss. Desert and newly established oases or plantations function as a network of interconnected sites supporting cultivated plant assemblages isolated from one another. Surveys of perennial crop species and farmer interviews in Old World desert (Aboragab 2010; Alnaeem 2013) and mountain oases (Alyahyai and Alkhanjari 2008; Gebauer et al. 2009) as well as oases in the New World (de Grenada and Nabhan 2013a) revealed that oases serve as refugia of some crop species beyond date palm diversity and serve as repositories of indigenous knowledge. Limited information from isolation to connectivity analyses indicated that agrobiodiversity is likely to decline and may disappear from the most isolated and from the fully integrated oases alike due to similar or different reasons. In both cases, oases are unable to support heritage perennial crop species and traditional farming systems over long time scales. As havens of agrobiodiversity in a constraining environment, degradation of oases is tantamount to high and irreversible genetic erosion (Diulgheroff 2006; Dansi et al. 2013).

4.4 The Soil–Water–Climate Nexus and Date Palm Vulnerability

Water and soil are the two important components of the oasis' local environment that are affected by agricultural and horticultural practices. The year-round flow and usually large amount of irrigation water is the most powerful factor influencing oasis soil due to its comprehensive involvement in the solution, erosion, transportation, and deposition of materials in soil (Boerma and Koohafkan 2008; Ben Salem et al. 2011; de Fraiture and Wichelns 2008). Moreover, due to its role in the growth of date palm and other crops, water plays an important medium for transporting nutrients both inside and outside the oasis agroecosystem (de Jong et al. 2005; Heidecke and Heckelei 2010). Material and energy flows in the oasis agroecosystem are also determined by climate-sensitive interactions between above- and below-ground micro- and macro-biological components and their traits (e.g., decomposers vs. mutualistic associations). Below-ground microbial and faunal communities interact with the aboveground realm in ways that depend on the nature of their association with perennial (e.g., date palm) and annual crop plants, whereas the aboveground communities exert strong control over root food webs primarily through the quantity and quality of plant matter inputs, which are the functions of plant community composition in the oasis. The latter component can be altered by herbivory through plant biochemical responses such as increased nitrogen content, and through plant community changes (e.g., annual vs. perennials; grasses vs. forage legumes) over time (Kittel 2012). Climatic vulnerability of an oasis agroecosystem may emerge from the compositional and functional diversity of its plant communities and complexity of their interactions (Floret et al. 1993; Chen et al. 2013; Gill et al. 2014). Biogeochemical processes and functional groups' behavior are strongly sensitive to their climatic controls, especially in a desert environment; however, ecosystem model intercomparison studies suggest that there is high uncertainty in our understanding of this vulnerability even at the broadest scales (Lovich and Bainbridge 1999; Issar et al. 2012). At a local oasis scale, the magnitude and expression of climate vulnerability are even less predictable because above- and below-ground dynamics are highly nonlinear and depend on site characteristics, such as soil fertility, soil and plant community composition, and components of agrobiodiversity in the oasis (Hubener and Kerschgens 2007; Baer and Risbey 2009; Kittel 2012). Such nonlinear ecological networks are potentially capable of sudden regime shifts and exceeding critical thresholds in their structure and function as was described earlier.

4.5 Vulnerability to Abiotic Stresses

The main physical, biological, and socioeconomic boundaries or envelops of oases agroecosystems vary over time and space, and all may become subjected to extreme fluctuations whose timing and magnitude under desert conditions are

uncertain and unpredictable (Goldman 1995; Issar et al. 2012; Kittel 2012; Kremer et al. 2014). The most obvious examples are extreme climatic and hydrologic events, such as droughts, storms, freezes, and floods; biological events, such as pest and disease outbreaks (see Sect. 4.5.2); and socioeconomic events, such as major price and market changes, and economic, social, and civil turmoil. Climates are changing worldwide at rates not seen previously in geological time. This directly affects food production itself and, indirectly the growth and reproduction of plant pathogens which reduce crop yield and quality. Currently, 20–25 % of harvested crops worldwide are lost to pre- and post-harvest diseases; climate change is expected to increase these losses as a result of extremes of environmental variables such as temperature, rainfall, humidity, and longer growing seasons (Preston et al. 2012; Shabani and Tylor 2012; Oliver and Morecroft 2014). Airborne and soilborne pathogens may become more virulent and devastate crops and produce (e.g., dates and other fruits in oases) due to expanding environmental envelope as a result of climate change (Dixon 2012; Shabani and Kumar 2013).

Threats of abiotic (and biotic) stresses to the millions of date palms in the Middle East and North Africa have been highlighted in a large number of published papers, report recommendations, and workshops (see Jaradat 2014b, c for a review). *Phoenix* spp. or cultivars of date palm with broad physiological optima will have better adaptation or resistance to abiotic stresses; while those with narrow physiological limits may not withstand extremes of heat, freezing, drought, salinity, mineral toxicity, or other abiotic stresses (Atkinson and Urwin 2012; Kittel 2012; Gill et al. 2014). However, such physiological tolerances do change with life stage in this and other perennial fruit species (Kurup et al. 2009; Shapcott et al. 2009; Elmodafar 2010; Molnar et al. 2013), so that vulnerability may be linked to a critical growth stage or may shift among factors during the lifespan of the date palm. Date palm cultivars or populations in oases within the core of the species' "climate envelop" may not be well adapted to its marginal climates (e.g., Elche in Spain, sub-Saharan Africa, or the Send valley). Such core populations or cultivars may not be viable under a climate shift that brings it near to, but still within, the limits of the species' current climate range. Under these conditions, empirical niche models would overestimate its persistence (Fiaboe et al. 2012; Kittel 2012; Shabani et al. 2013a, b).

A number of abiotic stresses, such as soil and water salinity, caused by the presence of excessive amounts of soluble sodium chloride salts that hinder or affect the normal function of plant growth, are increasingly becoming serious threats to the expanding date palm industry in several Middle Eastern and North African countries (Eljuhani 2010; Jaradat 2011). Moreover, excess irrigation and drainage waters may end up in shallow aquifers thus increasing the risks of hydromorphy and asphyxiation of date palms (Kraiem et al. 2012). More recently, drought, due to lengthy rainless periods and drying-up of many desert water wells, resulted in increased water and soil salinity. Salinity problems develop in oasis agroecosystems due to mismanagement of water and soil resources under high evapotranspiration demand of hyper-arid environments (Hubener et al. 2005). Although some farmers are learning how to manage their date palm orchards under increasingly saline conditions, the need for a holistic solution to the salinity problem is greater than ever.

Date palm is one of the most salt-tolerant fruit trees; however, large varietal differences have been found in the species (Alhammadi and Edwards 2009; Kurup et al. 2009; Tripler et al. 2007, 2011; Sperling et al. 2014); however, salt tolerance largely depends on growth stage, soil characteristics, and management options. Recent results of molecular analysis revealed that specific DNA fragments may characterize genes coding for tolerance to these abiotic stresses, and are expected to serve as markers for early evaluation and screening for salinity and drought tolerance in date palm (Chao and Krueger 2007; Kurup et al. 2009). Although salt-tolerant cultivars are potentially available, there is no systematic approach for their characterization at the molecular level. In-depth understanding of molecular, physiological, biochemical bases, and soil factors will be helpful in developing selection strategies for improving salinity tolerance (Alhammadi and Edwards 2009).

Micropropagation, a useful and easy method of producing and transferring disease-free date palm germplasm within and among countries and, theoretically, serves the purpose of genetic conservation (Friedrich and Kassam 2011; Bakheet and Taha 2013; Alghamdi 2001), may contribute to vulnerability of un-adapted germplasm to abiotic (and abiotic) stresses by narrowing the genetic base of one or a few date palm cultivars being grown over large plantation areas, to replace aging date palm trees, or renew old oases (Ghazouani et al. 2011; Bodian et al. 2012; Battesti 2013). Genetic diversity provides the basic substrate for evolution, yet few studies assess the impacts of climate change on intraspecific genetic variation. In this context, it is important to incorporate neutral and non-neutral genetic diversity when assessing the impact of climate change in order to predict the future distribution (Shabani et al. 2013a, b) and fate of *Phoenix* spp. or the oasis as an agroecological system (Pauls et al. 2013).

4.5.1 Global Climate Change

A "significant extinction of plant species is expected" when global average temperature increases by more than 3.5 °C (IPCC 2007); hence, the potential for loss of date palm agrobiodiversity, termination of evolutionary potential, and disruption of its environmental services must be taken more seriously. Perennial species adapted to desert or semi-desert conditions, such as *Phoenix* spp., may react to future climates through ecological plasticity or adaptation (Paul et al. 2013); failure to do so may render the species vulnerable and could result in loss of genetic diversity, extirpation, or even extinction. Therefore, the capacity to enrich genetic diversity of palms, in general, and date palm, in particular, becomes an essential component of their ecological dynamics in order to adapt them to future global climate change. Abundance and shifts in the species distribution induced by climate change may affect not only the survival of the species, but also the agrobiodiversity-related ecosystem services (Kittel 2012; Boyd et al. 2013; Brown et al. 2013). Global climate change will impact intraspecific genetic diversity through the following:

- Changes in the spatiotemporal distribution of genetic variants as the ranges of populations and species change,
- Changes in level of phenotypic plasticity of individuals and populations as they respond to new environmental conditions, and
- Evolutionary adaptation to changing environmental conditions; in many cases, these changes will reduce genetic diversity in populations and species, while in extreme situations genetic erosion will lead to reduced population viability and extinction (Pauls et al. 2013).

Intra- and interspecific interactions may impose constraints on the adaptive capacity of the date palm and could narrow its future range relative to its potential climatic limits based on its physiological traits alone. If changing population and community dynamics shift these constraints, the date palm may reveal latent capacity to fill niches that are consistent with its physiological limits (Jaiti et al. 2007; Kurup et al. 2009; Elmodafar 2010; Kittel 2012; Varsheny and Anis 2013). Genetic diversity analysis indicated that genes conferring resistance to abiotic stress and the unique sugar metabolism-related genes, with direct impact on fruit development and ripening in date palm, tend to be enriched in the chromosomal regions where the density of single-nucleotide polymorphisms is relatively low (Almssalam et al. 2013). Consequences of rapid climate change may point to the inability to timely and effectively value and quantify oasis agroecosystem services before they reach a tipping point, and the loss of unique and irreplaceable genetic diversity.

Accelerated degradation of oases, loss of ecosystem services, and loss of agrobiodiversity underline the need to find out whether the inherent buffering capacity provided by oasis agroecosystems will enhance societal capability to adapt to, and mitigate, anthropogenic climate change? Therefore, it is indispensable to make sure that oasis agroecosystems are sustainable by being resilient to current and future climate change (IPCC 2007; Baer and Risbey 2009), as well as to market and other social and economic pressures (Hoole and Berkes 2010; Jaradat 2011; Faboyede et al. 2013). The genetic diversity of the date palm and associated perennial fruit trees, forage, and annual crops are important components of that resilience and need to be enhanced by ensuring that the wide range of existing cultivars is not further diminished (Nabhan 2007; Jaradat 2014a, b). Additionally, it is important to restrict the ability of market forces to dominate or dictate the selection of the cultivars grown or favored in the future at a local or regional scale (Jaradat 2011).

Although current attention to climate change and concerns over its potentially disrupting social stability in relation to vulnerable oases agroecosystems is relatively new, historically, however, the inability to cope with a changing climate figured prominently in many instances where social collapse was linked to environmental change and resource degradation (Ilahiane 1996; Elbarasi and Saaed 2013; Jabbar and Zhou 2013). These and earlier documented examples of resource degradation (e.g., Timbuktu and Um Elma; See Jaradat 2011) demonstrate that the risk of climate change to social systems has as much to do with characteristics

of oases agroecosystems, particularly their capacity for adaptation, innovation, and conflict management, as well as to their biophysical environment (Chen et al. 2013; Nefzaoui et al. 2012). Tree-based production systems, such as those based on the date palm, are often indorsed (Molnar et al. 2013) because of their perceived biological, economic and social resilience in the context of anthropogenic climate change, and other production challenges. Recent climate warming intensified the impact of inadequate management practices in tree-based systems, including poor drainage and the absence of an effective water resource management system, and resulted in widespread salinization, loss of agrobiodiversity, and reduced genetic diversity (Masoud and Koike 2006).

Broad-scale shifts in the areas conducive to date palm cultivation are expected due to global effects of an altered future climate on date production (Shabani et al. 2012). Large parts of these areas may become unsuitable due to a number of climatic and attending social factors (Shabani et al. 2013a, b). Many crops and their wild relatives may not be able to adjust their distributions to new areas that would be suitable for their survival as a result of the rapid rate of current and projected climate change. Of particular concern is the impact of the general warming trend in tropical and subtropical ecosystems (Nexon 1951) where most palms, including date palm, exist. The impact of global climate change on genetic diversity within populations and species will be manifested through spatiotemporal changes in the distribution of genetic variants as the ranges of species and populations change; changes in levels of phenotypic plasticity of populations and cultivars as they respond to new environmental conditions; and evolutionary adaptation to changing environmental conditions (Pauls et al. 2013). These changes may reduce genetic diversity in populations and species, including date palm.

4.5.2 Environmental Impact

Horticulture, within the oasis context, has a complex and ever-changing relationship with the environment and natural resources; this characteristic makes attributing specific environmental impacts on the oasis and the date palm very difficult (Allam and Cheloufi 2013; Battesti 2013). Much of the natural resource base in old oases showed signs of degradation of soil, water, and agrobiodiversity resources (Gebauer et al. 2009; Jaradat 2011, 2014a; Mace et al. 2012). Worldwide, about 60 % of the ecosystem services evaluated are already being degraded or used unsustainably (Faboyede et al. 2013). Environmental problems faced by ancient desert oases, such as Siwa in Egypt (Aboragab 2010), are fast developing, and impacting the land and water resources, and consequently agrobiodiversity and genetic diversity of date palm and other species within these oases.

The oasis landscape determines the design of orchards, gardens, fields, primary, and secondary irrigation channels, especially where land topography is heterogeneous (de Grenade and Nabhan 2013a, b; Omrani and Ouessar 2011). In mountain oases (Gebauer et al. 2009), topography and soil give the gardens their slope

and substrate; however, these factors are moderated to some extent by management practices such as terracing and soil development and build-up. These physiographic features, in addition to water resources, may regulate the spatiotemporal level and fate of both agrobiodiversity and cultivar genetic diversity within an oasis.

Environmental variability and heterogeneity, beside topographical and ecoregional diversity, are important determinants of the magnitude and persistent of date palm genetic diversity (Friedrich and Kassam 2011; Jabbar and Zhou 2013; Bai et al. 2014), even within a relatively small geographical region such as Oman where 180 female and 48 male cultivars have been documented, most of which have adaptation to local ecogeographical conditions and management practices (Alyahyai and Alkhanjari 2008). This wide range of ecogeographical and genetic variability allows for extended harvest season with dates suitable for harvest at different times and for fresh consumption, storage, or processing. Unlike the inefficient non-photochemical energy dissipation in the extreme habitat of date palm cultivation in desert oases, the more efficient oxygen-dependent electron flow, which sustains the required electron flow for carbon metabolism, is elevated in date palm cultivars exposed to solute stress and high atmospheric water demands (Sperling et al. 2014). These enhanced photo-protective mechanisms are crucial for the durability of date palms to avoid solute toxicity and photo-inhibition caused by stomatal and non-stomatal photosynthetic restrictions, extreme atmospheric water demands, and high solar irradiance.

Environmentally friendly alternatives to pesticides, such as biological agents, are increasingly being used to control soilborne pathogens in date palm (Elhassani et al. 2007), especially the Bayoud disease for which no effective chemical control is available yet (Sedra 2013). The success of biological agent(s) in turning on plant defense mechanisms against pathogens depends on their ability to establish metabolically active populations that could mediate host protection and/or compete directly or indirectly with the pathogens for nutrient resources (Gomez-Vidal et al. 2009; Dixon 2012). Assessment of environmental impact of chemical applications, whether for pest control or as soil amendments, should be considered when planning integrated pest management programs and other strategies at the oasis level (Blumberg 2008; Ahmed et al. 2010; Boyd et al. 2013). Although integrated pest management programs rely on scientific and conceptual advances in the biological sciences, other disciplines and methodologies such as socioeconomic issues pertaining to community involvement, ecological theory and its application in relation to habitat fragmentation, searching behavior and food chain relationships, and the application of biotechnology in relation to tissue culture and insect biotype identification are being integrated in this system (Blumberg 2008; Gitau et al. 2009; Hoddle et al. 2013).

Computer simulation and modeling showed that climate change might determine the boundaries of future distribution of date palm at a country (Shabani et al. 2013a, b) and global scales (Shabani and Kumar 2013). The business-as-usual scenario regarding CO_2 emissions (A2; IPCC 2007) suggests that the suitable land area (defined as appropriate soil types) for its growth and production may

be reduced by two-thirds at a regional scale (Shabani et al. 2013a); however, at a global scale (Shabani and Kumar 2013), area could increase by sixfold at the end of the twenty-first century. Heat stress, as determined by physiological thresholds for vital biological processes, will be the single most important limiting factor in future distribution of the date palm. Horticulturists and planners can effectively utilize a modeling approach in order to minimize the impact of future climate change on date palm vulnerability and potential erosion of its genetic diversity. Although the simulation outputs can be based on the response of *P. dactylifera* to future climate, the results should be refined by incorporating physicochemical properties and spatial soil variation to meet the specific edaphic date palm growth and production requirements.

4.5.3 Water, Salinity, Drought and Heat

The cultivation and management of date palms, and production optimization of dates, in a world faced by diminishing water resources, both in quantity and quality, call for a new paradigm in allocating water resources based on requirements and consumption (de Fraiture and Wichelns 2008; Ben Salem et al. 2011; Battesti 2012). In addition, there is a need for reliable sensors, monitors, and indicators of salinity and drought stress (Sperling et al. 2014). Local farmers are increasingly losing control over water resources of their oases to national authorities who introduced hydropolitics in managing and distributing water resources among competing economies within and outside oases agroecosystems (Battesti 2012; Kendoucia et al. 2013). This change in the governance of water resources created shifts in water availability, changes in social norms and management practices, a more dynamic landscape, and potentially a shift in agrobiodiversity and genetic diversity at local and regional levels (Battesti 2012; Misra 2013; Oliver and Morecroft 2014). Problems in water governance, as a reflection of a power management structure, were the primary reasons behind low species diversity in some of the old (Kraiem et al. 2013; Mekki et al. 2013) and relatively new (de Grenade and Nabhan 2013b) oases. A larger degree of autonomy and integration was demonstrated when and where farmers have direct governance over their local water resources; as a result, higher agrobiodiversity and healthier agroecosystems were developed and maintained.

Unexpectedly, a limited and conflicting body of information, for the most part, is available on water relations and irrigation requirements of date palm in the main producing countries where the quantity and quality of water resources are declining (Ben Salem et al. 2011; Carr 2012; Alnaeem 2013). Water-use efficiency of date palm of about 1.3 kg fresh fruit m^{-3} irrigation water, on average, is rarely achieved and it varies among cultivars under natural (Heidecke and Heckelei 2010; Cohen et al. 2012) and controlled (Carr 2012) conditions. Irrigation malpractices under desert climate, occasionally (Kraiem et al. 2012), resulted in dumping the highly saline excess irrigation and drainage water in natural storage basins within

the oases ecosystem, thus impacting the ecosystem as well as exposing date palm trees to higher salinity stress. Therefore, improved water management by modernizing irrigation methods and by introducing effective drainage at the oasis level, and improved water-use efficiency at the cultivar level, can help mitigate salinity stress in particular, and climate change in general (de Fraiture and Wichelns 2008).

In general, the date palm is more vulnerable to salinity than to drought or heat stresses (Alhammadi and Edwards 2009; Kurup et al. 2009); at an oasis level, species richness, species diversity, and species composition significantly deteriorate with increasing salinity (Masoud and Koike 2006; Tripler et al. 2007, 2011). However, the hyper-arid climate, coupled with mismanagement of water and soil resources under high evapotranspiration demand (Hubener et al. 2005), are interconnected factors behind the growing salinity problems in oases agroecosystems (Potchter et al. 2008, 2012). High salinity of irrigation water reduces the growth of date palm mainly through osmotic stress and water relations; consequently, water potential imbalance impacts photosynthetic productivity when the date palm encounters water stress forcing stomatal limitations on photosynthesis; this course of events, in turn, exposes the date palm to excess solar irradiance and the need for photoprotection mechanisms under hot desert or semi-desert conditions (Sperling et al. 2014).

Vulnerability of date palm to salinity in desert oases can be viewed in relation to the disproportionate share of the global 20 and 50 % of cultivated and irrigated areas, respectively, being subjected to different magnitudes of salinity stress (Masoud and Koike 2006; Kraiem et al. 2012, 2013; Wang and Li 2012). Although some farmers are learning how to manage their date palm orchards under increasingly saline conditions, the need for a holistic solution to the salinity problem is greater than ever (Kurup et al. 2009; Zhou and Li 2013). Although date palm is one of the most salt-tolerant fruit trees, however, large intra- and interspecific differences have been documented (Alhammadi and Edwards 2009; Sperling et al. 2014). Salt tolerance in date palm, in particular, depends on growth stage, edaphic factors, and management practices. The ability of date palm genotypes to exclude Na^+ and Cl^- toxic ions from highly sensitive photosynthetic mesophyll tissues is the mechanism most recognized for salt tolerance or resistance (Sperling et al. 2014), while high K^+ concentration in the shoots constitutes a major protective factor because Na^+ toxicity is primarily caused by competition for K^+-binding sites in the mesophyll. However, management practices, such as drainage beyond the rooting zone, improved water- and nutrient-use efficiencies (Masoud and Koike 2006), the use of bio-drainage as an innovative natural drainage system that can reduce the heat island effect, stabilize sand dunes, and gradually build fertile topsoil in the oasis (Ghazouani et al. 2011), if properly and timely implemented can reduce date palm vulnerability to salinity (and sodicity) stresses. However, despite its outstanding agronomic and socioeconomic significance, attempts to screen and use available genetic diversity for salt tolerance have been limited and therefore of urgent priority.

Recently, abiotic stresses (e.g., soil and water salinity) are increasingly posing serious threats to the expanding date palm plantations in the Middle East and

North Africa. In addition, due to lengthy rainless periods and drying-up of many desert water wells, drought resulted in increased water and soil salinity in traditional oases agroecosystems (de Fraiture and Wichelns 2008; Ben Salem et al. 2011). The impact of extensive modern water extraction and irrigation techniques for human settlement, and consequently on agrobiodiversity and genetic diversity of date palm, is demonstrated in hyper-arid regions by the large quantitative and qualitative changes in vegetation cover that have occurred in several oases in the Middle East, North Africa, and elsewhere, during the past fifty years (de Jong et al. 2005; Huang et al. 2013; Kendoucia et al. 2013). The impact of these techniques is demonstrated by the disappearance of date palm orchards and associated agrobiodiversity due to the depletion of desert aquifers (Mekki et al. 2013), abandonment and declining oasis, (Marx 1999) or to seawater intrusion (Jaradat 2011).

The impact of climate change is expected to be sever on fossil water resources; it may be reduced by about 30 % by 2030 where major desert oases are located (Omrani and Ouessar 2011), thus rendering date palm diversity and agrobiodiversity vulnerable to salinity and drought stresses. Better water circumstances are found in mountain oases (e.g., in Oman), where almost 75 % of the traditional irrigation systems, known as aflaj, are still in operation and where farmers optimized an irrigation system capable of countering drought and salinity stresses (Gebauer et al. 2009), and thus were able to maintain large genetic diversity of date palm cultivars in mountain oases (Alyahyai and Alkhanjari 2008). In arid and semi-arid regions (e.g., southern and eastern margins of the Atlas mountain range, in Morocco), oases rely mainly on traditional water systems called khattara (Heidecke and Heckelei 2010; Kenducia et al. 2013). Recurring droughts, economic and anthropogenic factors related to their maintenance rendered about 50 % of these khattaras waterless with concomitant impact on date palm survival and vulnerability to drought. Potential water use may increase substantially due to advection in the environs of desert (Carr 2012) but not mountain oases (Gebauer et al. 2009) due to global warming and climate change.

Simulation studies (Shabani et al. 2012; Shabain and Kumar 2013a, b) suggested that rising temperature and drought stresses will play increasingly important roles in adaptation and distribution of date palm in the twenty-first century, and any future distribution will most likely be impacted by climate change. Recent examples from North Africa highlight the adverse effect of climate change on the fragile oasis agroecosystems, where, for example, ten oases in southern Morocco have lost about 40 % of their vegetation due to multiple abiotic stresses (i.e., drought, depletion of groundwater, high temperature, and sand encroachment; Bodian et al. 2012). Unique date palm orchards in southern Libya disappeared altogether along with their genetic diversity due to the depletion of desert aquifers (Alnaeem 2013; Kraiem et al. 2012, 2013). On the other hand, water and soil salinities present challenges in modeling water dynamics and growth of date palm as they highlight the need for response functions to salinity that can take into account the spatiotemporal dynamics in plant size, growth, fruit production, and water consumption relationships (Alhammadi and Edwards 2009; Kurup et al. 2009; Tripler et al. 2011).

Even though it is considered a salt-tolerant fruit tree and, it is more adapted to salinity stress than most other fruit trees, however, reports on salinity tolerance of the date palm are conflicting (Sperling et al. 2014). Nevertheless, date palm cultivars do differ in their responses to salt, particularly at the seedling stage. Traditionally, however, the date palm was considered a fairly salt-tolerant species, with a threshold electrical conductivity value for the saturated soil extract of about 4.0 dS m^{-1}. Recent evidence (Carr 2012) challenged this assumption suggesting that a reduction in osmotic potential, which restricts root water uptake, can be attributed to the presence of salts in the soil water, but not necessarily due to ion toxicity (Sperling et al. 2014). Long-term experiments (>20 years) showed that soil salinity of 4.7 dS m^{-1} (which is about 20 % of an earlier estimate) reduced fruit yield by about 50 %. Over time, accumulated salinity reduces the production of new leaves and rate of extension of new leaves, by about 50 % per year and 30 % per day, respectively. Eventually, a reduction in leaf canopy will lead to a drop in productivity, delayed fruit maturity, and eventual loss of salt-sensitive or vulnerable cultivars.

Water salinization and agroecosystem degradation are major environmental problems that may develop in response to water shortages arid regions (Masoud and Koike 2006; Wahba et al. 2007; Kraiem et al. 2012; Zhou and Li 2013). In groundwater-dependent oases, groundwater management and circulation control the overall water quality and ecosystem dynamics. Under such conditions, groundwater depth, flood events, and salt concentration are key factors controlling the growth, development, and yield of date palm in the oasis (Huang et al. 2013). Dynamic management practices are needed to avoid salinity and drought problems when different and changing ratios of groundwater and surface water are available for date palm irrigation (de Jong et al. 2005), or when conflicting water-use options do emerge (Elbarasi and Saaed 2013).

Large commercial plantations of elite date palm cultivars (e.g., Medjool and Deglet Noor) coupled with limited availability of fresh water in arid Mediterranean regions are behind the extensive use of low-quality water, including brackish and saline water, to meet the huge irrigation water demand for economic date production (de Fraiture and Wichelns 2008; Battesti 2012; Carr 2012; Cohen et al. 2012; Alnaeem 2013). The long-term effects on production, quality, and vulnerability of these cultivars are not well known or documented (Sperling et al. 2014). Large variations in a set of morphological, physiological, and molecular markers have been identified for tolerance to salinity (up to 27.3 dS m^{-1}) and drought among date palm cultivars (Kurup et al. 2009; Alhammadi and Edwards 2009). Recent results of molecular analyses revealed that specific DNA fragments may characterize genes coding for tolerance to these abiotic stresses, and are expected to serve as markers for early evaluation and screening for salinity and drought tolerance in date palm (Kurup et al. 2009). Nevertheless, the need is urgent to develop oasis-based screening methods (e.g., remote sensing) of date palm cultivars for salinity tolerance at different growth stages. Remote sensing and image analyses of satellite imagery were suggested (Masoud and Koike 2006) to provide evidence and locate possible changes at the oasis level due to

salinity; these and associated technologies that can detect and quantify spatiotemporal salinity extent in desert oases can be an effective strategy to be employed in combating current and future salinity stress in date palm (Alhammadi and Edwards 2009).

4.5.4 Eco-hydrology

The long-term evolution of an oasis is determined, to a large extent, by changes in eco-hydrology as influenced by spatiotemporal water resources and in relation to the land area and the vertical structure of crops which is dominated by the date palm (Ilahiane 1996; Marx 1999; Ghazouani et al. 2011; Mekki et al. 2013; Fig. 4.2), whereas the effect of human activities on oasis evolution is manifested through the direct and indirect impact on water resources, all of which effect oasis agrobiodiversity and genetic diversity of the date palm (Nabhan 2007). The eco-hydrology of some desert oases (e.g., in Timimoun and Touat, Algeria) evolved from being dependent on natural spring, to artisan wells, and finally to shallow and deep wells with increasing energy expenditure to lift water from successively greater depths (Battesti 2012). Deep, as well as shallow, desert aquifers are being depleted at a rapid pace and at an increasingly higher economic cost, and resulted in dried-up natural springs with adverse effects on oases agrobiodiversity and loss of date palm genetic diversity. Exploring the relationships between oasis landscape patterns and hydro-ecological processes in arid area, which are strictly influenced by water resource conditions, became a hot topic and difficult challenge in arid landscape ecology and one of the most important issues in the ecological protection and reconstruction of arid regions (Alkhashman et al. 2011; Ashkenazi et al. 2012; Bai et al. 2014).

A prerequisite for ecological protection and reconstruction of degraded oases is the identification of its relationship with oasis landscape patterns (Saaroni et al. 2004; Wang and Li 2012; Sawut et al. 2013). Hydrological factors are the key drivers of landscape changes as was demonstrated by the vanishing Ejina natural

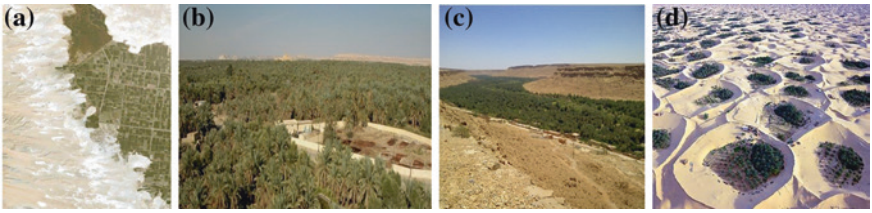

Fig. 4.2 Eco-hydrology and its effects on the design and potential vulnerability of desert oases. Farafrah oasis, Egypt, irrigated by shallow desert aquifers (**a**), Siwa oasis, Egypt, irrigated by saline underground water (**b**), Wadi Ziz, Morocco, irrigated by surface water (**c**), and Adjder oasis near Timimoun in Algeria, irrigated by very deep aquifer water sources (**d**) (*Source* Google Earth Images)

oasis (de Jong et al. 2005; Ben Salem et al. 2011; Fernald et al. 2014) where underground water table was mostly influenced by runoff of surface water, which in turn was influenced by anthropogenic factors such as population pressure, social and economic development, and water resources management. Where date palm irrigation depends on stored (surface) water, as in the case of Ouarzazate oasis in southeastern Morocco which depends on a reservoir fed by the Drâa river to recharge its aquifer (McGregor et al. 2009), the hydrological cycle is fine-tuned to maintain adequate water level in the aquifer and to provide a steady and adequate supply of irrigation water for the oasis (de Jong et al. 2005).

Water management in most desert oases faces several technical and anthropogenic constraints; the most critical of which is how to strike a balance between water availability for irrigation and its use for socioeconomic development (Omrani and Ouessar 2011). The numerous and diverse traditional date palm cultivars are being increasingly replaced by large-scale plantations of a limited number of elite date palm cultivars with higher added value (e.g., Deglet Nour in Tunisia; Karim et al. 2010; Mekki et al. 2013). This trend is stressing water resources in major date-producing areas and calling for the use of more efficient irrigation methods as a part of an overall management strategy of natural resources in these oases (Omrani and Quessar 2011). Engineering solutions to solve long-term irrigation problems in desert oases (e.g., drought, irregular water availability) may not be optimal if the natural eco-hydrology is radically changed due to building massive water works (e.g., damming a river; de Jong et al. 2005) or changes in the hydrological cycle (e.g., deterioration of aflaj and khattarah irrigation systems; Luedeling and Buerkert 2008; Heidecke and Heckelei 2010; Kenducia et al. 2013).

In such groundwater-dependent oasis agroecosystems, groundwater circulation controls the overall water quality and ecosystem dynamics (Huang et al. 2014). Local and national authorities need to conceptualize the interconnections between potential management options of deep aquifers and the functioning of oasis agroecosystems (Mekki et al. 2013). A compromise is urgently needed between the increasingly competing economic, developmental, water conservation, and water use for date palm irrigation options; it remains to be seen if indigenous knowledge can help maintain these traditional systems (e.g., aflaj-based irrigation systems in mountain oases and khattaras in North Africa) in the face of increasingly challenging anthropogenic and climate change stresses (Preston et al. 2012; Shabani et al. 2013a, b; Oliver and Morecroft 2014).

4.5.5 Heavy Metals

Date palms are increasingly being subjected to airborne and soilborne environmental pollutions and metal pollutant contamination, especially in urban areas where large numbers are planted for landscaping or gardening purposes (Aldjain et al. 2011). The date palm has been used as a bio-monitor of lead and other toxic elements in arid environments (Alshayeb et al. 1995). Heavy metal residues

(Fe > Pb > Zn > Ni > Cu > Cr) have been found on leaves and in fruit tissues with concentrations within the FAO-WHO safe limits recommended for human consumption (Alkhashman et al. 2011); however, these concentrations were higher in areas of heavy traffic and close to industrial sites and all were much higher than those obtained from a typical oasis environment. The ability to distinguish between airborne and soil contamination maybe of value in assessing the vulnerability of date palm cultivars to heavy metal toxicity (Hamurcu et al. 2010).

A limited number of studies concluded that the date palm can be used as a biomonitor of heavy metal contamination in areas subjected to industrial and traffic pollution (Aldjain et al. 2011). Further work is needed not only to assess the spatial distribution of metals in materials but also to examine variation at a smaller scale, with more intensive sampling and studies required to measure any change or increase in chemical elements in this area (Hamurcu et al. 2010; Alkhashman et al. 2011). Future samples should come from different depths in a range of sites throughout the investigated area in order to study the mobility of heavy metals at different soil and rooting depths.

4.6 Vulnerability to Biotic Stresses

Genetic diversity and agrobiodiversity are indispensable for maintaining agroecosystem services by reducing vulnerability to insects and diseases (Pauls et al. 2013). Traditional date palm cultivation over centuries accumulated a number of mismanagement practices including lack of insect and disease control measures, as well as crowding of trees, retention of old or unproductive trees, planting of mixed cultivars or seedlings, salt accumulation, poor drainage, insufficient irrigation, fertilization or tillage, competition with other crops, soil degradation, and inadequate irrigation water (Chao and Krueger 2007; de Jong et al. 2005; Ben Salem et al. 2011; Ghazouani et al. 2011; Bai et al. 2014); all of these mismanagement practices are potential factors rendering the date palm vulnerable to multiple stresses and genetic erosion of its diversity.

A surge in tree insects and diseases is one of the most perceptible indicators of increasing anthropogenic stresses on life systems (Bendiab et al. 1993; Getau et al. 2009; Ahmed et al. 2010; Ben Chaaban et al. 2011; Boyd et al. 2013). In an oasis agroecosystem, the date palm and other fruit trees are essential parts of sustainable, productive, and safe environments. Trade in date products is increasingly becoming an integral part of a modern, globalized economy but, if unregulated to protect against the spread of insects and diseases, has potentially severe price for some of the most important ecosystem services involving date palm trees (Boyd et al. 2013).

The date palm is plagued by many insects (Table 4.2) and diseases (Table 4.3), but the nature and severity of their impacts vary with cultivar, location, microenvironment, and cultural practices (Ghazouani et al. 2011; Tripler et al. 2011; Vinatier et al. 2012). Most reported diseases of date palm that can be associated

with a pathogen are attributed to fungi. However, recent reports highlighted a number of physiological disorders associated with phytoplasma (Table 4.4). The literature is replete with published papers, report recommendations, and workshops highlighting the threats of biotic stresses to the millions of date palms in the Middle East and North Africa. Two of these biotic stresses (i.e., the red palm weevil [*R. ferrugineus* (Olivier)] and Bayoud, caused by *F. oxysporum* f. sp. *albedinis*) are threatening the region's multimillion dollar date industry and the very survival of the date palm trees. The red palm weevil is causing severe damage in date palm orchards in eastern Arabia, Iraq, and Egypt; the insect is also considered as a major threat to *Phoenix theophrasti*, the native palm species in the island of Crete, Greece.

Historically, vulnerability to insects and diseases has been the single most important cause of major crop declines or genetic erosion (Goldman 1995; Blumberg 2008; Gitau et al. 2009); other causes can be categorized into several models of unsustainability, the most frequent involves a model of shocks or extreme unexpected events such as the widespread of the red palm weevil in the Middle East (Aldryhim and Albukiri 2003; Elshafie et al. 2011; Ju and Ajlan 2011) and Bayoud disease in North Africa (Bendiab et al. 1993; Elhassni et al. 2007; Elmodafar 2010; Sedra 2013). Climate change will likely affect the geographic distribution and virulence of many insects and diseases of date palms as evidenced by recent reports (Shabani and Kumar 2013a, b) and predictions of ecological niche models (Sutherst et al. 2000, 2007; Fiaboe et al. 2012). Climate change-based maps can provide indispensable information on predicted changes in certain regions that may become suitable for date palm cultivation under different risk levels of major insects and diseases, thus enabling decision-makers, oases dwellers, and plantation managers to anticipate the long-term implications of current management decisions (Shabani and Kumar 2013a, b). New geographical areas may potentially become more suitable for current date palm insects and diseases and new biotypes may emerge as a result of environmental changes across their distributional range. For example, local Algerian populations of Bayoud already showed genetic differentiation since these populations spread outside their original habitat and are established in new oases (Sedra 2013).

Genetic uniformity of date palm cultivars in North Africa (e.g., Medjool in Algeria and Morocco) exacerbated the impact of insects and diseases. It was estimated that Bayoud already destroyed more than 13 million trees in these two countries during the twentieth century. Recently, molecular markers have been identified in date palm as potential markers of resistance to this disease (Sedra 2013). Micropropagation of date palm, a useful, easy and rapid method of propagating and disseminating disease-free germplasm of elite cultivars within and among countries (Alghamdi 2001), the widespread demand for elite cultivars to be propagated using these methods may be contributing to genetic uniformity of such cultivars, increased vulnerability to insects and diseases, and loss of genetic diversity.

Climate change will likely affect the geographic distribution of pests and diseases as evidenced by the future geographic distribution of the red palm weevil predicted by ecological niche modeling (see Sect. 4.6.1). Areas where the insect was not reported yet were found to be suitable for its invasion as a result of climate change (Fiaboe et al. 2012). Date palm pathologists and entomologists acknowledge the immense challenges they have to face when considering the growing impact of a combination of climatic change and the increased activities and virulence of palm diseases and insects (Dixon 2012; Elshafie et al. 2011; Fiaboe et al. 2012). Making ecologically and economically comprehensive pest management decisions within an integrated pest management context is a complex process (Vinatier et al. 2012; Rahnama and Latifian 2013). A wide range of variables, including biological characteristics of targeted pests, levels at which pests can be tolerated, degree and cost of protection afforded by management options, and current and future biotic and abiotic stresses, as well as a number of interacting social, cultural, legal, and political factors that may outweigh those variables, need to be taken into consideration when formulating such decisions.

4.6.1 Insect Pests

Various insects attack date palms, but specific insect problems and their impact on date palm vulnerability vary with geographic area (Table 4.1). Several pest management methods, including chemical, biological, pheromone trapping, quarantine, and sanitation practices, are used to control insect pests of date palms (Fakeiro 2006; Vinatier et al. 2012; Hoddle et al. 2013). In particular, the biocontrol of the lesser date moth [*Batrachedra amydraula* Meryrick (Cosmop teridae = Batrachedridae)] on date palms serves as a model for non-chemical control in date palms (Habib and Essaadi 2007). Arguably, the red palm weevil [*Rhynchophorus ferrugineus* (Oliv.)] is the most important insect attacking date palm (Fig. 4.3); it was reported from almost half of the date palm-growing countries in the Middle East (Aldryhim and Albukiri 2003). The insect was reported first on *Cocos nucifera* in South Asia (Faleiro 2006) and moved to the Middle East through infested offshoots; it is considered as a lethal pest of about 17 palm species, including *P. dactylifera*, throughout their distributional range (Faleiro 2006; Fiaboe et al. 2012), especially in the Mediterranean region (Gadelhak and Enan 2005; Hazir and Buyukozturk 2013). The insect has spread from India to the Middle East, North Africa, and even to southern Europe, threatening date palm industry in several countries across this region (Ju and Ajlan 2011).

Initial and early infestations of mostly young (5–15 year old) trees are hard to discover due to lack of external symptoms (Elshafie et al. 2011; Faleiro 2006; Fiaboe et al. 2012), thus drastically reducing their potential life span and the production of insect-free offshoots and high-quality fruit. Some management malpractices, such as flood irrigation, help spread the insect. Pheromone traps have

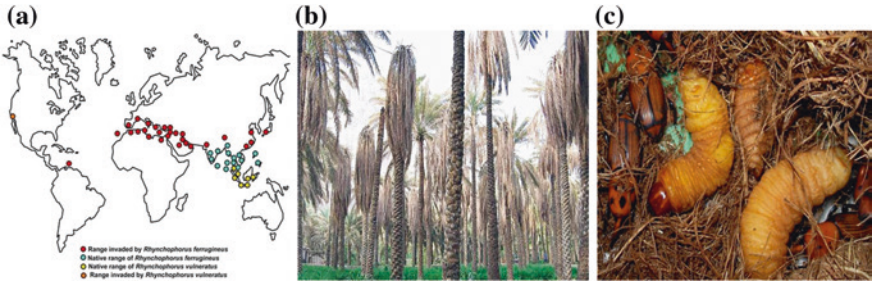

Fig. 4.3 Native range and geographical distribution of the red palm weevil (*Rhynchophorus ferrugineus*) and *Rhynchophorus vulneratus* (**a**), dead date palm trees infected by the red palm weevil in the United Arab Emirates (**b**), and the adult insect and its pupae extracted from an infected trunk (**c**) (*Source* Google Earth Images)

been used to monitor and mass trap the red palm weevil in an area-wide integrated pest management programs of about three million date palm trees (Aldryhim and Albukiri 2003; Alaied et al. 2006). However, conventional food-baited pheromone traps have to be periodically serviced by changing of food bait and insecticide solution, which is labor intensive. A bait-free method to 'attract and kill' red palm weevil adults has been recently developed for weevil control in date palm. Using Hook RPW in and around heavily infested plantations could substantially reduce the cost of an area-wide integrated pest management program due to elimination of trap servicing (Elshafie et al. 2011; Rahnama and Latifian 2013). Short-term and intensive integrated pest management system of the red palm weevil reduced the insect intensity and the use of insecticide applications, and resulted in ~90 % drop in palm eradication (Hoddle et al. 2013). New information on the flying ability of insect adults is being modeled using computer simulation (Avalos et al. 2014) as a part of a new initiative to control the red palm weevil (Mukhtar et al. 2011).

A strong correlation was found between genetic and geographic distances among the red palm weevil populations in parts of Arabia (Gadelhak and Enan, 2005), indicating speciation and specific adaptation to geographical conditions within its distributional range. Maximum entropy simulation models successfully predicted the known distribution of the red palm weevil, including the single North American occurrence in California, USA, as well as in various areas where the insect has been reported in the Middle East, North Africa, Southern Europe, and South Asia (Fiabo et al. 2012). Predictions based on the modeling approach may help prevent further spread of the insect to new regions and to safeguard non-infected date palms.

Three of the economically most important date palm insects are the Arabian rhinoceros beetle, the green pit scale insect, and the grater date moth (Table 4.1). The Arabian rhinoceros beetle (*Oryctes agamemnon arabicus*), an invasive species, is causing serious damage to date palm trees in the Tozeur and Kabili region of southern Tunisia, with >3000 ha of date palm already infected (Abdallah et al.

Table 4.1 Partial list of date palm insect pests (USA, United States of America; MENA, Middle East and North Africa)

Scientific name	Common name	Threat to	Country
Apate monachus	Bostrychid pest	Trees, rachis	USA
Arenipses sabella	Greater date moth	Bunch stalk, ripe fruit	
Asarcopus palmarum	Issid date bug	Tender leaves, foliage	MENA
Batrachedra amydraula	Lesser date moth	Flowers	Arabia
Brassolis sophorae	Brush-footed butterfly	Defoliation of fronds	Several
Cadra figulilla	Raisin moth	Stored fruit	
Coccotrypes dactylipedra	Date stone beetle	Unripe fruit	
Comstockiella sabilis	Palmetto scale insect		USA
Diceroprocta apache	Cicada	Fruit tissue	USA
Dinapate wrightii	Giant palm borer	Mature trees	
Dysmicoccus brevipes	Pineapple mealybug	Trunk, roots, ripening bunch	
Ectomyelois ceratoniae	Carob moth		USA
Eutetranychus palmatus	Date palm mite	Fruit	USA, MENA
Jebusea hammerschmidti	Palm stem borer	Trunk	Middle East, India
Lucanus cervis	Stag beetle	Trunk	
Microcerotermes diversus	Termite	Roots, fronds, trunk	Middle East
Neoderelomus piriformis	Palm flower weevil	Cross pollination	Canary Islands
Oligonychus afrasiaticus	Dust mite	Fruit	Old World
Ommatissus lybicus	Old world date bug	Mature dates	MENA
Oryctes agamemnon	Rhinoceros beetle	Roots, trunk	Worldwide
Oryctes sahariensis		Roots, trunk	Chad, Sudan
Oryzaephilus surinamensis		Fruit	Arabia
Palmaspis phoenicis	Green scale	Chlorosis of fronds, fruit drop	Sudan, Middle East
Parlatoria blanchardi	Parlatoria date scale insect	Leaves, bunch stalk, fruit	MENA
Phoenicococcus marlatti	Red date scale insect	Inflorescence, premature fruit drop	MENA

2013). Very little is known about its dispersal mode or genetic diversity (Blumberg 2008); however, its economic impact is already noticed in several date-producing countries. The green pit scale insect (*Palmapsis phoenicis* Rao) (*Asterolecanium phoenicis* Rao.), native of central Asia, is transmitted via offshoots across large areas in the Middle East (Ahmed et al. 2010). Recently, it become a real threat to date palm cultivation in Northern Sudan, with an infected area of about 5000 ha;

it continued to spread to new areas across several eco-regions of Sudan along and beyond the Nile valley (Ezebilo et al. 2013). Finally, the infestation by the larvae of the greater date moth (*Arenipses sabella*) caused the crown bending and dwarfing physiological disorder (see Sect. 4.6.3), which seemed to be cultivar-specific, especially in date palms of tissue culture origin (Sudhersan 2013).

4.6.2 Pathogens

The nature and severity of the many diseases afflicting the date palm (Table 4.2) are caused by fungi, and vary with cultivar, location, weather, and cultural practices (Feather et al. 1989; Elhassni et al. 2007; Gitau et al. 2009; Boyd et al. 2013; Chao and Krueger 2007). One of the most serious fungal diseases in North Africa is the Bayoud disease (Fig. 4.4); most infected trees die within 5–6 years of infection (Fig. 4.5). Local genetic differentiation in population of this pathogen exacerbates date palm vulnerability as it spreads to new oases and micro-environment (Sedra 2013). Genetic uniformity of date palm populations (e.g., Medjool in Morocco and Algeria) partly explains the high incidence and severity of the disease in North African oases (Bendiab et al. 1993; Elmodafar 2010), where an estimated 13 million date palm trees have been killed in these two countries during the twentieth century. A few resistant phenotypic variants have been identified in Morocco; however, mostly of poor fruit quality (Table 4.3).

Genetic diversity for natural and heritable resistance to Bayoud, which stems from constitutive multifactor defense mechanisms related either to mechanical

Table 4.2 Partial list of date palm diseases

Scientific name	Common name
Alternaria alternata	
Botryodiplodia theobromae (*Pat.*)	Offshoots deterioration
Bursaphelenchus cocophilus (*Cobb*).	Red-ring disease
Diplodia phoenicum	Diplodia disease
Fusarium moniliforme (*Sheldon*)	
Fusarium oxysporum f. sp. *albedinis*	Bayoud
Fusarium canariensis f. sp. *canariensis*	Bayoud
Fusarium proliferatum	Inflorescence rot
Fusarium solani	
Graphiola phoenicis	Graphiola leaf spot
Gliocladium roseum (*Link.*)	
Mauginiella scattae Cav.	
Mycospherella tassiana	Brown leaf spot
Omphalia tralucida	Omphalia root rot
Thielaviopsis paradoxa (*De Syn.*)	Heart rot disease

Fig. 4.4 Symptoms of Bayoud disease on date palm fronds (**a**), whole crown (**b**), loss of leaves and death of whole trees (**c**) and the pathogen *Fusarium oxysporum* f. sp. *albedinis*, in vitro (**d**) (*Source* Google Earth Images)

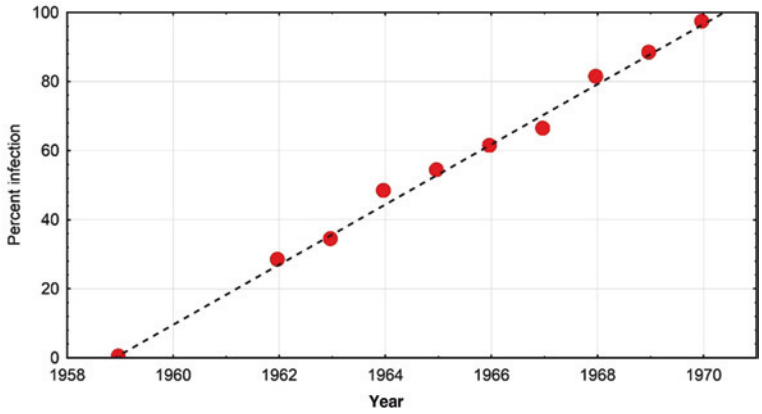

Fig. 4.5 Percent infection by Bayoud (*Fusarium oxysporum* f. sp. *albedinis*) in Medjool trees from Morocco during a 10-year period. Almost 50 % of infected trees were dead within 5 years of infection and all infected trees were dead 10 years after infection (Djerbi 1990)

Table 4.3 Resistant phenotypes and level of significance of the association between resistance to Bayoud disease and each of fruit color, fruit texture, and maturity class based on 35 different cultivars and selections of date palm from Morocco (Based on qualitative data in Sedra 2013)

Trait 1	Trait 2	Resistant variant	*P* (Fisher's exact test)
Fruit color	Resistance to Bayoud	Pale brown	0.06
Fruit texture	Resistance to Bayoud	Soft fruit	0.07
Maturity date	Resistance to Bayoud	Late maturity	0.03
Fruit color	Fruit texture		0.52
Fruit color	Maturity date		0.21
Fruit texture	Maturity date		0.45

properties of the cell wall or to chemical production and activity of pictinolytic, cellulolytic, and proteolytic enzymes, is very limited and represents the only practical method of disease control (Jaiti et al. 2007; Elmodafar 2010). Vulnerability to this disease can be appreciated by the fact that only six out of the hundreds of Moroccan date palm cultivars appear to be resistant to Bayoud (Table 4.3); however, their fruits are of poor quality. A highly complex breeding program, because of dioecy, the need for polygenic resistance, and yet complicated by the long lifespan of female trees, is expected to produce resistant progenies with high fruit quality from crosses between the few resistant and the many susceptible elite cultivars. Due to the strong morphological resemblance, and consequently a possible relationship between some cultivar groups selected for resistance to Bayoud disease (Elhoumaizi et al. 2002), simple morphological descriptors, although necessary to identify cultivar before the onset of fruiting stage, may help in accelerated breeding for resistance to this disease.

Progenies of Bayoud-resistant cultivars are characterized by a high level of electrophoretic polymorphisms in a number of allozymes (Bendiab et al. 1993); genetic linkage analyses may provide a useful method in identifying seedling populations derived from Bayoud-resistant cultivars and suitable for breeding programs. Monogenic resistance or any improvement due to only one defense component may not be durable due to high selective pressure on the pathogen (Boyd et al. 2013) and the emergence of new pathotypes (Feather et al. 1989; Elmodafar 2010; F). A complementary strategy to develop resistant genotypes should integrate genetic and biotechnological approaches, including callus and cell culture combined with in vitro selection (Elmodafar 2010).

Bayoud produces toxins involved in the expression of disease symptoms. Therefore, these toxins, if proved to be functionally linked to the disease, can be tested for the selection of resistant seedlings derived from callus or cell suspensions exposed to these toxins in vitro. Transgenic date palm plants can be regenerated from cell cultures carrying detoxification gene(s) to overcome fusarium's pathogenicity and virulence. Biocontrol measures, such as bacteria, yeasts, or saprophytic fungi to stop the growth of the pathogen or to enhance natural defense mechanisms of infected trees, have been suggested. Other biocontrol agents (e.g., in the genera Trichoderma, Penicillium, Gliocladium, Sporidesmium, Burkholderia, Bacillus, and Serratia) provided some levels of protection against soilborne pathogens including *Fusarium* spp. (Elhassni et al. 2007; Elmodafar 2010; Jung et al. 2012). As stated earlier, date palm cultivars (mostly of seedling origin) having natural resistance to Bayoud disease are rare and their fruits are of poor quality as compared to the susceptible, but high-quality cultivar (i.e., Medjool).

Two of the economically most important date palm diseases are the tissue decay, and the wilt and dieback; others are listed in Table 4.2. Tissue decay was detected in many of the freshly fallen fruit stalks caused by bunch drop (Cohen et al. 2010). A pathogenic fungus (*F. proliferatum*) was detected in most of the necrotic fruit stalks and isolates from infected fruit stalks generated necrotic lesions in fruit stalk tissue in vitro. However, fungicide treatments on trees in the

orchards were ineffective in reducing bunch drop. Wilt and dieback disease, an extremely diverse species complex (Gunn and Summerell 2002), attacks *P. canariensis* and *P. dactylifera*, with symptoms similar to those of Bayoud on date palm (Feather et al. 1989), which is caused by *F. oxysporum* f. sp. *canariensis* in association with *Gliocladium vermoeseni*. Although it was probably derived from a single lineage (Plyler et al. 2000), the pathogen has diversified in various regions of the world after being introduced through international germplasm movement and lack of quarantine (Feather et al. 1989; Krueger 2011; Sedra 2013). This diversity may have been caused by introduction of new races, wide dispersal of a single race, or adaptation of a local population to new environmental or host conditions. Diseases with symptoms similar to the wilt and dieback disease caused by *F. oxysporum*, and those of Bayoud, have been reported on *P. canariensis* from several countries (González-Pérez et al. 2004a, b).

Entomopathogenic fungi growing endophytically in *Phoenix* spp. modify plant defenses and may induce a primed state in date palm and eventually stimulate the tree growth (Gomez-Vidal et al. 2009). Primed plants were found to display endophytic colonization by entomopathogenic fungi which could induce a ‘‘primed’’ state in date palms. Primed plants display earlier and stronger activation of several cellular defense responses to biotic attacks and abiotic stresses. Such endophytes are candidates in biocontrol against date palm pests or pathogens.

4.6.3 Physiological Disorders

Occasionally, date palms show abnormal plant growth behavior with no indication of visible biotic stress (Table 4.4); it is therefore important to determine whether

Table 4.4 Partial list of physiological disorders and their causal agents

Name	Common symptoms	Causal agent	Remarks
Phytoplasma	Lethal yellowing	16SrIV group	
	Lethal decline	16SrIV-C	
	White tip dieback		Sudan
	Aster yellow	16Sr1 (Alwijam)	Arabia
	Stunning and yellow streaking of leaves	Candidatus phytoplasma	
		Cicadulina bipunctata (Milchar)	
	Root wilt disease	16SrXIV group	
	Slow decline	16SrXIV-A	
Texas Phoenix palm decline	Leaf yellowing	16SrIV-D	
	Flower abnormalities	Tissue culture (media and subcultures)	Several countries
	Bunch drop	Physiological causes	Israel

such disorder is a result of environmental conditions, genetic abnormalities, or some other biotic factors such as phytoplasma (Harrison et al. 2002). Broad physiological optima of the date palm will support the species tolerance, if not resistance, to stresses; however, physiological tolerances may change with life stage so that vulnerability may be linked to a critical time or may shift among factors during a tree's lifespan (Kittel 2012). In addition, several elements of its adaptive capacity may differ substantially for populations at the core of its distribution (i.e., Southern Mesopotamia and Arabia) as opposed to those at its northern (e.g., Spain) and southern (e.g., the Sind) distribution limits. Such populations may then have different responses to similar climatic change. Therefore, the species vulnerability may vary geographically with varying availability of suitable habitats, as well as with its genetically determined capacity to adjust to new local niches, which is location-specific (Kittel 2012).

Physiological disorders may include variegation of leaves, seedless fruit, broader leaves, abnormal spine structure, aborted embryos (e.g., no pollination in Barhee), bending stem, and compact growth (McCubbin et al. 2004). Punch drop, mainly in Medjool, is caused by a physiological disorder associated with rapid fruit stalk development and elongation. However, restraining the growth rate of the fruit stalk by reducing irrigation levels (up to 20 %) during the period of rapid fruit stalk elongation (of up to 5 cm per day; rendering it brittle) substantially reduces bunch drop levels (Cohen et al. 2010).

A few well-known phytoplasma-associated disorders are listed in Table 4.4. Several growth abnormalities or physiological disorders (e.g., crown bending, dwarfing, and terminal shoot bud death) were observed in a few tissue culture-derived date palms at the flowering stage (Suderhasan et al. 2013). Infestation by the greater date moth (*Arenepsis sabella*), followed by fungal infection on the wounds made by the insect larvae, has been implicated in these physiological disorders. These physiological disorders were noticed only in certain tissue-cultured date palm cultivars particularly Sultana, Suckary (Suderhasan 2013), and Barhee (Personal observations). Crosscut or V-cut (i.e., clean break in the tissues of the fruit stalk bases and on fronds) is a physiological disorder which consists of a slight to deep notch in the fruit stalk. This disorder results from an anatomical defect in the fruit stalks and fronds involving internal, sterile cavities leading to mechanical breaks during stalk or frond elongation. Fruits born on strands in line with the break shrivel and fail to reach full maturity.

When temperature falls below 0 °C, it causes serious metabolic disorders with some injury to date palm leaves characterized by a partial or total desiccation. Water of protoplasma freezes after released from the cells. During defrost, water invaded inter-cellular spaces, and the affected leaves turn brown and desiccated. The severity of damage is related to the intensity and duration of frost. Frost injury to the date palm groves may not cause a direct fruit loss; freezing produces loss of leaves so that the palm cannot support and mature the fruit crop in the following year (Cohen et al. 2004). Other physiological disorders caused by phytoplasma include bunch withering, crosscut, root wilt, lethal decline, lethal yellowing, Alwijam, white tip dieback, and slow decline (Eljuhani 2010). In addition, certain

physiological disorders, such as dwarfing, broader leaves with compact growth habit, twisted inflorescences, seedless fruits, and late flowering are commonly observed in tissue culture-derived date palm trees (Zabar and Borowy 2012).

Abnormal flower phenotypes may develop in older trees and include distortions of carpels and stigmas; fertilization will not be completed due to impaired pollen tube elongation before it reaches the ovary (Cohen et al. 2004). Other off-type phenotypes such as variegation, variation in leaf structure and in overall plant growth pattern, trees that do not form inflorescences, or trees that produce seedless parthenocarpic fruits, may develop in tissue culture-produced date palms; some of these phenotypes can only be detected either after planting in the field, or even following the juvenile growth stage such as the supernumerary carpels variant which was first implicated in the low fruit setting of tissue-cultured Barhee trees (Alghamdi 2001; Cohen et al. 2004). A similar abnormal phenotype was detected in Khallas, suggesting that both cultivars may be sensitive to tissue culture conditions than others (Gurevich et al. 2005). The similarities between 'Mantled' oil palm and 'Barhee' off-types, and the lack of detected genetic variation in both cases, suggest that an epigenetic mechanism expressed by an altered DNA methylation pattern may be responsible for the formation of the 'Barhee' off-type phenotype (Us-Camas et al. 2014). Average gene diversity, using AFLP, for Barhee propagated by offshoots was larger (0.78) than values obtained for commercially tissue-cultured Barhee (range from 0.00 to 0.57) (Gurevitch et al. 2005).

Somaclonal variations could be attributed to gene amplification, chromosomal irregularities, point mutation(s), or alteration(s) in DNA methylation (Saker et al. 2006; Sun et al. 2013). Moreover, in vitro culture environment may become mutagenic and trigger some of these mutations and the subsequent somaclonal variation. Apparently, the number of subcultures may determine percent plants showing somaclonal variations among trees derived from tissue culture. Genetic stability of tissue culture-derived date palm (e.g., Barhee) trees determines, to a large extent, its yield stability and overall yield potential (Zivdar et al. 2008).

4.7 Anthropogenic Stresses

The seemingly detached climate change and human activities (i.e., anthropogenic climate change) play different, but equally important, roles in oasis evolution at different temporal scales (Shabani et al. 2012); the first exerts continuous impact on oasis evolution and is manifested over large areas, whereas the second wields local and disconnected impact. Oases can be properly described as concentrations of natural resources scattered across desert vastness with sparse natural resources; however, some (e.g., in Sinai) are rather considered as human artifacts (Marx 1999); a point of view supported by ethnographic as well as historical accounts (Sagie et al. 2013). A desert location, without obvious prospects, can be transformed into an oasis if it serves a concealed strategic purpose of local or transient nomads (Fig. 4.6). On the contrary, a potentially fertile location may remain

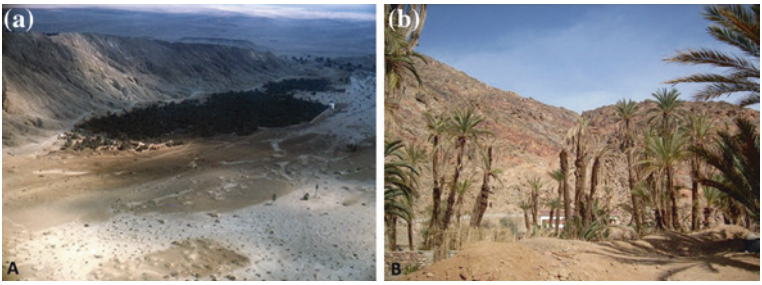

Fig. 4.6 Examples of permanent (**a**) and transient and declining (**b**) oases in the Sinai Peninsula (*Source* Google Earth Images)

neglected if unsuitable to the local nomads' interests, which may change over time, and therefore a seemingly thriving oasis may outlive its social usefulness and will be gradually abandoned (Marx 1999).

Although some of these oases survived for centuries, they should not be viewed as permanent geographical features, and others have emerged, disappeared, and shifted sites during a relatively short time. Under the harsh desert conditions (e.g., Sinai), farmers mitigate abiotic stresses (e.g., salinity) and declining production using local resources (e.g., sand and manure application); they may abandon vulnerable location altogether and establish date palms and other permanent and annual crops on a nearby, or a remote site (Marx 1999). Demographic, social, and economic factors in minor (de Grenade and Nabhan 2013a) and major date-producing countries (e.g., Iraq; Zabar and Borowy 2012) led, in a cascading fashion, to shortages in skilled labor, poor extension services, lack of inputs, vulnerable date palm populations, increased infestation of insects and diseases, decline in productivity, and degraded oases and plantations. Local and migrant labor shortages in some economies (e.g., oil-producing countries; Eljuhani 2010) contributed to a decline in date production and export revenues.

Anthropogenic drivers of climate change have multiple effects and threats on the oasis agroecosystem, including changes in agrobiodiversity, species composition, and ecosystem functioning; the long-term impact of single or multiple drivers depends on how fast they can decrease agrobiodiversity and restructure date palm populations. The age-old rigorous management of scarce water and land resources, in alliance with the date palm, culminated in the creation of desert and mountain oases across the vast deserts of the Middle East and North Africa (Floret et al. 1993; Aboragab 2010; Ashkinazi et al. 2012; Issar et al. 2012). The key to their sustainability was symbolized by human interactions that shaped oasis agroecosystems and enabled them to provide multiple socioeconomic and environmental services to meet the needs of local populations (King and Salem 2013). Perennial and annual crop species and cultivars have been carefully selected from natural or managed agroecosystems or from introductions over centuries of human selection and experimentation (Nabhan 2007; Ashkinazi et al. 2012; Issar et al. 2012). This diversity and its associated indigenous knowledge (see

Sect. 4.8.3) are indispensable assets for oases' inhabitants and constitute a strategic portfolio of livelihood options (Nixon 1951; Marx 1999).

Anthropogenic threats, particularly those acting as drivers of climate change, often have multiple effects, including changes in agrobiodiversity, species composition, and ecosystem functioning; the long-term impacts of these drivers depend on how fast they can decrease agrobiodiversity and restructure date palm populations (e.g., new monoculture plantations). Reduction in the within-species diversity of the date palm in response to economic pressure for higher productivity and concentration of the market on a few high-quality cultivars (e.g., Medjool in Morocco and Algeria, and Deglet Noor in Tunisia) due to consumer demand is spreading to other countries in large (e.g., Egypt; Elassar et al. 2005) or small date-producing countries (e.g., Jordan; Alkhashman et al. 2011). Marginalization of indigenous communities and the fast erosion of local cultures and indigenous knowledge are among the most important factors which influence peoples' livelihoods and sustainability of the oasis agroecosystems. For example, Deglet Noor occupies ~60 % of date palm plantations in Tunisia and Algeria, and continues to expand at the expense of other, less desirable, cultivars due to market forces. Likewise, traditional date palm propagation using offshoots of elite cultivars having desirable fruit quality traits resulted, even in the center of diversity of date palm, in genetic erosion and in the confinement of cultivars with distinctive fruit types to certain plantations (Battesti 2012). Traditional social water management system has been largely replaced by modern governing bodies with little or no coordination in some oases (e.g., Egypt, Morocco, Tunisia; Aboragab 2010; Ben Salem et al. 2011; Kraiem et al. 2012), while it persisted for millennia in others (e.g., Oman; Luedeling and Buerkert 2008; Genauer et al. 2009). The role of indigenous knowledge in managing crop, water, and land resources in a model mountain oasis agroecosystem is evident in maintaining high-quality irrigation water, elaborately built terraces, water distribution system designed to match crop needs during different growth stages, adequate drainage, and the lack of salinization (Luedeling and Buerkert 2008).

The massive propagation of elite date palm cultivars using tissue culture and potentially other mass propagation methods, at the expense of less popular, but genetically valuable cultivars, exacerbated vulnerability and genetic erosion of certain date palm cultivars (Jain 2011) and potentially exposes the massively tissue-propagated cultivars in monocultures to vulnerability threats imposed by biotic and abiotic stresses (Karim et al. 2010). Monoculture, especially of long-lived species such as the date palm, is a recipe for vulnerability to biotic and abiotic stresses (Karim et al. 2010) and the subsequent genetic erosion. Yet, selecting a small number of resistant cultivars in response to disease and insect stresses is a further threat to the diversity of the species. This may be even more damaging if the resistance to a particular disease or insect proved to be short lived because of a change in the virulence of the pest or changing climatic conditions (Fiaboe et al. 2012). Economic and social factors also impact the diversity of date palm; as a result, population composition as to the number of cultivars witnessed a sharp decline in recent years (Eljuhani 2010).

4.8 Socioeconomic Factors

Unlike in traditional oases, natural resources in new date palm plantations are primarily considered as objects of technical exploitation (Battesti 2012, 2013); new plantations are reduced to monocultures with the objective of producing high-quality dates for export as the only ecosystem service. Other ecosystem services are largely neglected or marginalized at best. The few remaining traditional social institutions, representing local communities, are charged with the oversight, control, and maintenance of common resources in the oasis agroecosystem (Koohafkan and Altieri 2011). These institutions derive their legitimacy and authority from increasingly eroding customary law and local norms (Hoole and Berks 2010; Faboyede et al. 2013; Sagie et al. 2013). Most agricultural products derived from the oasis are for family consumption, thus considered as guarantee of food security that is high in quality (Alfarsi and Lee 2008).

4.8.1 Land-Use Change, Vulnerability and Genetic Diversity

The magnitude and worth of ecosystem services in an oasis both react to spatiotemporal land-use changes (Sawut et al. 2013); therefore, farmers strive to maintain and fine-tune the delicate balance between number of date palms, other perennial trees, and land area allocated for field crops in some oases (Luedeling and Buerkert 2008). This balance is usually determined, if not totally dictated, by the long-term projection of reliable minimum water supply. In addition, the inherently low soil fertility of desert or semi-desert soils, where most new date palm plantations have been established, is compounded by many biological, ecological, and natural constraints (Goldman 1995; Ilahiane 1996; Khan and Pickett 2004; Li et al. 2013a; Chen et al. 2014) which represent ubiquitous challenges to the emerging date palm industry where a fine balance between inputs and outputs is needed to maintain long-term sustainability (Luedeling and Buerkert 2008). However, the water resource-dependent balance between sustainability and vulnerability, in most oases and new plantations, will be necessarily contingent on current and future trends in land-use change (Luedeling and Buerkert 2008; Fernald et al. 2014; Mamat et al. 2014).

Where irrigation indirectly depends on annual rainfall (McGregor et al. 2009; Ben Salem et al. 2011) or directly on surface or underground water resources (Battesti 2012; Alnaeem 2013), farmers mitigate recurring drought risks by allocating more land-use to annual crops that can be followed when water resources are not adequate beyond the long-term irrigation needs of date palms and other perennial fruit trees and crops (Luedeling and Buerkert 2008). Desert oases are increasingly subjected to threats of hyper-saline drainage water (e.g., Nefzaoua region in Tunisia) as a result of climate and land-use changes; and if soil types, topography, and irrigation and drainage methods are conducive (Kraiem et al. 2012).

Current and reliable information on land-use dynamics are crucial to provide necessary information to document salinity changes and may help predict and avoid future environmental degradation and loss of genetic diversity (Masoud and Koike 2006).

Land-use changes affect the wider environment and accelerate land degradation at different rates depending, among other factors, on physiography, land cover, and grazing intensity when livestock is integrated with date palm and other crop production in the oasis (Jabbar and Zhou 2013). As a powerful factor driving environmental change and ecosystem services, land use and land-use change need to be assessed simultaneously to better understand the interplay of relevant factors in sustainable land management, especially under the fragile oases (and plantations) agroecosystems (Lu et al. 2014). Besides its profound role in the availability and delivery of ecosystem services, land-use change is an important player driving environmental change in these agroecosystems (Boerma and Koohafkan 2008; Gill et al. 2014). Land allocation for the establishment of new date palm plantations, at the expense of natural and semi-natural ecosystems and their ecosystem services, represents a prominent land-use change during the past few decades in several Middle Eastern and North African countries. This change was driven, for the most part, by socioeconomic and market forces (Heidecke and Heckelei 2010; King and Salem 2013) and usually brought about some beneficial, but short-term, socioeconomic returns for local communities; however, it has also resulted in increased water consumption and significant risk of soil loss and degradation; the long-term impact of which on oasis agrobiodiversity and date palm genetic diversity cannot be overstated (FAO/IPGRI 2002; Diaz et al. 2003; Maxted and Guarino 2006; Hammer and Teklu 2008; van de Wouw et al. 2009).

In arid environments, where most oases have been developed and new date palm plantations are being established, water as resource and a supporting ecosystem service is usually limited; therefore, land-use decision-makers may need to incorporate trade-offs among land-use objectives, interactions among ecosystem services, and complex human–environment interactions to make the land management more adaptive across various spatiotemporal scales, especially in the case of new date palm plantations (Lu et al. 2014); consequently, changes in water resource allocation in response to land-use changes will result in far-reaching consequences on the long-term sustainability of oases and plantations as complex socioecological systems (Luedeling and Buerkert 2008; Battesti 2012; Baker et al. 2013; Mekki et al. 2013).

Water-related environmental degradation problems, within the context of the oasis, have been manifested, for example, in dieback of natural vegetation, surface runoff, groundwater depletion and pollution, desertification, secondary salinization caused by excessive irrigation, and agricultural non-point source water pollution. In extreme cases (Zabar and Borowy 2012), land-use change resulted in increased soil erosion, sand drift, and the development of new sand dunes, leading to desertification in the environs of oases and new plantations; these outcomes of land-use change are probable causes of date palm vulnerability and oases degradation.

4.8.2 Consumer Preferences: Impact on Genetic Diversity

The large genetic diversity in date palm populations and cultivars normally reported at the morphological (Elhoumaizi et al. 2002; Bodian et al. 2012; Ezebilo et al. 2013), biochemical (Jaradat 2014c), molecular levels (Adawy et al. 2004; Elassar et al. 2005; Elshibli and Korpelainen 2009; Karim et al. 2010), and fruit quality (Jaradat and Zaid 2004; Alobeed 2010) may be interpreted by some as if the date palm is neither perceptibly, nor probably, or even possibly vulnerable or threatened by genetic erosion. However, due to part to consumer preferences and market liberalization, the genetic richness of the date palm is threatened by genetic erosion which can be attributed to the wide adoption of a few elite cultivars to the exclusion of others. These elite cultivars produce the majority (~75 %) of commercially desirable fruit quality mostly destined for the expanding export market (Hamza et al. 2012). This situation is aggravated by the risk of biotic stresses; it constitutes a threat to agrobiodiversity and affects the livelihood of resource-poor farmers who depend on oasis farming as the main source of income.

The recent market liberalization for export of fresh or processed dates encouraged farmers to replace old cultivars in their oases, or start new plantations using elite cultivars and modern management practices and intensive inputs (Mekki et al. 2013); as a result, several local cultivars have been abandoned or disappeared altogether. Farmers may give up, or totally abandon growing locally adapted cultivars, particularly when a financially more attractive alternative market becomes available (Bediab et al. 1993; Goldman 1995; Elhoumaizi et al. 2002; Alobeed 2010). This is particularly important when isolated and remote oases, where unique date palm cultivars persisted for millennia, become connected with larger, local or export markets and economic centers by modern transportation systems (Nabhan 2007; Nabhan et al. 2010). This process plays an extremely important role in land-use change and the loss of valuable genetic resources and genetic diversity when traditional cultivars and management practices become irrelevant and unsustainable (Baker et al. 2013; Ezebilo et al. 2013).

Government policies in some countries (e.g., Tunisia) accelerated the loss of genetic diversity of old date palm cultivars (Omrani and Ouessar 2011) by encouraging and subsidizing the development of intensive management and irrigation systems to meet the growing demand of export markets for high-quality dates. In order to compensate for the dominance of a few elite cultivars in oases and in the market place, the collection, characterization, and evaluation of “common”, “traditional”, and “less desirable” cultivars is an important objective for their future use as genetic resources and sources of tolerance or resistance to biotic and abiotic stresses (Atkinson and Urwin 2012; Chen and Chen 2013).

4.8.3 Indigenous Knowledge of Agrobiodiversity and Genetic Diversity

Oases dwellers, as individuals, families, or clans, inherited, enriched, and passed-on a captivating body of indigenous knowledge in the diverse and complex fields of soil and water management, date palm, and other crops' husbandry, mitigation, and adaptation to biotic and abiotic stresses, sustainably extracting ecosystem services, and in balancing numerous and competing production objectives within delicately balanced and typically fragile agroecosystems (Boerma and Koohafkan 2008). Otherwise, these people, spatiotemporally, embraced, and advanced complex agroecosystems characterized by agronomic, ecological, economic, social, cultural, and political dimensions (Gill et al. 2014) where oases were molded as repositories of agrobiodiversity bounded by constraining and harsh environments. A contrasting image is demonstrated by farmers in marginal date palm-growing regions (e.g., Punjab in Pakistan; Ata et al. 2012; and Baha California in Mexico; Rouston 2012; de Grenade and Nabhan 2013a, b) who only have fundamental, if not rudimentary, level of knowledge about the production technologies of date palm, such as cultivar selection and propagation, irrigation and fertility management, and integrated pest management (Ata et al. 2012; Vinatier et al. 2012; Li et al. 2013a).

Oases established on the fringes of the major date palm production center (e.g., Baja California in Mexico; mountain oases in Oman), cumulatively harbor unique crop assemblages, and indigenous knowledge and practices that essentially differ from oases in the Old World. Potentially, such oases can be designated as sites of informal and formal in situ conservation of farmer-bred genotypes and traditional ecological knowledge of unique enough value that it may interest international conservation programs and agencies (de Grenade and Nabhan 2013b). A recent report (Rouston 2012) indicated that the date palm culture in Baja California is declining due to shortage in skilled horticulturists and laborers. However, people still harvest the fruit from largely unattended date palm trees to sell at local or regional markets, though the quality of the dates is highly variable and limits their market value.

Local organizations, such as farmer associations and cooperatives, are often able to manage oases natural resources more effectively, efficiently, and democratically than "officials" appointed by central governments who may have no practical knowledge of natural resources management and use (de Jong et al. 2005; de Fraiture and Wichelns 2008; Bai et al. 2014). For example, the French took… "an interest in the cultivation of the date palm in the Saharan region and so we have seen mainly in the south of Constantine, Algeria, the birth of many magnificent new oases, established from scratch right in the middle of a desert region" (Battesti 2012). In so doing, the state officials attached little or no value to the indigenous knowledge and ambitions of oasis dwellers (Kendocia et al. 2013); over the years, they replaced natural springs by mechanically drilled deep wells as sources of irrigation water, and introduced high external inputs for date production

with serious loss of indigenous knowledge and everlasting impacts on livelihoods, traditional management practices, date palm agrobiodiversity, and the local environment (Battesti 2012).

The need for documenting indigenous knowledge and values associated with life in the oases has become urgent as a result of the cascading effect of water scarcity leading gradual to loss of diversity, changes in oasis agroecosystems, and weakening of social institutions (Boerma and Koohafkan 2008; Elmodafar 2010; Misra 2013). Systematic and comprehensive documentation of indigenous knowledge on date palm and proper functioning of the oasis agroecosystem, cultivar identification, assessment of water and soil resources, and identification and prioritization of land-use options are integral components of the proposed documentation process. A fundamental question, however, is how to gather, document, and use this knowledge to reduce date palm vulnerability to biotic and abiotic stresses, and therefore, advance a sustainable rural economy and improve living conditions in the oases?

4.9 Combating Vulnerability

Vulnerability to changing environments is mainly determined by the genetic composition and genetic diversity of date palm populations as the underlying basis for adaptive capacity (Nefzaoui et al. 2012; Kittel 2012). However, phenotypic plasticity can modify adaptive capacity under a rapidly changing climate without relying on genetic diversity (FAO/IPGRI 2002; Chen et al. 2014; Jaradat 2014b). Nevertheless, both abiotic and biotic constraints on date palm populations tend to affect their adaptive capacity differently at the peripheries of date palm distribution (i.e., southernmost and northernmost distributional ranges) and at peripheries versus in their core populations in the Middle East and North Africa, thus applying different selective pressures and prompting environmentally determined genetic differentiation (Kittel 2012; Pauls et al. 2013).

Population response to abiotic stresses and internal dynamics in *Phoenix* spp. shape the species' response to climate change (Bai et al. 2014; Banga and Kang 2014). Climate changes to the physical environment in oases or plantations, and the ensuing shifting abiotic and biotic conditions may disrupt the integrity of these ecosystems differentially (Dixon 2012; Gill et al. 2014); such disruptions, and subsequent vulnerabilities, may initially impact primary producers, microbial decomposers, and energy and nutrient flow within the oasis or plantation agroecosystem (Elhassni et al. 2007; Oliver and Morecroft 2014).

The pollination systems of *Phoenix* spp. are highly diverse (e.g., hand, wind, and insect pollination; Meekijjaroenroj and Anstett 2003). Many *Phoenix* spp. are associated with specific pollinating weevils such as *Neoderelomus piriformis* which is associated with *P. canariensis*; along with other beetles they may be used as efficient pollinators of date palm and to generate progenies with wide genetic diversity. Patterns of pollen dispersal and estimates of genetic parameters of an

endemic species (e.g., *P. canariensis*) may prove useful for genetic conservation (Saro et al. 2014). The genetic structure of the effective airborne pollen cloud and the usually large variation in correlated paternity rates among maternal families in this (and similar) species call for large numbers of seed samples for ex situ conservation in order to minimize potential future vulnerabilities of collections with narrow genetic base (Francisco-Ortega et al. 2000; FAO/IPGRI 2002; Diulgheroff 2006; Chen et al. 2014). On the basis of observed spatial genetic structure and estimated average dispersion distance of the pollen cloud (Francisco-Ortega et al. 2000), the observed spatial genetic structure among adult palms and the estimated average pollination distance suggest a minimum separation distance of 70 m among mother trees for seed collection in order to maximize diversity among maternal families. The likelihood of interspecific genetic introgression through pollen flow from allochthonous *Phoenix spp*. on the basis of documented significant intraspecific pollen dispersal into isolated *P. canariensis* population (Saro et al. 2014) cannot be overlooked and should be taken into consideration in studies of spatial genetic structure.

Technical as well as socioeconomic considerations have to be addressed by the climate change community in dealing with biotic stresses in order to assess future vulnerabilities at local, regional, and global levels, and to inform farmers and policymakers accordingly (Suthers et al. 2000, 2007). Expert opinion, modeling of climatic responses, and comprehensive process-based simulation models are among the tools available for global change risk assessment and management, and therefore are of great value to assess vulnerabilities of *P. dactylifera* and related species (FAO/IPGRI 2002; Shapcott et al. 2009; Kittel 2012).

4.9.1 Recoupling Social and Ecological Systems

An oasis embodies a set of linked social–ecological systems that are dynamic and change continuously in response to internal or external stresses (Schlüter et al. 2014) and usually affect the delicate equilibrium in traditional oases agroecosystems (Boerma and Koohafkan 2008; Sagie et al. 2013; Gill et al. 2014). Moreover, interactions between these social–ecological factors govern the influence of global change factors on agrobiodiversity and genetic diversity within and among oases (Zimmerer 2010; Power 2010; Mace et al. 2012; Misra 2013). However, recent technical and socioeconomic developments have introduced critical changes in these traditional agroecosystems, especially in the composition, management, and scale of resource allocation for date palm production (Boerma and Koohafkan 2008; Koohafkan and Altieri 2011). Additionally, these developments placed increasing pressure on people's livelihoods and on the proper functioning of oasis agroecosystems (Gill et al. 2014). When viewed in the context of global change, a number of powerful social–environmental interactions are creating complex couplings in the current use and future fate of the biological diversity and genetic diversity within and among oases (Zimmerer 2010); these include economic

developmental strategies; market integration; changes in sociocultural, land-use, and technological domains, as well as the role of global food and environmental policies and institutions (de Fraiture and Wichelns 2008; Dixon 2012; Galhena et al. 2013). If the use of external inputs and the planting of elite and modern cultivars are inversely related to cultivating indigenous cultivars and crop species, then the advent of faster and easier transportation and access to local, regional, or global markets may have contributed to, or accelerated, the genetic erosion process of old date palm cultivars and the loss of agrobiodiversity from desert oases (Nabhan 2007; Nabhan et al. 2010). However, the persistence and relative stability of old date palm cultivars and traditional management practices, but not annual crops and some other fruit trees, in old (e.g., Siwa oasis in Northwest Egypt; Nabhan 2007; Elche, Spain; Rivera et al. 2008) and relatively new oases (Baja California oases in Mexico; de Grenade and Nabhan 2013a, b) are the exceptions.

Part of the emerging vulnerabilities, loss of agrobiodiversity, and erosion of genetic diversity in traditional oases can be attributed to decoupling of people from their local environment (Hoole and Berkes 2010). Monocultures in date palm plantations, considered as potential cause of genetic erosion, represent another dimension of the same phenomenon (Battesti 2012). Therefore, memory mapping and interviews within and outside traditional oases may help reveal the age-old insightful wisdom of lost traditions and a strong desire to return to the old way of life, not to exploit agroecosystems but to restore cultural practices and acquire certain ecosystem services (Hoole and Berkes 2010). An assessment of agrobiodiversity and genetic diversity, on the basis of early and current records using time-series techniques, is an informative quantitative method to estimate the relative efficiency of in situ conservation in the face of vulnerability and genetic erosion over time (Nabhan 2007).

Indicators for assessing the conservation and sustainable use of non-renewable desert resources, which support oases agricultural and economic development, will lead to further improvements in evaluating long-term effects of constraints and incentives on the management of deep fossil aquifers (Kraiem et al. 2012) and on long-term objectives to conserve agrobiodiversity and genetic diversity (Gebauer et al. 2009; Allam and Cheloufi 2013). The often divided natural and human systems can be envisioned as a single, coupled social–ecological system (Schlüter et al. 2014) where two-way feedback interactions among subsystems highlight the interdependent and coevolutionary nature of such interactions. This was evident in the case of social and cultural disconnect between farmers and extension agents, especially in marginal date palm-growing regions of the world (Ata et al. 2012), where a widening knowledge-gap about modern management techniques exacerbated and compounded the impact of biotic and abiotic stresses on small-scale, traditional date palm groves and gardens (Boerma and Koohafkan 2008; Elmodafar 2010; Misra 2013).

An integrated assessment of public policies designed for natural resources management can be achieved at the oasis level by incorporating biophysical and socioeconomic aspects of date palm production process (Mekke et al. 2013). Public policies often failed in managing regional or oasis-specific water resources issues

due to lack of consideration for individual initiatives and to the long-term uncertainties of such policies (Omrani and Ouessar 2011; Battesti 2012); under such circumstances, more often than not farmers' practices have been driven by economic rather than cultural factors.

4.9.2 Germplasm Enhancement

Future climate change is expected to adversely affect the survival, distribution, adaptation, and sustainable productions of the date palm whether in traditional oases or in plantation agroecosystems (Shabani and Kumar 2012; Shabani et al. 2013a, b). Population dynamics determine the species vulnerability to climate change and the ensuing biotic and abiotic stresses (Kittel 2012). Direct impact of climate change on reproduction (e.g., production of viable and numerous offshoots, pollen grain viability and fecundity) and mortality rates (e.g., due to insect and disease attacks) alter population viability across a range of timescales (e.g., fertilization, embryogenesis, seedlings, and mature trees), and may have different effects on male and female trees (Meekijjaroenroj and Anstett 2003).

Therefore, germplasm enhancement and proper management and utilization of current genetic diversity and germplasm resources become urgent for the development and improvement of date palm cultivars (Jain 2011). Potential advantages of clonal reproduction of date palm as revealed by the relationship between its mating system, growth form, and genotypic diversity, include facilitated resource uptake in heterogeneous environments, persistence under suboptimal environmental conditions and increased attraction of pollinators by increased floral display (Honnay and Jacquemyn 2008). However, this strategy may incur fitness (i.e., fruit yield) costs, which are associated with the effects of large clonal individuals and floral displays, patterns of pollen dispersal (*excluding artificial pollination*), and the rate of sexual reproduction (i.e., offshoot production).

Breeding of fruit tree species, such as the date palm, is challenging because of their longevity, alternate reproductive methods, and breeding system with separate male and female plants (Khanam et al. 2012). The discovery and valuation of genetic variation among cultivars, through germplasm enhancement, are prerequisites for the development of improved date palm cultivars; molecular analyses' techniques have been instrumental in cultivar identification, probing genetic diversity and exploring phylogenetic relationships; when followed by marker-assisted selection, molecular techniques can optimize the breeding and cultivar production process. However, the Old World nomenclature norms produced a plethora of local vernacular names for the same cultivar, or in one "descriptive" name for many cultivars in several oases or even countries (Bendiab et al. 1993; Bodian et al. 2012; Jaradat 2011). This phenomenon complicates the processes of selecting germplasm from the highly disputed number (1500 to >5000) of "named" cultivars around the world (Battesti 2013) for genetic enhancement and for subsequent phenotypic and genotypic analyses, and may render the process inefficient

and incomprehensive (FAO/IPGRI 2002; Maxted and Guarino 2006). Therefore, extensive characterization, evaluation, and documentation of date palm genetic resources are prerequisites for its rational, sustainable, and dynamic utilization. Two of the most important steps in germplasm enhancement are characterization (i.e., assessment of the presence, absence, or intensity of a specific phenotypic trait the expression of which is little or not influenced by varying environmental conditions) and evaluation (i.e., assessment of plants for potentially useful genetic traits, many of which may be environmentally variable such as insect or disease resistance, fruit quality, flavor).

Farmers, if economically motivated, may actively participate in the enhancement and conservation of date palm (and other crops) genetic resources. Intercultivar diversity (e.g., for maturity dates, fruit types, physiological thresholds) can help lower the risk of total failure due to natural (biotic and abiotic) disasters; farmers may have a greater incentive to grow many and diverse cultivars if they appreciate the long-term benefits of on-farm diversity (Jaradat 2011). Also, farmers may consider a trade-off between extra income from monocultures and environmentally adaptable date palm cultivars capable of producing steady and reliable annual yield (Baker et al. 2013; Ezebilo et al. 2013; Mekki et al. 2013). Farmers who grow date palm for family consumption often appreciate dates with different tastes and are likely to grow different cultivars to meet their own demand (Jaradat and Zaid 2004; Alobeed 2010). In general, the more the diversity of date palm cultivars in a family garden or a grove, the smaller the unit yield value in the market place (Hamza et al. 2012); however, family size may dictate the number of cultivars grown by farmers, and therefore, their role in germplasm enhancement, conservation, and utilization.

Traditional and non-elite date palm cultivars may have genes or gene complexes of potential use in meeting future breeding and improvement needs and challenges (Bodian et al. 2012; Dansi et al. 2013; Elshibli and Korpelainen 2008, 2009; Jaradat 2014b), but their presence is largely unknown. Therefore, traditional farmers should be encouraged or even subsidized to (re)plant their orchards and home gardens with locally produced, highly heterozygous, and heterogeneous offshoots or seedlings (Ledig 1986). Replacement of old, non-productive, or dead trees with only elite and mostly non-adapted cultivars (e.g., in new plantations in Egypt, Tunisia, and Sudan; Elassar et al. 2005; Hamza et al. 2009; Elshibli and Korpelainen 2008, 2009), will diminish genetic diversity and hasten genetic erosion of locally adapted cultivars.

Relatively low polymorphism and lack of apparent groupings among date palm cultivars reported in several studies and from geographically diverse countries (e.g., Iraq, Tunisia, and Morocco) can be attributed, at least in part, to historical event(s) during the early years of germplasm introduction(s) from the center of diversity in Arabia (Zohary and Hopf 2000; Zohary 2004; Battesti 2013; Sedra 2013); supposedly, those introductions had narrow genetic base and, for millennia, received more or less uniform traditional management practices (Brown et al. 2013; Ben Salem et al. 2011; Tripler et al. 2011; Bai et al. 2014). Apparently, the spatiotemporal and strong selection for fruit quality attributes may have targeted

a small portion of the date palm genome leaving the largest portion under a slow and mild natural selection. Throughout the history of farmer-led improvement, date palm opportunistic seedlings, known as khalts or "mixtures of genotypes," have been singled out and valued as sources of genetic diversity, especially for qualitative traits including fruit quality, adaptation to innovative management practices, and changing climates (Sedra 2013). Due to their large genetic diversity for many qualitative and quantitative traits, seedling populations comprise a valuable genetic resource for the date palm improvement (Majourhata et al. 2002; Johnsn et al. 2013) and to reverse the narrowing genetic base of the species (Brown et al. 2013).

A few interspecific barriers in the genus *Phoenix* (Chevalier 1952; González-Pérez et al. 2004a, b; Gros-Balthazard 2013) allow for the production of a wide range of natural or artificial hybrids, such as those between the dwarf *P. roebelenii* and the imposing *P. canariensis*; other hybrids (e.g., with *P. pusilla*) are infertile because seed development is arrested at the khalal stage. Spontaneous gene flow, or deliberately done through artificial pollination, between sympatric *Phoenix* spp. populations (Gros-Balthazard 2013) give rise to swarms of hybrids. Farmers recognized the horticultural worth of some of these hybrids as potential elite cultivars and to widen the genetic diversity of the cultivated species. Moreover, the metaxenic effect of pollen from "exotic" male sources may improve the fruit quality (Alkhalifah 2006; Johnson et al. 2013). Putative hybrids between date palm and *P. canariensis*, such as *P. macrocarpa* and *P. intermedia*, represent introgressions that make it difficult to delimit species boundaries (Dransfield et al. 2008); even temporal interspecific barriers can be circumvented by storing the pollen for months. Genes or gene complexes of potential use in germplasm enhancement and in meeting future challenges may well be present in old date palm cultivars, seedling populations, wild relatives, and other *Phoenix* spp. For example, the genetic distinctiveness of *P. atlantica*, coupled with the presence of unique alleles and extensive infra-specific genetic variation suggest that a significant period must have elapsed since *P. atlantica* diverged from date palm; however, this should be seen in the context of a sound phylogenetic analysis of *Phoenix* spp. followed by a more comprehensive population sampling of *P. dactylifera* from North Africa and its "presumed" wild populations (Henderson et al. 2006). Recent findings (González-Pérez et al. 2004a, b) indicated that the genetic discontinuities based on molecular analyses support the recognition of *P. atlantica* as a distinct species; it has been isolated for a long time with no gene flow despite little or no morphological differentiation from its mainland relative, *P. dactylifera*. Nevertheless, *P. atlantica* may provide unique genes to the date palm industry, which is experiencing a narrowing genetic base, to develop Bayoud-resistant cultivars, currently a devastating disease in North Africa (Henderson et al. 2006).

The diversity in reproductive phenological cycles of the large number of cultivars and seedlings planted in the same or different oases constitute valuable genetic resources for traits associated with extended harvest season and fruit quality traits (Alyahyai and alkhanjari 2008). The marginal date palm populations in Elche in Spain, with presumed North African origin (González-Pérez et al. 2004a, b;

Rivera et al. 2008) display extremely high levels of diversity for phenotypic traits, including trunk morphology, fruit traits, leaf shape, and leaf color. The minor local types add to the agrobiodiversity and genetic diversity of date palm in this region. These include germplasm of *P. iberica*, a wild species from the valleys near the Mediterranean; it has glaucous leaves, stout stems, and small dates with thin flesh. This minor species displays vegetative characteristics similar to those of the cultivars Medjool and Barhee; however, the fruits are intermediate between those of *P. theophrasti* and *P. sylvestris*. A group of cultivars well known for their green leaves and small fruits, which normally ripen under the marginal climate of Elche, has been described as *P. chevalierii* (Rivera et al. 2008). The dates in the Baja California (de Grenade and Nabhan 2013a, b) represent a wide range of fruit and seed types and may be of value for germplasm enhancement; these include large or small, large fat seeds, long narrow seeds; red, yellow, dark, black, caramel colored fruit; and sweet and astringent fruit. However, although most of them are inferior to the commercial date cultivars reproduced as clones from a few seedling-derived cultivars, male genotypes may offer a genetic resource to assess metaxenic effects (Alkhalifah 2006) and could lead to the selection of male cultivars specific to the pollination of given female cultivars, improved yield, larger fruit size, and even the production of seedless dates.

4.9.3 Push–Pull Strategy to Combat Biotic Stresses

The development of a reliable, robust, and sustainable push–pull strategy for insect control (e.g., red palm weevil) requires a thorough scientific understanding of the insect biology and the behavioral and chemical ecology of its interaction with the date palm and other potential host(s), conspecifics, and natural enemies, if any (Vinatier et al. 2012). The specific combination of components differs based on the specificity, sensory abilities, and mobility of the insect different biotypes (Faleiro 2006).

Behavioral manipulation is through baits, repellants, or both, as components of the push–pull strategy, where the source stimuli can be masked by applying natural or synthetic analogs in sufficiently high concentrations and over a broad area to effectively prevent the insect from finding the target resource (e.g., the date palm or the fruit; Khan and Pickett 2004). The insect is "pushed" or repelled away from the target by stimuli that can mask the "chemical appearance" of the host; at the same time, it is "pulled" toward traps (or trap crops) using strong stimuli where it is controlled using appropriate pesticides (Khan and Picket 2004). The repellent(s) and attractive stimuli can be deployed at tandem to maximize the effects of this strategy. This strategy is a fundamental part of the integrated pest management system which attempts to reduce the damaging effects of disease and insect populations that become pests through detrimental abundance. Virulence or sudden surges, either of endemic or exotic insects and diseases, have caused major unexpected crop failures and losses (Banga and Kang

2014). Such losses have often resulted in the long-term decline or disappearance of affected crops that have long been components of a region's farming system (Dixon 2012).

The danger from insect migration (e.g., the widespread of red palm weevil through offshoots from the Southeast Asia to the Middle East is a case in point) has grown steadily due to dramatic increase of movement of people and goods, and due to lax quarantine and legislative measures in developing countries. Tissue culture, coupled with biotechnological detection methods (e.g., acoustic devices) may help avoid further spread of the insect. A most successful push–pull strategy is one that undertakes a holistic approach in exploiting chemical ecology and agro-biodiversity in a particular oasis, can rely on technologies appropriate to the oasis agroecosystem, and can be easily adopted by farmers at a regional scale (Sakar et al. 2000; Gurevich et al. 2005; Jain 2007).

4.10 Challenging Threats of Genetic Erosion

Genetic erosion of date palm germplasm, defined as total loss of the crop, cultivar, or allele (van de Wouw et al. 2009), ignores the dynamic nature of the oasis agroecosystem and population genetic processes by considering what has been lost, and not what it was replaced with. On the other hand, a reduction in the total number of crops, cultivars, or alleles in the oasis is a better indicator for genetic erosion and is reflected on a reduction in richness (FAO/IPGR 2002; Maxted and Guarino 2006), which, incidentally, can be compensated for by new and additional genetic diversity within the oasis agroecosystem (Moonen and Bareri 2008). In this case, richness might only poorly reflect increased levels of genetic uniformity because rare cultivars or alleles contribute to genetic diversity as much as their common counterparts (Pinaud 2010). The intensity of documentation (whether based on actual field surveys or farmer interviews) will determine the level of richness found within and among oases (Nabhan 2007; Battesti 2013). On the other hand, a reduction in evenness can be considered an indication of genetic erosion, whether based on diversity indices used in vegetation ecology (e.g., Shannon's diversity index), or those used in population genetics (e.g., Nei's gene diversity index).

Unlike reduction in richness, increased dominance of one or a few crop species, genotypes or alleles will cause a marked reduction in evenness (Maxted and Guarino 2006; van de Wouw et al. 2009); therefore, rare alleles contribute little to this reduction in diversity although it is known that the risk of losing alleles (or even cultivars) is higher when allelic distributions are skewed. Nevertheless, clonal propagation, using offshoots or tissue culture, maintains heterozygosity and genetic purity, particularly of female date palm cultivars; however, it promotes genetic uniformity, may accelerate genetic erosion or enhance vulnerability to biotic and abiotic stresses, including environmental stresses that may be triggered by future climate change (IPCC 2007). Therefore, the maintenance, if not enhancement, of genetic variation within and among oases (and modern

plantations) remains a central objective in the production of genetically diverse populations of date palm.

The recently (~300 years ago) introduced *P. dactylifera* into the Baja California peninsula (de Grenade and Nabhan 2013a, b) became a keystone species, and in many cases it grows along with or already replaced the native fan palms (*Washingtonia filifera* and *W. robusta*). In these, and similar situations (e.g., *P. atlantica* in Morocco and *P. sylvestris* in the Indian subcontinent), the introduction of *P. dactylifera* may become a serious threat to the genetic integrity and conservation of endemic *Phoenix* species; the latter may become at risk from genetic introgression or from cross-breeding depression (González-Pérez et al. 2004a, b). In addition, extreme cases of invasiveness are increasingly becoming an issue of great global concern (Holmquist et al. 2011; Forsman 2014), especially in light of extensive human movement and trade globalization of date palm germplasm. A similar situation is encountered at Elche, southern Spain, where the date palm populations, probably established by the Phoenicians some 3000 years ago, are considered as representatives of authentic *P. dactylifera* and *P. canariensis* (González-Pérez et al. 2004a, b). The genetic constitution of *P. canariensis* seems to be a subset of that found in *P. dactylifera* (Gros-Balthazard 2013), and the large genetic similarity between both species strongly suggest that *P. canariensis* is recently derived from a common ancestor closely related to date palm. Although both species can be distinguished from each other at the molecular and biochemical levels, swarms of mixed populations, genotypically resemble *P. canariensis* thus threatening the genetic integrity and future degradation of genetic diversity of *P. canariensis*.

Molecular evidence of hybridization between the endemic *P. canariensis* and the widespread *P. dactylifera* in the Canary Islands (Fig. 4.7) was detected using DNA markers (González-Pérez et al. 2004a); this gene flow may put the endemic species at risk if hybrid progeny and progeny from advanced hybridization are vigorous and fertile; or the common species may become at risk if the hybrid progenies are sterile or have reduced vigor (Gros-Balthazard 2013; Saro et al. 2014).

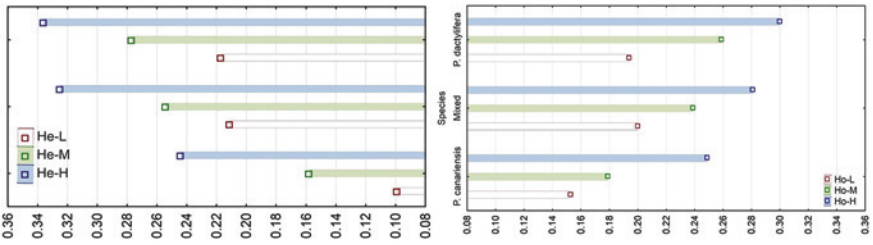

Fig. 4.7 Low (L), medium (M), and high (H) estimates of observed (Ho) and expected (He) heterozygosity based on allozyme variation in pure genotypes of *P. canariensis*, and *P. dactylifera* and in a mixed population of both species. Estimates of observed and expected heterozygosity for *P. dactylifera* were always larger than those of *P. canariensis*; whereas estimates for the mixed populations were intermediate (Based on data compiled from González-Pérez et al. 2004b)

4.10.1 Quantitative Indicators and Measures

Compared to other perennial fruit trees and their wild relatives, a relatively small part of the total genetic diversity in *Phoenix* spp., including that of *P. dactylifera*, has been characterized, evaluated, and used for date palm breeding and improvement (Glilcan 1997; Johnson et al. 2013). However, recently discovered polymorphisms (Aldous et al. 2011) and molecular markers (Billotte et al. 2004) will be available for cultivar identification, pedigree analysis, germplasm diversity assessment, and genetic mapping studies. Molecular markers, compared with phenotypic or biochemical markers, are more precise and can accurately identify cultivars and quantify their genetic diversity and phylogenic relationships (Diaz et al. 2003; Khanam et al. 2012); these markers have been extensively used to study the genetic variation of date palm cultivars (Table 4.5; Fig. 4.8). These include randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellite markers, restriction fragment length

Table 4.5 Characteristics of molecular markers used in date palm genetic diversity studies (Compiled from several references)

Characteristic	AFLP	RAPD	RFLP	iSSR	SSR
	Dominant	Dominant	Codominant	Dominant	Codominant
Polymorphism	Medium	Low	Low	High	High
Template quality	Low	Low	High	Medium	Low
Cost	Medium	Low	High	Medium	Medium
Skill needed	Medium	Low	Medium	Medium	Low
Reliability	Medium	Low	High	Medium	High

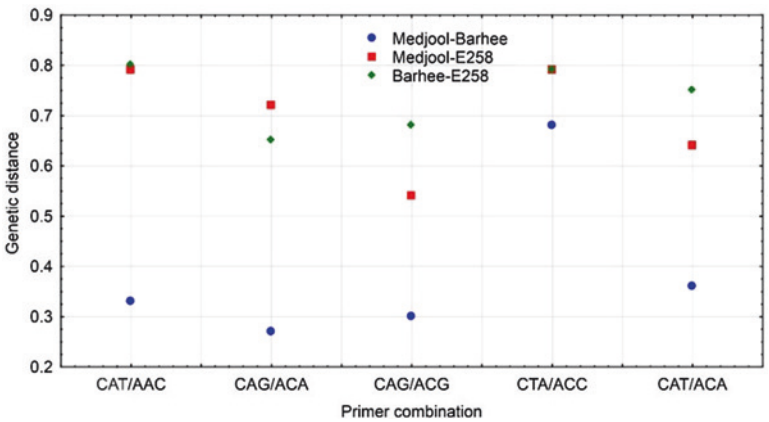

Fig. 4.8 Genetic distances between two date palm cultivars (Medjool and Barhee) and a hybrid E-258 based on five primer combinations. Medjool and Barhee were closer to each other (except when CTA/ACC) than to the hybrid, although each cultivar originated in geographically distant part of date palm center of origin (Based on data from Diaz et al. 2003)

polymorphisms (RFLP), inter simple sequence repeat (iSSR), and simple sequence repeat (SSR). Each method has its advantages and disadvantages and limitations; however, nuclear microsatellites or SSR seem to fulfill most requirements for an accurate analysis of date palm diversity and phylogeny (Fig. 4.9). A number of newly isolated microsatellite markers are expected to provide a valuable and highly informative resource for genetic mapping and diversity analysis in the species (Cornique and Mercier 1994; Akkak et al. 2009; Aldous et al. 2011; Racchi et al. 2013).

The relative magnitude of gene differentiation which is a measure of population differentiation among subpopulations, whether based on phenotypic (G_{ST}) or molecular (F_{ST}) markers, depends on total genetic variation (H_T); if the latter is small, then G_{ST} or F_{ST} may become large even if the absolute gene differentiation is small (Jaradat 2014c, in press). In addition, a clear distinction between molecular markers representing sex-linked and those representing autosomal-linked chromosomes stems from different relationships between population differentiation based on molecular markers and each of heterozygosity and fixation index; the larger variation in the fixation index F_{ST} (which measures the amount of genetic variance that can be explained by the population structure) when F_{IS} and the differences between observed (*Ho*) and expected (*He*) heterozygosity were relatively small (Jaradat 2014b, c, in press). Remarkably, negative fixation indices have been reported for date palm populations from Libya (Racchi et al. 2013) and Sudan (Elshibli and Korpelainen 2009) on the basis of quantitative molecular markers, and were attributed to excess heterozygosity in these populations.

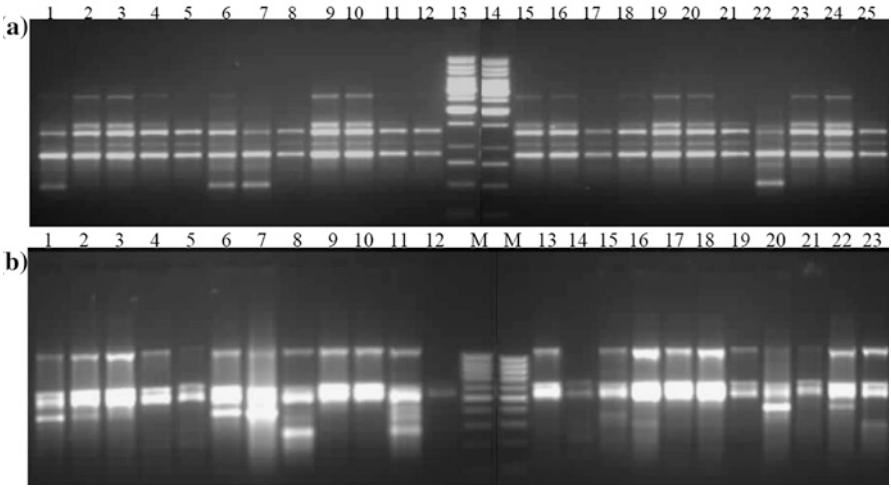

Fig. 4.9 Genetic diversity as revealed by RAPD (**a** lanes 13 and 14 represent standard 1-Kb DNA ladder) and ISSR banding profiles (**b** lanes M and M represent standard 1-Kb DNA ladder) for five male and 18 female date palm cultivars from eight Middle Eastern countries (See Fig. 4.10 for cultivar names) (*Courtsey* Haider et al. 2012)

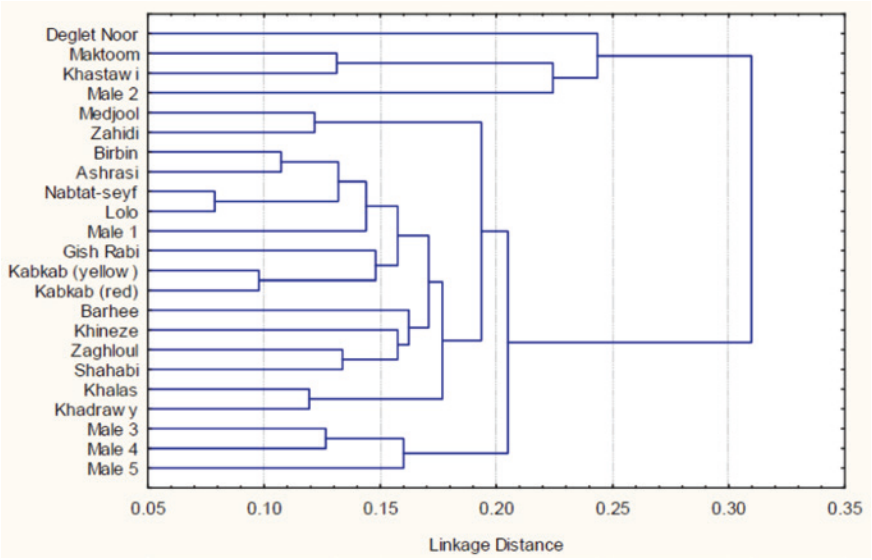

Fig. 4.10 Dendrogram based on RAPD and ISSR banding profiles of five male and 18 female date palm cultivars from eight Middle Eastern countries (see Fig. 4.9) (*Courtsey* Haider et al. 2012)

The generally accepted course of events leading to date palm "domestication" must have involved selecting for specific tree and fruit traits; therefore, the population genetic structure must have been influenced by selection for high fruit yield, better quality, maturity, and regular, long-term, and annual productivity. Population genetic structure refers to variation in size, maturity, and connectedness among the various populations of a species in a region; it takes into account the numbers and age range of individual trees and their spatiotemporal distribution within oases and plantations (Brown et al. 2013; Chen et al. 2014). In date palm populations, genetic structure is governed by the following:

- Environmental characteristics of the region of cultivation (i.e., oasis or plantation),
- Isolation by distance, and
- Biological characteristics of the date palm tree.

However, other factors, such as the history of cultivation and the cultural practices, must have played strong roles in the structuring of its genetic diversity (Millar and Libby 1991; Namoff et al. 2010). Worldwide, the genetic structure of date palm populations has been shaped by factors other than geographical distances between populations; these include seed dispersal, especially during the initial phase following its domestication and spread in the Old World, and the exchange of vegetative propagules (e.g., offshoots, vitroplants, etc.) and pollen (Azeqour et al. 2002; Gurevich et al. 2005; Saro et al. 2014). However, different factors and (micro)-evolutionary processes may have influenced genetic diversity and genetic

structure after population fragmentation when genetic diversity and population genetic structure were compared among different stages of life history (Khanam et al. 2012). High genetic diversity may result from natural selection, which favors excess heterozygozity, together with a combination of a reproductive system and seed or pollen dispersal mechanisms that favor gene flow between populations (Elshibli and Korpelainen 2009b).

Upon domestication, the population genetic structure of date palm became almost entirely dependent on human interference and on the oasis environment as a man-made habitat. Countless generations of horticulturists and oasis dwellers significantly influenced the diversity and genetic structure of local date palm populations. Orchards within an oasis, a garden, or a plantation, may vary in size, distances, density, or are composed of one or numerous closely related or genetically different cultivars. Depending on the mode of reproduction, this structuring can have wide ranging effects on the genetic diversity and genetic structure of the date palm. Evidently, large oases, orchards, unlike modern plantations, most probably encompass more diverse date palm cultivars and populations than the small ones; therefore, their genetic structures may differ from those of small oasis and gardens or plantations. Unexpectedly, close relationships based on molecular markers have been found among date palm cultivars of distant geographic origins in the Middle East, but not among those from North Africa (Alrugaishi et al. 2007). Moreover, in a relatively small geographical and marginal date palm-growing region (Elkichaoui et al. 2013), moderate level of polymorphism (49 %) but relatively large (76–93.5 %) levels of genetic similarity were found among a few date palm cultivars.

A combination of stable and reliable vegetative descriptors and molecular markers revealed substantial genetic variation between soft, semisoft, and dry date palm cultivars; most (93 %) molecular variance was partitioned within subpopulations (Alhammadi and Edwards 2009). Genetic diversity of date palm based on molecular markers in Sudan (Elshibli and Korpelainen 2008), Tunisia, Karim et al. (2010); Egypt (Bekheet and Taha 2013), and based on fruit quality traits in the Gulf Cooperation Council countries (Jaradat and Zaid 2004) is mostly partitioned within, rather than among populations, whereas heterozygosity and low genetic differentiation were attributed to human impact and ecological determinants (Elshibli and Korpelainen 2009).

4.10.2 Qualitative Indicators and Measures

Sexual expression in palms, which impacts genetic diversity, is spatially separated at the following levels (Chao and Krueger 2007; Younis et al. 2008):

- Within flowers (in-between floral organs),
- Within flower clusters (in-between flowers),
- Within inflorescences (in-between partial inflorescences),

- Within palms (in-between inflorescences), and
- In-between palms levels.

The complexity of sexual expression in palms and its impact on genetic diversity becomes clearer when the spatiotemporal separation of male and female functions is considered as in *P. dactylifera* (Adawy et al. 2004; Younis et al. 2008). The distribution of *Phoenix* spp. genetic diversity is not random or uniform over time and space; therefore, estimates of its genetic diversity differ among oases and populations, while several key historical, geographical, ecological, and anthropogenic factors determine its spatiotemporal distribution. The impact of these factors and their interaction is reflected on the level of population differentiation and, especially on fruit quality traits (Jaradat and Zaid 2004; Alobeed 2010; Aldjain et al. 2011; Battesti 2013).

In spite of recent developments, traditional oases will continue to play a vital role in the maintenance and enrichment of date palm genetic resources and their genetic diversity through multiple processes and dynamic conservation practices. A better understanding of the intraspecific genetic variation in date palm and its distribution in oasis agroecosystems will be necessary for the proper conservation and sustainable use of its genetic diversity. If properly designed and implemented, strategies for the study, productive conservation, and sustainable use of date palm agrobiodiversity will minimize anthropogenic disturbance, interference, and impact; optimize oasis agroecosystem functions; and result in integrated protection of environmental resources of the fragile oasis agroecosystems (Jaradat 2011, 2014b, c).

In situ or on-farm (i.e., oasis, grove or home garden) conservation to promote genetic diversity of date palm was encouraged in a few Middle Eastern and North African countries as a prospective strategy of genetic resources conservation and to maintain a genetic resource base for the future. If farmers are motivated, economically or otherwise, they may be willing to participate in activities and practices leading to date palm genetic resources conservation. Farmers may have a greater incentive to grow many and diverse cultivars to help lower the risks of total crop failure in the case of disease or insect epidemics or natural disasters. Farmers may be willing to give up extra income or yield to obtain a more stable and environmentally adaptable date palm cultivar (Ezebilo et al. 2013). Farmers who grow date palm for own consumption often have different tastes and are likely to grow different cultivars to meet their specific needs and preferences (Glilcan 1997; Jaradat and Zaid 2004). Family size may dictate the number of date palm cultivars; the more the diversity of date palm cultivars in a family garden, the smaller the unit value will be.

Modern biotechnology may contribute significantly to this effort by providing complementary in vitro conservation options through tissue culture, DNA or RNA sequences, and other techniques (Gurevich et al. 2005; Bekheet and Taha 2013). Genetic resource collections in the form of frozen tissues, purified DNA samples, frozen viable cell cultures, and derivatives such as RNA, cDNA, and genomic libraries all represent valuable components of a comprehensive storage strategy. However, safety duplicates of the living collections and alternate

conservation strategies are needed to safeguard the large number of cultivars (Jain 2011; Bekheet and Taha 2013), especially those threatened by genetic erosion or vulnerable to biotic and abiotic stresses. Two elite cultivars (i.e., Medjool and Barhee) have become the most important cultivars produced, and virtually conserved, via tissue culture; however, this trend may contribute to narrowing their genetic resources base and subject them to adverse biotic and abiotic stresses (Atkinson and Urwin 2012; Chao and Krueger 2007).

Challenging threat of genetic erosion, imposed by diseases and insects, through selection and breeding is a long-term and demanding process (Faleiro 2006; Ahmed et al. 2010). In order to collect offshoots or vitroplants from hundreds, if not thousands of date palm cultivars distributed all over the world for germplasm enhancement, genetic diversity studies, or breeding purposes, many logistical difficulties will have to be solved such as transferring, assemblage, and maintaining of offshoots. Date palm offshoots or plant tissue materials from certain countries in North Africa are prohibited from direct entry because of Bayoud disease, but may be imported after growing them first under quarantined conditions. Bayoud was first observed around 1890 in Morocco (Feathers et al. 1989), and by 1950 almost 10 million date palm trees had been killed by the disease (Elmodafar 2010). Development of resistant cultivars is the only reliable method to control the disease. Although quarantine measures have been enforced to prevent its spread beyond North Africa, the pathogen was found on *P. dactylifera* and *P. canariensis* in California (Feathers et al. 1989), thus posing a great threat to date palm industry in the US. Resistance to Bayoud and high fruit quality seem to be genetically independent traits (Bendiab et al. 1993) that can be combined in one genotype (e.g., Medjool). Khalts, especially those with high fruit quality already growing in geographical regions where the pathogen persisted for many years, are promising sources of resistance to the disease.

Linkage maps may facilitate the breeding processes and identify genotypes that combine disease resistance and high fruit quality. Such linkages are used to verify phenotypic and genotypic identity of plants derived from in vitro culture (vitroplants) in relation to the explant (Bendiab et al. 1993; Diaz et al. 2003) and to identify genetic variation at different stages of the breeding program. Genotypic authenticity (i.e., true-to-type) of vitroplants cannot be easily verified due to somaclonal variation generated during in vitro culture (Kunert et al. 2003; Eshraghi et al. 2005). Yet, the final phenotypic characteristics of selected genotypes have to match those of the envisioned "ideotype" (Elhoumaizi et al. 2002). However, mass propagation, using in vitro culture or any other mass-production method, exerts strong selection, mostly on fruit traits, and decrease their genetic variance and heritability estimates; on the other hand, traits that are closely associated with fitness (i.e., yield) most probably will have higher genetic variance, but lower heritability estimates than weakly selected traits.

Although most variation estimated for multiple fruit quality traits at a regional level was found to reside among populations, substantial differences were found in genetic diversity components among and within populations (Jaradat and Zaid 2004). However, several studies, based on isozyme and microsatellite markers,

reported larger within-population than between-population genetic diversity levels of date palms in several North African countries and Sudan (Elshibli and Korpelainen 2008, 2009; Hamza et al. 2012). Therefore, it is postulated that the long-term intra- and inter-country selection for specific traits resulted in a highly diverse germplasm in the center of origin and center of diversity of date palm. Detailed analyses of date palm populations originating from different geographic locations will help in understanding their genetic structures and will reveal the extent of gene flow (through seed or offshoot movement and exchange) between populations and its impact on population structure, adaptation to biotic and abiotic stress, yield, and fruit quality.

4.11 Synthesis of Research Findings, Needs, and Priorities

Whether the global diversity of *P. dactylifera* and related feral and wild species has been perceptibly, probably or possibly reduced by genetic erosion, is open to speculations. A quantitative and accurate estimate of the magnitude of genetic erosion and the loss of diversity at several hierarchical levels of the population structure that might have happened in *Phoenix* spp. in general and *Phoenix dactylifera* in particular are contingent on understanding its past genetic diversity and on information about the level of its vulnerability to stress factors. Unavailability of historic data, information and germplasm (e.g., old cultivars) material limit the scope and extent of genetic erosion studies. Scientifically, it is improbable to extrapolate to a global level results and information from locally or regionally conducted genetic diversity studies, and to make inferences about genetic erosion and loss of germplasm. In the recent past, genetic erosion of date palm in the form of loss of traditional cultivars might have happened in response to anthropogenic, climatic, biotic and abiotic stress, or economic factors.

Research and development, with significant consequences on the future of date palm agrobiodiversity and genetic diversity, its vulnerability and ability as a species to withstand current, and anticipated biotic and abiotic stresses, may revolve around the ability of the date palm to interact with other components of the agrobiodiversity complex within oasis agroecosystems, and is concerned with the following interrelated questions: what are the practical implications of these interactions:

- How is the genetic diversity partitioned within and among populations and within and among traditional oasis and modern plantations;
- What are the scientific and practical implications for the conservation of this genetic diversity;
- What are the benefits and the dangers of mass vegetative reproduction of date palm through tissue culture;
- What are the consequences of this technology on total diversity and vulnerability of the species; and
- Where are the “hot-spots” of key tree traits for biotic and abiotic stress tolerance, and for fruit quality traits, and how to utilize their genetic resources efficiently?

Agrobiodiversity; global climate change; management and scale; and social–environmental adaptation, vulnerability, and resilience are four major nodes within the overall multidimensional structure of an oasis agroecosystem. Several questions are emerging concerning their interactions and impact on the date palm and its genetic diversity, including questions about intensification in response to economic drivers; water resources management in relation to expanding plantations and climate change; land-use change in relation to new plantations and date palm monocultures; and socioeconomic developments in key date palm-producing countries and the loss of indigenous knowledge and declining local and migrant work force.

Climate change is increasingly recognized by entomologist, pathologists, and horticulturists as a threat to date palm survival, fruit production, and fruit quality. Comprehensive assessment of date palm vulnerability to stresses imposed by climate change, desertification, and salinity stress requires knowledge of the extent and distribution of its genetic diversity within wild and domesticated genetic resources, both of which depend on the species evolution and its unique breeding system; past genetic bottlenecks; and ecological, geographical, and anthropogenic factors. Wild relatives of *P. dactylifera* are valuable sources of genes and gene complexes that can be used in breeding new date palm cultivars with unique combinations of qualitative and quantitative traits, including metaxenic effects of male wild species. In-depth analyses of multiple qualitative and quantitative traits will help to define the boundaries between *P. dactylifera* and related species.

The combined use of morphology and molecular markers will most likely lead to a deeper understanding of the complex relationships between established cultivars, feral genotypes, and wild relatives of the species. In order to identify and conserve the remaining unique populations of *Phoenix* spp. and to evaluate the extent to which they are endangered by anthropogenic and ecological interacting factors, we need an efficient management and research strategy to enhance the genetic resources of cultivated and wild relatives of date palm. Accurate estimates of phenotypic diversity in structural and yield components traits (i.e., trunk, crown, fruiting, and fruit quality attributes), and their partitioning within and among the gene pool of date palm in its center of origin and center of diversity, are requisites for a flourishing date palm industry. The most effective strategy for capturing adequate genetic diversity, if the proportion of among-population variation is larger than the within-population variation, is to target the maximum number of populations that can be practically conserved.

New advances in biotechnology and genetic manipulation, unlike conventional breeding, may allow for the transfer of selected gene(s) to a specific genotype (e.g., for disease resistance) in only a single generation. Enhancement of date palm genetic diversity, a vital component of oasis' resilience, can be achieved by ensuring that the current range of existing cultivars will not be reduced further by biotic and abiotic stresses, and by thwarting the drive of market forces to dictate or encourage the few types of cultivars favored by consumers and the export industry. Coordinated regional and international efforts are needed to establish a comprehensive DNA fingerprint database and phylogeny maps for the 1500–5000

date palm cultivars known by different unique, multiple, or vernacular names and descriptors. Stable phenotypic and molecular markers that may accurately assist in identifying cultivars and specific traits of high economic and horticultural value are required for future advances in developing elite date palm cultivars. Genetic diversity has been generally assessed in relatively few date palm cultivars with a limited geographical coverage, and rarely was assessed over time for the same cultivars or their progenies. Detailed information is already available on genome-wide structural parameters of genes and genome of the date palm. However, in order to position its genome in an evolutionary context, additional sequencing is needed to account for the high levels of heterozygosity of recessive alleles and to achieve high coverage of quantitative trait mapping. A thorough knowledge of spatial patterns of genetic differentiation of date palm populations is key to understanding important processes, such as evolutionary mechanisms of population differentiation and ecological or conservation consequences of loss of genetic diversity.

Information derived from the mating system and long-distance gene flow in heart of palm (*Euterpe edulis* Mart.), for example, suggested that population dynamics can significantly affect evolutionary factors such as selection and genetic drift, and consequently may have important effects on the genetic structure of *P. dactylifera* populations. Therefore, worldwide date palm genomes need further study to identify proper markers that may assist in selecting horticulturally exceptional and economically important cultivars. Detailed analyses of date palm populations originating from different geographic locations will promote the understanding of their genomes and will reveal the true magnitude of gene flow between populations. Recently, developed sequence data from multiple genomes have provided the largest resource of polymorphic markers to date. A small subset of these markers is expected to differentiate between the thousands of available date palm cultivars around the world.

Many factors, singly or in combination, partially shape agrobiodiversity in the oasis; these include oasis physiography, spatial isolation and access to markets, industrialization of agriculture, urban development, water quantity and quality, proximity to urban centers, and land area under date palms. In-depth research on ecological, economic, and social efficacy of adaptation strategies in relation to conservation, adaptation, and mitigation objectives is required to develop adaptation strategies relating to oases and date palm responses to biotic and abiotic stresses. Currently, the complex interactions between social and ecological systems within an oasis are not well understood. A better understanding of these interactions, however, is essential for the development of sustainable management approaches, to implement appropriate adaptation and mitigation strategies, and to explore and model these complex interactions as a prelude to germplasm enhancement and a safeguard against vulnerability and genetic erosion.

A better appreciation of the factors that are a threat to the survival and husbandry of date palm, as well as to the development of appropriate diagnostic, monitoring and sustainable management techniques, can optimize production and help identify and manage emerging climatic, disease, and insect threats. The

role of victors of many date palm diseases, physiological disorders, and phenotypic abnormalities is still unconfirmed or unknown. Accurate taxonomy and biotype identification of suspected pest taxon are important for the development of integrated pest management programs, especially where biological control is an option.

Strategies for the study, productive conservation, and sustainable use of date palm agrobiodiversity, if properly designed and implemented, will minimize anthropogenic disturbances, interference, and impact; it will optimize ecosystem functions; and may result in integrated protection of environmental resources of the usually fragile oasis agroecosystems. Oases which can be designated as protected heritage areas will serve as model sites where the conservation and traditional use of their unique crop assemblages, genetic resources, traditional management practices, and indigenous knowledge can be combined as a human legacy.

Substantial investments in advanced and practical forms of mitigation are prerequisites to safeguard the thousands of date palm cultivars, especially in "monocultures" and modern plantations, against potential vulnerabilities to multiple threats. Building relational databases on *Phoenix* species, tree and fruit phenotypic and biotechnological attributes of populations and cultivars, and the development of a "Digital Atlas" will help document and provide online information for research, conservation, and sustainable utilization of date palm genetic resources. The development of alternative markets for date palm by-products will create incentives for farmers to enhance its genetic resources, grow more and diverse date palm cultivars, encourage the development of a wide range of products based on phenotypic and fruit trait diversities, and enhance the role of date palm as a functional genetic resource.

The date palm provides indispensable ecosystem services beyond its unique fruit; evidence-based management strategies, if timely implemented, will protect this irreplaceable genetic resource against current and potential threats and enhance its genetic diversity.

4.12 Conclusions

Genetic erosion in cultivated species, no matter how slow and complex the process was, most probably did occur as a consequence of agricultural development; and in the case of the date palm, sound scientific and circumstantial evidences supporting this premise are not difficult to find. Ecological and socioeconomic factors are affecting the delicate balance of oasis agroecosystem directly, and indirectly impacting the genetic diversity of the date palm. Hazards include genetic erosion due to advances in clonal propagation, inappropriate agronomic practices, land degradation, frequent droughts, aquifer depletion, soil and water salinity, desertification, sand dune encroachment, and the introduction of exotic plant species into

remote oases. In addition, date palm agrobiodiversity and production potential are threatened by a number of biotic and abiotic stresses.

Date palm orchards in North Africa are aging; almost half of the Tunisian productive date palms are more than 50 years old, while renewed oases and new plantations are competing on declining water resources with other sectors of the local economy. Almost one-third of productive trees in Algeria are beyond the limits of their productive years, and along with Moroccan date palms, and have been devastated and still are vulnerable to the Bayoud fungal disease. Similarly, date palms and wild *Phoenix*. Spp. in the Middle East are threatened by the red palm weevil which drastically shortens the adult productive life of otherwise productive trees. Interspecific mating within the *Phoenix* spp. between endemic and *P. dactylifera* as an introduced species will have some implications for genetic diversity, vulnerability, and genetic erosion of *P. dactylifera*; if hybrid progeny and progeny from advanced hybridization are vigorous and fertile, the endemic species is at risk from genetic assimilation; however, if hybrid progenies are sterile or have reduced vigor, then the introduced is at risk from outbreeding depression.

Ample opportunities are available for date palm research and development through biotechnological advances, especially to identify and quantify genetic diversity components in the species, identify and clone genes and gene complexes for biotic and abiotic stresses, and utilize the generated information for the advancement of date palm industry. The assessment of interspecific hybridization and introgression between species and subspecies, although still lacking, is becoming more important for the implementation of appropriate genetic conservation strategies, and for the assessment of overall vulnerability of existing date palm genetic diversity and for the status of agrobiodiversity in the oasis.

Broad-scale shifts, based on modeling studies, possibly are expected in areas favorable for date palm cultivation and how different areas of the world may be affected due to climate change based on broad regional scale changes over the next hundred years using coarse scale climate data. Such modeling is useful in planning for genetic resources conservation as well as for future strategies to minimize economic impacts in areas that may be adversely affected, while preparing to take advantage of new opportunities in regions where favorable climates may prevail in the future.

The date palm is considered a renewable natural resource because it can be replaced in a relatively short period of time or used through conservation efforts without depletion. Countries which hold significant amounts of genetic diversity of date palm have a greater responsibility to conserve and safeguard date palm germplasm and utilize it for genetic improvement and development of adapted cultivars for domestic and foreign markets. If properly designed and implemented, a holistic approach to the conservation of genetic resources and agrobiodiversity will minimize anthropogenic disturbances, interferences, and impacts; optimize ecosystem functions; and result in integrated protection of environmental and natural resources of the usually fragile oasis agroecosystems, including the genetic diversity of the date palm.

Accurate estimates of genetic diversity and its partitioning, at the oasis, plantation, country, and global levels, especially for fruit quality traits, tolerance to biotic and abiotic stresses within, and among gene pools of Phoenix spp. in its center of origin and center of diversity are important considerations for the future of a successful date palm industry. The proficiency in using genetics and genomics to discriminate between cultivars and to predict seedling sex are perhaps the two most immediate challenges in applying biotechnology to date palm improvement and development. A suitable conservation and propagation approach for date palm genetic diversity requires a holistic approach which combines different ex situ and in situ conservation methods in a complementary manner. However, issues such as consideration of biological characteristics, identification of conservation objectives, available methodologies, socioeconomic factors, and organizational and funding concerns, need to be taken into consideration. Although field genebanks provide easy access to conserved material for use, they run the risk of destruction by natural disasters, biotic and abiotic stresses, and climate change. Therefore, a complementary strategy for conservation of date palm genetic diversity should employ a combination of methods including nature reserves, field genebanks, tissue cultures, and others, as no single method can conserve all the diversity.

Some cultivars have become prominent in world markets due to their unique quality traits; these include Deglet Noor from Tunisia, Medjool from Morocco, Barhee, Halawy, Khadrawi, and Zahidi from Iraq, and Hayany from Egypt. The slow rate of vegetative propagation of these cultivars by offshoots may not meet the growing global market demand. Therefore, extensive efforts are needed to propagate them through other means provided that their genetic diversity is not compromised or diminished. In vitro propagation through tissue culture requires that all progeny plants remain phenotypically and genetically identical to their progenitors. However, the process of tissue culture, and potentially other micropropagation methods, is known to result in the production of 'off-types', (i.e., plants that differ visually from the original cultivar). This phenomenon, known as somaclonal variation, is caused by genetic and epigenetic alterations generated during the in vitro process.

Future research on date palm will be carried out, with high probability, not only in the date palm-producing countries of the Middle East and North Africa, where dates are an important economic commodity and the date palm is a traditionally an important fruit tree, but also in countries where advanced scientific and analytical capabilities are available, and where an emerging date palm industry is founded on advanced principles of genomics and precision agriculture.

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Chapter 5
The Genetic Diversity, Conservation, and Use of Passion Fruit (*Passiflora* spp.)

Carlos Bernard Moreno Cerqueira-Silva, Fábio Gelape Faleiro, Onildo Nunes de Jesus, Elisa Susilene Lisboa dos Santos and Anete Pereira de Souza

Abstract The characterization of genetic variability is important for conservation and biodiversity as well as the strategies and research techniques that contribute to such characterizations of flora, including the use of morpho-agronomic descriptors and molecular markers. In this chapter, we present and discuss the issues related to the genetic diversity of passion fruit (*Passiflora* spp.) to provide the reader with an updated view on the advances and challenges associated with the characterization, conservation and genetic diversity of the genus *Passiflora*. *Passiflora*, whose species are commonly known as passion fruits, stands out in the family Passifloraceae both for its number of species (approximately 520) and its ecological and economic importance. Passion fruits grow in various countries, and they are diversely represented in the Americas; in particular, Colombia and Brazil grow approximately 170 and 150 species of *Passiflora*, respectively. Despite increasing interest

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in this genus, genetic characterization, and breeding programs remain modest, especially considering the number of species not yet studied. Because almost all passion fruit diversity estimates derive from accessions maintained in germplasm banks using ex situ conservation to reduce the loss of species genetic variability, the scientific community must increase the number of these accessions. In addition, an urgent need exists for estimations of the diversity of natural populations and expanded analyses of passion fruit accessions present in germplasm banks, to provide more realistic estimates regarding the diversity of *Passiflora* and its representation in germplasm banks, both for conservation and biodiversity.

Keywords Conservation and management of biodiversity · Ex situ conservation · Genetic variability · Molecular markers · Passifloraceae

5.1 Introduction

5.1.1 The Characterization, Conservation, and Use of Biodiversity

Biodiversity is the assemblage of all genes, species, and ecosystems present in a specific area or on the planet, where diversity is the result of evolutionary processes (Nass 2011; Frankham et al. 2008). Studies devoted to characterizing the components of biodiversity are justified by the importance and ecological interest of their potential and immediate use by humans (Nass 2011), especially when certain specimens are known biological resources (or, more specifically, genetic resources).

Because the number and genetic variability of many species are rapidly declining as a direct or indirect consequence of human actions (Frankham et al. 2008), and the demand for food products and other derivatives (e.g., biofuels and new drugs) is growing rapidly (Lee et al. 2014), a notable need exists for research that helps establish conservation strategies as well as manage and make use of the variation in available genetic material. In this context, genetic resources are resources of natural raw material (genetic variability), both for breeding programs and conservation strategies.

Over recent decades, the use of molecular techniques in studies related to the characterization of genetic variability has grown exponentially, especially the use of molecular markers (Fig. 5.1). Of the major applications for molecular markers, Cerqueira-Silva et al. (2014a, b) and Faleiro (2007) highlighted the following: (i) the estimation of intraspecific and interspecific genetic diversity; (ii) the determination of evolutionary relationships and phylogenetic classifications; and (iii) the identification, characterization, and mapping of genes. All of these applications have contributed to the characterization, conservation, and use of biodiversity.

Rapid advances in molecular techniques and the consequent reductions in their costs have produced increasingly robust research, not only with regard to

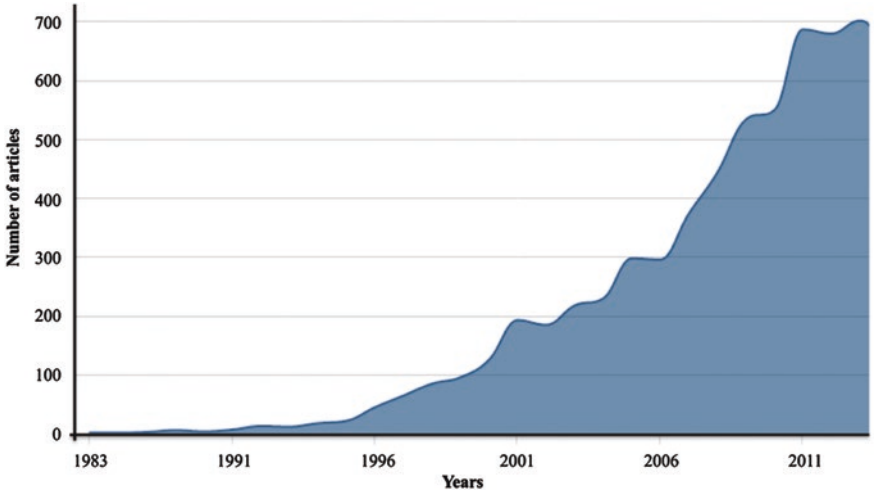

Fig. 5.1 The number of articles published using molecular markers to estimate the genetic diversity of passion fruit species

commercial species but also wild species with little or no direct/immediate economic effects (Phillips 2014; Ferreira and Rangel 2011). The quantitative and qualitative growth of information generated from molecular-genetic studies obtained using modern approaches, e.g., next-generation sequencing (NGS) and genotyping-by-sequencing (GBS), has greatly expanded the possibility of the associations between genotypic and phenotypic data.

The following sections present and discuss issues related to the genetic diversity of passion fruit (*Passiflora* spp.). This chapter seeks to provide the reader with an updated view of the advances and challenges associated with the characterization, conservation, and use of the genetic diversity present in the genus *Passiflora*.

5.1.2 The Diversity and Distribution of the Genus Passiflora

The family Passifloraceae Juss. ex DC. corresponds to a group of species with highly variable leaves and flowers (Ulmer and MacDougal 2004), which are often considered lianas or climbing plants with tendrils and occasionally trees or shrubs without tendrils (Cervi 1997). Estimates of the number of species in the Passifloraceae family vary between 520 (Bernacci 2003; MacDougal and Feuillet 2004) and 700 (Feuillet 2004), and the number of genera varies between 18 (Feuillet 2004) and 23 (Barroso 1978). These variations are the result of taxonomical uncertainties, the use of synonyms, and inconstant descriptions of new species (Wetzel et al. 2011). Despite the taxonomical uncertainties, within the family Passifloraceae the genus *Passiflora* is noted for its species diversity

(approximately 520; MacDougal and Feuillet 2004). Species of this genus are commonly known as "passion fruits" and "passion flowers," In general, passion fruits are allogamous plants that exhibit self-incompatibility (Bruckner et al. 2005). However, certain species are self-compatible and can reproduce via self-fertilization, like some passion fruit species of the subgenus *Decaloba* (Varassin and Silva 1999).

With regard to their geographical distribution, approximately 96 % of passion fruit species are widely distributed across tropical and subtropical regions, especially in South America. In this context, countries such as Colombia and Brazil (with approximately 170 and 150 passion fruit species, respectively) are considered the diversity centers of the genus *Passiflora* (Bernacci et al. 2014; Ocampo et al. 2010; Fajardo et al. 1998). Although fewer passion fruit species are located outside the Americas, they have been observed in India, China, Southeastern Asia, Australia, and the Pacific islands (e.g., *Passiflora aurantia*, *P. cinnabarina*, *P. herbertiana*, *P. cupiformis*, *P. henryi*, *P. jugorum*, *P. moluccana,* and *P. siamica,* Cerqueira-Silva et al. 2014a).

Despite the species richness and wide distribution of the genus *Passiflora* across tropical regions, the lack of ecological and genetic research concerning most passion fruit species has become a risk factor for the conservation of their biodiversity. Various research groups have performed basic research devoted to advancing knowledge related to the geographic distribution of *Passiflora* (Scherer 2014; Ocampo et al. 2010; Ocampo-Perez et al. 2007), including the promotion and discussion of hypotheses related to patterns of distribution, ecological and evolutionary relationships, and the identification of the species at the greatest risk for extinction. Specific to the passion fruit species of Brazil, a recent study of 58 species indicated that most of the species were present in only two (12 species) or one (23 species) of the five biomes found in Brazilian forests (Scherer 2014).

Taking a macroecological approach in which both geographical distribution and climatic characteristics are considered, the occupation pattern of the climatic niches estimated for *Passiflora* shows that passion fruits restricted to homogeneous environments with high temperatures and significant rainfall are more susceptible to climate change (e.g., *P. vespertilio*, *P. micropetala*, *P. rubra*, *P. mansoi*, *P. ceratocarpa*, *P. candida*, *P. foetida*, *P. vitifolia*, *P. coccinea*, *P. nitida*, *P. riparia*, *P. ambigua,* and *P. quadrangularis*; Scherer 2014). The same author argued that more widely distributed *Passiflora* species in heterogeneous environments with rainfall concentrated during the warmer months (e.g., *P. setulosa*, *P. mendocaei*, *P. ishnoclada*, *P. caerulea*, *P. tenuifila*, *P. urubicensis*, *P. elegans,* and *P. actinia*) are at less risk for extinction and more likely to preserve the evolutionary history of the genus.

According to the information provided combined with the constant threat of genetic erosion or even extinction due to the fragmentation and reduction of forests via human activity, genetic estimates that provide information concerning variability and genetic structure are important for the conservation, management, and use of *Passiflora* (Faleiro et al. 2011a, b; Ocamp et al. 2010).

5.2 Genetic Variability and the Conservation of Passion Fruit

5.2.1 Diversity Studies Based on Morphological and Agronomic Descriptors

The wide morphological variability observed among passion fruits is one of its striking features, encompassing both interspecific and intraspecific variation. All of this variability is related to the wide geographical distribution of the genus and the evolutionary factors that resulted from the interactions between passion fruits and pollinators, seed dispersers, pests, and pathogens. The wide variation present in both the flowers and fruits of passion fruit emphasizes their beauty and potential influence on the ecological relationships of species (Fig. 5.2).

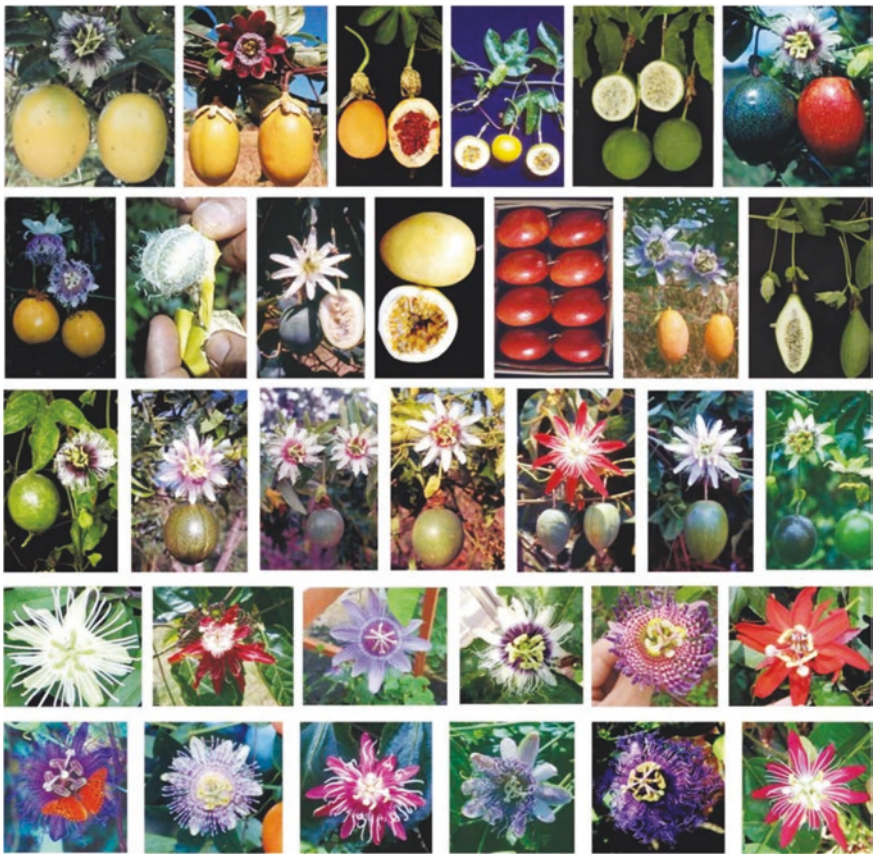

Fig. 5.2 The diversity of forms and colors in the fruits and flowers of passion fruits. Accessions conserved in the germplasm bank of Embrapa Cerrados. Credits: F.G. Faleiro and NTV Junqueira, researchers from Embrapa, Brazil

Most studies on the diversity of the genus *Passiflora* have been conducted to support pre-improvement actions, especially the pioneering studies of Oliveira (1980), Maluf et al. (1989), Oliveira et al. (2012), Ocampo and Coppens-d'eeckenbrugge (2009), Araujo et al. (2008), Castro et al. (2012), and Jesus et al. (2014), who proposed the establishment and use of morphological and agronomic descriptors to characterize passion fruit diversity. When characterizations of diversity primarily stem from the analysis of accessions held in germplasm banks and collections, the results from these studies contribute more to the advancement of breeding programs than to that of the population of *Passiflora* species.

Together, the variability estimates presented by various authors based on morphological and agronomic characteristics have indicated wide intraspecific variability among the *Passiflora* species (Ocampo et al. 2013; Faleiro et al. 2005, 2011a, b; Cerqueira-Silva et al. 2009). Studies have also observed preferred crosses, with the aim of enhancing the segregation or maintenance of characteristics of interest. Estimates of pathogen resistance, observed in various genotypes as reactions to anthracnose, woodiness virus, scab, fusarium, and bacterial blight, also support the genetic variability within and among *Passiflora* species (Batistti et al. 2013; Oliveira et al. 2013a; Cerqueira-Silva et al. 2008; Junqueira et al. 2003).

5.2.2 Genetic Variability Estimates Based on Molecular Markers

Estimates of genetic variability based on the polymorphisms of molecular markers are increasingly common among *Passiflora*. The first of these studies used isozyme markers (Segura et al. 1998) and randomly amplified polymorphic DNA (RAPD; Fajardo et al. 1998) to estimate the intraspecific and interspecific genetic diversity of passion fruit accessions. Since that first study, a variety of molecular markers have been used to (i) estimate the diversity of wild and cultivated accessions (Bernal-Parra et al. 2014; Cerqueira-Silva et al. 2014b, Oliveira et al. 2013, Ortiz et al. 2012, Santos et al. 2011); (ii) construct genetic maps (Pereira et al. 2013; Oliveira et al. 2008; Carneiro et al. 2002); (iii) characterize and confirm hybridizations (Santos et al. 2012; Conceição et al. 2011; Junqueira et al. 2008); and (iv) analyze either the recovery of recurrent genomes in plants obtained from backcrossing in a molecular marker-assisted program (Fonseca et al. 2009; Bellon et al. 2014) or the effect of selection on genetic variability (Costa et al. 2012). A recent compilation of publications regarding the use of molecular markers in *Passiflora* found that at least eight different types of markers have been used to characterize diversity, with the RAPD markers used most prevalently (approximately 50 % of all passion fruit diversity studies; Fig. 5.3; Cerqueira-Silva et al. 2014a). Although these markers are informative, they do not maximally exploit the available genetic information because it is impossible to distinguish heterozygote genotypes. In the last few years, however, the number of studies using other markers (e.g., microsatellites) has increased at the expense of studies using dominant markers (e.g., RAPD).

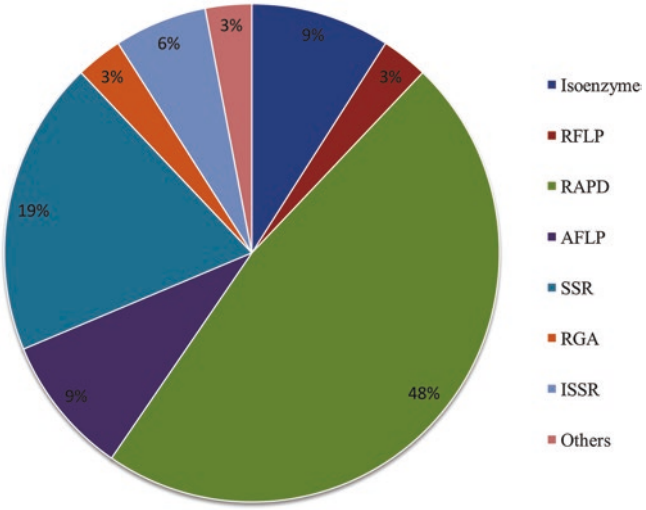

Fig. 5.3 The percentages of articles published using various molecular markers to estimate the genetic diversity of passion fruit

Although a growing number of studies have been devoted to characterizing the genetic diversity of passion fruits, estimates from primary scientific publication databases indicate that fewer than 15 % of passion fruit species have available genetic diversity data based on molecular markers (Cerqueira-Silva et al. 2014a). In addition, no population studies have been conducted that enable the evaluation of diversity patterns and the natural variability available within and among *Passiflora* species. In this context, the available knowledge, similar to that of morpho-agronomic evaluations, is the almost exclusively the result of the characterization of the accessions maintained in germplasm banks and collections.

Oliveira et al. (2005) and Padua et al. (2005) were the first research publications devoted to the development and characterization of microsatellite (or Simple Sequence Repeat, SSR) markers for *P. edulis* and *P. alata*, respectively. These publications marked an important step for population studies of *Passiflora*. However, new microsatellite markers for wild and commercial passion fruit species were not developed until 2012 (Cerqueira-Silva et al. 2012, 2014b, c; Penha et al. 2013; Cazé et al. 2012) when approximately 450 microsatellite markers were developed and characterized (Cerqueira-Silva et al. 2014a). Despite the morphological variability and high potential for polymorphisms observed in the microsatellite locus, the results obtained using these markers have mostly indicated the existence of low-to-moderate genetic variability in passion fruit species (Table 5.1).

Is also important to highlight that the SSR markers available for *Passiflora* were developed from the genomic DNA of only six species: *P. alata*, *P. edulis*, *P. cincinnata*, *P. contracta*, *P. pohlii,* and *P. setacea* (Cerqueira-Silva et al. 2012, 2014c; Penha et al. 2013; Cazé et al. 2012; Pádua et al. 2005; Oliveira et al. 2005, 2008).

Table 5.1 The average number of alleles (Na) and observed (H_O), and expected (H_E) heterozygosity values in the characterization of microsatellite loci from passion fruit species and studies of genetic diversity

Species	Microsatellite loci characteristics			References
	Na	H_O	H_E	
P. alata	3.1	0.26	0.53	Pádua et al. (2005)
P. edulis	7.6	0.58	0.62	Oliveira et al. (2005)
P. cincinnata	5.0	0.52	0.52	Cerqueira-Silva et al. (2012)
P. contracta	4.9	0.53	0.61	Cazé et al. (2012)
P. edulis	1.0	–*	–*	Ortiz et al. (2012)
P. cincinnata	3.3	0.26	0.36	Cerqueira-Silva et al. (2014c)
P. edulis	3.4	0.31	0.36	Cerqueira-Silva et al. (2014c)
P. setacea	2.8	0.34	0.41	Cerqueira-Silva et al. (2014c)
P. cincinnata	6.0	0.42	0.52	Cerqueira-Silva et al. (2014b)
P. edulis	6	0.43	0.50	Cerqueira-Silva et al. (2014b)
P. setacea	3	0.25	0.36	Cerqueira-Silva et al. (2014b)
P. alata	–*	–*	–*	Silva et al. (2014)
P. ligularis	12.2	0.98	0.96	Bernal-Parra et al. (2014)

*These studies did not observe polymorphic loci or analyze binary data (e.g., dominant markers); therefore, estimations of Na, H_O, and H_E were not possible

Therefore, efforts to increase the number of loci available for future genetic studies of *Passiflora* must be maintained.

Despite the economic importance of *Passiflora* (including alimentary, pharmaceutical, and ornamental uses), little is known about this genome. A better understanding of it is important to the efficient use of its genetic resources (Santos et al. 2014). Genomic information would enable the use of molecular markers, such as single nucleotide polymorphisms (SNPs), thereby expanding our knowledge of the genetic relationships between different species.

In addition to the identification of microsatellite markers and the development of new primer pairs, cross-amplification (which occurs when the DNA regions that flank the microsatellite loci are sufficiently conserved and enable the amplification of a microsatellite locus in different species with the use of the same primer pair) has been used to provide quick and cost-effective sets of microsatellite markers to genetically characterize passion fruit accessions and populations (Paiva et al. 2014; Cerqueira-Silva et al. 2014b, c; Silva et al. 2014; Oliveira et al. 2013b). Cerqueira-Silva et al.'s (2014b) recent experimental data and compilations of other results show that at least 20 *Passiflora* species have characterized microsatellite loci. Included among these species are the major commercial species in Brazil, *P. edulis* and *P. alata*, which have approximately 300 and 170 characterized loci, respectively. The same authors showed that 12 other passion fruits (*P. malacophylla*, *P. galbana*, *P. watsoniana*, *P. cincinnata*, *P. tenuifila*, *P. gibertii*, *P. setacea*, *P. foetida*, *P. morifolia*, *P. suberosa*, *P. rubra,* and *P. laurifolia*) have approximately 70 characterized microsatellite loci each. Cerqueira-Silva et al.'s (2014b)

cross-amplification assay results showed that 23 microsatellite loci are conserved among 14 species, which confirms the potential use of these markers in intraspecific and interspecific genetic studies.

Because most diversity studies of *Passiflora* have been conducted using a few representative genotypes, we must highlight Bernal-Parra et al. (2014) who examined 41 accessions of *P. ligularis*; Cerqueira-Silva et al. (2014b) who examined a total of 116 accessions of *P. edulis*, *P. cincinnata,* and *P. setacea*; Ortiz et al. (2012) who examined 70 accessions of *P. edulis*; and Santos et al. (2011) who examined a total of 45 accessions of *P. edulis* and *P. alata*. Although these studies identified selectable genetic variability and the absence of genetic structures associated with the geographical origin of access, contrasting results regarding the magnitude of variability estimated for passion fruit species can be found in the literature. One such example is the absence of polymorphisms observed at 17 microsatellite loci used to evaluate *P. edulis* accessions (Ortiz et al. 2012) and the high variability observed at five microsatellite loci (mean = 12.2 alleles per locus; heterozygosity = 0.98) used to evaluate *P. ligularis* accessions (Bernal-Parra et al. 2014); both of these studies were performed in Colombia to support pre-breeding actions. Because of the lack of studies from natural populations, determining the representativeness of the group diversity of accessions available in germplasm banks is difficult.

5.2.3 *Strategies and Actions for the Conservation and Use of Passion Fruit*

The practical implementation of strategies and actions dedicated to conserve passion fruit remains incipient, and it is generally limited to the ex situ conservation of accessions in germplasm banks and collections. Approximately, 50 such collections are estimated to be spread across 32 countries; together, these collections represent at least 1200 passion fruit accessions (Faleiro et al. 2011a, b; Ferreira 2005). Approximately, 95 % of these accessions are located in germplasm banks in only nine countries: Brazil (32 %), Ecuador (30 %), Peru (14 %), Colombia (8 %), France (3 %), and USA, Costa Rica, Jamaica, and Kenya (2 % each; Cerqueira-Silva et al. 2014a; Ferreira 2005).

Embrapa, the Brazilian Agricultural Research Corporation (https://www.embrapa.br), has three passion fruit germplasm banks (BAG passion fruit; Fig. 5.4). These banks are located across three units: Embrapa Cerrado (the Cerrado Biome), Embrapa Cassava and Fruits (the Atlantic Forest Biome), and Embrapa Semi-Arid (Biome Caatinga). This distribution across different biomes is interesting with regard to the conservation of *Passiflora* genetic resources. In addition to the Embrapa BAGs, other important collections are located at universities and state/federal institutions in Brazil.

Despite the importance of these collections, accession loss during storage remains common. This loss includes problems with in vivo maintenance (including

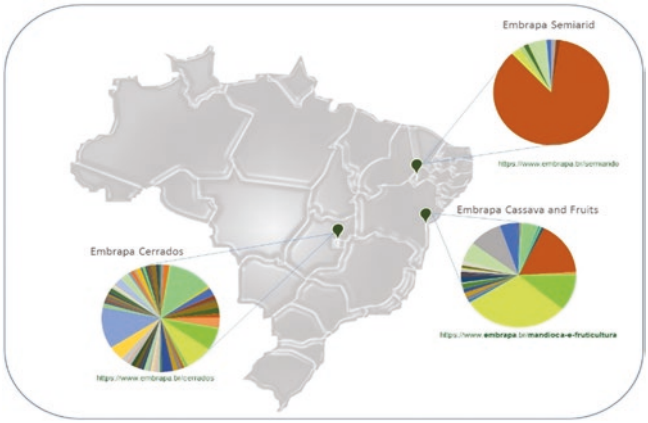

Fig. 5.4 Major germplasm banks of *Passiflora* from Embrapa. The *colors* in the pie charts represent the same species conserved in other germplasm banks. Within each graph, the *colors* represent the numbers of species preserved

improper species adaptation, and phytopathological problems) and difficulties with appropriate protocols to ensure the viability of stored seeds. These limitations can be overcome with a better understanding of the physiological ecology of the species, the type of seed (orthodox, recalcitrant or intermediate), the appropriate conditions for storage (ideal temperature and humidity), and new conservation strategies (e.g., in vitro tissue culture techniques and cryopreservation; Fig. 5.4). Cryopreservation has been used for long-term conservation at ultra-low temperatures (−196 °C) that suspend cell division, metabolic, and biochemical activities (Radha et al. 2012), thereby enabling the long-term storage of vegetative and reproductive structures (González-Benito et al. 1998; Solomon 2002) as well as species with recalcitrant or intermediate seeds (Santos 2000).

The first step to preserve the close relationship between conservation and biodiversity studies of *Passiflora* is research dedicated to identifying and prospecting species. The second step is the characterization of accessions in germplasm banks and collections. The first step is crucial to maintain more representative variability in germplasm banks and reduce the number of misidentified accessions. The second step is essential for their effective use to support pre-breeding actions (i.e., activities that involve identifying genes and traits in wild species as well as their potential incorporation into agronomically suitable materials) and breeding (i.e., the use of selection and recombination methods to obtain genetic gains; Fig. 5.5).

In this context, the contribution of genetic characterizations to estimate and understand the diversity of populations or groups of regional passion fruit accessions have enabled (i) the identification of converging and diverging crosses of commercial (e.g., *P. edulis*) and wild species (e.g., *P. setacea*, *P. cincinnata,* and *P. trintae*; Cerqueira-Silva et al. 2010, 2014c); (ii) the confirmation and characterization of interspecific hybrids (Santos et al. 2012; Conceição et al. 2011;

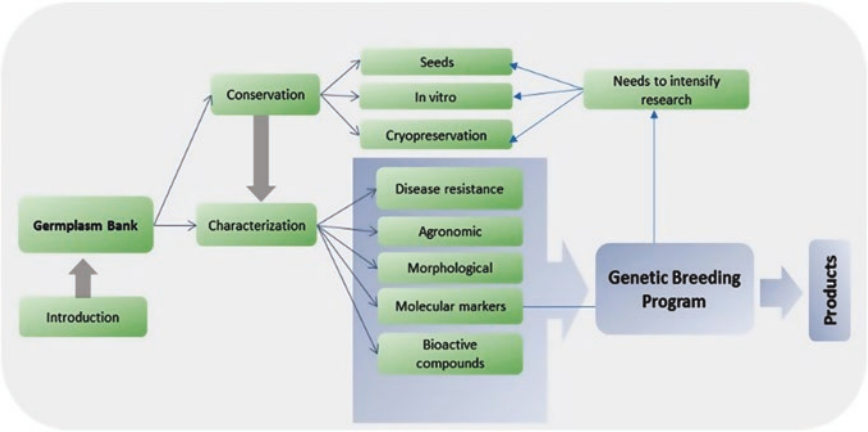

Fig. 5.5 A simplified flowchart of specific conservation strategies, including the characterization and use of accessions conserved in germplasm banks

Junqueira et al. 2008) that decrease loss and reduce costs via the restricted maintenance of seedlings; and (iii) the characterization and selection of specimens with a higher genomic contribution from the recurrent parent, which reduces the number of backcrossing cycles required to obtain the hybrid of interest (Fonseca et al. 2009; Bellon et al. 2014).

The use of wild germplasm is a major interest among passion fruit researchers (Faleiro and Junqueira 2009) because of the importance of introducing the characteristics found in several wild Brazilian *Passiflora* species into commercial species (Faleiro and Junqueira 2009; Faleiro et al. 2011a, b). Wild species are used intensively in passion fruit breeding programs; these species have been tested as rootstocks to obtain resistances to soil fungi and early death as well as to diversify production systems with new functional foods for *in natura* consumption and use as ornamental and medicinal plants (Faleiro et al. 2011a, b, 2012a, b).

Agronomical evaluations of wild *Passiflora* germplasm have indicated the potential of certain species for disease resistance, cold tolerance and improved physical, chemical, or flavor characteristics of passion fruit pulp, thereby enhancing its functional properties (Faleiro and Junqueira 2009). Self-pollinating species such as *P. tenuifila*, *P. elegans*, *P. capsularis*, *P. villosa*, *P. suberosa*, *P. morifolia,* and *P. foetida* have also been identified as well as those with shorter androgynophores that reduce stigma length in relation to the crown, thereby facilitating pollination due to smaller insects. These features increase productivity, decrease the labor costs entailed by manual pollination, and reduce the negative effects of African bees (Faleiro and Junqueira 2009).

Research performed at Embrapa on genetic compatibility, crossability rates, anthesis periods, pollen viability periods, and stigma receptivity have produced several fertile interspecific hybrids through artificial crossings (Junqueira et al. 2008).

Fig. 5.6 Different uses and cultivars of passion fruit. Credits: F.G. Faleiro, NTV Junqueira, researchers from the Embrapa, Brazil

Furthermore, DNA molecular marker-aided backcrossing has been used to recover commercial features while maintaining resistance and other genes of interest (Fonseca et al. 2007; Bellon et al. 2014). Hybrids involving three or more species have also been obtained to pyramid genes for disease resistance. Beyond genetic breeding, certain wild species, and released cultivars have ornamental potential and direct consumption uses (Fig. 5.6). The passion fruit breeding program at Embrapa has worked with regard to the population selection of wild species to increase fruit size for the fresh fruit market and produce ingredients for sweets and ice cream.

5.3 Perspectives and Challenges for the Conservation and Use of *Passiflora* spp.

Research involving the prospection, conservation, and characterization of commercial and wild passion fruit are strategically important for both conservation and breeding. Thus, the absence of phenotypic and molecular characterizations for most species and accessions of passion fruit is a challenge to introducing information from genetic diversity estimates into current breeding programs as well as to managing and conserving this biodiversity. In this context, we believe that the following research activities should be prioritized: (i) Population genetics should measured to understand the genetic diversity and structure of passion fruit; (ii) Phenotypic characterizations should be conducted to contribute to interspecific crosses and studies of the association between phenotypic and molecular data; and (iii) New accessions should be identified, especially wild species, to increase the representation within germplasm banks.

The identification of new microsatellite loci for *Passiflora* species and the use of cross-amplification strategies to popularize the use of these markers should increase the amount of passion fruit research. As Table 5.1 shows and recent studies

(Cerqueira-Silva et al. 2014a, b) have discussed, however, additional research should be performed to identify and characterize microsatellite loci because the current results indicate low allelic diversity among SSR loci from wild and commercial passion fruit. Recent research involving *Passiflora* species has used biotechnology (Faleiro et al. 2012a, b) and genomics; investigations of commercial passion fruit species have developed and characterized the first SNPs for *P. alata* (Pereira et al. 2013). Various research groups are providing information concerning the genomes of passion fruit species, and interesting results are already available from Santos et al. (2014), Cutri and Dornelas (2012) and Yotoko et al. (2011). These genomics studies have the potential to enhance the development of new markers [e.g., SSR markers, expressed sequence tags (ESTs), and SNPs] and expand discussions of the structure, organization, and evolution of the *Passiflora* genome.

Moreover, the development and use of large-scale genotyping should enable the use of genome-wide selection strategies, thereby enhancing the association of molecular diversity data with characteristics of agronomic interest.

5.4 Conclusions

Despite the advances observed over recent decades with regard to the characterization and use of the diversity within *Passiflora*, the scarcity of genetic and population information enables only a preliminary understanding of the distribution and magnitude of the genetic variability within passion fruit species. Given the increase of human interventions in the environment and the abiotic effects driven by climatic changes, population genetics remains open to studies regarding the conscientious management of *Passiflora* biodiversity.

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Chapter 6
Genetic Diversity and Erosion in *Hevea* Rubber

P.M. Priyadarshan

Abstract Genetic diversity keeps the soil fertile, recycles all nutrients, and cleans the air and water. The richer the genetic baggage, the higher shall be the capacity to fight different fungi, virus, or bacteria. Like other essentials, rubber is an industrial commodity that is indispensable to humans with more than 55,000 vivid products made from it. *Hevea*, rubber originated in Amazon, where 1652 plants belonging to 107 species in 37 different families are found in about 630 m^2. *Hevea*—rubber originated in such a biologically diversified environment. From the story of the first transfer of rubber seeds from Brazil to Asia, it is difficult to evaluate how narrow the genetic base initially was for what has now become the "Wickham" domesticated population. Much importance was conferred to a small number of 22 seedlings disseminated from Singapore to Malaysia after 1876, and the original Wickham stock was collected in only one Brazilian site, Boïm, on the Western banks of the Tapajoz River. Though generation wise assortative mating as the prime breeding tool was applied to these accessions and Wickham collection, much genetic variation could not be tapped for commercial purpose. The molecular marker systems (all three generations markers) are being applied in *Hevea* rubber. Of these, SNPs are the new generation markers used for Marker-Assisted Selection (MAS). A saturated linkage map of *Hevea brasiliensis* has been accomplished and the whole genome size was calculated as 6×10^8 base pairs. Selection was indirectly toward nuclear DNA polymorphism, while evolving modern clones. mtDNA of Wickham clones has lesser variation because their female progenitors are all primary clones (either PB 56 or Tjir 1). Chloroplast genomes are sufficiently large and complex to include structural and point mutations that are useful for evolutionary studies from intraspecific to interspecific levels. Populations were subjected to several rounds of controlled crossing that further narrowed the diversity. But the strategy followed by the breeders to select only the desirable

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genotypes and to reject the unwanted ones (without assessing the utility other than yield) is the main reason that reduced diversity. Much work at the molecular level had been carried out like for Tapping Panel Dryness, latex production, defense genes and alike. Setting up of a molecular library for *Hevea* and scientists working worldwide contributing to this library will be a good option to study and document molecular diversity.

Keywords Genetic erosion · *Hevea* rubber · Allied species · Amazon · mtDNA · cpDNA · Molecular diversity · Breeding · Trees

6.1 Introduction

Nearly 10,000 different plant species have been used by humans for food and fodder production since the agriculture began 12,000 years ago (Wadley and Martin 1993). Farming first began in the Fertile Crescent, which stretches from Israel north to southeast Turkey and then curves southeast to the Persian Gulf (Zeder 2008). (see http://www.ngdc.noaa.gov/paleo/ctl/10k.html for further details). However, agriculture was invented independently in other parts of the world as well. World population was only five million then, which is seven billion today. But as of now, just 150 crops feed most human beings on the planet, and just 15 crops provide 90 % of food energy—wherein, wheat, rice, maize, and potato alone provide 60 % (Xu 2010; Ji et al. 2013). Most of a crop's varietal diversity has been lost through genetic erosion due to extensive cultivation of a few so-called high yielding varieties. In these species, a combination of genes defines the characteristics of species morphology and their capacity to interact with the world. The gene content and diversity among plant species are intriguing. The total amount of genes differs as in bacteria with as many as 1600 genes (Cole et al. 2001) and mammals with 30,000 (Waterston et al. 2002). Among flowering plants, rice has 41,000 genes (Sterck et al. 2007), with *Paris japonica* having the biggest genome (Pellicer et al. 2010). This genetic baggage is passed through each generation and causes a species to evolve ways and means to confront with natural selection.

Genetic diversity plays a crucial role in the stability of our ecological system. Every species fulfills a role in the earth's biosphere and assures ecological survival. By this, biodiversity keeps the soil fertile, recycles all nutrients, and cleans the air and water. The richer the genetic baggage, the higher shall be the capacity to fight different fungi, virus, or bacteria. It is the diversity of genetic baggage that makes natural extinction so rare. Basically, biodiversity provides everything humans need to survive like food, fresh air, clean water, clothing, medicine, wood, and various raw materials for industrial uses. A rich ecological environment is indeed very complex, and is impossible for humans to recreate. Genetic erosion is wiping out millions of years of evolution and a loss in biodiversity is irreparable. In recent years, there is a fillip which contests that Genetically Modified Organisms (GMOs) can bring in genetic diversity. With the advent of transgenic

plants, the problem of gene flow that may turn as a cause for ecological risk stems special significance (Hammer and Teklu 2008). This contention requires ample debate.

Genetic erosion is the loss of genetic diversity—often magnified or accelerated by human activities. In native plant populations, genetic erosion results from habitat loss and fragmentation, but it also can result from a narrow genetic base in the original collections or by practices that reduce genetic diversity. The loss of biological diversity has been measured traditionally by frequency of species extinctions. In general, genetic erosion is loss of genetic diversity within a species. It can represent the loss of entire populations genetically differentiated from others, the loss or change in frequency of specific alleles within populations or over the species as a whole, or the loss of allele combinations. Quite often, cultivation of a limited number of high-yielding genotypes can also lead to genetic erosion.

6.2 Genetic Diversity in *Hevea* Rubber

Like other essentials, rubber is an industrial commodity that is indispensable to humans with more than 55,000 vivid products made from it. The *para* rubber tree, the Euphorbiaceous *Hevea brasiliensis* (Willd. *ex* A. Juss.) Müll Arg, is the chief contributor to natural rubber production worldwide (Priyadarshan and Clément-Demange 2004). Rubber is synthesized in over 2500 plant species, confined to 300 genera of seven families: Euphorbiaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Moraceae, Papaveraceae, and Sapotaceae (Backhaus 1985; Lewinsohn 1991; Cornish et al. 1993). At least two fungal species (*Lactarius deceptiva* and *Peziza* sp.) are also known to make natural rubber (Stewart et al. 1955). The Euphorbiaceae family is extremely diverse and considered to be polyphyletic (Webster 1994). Much of the diversity of the center of origin (Amazon basin, Brazil) is being lost due to extensive deforestation (Fig. 6.1).

Today, the Amazon River is the most voluminous river on Earth, (eleven times the volume of Mississippi) that drains an area equivalent in size of United States. Every day, up to 500 billion cubic feet of water (5,787,037 cu ft/s) flows into the Atlantic. The Amazon River Basin is home to the largest rainforest on Earth. The basin covers some 40 % of the South American continent and includes parts of nine South American countries, viz., Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, Suriname, and French Guiana. The Amazon basin consists of enormous trees, some exceeding a height of 100 m, with an incredible number of species growing side by side in the greatest profusion arranged in different strata. For example, in Manaus (Brazil), 1652 plants belonging to 107 species in 37 different families were found in about 630 m^2. Ducke (1941) estimated 2500 tree species and as many as 100 arboreal species have been counted on a single acre of forest with hardly any one of them occurring more than once. Papers of Seibert (1947) and Schultes (1945) further confirm this enormous diversity. Latest analysis suggests that lowland Amazonia harbors 3.9×10^{11} trees and ~16,000

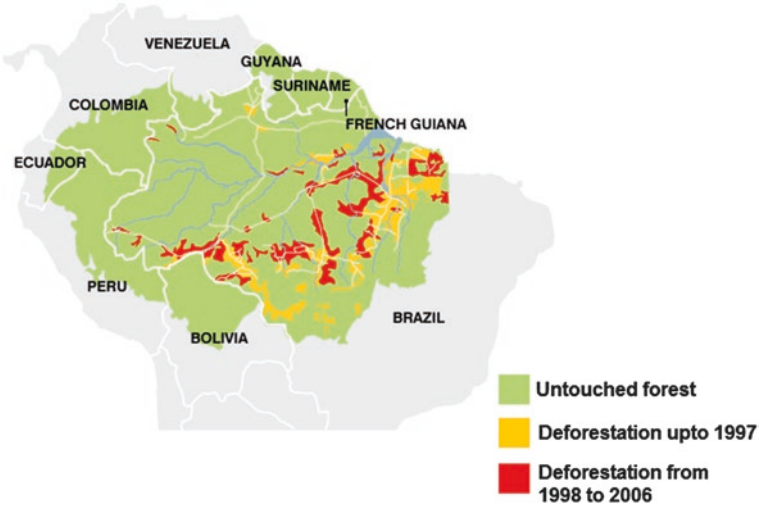

Fig. 6.1 Loss of diversity in the centre of origin (Amazon basin, Brazil) due to extensive deforestation

tree species (Steege et al. 2013). According to the mathematical model used in the study, roughly 6,000 tree species in the Amazon have populations of lesser than 1000 individuals, which automatically qualifies them for inclusion in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. The problem is that these species are so rare that scientists may never find them. The Amazon forest has a strikingly layered structure. The canopy of sun-loving giants, soar to as much as 40 m above the ground and a few, known as emergents, rise beyond such canopies, frequently attaining heights of 70 m. Their straight, whitish trunks are covered with lichens and fungus. A characteristic of these giant trees is the buttresses, or basal enlargements of their trunks, which presumably help stabilize the top heavy trees during infrequent heavy winds. Further characteristics of the canopy trees are their narrow, downward-pointing "drip-tip" leaves that easily shed water. Flowers are inconspicuous. Among the canopy species, prominent members include the rubber tree (*H. brasiliensis*), the silk cotton (*Ceiba pentandra*), the Brazil nut (*Bertholletia excelsa*), the sapucaia (*Lecythis*), and the sucupira (*Bowdichia*). Many creatures, including monkeys and sloths, spend their entire lives in this sunlit canopy.

The Amazon basin covers a surface area of 4,100,000 km^2 (1,583,000 square miles), of which around 3.4 million km^2 (1.3 million square miles) are presently forested (Schroth et al. 2004). Accounting for parts of the Amazon outside Brazil, the total extent of the Amazon is estimated at 8,235,430 km^2 (3,179,715 square miles). By comparison, this is equivalent to the land area of the USA (including Alaska and Hawaii) which is 9,629,091 km^2 (3,717,811 square miles). In total, the Amazon River drains about 6,915,000 km^2 (2,722,000 square miles), or roughly 40 % of South America (Schroth et al. 2003).

Amazonian evergreen forests account for about 10 % of the world's terrestrial primary productivity and 10 % of the carbon stores in ecosystems (Melillo et al. 1993)—of the order of 1.1×10^{11} t of carbon (Tian et al. 2000). Amazonian forests are estimated to have accumulated 0.62 ± 0.37 t of carbon ha^{-1} $year^{-1}$ between 1975 and 1996 (Tian et al. 2000). Fires related to Amazonian deforestation have made Brazil one of the top greenhouse gas producers. Brazil produces about 300 million tons of CO_2 a year; 200 million of these come from logging and burning in the Amazon. Despite this, Brazil is listed as one of the lowest per capita (rank 118) in CO_2 emissions according to the US Department of Energy's Carbon Dioxide Information Analysis Center (CDIAC).

From the story of first transfer of rubber seeds from Brazil to Asia (Dean 1987; Baulkwill 1989), it is difficult to evaluate how narrow the genetic base initially was for what has now become the "Wickham" domesticated population. Much importance was conferred to a small number of 22 seedlings disseminated from Singapore to Malaysia after 1876; but a significant part of the Wickham seedlings which germinated in Kew Gardens was then sent to Ceylon (now Sri Lanka), raised and disseminated to different countries, especially India (Fig. 6.2). However, it must be underlined that the original Wickham stock was collected in only one Brazilian site, Boïm, on the Western banks of the Tapajoz River, not far from Santarem. All these contentions are debatable (Thomas 2001). Directional selection applied to such populations for more than a century, and the limitation of the low fruit-set in *Hevea* probably further contributed to reduce the genetic base.

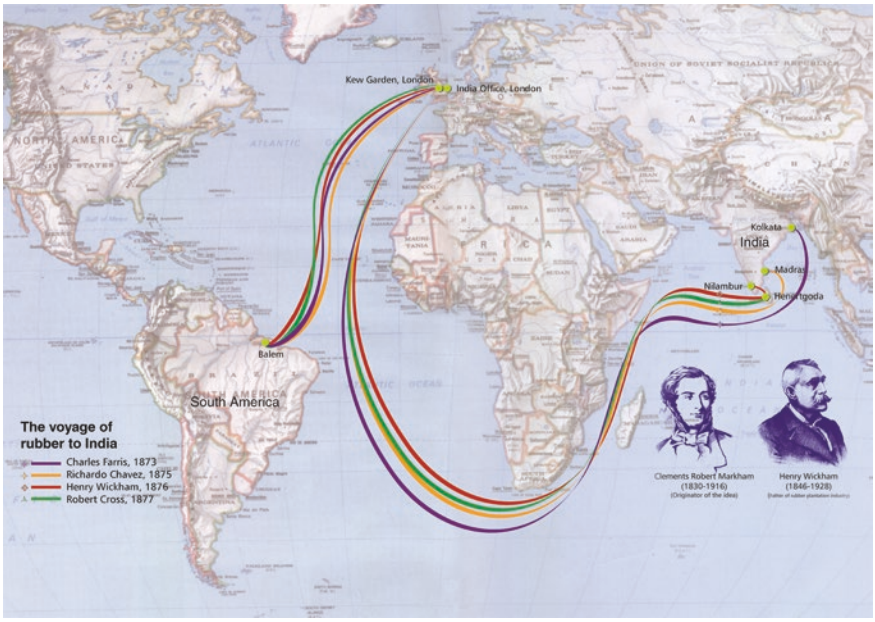

Fig. 6.2 Voyage of *Hevea* rubber to East Asia

Genetic diversity can now be compared to that of the available wild Amazonian populations by use of molecular genetic markers (MGMs).

The "Wickham" population developed in Asia and issued from the collection of seeds in Brazil by Wickham in 1876, has been the basis for rubber domestication and was reputed to have a narrow genetic base.

This justified the organization of other collections and transfers of wild germplasm from Amazonia to the main rubber producing countries, mainly for *H. brasiliensis* but also for allied species. Moreover, Ford and Firestone in Latin America, as well as Brazilian research contributed to create a stock of selected "Amazonian" and "Wickham × Amazonian" germplasm (F, FX, MDF, FDR, IAN, and IAC clones).

Many other introductions from Brazil to Asia and also Africa were carried out between 1876 and 1974, including some species different from *H. brasiliensis* (Dijkman 1951; Brookson 1956; Baptist 1961; Wycherley 1968; Hallé and Combe 1975; Nicolas 1976; Ong et al. 1983; Tan 1987; Ong and Tan 1987). All collections were quantitatively rather limited, especially for *non-brasiliensis* species.

Since the introduction of rubber to southeast Asian countries by Wickham and Cross in 1877 through Kew Botanic Gardens, there have been attempts to collect new material and increase the genetic diversity. Between 1945 and 1982, at least 10 collections from Brazil (mostly Rondonia) were undertaken (Gonçalves et al. 1982). During 1951–1952, 1614 seedlings of five *Hevea* species (*H. brasiliensis*, *H. guianensis*, *H. benthamiana*, *H. spruceana, and H. pauciflora*) were introduced in Malaysia (Tan 1987). Seeds of different Hevea species were also imported from the Schultes Museum at Belem, Brazil in 1966 to Malaysia. In Sri Lanka, 11 clones of *H. brasiliensis* and *H. benthamiana* and 105 hybrid materials were imported during 1957–1959, through collaboration of USDA, IAN (Brazil), and Liberia. Many of these clones were later given to Malaysia which were used for further breeding programs at RRIM (Tan 1987). It is very evident that *Hevea* rubber was evolved because of evolution spanning over thousands of years. Today, the cultivation of *Hevea* rubber spans several continents and new environments that are both ideal and with constraints (Priyadarshan 2003) (see Table 6.1).

6.2.1 IRRDB Explorations

With the initiatives taken up by the International Rubber Research and Development Board (IRRDB), 64,734 seeds, 1413 m of budwood from 194 high-yielding trees and 1160 seedlings were collected during 1981 from Acre, Rondonia, and Mato Grosso states of Brazil, from 60 different locations spread to 16 districts (Nicolas 1981; Nouy 1982; Tan 1987; Simmonds 1989). Of this, 37.5 % of the seeds went to Malaysia and 12.5 % to Côte d'Ivoire while half of the collections were retained in Brazil. The clonal selections were brought to

Table 6.1 Climatic features of rubber growing countries

Country	General climate
Brazil	Range: equatorial, tropical, semi arid, high land tropical and sub tropical. Annual average temperature in the Amazon region is 22–26 °C Brazil is in the south of the equator, seasonal changes are vice-versa compared to north of the equator. Plateau of Sao Paulo is non-traditional area for rubber
China	Extremely diverse, tropical in south to sub arctic in the north, with great climatic differences resulting from the monsoon, the expanse of the land mass, and the considerable differences in altitude. Typhoons are prudent in southeast China between July and September. China is a non-traditional zone for rubber
Côte d'Ivoire	Tropical along coast, semi arid in far north; three seasons—warm and dry (November to March), hot and dry (March to May), hot and wet (June to October); Three main climatic regions: the coast, the forest and the savannah. Low rainfall areas in north (less than 1300 mm) are non-traditional experimental zone for rubber
India	Tropical monsoon type with winter (November to January), Summer (March to May), southwest monsoon season (June to Sept.) and post monsoon or northeast monsoon season (Oct. to Dec.). Most of the rainfall brought by southwest monsoon. Because of the geographical diversity of India, regional climate conditions in the extreme north, east and west vary from the general conditions given here. Specific areas of west, east and northeast are non-traditional for rubber
Indonesia	Tropical, climate even all year around. Heavy rainfall usually between Dec. and Jan. The equatorial position of the country makes opposite climates in the north and the south
Liberia	Tropical; hot, humid; dry winters with hot days and cool to cold nights; wet, cloudy summers with frequent heavy showers.
Malaysia	Tropical, annual southwest (April to October) and northeast (October to February) monsoons
Nigeria	Varies; equatorial in south, tropical in center, arid in north. Two principal wind currents affect Nigeria; the *harmattan*, from the northeast, is hot and dry and carries reddish dust from the desert and causes high temperatures during the day and cool nights. The southwest wind brings cloudy rainy weather
Sri Lanka	Tropical monsoon; northeast monsoon (December to March); southwest monsoon (June to October)
Thailand	Tropical; rainy, warm, cloudy southwest monsoon (mid-May to September); dry, cool northeast monsoon (November to mid March); southern isthmus always hot and humid. North and northeast areas are non-traditional for rubber
Vietnam	Tropical in south; monsoonal in north with hot, rainy season (mid-May to mid September) and warm, dry season (mid-October to mid March). Diverse range of latitude, altitude and weather patterns produces enormous climatic variation. North Vietnam like China has two basic seasons: a cold humid winter from Nov. to April, and warm, wet summer for the reminder of the year. The northern provinces share the climate of the north, while the southern provinces share the tropical weather of the south. South Vietnam is relatively warm. Central highlands and the coastal regions are non-traditional areas for rubber

Sources www.britannica.com; www.worldatlas.com; www.wmo.ch; www.usda.gov; www.iwmi.org

Malaysia and Côte d'Ivoire after quarantine measures (of one year in Guadalupe Island) for South American Leaf Blight (SALB—*Microcyclus ulei*). IRRDB supports germplasm centers based in Malaysia and Côte d'Ivoire to conserve these materials. After the establishment of two IRRDB Germplasm Centres in Malaysia and Côte d'Ivoire, other IRRDB member countries were supplied with budwood from this material according to their request. Malaysia alone established 8900 seedlings and 109 clones from this exploration (Pushparajah 2001). Crosses between Wickham and Amazonian accessions could introduce more variation. Breeding at Institut de Recherches sur le Caoutchouc en Afrique (IRCA), Côte d'Ivoire, under the auspices of CIRAD, involve utilization of Amazonian accessions (Clement-Demange et al. 1998).

The field evaluation of this wild Amazonian germplasm showed that the latex yield was as low as about 10 % of GT1, one of the most cultivated clones. Attempts to improve it through Wickham × Amazonian crosses resulted in recombinants with a still low yield, ranging between 30 and 50 % of the level of GT1, probably due to the important genetic gap lying between the two populations. Conversely, a wide variability was found within these crosses for growth, enabling the selection of very vigorous Wickham × Amazonian clones. A clear difference in branching habit could be observed between accessions from Acre and Rondonia, which more often have tall trunks with poor branching located at high height, and those from Mato Grosso, which display abundant branching at low height. Obviously, this wild Amazonian germplasm is bearing an important genetic burden in terms of unfavorable alleles. From the evaluation of the IRRDB 1981 germplasm in Côte d'Ivoire, a working population of 287 accessions was selected, taking into account genetic diversity but mainly based on yield; the average yield level of this population is estimated at 36 % of the level of GT1 (Nicolas et al. 1988; Clément-Demange et al. 1998). Four genetic groups of this population could be the base of a population pre-breeding work aimed at improving their yield level before testing them by crossing with the Wickham population.

In 1995, an expedition was launched by RRIM to collect rubber seeds from Brazil. From this collection, about 50,231 seedlings were planted in Malaysia, including allied species (RRIM Annual Report 1997; MRB Annual report 1999). In order to enlarge genetic variability of *Hevea*, some research was carried out on mutation breeding (Ong and Subramaniam 1973; Markose et al. 1977), and on polyploidization of the $2n = 36$ *H. brasiliensis* species' (Mendes and Mendes 1963; Shepherd 1969; Zheng et al. 1980, 1981). An artificial triploid has been produced by crossing a diploid and a tetraploid (Saraswathyamma et al. 1988). Naturally occurring triploids have been reported (Nazeer and Saraswathyamma 1987). The existence of some putative genetically dwarf or semi-dwarf genotypes was mentioned (Ong et al. 1983); *H. camargoana* would have a dwarf growth habit (Gonçalves et al. 1982). It was attempted to associate some MGMs with the dwarfing trait (Venkatachalam et al. 2004).

6.3 *Hevea* as a Species Complex

The genus *Hevea* is basically comprised of 10 species: *H. brasiliensis*, *H. guianensis*, *H. benthamiana*, *H. pauciflora*, *H. spruceana*, *H. microphylla*, *H. rigidifolia*, *H. nitida*, *H. camporum*, *and H. camargoana* (Webster and Paardekooper 1989; Wycherley 1992; Schultes 1990). All species originated in Brazil. Seven species are found in the upper Rio Negro region, considered to be the centre of origin of the genus. *H. brasiliensis* is found in Southern areas outside of this centre, in the upper Rio Madeira, where five other species are represented. It has generally been assumed that the species are freely inter-compatible (Seibert 1947; Baldwin 1947). Pires (1981) observed natural hybrids of *H. camargoana* × *H. brasiliensis*, and Gonçalves et al. (1982) analyzed progenies issued from hand pollination from this type of crossing. Consequently, *Hevea* species might be considered as a species complex, due to the absence of a strict barrier to recombination between species. Many efforts led to the identification of certain types which were formerly presented as other possible species. *H. paludosa* was identified in Brazil by Ule in 1905 and is often considered as an eleventh species (Gonçalves et al. 1990; Priyadarshan and Gonçalves 2003). An elaborate description of taxonomical and botanical aspects of *Hevea* has been reviewed by Schultes (1977, 1987, 1990) and Wycherley (1992).

All *Hevea* species have $2n = 36$ chromosomes, with the exception of one triploid clone of *H. guianensis* ($2n = 54$) and the existence of one genotype of *H. pauciflora* with $2n = 18$ (Baldwin 1947; Majumder 1964). Although *Hevea* behaves as a diploid, it is believed to be an amphidiploid ($2n = 36$; $x = 9$) that stabilized during the course of evolution. This contention is supported by the observance of tetravalents during meiosis (Raemer 1935; Ong 1975; Wycherley 1976). In situ hybridization studies revealed two distinct 18S-25S rDNA loci and one 5S rDNA locus, suggesting a possible allotetraploid origin with the loss of 5S rDNA during the course of evolution (Leitch et al. 1998). But locus duplications are infrequent in Hevea genome, and they could have occurred due to chromosomal modifications posterior to the polyploidization event (Seguin et al. 2003); consequently, the two unknown ancestral genomes of *Hevea* would have strongly diverged (Priyadarshan and Clément-Demange 2004; Priyadarshanet al. 2008).

The species are inter-crossable (Clement-Demange et al. 2000). Schultes (1977) and Wycherley (1992) refer the readers to excellent reviews on this subject. The taxonomic considerations from 1874 to 1970 delineated the genus with several species at different occasions. Though even 24 species were considered during 1906, the species concept crystallized with nine species in 1970 (Schultes 1977). A tenth species, *H. camargoana* was added during 1971 (Schultes 1987). Brazil considers 11 species including *H. paludosa* (Pires 1973; Goncalves et al. 1990). Three botanists shall be considered principal workers on species delineation—Baldwin, Seibert, and Schultes—who during their classical exploratory studies contributed significantly toward the botany of Hevea. A Harvard University Gazette Archives says “Schultes’ field work, conducted mostly in the Colombian

Amazon beginning in 1941, made him a leading voice in the field and one of the first in the 1960s to warn about destruction of the rainforests and disappearance of their native people" (see www.harvard.edu).

A summary of the salient features of different species of *Hevea* is presented in Table 6.2. All species have 36 chromosomes ($2n = 36$ $x = 9$). *H. brasiliensis* behaves as an amphidiploid (Ong 1979). However, this contention is disputed at the molecular level. In situ hybridization studies revealed two distinct 18S-25S rDNA loci and one 5S rDNA locus (Leitch et al. 1998), suggesting a possible allotetraploid origin with the loss of 5S rDNA during the course of evolution. Hence,

Table 6.2 Allied species of the genus *Hevea*—occurrence and features

Species	Occurrence	Notable features[1]
H. benthamiana Muell.-Arg.	North and West of Amazon forest basin, upper Orinoco basin (Brazil)	Complete defoliation of leaves Medium size tree Habitat: swamp forests
H. brasiliensis (Willd. ex. A. de. Juss.) Muell.-Arg.	South of Amazon river (Brazil, Bolivia, Ecuador, Peru)	Complete defoliation of leaves From medium to big tree size Habitat: well drained soils
H. camargoana Pires	Restricted to Marajo island of Amazon river delta (Brazil)	Possibility of natural hybridization with *H. brasiliensis* from 2 to 25 m tree height Habitat: seasonally flooded swamps
H. camporum Ducke	South of Amazon between Marmelos and Manicoré rivers tributaries of Madeira river	Retain old leaves until new leaves appear. Maximum 2 m tall Habitat: dry savannahs
H. guianensis Aublet	Throughout the geographic range of the genus (Brazil, Venezuela, Bolivia, French Guyana, Peru, Colombia, Surinam, Ecuador)	Retain old leaves until new leaves and inflorescences appear. Grows at higher altitudes (1100 m MSL) Medium size tree Habitat: well drained soils
H. microphylla Ule	Upper reaches of Negro river in Venezuela. It is not found in other region of geographic range of the genus	Complete defoliation of leaves. Small trees. They live on flooded area (igapós) Habitat: sandy or **lateritic** soils
H. nitida *Mart. ex Muell.-Arg.*	Between the rivers Uaupes and Icana tributaries of the upper Negro river (Brazil, Peru, Colombia)	Inflorescences appear when leaves are mature. Small to medium size trees (2 m) Habitat: **quartzitic** soils
H. pauciflora (Spr.ex Bth.) Muell.-Arg.	North and West of Amazon river (Brazil, Guyana, Peru). Distribution discontinuous due to habitat preferences	Retain old leaves until new leaves and inflorescences appear. No wintering. Small to big size trees Habitat: well drained soils, rocky hill sides

(continued)

Table 6.2 (continued)

Species	Occurrence	Notable features[1]
H. rigidifolia (Spr. ex Bth.) Muell-Arg.	Among Negro river and its **affluents**. Uaupes and Içana rivers (Brazil, Colombia and Venezuela)	Retain old leaves even after inflorescences appear. Small tree from savannahs. Sometime tall, with small crown on the top Habitat: well drained soils
H. spruceana (Bth.) Muell.-Arg.	Banks of Amazon, Rio Negro and lower Madeira (Brazil)	Retain old leaves until new leaves and inflorescences appear. Flowers reddish purple. Medium size tree Habitat: muddy soils of islands
H. paludosa Ule[2]	*Marshy areas of Iquitos, Peru*	Small leaflets, narrow and thin in the fertile branches; up to-30 m height Habitat: marshy areas

After Wycherley (1992), Schultes (1977), Goncalves et al. (1990), Pires (1973) and Brazil (1971)
[1]Wintering characteristics mentioned here has a bearing on the incidence of fungal diseases especially secondary leaf fall (Oidium) since retention of older leaves may make the tree 'oidium escape'. Dwarf types are desirable of the possible wind fastness. All species are diploid ($2n = 36$) (Majumder 1964), and are crossable among themselves (Clement-Demange et al. 2000)
[2]Pires (1973) considered 11 species including *H. paludosa*; Brazil (1971) considers 11 species

as long as a potential ancestor with $2n = 18$ is unknown, rubber tree will be considered as an amphidiploid. The genus *Hevea* could eventually be considered as a species complex.

6.3.1 Distribution of Allied Species

The distribution of allied species of *Hevea* is wide among the countries of South America (Fig. 6.3a, b). *Hevea* species are indigenous to Bolivia, Brazil, Colombia, French Guiana, Guayana, Peru, Surinam, and Venezuela. All species occur in Brazil, the center of origin. Four species have been found in Colombia and three occur in Venezuela. Two occur in Bolivia and French and British Guyanas. *H. guianensis* is the most widely adapted species (Fig. 6.3a) (Priyadarshan and Goncalves 2003). These species of *Hevea* were evolved in Amazonian forests over 100 thousand years ago (Clement-Demange et al. 2000). It is pertinent that species adaptation to a particular area is as per climatic and edaphic requirements. Species like *H. camporum*, *H. paludosa and H. rigidifolia* shows only limited adaptation. The specific adaptation needs to be closely studied, with reference to climatic and edaphic factors, when clones are to be developed for new environments especially for marginal areas. It is worthwhile to note that except *H. benthamiana* (F 4512, F 4542), none of the other species has been actively utilized for the improvement of rubber tree.

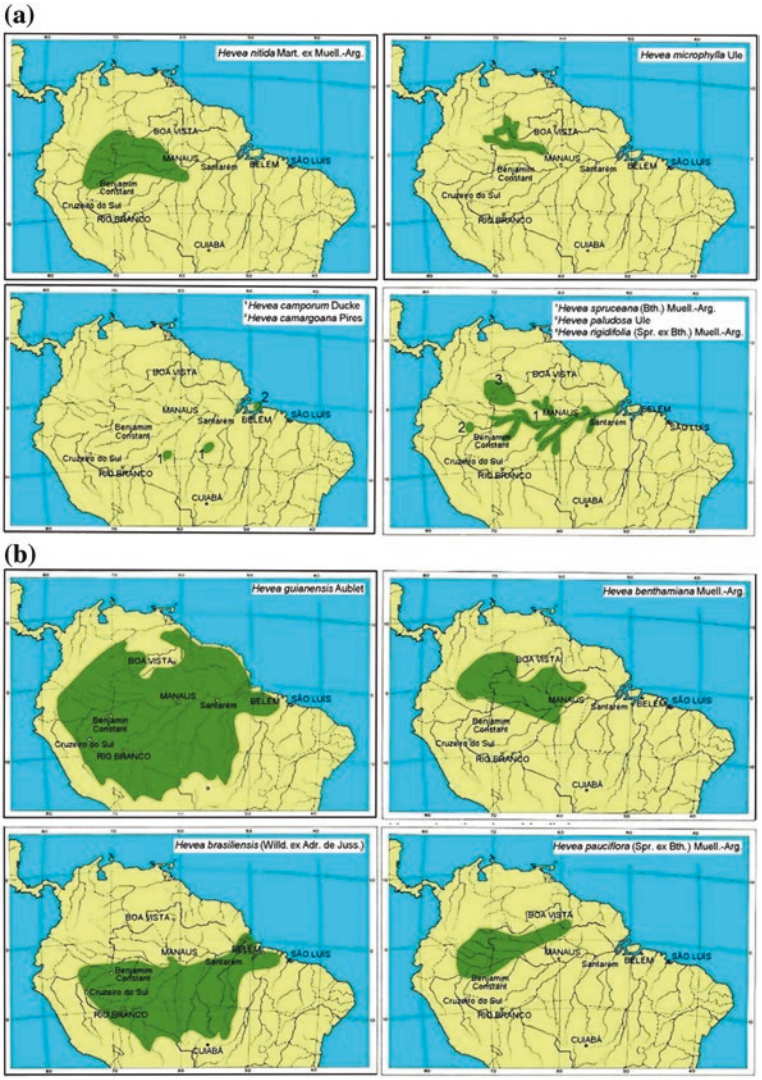

Fig. 6.3 The distribution of allied species of *Hevea* among the countries of South America

6.4 Molecular Diversity and Genomics

The association between DNA sequence variation and heritable attributes has helped to define variations in plants at the molecular level. However, identification and utilization of recombinants with desirable traits is time consuming and laborious in rubber due to long generation time and larger size of the crop. With

the advent of DNA markers, localization of desirable traits has become routine. The molecular marker systems can be broadly classified into three viz., first generation (RFLPs, RAPDs and modifications); second generation (simple sequence repeats—SSRs, Amplified Length Polymorphism—AFLPs) and third generation markers (Expressed Sequence Tags—ESTs, Single Nucleotide Polymorphism—SNPs) (Gupta et al. 2001). Of these, SNPs are the new generation markers used for Marker-Assisted Selection (MAS). All marker systems, except SNPs have been applied in *Hevea* to facilitate identification and characterization of genes (Saha and Priyadarshan 2012). Recently, a saturated linkage map of *H. brasiliensis* has been accomplished (Lespinasse et al. 2000a). Efforts were on for breeding *Hevea* at the molecular level ever since Low and Bonner (1985) characterized nuclear genomes containing 48 % of most slowly annealing DNA (putative single copy) and 32 % middle repetitive sequences with remaining highly repetitive or palindromic ones. Also, the whole genome size was calculated as 6×10^8 base pairs.

Low and Bonner (1985) characterized *Hevea* nuclear genome as containing 48 % of slowly annealing DNA (putative single copy) and 32 % middle repetitive sequences with remaining highly repetitive or palindromic DNA. The whole nuclear genome size was first estimated as 6×10^8 base pairs. Estimation with flux cytometry demonstrated 1.9×10^9 base pairs for *H. brasiliensis*, *H. benthamiana*, *H. guianensis*, *H. pauciflora*, *and H. spruceana* (Seguin et al. 2003). The evolution of cytoplasmic genome was slower, due to the lack of genetic recombination through meiosis. The estimated mean molecular size of chloroplast DNA (ct DNA) is 152 kb (Fong et al. 1994). Differentiation of the genus into species appears to be linked with the evolution of the Amazonian forest over the last one hundred thousand years. Alternations of humid and semi-arid periods responsible for the forest extension or fragmentation resulted in the formation of forest islets. These are assumed to have become zones of protection and differentiation under local selection pressures.

Seguin et al. (2003) proposed a general organization of *H. brasiliensis* germplasm with 6 genetic groups: **group 1** made up with the two districts AC/T (Tarauaca) and AC/F (Feijo) in the western part of Acre, and with the Calima component of the Schultes collection; **group 2** made up with the three districts AC/B (Brasileia), AC/S (Sena Madureira), and AC/X (Xapuri) in the eastern part of Acre; **group 3** made up with the six following districts of Rondonia: RO/A (Ariquemenes), RO/C (Calama), RO/CM (Costa Marques), RO/J (Jaru), RO/JP (Jiparana), RO/OP (Ouro Preto), the district MT/VB (Vila Bella) of Mato Grosso, and accessions Madre de Dios Firestone (MDF) from the Firestone collection in Peru; **group 4** made up with three districts MT/A (Aracatuba), MT/C (Juruena), and MT/IT (Itauba) of Mato Grosso, and the district RO/PB (Pimenta Bueno) of Rondonia; **group 5** made up with the Palmira component of the Schultes collection; and **group 6** made up with the domesticated Wickham population (Fig. 6.4). Even if no prediction can be made about the progenies of crosses between these groups, they can be used as a base for managing the genetic variability in the long term and organizing the recombination process. Methodological researches have been carried out in order to select the genotypes for making up a collection of

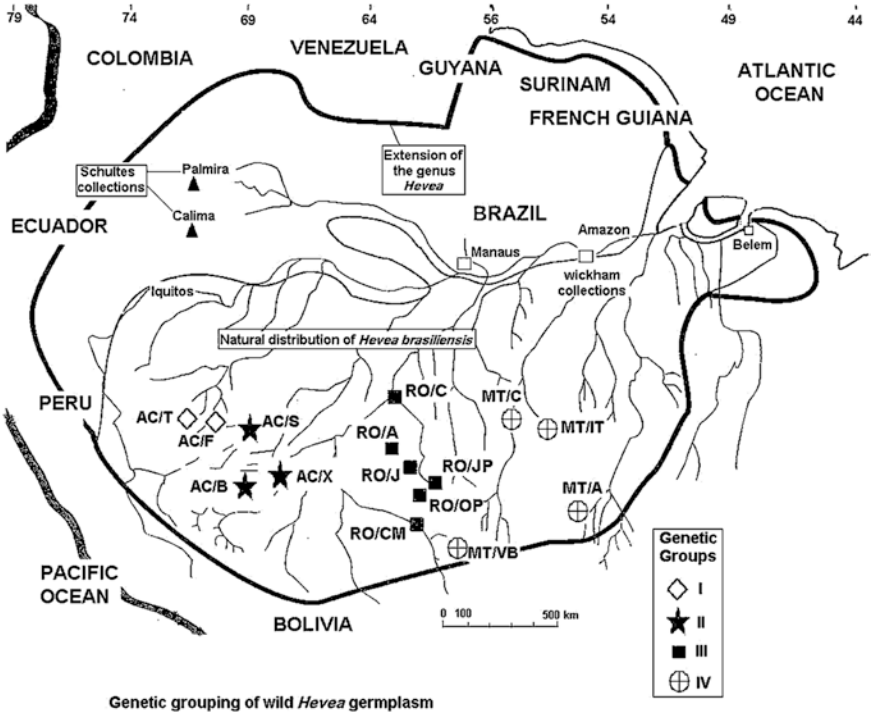

Fig. 6.4 Molecular genetic grouping of Amazonian accessions (after Besse et al. 1994)

reduced size of the Amazonian germplasm, representative of the predominant part of the total variability of this germplasm, according to the concept of "core collection" (Hamon et al. 1998). The germplasm characterization and diversity analysis studies coordinated by CIRAD were funded by the European Union from 1985 to 1997. On the contrary, Lekawipat et al. (2003) used 12 microsatellite markers to detect DNA polymorphism among 108 accessions of *H. brasiliensis* including 40 Wickham clones and 68 wild accessions (1981 Amazonian accessions). Genetic similarity values between genotypes calculated from all the microsatellite markers were used to produce a dendrogram of the relationship among accessions, using the unweighted pair-groups method with arithmetic average. A total of 170 alleles were detected. The number of alleles ranged from 5 to 21, with an average of 14 alleles per marker. The results clearly demonstrated that wild accessions are more polymorphic than cultivated Wickham clones and could be divided into three clusters, depending on the geographical origin of collection areas such as Acre, Rondonia, and Mato Grosso state. Despite the narrow genetic basis of Wickham clones, their high level of polymorphism could be detected.

6.4.1 Gene Flow and Paternity Identification

Pre-breeding of the Amazonian genetic groups was considered based on recombination through seed gardens. For methodological purposes, one seed garden made up with 50 Amazonian genotypes and GT1 clone, planted at CNRA (Côte d'Ivoire) was subjected to the analysis of gene flux and paternity identification with isozymes and microsatellites (Blanc et al. 2001; Lidah 2005). Paternity identification with microsatellites was carried out with the Cervus software (Marshall et al. 1998). A high level of confidence was found for paternity identification carried out with eight microsatellite markers. The distribution of the contribution of the different genotypes to pollination was found highly unequal, with four genotypes accounting for 40 %, 14 genotypes accounting for 80 %, and 25 genotypes accounting for 95 % of the total fertilization of the seed garden. The variation of selfing rate was assessed among the genotypes with an average of 5 %, and no selfing was found on GT1 as expected for a male-sterile clone. The isolation of the seed garden was confirmed since no allele other than those belonging to the parental population was found. The efficiency in paternity identification which is made possible by microsatellites suggests the new possibility to exercise selection on seedlings raised from natural pollination and to identify paternity a posteriori only on the best trees. Here, male-sterile clones GT 1 and BPM 24 can be used to ensure cross-pollination.

6.4.2 Breeding Without Breeding (BwB)

The classical breeding methods used by tree breeders rely on pre-determined mating designs. El-Kassaby et al. (2006) has introduced a scheme of Breeding without Breeding (BwB) that allows the assemblage of full-sib (FS) and half-sib (HS) families from seed orchards' naturally pollinated offspring without conducting any crosses. This scheme circumvents artificial mating, focusing instead, on a subset of randomly sampled, maternally known but paternally unknown offspring to delineate their paternal parentage. This method calls for highly informative molecular markers (e.g., SSRs), for pedigree reconstruction (El-Kassaby and Lstibůrek 2009). SSRs are now in a development stage in *Hevea* rubber (Garcia et al. 2011; Li et al. 2012). But this situation shall improve with time. In *Hevea*, well organized breeding orchards that permit pollen from only hetero-neighbors can be subjected for raising such FS and HS families. A three-dimensional hetero-neighbors' layout, as proposed by Simmonds (1986), can be well suitable for such an exercise (Fig. 6.5). This can be used for both breeding FS offspring and for collection of polyclonal seeds. For this, large polyclonal orchards are necessary that can produce thousands of seeds every year. Alternately, a clone evaluation garden laid under completely randomized design (CRD) can also be used for collecting HS seeds. Such HS families shall be raised in closer spacing (2–3 m) that can

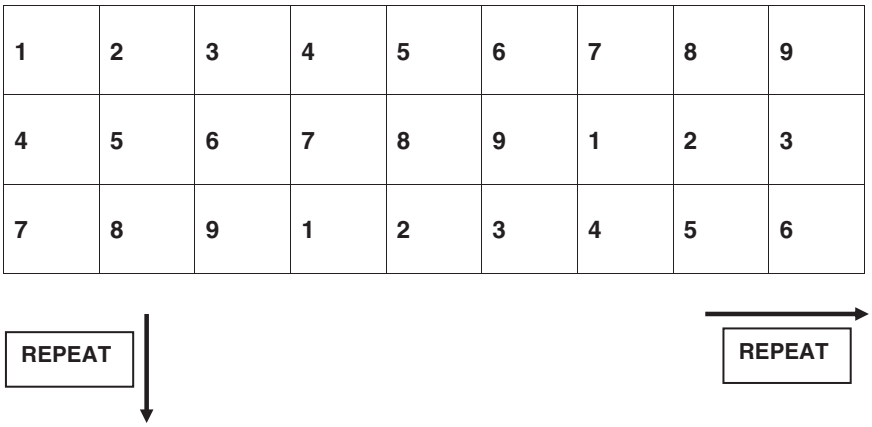

Fig. 6.5 A three dimensional hetero-neighbours' layout for breeding orchard (after Simmonds 1986)

be subjected for yield screening upon attainment of 50 cm girth. A mistake usually being committed by the breeders is to select the early yielding genotypes and reject the ones that are yet to be tapped. This exercise has indirectly culminated in the selection of clones with faster girth increment that reduces gestation period. However, a point to be remembered here is that the left over set may contain high-yielding recombinants, which may attain maturity a little late. While exercising clone selection, both early yielding and late yielding clones are a necessity, to present before the planters an array of clones with vivid attributes to choose from. If SSRs that are linked to QTLs for high yield can be used, then, the exercise can minimize screening process to a great extent. In this way, this method allows the capture of 75–85 % of the genetic response to selection attained through conventional programs without the need to do any controlled pollination or possibly no experimental field testing: both considered to be the most resource-demanding activities in breeding programs. The selections borne out of these HS evaluations can be further confirmed through clonal nursery trials following line RBD with a reference clone. Simultaneously, these selections can be propagated and given for block trials in government owned areas with a reference clone. In this way, a quick derivation of clone can be achieved. For all this, DNA profiles of all available clones is a prerequisite to ascertain the parentage.

6.4.3 Genetic Mapping

The availability of numerous MGMs (Molecular Genetic Markers) led to the development of genetic linkage mapping based on the analysis of the percentage of crossing-over between the loci of two markers during meiosis (a genetic and not a physical distance), and the ranking of the different loci on the different

chromosomes of one species. Due to the heterozygotic nature of rubber clones, the construction of genetic linkage maps in *Hevea* requires specific methodology. Unlike annual crops, a cross between two heterozygous parents in *Hevea* can yield information up to four alleles, which are segregated further. The first comprehensive genetic linkage map of *H. brasiliensis* has been built recently, mainly by use of RFLP markers but also AFLPs, microsatellites and isozymes (Lespinasse et al. 2000a). This was accomplished through a double pseudo-test cross as per the methodology of Grattapaglia and Sederoff (1994) and a map was constituted separately for each parent. Further, homologous markers segregating in both parents were ascertained and consensus map prepared. The parents used were PB260 (PB5/51 PB49) and RO38 (F4542 AVROS363). F4542 is a clone of *Hevea benthamiana* species. The F_1 synthetic map of 717 markers was distributed in 18 linkage groups (LG) corresponding to the 18 chromosomes. This comprised of 301 RFLP, 388 AFLP, 18 microsatellite and 10 isozyme markers. The genetic length of the 18 chromosomes was fairly homogeneous, with an average map length per chromosome of 120 cm. Many AFLP markers were seen in clusters, which were attributed as reduced recombination frequency regions. Though the RFLP markers were well distributed all over the 18 LG, these were insufficient to saturate the map. AFLPs and a few microsatellites together contributed to saturating the map. A partially nonrandom arrangement of duplicate loci observed in RFLP profiles indicate that they have homology descending from a common ancestor (Lespinasse et al. 2000a). The origin of such duplications is still unknown and *H. brasiliensis* continues to behave as a diploid.

In yet another study, Souza et al. (2011) found 603 microsatellite markers, with 309 of them (51 %) showing polymorphism. Chi-square test was carried out on the genotyping polymorphic loci showed that 110 loci followed a segregation ratio of 1:1, 28 followed a ratio of 1:2:1 and 87 (38.7 %) followed a ratio of 1:1:1:1. The map consists of 225 markers, distributed in 23 LG and 2471.2 cm in length with an average genetic distance of 11 cm between adjacent markers. The largest group has 215.9 cm (18 markers) and the smallest has 2.71 cm (2 markers). This reflects a real polymorphism in a FS cross.

Genetic linkage maps associated to phenotyping studies (field evaluation of the genotypes) can generate phenotypic comparisons between a huge number of classes of alleles and lead to the identification of QTLs. The research developed on the cross PB260 × RO38 was targeted to understanding the genetic determinism of the resistance of this cross to SALB, first with manual infection at the laboratory level (Lespinasse et al. 2000b). Eight QTLs, with one predominant on linkage group g13, were identified for resistance in RO38 map through Kruskel-Wallis marker-by-marker test and interval mapping method (Lander and Botstein 1989; Oojen van et al. 1992). The F_1 consensus map confirmed results obtained in parental maps. It was further rationalized that the resistance alleles of RO 38 have inherited from its wild grandparent (*H. benthamiana*) and no favorable allele came from AVROS 363, the Wickham parent. Eight different QTLs for five strains of fungus were found available in RO38, with specificity of resistance to different strains. Field evaluation against the pool of *Microcyclus* strains available in French Guyana was carried out under the real infestation conditions, and it confirmed the presence of the

predominant QTL in g13 previously found under controlled infestation (Le Guen et al. 2003). Then it was shown that this major QTL was no more efficient against two widely virulent and highly aggressive strains; for one of them, another QTL located on the linkage group g12 was able to reduce the aggressiveness. This genetic mapping and QTL approach is currently being continued with other crosses for analyzing the genetic determinism to different sources of South American Leaf Blight (SALB) resistance. Research for identifying and cloning the real genes responsible for this QTL in linkage group g13 is undertaken at CIRAD in the framework of the building of a bacterial artificial chromosome (BAC) bank and of a physical map of the rubber tree genome based on the clone RO38 that inherited the resistance trait from F4542. Among other applications, this will make possible the search for the DNA fragments bearing the QTL g13 and the development of the "chromosome walking" technique towards genes associated with QTL g13 on these fragments. This physical map with a high density of MGMs (fine mapping) will also allow one to assess the stability of linkage between the neighboring genetic markers.

Mantello et al. (2012) studied new genomic microsatellite markers developed and characterized in *H. brasiliensis* and evaluated their transferability to other *Hevea* species. They constructed di- and trinucleotide-enriched libraries. From these two libraries, 153 primer pairs were designed and initially evaluated using nine genotypes of *H. brasiliensis.* A total of 119 primer pairs had a good amplification product, 90 of which were polymorphic. A total of 46 polymorphic markers were characterized in 36 genotypes of *H. brasiliensis*. The expected and observed heterozygosities ranged from 0.1387 to 0.8629 and 0.0909 to 0.9167, respectively. The polymorphism information content (PIC) values ranged from 0.097 to 0.8339, and the mean number of alleles was 6.4 (2–17). The microsatellites were also tested in six other *Hevea* species. The percentage of transferability ranged from 82 to 87 %. Locus duplication was found in *H. brasiliensis* and also in five of other species in which transferability was tested. Six other species from the genus *Hevea* (*H. guianensis*, *H. rigidifolia*, *H. nitida*, *H. pauciflora*, *H. benthamiana and H. camargoana*) being two different genotypes of *H. pauciflora*, were used to evaluate the transferability of the markers. All loci were tested under the same PCR conditions used for *H. brasiliensis*. Of the 46 loci tested, 40 (87 %) were amplified for *H. guianensis* and *H. pauciflora*—(112CNSG), 39 (85 %) were amplified for *H. camargoana*, *H. nitida and H. pauciflora*—(116CNSG), and 38 (82 %) were amplified for *H. benthamiana*. This high percentage of transferability may be useful in the evaluations of genetic variability and to monitor introgression of genetic variability from different *Hevea* species into breeding programs.

6.4.4 Expressed Genes in Hevea

Lekawipat (2004) performed a genetic diversity analysis of *H. brasiliensis* germplasm over 66 Amazonian and 40 Wickham accessions, by use of non-expressed MGMs (12 microsatellites) and also 17 markers of expressed genes (Single Strand

Confirmation Polymorphism—SSCP, based on PCR and the secondary conformation structure of single strand DNA on non-denaturing acrylamide gel, aimed at mutation detection in expressed genes). It was found that microsatellites could detect higher polymorphism than gene specific primers of SSCP in rubber accessions. SSCP markers could not differentiate the Wickham and the Mato Grosso accessions.

In reproductive biology, rubber flower and inflorescence development have been characterized; one important gene regulating flower induction and development (*leafy/floricaula*) was cloned and its expression was analyzed and localized by in situ hybridization (Dornelas and Rodriguez 2005). In post-germination changes in rubber seeds, proteomics (2D-Page and mass spectrometry methods) were implemented for examining the changes in protein expression from the mature seed to the germinated seed (Wong and Abubakar 2005). NMR spectroscopy was used for characterizing *cassiicolin*, the toxin of *Corynespora* (Barthe et al. 2007). Suppression Substractive Hybridization (SSH) technique is currently widely implemented between different couples of mRNA samples for the production of molecular resources by Real-Time-Polymerase Chain Reactions (RT-PCR) in the form of substracted cDNA libraries. SSH is enormously useful in addressing the issues related to Tapping Panel Dryness (TPD). Although a great deal of effort has been made to study TPD in rubber tree, the molecular mechanisms underlying TPD remain poorly understood. Identification and systematic analyses of the genes associated with TPD are the prerequisites for elucidating the molecular mechanisms involved in TPD. Li et al. (2010) made an attempt to decipher the intricacies of TPD with the help of SSH. To identify the genes related to TPD in rubber tree, forward and reverse cDNA libraries from the latex of healthy and TPD trees were constructed using SSH method. Among the 1106 clones obtained from the two cDNA libraries, 822 clones showed differential expression in two libraries by reverse Northern blot analyses. Sequence analyses indicated that the 822 clones represented 237 unique genes; and most of them have not been reported to be associated with TPD in rubber tree. The expression patterns of 20 differentially expressed genes were further investigated to validate the SSH data by reverse transcription PCR (RT-PCR) and real-time PCR analysis. According to the Gene Ontology (GO) convention, 237 unique genes were classified into 10 functional groups, such as stress/defense response, protein metabolism, transcription and post-transcription, rubber biosynthesis, etc. Among the genes with known function, the genes preferentially expressed were associated with stress/defense response in the reverse library, whereas metabolism and energy in the forward one. Systematic analyses of the genes related to TPD suggest that the production and scavenging of reactive oxygen species (ROS), ubiquitin proteasome pathway, programmed cell death and rubber biosynthesis might play important roles in TPD.

MicroRNAs (miRNAs) are set of RNAs that are induced by abiotic stress and regulate gene expression by targeting the cleavage or translational inhibition of target messenger RNAs. Gébelin et al. (2013) studied sequences of miRNAs expressed in latex cells to identify TPD-related putative targets. Deep sequencing of small RNAs was carried out on latex from trees affected by TPD using Solexa technology. The most abundant small RNA class size was 21 nucleotides for TPD

trees compared with 24 nucleotides in healthy trees. By combining the LeARN pipeline, data from the Plant MicroRNA database and *Hevea* EST sequences, 19 additional conserved and four putative species-specific miRNA families were identified that were not found in previous studies. The relative transcript abundance of the *Hbpre-MIR*159*b* gene increased with TPD. This study revealed a small RNA-specific signature of TPD-affected trees. Both RNA degradation and a shift in miRNA biogenesis are suggested to explain the general decline in small RNAs and, particularly, in miRNAs.

Collection of Expressed Sequence Tags (ESTs, or small and partial 5'-end-sequences of expressed genes) that are related with varied metabolic aspects are developed to study genes expressed in latex cells (Garcia et al. 2011; Triwitayakorn et al. 2011; Li et al. 2012; Salgado et al. 2014; Cubry et al. 2014). Entries of these banks are compared with public databases of already known genes for identifying the putative functions of the corresponding genes. These EST banks will also create the way for macro- or microarray-based studies of *Hevea* gene expression. The 'Latex Lambda Triplex' EST-cDNA library (Ko et al. 2003) published in the EMBL/GenBank databases (858 entries) showed that about 16 % of the database matched ESTs encoding rubber biosynthesis-related proteins. Rubber biosynthesis-related genes appeared to be expressed at the maximum, followed by defense-related genes and other protein-related genes (Han et al. 2000). Another EST bank was developed from a latex cDNA library in Malaysia (Chow et al. 2001), with a current number of more than 10,000 entries. Published DNA sequences of the latex allergens were matched against these ESTs, thereby indirectly providing a ranking of the allergens depending on their concentration in the latex. More than 1000 ESTs matched with the sequences of rubber elongation factor (*REF*, or *Hev.b.*1) and small rubber particle protein (*SRPP*, or *Hev.b.*3).

Genes responsible for the synthesis of *rubber transferase*, the key enzyme for polymerisation of polyisoprene (natural rubber), appears to be among the most abundantly expressed genes in the latex (Cornish and Xie 2012). By using sequence information from the conserved regions of cis-prenyl chain elongating enzymes that were cloned earlier, Asawatreratanakul et al. (2003) isolated and characterized cDNAs from *H. brasiliensis* for a functional factor participating in natural rubber biosynthesis. Sequence analysis revealed that all of the five highly conserved regions among cis-prenyl chain elongating enzymes were found in the protein sequences of the *Hevea* cis-prenyltransferase. Northern blot analysis indicated that the transcript(s) of the *Hevea* cis-prenyltransferase were expressed predominantly in the latex as compared with other *Hevea* tissues. *Hevein*, a chitin-binding protein, one of the defense proteins that play a crucial role in the protection of wound sites from fungal attack, is also involved in the coagulation process; it belongs to a multigene family, and the specificity of its expression in the latex is under investigation (Broekaert et al. 1990; Pujade-Renaud et al. 2005). Nearly 12.6 % of the proteins available in the latex are defense related (Han et al. 2000). Among 200 distinct polypeptides (Posch et al. 1997), mainly three rubber synthesis-related genes are expressed in the latex: *REF* (Dennis and Light 1989; Goyvaerts et al. 1991), *hydroxy*-3-*methylglutaryl coenzyme A reductase* (*HMGR*)

(Chye et al. 1992), and *SRPP* (Oh et al. 1999). The most abundantly expressed gene is *REF* (6.1 %) and then *SRPP* (3.7 %) (Han et al. 2000). References and partial or full-length sequences of these cloned genes can be found in the EMBL/GenBank databases.

Unlike photosynthetic genes, transcripts involved in rubber biosynthesis are 20–100 times greater in laticifers than in leaves (Kush et al. 1990). On the other hand, transcripts for chloroplastic and cytoplasmic forms of *glutamine synthetase* are restricted to leaves and laticifers, respectively (Kush et al. 1990), indicating thereby that the cytoplasmic form of *G. synthetase* plays a decisive role in amino acid metabolism of laticifers. The transcript levels of hydrolytic enzymes viz., *polygalacturonase* and *cellulase*, might be taken as indicators for a better development of the laticifers. Genes expressed in the latex of *Hevea* can be divided into three groups based on the proteins they encode: (1) defense-related proteins such as *hevein, chitinase, β*-1,3-*glucanase*, and *HEVER*; (2) rubber biosynthesis-related proteins such as *REF*, *HMGR* (*hydroxymethylglutaryl-coA reductase*) and *hydroxymethylglutaryl-coA synthase* (*HMGS*), *cis-prenyltransferase* (*CIS*), *geranylgeranyl diphosphate* (*GGPP*) *synthase*, *small rubber particle protein* (*SRPP*), *isopentenyl diphosphate* (*IPP*) *isomerase*; and (3) latex allergen proteins such as *Hev.b.*3, *Hev.b.*4, *Hev.b.*5, *Hev.b.*7. Biological functions of the allergenic proteins are largely unknown (Oh et al. 1999).

Mantello et al. (2014) performed RNA sequencing (RNA-seq) of *H. brasiliensis* bark on the Illumina GAIIx platform, which generated 179,326,804 raw reads on the Illumina GAIIx platform. A total of 50,384 contigs that were over 400 bp in size were obtained and subjected to further analyses. A similarity search against the non-redundant (nr) protein database returned 32,018 (63 %) positive BLASTx hits. The transcriptome analysis was annotated using the clusters of orthologous groups (COG), GO, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Pfam databases. A search for putative molecular marker was performed to identify SSRs and SNPs. In total, 17,927 SSRs and 404,114 SNPs were detected. Finally, we selected sequences that were identified as belonging to the mevalonate (MVA) and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways, which are involved in rubber biosynthesis, to validate the SNP markers. A total of 78 SNPs were validated in 36 genotypes of *H. brasiliensis*. This new dataset represents a powerful information source for rubber tree bark genes and will be an important tool for the development of microsatellites and SNP markers for use in future genetic analyses such as genetic linkage mapping, quantitative trait loci identification, investigations of linkage disequilibrium and marker-assisted selection.

6.5 Nuclear versus Cytoplasmic Genetic Diversity

Besse et al. (1994), using 92 clones of Amazonian origin and 73 Wickham clones did an assessment of RFLP profiles. Interestingly, accessions of Brazil Amazonia could be categorized into genetic groups according to their geographic origin

(Acre, Rondonia, Mato Grosso). On the other hand, cultivated clones conserved relatively high level of polymorphism, despite narrow genetic base and continuous assortative mating and selection. As expected, polymorphism is very prudent among allied species of *Hevea*. A comparison of isozyme analysis (Lebrun and Chevallier 1990) with DNA markers showed much similarity (Besse et al. 1994). Identification of all Wickham clones could be done with 13 probes associated with restriction enzyme *Eco RI* (Besse et al. 1993). The cultivated clones are genetically close to the Mato Grosso genotypes. Rondonia and Mato Grosso clones are more polymorphic as per RFLP data (Besse et al. 1994). A Rondonia clone (RO/C/8/9) showed eight specific restriction fragments and a unique malate dehydrogenase (MDH) allele, indicating this clone is of interspecific origin. Such molecular markers are useful in rubber tree breeding since no distinct morphological traits exist. Mitochondrial DNA (mtDNA) polymorphism was analyzed in 345 Amazonian accessions, 50 Wickham clones and two allied species (*H. benthamiana*, *H. pauciflora*) (Luo et al. 1995). While the variation in wild accessions was considerable, the cultivated clones formed only two clusters.

6.5.1 Potentiality of mtDNA

The aforesaid observations amply indicate that the selection was indirectly towards nuclear DNA polymorphism, while evolving modern clones. Luo et al. (1995) argue that the geographic specificity towards nuclear and mtDNA polymorphisms are due to great level of genetic structuring among natural populations in the Amazon forests in relation to hydrographic network. In wild accessions, seed dispersal and selection are as per the environmental conditions. If this is true, we observe that much of the variations produced in the natural habitat are being lost due to selection pressure of environmental factors. This is a matter of concern since the wild accessions have not rendered much contribution in evolving high yielding clones so far, after introduction to other parts of the globe. On the other hand, Wickham clones exhibited high nuclear DNA polymorphism, perhaps due to breeding under different climates. It is presumable that the nuclear genome has been forced to enhance variation to suit the diverse hydrothermal situations of newly introduced areas, resulting in selection of rightly adapted clones under a given environment. mtDNA of Wickham clones has lesser variation because their female progenitors are all primary clones, naturally bred under the similar environmental conditions of Malaysia and Indonesia. These clones were introduced later into India and Sri Lanka for further breeding programmes. Moreover, cytoplasmic donors for most of the improved clones are either PB 56 or Tjir 1 (Fig. 6.6). While the cytoplasm of PB 56 is transferred through PB 5/51, the cytoplasm of Tjir 1 was through RRII 105, RRIM 600 and RRIM 605. In conventional breeding systems followed in rubber, the best parents of one generation are used as parents for the next cycle of breeding (Simmonds 1989). Obviously, this is the reason for the mtDNA profile showing only two clusters. A possible explanation

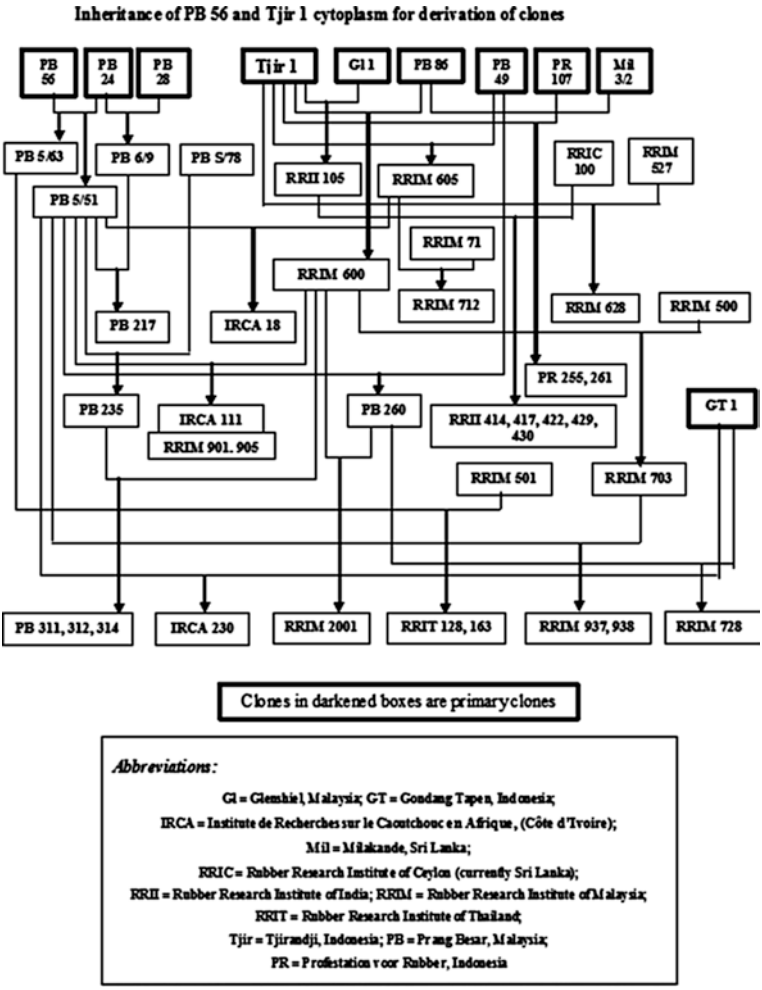

Fig. 6.6 Cytoplasmic donors for improved clones (PB 56 or Tjir 1)

for greater polymorphism in mtDNA in wild accessions is that they must have been evolved through interspecific hybridization. mtDNA polymorphism in wild accessions needs to be exploited fully. A molecular survey of available Amazon accessions and isolation of competent molecular variants in their progeny are the possible exercises that would give meaningful results.

Plant mitochondrial genomes encode tRNAs, rRNAs, proteins and ribosomal proteins and range in size from 200 Kb in *Brassica hirta* (Palmer and Herbon 1987) to 2.74 Mb in *Cucumis melo* (Rodríguez-Moreno et al. 2011). Mitochondrial genome expansion in land plants is primarily due to large intergenic regions, repeated segments, intron expansion and incorporation of foreign DNA such as plastid and nuclear DNA (Turmel et al. 2003; Bullerwell and Gray 2004).

Accumulation of repetitive sequences in plant mitochondrial genomes cause frequent recombination events and dynamic genome rearrangements within a species (Chang et al. 2011; Allen et al. 2007). Several mutations by gene rearrangement of the mitochondrial genes were found associated with cytoplasmic male sterility (CMS) such as the T-urf13 gene in maize (Dewey et al. 1981), pcf gene (a fusion of atp9 and cox2 portions) in petunia Young and Hanson 1987), cox1 in rice (Wang et al. 2006) and mutations in ATPase subunits in sunflower (Laver et al. 1991) and Brassica (Landgren et al. 1996). RNA processing also plays an important role in controlling CMS as evidenced in *orf*355/*orf*77 (*atp*9) and *T-urf*13 in maize (Gallagher et al. 2002; Dill et al. 1997). With the development of next generation sequencing (NGS) technologies, new strategies have been used to obtain plant mitochondrial genomes. A combination approach of shotgun and paired-end NGS sequencing from non-enriched whole genome DNA libraries have been successfully used to obtain the mitochondrial genomes.

Clone BPM 24 exhibits CMS, inherited from the variety GT 1. Shearman et al. (2014) constructed the rubber tree mitochondrial genome of a cytoplasmic male sterile variety, BPM 24, using 454 sequencing, including 8 kb paired-end libraries, plus Illumina paired-end sequencing. They further annotated this mitochondrial genome with the aid of Illumina RNA-seq data and performed comparative analysis. Shearman et al. (2014) then compared the sequence of BPM 24 to the contigs of the published rubber tree, variety RRIM 600, and identified a rearrangement that is unique to BPM 24 resulting in a novel transcript containing a portion of atp9 (Fig. 6.7). The novel transcript is consistent with changes that cause CMS through a slight reduction to ATP production efficiency. The exhaustive nature of the search rules out alternative causes and supports previous findings of novel transcripts causing CMS.

6.5.2 Potentiality of cpDNA

Chloroplast genomes are sufficiently large and complex to include structural and point mutations that are useful for evolutionary studies from intraspecific to interspecific levels (Neale et al. 1988; McCauley 1992; Graham and Olmstead 2000; Provan et al. 2001). Since the first complete chloroplast (cp) genome sequence of liverwort (*Marchantia polymorpha*) was reported in 1986 (Ohyama et al. 1986), more than 150 chloroplast genomes have been sequenced and characterized thus disclosing an enormous amount of evolutionary and functional information of chloroplasts. In chloroplasts, transcripts undergo a series of RNA processing steps such as intron splicing, polycistronic cleavage, and RNA editing. RNA editing is a mechanism to change genetic information at the transcript level by nucleotide insertion, deletion or conversion (Bock 2000; Knoop 2010). The chemical composition of natural rubber is cis-polyisoprene, a high-molecular weight polymer formed from sequential condensation of isopentenyl diphosphate (IDP) units catalyzed by the action of rubber transferase (Cornish 2001). IDP is also an important intermediate

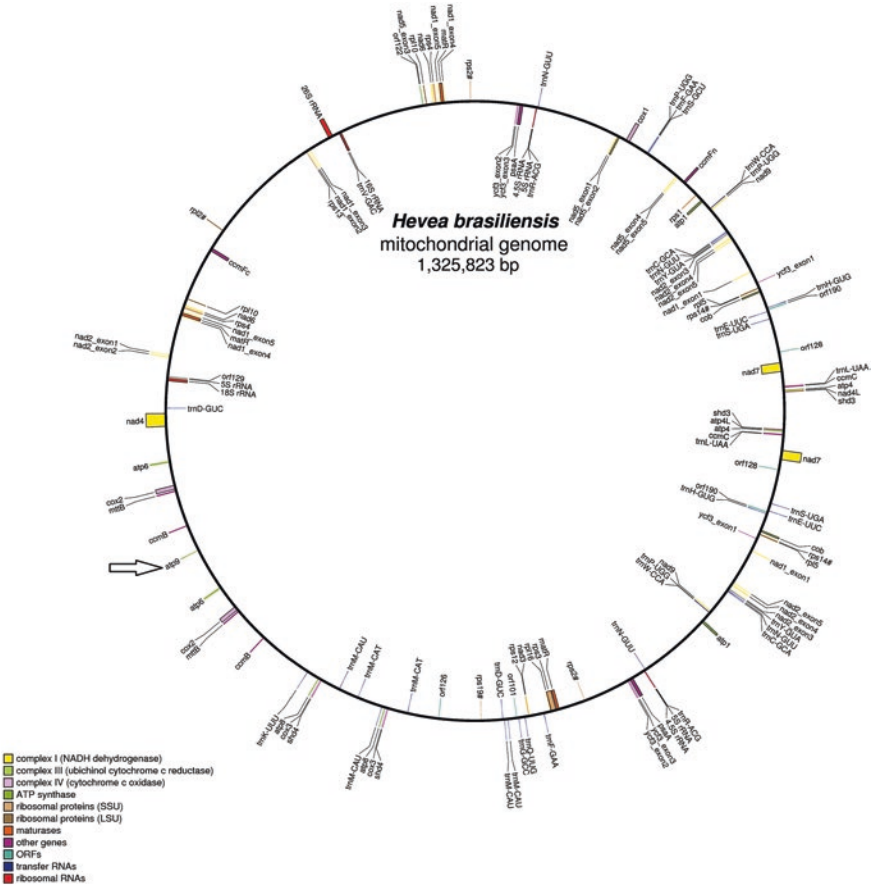

Fig. 6.7 Annotated representation of the rubber tree mitochondrial genome (after Shearman et al. 2014)

for biosynthesis of essential oils, abscisic acid, cytokinin, phytoalexin, sterols, chlorophyll, carotenoids and gibberellins (Chappell 1995a; McGarvey and Croteau 1995; Lichtenthaler et al. 1997; Cornish 2001). There are two IDP biosynthesis pathways: the MVA pathway which occurs in cytosol (Chappell 1995b); and the 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-Derythritol 4-phosphate (MEP) pathway which occurs in plastids (Lichtenthaler 1999; Ko et al. 2003). One approach to improving rubber production in *H. brasiliensis* would be to engineer chloroplasts and modify metabolic flux to produce more biosynthetic intermediates. The availability of the complete chloroplast genome sequence should also facilitate the chloroplast transformation technique. The improved transformation efficiency and foreign gene expression can be achieved through utilization of endogenous flanking sequences and regulatory elements (Birch-Machin et al. 2004; Maliga 2004; Tangphatsornruang et al. 2010). Transformation of chloroplast genome offers a number of advantages

over nuclear transformation including a high level of transgene expression, polycistronic transcription, lack of gene silencing or positional effect and transgene containment (Daniell et al. 2002; Maliga 2002, 2004; Bock 2007). Tangphatsornruang et al. (2011) reported the complete chloroplast genome sequence of rubber tree as being 161,191 bp in length including a pair of inverted repeats of 26,810 bp separated by a small single copy region of 18,362 bp and a large single copy region of 89,209 bp. The chloroplast genome contains 112 unique genes, 16 of which are duplicated in the inverted repeat. Of the 112 unique genes, 78 are predicted protein-coding genes, four are ribosomal RNA genes and 30 are tRNA genes. Relative to other plant chloroplast genomes, Tangphatsornruang et al. (2011) observed a unique rearrangement in the rubber tree chloroplast genome: a 30-kb inversion between the *trnE*(*UUC*)-*trnS*(*GCU*) and the *trnT*(*GGU*)-*trnR*(*UCU*). A comparison between the rubber tree chloroplast genes and cDNA sequences revealed 51 RNA editing sites in which most (48 sites) were located in 26 protein-coding genes and the other 3 sites were in introns. Phylogenetic analysis based on chloroplast genes demonstrated a close relationship between *Hevea* and *Manihot* in Euphorbiaceae.

Shotgun genome sequencing of *H. brasiliensis* using pyrosequencing technology revealed the complete chloroplast genome sequence (Tangphatsornruang et al. 2011). Gene content and structural organization of the rubber tree chloroplast genome is similar to that of *M. esculenta*, with an exception of the 30-kb fragment rearrangement. By comparing the rubber tree chloroplast genes and the cDNA sequences, the distribution and the location of RNA editing sites in the chloroplast genome could be determined (Tangphatsornruang et al. 2011). The phylogenetic relationships among angiosperms, based on ct DNA sequences including those of the rubber tree ct DNA provided a strong support for a monophyletic group of the eurosid I and demonstrated a close relationship between *Hevea*, *Manihot*, *Jatropha* and *Populus* in *Malpighiales*.

As a synthesis of these diversity studies, good relationships were found between the results issued from the different genetic markers. Even if the contribution of isozymes is important by itself, molecular markers provided important clarifications for the distinction of different groups. There would be no barrier to migration of *Hevea* genes within the Amazonian basin. However, the wideness of the area and the limited dispersion of *Hevea* seeds allowed the preservation of the current structure, which is assumed to have initially resulted from the fragmentation of the Amazonian forest during the pleistocene period, according to the refuge theory presented by Haffer (1982). Moreover, the *Hevea* germplasm genetic structure clearly appears as geographically structured in relationship with the hydrographic network of the Amazonian forest, which confirms the role of rivers and inundated zones in the transport of seeds and dissemination of the species (Besse et al. 1993; Luo et al. 1995; Seguin et al. 1996). The mtDNA of Wickham population has lesser variation since their female progenitors are restricted to a very small set of primary clones. Cytoplasm donors for most of the improved clones are either PB56 or Tjir1. Obviously, this is the reason for the mtDNA profile showing only two clusters (Priyadarshan and Gonçalves 2003). Possible explanation for greater polymorphism in mtDNA of wild accessions is that many might have been evolved through interspecific hybridization.

6.6 Conclusions and Prospects

As said earlier, genetic erosion can result from a narrow genetic base in the original collections or by practices that reduce genetic diversity. That the original 22 seedlings of Wickham collection, as it is believed till date, is the base population from which the day-to-day *Hevea* clones were evolved had been genetically narrow to enrich the *Hevea* gene pool. In addition, these populations were subjected to several rounds of controlled crossing that further narrowed the diversity. Moreover, the strategy followed by the breeders to select only the desirable genotypes and to reject the unwanted ones (without assessing the utility other than yield) is the main reason that reduces diversity. Concerted efforts to infuse the Amazonian germplasm through controlled crossings never met with enriching the diversity as desired as expected. This is because selection was, and is always been in favor of higher yield only. Preserving other genotypes/entries can not be accomplished due to space constraints unlike annual species. This drawback needs to be addressed resolutely if the diversity of *Hevea* rubber is to be increased. Genetic diversity not produced or preserved is equivalent to genetic diversity lost. The total number of clones is not more than hundred that are being cultivated world wide for natural rubber production.

Molecular characterization of *Hevea* has not been done systematically. Only molecular diversity of Amazonian accessions and a few clones had been studied to an extent. A very systematic study of all *Hevea* clones at molecular level is appreciable, since the wisdom of understanding differences in morphological and molecular diversity has accumulated of late. QTL mapping is yet another area that needs to be undertaken with international coordination. As mentioned in this article, much work at the molecular level had been carried out like for TPD, latex production, defense genes and alike. Only growth related traits have been attempted for QTL mapping (Souza et al. 2013). But a sincere and systematic effort to tie up other variations with QTLs for yield had not been done so far. This exercise is difficult but not impossible. This systematic exercise can only elucidate the intricacies underlying diversity of *Hevea* rubber. Such an exercise can lead to setting up of a molecular library for *Hevea* and scientists working worldwide can contribute to this molecular library. The deposition of microsatellites, SSRs and ESTs is not enough, but a library that includes genes for QTLs is most warranted. The contribution of Rahman et al. (2013) on gene sequencing of *Hevea* is a sincere and systematic step towards this. The attempts of Saldago et al. (2014) did transcriptome analysis in *Hevea*. Such investigations with modern methodologies are encouraging, since utilization of such technologies almost rarely happens in tree research. This tempo needs to be accelerated further, should there be a comprehensive gene library for *Hevea* rubber.

One of the early contributors to the science of plant genetic resources, Harlan (1970) remarked: ‘The varietal wealth of the plants that feed and clothe the world is slipping away before our eyes, and the human race simply cannot afford to loose it’, and he also predicted a ‘genetic wipe out of centers of diversity’ (Harlan 1975).

Genetic wipe out has not really happened but the modern varieties have replaced traditional varieties or land races. One of the primary duties of a Plant Breeder is to evolve, document and manage genetic diversity. As such, there are no land races in *Hevea* rubber, but only modern clones. In this context, how much genetic diversity is getting conserved, cataloged and utilized and how much genetic erosion happens are the options left to one's own wisdom.

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Chapter 7
Estimating Genetic Erosion in Threatened Conifers: The Example of *Picea chihuahuana* Martínez

Christian Wehenkel, Cuauhtémoc Sáenz-Romero and Juan Pablo Jaramillo-Correa

Abstract The necessity of genetic diversity for evolution and the relationship between heterozygosity and population fitness are important arguments for conserving genetic diversity. The loss of genetic diversity can be detrimental to the short-term viability of individuals and populations, and to the evolutionary potential of populations and species. Genetic erosion can be defined as the permanent reduction in richness or evenness of common local alleles or as the loss of combinations of alleles over time in a defined area. Various international and intergovernmental organizations and networks have therefore recognized the need to assess and monitor plant genetic erosion in order to prevent such effects. The rare tree species Chihuahua spruce (*Picea chihuahuana* Martínez), which is endemic to Mexico, is an excellent model for estimating genetic erosion. The species occurs in about 40 isolated relict populations in the Sierra Madre Occidental, in the northwest of the country. Here, we will review a study assessing the degree of genetic erosion that was evaluated in five populations of *P. chihuahuana* M. in the State of Durango (Mexico), by comparing genetic diversity across diameter classes (which were assumed to be a surrogate for age classes). In the two largest populations, there was a moderate loss of genetic diversity at *AFLP* loci from older trees to saplings, and to young seedlings. Significant genetic erosion was only detected in

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the very small population of San José de las Causas (*SJ*). Hence, if genetic diversity at *AFLP* loci reflects diversity in the whole genome, genetic erosion per se does not explain the relict status of Chihuahua spruce, except for very small populations, such as *SJ*. However, further researches with candidate genes are necessary to assess the putative loss of evolutionary potential in these stands. Activities that increase population size should be helpful to preserve genetic diversity.

Keywords Loss of genetic diversity · Diameter distribution · Covariation · Permutation test · Climatic change

7.1 Introduction

7.1.1 Genetic Erosion

The necessity of genetic diversity for evolution and the relationship between heterozygosity and population fitness are important arguments for conserving genetic diversity (Reed and Frankham 2003). In the short term, genetic diversity is related to increased inbreeding, which at its turns affects individual fitness. In the longer term, standing genetic variation can be associated with a species' ability to respond to changing selection pressures (Young et al. 1996; Reed and Frankham 2003; De Carvalho et al. 2010; Kremer et al. 2012). On the other hand, genetic erosion can be detrimental to the viability of individuals and the evolutionary potential of populations and species, thus affecting the direct use of genetic resources (Brown et al. 1997).

Various international and intergovernmental organizations and networks [e.g., the World Conservation Union (IUCN), Species Survival Commission, Convention on Biological Diversity (CBD), UNEP World Conservation Monitoring Centre (UNEP/WCMC), Organisation for Economic Co-operation and Development (OECD), European Union (EU), Bioversity International (formerly IPGRI) and FAO] have recognized the need to assess and monitor genetic erosion, in order to prevent such effects (Diulgheroff 2006). A literature review has shown that surprisingly few studies have measured and assessed this important process in forest tree communities (e.g., Lee et al. 2002), particularly for subtropical threatened taxa.

Genetic erosion can be viewed as the "*loss of genetic diversity, in a particular location and over a particular period of time, including the loss of individual genes, and the loss of particular combinations of genes such as those manifested in landraces or varieties. It is thus a function of change of genetic diversity over time*" (FAO/IPGRI 2002). Maxted and Guarino (2006) suggested that genetic erosion may also be defined as a "*permanent reduction in richness or evenness of common local alleles or the loss of combination of alleles over time in a defined area*".

However, it must be noted that these definitions do not specify whether genetic erosion is caused by adaptation (selection), genetic drift, or inbreeding. For instance, the latter includes the total number of variants and their relative frequencies, as two important components of diversity that are well-balanced, and considered in the Simpson index (Simpson 1949) and in the "effective number" of variants (υ_2) ($\upsilon_2 = 1/\Sigma p_i^2$) (Gregorius 1978). As a complement, Brown et al. (1997) provided a useful list of features or indicators for estimating the potential risk of genetic erosion, namely: (i) the number of subspecific entities, (ii) population sizes, numbers, and isolation, (iii) environmental amplitude, (iv) genetic diversity at marker loci, (v) quantitative genetic variation, (vi) interpopulation genetic structure, and (vii) amount and patterns of mating.

In this chapter, we will discuss one of the few documented examples of genetic erosion found the endangered, rare, relictic, and fragmented endemic Mexican spruce, *P. chihuahuana* M., in Durango State, northwestern Mexico. It studied five populations, by comparing genetic diversity among diameter at breast height (*DBH*) classes (as a surrogate variable for age classes), estimated using dominant gene markers (*AFLP*) and Gregorius' total population differentiation (δ_T) (Gregorius 1987). These populations bear less adult trees and should be more affected by the ongoing climate change than the northern ones, and thus represent ideal models to estimate genetic erosion. Results were previously reported in Wehenkel and Sáenz-Romero (2012).

7.1.2 Genetic Diversity and Structure of Picea chihuahuana Martínez

The rare tree species Chihuahua spruce (*P. chihuahuana* Martinéz), an endemic of Mexico, is an excellent model for estimating potential genetic erosion (Ledig et al. 1997). This species occurs in about 40 isolated relict populations at elevetions between 2,155 and 2,990 m above sea level in the Sierra Madre Occidental in the states of Durango and Chihuahua, in Northwestern Mexico. The size of the populations varies from 21 to 5546 individuals, including trees, saplings, and seedlings (Ledig et al. 2000; Farjon 2001).

As this species is a relict stranded by a warming climate during the current interglacial period, Mahlman (1997) and Ledig et al. (2000) proposed that Chihuahua spruce can serve as a signal species for the projected climate change in the twenty-first century. It has thus became emblematic of the challenges that Mexico will face in implementing management actions, such as assisted colonization, to prevent extinctions due to global warming (Ledig et al. 2010). Some studies have been carried out to establish the genetic diversity and structure of this species (Ledig et al. 1997, 2004; Jaramillo-Correa et al. 2006; Wehenkel and Sáenz-Romero 2012; Wehenkel et al. 2012; Quiñones-Pérez et al. 2014a, b; www.mapforgen.org).

Ledig et al. (1997) analyzed 24 loci in 16 enzyme systems to estimate genetic diversity (H_e) and the number of alleles per locus (A) in 10 populations comprising 15 to 2441 mature trees, based on seeds and sample sizes of 7.7–22.9 trees per locus. They concluded that "*if genetic diversity at isozyme loci reflects diversity in the genome as a whole, lack of diversity* per se *is not the reason for the relictual status of Chihuahua spruce.*" These authors also found that H_e and A were closely related to the logarithm of the number (N) of mature trees in the population ($r_{He,N} = 0.93$, $P = 0.004$; $r_{A,N} = 0.78$, $P = 0.047$), which confirms the theory relating population size and genetic diversity (Frankham 1996).

Jaramillo-Correa et al. (2006) determined and observed numbers of mitotypes and chlorotypes, and mitochondrial and chloroplast diversity estimates (H; equivalent to the expected heterozygosity; H_e, for diploid data) for 16 Chihuahua spruce populations, based on seeds and sample sizes of 8–10 mature trees per population. These authors found that none of the 16 *P. chihuahuana* populations surveyed was polymorphic for the *mtDNA* markers, while for the *cpDNA* markers, three of the 16 stands surveyed were fixed for a particular chlorotype. In addition, they noted that the diversity in *cpDNA* decreased from northern to southern latitudes (*Lat*) ($r_{H,\mathrm{Lat}} = 0.626$; $P < 0.01$) and that genetic and geographic distances were not related.

However, a marginal correlation was observed when comparing the diversity in *cpDNA* with the population census ($r_{H,\ln(N)} = 0.064$; $P = 0.863$) and with all other ecological or demographic factors previously considered (Ledig et al. 2000) in the stands surveyed therein. Jaramillo-Correa et al. (2006) therefore assumed that *P. chihuahuana* has been subjected to strong bottlenecks and has suffered from genetic drift in the recent past (i.e., during the Holocene). Thus, according to the previous definition, genetic erosion has proceeded in the species as a whole, at least since the end of the last glacial period. However, the question remains as to whether genetic erosion took place recently (or is still taking place) in single isolated populations, because not all fragmentation events lead to reduced genetic variation in plants (Young et al. 1996).

7.2 Genetic Erosion in Populations of *Picea chihuahuana* M.

7.2.1 Populations Studied and Methodological Approach of Detecting Genetic Erosion

The study was conducted in the State of Durango which occupies about 23 % of the Sierra Madre Occidental ecosystem. Branches were collected from each of 254 randomly chosen specimens of *P. chihuahuana* M., distributed in five populations as follows: (a) Paraje Piedra Rayada (*PPR*), (b) Quebrada de los Durán (Arroyo del Indio Ignacio) (*QD*), (c) La Pista (*LP*), (d) Santa Barbara (Arroyo del Infierno)

(*SB*), and (e) San José de Causas (*SJ*), which covers most of the latitudinal range of the species in Durango State, Mexico (Figs. 7.1 and 7.2). In addition, the diameter at breast height (*DBH*) of trees and saplings, and the diameter at ground level of seedlings were assessed for every individual studied.

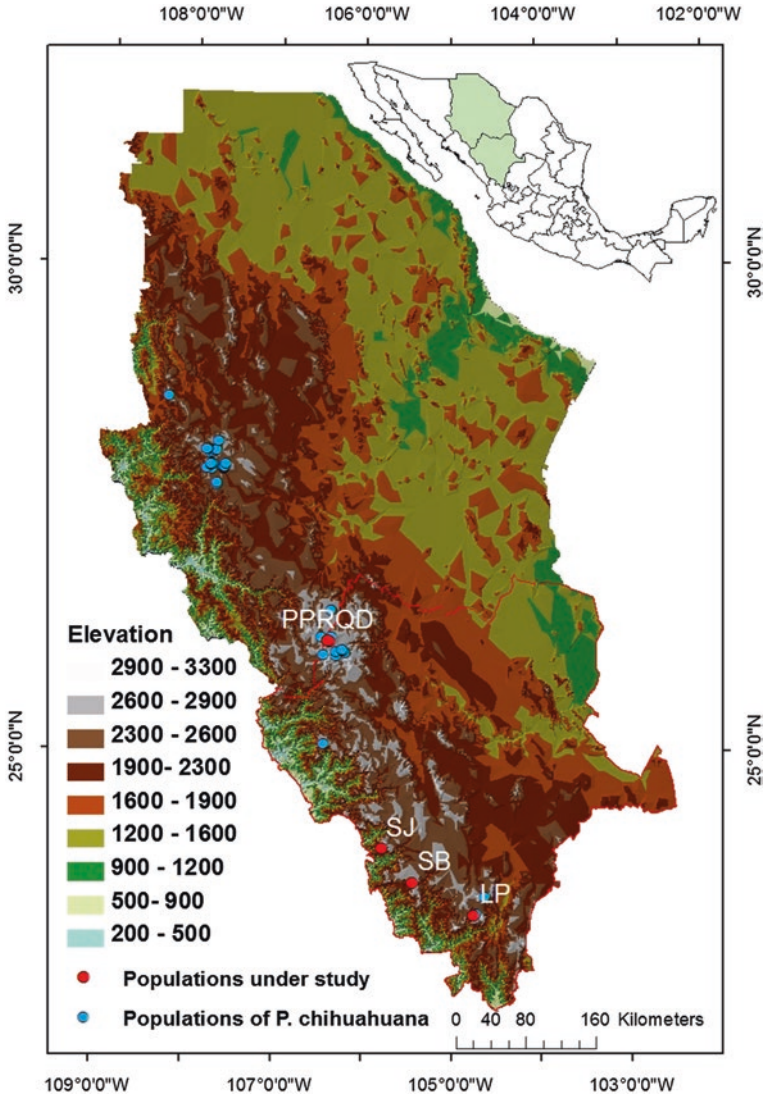

Fig. 7.1 Map of the 40 already detected populations of *Picea chihuahuana* M. and the five locations of the populations under study: Paraje Piedra Rayada (*PPR*), Quebrada de los Durán (Arroyo del Indio Ignacio) (*QD*), La Pista (*LP*), Santa Barbara (Arroyo del infierno) (*SB*), and San José de Causas (*SJ*) (*red circles*), in the State of Durango, Mexico (Elevation in m)

Fig. 7.2 Individuals of *Picea chihuahuana* M. in Paraje Piedra Rayada (*PPR*) and Quebrada de los Durán (Arroyo del Indio Ignacio) (*QD*), Durango, Mexico

Because *P. chihuahuana* is an endangered species (IUCN Red List of Threatened Species 2011; www.iucnredlist.org), the sample trees were not cored for growth ring counting to obtain the actual ages (permission to core the stems was not obtained from federal authorities). However, Gordon (1968) and

Narvaéz-Flores (1984) - in Ledig et al. (2000) - reported a positive relationship between tree diameter, height, and tree age in Chihuahua spruce. According to Ledig et al. (2000), "*P. chihuahuana probably grows slowly, about 0.25 to 0.75 m per year, on average, over its first 100 years*"; this was estimated from a regression of height and age of 29 trees. DBH was therefore used as a surrogate for age (see below for more details). Ledig et al. (2000) also provided detailed descriptions of the first four populations shown in Table 7.1 along with some demographic and ecological parameters.

Given that DBH can represent the approximate tree age (Seymour and Kenefic 1998; Nord-Larsen and Cao 2006) in Chihuahua spruce (Ledig 2000), it was assumed that each diameter class reflects natural regeneration during a particular period within the past 160 years. For instance, the dc of 5 cm was assumed to represent the natural regeneration in the past 20 years, the dc of 75 cm the natural regeneration that occurred about 130–160 years ago, etc. This enabled Wehenkel and Sáenz-Romero (2012) comparing the genetic diversity of Chihuahua spruce across different time periods for a defined area.

According to Wehenkel and Sáenz-Romero (2012), DNA data from frozen needles were obtained by the amplified fragment length polymorphism (*AFLP*) technique. *AFLP* fingerprints were generated using a modified protocol described by Vos et al. (1995). As for other dominant markers, each band detected (presence) at each given position (locus) corresponds to a dominant genotype (plus phenotype).

Further methodological details for measuring Gregorius' total differentiation (δ_T) can be found in Simpson (1949) and Gregorius (1987); for determining the proportion of polymorphic fragments (pr_{poly}) and down-weighted marker values (*DW*) in Schönswetter and Tribsch (2005); for computing arithmetic the mean

Table 7.1 Locations of the five populations of Chihuahua spruce, the number of trees (>2 m high) counted for the first four populations by Ledig et al. (2000), the Gregorius' total differentiation (δ_T), proportion of polymorphic fragments (pr_{poly}), the down-weighted marker value (*DW*), and the arithmetic mean genetic distance calculated with all pairs of individuals ($d_{0,ind,m}$) in each population

Code	Location	Number of trees	Geographical coordinates	δ_T	pr_{poly}	*DW*	$d_{0,ind,m}$
PPR	Paraje Piedra Rayada, Guanaceví	3564	26°08′48″N 106°22′53″W	0.183	0.856	84.4	0.091
QD	Quebrada de los Durán, Guanaceví	2628	26°07′15″N 106°24′17″W	0.162	0.803	63.9	0.115
LP	La Pista, Mezquital	919	23°19′4.5″N 104°44′42.6″W	0.150	0.755	68.8	0.095
SB	Santa Barbara, Pueblo Nuevo	148	23°39′50″N* 105°26′08″W	0.117	0.655	52.0	0.128
SJ	San José de las Causas, San Dimas	*ca.* 120	24°01′05″N 105°47′06″W	0.136	0.677	50.0	0.117

*Geographical coordinates reported by Ledig et al. (2000) are incorrect

genetic distances calculated with all pairs of individuals in each cohort ($d_{0,\mathrm{ind,m}}$) and with four randomly chosen pairs of individuals in each cohort to compensate for the different sample sizes ($d_{0,\mathrm{ind,m(4)}}$) in Weir et al. (2006) and Gregorius et al. (2007); and for calculating covariation (C) as well as for statistical tests in Gregorius et al. (2007). Those methods were used by Wehenkel and Sáenz-Romero (2012).

7.2.2 *Significant Genetic Erosion Detected in the Very Small Population*

Wehenkel and Sáenz-Romero (2012) reported that a significant loss of genotype diversity was only detected among the 319 *AFLP* loci studied in the very small San José de Causas (*SJ*) stand (Fig. 7.3b and Table 7.2); although the demographical stem-number distributions were almost balanced when using 10-cm *DBH* classes (Fig. 7.5). One of the main reasons for the loss of diversity in *SJ* may be the smaller proportion of mature and reproductively competent individuals (Reed and Frankham 2003), which can be explained by the detection of significantly less genetic differentiation (and thus higher relatedness) between individuals in the younger generations (Fig. 7.4 and Table 7.2).

Hence, the results of Wehenkel and Sáenz-Romero (2012) are consistent with the findings of Ledig et al. (1997), i.e., if genetic diversity at a set of loci reflects the diversity in the whole genome, then genetic erosion per se does not explain the relict status of Chihuahua spruce, except in the very small populations, such as *SJ*. This population, consisting of about 120 trees, and has probably fallen below the level of a minimum viable population size when demographic and environmental stochasticity, as well as natural catastrophes, are ignored. The standing genetic diversity of this population would therefore not be sufficient to prevent a dangerous accumulation of inbreeding depression, while future mutations should not compensate for the loss of alleles due to genetic drift (Wright 1938; Millar and Libby 1991; Frankham et al. 2002; Bücking 2003).

It is important to note that the trend of genetic erosion in the population *SJ* could be reversed if pollen and/or seedlings originating from older trees in *SJ* (genetically more variable than younger individuals) or from neighboring populations are (re)introduced (Wehenkel and Sáenz-Romero 2012). It may be argued that the introduction of foreign alleles may cause outbreeding depression. However, there is probably a greater risk of allowing this population to continue its genetic decline in what could become a vortex of extinction (Frankham et al. 2002). Furthermore, given that all populations studied therein have shown identical mtDNA signatures and similar cpDNA patterns, they should be derived from the same ancestral stand and bear similar adaptive alleles. Therefore, the risk of outbreeding depression should be low. It must also be noted that some models predict that gene flow fosters adaptation in forest trees (see Kremer et al. 2012 for a

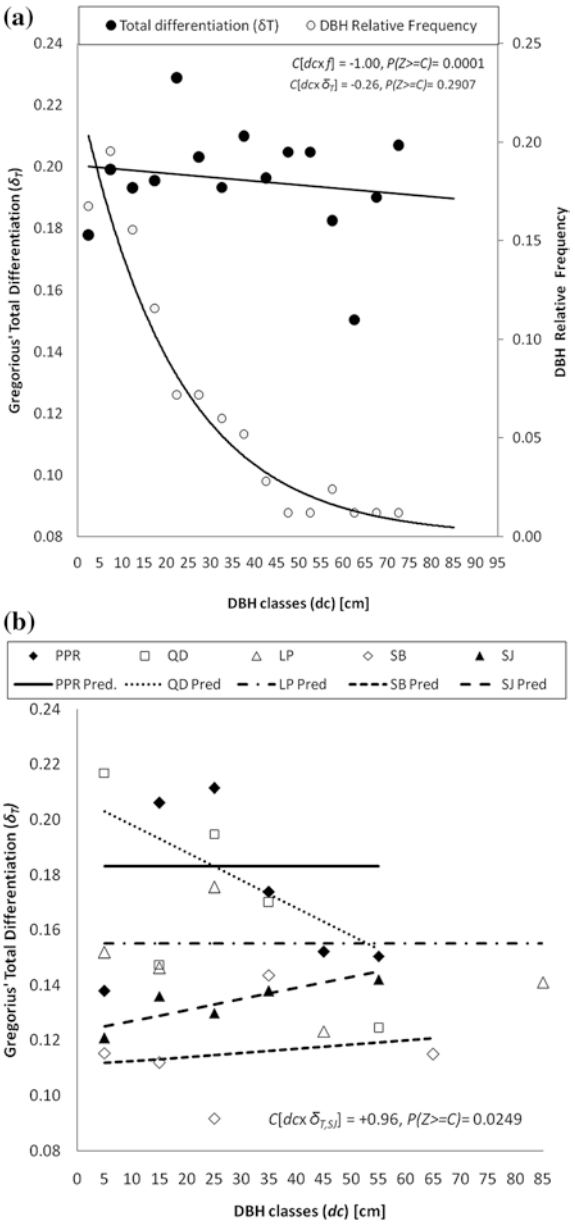

Fig. 7.3 Relationships between DBH classes and mean values of Gregorius' total differentiation (δ_T), **(a)** across all populations and for observed relative diameter distribution (*f*), and **(b)** total differentiation (δ_T) for each population [Paraje Piedra Rayada (*PPR*), Quebrada de los Durán (Arroyo del Indio Ignacio) (*QD*), La Pista (*LP*), Santa Barbara (Arroyo del infierno) (*SB*) and San José de Causas (*SJ*)] for all 254 individuals of *Picea chihuahuana* M. studied in Durango, Mexico

review), and although these effects might be different for the rear edge populations (such as those surveyed by Wehenkel and Sáenz-Romero 2012), this could be an ideal opportunity to empirically test the putative responses of these threatened stands to future environmental changes.

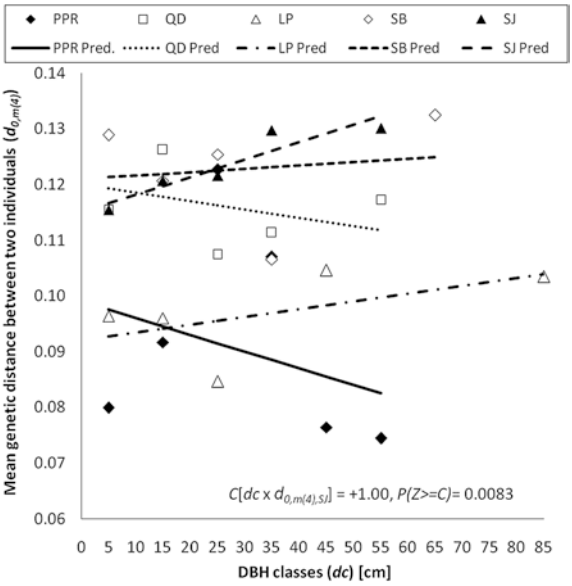

Fig. 7.4 Relationships between DBH classes and mean values of mean genetic distance between two individuals with four randomly chosen pairs of individuals in each cohort ($d_{0,m(4)}$) for each population [Paraje Piedra Rayada (*PPR*), Quebrada de los Durán (Arroyo del Indio Ignacio) (*QD*), La Pista (*LP*), Santa Barbara (Arroyo del infierno) (*SB*), and San José de Causas (*SJ*)] of *Picea chihuahuana* M. studied in Durango, Mexico

Table 7.2 Covariation (*C*) between diameter classes (*dc*) and population size (N_P), and observed degrees of Gregorius' total differentiation (δ_T), proportion of polymorphic fragments (pr_{poly}), down-weighted marker values (*DW*), and arithmetic mean genetic distances calculated with all pairs of individuals in each cohort ($d_{0,ind,m}$) and with four randomly chosen pairs of individuals in each cohort to compensate for the different sample sizes ($d_{0,ind,m(4)}$) per dc for each population [Paraje Piedra Rayada (*PPR*), Quebrada de los Durán (Arroyo del Indio Ignacio) (*QD*), La Pista (*LP*), Santa Barbara (Arroyo del infierno) (*SB*), and San José de Causas (*SJ*)] of *Picea chihuahuana* M. studied in Durango, Mexico

dc	index	δ_T	pr_{poly}	*DW*	$d_{0,ind,m}$	$d_{0,ind,m(4)}$
PPR	*C*	−0.31	0.05	0.41	−0.65	−0.36
	$P(Z \geq C)$	0.3250	0.4718	0.3026	0.1704	0.3118
QD	*C*	−0.85	−0.81	−0.86	−0.97	−0.28
	$P(Z \geq C)$	0.0830	0.1510	0.1255	0.0503	0.3920
LP	*C*	−0.62	0.39	0.37	0.79	0.78
	$P(Z \geq C)$	0.2161	0.3527	0.3676	0.1258	0.1758
SB	*C*	0.32	−0.86	−0.81	−0.67	0.19
	$P(Z \geq C)$	0.3680	0.0998	0.1176	0.2397	0.4749
SJ	*C*	0.96	0.62	0.88	0.87	1.00
	$P(Z \geq C)$	**0.0249**	0.2320	0.0843	0.0749	**0.0083**
total	*C*	−0.21	−0.64	0.26	−0.02	0.08
	$P(Z \geq C)$	0.3228	0.0467	0.2599	0.4842	0.4320
N_P	*C*	0.99	0.99	0.96	−0.80	−0.80
	$P(Z \geq C)$	**0.0165**	**0.0171**	0.0255	0.1161	0.117

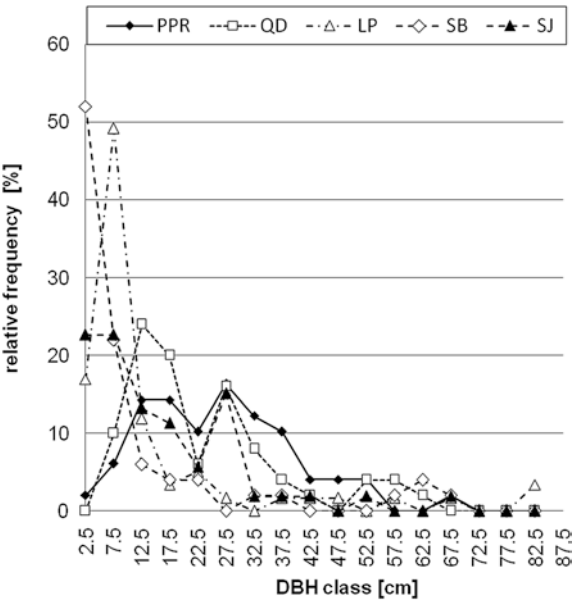

Fig. 7.5 The observed relative diameter distribution in the diameter classes of 2.5–82.5 cm in the populations Paraje Piedra Rayada (*PPR*), Quebrada de los Durán (Arroyo del Indio Ignacio) (*QD*), La Pista (*LP*), Santa Barbara (Arroyo del infierno) (*SB*), and San José de Causas (*SJ*) populations of *Picea chihuahuana* M., Durango, Mexico

7.2.3 Implications for Management and Conservation

Traill et al. (2007) reported in a meta-analysis based on 141 sources and 212 species that the minimum viable population size is context-specific and is on average 4,824 individuals for plant species (95 % CI = 2,512 − 15,992). Such an estimate suggests that all populations of Chihuahua spruce have low chances of survival without assistance, given that the maximum population size currently observed is only 3564 trees (>2 m tall; Ledig et al. 2000). Therefore, all activities that increase population size would be helpful regarding the genetic structure and diversity of the species as a whole. Such activities might include the following: (i) protect natural regeneration against livestock, wild animals and forest fires, (ii) establish artificial regeneration with autochthonous reproductive material in well-selected locations in the vicinity of (but not inside) the particular population, (iii) remove competing vegetation (including other tree species) in the vicinity of the particular population, (iv) support putative biotic dispersal vectors, and (v) promote the establishment of local mycorrhiza and other microorganisms that might help increase nutrient intakes in seedlings and saplings in and around populations.

Moreover, it is necessary to continue monitoring the population sizes and genetic diversity of the existing stands in situ. Then, if genetic erosion, or any other significant stochastic force, is detected in a population, the most obvious tactic should be to restore gene flow to this population (Ledig et al. 1997). However, because of differences in the genetic population structure, seed transfer from the northern to the central or southern populations and vice versa should be avoided (see Jaramillo-Correa et al. 2006).

Interestingly, for the small La Pista (*LP*) population and the very small Santa Bárbara (*SB*) stand, the mean genetic diversity did not decrease throughout the *DBH* classes (and thus age classes) (Wehenkel and Sáenz-Romero 2012). Perhaps the purging of lethal alleles and facultative selfing suspected in many threatened spruces (Ledig et al. 2002, 2005; Aleksić and Geburek 2013) occurred earlier during a bottleneck event for these particular locations, thus maintaining higher mean levels of genetic diversity. It is also possible that an array of well-adapted individuals that retained a well-represented genetic diversity survived and sustained these populations since the last bottleneck. Because of the limitations imposed by the dominant marker used, Wehenkel and Sáenz-Romero (2012) could not determine whether the fitness and constant genetic diversity of these individuals were caused by high degrees of heterozygosity (Ledig 1986), which should be thus explored further. However, the results reported by Ledig et al. (1997) argue against this possibility given the excess of homozygosity found for eight of the 13 isozyme loci surveyed. In which case, it could be argued that any possible loss of genetic diversity in *LP* and *SB* due to genetic drift and inbreeding was compensated by incoming gene flow from the neighboring populations, such as proposed for isolated stands in the endemic and endangered Serbian spruce (Aleksić and Geburek 2013).

7.2.4 Adaptation to Climatic Change

The predicted reduction and eventual disappearance of a suitable habitat for *P. chihahuana* due to climatic change (Ledig et al. 2010) imposes an additional risk of extinction. Management actions such as migration (also called assisted colonization), e.g., establishing *ex situ* conservation plantations outside the present distribution, at localities where it is predicted that suitable habitat will occur, should be carried out, giving priority to seedlings grown from seed collected from older trees. According to Wehenkel and Sáenz-Romero (2012), these mature trees should be the most genetically diverse.

A priority area to establish *ex situ* conservation plantations would be the northwest corner of the State of Durango, near its border with the State of Chihuahua, where it was predicted that suitable climatic habitat will arise in the near future (i.e., between 2030 and 2060; Ledig et al. 2010). Also, in this area will collide projected suitable climatic habitats for what was identified as the northern mitotype and the central–southern mtDNA variant (Jaramillo-Correa et al. 2006). Since there are no available provenance test results so far, and then, it is not known if both mitotypes reflect genetic differentiation for quantitative traits of adaptive relevance, a conservative approach would be to keep separated both mitochondrial lineages at the *ex situ* conservation planting sites to prevent potential outbreeding depression.

Such *ex situ* conservation plantations should be big enough to equate a genetically viable effective population size (see Traill et al. 2007), where the population size might be large enough for at least to theoretically compensate the loss

of genetic diversity due to genetic drift with new genetic variants provided by mutation (Millar and Libby 1991). As a general suggestion, it could be proposed to establish Forest Genetic Resource Conservation Units (FGRCUs), with a minimum of 4,660 trees at reproductive age, in order to maintain an average heterozygosity similar to that found across Mexican conifers (Sáenz-Romero et al. 2003). For the *P. chihuahuana* case, however, the FGRCU would be an *ex situ* plantation, instead of a designed natural stand. Nevertheless, further research might be needed to determine how many mature trees should be sampled to collect the seed to establish such a plantation. In any case, as a starting point, and considering the lack of information and the urgency to preserve *P. chihuahuana*, it seems reasonable to aim for *ex situ* conservation plantations of a minimum of 5,000 individuals at the above-mentioned border of Durango and Chihuahua States as soon as possible.

7.2.5 Future Research Needed

Estimations of current and historical gene flow among populations seem essential to enrich the current conservation programs for *P. chihuahuana*. Ideally, assisted migration should only include populations that have been or are still exchanging genetic material, and their identification can easily be done by scanning the genome with anonymous markers (such as AFLPs) and/or by transferring some of the genetic tools developed for other spruces. Recently, two different SNP arrays comprising more than 15,000 markers originally designed for *P. glauca* (a boreal conifer whose genome was recently published; Birol et al. 2013) were tested in seven spruce taxa, including *P. chihuahuana* (Pavy et al. 2013). Although the number of segregating markers was very low in this last species (i.e., 322), they are still more than enough to estimate basic population-genetics and demographic figures, including gene flow and inbreeding. Further re-sequencing of genes directly derived from these genomic resources might allow the estimation of other demographic parameters, such as the time and intensity of the past bottlenecks, and the times of divergence of the modern populations (e.g., Aleksić and Geburek 2013). Altogether, these parameters could be integrated into simulation frameworks to predict possible outcomes under different climate change scenarios, and to propose precautionary measures when needed.

Genomic tools can also be used to monitor effective population sizes, genetic diversity and inbreeding across time. Indeed, under a population decline, or an extinction vortex scenario, these parameters are expected to change rapidly from one generation to next (Frankham et al. 2002). Therefore, developing DNA arrays that are easy to apply would allow the direct estimation of these parameters once a new cohort is established and/or prior to the introduction of individuals (or pollen) from other populations, which could maximize the amount of preserved genetic diversity. Furthermore, the combination of these analyzes with test plantations in the field could help identify divergence patterns of adaptive relevance.

Nevertheless, because a significant loss of diversity had occurred in some of the studied Chihuahua spruce populations at different gene loci, a globally directed force, such as climate, which should operate as a directional selective force, should be less relevant than most stochastic pressures. Indeed, genetic drift and inbreeding due to reduction of the effective population size would be expected to have a greater influence (Franklin 1980). However, the search for putative adaptive gene variants that still remain in the populations, and which are related to other variables than climate (i.e., soil type), should be helpful to reinforce any future conservation efforts in order in correctly account for the evolutionary potential of each particular stand.

7.2.6 Conclusions

Genetic erosion was documented at one of the smallest population of the southern distribution of *P. chihuahuana*. Considering the progressive loss of genetic diversity along the DBH classes (a surrogate of age classes), the insufficient recruitment of young plants and the multiple threatening factors (illegal logging, grazing, forest fires), and climatic change, there is no reason to believe that such population would not go extinct, unless there is an active management for conservation. Among other conservation actions, it should be considered enrichment to perform some planting to increase genetic diversity and population size, and to develop *ex situ* conservation, by planting in sites where it is predicted that will occur the climate for which populations are adapted (as a measure for adaptation to climatic change).

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Chapter 8
Genetic Erosion and In Situ Conservation of Lima Bean (*Phaseolus Lunatus* L.) Landraces in Mesoamerican Diversity Center

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Abstract To understand the role of a geographical region in the in situ conservation of the genetic diversity of any crop, it is necessary to analyze the current conservation status of the crop and any genetic changes that have occurred within the last few decades in the region. Lima bean (*Phaseolus lunatus* L.) is an important crop in the Mayan agriculture of the Yucatan Peninsula, Mexico, its Mesoamerican center of diversity. In this region, 3 of the 21 landraces dominate 71.24 % of the

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cultivated area, and 12 are rare landraces grown in only 6.29 % of the area. This chapter analyzes the risk of the genetic erosion in Lima bean landraces from the Yucatan Peninsula using molecular markers, with the objective of generating data to develop comprehensive in situ conservation programs for the crop. Molecular analyses showed that the many landraces that are planted by only a few peasants contained higher levels of genetic diversity compared with the three most abundant landraces. Also, they showed that the landraces planted in 1979 have higher levels of genetic diversity than those planted in 2007 and that, over the last 30 years, the genetic make-up of this crop has shifted. If current trends in the cultivation of the Lima bean landraces continue, many will no longer be planted within two to three generations, contributing to further genetic erosion. The establishment of evidence-based programs for the in situ conservation of Lima bean landraces is urgently needed in this center of genetic diversity.

Keywords ISSR markers · Landraces · Loss of genetic diversity · SSR markers · Traditional mayan agriculture · Yucatan peninsula

8.1 Introduction

Mexico forms part of the Mesoamerican center of domestication (Vavilov 1926). The ecological, productive, and cultural conditions of the traditional agroecosystems in Mexico have helped to conserve a large number of domesticated species. These conditions have also maintained these species as part of a dynamic scenario for the development of new crops and the evolution of species, processes that favor high levels of variation and genetic contact with wild relatives (Hernández-Xolocotzi 1973). Genetic erosion, the loss, or reduction of genetic diversity between and within populations of the same species over time (Jarvis et al. 2000), is a significant issue affecting diversity in crop domestication areas as Mexico: (1) concentrate the highest genetic diversity; (2) traditional growers conserve ancestral landraces (i.e., the local populations of cultivated species generated by traditional farmers), along with the knowledge and cultural practices that created this diversity; and (3) the presence of wild–crop introgression (Bellon and Taylor 1993; Brush 1991). Most often resulting from agricultural, economic, and social changes (FAO 1996), genetic erosion in domesticated species has been evaluated at the level of landrace (Hammer and Laghetti 2005; Tsegaye and Berg 2006) because it constitutes the primary available genetic pool for hybridization and genetic improvement programs (Harlan and De Wet 1971). Several decades ago, Frankel and Bennett (1970) noted the importance of genetic erosion within traditional agricultural systems on the word. They stated that many genetic reservoirs for crop plants were rapidly disappearing, and the detailed five principles: (1) diversity in crops exists because of adaptation by localized populations; (2) traditional agriculture that continues in centers of diversity maintains high, stable diversity; (3) modern agricultural technology, including modern varieties, is a recent phenomenon

and leads to instability; (4) competition between local and introduced varieties results in displacement of local varieties; and (5) displacement of local varieties reduces the genetic variability of the entire crop gene pool.

In Mexico, the milpa is the most important traditional agricultural system. It is an ancestral Mesoamerican dry land farming system based on human energy by which vegetation is cyclically slashed and burned to plant a group of basic crops. In the milpa, after 2–4 years of cultivation (depending on soil fertility), the land is allowed to rest for 5–15 years before a new cycle is begun (Hernández-Xolocotzi 1992; Pérez-Toro 1945). The conservation of patches of vegetation that are cyclically cultivated is, in turn, the mainstay of the milpa's productivity, as it assures the recovery of soil fertility and maintains the habitat for a large part of the plant genetic resources integrated into the milpa agroforestry production system (Colunga-GarcíaMarín and May-Pat 1993; Hernández-Xolocotzi 1992). The three principal crops of the milpa are corn (*Zea mays* L.), beans (*P. vulgaris* L., *P. coccineus* L., *P. polyanthus* Greenman, *P. lunatus* L.), and squash [*Cucurbita moschata* (Duch) Duch ex Poir; C. argyrosperma Huber]. Alongside these basic crops, many other secondary species are cultivated, such as chilli peppers (*Capsicum* spp.), batata (*Ipomoea batatas* L.; sweet potato), tomato (*Solanum esculentum* L.), and cassava (*Manihot sculenta* Crantz). Within any of these crops, a great intraspecific diversity is reflected in the existence of a large number of landraces. It is the case of the Lima bean (*P. lunatus*).

8.2 Lima Bean (*Phaseolus Lunatus* L., Fabaceae)

Lima bean (*Phaseolus lunatus* L.) is one of five domesticated species of the genus *Phaseolus* that has evolved in the neotropics for at least 6000 years (Kaplan and Lynch 1999). It is, after common bean (*P. vulgaris)*, the second most important commercial species of *Phaseolus* beans around the world (Baudoin et al. 2004). Its primary genetic pool has wild (*P. lunatus* var. *silvester*) and domesticated (*P. lunatus* var. *lunatus*) forms (Baudet 1977). Recent studies using *cpDNA* and *ITS* polymorphisms (Motta-Aldana et al. 2010; Serrano-Serrano et al. 2010, 2012; Andueza-Noh et al. 2013) have indicated that the organization of the genetic diversity of *P. lunatus* comprised three major gene pools: Andean (A), Mesoamerican I (MI), and Mesoamerican II (MII), all containing both wild and domesticated populations. Three cultigroups (cv-gr) are recognized in the domesticated forms (Baudet 1977): (1) Potato, with small, round seeds; (2) Sieva, with medium-sized, kidney-shaped seeds; and (3) Big Lima, with large, flat seeds. The Potato and Sieva cultigroups represent the MI and MII groups, and the Big Lima represents the Andean. The domestication area of the Andean gene pool has been located between Ecuador and northern Peru (Motta-Aldana et al. 2010; Serrano-Serrano et al. 2012). Evidence generated recently indicates a domestication event for MI in western Mexico (Motta-Aldana et al. 2010; Serrano-Serrano et al. 2012; Andueza-Noh et al. 2013). Although domestication of MII has yet to be defined,

recent evidence suggests that, if present, it will be located in the region between Guatemala and Costa Rica (Andueza-Noh et al. 2013).

8.2.1 Lima Bean in the Yucatan Peninsula, Mexico

The Lima bean landraces, called Ibes in Mayan, represent the fourth main crop for the Maya of the Yucatan Peninsula, the region with the highest morphological variation of landraces in all Mexico (Ballesteros 1999). This crop is planted into the milpa (Fig. 8.1a), principally and, less frequently, in home gardens (Fig. 8.1b). At present, there are four geographic areas in the Yucatan Peninsula where the milpa continues to be the most important economic activity (Fig. 8.2). These areas correspond to four of the 13 cultural-geographic zones established by Adams and Culbert (1977) for the origin of the Maya lowland civilization: (1) northeastern Campeche (*NECAMP*), in "Los Chenes" zone, (2) southern Yucatan (*SYUC*), in the "Puuc" zone, (3) southeastern Yucatan (*SEYUC*), located within the "Northern Plains" zone, and (4) central eastern Quintana Roo (*CEQROO*), within the "Río Bec" zone (Fig. 8.2).

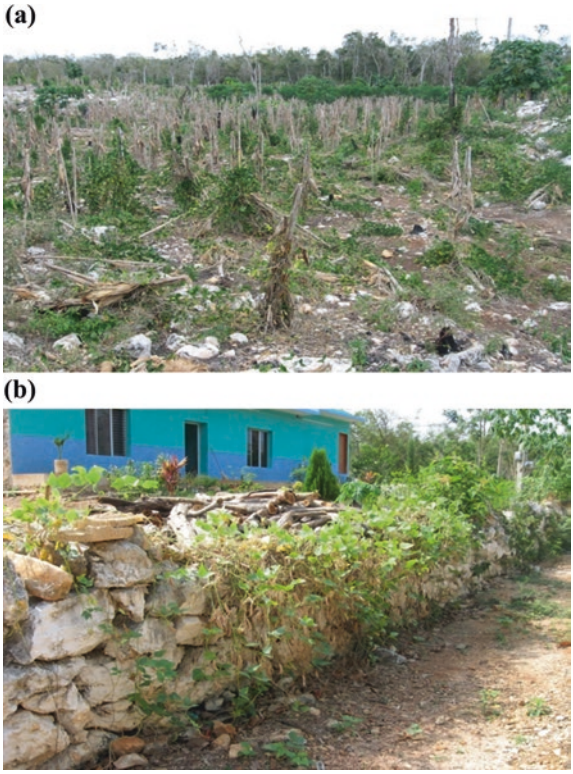

Fig. 8.1 Lima bean cultivated in two different agricultural systems in the Peninsula of Yucatan, Mexico. **a** Lima bean planted over maize plant in the traditional Mayan milpa. **b** Lima bean planted over a traditional Mayan backyard (albarrada) of a home garden

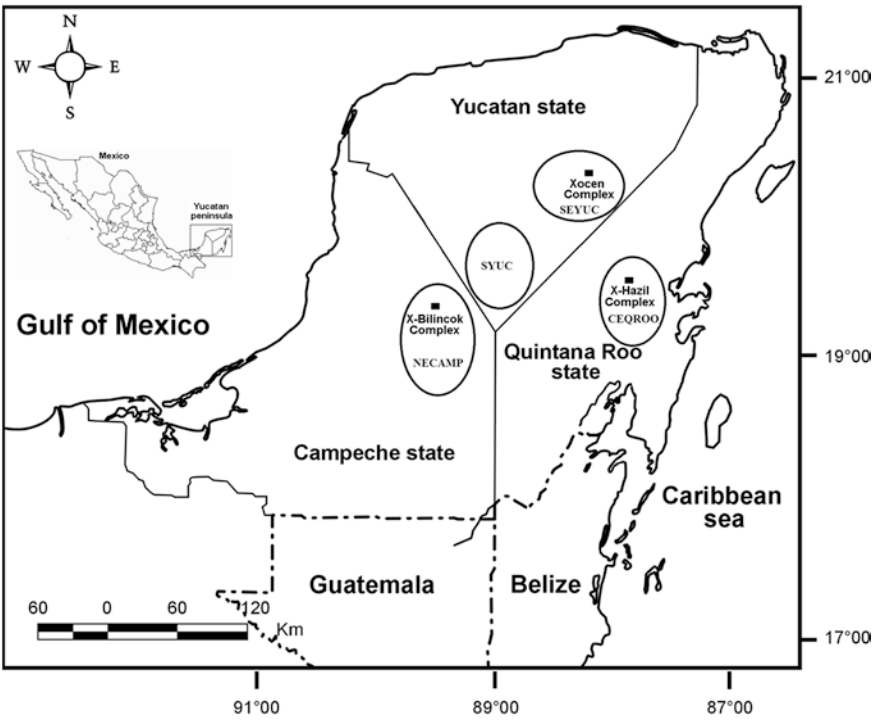

Fig. 8.2 Four agricultural regions where the Mayan milpa is an important traditional agricultural system. *SYUC* southern Yucatan, *NECAMP* northeastern Campeche, *SEYUC* southeastern Yucatan, *CEQROO* central eastern Quintana Roo. Also, this Figure shows the three wild-weedy-domesticated complexes (X-Bilincok, Xocen and X-Hazil) of Lima bean studied by Dzul-Tejero (2011)

Using morphophenological and ethnobotanical data, Martínez-Castillo et al. (2004) found 25 landraces of Lima bean with Potato, Sieva and intermediate forms of both cultigroups and characterized their relative abundance based on the percentage of cultivated area and the number of farmers that plant each landrace. Using a sample of 160 traditional Mayan farmers from the four agricultural zones before mentioned, these authors found that, of 25 landraces planted, three accounted for 71.24 % of the cultivated area. Most of the remaining 22 landraces were rare, meaning each accounted for less than 2 % of the cultivated area; in many cases, they were grown by a single farmer. This situation indicates that the crop is at serious risk for genetic erosion, which is increased by three factors: (1) environmental factors such as drought and hurricanes, which have led to loss of seed; (2) intensification of the traditional Mayan agriculture, which displaces cultivation of these landraces; and (3) increasing rural population and socioeconomic changes have led to migration of Mayan growers to tourist centers, with consequent abandonment of agricultural activity and changes in the traditional Mayan diet (Cuanalo and Arias 1997; Ku-Naal 1995; Reyes and Aguilar 1992).

Fig. 8.3 Groups of Lima bean landraces analyzed. Line *A* (abundant landraces): Mulición, Sac, Putsica-sutsuy; line *B* (common landraces): *Bacalar*, *Nuk*, *Chak-saac*, *Mejen*, *Chak-petch*, *Balche*; line *C* (rare landraces): *Box-petch*, *Balam-pach*, *Tsisibal*, *Kan*, *Chak-mejen*, *Madzakitam*; line *D* (rare landraces): *Pool-santo*, *Tabaco*, *Box-uolis*, *Chak-uolis*, *Chak-chí*, *Chocolate*. Landraces are named from *left* to *right*. Culti-groups: P (cv-gr Potato), S (cv-gr Sieva), I (intermediate forms between Potato and Sieva) (Reproduced from Martínez-Castillo et al. 2008)

8.3 Genetic Erosion in Lima Bean from the Yucatan Peninsula: Evidence from *ISSR* Molecular Markers

Since Zietkiewicz et al. (1994) invented the Inter-Simple Sequence Repeats (*ISSR*) technique, it has proven to be a rapid, simple and inexpensive way to assess genetic structure and diversity (Culley et al. 2007; González et al. 2005), to analyze genetic relationships among cultivars (Prevost and Wilkinson 1999; Martins et al. 2003), and to study evolutionary processes (Galván et al. 2003). The *ISSR* technique allows the detection of polymorphism without previous knowledge of *DNA* sequences. It amplifies *DNA* using the polymerase chain reaction (*PCR*) and a single primer composed of a microsatellite (*SSR*) sequence, anchored at the 3′ or 5′ end by two to four arbitrary, often degenerate, nucleotides (Zietkiewicz et al. 1994). The *ISSR* is a dominant marker so that the heterozygote cannot be directly distinguished from the dominant homozygote phenotype (band) at individual loci and consequently, the estimation of allele frequencies using *ISSR* markers presents some statistical difficulties (Lynch and Milligan 1994). These difficulties have

been resolved using appropriate estimators for the analysis of dominant markers such as the Shannon diversity index (*I*) (Shannon and Weaver 1949), percentage of polymorphic loci (*%P*), Nei's genetic diversity index (*h*) considering the Taylor expansion (Lynch and Milligan 1994) and with new methods as average heterozygosity (*H*) using the Bayesian approach proposed by Zhivotovsky (1999).

On the basis of the plant material and data collected by Martínez-Castillo et al. (2004, 2008) used 90 *ISSR* loci (Table 8.1) to analyze the genetic diversity of 21 Lima bean landraces of differing relative abundances: (a) three abundant landraces, each grown on more than 16 % of the total cultivated area and planted by 10–33 producers in four agricultural zones; (b) six common landraces, each grown on 3–5 % of the cultivated area and by 5–14 producers; and (c) 12 rare landraces, each planted on less than 2 % of the total area and grown by 1–4 farmers (Fig. 8.3, Table 8.2). These authors found that the Yucatan Peninsula has high levels of *h*genetic diversity ($h = 0.28$) in comparison with others studies. Using alloenzymes, Maquet et al. (1997) reported an $= 0.26$ for the *P. lunatus* base collection of the Germplasm Bank of the International Center for Tropical Agriculture (CIAT-Colombia) and they stated that this is a significant level and higher than reported for other plants that, like *P. lunatus*, are mixed-mating or short-lived perennial species ($h = 0.12$) (Hamrick et al. 1991). Using *RAPD* markers, Nienhuis et al. (1995) found a lower genetic diversity for domesticated Mesoamerican forms ($h = 0.11$). Also, using *AFLP* molecular markers, Castiñeiras et al. (2007) found a lower genetic diversity in landraces planted in Cuban home gardens ($h = 0.119$). Compared with all these studies, results from Martínez-Castillo et al. (2008) could be reflecting the high genetic diversity maintained by Mayan farmers in the milpa of the Yucatan Peninsula, México (Table 8.3).

Within the domesticated gene pool from the Yucatan Peninsula, Martínez-Castillo et al. (2008) found that the common group of landraces had the highest genetic diversity (except for *%P*), although the differences between the three groups (abundant, common, and rare landraces groups) considered were not statistically significant (Table 8.3). The rare landraces group had genetic diversity values (h and I) slightly lower than the common landraces group, but higher for *%P* (Table 8.3), probably because nine of the 12 rare landraces were represented by only one accession (Table 8.2), whereas all the common landraces were

Table 8.1 Characteristics of four ISSR primers to estimate the diversity and genetic relationships of *P. lunatus* landraces from the Yucatan peninsula, Mexico (Martínez-Castillo et al. 2008)

Primer code	Primer sequence	Annealing temperature (°C)	Number of loci analyzed	Polymorphic bands	Monomorphic bands
15	$(GACA)_3$ RG	42	21	15	6
16	YR $(GACA)_3$	42	16	13	3
30	$(GACAC)_3$ AG	54	20	15	5
32	$(GACAC)_3$ RG	54	33	28	5

*R = A or G

Table 8.2 Local name, culti-group, number of accessions, relative abundance, percentage of cultivated area, and agricultural regions of 21 landraces of Lima bean (*P. lunatus*) of the Yucatan peninsula, Mexico (Martínez-Castillo et al. 2008)

Local name	Culti-group	Number of accesions used	Relative abundance	% of cultivated area	Agricultural regions
Mulición	Potato	5	Abundant	29.61	All regions
Sac	Intermediate	5	Abundant	25.13	All regions
Putsica-sutsuy	Intermediate	5	Abundant	16.5	All regions
Bacalar	Sieva	5	Common	5.82	CEQROO
Nuk	Sieva	5	Common	4.12	SYUC
Chak- saac	Sieva	5	Common	4.1	CEQROO, SEYUC
Mejen	Sieva	5	Common	3.00	SYUC
Chak- petch	Sieva	5	Common	1.79	CEQROO, SEYUC
Balche	Sieva	5	Common	0.92	CEQROO
Box-petch	Intermediate	1	Rare	1.85	CEQROO, NECAMP
Balam-pach	Potato	1	Rare	1.1	SEYUC
Tsisibal	Potato	2	Rare	1.1	SEYUC
Kan	Potato	1	Rare	1.01	SEYUC
Chak-mejen	Sieva	2	Rare	0.32	NECAMP
Madza-kitam	Sieva	1	Rare	0.31	SEYUC
Pool-santo	Intermediate	1	Rare	0.26	CEQROO, SEYUC
Tabaco	Sieva	1	Rare	0.16	CEQROO
Box-uolis	Potato	1	Rare	0.08	CEQROO
Chak-uolis	Potato	4	Rare	0.06	CEQROO, SEYUC
Chak-chi	Sieva	1	Rare	0.02	SEYUC
Chocolate	Sieva	1	Rare	0.02	CEQROO

Agricultural regions: *SEYUC* southeastern Yucatan, *CEQROO* central eastern Quintana Roo, *SYUC*, southern Yucatan, *NECAMP* northeastern Campeche

Table 8.3 Estimators of genetic diversity of Lima bean landraces groups from the Yucatan peninsula, Mexico, using 90 ISSR loci (Martínez-Castillo et al. 2008)

	Percentage of polymorphic loci (% *P*)	Shannon's diversity index (*I*)	Nei's gene diversity (*h*)	Bayesian average heterozygosity (*H*)
Total domesticated gene pool	78.9	0.33	0.29	0.31
Groups of landraces				
Dominant landraces	26.7	0.17 a	0.13 a	0.27 a
Common landraces	58.9	0.33 a	0.26 a	0.37 a
Rare landraces	66.7	0.27 a	0.24 a	0.28 a

Groups with the same letter are not different significantly (a = 0.05)

represented by at least five accessions. The minimal abundance of the rare landraces is the main factor that most increases their risk for genetic erosion since it can lead to their local extinction. During a travel made in 2007 to collect germplasm of Lima bean landraces, a farmer from southeastern of the Yucatan reported that he had lost his seed of *Pool-santo* and *Chak-chí* landraces in the 2006 agricultural cycle due to a lack of rain. In another case, a farmer from Central-east of Quintana Roo stopped planting the *Chocolate* and *Tabaco* landraces in 2005 because he became sick that year and did not cultivate his milpa. This farmer was the only one who had these two rare landraces and these have not been collected again until now. Two factors that could reduce the risk of genetic erosion in some of the rare landraces are dark seed color and their mixed management by Mayan farmers. Both aspects favor the entrance of wild alleles through formation of wild–weedy–domesticated complexes and the generation of weedy forms (Martínez-Castillo et al. 2004). A special case in the use of seed mixtures is the *Bacalar* landrace, which has become a kind of "genetic dump" as it contain seeds similar to many different landraces, such as *Mejen, Nuk,* and *Pool-santo*, including weedy forms (Fig. 8.4).

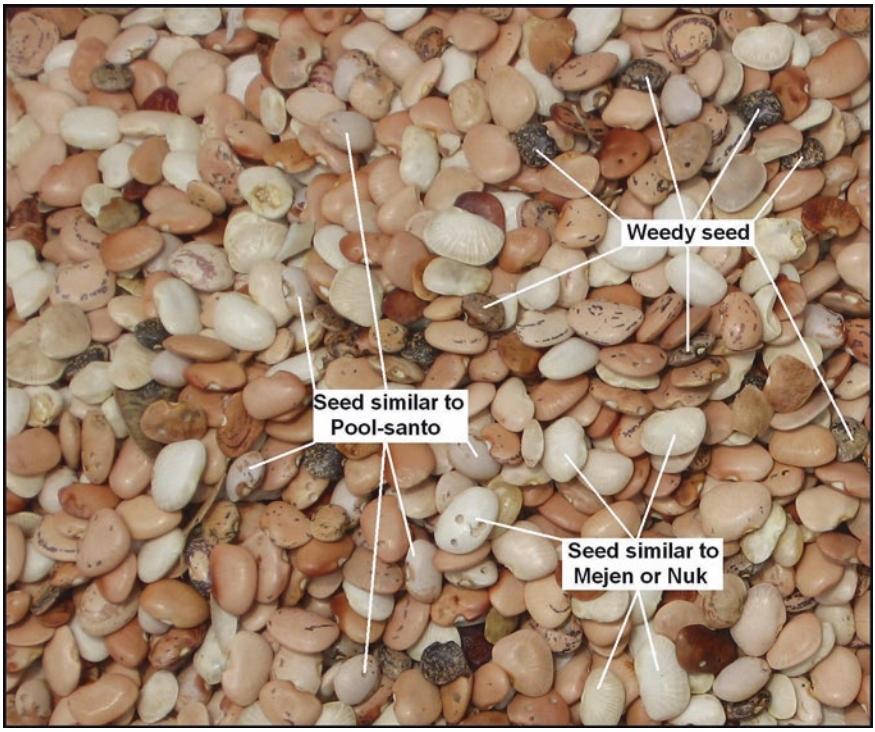

Fig. 8.4 Weedy forms found into a *Bacalar* seed lot from central-east Quintana Roo (Reproduced from Martínez-Castillo et al. 2008)

Martínez-Castillo et al. (2008) showed that the abundant landraces group had the lowest values of genetic diversity among the three groups for all estimators, except for *H* that equaled the value for the rare landrace group (Table 8.3). These low values could reflect a germplasm selection influenced by external market demands. Martínez-Castillo et al. (2004) reported that one of the main selection criteria for the three most abundant landraces (*Mulición*, *Sac,* and *Putsica-sutsuy*) is production of seed for sale; thus, Mayan farmers currently tend to plant white-seeded landraces (*Mulición*, *Sac*, *Mejen*, *Nuk*). This tendency leads to selection against weedy forms that are produced from crosses between landraces and the wild populations surrounding the milpas, consequently limiting introgression of wild alleles and increasing the risk of genetic erosion. In relation to the dominant Lima bean landraces, Debouck (1979) collected at least 10 different landraces in northeastern Campeche in 1979, but currently only three have been observed, and these are dominated by *Mulición* and *Sac*. Informal interviews with Mayan growers suggest that this loss of landraces is associated with the introduction of mechanized agriculture and monoculture of improved varieties of corn. Recent field observations indicate that even the planting of abundant Lima bean landraces such as *Mulición* and *Sac* is decreasing in response to low prices. A similar case is happening in southern Yucatan, where the *Mejen* landrace has been replacing the other landraces with seeds that are not white (Martínez-Castillo et al. 2004). Recently, the area sown with *Mejen* has decreased because of low market demand. Even though in the 2004 study, *Mejen* was considered as a landrace, evidence suggests that it could be an improved variety introduced approximately 25 years ago: (1) it was not found by Debouck in 1979, (2) it is a variety planted as a monocrop (a rarity in traditional Mayan agriculture) and is not associated with maize as are all the other landraces, and (3) it is a variety with a very short production cycle that depends on a lot of water, a limited resource in the Yucatan Peninsula. This decrease in the number and density of planted populations may mean that a new genetic bottleneck is soon to come for the abundant landraces.

8.4 Genetic Erosion in Lima Bean from the Yucatan Peninsula: Temporal Analysis Using *SSR* Molecular Markers

The microsatellite (*SSR*-Simple Sequence Repeat) marker, a codominant marker that is highly polymorphic and discriminating and distributed throughout the genome (Tautz and Renz 1984), have proven useful in studies of genetic structure and diversity in cultivated plants (Martínez-Castillo et al. 2006, 2007; Zhou et al. 2006). Using nine *SSR* polymorphic loci (Table 8.4), Martínez-Castillo et al. (2011) temporally analyzed genetic erosion in Lima bean landraces from northeastern Campeche (*NECAMP*) (Fig. 8.2). These authors analyzed material collected during two different years: (1) seeds of 23 accessions that were collected in

Table 8.4 Characteristics of nine SSR loci used to estimate the genetic erosion of the Lima bean accessions collected in 1979 and 2007 in northeast Campeche, Mexico (Martínez-Castillo et al. 2011)

Primer code	SSR sequence	5′–3′	Primer sequence	Annealing temperature (°C)	Base pairs
GATS91	$(GA)_{17}$	Left Right	GAGTGCGGAAGCGAGTAGAG TCCGTGTTCCTCTGTCTGTG	53	229
AG1	`$(GA)_8$GGTA$(GA)_5$	Left Right	CATGCAGAGGAAGCAGAGTG GAGCGTCGTCGTTTCGAT	52	132
BM140	$(GA)_{30}$	Left Right	TGCACAACACACATTTAGTGAC CCTACCAAGATTGATTTATGGG	55	190
BM156	$(CT)_{32}$	Left Right	CTTGTTCCACCTCCCATCATAGC TGCTTGCATCTCAGCCAGAATC	52	267
BM211	$(CT)_{16}$	Left Right	ATACCCACATGCACAAGTTTGG CCACCATGTGCTCATGAAGAT	52	186
BM202	$(GA)_9$GT$(GA)_4$	Left Right	ATGCGAAAGAGGAACAATCG CCTTTACCCACACGCCTTC	50	156
BM170	$(CT)_5$CCTT$(CT)_{12}$	Left Right	AGCCAGGTGCAAGACCTTAG AGATAGGGAGCTGGTGGTAGC	50	179
BM183	$(TC)_{14}$	Left Right	CTCAAATCTATTCACTGGTCAGC TCTTACAGCCTTGCAGACACT	52	149
BM197	$(GT)_8$	Left Right	TGGACTGGTCGATACGAAGC CCCAGAAGATTGAGAACACCAC	54	201

1979 by Dr. Debouck in Nohalal town (in northeastern Campeche) were obtained from the Germplasm Bank of the Centro Internacional de Agricultura Tropical (CIAT-Colombia) (Fig. 8.5a), and (2) seeds of 21 accessions were collected directly in 2007 by Dr. Martínez-Castillo in Nohalal and three adjacent Mayan towns: Chunyaxnic, X-Bilincock, and Yaax-haltun (Fig. 8.5b).

First, Martínez-Castillo et al. (2011) analyzed genetic erosion in the same Mayan town (Nohalal) for the two collected years (1979 vs 2007). The authors found that all the genetic diversity estimators gave higher values for 1979 (Table 8.5). These results can be a consequence of the number and kind of landraces collected during each year. In 1979, Dr. Debouck collected 10 landraces at least, whereas in 2007 only three landraces were collected, and many of the accessions were seeds with white testa (Fig. 8.5b). At present, white seed landraces (*Sac*, *Mulición* and *Mejen*) dominate Lima bean production in the Yucatan Peninsula (Martínez-Castillo et al. 2004). Secondly, the authors analyzed the genetic erosion between the accessions collected in 1979 in Nohalal compared with all accessions collected in 2007 in Nohalal and the three neighboring towns. Values of *%P* and the number of alleles per locus (N_a) between the two collected years did not differ, meaning that the allelic richness is similar in both years. However, the number of effective alleles (N_e) and *h*, whose values were higher in the accessions collected in 1979 (Table 8.5), did differ for the two groups, indicating that the genetic diversity was greater in 1979. Importantly, including

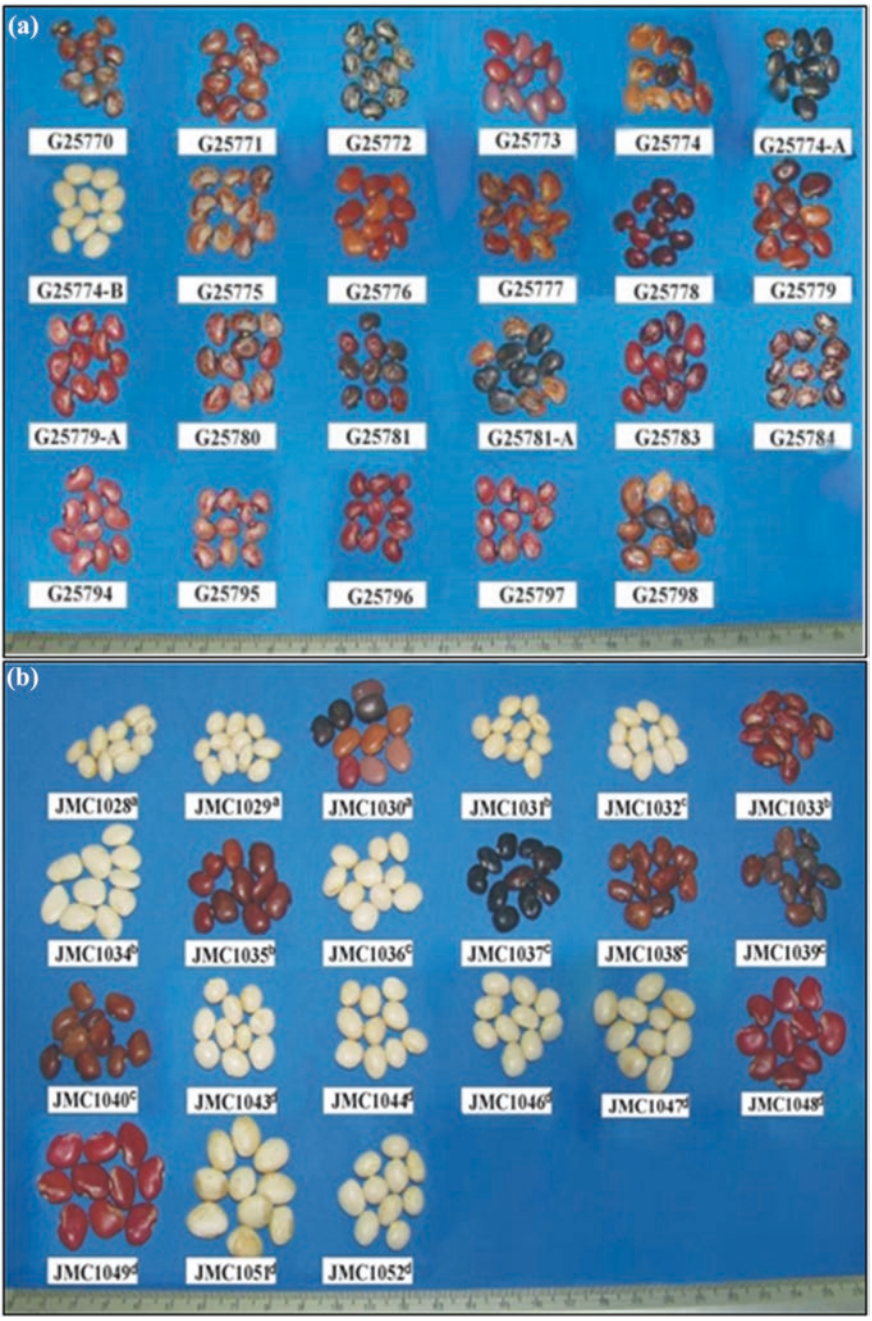

Fig. 8.5 **a** Accessions of Lima bean analyzed from Nohalal-1979. **b** Accessions of Lima bean analyzed from 2007. In Fig. 8.5b, letter after the accession represent the town where that accession was collected: **a** Yaax-haltun, **b** Chunyaxnic, **c** X-Bilincok, **d** Nohalal (Reproduced from Martínez-Castillo et al. 2011)

Table 8.5 Estimators of genetic diversity of the Lima bean accessions collected in northeastern Campeche analyzed by collected year (1979, 2007) (Martínez-Castillo et al. 2011)

Collected year	Percentage of polymorphic loci (*% P*)	Observed number of alleles (*na*) (SD)	Effective number of alleles *(ne)* (SD)	Nei's genetic diversity index (*H)* (SD)
1979	44.44	1.55 (0.73)	1.34 (0.50)	0.18 (0.24)
2007-Nohalal	5.00	1.04 (0.01)	1.00 (0.02)	0.01 (0.01)
All-2007	44.44	1.56 (0.73)	1.05 (0.08)	0.05 (0.07)

SD standard deviation

accessions collected in the three other Mayan towns generated a decrease in the differences observed between the genetic diversity estimators, in relation to the previous analysis (Nohalal-1979 vs Nohalal-2007) (Table 8.5). This decrease could be due to the fact that the germplasm collected in 2007 in the three towns contained accessions of other landraces that were not found in Nohalal in 2007. These landraces may have high levels of genetic diversity that could compensate for the lower levels of genetic diversity present in the landraces collected in 2007 in Nohalal. These landraces are no longer planted in Nohalal, even though the four Mayan towns are neighbors. The main reason for this could be the agricultural intensification present in Nohalal over the last 30 years. The Nohalal Mayan farmers have better soils that could be used to plant improved varieties of maize and other species with high commercial value. In the case of the Lima bean landraces, only those with commercial value (e.g., *Sac* and *Mulición*) are considered for planting in these areas.

To understand better the genetic erosion process in the landraces from northeastern Campeche between 1979 and 2007, Martínez-Castillo et al. (2011) also analyzed the genetic differentiation among the landraces between these two collected years. An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) revealed that a great proportion (82.2 %) of the total variation can be explained by differentiation among the two temporal groups of accessions (1979 vs all-2007), with only 12.9 % of the total among accessions within years. To confirm these results, the authors analyzed the genetic relationships among the all accessions. An Unweighted Pair Group Method with Arithmetic mean (UPGMA) analysis (Fig. 8.6) indicated a grouping of the accessions in accordance with the years of collection, with high bootstrap values supporting each group. This result indicated that the genetic erosion is not only quantitative as discussed earlier, but also qualitative. Thus, over the last 30 years, there has been a shift in the genetic make-up of this crop in *NECAMP*. This result is also similar to other studies that have indicated that plant breeding can generate a qualitative change in the genetic diversity of crops (Khlestkina et al. 2004; Le Clerc et al. 2005; Mantegazza et al. 2008; Xiu-Quiang et al. 2007).

Finaly, Martínez-Castillo et al. (2011) used the Bottleneck program (Luikart and Cornuet 1997) to look for a possible bottleneck event between 1979 and 2007, first for each year of collection (accessions from 1979 in Nohalal, accessions

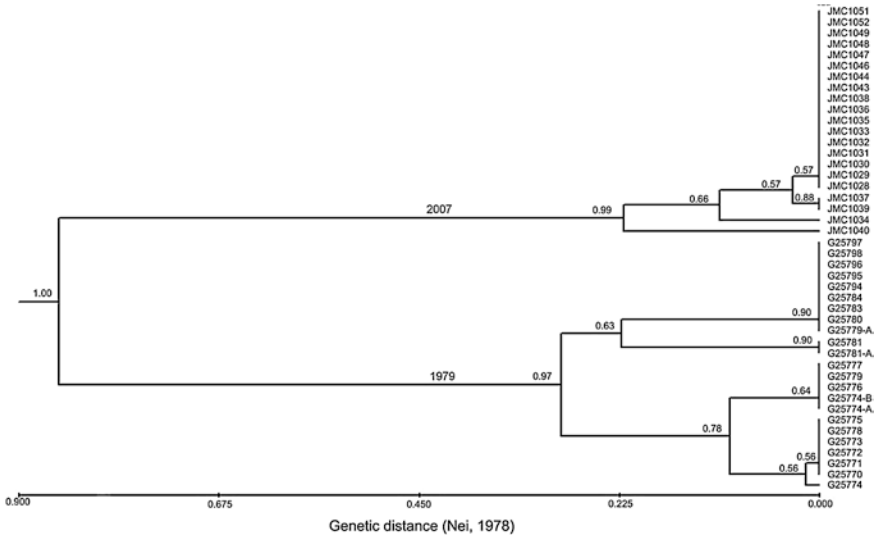

Fig. 8.6 Dendrogram (UPGMA) based on Nei's genetic distance (1978) of 44 accessions analyzed using 9 SSR loci. The numbers at the nodes are the proportion of similar replicates supporting each node (Reproduced from Martínez-Castillo et al. 2011)

collected in 2007 in Nohalal, and accessions collected in 2007 in Nohalal, and the other three adjacent towns). For this analysis, Wilcoxon sign-rank tests were not significant, indicating there is no excess in gene diversity under any of three mutation models (Stepwise Mutation Model [*SMM*], Two-Phased Model [*TPM*], Infinite Allele Model [*IAM*]) considered in the Bottleneck program and thus no bottleneck event in these three gene pools. Only the Lima bean accessions collected in 1979 in Nohalal yielded values close to $\alpha = 0.05$, indicating a possible bottleneck event. It is important to remember that the accessions collected in Nohalal in 1979 were obtained from the CIAT gene bank where the rejuvenation process of the seed alone can lead to genetic erosion. Even considering this factor, these accessions had higher genetic diversity than did all the accessions collected in 2007 ($h = 0.18$ vs $h = 0.05$, respectively).

8.5 Genetic Diversity in Lima Bean from the Yucatan Peninsula Compared with the Highland Mayan Subarea

Mayan culture is one of the most important of Mesoamerica. The Mayan geographical region has been divided into two subareas: lowlands and highlands (Ruz 1981; Sharer 1999). The Yucatan Peninsula is part of the Mayan lowlands;

Chiapas state (Mexico), and Guatemala (except the Peten area) are part of the Mayan highlands. Whereas the genetic diversity of Lima bean planted in the Mayan lowlands is welldocumented (Martínez-Castillo et al. 2004, 2008, 2011), until 2012 there was no molecular data for Lima bean from the Mayan highlands. Considering this, using 73 *ISSR* loci, Camacho-Pérez (2012) analyzed the genetic diversity in the entire Mayan region and in each subarea (23 accessions from each subarea). Using a Bayesian approach (Zhivotovsky 1999), Camacho-Pérez found high levels of genetic diversity in the Mayan area ($H = 0.45$), with higher levels in the lowlands ($H = 0.44$) than in the highlands ($H = 0.36$). This author also found a high genetic differentiation between the two subareas (AMOVA = 65 % of total variation between groups) and a grouping pattern based on the presence of the two subareas. These results confirmed the importance of the Yucatan Peninsula as a center of genetic diversity for the Lima bean landraces.

8.6 Gene Flow and Genetic Introgression: Factors that Counter Genetic Erosion in Lima Bean in the Yucatan Peninsula

In the Yucatan Peninsula, inter-landrace gene flow and natural introgression of wild alleles may prevent the genetic erosion of Lima bean landraces. Martínez-Castillo et al. (2004) reported the planting of up to seven landraces in a single milpa and existence of a wide variety of hybrid seeds. Using eight SSR markers, Martínez-Castillo (2005) observed very high gene flow levels between landraces in each of the agricultural regions of the Yucatan Peninsula. On the other hand, Martínez-Castillo et al. (2006) reported high genetic diversity levels in the wild populations of Lima bean from this region, and Martínez-Castillo et al. (2007) documented wild–domesticated gene flow and weedy forms derived from this flow (Fig. 8.7). Ethnobotanical observations showed that these weedy forms generated by introgression are being incorporated by Mayan farmers into their cultivated gene pools (Fig. 8.4). It is considered that the natural wild–domesticated

Fig. 8.7 Morphological variation in wild and weedy seeds collected in the Yucatan Peninsula, Mexico

introgression has played a vital role in the evolution of domesticated species and continues to be an important factor in increasing genetic diversity in modern crops (Arnold 1992; Harlan 1965; Jarvis and Hodgkin 1999; Slatkin 1987).

Considering before mentioned, and the important role that traditional farmers have played in this microevolutionary process, Dzul-Tejero (2011) used 11 *SSR* loci to assess levels of introgression in three wild–weedy–domesticated complexes of Lima bean from three Mayan milpas of the Yucatan Peninsula (Fig. 8.3) analyzes its impact on the genetic diversity of this crop. A test of assignment of individuals using the *STRUCTURE* program (Pritchard et al. 2000) indicated that the complex with the lowest level of introgression was one where the farmer actively selected against wild plants and introgressed seed (Fig. 8.8). This complex was Xocen located in southern Yucatan-*SEYUC*- (Fig. 8.3). By contrast, the complex with the highest level of introgression was one where the farmer had been consciously selecting a weedy morphotype for 15 years and had already incorporated into his diet (Fig. 8.8). This complex was *X-Hazil* located in Central-east Quintana Roo -*CEQROO*- (Fig. 8.3). Dzul-Tejero (2011) also found that the genetic diversity was higher in the complex with the higher level of introgression. These results confirmed the importance of genetic introgression to maintain and increase the levels of genetic diversity in crops.

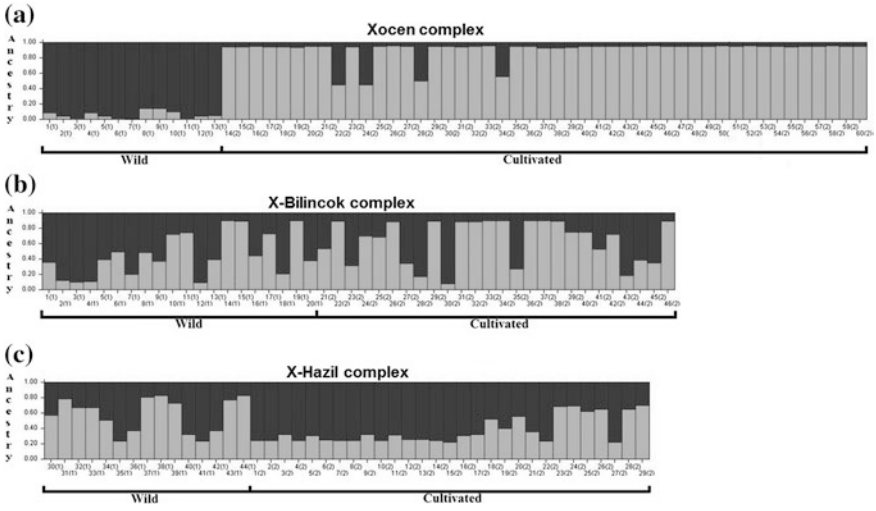

Fig. 8.8 Wild to crop introgression in three wild-weedy-domesticated complexes from Yucatan peninsula, analyzed using STRUCTURE program and 11 SSR loci. **a** Xocen complex from SEYUC; **b** X-Bilincok complex from NECAMP; **c** X-Haxil complex from CEQROO

8.7 Conclusions

Studying the genetic erosion in Lima bean landraces in the Yucatan Peninsula is important because this region is likely playing an important role in the in situ conservation of the Mesoamerican gene pool of this crop (Ballesteros 1999; Camacho-Pérez 2012; Martínez-Castillo et al. 2004). Martínez-Castillo et al. (2008) showed that many landraces of Lima bean are at higher risk of genetic erosion because, with few farmers planted these landraces and with moderate genetic diversity, they represent the greatest loss of unique alleles if these landraces go to local extinction. On the other hand, the abundant landraces have the lowest genetic diversity levels and are thus at great risk of genetic erosion due to selection criteria imposed by an external market, too. Although the high gene flow levels observed in the domesticated gene pool (Martínez-Castillo 2005) and the existence of genetic introgression between domesticated and wild populations (Dzul-Tejero 2011) could be limiting the genetic erosion of the Lima bean landraces, it is important to consider that the landraces are not just a group of unrelated alleles. Instead, each is a package of alleles selected during centuries by traditional Mayan farmers to cope with different environmental restrictions. The loss of landraces could mean a great loss in the genetic diversity of this crop and in the production and surviving strategies of the Mayan farmers of this part of Mexico.

The temporal analysis done by Martínez-Castillo et al. (2011) confirmed the high risk of genetic erosion faced by Lima bean from the Yucatan Peninsula, indicating that a smaller number of landraces planted means also a minor level of genetic diversity present in the crop. Indeed, molecular data showed the existence of a decrease in the genetic diversity in between 1979 and 2007 as well as a great shift in the allelic composition. This genetic shift might possibly be a consequence of the introduction of improved varieties of *P. lunatus* or by changes in the Mayan criteria selection of germplasm or both. In the same way as in the Mayan town of Nohalal, the landraces may be disappearing in many other Mayan towns from this part of Mexico. This loss is not only a consequence of factors associated with agricultural intensification or the incorporation of the Mayan farmers into nonlocal market system. A series of environmental, socioeconomic and cultural factors are participating, too. For example, the Dean hurricane in 2007 in the south central state Quintana Roo caused seed loss of many cultivated species, among them, many rare landraces of *P. lunatus*. At present, several of these landraces have not been collected by our research group.

One little-studied factor in the genetic erosion of crops is a change in the food preferences of rural populations. For Lima bean in the Yucatan Peninsula, such preference takes three forms: (1) young adults and children do not eat them, (2) only the elderly plant many of the rare landraces for their own use, and (3) cowpea [*Vigna unguiculata* (L.) Walpers, locally known as *x-pelon*], introduced to the region from Africa in the twentieth century, has been replacing *P. lunatus*. Because the process of reintroducing a crop plant is a long one, Esquivel and Hammer (1988) in a study in Cuba proposed maintaining Lima bean landraces as part of the

traditional horticultural system. In several Mayan towns of the Yucatan Peninsula, some landraces are planted into home gardens (Fig. 8.2), including the *X-Konan jonal* landrace (keeper of the house in Mayan, because this landrace can be planted in either home gardens or the milpa). However, such dual-planting of a landrace is not a common agricultural practice. On the other hand, loss of landraces is also apparently linked to different generations of human populations. Mayan farmers that plant a large variety of rare landraces are elderly; their death almost surely means the loss of these landraces.

8.8 Prospects

If the data about relative abundance reported by Martínez-Castillo et al. (2004) reflect the current condition of the domesticated Lima bean pool in the Yucatan Peninsula, then this species is at very high risk of genetic erosion since this region is one of its main centers of genetic diversity in Mesoamerica (Ballesteros et al. 1999; Camacho-Pérez 2012; Martínez-Castillo et al. 2004). If current trends continue in the region, many Lima bean landraces may cease to be grown into the milpa in two to three generations. To prevent the loss of this valuable germplasm, in situ conservation programs are needed to implement (1) an emergency collecting effort to save all landraces ex situ, as a backup for in situ conservation activities, (2) in situ conservation of landraces and their alleles, (3) conservation of wild–weedy–domesticated complexes that allow introgression of wild alleles into landraces, and (4) reintroduction of rare landraces and programs to promote their planting and acceptance among young Mayan producers and their families. To do this, areas need to be selected that favor in situ conservation while considering the natural, economic, social and cultural factors that contribute to this conservation. In the case of the Yucatan Peninsula, we consider that the corridor *SEYUC–CEQROO* is a good area for this in situ conservation, not only for *P. lunatus*, but also for the many other domesticated plants of the Mayan milpa of this region of Mexico. Our research group is presently collecting the landraces and wild populations of *P. lunatus* in the Yucatan Peninsula, to create a core collection representative of this important crop in the traditional Mayan agriculture of the Yucatan of Mexico.

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Chapter 9
Agrobiodiversity: The Importance of Inventories in the Assessment of Crop Diversity and Its Time and Spatial Changes

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Abstract In general, the absence of detailed knowledge of world biodiversity prevents the application of the methodological tools that could successfully assist in biodiversity conservation. Inventories are seen as a first step to assessing the biodiversity with respect to its richness and distribution patterns and to monitor its changes. Nevertheless, currently no comprehensive global inventory of species diversity exists. Our knowledge of biodiversity encompasses only 20 % of the total estimated number of species. Similar gaps could also be identified in the current understanding of crop diversity with a particular emphasis on the intraspecific diversity where a wide and comprehensive inventory is urgently required. Surveys

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are pivotal for the accumulation of knowledge required to populate agrobiodiversity inventories that are essential tools for creating effective mechanisms to monitor changes in the crop diversity and to estimate genetic erosion of predominantly threatened components of diversity, i.e., landraces. Our work aims to review the current state of agrobiodiversity inventories with particular emphasis on crop species and their intraspecific diversity. The complexity of crop diversity and the limitations of our knowledge with that respect are discussed. The need of inventorying and surveying at the species and below-species levels is reviewed. The ambiguity of landraces definition, which is a major component of intraspecific crop diversity, along with the distinct needs to design and execute their inventory strategies is debated. Crop diversity has a prospective use for agriculture and food production sustainability, crop improvement or crop adaptation to climatic changes, and therefore needs to be inventoried and protected against erosion and extinction. Finally, we present some inceptive attempts to advance ex situ and in situ landrace inventories.

Keywords Biodiversity · Agrobiodiversity · Crop wild relatives · Landraces · Inventories · Surveys · Collections

9.1 Introduction

Humankind is presently facing a global challenge to provide forthcoming generations with continuing and sustainable development that would guarantee present and future food quality and security, while assuring at the same time conservation of biological diversity. Nowadays, biodiversity is threatened by increasing rates of species extinction and geographical and genetic drift that are further accelerated by climate changes (Thuiller et al. 2005; Parry et al. 2007). The Convention on Biological Diversity (CBD) (UN 1992) defined the following as major challenges: completing the inventory of biodiversity, promoting its conservation and rational use, and fair share of biodiversity benefits. The failure to accomplish the 2010 biodiversity targets for the Millennium (CBD 2010) created an urgent need to intensify the efforts to improve biodiversity inventorying and to monitor its changes. This need for the intensification of efforts is addressed in the 2011–2020 strategic plans for biodiversity, called the Aichi Biodiversity Targets (CBD 2010). However, the task to monitor biodiversity changes is too broad and aims to achieve consensus on the essential biodiversity variables (EBV). The development of the EBV was meant to be a methodological tool that allows performing worldwide

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monitoring programs that could bridge the gaps detected in the strategic plan for biodiversity conservation (Pereira et al. 2013). To provide a genuine value these programs have to possess several attributes including (a) precise knowledge of biodiversity and the number of species existing on Earth; (b) complete inventories that could help to estimate biodiversity patterns based on *taxa* accounts at different taxonomic ranks; (c) generate reliable data on interspecific and intraspecific diversity; (d) standard methodology aiming to perform cost-effective biodiversity surveys of target areas and groups; (e) effective systematization of data facilitating retrieval and use of the information.

Biodiversity has several components and can be inventoried at species, ecosystems, and genetic levels (Tor-Björn Larsson 2001). Nonetheless, biodiversity is most frequently measured at the species level. In this context, the species inventories are seen as the milestone to assess biodiversity and monitor its change, including extinctions and erosion. However, the absence of detailed assessment of the biodiversity with respect to its richness and distribution patterns is a major issue. The actual knowledge of biodiversity is mainly based on species numbers resulting from 250 years of taxonomists' efforts. Using this approach Earth's biodiversity is estimated for 1.2–1.9 million species (Lecointre and Le Guyader 2001). However, it is of common sense that this does not fully represent the actual biodiversity. Several appraisals predicting the number of unknown species were made, with ambiguous success, since the global species richness is calculated indirectly, frequently relying on highly controversial estimations and assumptions, which resulted in a very broad range stretching between 3 and 100 million species (Erwin 1982; Hambler 2004; May 2010). Heywood (1996) estimated that the total number of animals and plants was between 13 and 14 million species. Using a newly developed methodology based on existing species inventories and statistical methods, weighing the velocity of new species discovery, Mora et al. (2011) estimated Earth biodiversity for 8.5 million species. According to the actual assessment of species diversity endorsed by the International Union for Conservation of Nature (IUCN), the appraised number of described species is only 1,729,693 (IUCN 2013). Taking into consideration this number, present Earth's biodiversity based on taxonomic inventories would reach only 14–22 % of the estimated species diversity. Thus, it is paramount to increase our understanding of less known taxonomic groups in different ecosystems and parts of the world and with respect to current knowledge of agrobiodiversity. Mora's method based on statistical predictive analysis of inventories can be applied to estimate the total number of *taxa* in different diversity groups and conceivably could be applied to estimate crop diversity of less documented groups, i.e., landraces.

The urgency of biodiversity assessment is dictated by the uncertainty of our overall knowledge of the matter and by the increasing levels of biodiversity losses. The most recent estimation of biodiversity loss predicts that up to 311 species per day may be extinct in the next 30 years due to pressure of global changes of native habitats (Khera et al. 2001). Based on the actual rates of species inventory (6200 species per year) and estimated extinction rates (110,715 species per year according to Khera et al. (2001) or 0.72 % of species extinction per year (61,200 taxa)

in the case of the Mora's estimations (Mora et al. 2013) one can conclude that numerous species will disappear even before they could be discovered (Carbayo and Marques 2011). The same trend also affects agrobiodiversity, both at the species or intraspecific levels. The number of crop species is relatively well established, especially when compared to other groups such as insects, fungi, bacteria, including soil microorganisms or crop wild relatives (CWR), which remain largely insufficiently inventoried (Maxted et al. 2007, 2012). Understanding the enormous gaps in biodiversity inventorying has prompted the development of several programs, such as the Rapid Assessment Programs (RAP, https://learning.conservation.org/biosurvey/Pages/default.aspx), aiming to build methodologies and capacity for systematic data collection and dissemination. This program targets biodiversity hotspot regions and particularly the areas where biodiversity data and knowledge are lacking. Created in 1990, the RAPs have conducted 66 surveys of terrestrial and marine biodiversity in different parts of the world. Unfortunately, most of these surveys did not target the inventory of agrobiodiversity. A smaller number of the EBV required to monitor changes in biodiversity and/or agrobiodiversity over time (Pereira et al. 2013), particularly in the high diversity regions would be possible only when a solid knowledge of biodiversity is established.

9.2 Definition and the Complexity of Agrobiodiversity

This paper reviews the current knowledge related to agrobiodiversity with particular emphasis on the available inventories of crop diversity. The systematic study of crop diversity and its genetic resources was initiated by De Candolle (1884) and Vavilov (1926, 1951, 1957). The latter researcher proposed a theory of the centers of origin and domestication of cultivated species. These centers of domestication have been reviewed by Harlan (1992) and their geographical distribution has slightly changed for areas that are essentially coincidental with the Vavilov's original centers and the actual biodiversity hotspots.

Agrobiodiversity and agro-diversity are two somewhat similar yet diverse terms used to classify biodiversity related to agriculture. Agro-diversity according to Brookfield and Padoch (1994) is defined as "many ways in which farmers use the natural diversity of the environment for production, including not only their choice of crops but also their management of land, water, and biota as a whole." Thus, this notion pays attention to the multitude of interactions in the agroecosystem, including management practices, farmers' resource endowments, biophysical resources, animal breeds, and crop species. This broad understanding of agro-diversity underlines the key role of farmers in the creation and maintenance of agro-diversity, e.g., animal and plant species, breeds, landraces, and varieties and also the importance of nonbiological components (e.g., farming systems and practices, soil, water and nutrients) of the agroecosystem in its evolution exemplifying the spatial and temporal dimensions of crop diversity.

The term agrobiodiversity is older and specifically refers to all biological diversities present on the lands modified and used for agricultural purposes (Brookfield and Stocking 1999). The concept of agrobiodiversity includes species playing different roles in the agriculture, such as: (a) crop and animal species involved and used to produce food, feed, or raw materials; (b) crop wild relatives, semidomesticated and nondomesticated species used for food or crop improvement; and (c) wild species and organisms that are not directly involved in the agricultural production, but can diminish the agroecosystem productivity (e.g. weeds, pests, diseases) are fundamental to its health and sustainability (e.g., soil fauna, flora and microorganisms, auxiliary species, etc.). However, if non-harvested or nondomesticated diversity can exist outside agroecosystems without a need of direct human intervention, including the second and third components of agrobiodiversity, the same is not entirely true for the domesticated species. Their survival depends of farmers' cultivation, management, and conservation efforts in a close association with the agroecosystems' multifunctionality features. In this paper the term "agrodiversity" will be used with regard to crop diversity.

The strong dependence of agro-diversity (crop and bred species) on the continuous cultivation and use is also an important factor exacerbating susceptibility to genetic erosion and extinction. We subscribe to the broad description of agrodiversity proposed by Khosbakht and Hammer (2008) defined as "a part of overall biological diversity, and composed by cultivated crop species, domesticated by mankind, including species used in amenity horticulture, comprising ornamentals and plants connected with gardening and landscaping." Agro-diversity cannot be also dissociated from the crop wild relatives (CWR) (Maxted et al. 2007) as they are an important diversity component potentially contributing to assurance of safety of crop genetic resources due to their close genetic relationship. For purposes of the present work we will thoroughly analyze the current state of crop diversity related to the species cultivated for food.

The agro-diversity and its richness can be measured by the number of crop species or their intraspecific entities evolving in the field. Thus, the present work will focus on the current knowledge about agro-diversity with particular emphasis on the inventories of crop species intraspecific diversity, on their potential to assess the spatial and temporal changes affecting crop diversity, and consequently allowing for estimation of the incidence of genetic erosion and extinction phenomena.

9.3 Current Knowledge on Agro-Diversity

The current knowledge of agro-diversity and specifically of its crop genetic resources required for production of nourishment or raw materials, and having past, present, or future potential interest to mankind is affected by the same set of issues pertinent to the assessment of overall biodiversity, specifically the need to: (a) clarify the concepts of crop species and its intraspecific categories classified as agro-diversity; (b) prioritize agro-diversity groups to be inventoried based on

the evaluation of their early knowledge determined by the need to perform a rapid assessment; (c) perform inventory of specific agro-diversity groups or categories to establish the rates of their conservation or loss due to erosion or extinction; (d) prioritize regions and areas for inventorying; and finally (e) improve access to the agro-diversity data. The situation is aggravated by a need to account for the complexity that represents the above- and below-species ranks of agro-diversity, for instance diversity of farmer systems (e.g., agrosystems), interspecies (e.g., hybrids and ruderal forms) and intraspecific diversity (e.g., varieties, landraces, ecotypes and populations, cultivated forms).

The foremost issue requiring clarification is the need to summarize present knowledge of agro-diversity based on the general diversity indices, e.g., the number of crop species. In the definition of crop species the most relevant aspects are the domestication and active processes of plant cultivation. Mansfeld (1959) argued that intentional cultivation should be considered the main criterion to categorize a plant among crop species. Meyer et al. (2012) added also domestication to the list of main criteria. The number of species constituting agro-diversity would increase up to 14% of the known higher plants, reaching 35,000 species, if the inventory encompasses all species used in agriculture for different purposes, including amenity horticulture (28,000 species) (Khosbakht and Hammer 2008). The present work intends to analyze the current status of agro-diversity inventorying with emphasis on the crops species for food. The number of species grown for food has been estimated as 7,000 by Hammer (1995) applying the Mansfeld (1959) approach. This number includes species historically and contemporarily cultivated and used in agriculture for food production. Meyer et al. (2012) concluded that lasting for millennia a very dynamic process of domestication of species grown for food affected 158 of 620 known plant families, when crop species reached different degrees of taming and can be classified as domesticated, semidomesticated, and undomesticated plants. Diversity and the number of crop species for food could be even higher as a consequence of the results of agro-diversity surveys performed in centers of domestication, remote areas of the world, or and marginal agroecosystems where neglected and underutilized crops of some less known plant domestication processes could be rediscovered. For instance, in remote areas of Portugal several native semidomesticated or undomesticated species are sporadically cultivated for food, including *Tamus edulis* Lowe common name ‘norça’, *Arum italicum* Mill. subsp. *canariense* (Webb & Berthel.) P.C. Boyce, common name ‘bigalhó’, *Raphanus raphanistrum* L., common name ‘rinchão’, or *Melanoselinum decipiens* (Schrad. & J.C. Wendl.) Hoffm on the Archipelago of Madeira, and *Brassica napus* L. var. *napus*, common name ‘raba’, and *Raphanus sativus* L. var. *radicula*, common name ‘rabão’ in the Portugal mainland. Other examples include wild edible greens (Dogan 2012; Klados and Tzortzakis 2014) and numerous aromatic and medicinal plants promoted worldwide, of which several eventually became crops (Canter et al. 2005).

Another factor contributing to the fluctuation of the number of crop species and other *taxa* composing agro-diversity is persisting discrepancies in the recognition of taxonomic nomenclature of some crop groups, such as cabbage, wheat, or oats.

Several taxonomic systems distinguishing different *taxa* still coexist within these groups (Dorofeev et al. 1979; van Slageren 1994; Ochsmann 2004; Ladizinsky 2012), hampering performance of inventory of agro-diversity, while universally accepted standardization of taxonomic nomenclature is lacking. Attempts to overcome the present problems involving the current nomenclature classification of crop species involve two approaches: development of the International Code of Nomenclature for Cultivated Plants (ICNCP) (Trehane et al. 1995) and development of a novel classification and naming system based on genome similarity (Marakeby et al. 2014). Authors of the latter method claim that the system is suitable for automatic assignment of codes to any genome-sequenced organism without a need for any phenotypic or phylogenetic analysis. We are of the opinion that if this classification system allows to deal with the need of fast assessment and inventory of enormous agro-diversity, it ought to be applied with caution and validated because bidimensional genetic information cannot integrate all complexity of information composing plant phenotype expression (at the biochemical, physiological, or morphological levels). Furthermore, in the case of landraces this system cannot encompass several other criteria required for their recognition and inventorying. For instance, several authors showed that diversity of landraces has a complex polygenetic basis that results from a different weight of morphological (Pinheiro de Carvalho et al. 2008; dos Santos et al. 2009; Terzopoulos and Bebeli 2010; Freitas et al. 2011) and physiological or biochemical (Gouveia et al. 2014; dos Santos et al. 2012) traits, or could be a result of complex relationship with agrosystem features (Hadado et al. 2009). These traits have complex heritability and their architecture and variation at phenotypic level are not always the sum of gene expression, especially in traits with a polygenetic control (Delker and Quint 2011).

Although agro-diversity at the level of species richness is quite reasonably established, some efforts are still necessary to assess the number of crop species of some groups and to make inventory of minor and local crops (Hambler 2004; Dogan 2012; Meyer 2012). These efforts are required to calculate the richness of plant genetic resources and to perform a global assessment of agro-diversity (Khosbakht and Hammer 2008) with evaluation of the degree of threat to cultivated species. The number of crop species used for food production is a matter of some discussion, both with respect to the number of domesticated species (Meyer et al. 2012), number and importance of crop species grown for food (Wilkes 1993), as well as richness, abundance, and composition of crop species relevant for food supply worldwide (Khoury et al. 2014; Groombridge and Jenkins 2002; Meyer et al. 2012). Wilkes (1993) argued that only 1,500 species could be used in agriculture for food production. This number contrasts with the 7,000 species inventoried by Hammer (1995) and 2,500 species estimated to have undergone the domestication process (Meyer et al. 2012). Crop species play different roles in agriculture, with a great majority of them having actually limited contribution for food production. Additionally, some of them could have a paramount importance in conservation of genetic resources and safety of food production. Since the end of the nineteenth and beginning of the twentieth century, a fast decline in the

overall number of crop species used in agriculture for food and feed production has been reported. These changes are related to the globalization and mechanization of agriculture, development of new breeding methodologies (Vavilov 1997), which resulted in release of modern and more productive varieties and decelerated domestication of new crops (Meyer et al 2012). Applying criteria of actual use and production, 203 species, of which 66 species were quoted in the FAOStat and considered global crops, 95 minor but locally important crops, and 42 underutilized or semidomesticated species were identified. The major crops partially differ from the 103 crop species worldwide recognized as securing 90 % of food production for humankind (Prescott-Allen and Prescott-Allen 1990; FAO 2010). Groombridge and Jenkins (2002) have composed an inventory with these species that include 109 crops that according to FAO supply the highest percentage of calories, protein, or fat per capita in at least ten countries. This inventory also includes 33 crop species that were not refereed by Meyer et al. (2012), including 17 major crops. However, all these data raise serious concerns about reduction of agro-diversity at the species level (Esquinas-Alcazar 2005; Brown 2008) since many of domesticated species are presently not included in the production systems. Due to a domestication syndrome and their reproductive features (Brown et al. 2008; Meyer et al. 2012) they could suffer different degrees of vulnerability or even face extinction.

Another key issue for the inventorying of agro-diversity and evaluation of undergoing genetic erosion processes is related to the recognition of below-species categories playing an important role in its richness and contributing to crop stability, quality, and improvement. The recognition of these categories can significantly change the overall perception of agro-diversity. In our mind, these intraspecific categories (Cleveland et al. 1999) would include botanical varieties, races, agronomic varieties, and landraces (regional, local or farmers varieties). Not all of these categories are commonly recognized and, hence, several attempts to delimit their definition and develop a hierarchal classification system had been undertaken (Spooner et al. 2010). We will focus on the intraspecific diversity aspects of landraces. Different definitions of landraces are provided in the literature (Zeven 1998; Brown 1999; Camacho Villa et al. 2006; Stolton et al 2006; Pinheiro de Carvalho et al. 2013). Crop species are relatively well established entities that result from a dynamic domestication process started about 10,000 years ago. This process was associated with a domestication syndrome that included a considerable variation in reproductive strategies (Meyer et al. 2012) contributing to the genetic isolation of tamed species from their closely related wild species. However, in some cases partial domestication or incomplete reproductive isolation did not prevent from crossbreeding with CWRs of gene pool 1, other close-related wild species, or crop species from the same plant complex, which resulted in formation of ruderal forms and hybrids (Ellstrand 2001; Munster and Wieczorek 2007). On the other hand, the continued process of adaptation of crops to a number of different environments, agroecological conditions and production needs, and the related farmers' traditional knowledge have contributed to a preservation and even augmentation of the enormous diversity that landraces and other

traditional varieties may offer. Landraces are characterized by a complex structure, high diversity, and are different from populations of a particular variety managed by farmers under the same cultivation practices (Brown 1999). Therefore, the intraspecific diversity is a result of a complex process of crop adaptation, intentional or unintentional selection, and consequently its maintenance and evolution depended upon continuous use in cultivation. This process increased the agro-diversity indices without interruptions until recently, reaching its climax in the first half of the twentieth century (Damania 2008). A dramatic reduction and erosion in the last several decades, especially after modernization of agriculture triggered by the Green Revolution (Almekinders and de Boef 1999) has been reported. Novel breeding technologies introduced during the Green Revolution released a plethora of substantially improved varieties among the major crops, i.e., wheat dwarf varieties (Motzo and Guita 2007), which radically increased food supply to the mankind. The development of these varieties is highly dependent of crop genetic diversity to keep their productivity and assure a genetic gain. However, through a process of modernization and industrialization of agriculture these bred varieties have replaced and genetically eroded landraces in different parts of the world. Since landraces are below-species intermediate entities, depending on their reproductive mechanism and strategy, they can easily outcross with other improved or non-improved varieties or genetically modified varieties (GM) of the same crop giving origin to "creolized" or contaminated varieties (Quist and Chapela 2001; Stolton et al. 2006; Love and Spaner 2007). These processes are the underpinning reason of genetic erosion and pollution (unintentional introgression of genetically modified traits) of many landraces that are the chief and most threatened component of the overall agro-diversity (Abberton and Warren 2006; Maxted and Guarino 2006; Bettencourt et al. 2008).

9.4 Why We Need to Inventory the Agro-Diversity?

An inventory represents a detailed and itemized list recording species and their taxonomic names in a given place at a given time. Therefore, inventories are focused on diversity at the species level. The inventories report existing species richness in a given community, ecosystem, or region of the world. In the case of agro-diversity the community and ecosystems represent farmer's fields and agrosystems.

The biodiversity assessments can be performed based on richness patterns or diversity indices. These are the most common ways to measure biodiversity and are essential to assess and monitor its changes, including the changes in agro-diversity instigated by the man or by the global changes. Actually, many biodiversity assessments are based on or put emphasis on the species inventories (Wilson 2000) using the work of taxonomists, field biologists, or herbarium collections. The oldest taxonomic inventories that can be readily used to assess biodiversity are 250 years old (Wilson 2000) and appeared long before the advent of the

taxonomic scientific nomenclature. However, these inventories are primarily based on single samples and generally provide qualitative (species presence or absence) rather than quantitative (number of specimens or traits variation) information. They typically have limited application for the assessment of diversity changes or, in the case of agro-diversity, for assessment of crops' genetic erosion. As referred earlier, enormous gaps detected in our knowledge regarding biodiversity prompt executors of some projects (i.e., the RAP or the People, Land Management and Environmental Change projects (PLEC) (http://archive.unu.edu/env/plec/about.html) to assess biodiversity (including agro-diversity) by surveys of species richness in different habitats and ecosystems of biodiversity hotspots, domestication centers, traditional communities, and farming systems.

Several diversity indices can be used to measure agro-diversity (Simpson 1949; McCune and Grace 2002). They provide quantitative outputs and are applicable to different entities, e.g., crop species, agronomic varieties, or landraces, present in the region. The inventories for calculation of these indices ought to reflect the abundance of individuals of different inventoried entities. The use of regular species inventories allows to determine crop richness, while the measurements of other components of agro-diversity, for instance intraspecific and landraces diversity that use other indices, require quantitative records of abundance, appraisal of ecogeographic regions or agroecological zones covered by landraces or varieties, knowledge of population sizes, traits, and inherent genetic diversity (Brown 1999). The richness patterns (Hubbell 2001) describing agro-diversity typically provide the number of different crop species or other taxonomically recognized entities, present in the inventory for a specific region. The evenness (Margalef 1958) of the estimation of agro-diversity describes the relative abundance of each category, e.g., species or landraces by accounting the number of individuals belonging to each category of the inventory or assessment. The assessment of intraspecific agro-diversity in general, and of landraces in particular, is almost always based on reports of field missions collecting germplasm and passport data of accessions that are included in the gene bank documentation systems. These reports do not collect quantitative data regarding the population size, different morphotypes, or traits that compose a landrace. However, since determination of these data at the intraspecific level could be challenging, the relative species abundance estimating occurrence of crop species is often used (Hubbell 2001). A full diversity index could be eventually based on the account of the entities such as morpho- or ecotypes that compose landraces or the distribution and frequency of traits or gene alleles composing their populations (Brown 2008; Brown and Brubaker 2002). The agro-diversity indices are higher and have higher weight when the number of entities and their abundance expressed as number of individuals increases. To monitor ongoing changes, such full agro-diversity inventories require thorough knowledge of the structure of diversity at different levels, including landrace diversity and structure. Inventory of intraspecific agro-diversity meant for calculation of the diversity indices requires a profound understanding of crop structure, phenotypic variability, and identification of the key traits that facilitate recognition of its entities. At the same time agro-diversity as well as biodiversity have two

coordinates, spatial (agroecological zones, ecogeographic units, or farmer's plots) and temporal scales, which dimensions are given by alpha (α), measuring average diversity in a sampling unit (e.g., quadrate area or farmer's field), beta (β)—measuring changes in diversity composition (e.g., species, races, varieties, or landraces) between sampling units, and gamma (γ) diversities that measure diversity detected in several units of a larger unit, such as ecogeographical regions or even a center of origin (Whittaker 1965; Vellend 2001; Condit et al. 2002; Love and Spaner 2007).

However, the majority of agro-diversity inventories record only the number of crop species. Such inventories could be incomplete even at the species level since they often focus only on major crops without performing their full identification. For instance, the Portugal Country Report to the FAO International Technical Conference on Plant Genetic Resources reported 821 species or *taxa* (Varela et al. 1995) but the records appear to be incomplete and contradictory. Inventories generated by gene bank documentation systems deliver information on species maintained under ex situ conditions, including agroecological zones or ecogeographic regions. In some cases they also provide information on the accessions belonging to landraces (Pinheiro de Carvalho et al. 2013) creating a valuable instrument to assess changes over time of this agro-diversity component and to detect ongoing genetic erosion and extinction processes. In Portugal, according to the Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture (FAO 2010), a total of 45,375 accessions were conserved, of which 59.7 % were cereals species, 23.3 % grain legumes, 4.4 % forages and pastures, 10.6 % vegetable species, 1.6 % aromatic and medicinal plants and 0.4 % fiber crops. Regrettably, scarce information is provided on landraces represented by these accessions.

Another way to measure genetic diversity within production systems is to inventory where and how many landraces are still an important part of crop food production. However, little information has been made available regarding the actual numbers of traditional varieties maintained in farmers' field (FAO 2010).

Access to the reports of taxonomical missions as well as to data of agro-diversity surveys and the improvement of the data quality can bridge the gaps in knowledge of agro-diversity at different levels and could increase accuracy of estimations of the global richness patterns and diversity indices. The first large-scale, long-term agro-diversity missions were performed by (Vavilov 1926, 1951) in the 1930s of the twentieth century (Pistorius 1997). Afterwards, national programs for genetic resources supported by several gene banks and the International Board on Plant Genetic Resources (IBPGR, presently Bioversity International) conducted more than 500 collection missions (http://bioversity.github.io/geosite/) in the first decades of existence (1974–2012). They significantly contributed to a generation of agro-diversity reports and inventories that considerably increased available information (Lawrence 1984) along with numerous Directories of Germplasm Collections that were, at that time, made widely and freely available. The use of the GRIN-Global and other platforms for documentation of gene bank collection (Arnaud et al. 2010) that offer access to tools allowing the georeferentiation and

mapping, for instance the geographic information systems (GIS), Cybertracker tool (Germeier et al. 2012) or Google Earth, will improve access and handling of information on agro-diversity, available in the global documentation systems, e.g., the European Search Catalogue (EURISCO)—portal providing information about Plant Genetic Resources for Food and Agriculture (Genesys), the FAO World Information and Early Warning System (WIEWS), and the Plant Genetic Resources Diversity Gateway (PGR Diversity Gateway). These improvements will contribute to a partial bridging of some of the detected gaps and will offset scarcity of information in agro-diversity inventories (Pinheiro de Carvalho et al. 2013). They will also support efforts of monitoring of the agro-diversity changes over time and spatial dimensions. Yet, information systems combining quality inventory data for agro-diversity (landraces and crop wild relatives), ex situ, in situ, and on-farm would be the preferred route as the baseline system for monitoring its changes. Such system would allow monitoring of many aspects of the landrace structure and the undergoing changes of the evolving agrosystem.

9.5 Structure Requirements for the Inventories of Landraces Diversity

A better understanding of intraspecific diversity is required to inventory agro-diversity, implement ex situ and situ (on-farm) conservation strategies and to assess its changes in space and time. The importance of landraces as component of agro-diversity has been already extensively discussed (Zeven 1998; Camacho Vila et al. 2006; Newton et al. 2010). The word “landraces” was used for the first time by E. von Proskowetz and F. Schindler in 1890 to designate “primitive cultivate forms” as genetic resource (Zeven 1998). Since then many definitions of landraces have been proposed. Zeven (1998) reviewed landrace descriptions and defined “autochthonous landrace” as “a variety with a high capacity to tolerate biotic and abiotic stress, resulting in high yield stability and an intermediate yield level under a low input agricultural system.” This definition was subsequently rediscussed by adding or reviewing features of landraces and criteria for their delimitation (Camacho Villa et al. 2006; Newton et al. 2010; Negri et al. 2012). Other definitions put emphasis on several common attributes that can be used for the identification of landraces. These attributes involve a specific geographical distribution, local production of seeds or propagation material and farmers use based on the agronomic value for instance, capability to satisfy farming concerns (e.g., soil and edaphic conditions or yield qualities), adaptation to local agroecological conditions (Brush 1999), genetic heterogeneity and variability (Brown 1999) in the sense of morphoagronomic characters, and tolerance to abiotic or biotic stresses (Qualset et al. 1997), which have associated phenotypic traits, biochemical and genetic markers. They are a result of selection performed by local farmers with respect to a limited number of landrace features (Swanson and Goeschle 2000). Until 1995 landraces as an entity of crop diversity were not included in

the International Code Nomenclature of Cultivated Plants (ICNCP) (Trehane et al. 1995), while later inclusion (Spooner et al. 2006) did not completely describe the nature of landraces (Zeven 1998). Owning to their structure, landraces represent a specific and complex equilibrium between populations or genotypes within landraces population. The presence of these genotypes at the phenotype level is not always evident, which may complicate inventory efforts. The recognition of the role of landraces as a key element of agro-diversity is not always consensually accepted (Wood and Lenne 1997; Louette 1999). Their definition, even for the inventory purposes, needs additional standardization with regard to the nomenclature, landrace structure, and definition of a number of key traits or characters to achieve an effective inventorying. The above-mentioned attributes of landraces should be covered by agro-diversity inventories that need to be tuned specifically for the diversity of landraces, while the methodologies for their identification and quantification have to be developed in a standardized way; that ought to include at least geographic origin, historical, ethnographic, traditional knowledge and use value sensu (Pardey et al. 1998), cultivation practices, adaptation to local agroecological conditions, and identification of the key traits. Further to that, landrace inventories have to quantify other parameters, i.e., chorology and production areas, agroecology data, number of populations maintained under the same practices, estimation of individuals, classification of conservation status based on adapted criteria from the management of wild diversity. Early inventories have intrinsic problems since they did not take into account several attributes of landraces but performed identification based on vernacular names or equalized landraces to landraces' population (cultivated form) in the farmers' field. The complications to access passport and evaluation data from the gene banks documentation systems make impossible to fully understand these inventories and hence to assess the landraces diversity (Pinheiro de Carvalho et al. 2013) and monitor ongoing changes. A recently published Romanian inventory of landraces that includes a red list of criteria evaluating conservation status already addresses several of the requirements (Antofie et al. 2010) facilitating evaluation of diversity changes. Therefore, it could serve as a foundation for development of subsequent inventories. In addition to the described attributes, proper documentation and understanding of information about farmer's knowledge of the crops is of critical importance. Therefore, Bioversity International and The Christensen Fund (2009) have developed the descriptors for farmers' knowledge of plants. To assist in the documentation of landraces, a group of experts developed the Descriptors For Web-Enabled National In Situ Landrace Inventories (Negri et al. 2012). This document offers the most complete set of information to be collated and recorded while inventorying landraces. It also reduces variability of the methods and tools used to perform landrace inventory as agreed on during execution of the PGR Secure project (http://www.pgrsecure.org/). Such approach facilitates understanding and cross-analysis of the current inventories. Information on landraces is provided by the Plant Genetic Resources Diversity Gateway for the conservation and use of landraces and crop wild relative traits, for short—PGR Diversity Gateway. This is a central system for landraces and CWR information that includes trait information, passport,

and characterization and evaluation data available from national inventories and checklists and conservation strategies (Dias 2012). The Plant Genetic Resources Diversity Gateway aims to promote and facilitate the use of landraces and crop wild relatives in breeding and crop improvement by providing also QTL information of potential value to breeders and other users of germplasm. It also delivers information at the national and regional levels that could be used by conservation managers, scientists, and policy makers (http://pgrdiversity.biodiversityinternational.org). The primary value of any inventory is to make information on diversity and crop genetic resources available in public domain thus promoting the sustainable use of germplasm in plant improvement, research, and training. A wider pool of public information on ex situ and in situ conserved accessions or populations would increase the probability of finding materials of potential interest for further crop improvement. Agro-diversity inventories, that encompass landraces could additionally target assessment of crop diversity based on its evenness, abundance and other diversity indices related to α, β, γ diversities, providing a powerful tool to survey and monitor the time and space changes affecting this agro-diversity component. Monitoring of agro-diversity inventorying simplifies gathering of scientific information essential to support conservation and to promote increased use of suitable landraces contributing to mitigation of nutrition needs and increase food security. Monitoring is also a part of the inventory toolkit for informed decision-making in support of continuous conservation and enhanced used by national and international policy makers.

9.6 Goals and Strategies of Agro-Diversity Inventorying

The agro-diversity inventories have at least two main goals, which are to perform a survey of: (1) agro-diversity through establishing the number of crop species and (2) crop diversity through the evaluation of its intraspecific diversity, namely diversity of landraces. When supported by diversity indices and data representing the structure of the categories inventories provide information and consequently contribute to conservation of agro-diversity and promotion of its sustainable use in food production or breeding programs.

As previously mentioned, the current knowledge of agro-diversity suggests that crop species awaiting inventory are likely to be in smaller percentage than the number of unknown species of overall biodiversity. They will be composed of minor, neglected, or underutilized crops. The majority of these crop species could be perhaps concentrated the world's biodiversity hotspots and underexplored areas (Aravindakshan and Sherief 2010). Therefore, the agro-diversity inventory of species richness needs to be focused on the Centres of Crop Origin and Domestication (Vavilov 1927, 1997). The crop domestication process appeared to be more complex than initially described by Vavilov with different levels of species domestication (Meyer et al. 2012) and more worldwide distribution (Harlan 1992; Meyer et al. 2012) that determines the need to perform crop species

inventorying also in marginal agrosystems and remote areas, where industrialization of agriculture did not reach the status of intensive monoculture. In these latter, inventorying efforts, historical and ethnographic records, assistance of ethnobotanists and sociologists who trace handling not fully domesticated species by protohumans (Crosby 1983; Fraser et al. 2011) could be an advantage. One we can argue that the majority of these crop species lost their place in modern agriculture after the advent of the Green Revolution with only 103 crops presently playing a significant role in human nourishment. First of all they provide the needed calories, second, serve as resource for assuring food safety and sustainability, alternative sources of food, genetic material for breeding purposes or biofuncional components. Importance of these crops in the contribution to overall agrobiodiversity must not be also underestimated. At the same time, additional efforts to inventory crop species at the national level are also required in some countries, for instance in Portugal, where a complete inventory still does not exist.

Some surveying programs can support the efforts of improvement of the agrodiversity inventorying. One of them is the Rapid Assessment Program, which promoted more than 66 RAPs evaluations of Earth biodiversity, primarily focusing on the regions of biodiversity hotspots. Some of these hotspots, especially in the subtropical regions, overlap with the agro-diversity hotspots identified by Vavilov (1927; 1951; 1957; 1997) and more recently by Harlan (1992) and Meyer et al. (2012). Nevertheless, the main goal of these assessments is not exclusively focused agro-diversity. The RAPs assessments performed in the regions considered as the biodiversity hotspots and primary or secondary centers of crop diversity, like New Guinea or the Andean Peru, could provide additional information for the agro-diversity inventories. In the majority of cases the RAP assessment is limited to the species counts and identification. However, the assessment of the Cordillera de Vilcabamba in Peru that surveyed major local and commercial crops recognized 80 cultivated species, 55 of them identified at species level and 30 at the variety level including crops such as manioc (*Manihot esculenta* Crantz), sugarcane (*Saccharum officinarum* L.), banana (*Musa acuminata* Colla) and plantain (*Musa* × *paradisiaca*), maize (*Zea mays* L.), groundnut peanut (*Arachis hypogaea* L.), and cotton (*Gossypium hirsutum* L.) (Alonso et al. 2001). Unfortunately, this assessment did not provide data about identification of these varieties with regard to the delimitation of their structure, evenness, and abundance. In the case study of the Archipelago of Madeira, a small Portuguese Autonomous Region (780 km^2) in the Atlantic, located on the intersection of former routes of the transfer of genetic resources between continents, the most recent inventories identified 111 crop species, among them four semidomesticated and nondomesticated crops and significant number of neglected crops, plus 432 crop wild relatives. This rich agrodiversity is sustained by a variety of agroecological conditions that involve four bioclimatic levels, seven agroecological zones, agrosystems and related practices distributed among low input horticulture (45.1 % of agricultural land), vineyards (15.8 %), subtropical orchards (11.9 %), tropical roots and tuberous crops (7.6 %), pastures (7.0 %), temperate orchards (3.0 %) and family-based horticulture (2.6 %) (DREM 2012).

For plant biologists in general and for gene bank curators, plant breeders and conservationists, in particular, the inventorying of intraspecific agro-diversity is a challenging task that can have an enormous importance to define their research programs, to monitor the changes in agro-diversity and to implement measures minimizing losses or promoting the sustainable use of the available genetic resources and materials with desirable traits. To achieve these goals agro-diversity inventories need to be performed based on the assessment of landrace diversity. Landraces are the most important component of intraspecific agro-diversity, but also the most threatened one. They are impacted by several presently ongoing processes, including its disappearance by abandonment, cultural erosion exacerbated by the loss of traditional knowledge, decline of rural populations and genetic erosion or pollution when uncontrolled exchange of germplasm takes place without precaution to protect local genetic resources (Brush 1999; Stolton et al. 2006; Brown 2008). Abandonment of agricultural activities is also aggravated by massive migration of young villagers to the urban areas. As a result, aging farmers who decided to stay on the land cultivate crops to satisfy only their own consumption needs. In addition, a progressive abandonment of landraces and increased replacement by commercial, improved varieties resulting in disappearance of the traditional agricultural systems and the knowledge associated with landraces development, maintenance, management and use has been reported in many parts of the world (Rocha et al. 2008).

Genetic erosion can be defined as "the loss of genetic diversity, in a particular location and over a particular period of time, including the loss of individual genes, and the loss of particular combinations of genes such as those manifested in landraces" (FAO/IPGRI 2002). The major driving forces of genetic erosion are the abandonment, decrease of production areas, and the loss of uses associated with landraces. Hence, genetic erosion is a function of diversity change, both over time and in space dimensions. Genetic pollution of landraces may be defined as a gene flow and introduction of alien genetic diversity into a host genome with potentially harmful effect. The major concern with that respect is pollution due to cross pollination with improved or genetically modified varieties. The inventories of landraces could help in the survey of both ongoing process, only if accomplished with required structure and information.

In recent times, several attempts have been made to estimate the agro-diversity loss resulting from the landraces abandonment or replacement by the improved varieties. Zeven (1998) argued that these irreversible processes started in Europe after WWII and affected major agriculture crops. The development of aggressive breeding programs triggered the presently enduring processes, first in the western countries, including USA and after the Green Revolution it affected the developing countries as well. The measurement of landrace loss is a difficult task because of the absence of early landrace inventories. The Small Seeds Genebank of USDA estimated that 93 % of varieties of 66 crop species become extinct. According to the FAO estimates 75 % of genetic diversity of 103 world crops has been lost during the last century. The same reports shown that Germany and South Italy lost 90 and 75 % of "historical crop diversity", respectively (Stolton et al. 2006). At

the same time, remaining landraces and varieties are progressively contaminated by genetically modified varieties. Genetic pollution has been traced in maize, plums, and rice landraces around the world (Quist and Chapela 2001; Stolton et al. 2006). Based on the analyses of current trends of agro-diversity changes, Zeven (1998) concluded that landraces ultimately will disappear from the farmers' fields and will be completely replaced by bred and improved varieties. This is very unfortunate since there is a common consensus among the researchers that landraces are essential for food security (Stolton et al. 2006), sustainability of agriculture (Annicchiarico and Pecetti 2003; Netwon et al. 2010), diversification of local rural economy (Bardsley and Thomas 2005), and plant breeding (Motzo and Guinta 2007). In situ (on-farm) or ex situ conservation of landraces is a major challenge faced by national and international organizations attempting to prevent extinction of the genetic resources. Inventorying of landraces could substantially strengthen the conservation efforts. Signatory countries of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) under Art 5 committed to perform surveys and inventories of plant genetic resources for food and agriculture, taking into account the status and degree of variation in the existing populations, including those that are of potential use and assess any threats to them (FAO 2009).

The goal of In situ (on-farm) conservation include reduction of the "genetic erosion threat" of local (e.g., autochthonous) genetic resources, development of an economic interest for food products, and dissemination of information about local genetic resources (Torricelli et al. 2009). For instance, in Italy cultivation of landraces of emmer wheat (*Triticum dicoccum*) generates incomes to local farmers, creates opportunity for development of new products, and contributes to protection of the environment. The national and international valorization of the traditional products resulting from the utilization of landraces and associated traditional knowledge, converting the agro-diversity conservation and the sustainable use into a profitable economic activity capable of generating income are key elements strongly contributing to the sustainability of the whole process of maintaining, using, further developing and harnessing the economic and cultural value of agro-diversity (Bettencourt 2008). Calculations of economic value of agro-diversity in Archipelago of Madeira using Costanza et al. methodology (1997) reported an annual income of 34.3 million euros per year that accounted for both food production and the ecological services (Pinheiro de Carvalho 2014).

To be an effective tool, landrace inventories ought to have a precise scope and appropriate inventorying strategy. The inventory needs to classify the populations or accessions allowing for their clear identification as agro-diversity category, e.a. landraces, by providing information about its structure and features and permitting the calculation of related diversity indices. If the inventory sample properties, especially the number of categories, is represented by only one or few records it should be used to estimate the agro-diversity richness, for example, species or landrace richness in the sampled unit. However, the agro-diversity measures are affected not only by the number of categories, but also by the number of individuals, the samples heterogeneity, diversity of sampled environment sites, agriculture practices, and agrosystems that can become higher when gradients of these factors are used

to delineate an inventorying strategy. Consequently, depending on the purpose of the inventories, the diversity assessment should involve determination of the entities richness, evenness, abundance, and frequency of specimens or specific traits. All these parameters are quantified on the basis of defined sampling units of landrace provenance, which may consist of a single farm field, assembly of farms' fields, geographic units (counties, district or province), or different agroecological zones. In these inventorying efforts agro-diversity categories and their boundaries ought to be well-defined and delimited. This is not a trivial task since typically the work is being done on large spatial units and not all present individuals can be easily observed and identified. In these efforts various tools such as the cybertracker (Germeier et al. 2012) or georeferentiation and mapping using geographic information systems (GIS) could play an important role in assisting in definition of sampling strategies. The selection of adequate sampling methods and sizes of sampling may contribute to overcoming these limitations but the sampling must be validated using smaller areas. To generate the requested information landraces have to be characterized and sampled in the field using between 20 and 50 randomly selected plants. The number of plant samples depends of crop species and population sizes. Typically, a sample of 4,000 to 12,000 seeds is recommended for conservation of landrace diversity for gene bank purposes (Rao et al. 2006), which for the majority of crop species would correspond to amount of seeds collected from 20 to 100 or more individual plants. Plants in the field that are used to identify key traits and to measure variation of the traits could be also used to analyze the frequency of marker genes. Further information on sampling of landrace diversity can be found in Guarino et al. (2011). Re-collecting methods can be employed to validate information or to bring samples of different sizes to a common footing (Rocha et al. 2008). More recently, Crossa (2011) explored and subsequently optimized strategies for determination of adequate sample size in order to maintain the representativeness of the original diversity when collecting and regenerating genetic resources. The concept of variance of the effective population size is important for the measurement of genetic representativeness and has been successfully applied for genetic conservation. If inventorying with the determination of species richness or other diversity indices of the sampled crop is meant to represent agro-diversity from a center of domestication, secondary centers, remote areas, or geographic region for purposes of comparison with other similar units or for further assessment with the intention of monitoring the diversity changes, the sampling efforts need to be standardized in an appropriate way. Standardization will empower monitoring of diversity changes over time using a small number of essential variables in a similar way to the EBV (Pereira et al. 2013) applied for the biodiversity monitoring.

9.7 Advances in the in Situ and Ex Situ Landrace Inventories

Habitually, farmers have been mindful with regard to the crops they cultivated in the field and they were selecting the best individuals for production in consecutive seasons. In general, this knowledge was typically not methodically

documented but rather passed by viva voce (word of mouth) from generation to generation. Fortunately, in some instances the valuable information regarding landraces was recorded by the scientists, crops experts, and students. For example, the Plant Inventory Book contains information on plant material introduced into the US National Plant Germplasm System between 1898 and 2008 totaling 217 inventories (http://ars.usda.gov/Services/docs.htm?docid=18722). Currently, the US NPGS retains a summary of 300 accessions available with sample status for landraces (http://www.ars-grin.gov/cgi-bin/npgs/acc/query.pl). In Europe despite the absence of detailed landraces inventories Papadakis (1929) and Vasconcellos (1933), elaborated comprehensive morphological descriptions provided information on a significant number of the Greek and Portuguese cereal landraces cultivated in the beginning of the twentieth century. Presently, the Portuguese wheat diversity is represented by 116 landraces.

Nowadays, many landraces are conserved in the world gene banks and the register of record accessions can be found on the Internet through accessible databases that include search option by the improvement status (Bioversity International 2008; FAO 2008; The Nordic Genebank 2008; national gene banks of the United States (GRIN) and Canada (GRIN-CA); and Genesys). Since the 70s of last century the world system of gene banks commenced assembling intensively collections of plant germplasm that reach 7,420,236 accessions in 2010, globally (Pinheiro de Carvalho et al. 2013). These records offer the most complete set of data and full inventory of diversity of the early landraces collected during the field missions. They also provide associated information about collection places, names, and other passport data and could be the base for assessing changes in agro-diversity over time. Biodiversity International maintains a database of original passport data of more than 220,000 samples collected around the world between 1974 and 2012 (Bioversity International 2014). According to the WIEWS data referred in our previous paper (Pinheiro de Carvalho et al. 2013) the number of cereal landrace accessions maintained in the gene banks reached 179,514 for wheat, 124,083 for barley, 19,775 for oat, and 7,662 for rye. Complications with retrieving data can seriously limit their use for inventorying proposes. Additionally, a high number of accession samples of unknown status prohibit efficient assessments of the existing duplicates or even understanding of the used sampling strategies. While in some gene banks (e.g., Index Seminum Gaterslebensis, Knüpffer 1999a) there are landraces listed with names, many other gene banks provide only information regarding geographic denomination (Knüpffer 1999b).

Despite the advances in the knowledge of landraces till date complete landrace inventories for every European country are still not available. This lack of information severely hampers the ability to effectively conserve and use these genetic resources. In an attempt to individualize the constraints for such knowledge gap, Maxted et al. (2012), conducted a Europewide survey (33 countries) focusing on whether the European countries had a National Landraces Conservation Strategy in place and, if not, what constraints were impeding the progress. Italy was the first European country that in 1997 commenced protection of genetic resources and landraces through regional and national policies. However, so far only one country

(Switzerland) has a National Landraces Conservation Strategy in place, 23 countries have them partially completed and 8 countries have not yet started formulation of a strategy. Many of the uncompleted European landrace inventories have been reported (Veteläinen et al. 2009). The following countries are presently updating their inventories: Bulgaria, Denmark, Finland (cereals landraces), Germany, Greece, Hungarian, Portugal, Romania, Russia (cultivated plants); inventories are being revised in Sweden and UK. Nevertheless, efforts have been made to bridge the existing knowledge gaps. Scholten et al. (2009), published an overview of traditional varieties of wheat, forage, and other crops (barley, cabbage, oats, etc.) maintained as landraces as well as, in some cases, indicated number of growers, area sown, and crop production. A general outlook at the distribution of landraces in Romania has been also presented by Strajeru et al. (2009). In Portugal, DRAPC (2014) listed a total of 161 regional varieties of Pomoideae, most of them still present in farmers' fields. An inventory performed by the ISOPlexis Genebank to assess the landrace diversity of nine predominant crops (wheat, bean, corn, sweet potato, potatoes, taro, tomatoes, apples, and banana) of the Archipelago of Madeira reported that during the last 20 years overall diversity in field was reduced from 124 to 80 landraces. The most complete inventory of landraces was done in Italy within the "PGR Secure project" (www.pgrsecure.org) where the Department of Applied Biology at the University of Perugia compiled "The First Italian Inventory of In Situ Maintained Landraces" (Negri et al. 2013). The inventory includes all

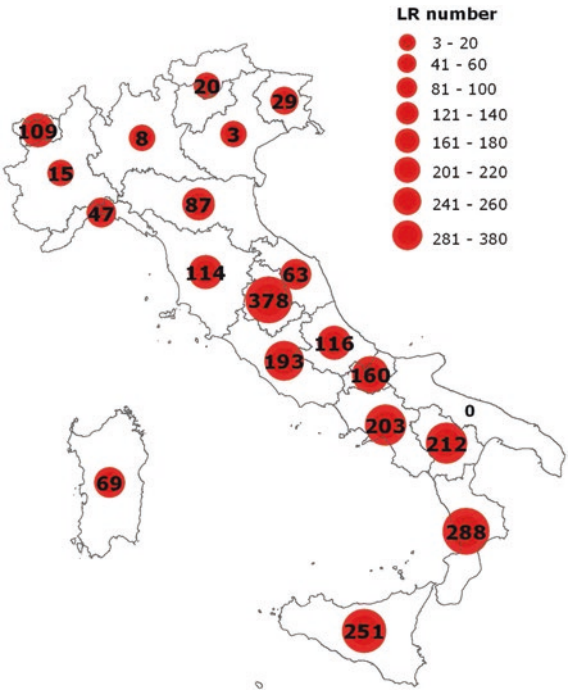

Fig. 9.1 Number of landraces by region in Italy (from Pacicco et al. 2013)

landraces that have been recorded by the Italian regions and autonomous provinces during the last two decades and reports data until January 2013.

In the Italian inventory a total of 4,806 accessions belonging to 2,365 landraces were considered (Fig. 9.1). A total of 329 species are nowadays cultivated as landraces including fruit trees, vegetables, grain legumes, forage crops, cereals, ornamental plants, and other species (Pacicco et al. 2013).

Fruit trees accounted for 73 % of the total, while grain legumes and vegetables comprised 12 and 9 % of the total, respectively (Fig. 9.2) (Pacicco et al. 2013).

Recently, the PGR Secure project (http://www.pgrsecure.org) has started updating these inventories and they are now reachable/ linked to or are searchable through the PGR Diversity Gateway (http://pgrdiversity.bioversityinternational.org/National_Inventories). The PGR Diversity Gateway, now comprising of several national inventories (landraces and CWR), links to the Austrian, German and the United Kingdom landrace national inventories and holds the "European specific landrace conservation strategy for target crops (*Avena*, *Beta*, *Brassica* and *Medicago*)" and a few national conservation strategies derived from the inventories of respective countries (http://pgrdiversity.bioversityinternational.org/National_Inventories, and http://pgrdiversity.bioversityinternational.org/Conservation_Strategies).

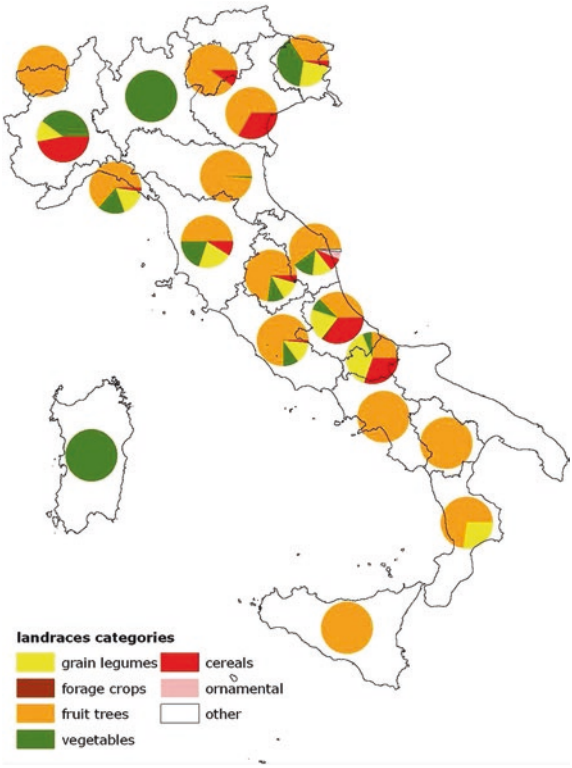

Fig. 9.2 Landrace crop type by region in Italy (from Pacicco et al. 2013)

9.8 Concluding Remarks

Agro-diversity inventorying facilitates the assessment of the genetic erosion and can assist the efficient conservation of plant genetic resources that is the foundation of plant improvement that has an immediate impact in agriculture production.

Even though positive actions have been taken worldwide toward agro-diversity inventorying the complexity of its assessments has given incomplete results. In order to perform inventory of crop genetic resources it is necessary to define the number of crop species which can be increased or decreased due to the inclusions of minor species cultivated in marginal areas or newly domesticated species or taxonomic discrepancies. Intraspecific diversity is playing an important role in crop species diversity and adaptation to different agroecological, and is fundamental for breeding, genetic resources conservation, and food safety. This diversity plays an important role agriculture sustainability considering the natural development of new types due to gene flow that follows cross pollination in several crop species.

Agro-diversity could be measured by several indices that are applicable to different entities and in different time and spatial scales. However, the majority of agro-diversity inventories records only the number of crops and crop species and could be incomplete even at the species level. So these inventories do not provide the required information to assess the changes in agro-diversity.

Collection missions organized during the twentieth century significantly contributed to the generation of agro-diversity reports and inventories, while global documentation systems made the obtained data widely available. Improvement of access to information will reduce efforts needed to monitor the agro-diversity changes over time and spatial dimensions.

A comprehensive understanding of intraspecific diversity is required to assess agro-diversity, to implement complementary ex situ and in situ conservation strategies and breeding programs and recognize the agro-diversity changes in space and time dimensions. Landrace inventories have to include and quantify other parameters related to their structure, production, cultivation and conservation status, use and ethnographic elements. Distinctive descriptors have been developed to document the farmers' knowledge on crop plants and for the Web-Enabled National In Situ Landrace Inventories to facilitate understanding and to allow cross-analyses of the inventories.

Depending on the purpose of inventories and agro-diversity assessments the measure of diversity needs to quantify the entity richness, specify individuals needed to be sampled and exactly quantified in a way to represent the defined sampling unit. The selection of adequate sampling methods and sizes of sampling may assist in overcoming some limitations. Nevertheless, sampling must be validated for smaller areas. If species richness or other diversity measures of the obtained sample are meant to represent the agro-diversity of the center of origin, secondary centers, ecogeographical units or agroecological zones with the purpose of comparison with other similar units, the sampling efforts need to be standardized in

an appropriate way. Such measurements of agro-diversity will allow comprehending the extinction, erosion, and pollution events affecting this diversity at different ranks.

Several landraces have been conserved in the world's gene banks and could be found in online accessible databases that include search by the improvement status. Complete inventories for every European country are still lacking. Absence of information severely hampers the possibility of effective conservation and utilization of the genetic resources. Many European inventories have been reported and described in Bioversity International Technical Bulletin, and more recently through the PGR Secure project and the PGR Diversity Gateway, making expectable that actual gaps and deficiencies in landraces inventories could be.

Landraces are a very important yet vulnerable to genetic erosion plant genetic resource and its inventorying will assist in their efficient conservation ex situ and on-farm and use in plant breeding programs or in diversification and sustainability of local agriculture.

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Chapter 10
Genetic Diversity and Conservation of Olive Genetic Resources

Concepción M. Díez, Juan Moral, Diego Barranco and Luis Rallo

Abstract The olive (*Olea europaea* subsp. *europaea*) is indigenous to the Mediterranean Basin and is the most economically important oil tree crop in temperate areas. Olive cultivars (*Olea europaea* subsp. *europaea* var. *sativa*) have been empirically selected and vegetatively propagated in all the traditional olive-growing countries. However, the domestication history of the olive and its relationship with its wild ancestor (*Olea europaea* subsp. *europaea* var. *sylvestris*) remain puzzling. The knowledge of the relationship between cultivars and wild olives is critically important for conservation purposes, for breeding programs, for the design of genome association studies, and to untangle the population history. In this chapter, we examine the characterization of olive genetic resources (wild and cultivated) in the main olive-growing regions of Spain using microsatellite (SSR) markers. We observed significant differentiation between the cultivars from south and northeast Spain, which possibly indicate independent selection processes. In addition, our results revealed differential relationships and admixture

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events between the wild and cultivated olives depending on their region of origin. Finally, we describe how the new olive-growing systems, which are more intensive and mechanically harvested, are leading to a reduction in the number of cultivars used in new plantations. Coordinated efforts involving the application of ex situ and in situ conservation approaches are needed to evaluate and preserve the wealth of genetic legacy present in both the wild and cultivated olive. These actions are urgent, given the rapid expansion of new olive plantations and the severe effects of climate change that are predicted for the Mediterranean Basin.

Keywords *Olea europaea* L. · Domestication · Traditional cultivars · Microsatellites · Genetic erosion · Germplasm banks

10.1 Introduction

The olive tree (*Olea europaea* subsp. *europaea*) and its main products, oil and table olives, are deeply rooted in the history of Mediterranean societies due to their economic and cultural importance. Since ancient times, commercial shipping has extended olive-growing westward across the Mediterranean Basin. The olive remains an important species worldwide because it is the most economically important oil tree crop in temperate areas, with 10.2 million ha under cultivation (FAO 2012). Reflecting the historical importance of olive cultivation in the Mediterranean Basin, the leading producers of olives are Spain, Italy and Greece (Vossen 2007). However, olive is also a crop that is increasingly being cultivated in Argentina, Australia, Chile, China, and the United States (FAO 2012).

Cultivated olive (*Olea europaea* subsp. *europaea* var. *sativa*) consists of a broad diversity of clonally propagated cultivars (Fig. 10.1) (Rallo 2005; Haouane et al. 2011; Trujillo et al. 2013). Olive cultivars often grow near its wild ancestor (*Olea europaea* subsp. *europaea* var. *sylvestris*), called "oleaster," which is indigenous to most areas of the humid and subhumid thermo-Mediterranean with low occurrences of frost (Rivas-Martinez and Gandullo 1987; Carrión et al. 2010).

Despite the close geographic link between cultivated and wild olives, the genetic relationship between the two is somewhat puzzling. According to archeological remains, olive was first grown in the eastern Mediterranean Basin—approximately 6000 years ago (Zohary and Spiegel-Roy 1975; Kaniewski et al. 2012). The analysis of chloroplast DNA also indicated the Syrian-Turkish border as the primary domestication center (Besnard et al. 2013). Soon after domestication, the discovery of clonal propagation techniques may have boosted the expansion of olive in the Mediterranean Basin, along with other long-lived perennial crops such as grape and fig (Zohary and Spiegel-Roy 1975; Kaniewski et al. 2012). Clonal propagation was remarkably effective, because approximately 90 % of the olive cultivars across the Mediterranean basin share the same "eastern-like" chlorotype (Besnard et al. 2013). Under this "single domestication" scenario, it is possible that local wild olives acted as pollen donors to the primary domesticated

Fig. 10.1 Olive cultivars present a large diversity. As an example, we can appreciate the variety of fruit morphologies and phenological stages showed by a handful of olive cultivars, collected the same day (24th of October), in the World Olive Germplasm Bank of Cordoba, Spain (Picture courtesy of D. Barranco)

cultivars, thus reducing the possible deleterious effects associated with inbreeding and most likely producing better locally adapted cultivars.

An alternative or complementary scenario for olive domestication posits the existence of several primary domestication centers throughout the Mediterranean Basin; these centers may coincide with quaternary long-term refugia (Breton et al. 2009; Besnard et al. 2013). Two observations support the multilocal domestication hypothesis. First, putative quaternary refugia show the highest plastid DNA (ptDNA) diversity for wild olives, suggesting that they could have been an essential foundation for cultivated olive (Besnard et al. 2013). Second, two minor haplotypes, E2, and E3 occur only in wild germplasm and cultivars from the central and western Mediterranean Basin, implying that they arose separately from any putative site of single domestication (Besnard et al. 2013).

Regardless of the primary origin of olive cultivars, once the superior genotypes were propagated clonally, they were able to spread via migration. The migration history of olives is particularly complex; the Phoenicians, Greeks, and Romans were thought to have expanded olive cultivation from east to west through both the northern and southern coasts of the Mediterranean Basin (Zohary and Spiegel-Roy 1975; Kaniewski et al. 2012). The migration of clones has led to confusion in the cultivar identity and nomenclature, such that most of the ~1200 (Bartolini et al. 1998) Mediterranean cultivars are of uncertain pedigree. Moreover, each traditional olive-growing country has its own cultivars, and these cultivars are typically only shared in border areas (Rallo 2005; Trujillo et al. 2013).

Identification of existing cultivars represents the first step in their cataloging. Only morphological descriptors were used for identification purposes until the 1980s. The main shortcoming for the use of these characters is the influence of environment on the expression of morphological traits (Rallo 2014).

In Andalusia, Spain, a systematic pomological characterization, including 55 morphological qualitative descriptors from tree (3), shoot (3), leaf (11), inflorescence (4), fruit (16), and stone (18) from 511 trees sampled in 83 localities found

out 197 different denominations, allowed the discrimination of 156 different cultivars and the establishment of synonyms, homonyms, and wrong denominations. This work (Barranco et al. 1984) provided a general elaiography of the most important olive region in the world and evidenced the usefulness of a morphological schedule for cataloging cultivars. This schedule was the base of the descriptors adopted by the International Union for the Protection of New Varieties of Plants (UPOV) for the olive. A simplified morphological schedule with only 27 descriptors have been used for cataloging the main 139 cultivars of the world for the IOC (Barranco et al. 2000), 262 cultivars from Spain (Barranco et al. 2005), 91 cultivars from France (Moutier et al. 2004), 202 in Italy (Muzzalupo 2012), and 56 cultivars in Tunisia (Trigui and Msallem 2002). Therefore, a systematic and simplified morphological schedule carried out by trained workers appears as a useful tool for cataloging olive cultivars (Rallo 2014).

The use of molecular markers for genotyping olive cultivars started with isozymes in the 1980s (Pontikis et al. 1980). The advent of DNA markers and their use for genotyping olive started in the mid of 1990s. Since that time, genotyping and studies on variability of olive cultivars increased exponentially. Critical review of the numerous elaiographical lists and the modern research tools used, particularly DNA and molecular markers, lead to a final exhaustive report on characters used for olive classification (Ganino et al. 2006).

A strategy based on a consensus list of minimum morphological characters (Barranco et al. 2000, 2005) and simple sequence repeat (SSR) (Baldoni et al. 2009) is in development. Works carried out in the Germplasm Banks of Marrakech (Haouane et al. 2011) and Córdoba (Trujillo et al. 2013) illustrate on the power of this strategy to identify in a short delay the accessions of cultivars' collections.

10.2 The Characterization of Olive Genetic Diversity: The Case of Cultivated and Wild Olives in Spain

10.2.1 Background

The diversity of cultivars in olive-growing countries is progressively changing. The clonal propagation of olive was performed by farmers using large propagules such as hardwood cuttings, suckers, or spheroplasts. Currently, olive is propagated by the nursery industry using small semi-hardwood leafy cuttings. This change has facilitated the movement of cultivars to areas that are far from their traditional growing regions. However, the nursery industry is only propagating selecting outstanding traditional cultivars and some newly bred cultivars. For example, in Spain, only six oil cultivars (Arbequina, Arbosana, Frantoio, Hojiblanca, Koroneiki, and Picual) and four table olive cultivars (Gordal Sevillana, Hojiblanca, Manzanilla Cacereña, and Manzanilla de Sevilla) represent more than 90 % of the commercialized nursery plants (Rallo and Muñoz-Díez 2010). Similar trends have been reported in most countries.

This reduction in the number of olive cultivars used in the new plantations might lead to progressive genetic erosion phenomena. Genetic erosion is defined as "*the permanent reduction in richness or evenness of common local alleles or the loss of combination of alleles over time in a defined area*" (Maxted and Guarino 2006).

A major emphasis on the exploration, cataloging, conservation, and evaluation of olive genetic resources is necessary to counteract possible genetic erosion phenomena. These types of studies are being carried out in Spain (Barranco and Rallo 2000; Barranco et al. 2005) and other countries (Khadari et al. 2003; Gemas et al. 2004; Bracci et al. 2009; Haouane et al. 2011; Yoruk and Taskin 2014), thus increasing the worldwide cultivar germplasm banks and accessions (Bartolini and Cerreti 2008).

Among the olive genetic resources, very little attention has been paid to oleasters, despite their importance as a source of genetic variability. In recent years, various studies have focused on the genetic variation of wild olive populations and their relationships with cultivars using different molecular markers (Lumaret and. Ouazzani 2001; Besnard et al. 2002, 2007; Lumaret et al. 2004; Breton et al. 2006; Belaj et al. 2007). Detailed analyses at a smaller scale may produce new insights in olive domestication and provide a better understanding of the distribution of genetic diversity at regional levels (Baldoni et al. 2006). In addition, the comparison of the genetic diversity between the wild and cultivated forms in specific areas might allow us to evaluate the potential loss of genetic variability as a consequence of domestication and the posterior intensification of agricultural systems.

In this chapter, we illustrate the characterization of olive genetic resources (wild and cultivated) in the main olive-growing regions of Spain using SSR markers and extending the study previously carried out by Belaj et al. (2010). Spain is the first olive oil producing country in the world and offers optimal conditions to perform this study for two main reasons. First, there is a rich diversity of traditional cultivars that have been systematically surveyed and characterized by morphological descriptors and molecular markers (Barranco et al. 2000, 2005; Trujillo et al. 2013). Second, Spain includes the most important reservoir of genetic variability for wild olive (Rubio de Casas et al. 2006; Carrión et al. 2010; Besnard et al. 2013).

The comparison between cultivated and wild populations at a regional scale may shed light on the following: (1) the genetic diversity of wild and cultivated olives; (2) their genetic differentiation and relationships; (3) the occurrence of gene flow between wild and cultivated olives, and (4) the genetic structure of wild and cultivated forms.

10.2.2 Sampling and Methodological Approach

We included wild and cultivated olives from the six main olive-growing regions of Spain (Barranco et al. 2005): west (W), southwest (SW), south central (SC), southeast (SE), east (E), and northeast (NE) (Fig. 10.2). In total, we analyzed 331 samples, of which 93 were traditional cultivars and 238 were wild olives (Table 10.1).

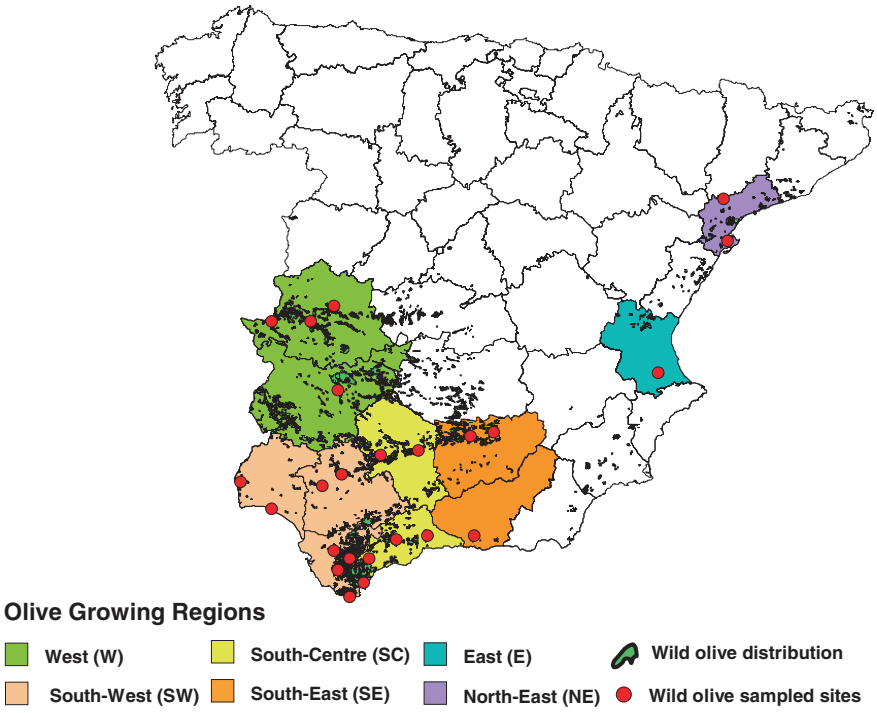

Fig. 10.2 Geographical regions and wild olive populations sampled in this study

Table 10.1 Status, origin, and genetic variability parameters for the wild and cultivated olives included in this study

	Group	Status[a]	Origin	*n*	Am	Ar	Au	Ho	He	Fis
(a)	C	C		93	9.14	9.12	8	0.73	0.69	−0.06
	W	W		238	15.93	13.32	103	0.65	0.77	0.16
(b)	W	C	West	15	4.79	3.83	2	0.71	0.64	−0.11
	SW	C	Southwest	22	6	4.08	0	0.76	0.67	−0.14
	SC	C	South-Center	12	4.64	3.84	0	0.73	0.65	−0.12
	SE	C	Southeast	22	4.71	3.6	1	0.73	0.64	−0.15
	E	C	East	8	5.21	4.71	0	0.7	0.68	−0.02
	NE	C	Northeast	14	6.36	4.93	0	0.75	0.73	−0.03
	W	W	West	43	9.43	4.87	1	0.64	0.72	0.11
	SW	W	Southwest	85	13.43	5.6	23	0.66	0.77	0.14
	SC	W	South-Center	53	11.14	5.47	8	0.65	0.77	0.16
	SE	W	Southeast	22	9.57	5.58	4	0.66	0.76	0.13
	E	W	East	11	6.79	5.35	8	0.66	0.76	0.13
	NE	W	Northeast	24	8.36	5.05	1	0.67	0.73	0.08

[a]*C* Cultivated, *W* Wild , *n* sample size, *Am* average number of alleles per locus, *Ar* allelic richness, *Au* number of unique alleles; *Ho* observed heterozygosity, *He* expected heterozygosity, *Fis* inbreeding coefficient

Table 10.2 Diversity parameters of the 14 SSR markers used in this study

Marker	Na	Ho	He	An	PIC
ssrOeUA-DCA3	15	0.767	0.757	−0.008	0.740
ssrOeUA-DCA9	23	0.921	0.909	−0.007	0.904
ssrOeUA-DCA11	16	0.703	0.741	0.026	0.716
ssrOeUA-DCA13	8	0.580	0.748	0.140	0.713
ssrOeUA-DCA15	7	0.253	0.682	0.467	0.623
ssrOeUA-DCA16	39	0.912	0.922	0.005	0.918
ssrOeUA-DCA18	13	0.918	0.877	−0.024	0.867
GAPU59	15	0.718	0.737	0.011	0.700
GAPU71B	6	0.724	0.716	−0.002	0.670
UDO99-011	16	0.827	0.838	0.011	0.823
UDO99-019	8	0.327	0.552	0.254	0.503
UDO99-024	17	0.602	0.799	0.147	0.775
UDO99-039	24	0.494	0.875	0.279	0.865
UDO99-043	24	0.729	0.908	0.109	0.903
Mean	16.5	0.677	0.790		0.766

Number of alleles (*Na*), expected (*He*) and observed (*Ho*) heterozygosity, null allele frequency (*An*), and Polymorphic Information Content (*PIC*)

Total DNA was extracted from young leaves and genetically characterized using 14 SSR markers (Table 10.2). These markers had previously been used to distinguish among cultivars in the World Olive Germplasm Bank of Cordoba (WOGBC), Spain due to their high resolution. They were also used in previous studies to describe the genetic patterns between wild and cultivated olives (Erre et al. 2009; Belaj et al. 2010; Diez et al. 2011, 2012). The SSR amplification was performed in a total volume of 20 µl, containing 2 ng of genomic DNA, 1X supplied PCR buffer (Biotools, Spain), 200 µM of each dNTP (Roche), 0.25 units of Taq DNA polymerase (Biotools, Spain), and 0.2 µM of forward (fluorescently labeled) and reverse primers. The PCR reactions were carried out in a thermal cycler (Perkin-Elmer-9600) using the following program: denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 20 s, 50 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 7 min. The detection of amplification products was performed with an ABI 3130 Genetic Analyzer using the internal standard GeneScan 400 HD-Rox. Two cultivars, Arbequina and Frantoio, were used as controls in all runs.

We characterized the overall genetic diversity of our samples by calculating the number of parameters per microsatellite locus using the PowerMarker V3.23 software package (Liu and Muse 2005). The parameters were as follows: average number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), and Polymorphism Information Content (PIC) (Botstein et al. 1980). The presence of null alleles (An) was estimated using the Cervus software package (Marshall et al. 1998).

We also evaluated the genetic diversity of the groups of samples by comparing the average number of alleles per locus (Am), allelic richness (Ar) (Petit et al. 1998), observed (Ho) and expected (He) heterozygosity, and inbreeding coefficient (Fis) with the software Fstat v2.9.3.2 (Goudet 1995). The unique alleles (Au) (alleles present only in a group) were determined using Microsat (Minch et al. 1996). We applied two-way AMOVA and calculated pairwise Fst values to study the distribution of the molecular variance in our set of samples using Arlequin 3.5.1.3 software (Excoffier et al. 2005).

In addition, we studied the relationship among genotypes that were grouped according to their status and geographical origin. To study this relationship, we built an unrooted phylogenetic tree based on the Cavalli-Sforza and Edwards chord distance (CS), and the Fitch-Margoliash least squares algorithm implemented in the FITCH program of the PHYLIP 3.6b software package (Felsenstein 1989). The robustness of the tree nodes was evaluated using 10,000 bootstrap (BS) replications.

To detect clusters of genetically similar genotypes and to estimate the individual coefficients of admixture with regard to the detected clusters, we used a Bayesian clustering method described in Corander et al. (2003) implemented in the software Bayesian analysis of population structure (BAPS) (Corander et al. 2008). BAPS uses a stochastic optimization algorithm for analyzing Bayesian models of population structure, which greatly improves the speed of the analysis compared to traditional MCMC-based algorithms. Furthermore, comparison tests have shown that BAPS has comparable statistical power to STRUCTURE software and increased power over small geographical distances (Corander and Marttinen 2006; Latch et al. 2006). When testing for population clusters, we ran 10 replicates for every level of K (K is the maximum number of clusters), up to $K = 12$. When estimating individual ancestry coefficients via admixture analysis, we utilized only clusters that had at least 10 individuals present within them. In addition, we used the recommended number of reference individuals (200) and 100 iterations to estimate the admixture coefficients of the reference individuals.

10.2.3 Genetic Diversity of Wild and Cultivated Olives in Spain

Our study uncovered abundant allelic variation and high overall genetic diversity in both cultivated and wild olives. A total of 231 alleles were found across the 14 SSR markers. The average number of alleles per locus was 16.5, with a maximum of 39 alleles (ssrOeUA-DCA16) and a minimum of six alleles (GAPU71B). The average PIC value was high (0.766), which was similar to the values of other studies that used these markers (Díez et al. 2011; Trujillo et al. 2013). The expected heterozygosity (He) was larger than the observed heterozygosity (Ho), possibly due to the presence of null alleles in some of the markers (Table 10.2).

Because domestication involves the selection of individuals with outstanding agronomical performance, much of the genetic diversity present in the wild ancestors of the crops was lost. For instance, some annuals such as soybean, maize, and wheat have lost 34, 38, and 70–90 % of the genetic diversity that was present in their wild ancestors, respectively (Tenaillon et al. 2004; Hyten et al. 2006; Haudry et al. 2007). The following results of this study were in agreement with this premise: (i) both forms, wild and cultivated olives, shared only ~52 % of the alleles; (ii) the wild olives presented 10 times more unique alleles than the cultivars (103 vs. 8; Table 10.1a); and (iii) the allelic richness (Ar), which allows the comparison between groups independent of their sample size, was higher in the wild than in the cultivated olives, although this latter comparison was not significant (Wilcoxon rank test; $p = 0.064 > 0.05$). This lack of significance may most likely be related to the fact that the transition from wild to cultivated forms appears to be smoother in long-lived perennials than in annual plants. For example, no genetic bottleneck was detected between traditional cherry cultivars and wild cherries (Mariette et al. 2010). Similarly, traditional cultivars of grape and apple showed as much genetic variation as their wild relatives (Myles et al. 2011; Cornille et al. 2012). Two distinctive features of perennial plants may contribute to lessen their domestication bottlenecks. First, long-lived plants are generally open-pollinator species, a characteristic that might have favored the gene flow between wild and cultivars with the consequent maintenance of high levels of genetic diversity (Miller and Gross 2011). Second, perennial crops are typically clonally propagated, and this technique decreases the number of generations between the cultivars and their wild ancestors, and consequently, the differences between them (Mckey and Elias 2010; Miller and Gross 2011). Moreover, clonal propagation facilitates the existence of overlapping generations, which also contributes to this slight differentiation.

In our study, approximately 11 % of the molecular variance was due to differences between the cultivated and wild forms (Table 10.3).

Notably, the Fst values were significant for all the cultivated and wild comparisons except for the pairs of groups from the E and the NE (Table 10.4). As an additional distinctive feature between the cultivated and wild forms, the cultivars showed a negative Fis value, indicating an excess of heterozygotes; by contrast, the wild groups favored homozygosity, with Fis > 0.0 (Table 10.1a, b). Using SSR markers, several authors also reported the same trend (Breton et al. 2006; Belaj et al. 2007, 2010; Erre et al. 2009), but others found similar Fis values for both wild and cultivated olives (Yoruk and Taskin 2014). While the pervasive character of this opposite trend in Fis values still needs further confirmation, it might be the outcome of several processes. First, the indirect selection of highly heterozygous genotypes during domestication may occur because it is possible that they exhibit better agronomical performance or hybrid vigor. However, the existence of this phenomenon in olive remains unclear (Biton et al. 2012). Second, the accumulation of somatic mutations may occur during myriads of generations of clonal reproduction in cultivars, especially in highly variable and neutrally evolving genomic regions, such as SSRs. These regions might accumulate mutations

Table 10.3 AMOVA considering the variation at three hierarchical levels, groups (wild versus cultivated), geographical populations within groups and among individuals within geographical populations

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	*p*-value
Between groups (wild vs. cultivated)	1	191.06	0.655 = Va	11.20	$p < 0.001$
Among geographical populations, within groups	10	150.90	0.195 = Vb	3.33	$p < 0.001$
Among individuals within geographical populations	650	3249.26	4.998 = Vc	85.47	$p < 0.001$
Total	661	3591.23	5.848		

Table 10.4 Pairwise Cavalli-Sforza (1967) distance (upper diagonal) and Fst values (lower diagonal) between olive groups. Cultivated and Wild groups are identified with red and green color, respectively

	W	SW	SC	SE	E	NE	W	SW	SC	SE	E	NE
W		0.019	0.020	0.016	0.042	0.082	0.107	0.100	0.092	0.103	0.077	0.092
SW	0.003		0.018	0.017	0.038	0.062	0.095	0.081	0.073	0.086	0.059	0.078
SC	0.009	0.011		0.012	0.033	0.066	0.113	0.098	0.089	0.096	0.072	0.082
SE	−0.003	0.009	−0.003		0.041	0.067	0.114	0.102	0.094	0.100	0.073	0.088
E	**0.032***	0.047	0.019	0.038		0.056	0.100	0.094	0.083	0.089	0.061	0.064
NE	**0.118**	**0.113**	**0.098**	**0.109**	**0.043**		0.092	0.077	0.071	0.081	0.052	0.030
W	**0.186**	**0.176**	**0.192**	**0.198**	**0.166**	**0.147**		0.018	0.020	0.032	0.056	0.066
SW	**0.157**	**0.143**	**0.155**	**0.165**	**0.138**	**0.119**	**0.011**		0.014	0.025	0.048	0.054
SC	**0.152**	**0.135**	**0.152**	**0.162**	**0.129**	**0.117**	**0.011**	**0.007**		0.022	0.040	0.050
SE	**0.148**	**0.135**	**0.141**	**0.155**	**0.116**	**0.117**	**0.031**	**0.024**	0.01		0.053	0.054
E	**0.089**	**0.081**	**0.085**	**0.089**	0.046	**0.038**	**0.064**	**0.049**	**0.037**	**0.031**		0.052
NE	**0.131**	**0.134**	**0.115**	**0.133**	**0.057**	0.018	**0.114**	**0.092**	**0.090**	**0.077**	**0.032**	

*Significant values in bold

without necessary phenotypic consequences in crop morphology and agronomic performance (Mckey and Elias 2010; Miller and Gross 2011; Díez et al. 2011). Finally, differential autogamy rates may occur in the wild and cultivated olive forms.

Despite the cultivated olive being considered as almost a strict out-crosser (Diaz et al. 2006), certain self-compatibility rates have been found for some cultivars (Guerriero and Bartolini 1995; Koubouris et al. 2014). Although higher self-compatibility rates in the wild progenitor than in the crops are not frequent in long-lived perennials (Miller and Gross 2011), this possibility has never been studied in olive; further, its possible relationship with the domestication process has also not been explored.

10.2.4 Genetic Relationships Between Wild and Cultivated Olives at a Regional Level

Although most of the molecular variance was due to differences between the wild and cultivated forms (~11 %) and between samples (~85 %), a subtle but significant proportion (3.3 %) of the molecular variance was due to differences among samples arranged according to their areas of origin (Table 10.3). This geographical differentiation pattern was clear among the wild groups but not in the cultivated groups. For example, the Fst values were significant between the wild olive groups and were more important for those not geographically adjacent (Table 10.4). By contrast, no significant Fst values were found between most of the cultivated populations. The recent movement of cultivars linked to human migration might have blurred the geographical fingerprint that was once present in the traditional cultivars (Baldoni et al. 2006). Only the cultivars from the east and northeast regions showed significant Fst values compared to all the other cultivars. Previous studies based on RAPD markers that analyzed Spanish cultivars reported the distinctiveness of the olive cultivars from the east and northeast compared to those from the rest of the country, suggesting they might be derived from different domestication processes (Belaj et al. 2004, 2010).

The dendrogram and the Bayesian analyses demonstrated the differences between the wild and cultivated samples. Again, the only pairs of cultivated and wild groups that were closely related were mostly those from the east and northeast regions (E and NE). These samples had an intermediate position between the wild and cultivated olives in the dendrogram (Fig. 10.3), and formed a distinctive genetic cluster in the Bayesian analysis (Fig. 10.4). Thus, summarizing the results from the Bayesian analysis and the dendrogram, our samples clustered into the following three well-supported (BS values > 95 %) groups: (i) cultivars from the western and southern regions; (ii) cultivars and wild populations from the east and northeast; and (iii) wild populations from the western and southern regions of Spain.

The genetic similarity between local cultivars and wild olives has been previously used as a proxy to support or reject the local domestication of these cultivars (De Caraffa et al. 2002; Baldoni et al. 2006). According to the dendrogram and the Bayesian analysis, the cultivars from the south (SW, SE, and SC) and west of Spain were minimally related to their local wild olives. Díez et al. (2011) found similar patterns when analyzing ancient olives from southern Spain, suggesting that the beginnings of olive growing in some areas of the west Mediterranean Basin could be based on the grafting of not necessarily autochthonous cultivars over local oleasters (Díez et al. 2011). In line with this hypothesis, almost all the cultivars from southern Spain presented the same haplotype (E1.1), which is broadly represented in wild and cultivated olives from the eastern Mediterranean Basin, where olive was likely primarily domesticated (Besnard et al. 2013).

Conversely, most of the cultivars from east (E) and northeast (NE) Spain were closely related to the local wild forms. This finding might suggest that these

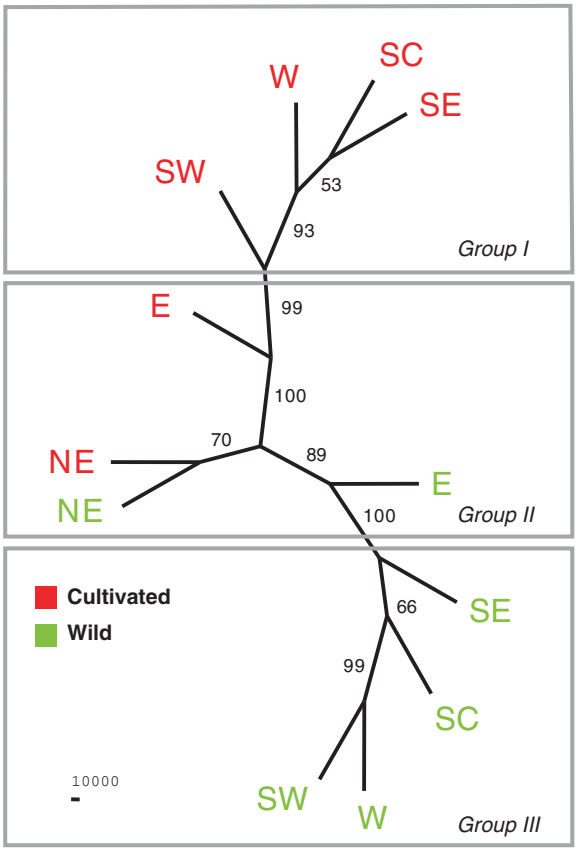

Fig. 10.3 Dendrogram showing the relationships between cultivated and wild olive samples arranged according to their geographical origin; West (*W*), Southwest (*SW*), South-Center (*SC*), Southeast (*SE*), East (*E*), and Northeast (*NE*). Bootstrap values are given in percentages over 10,000 replicates

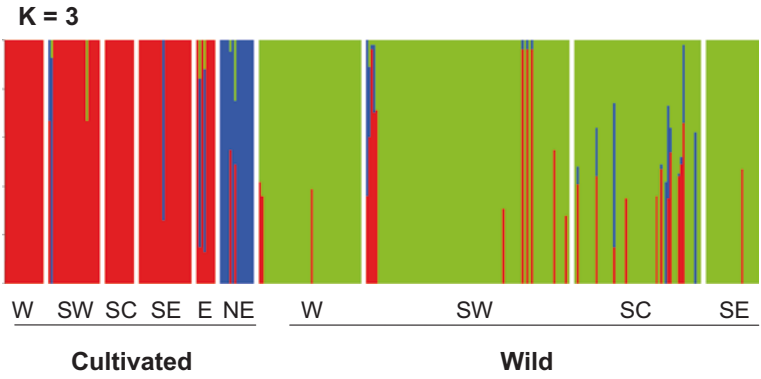

Fig. 10.4 Proportions of ancestry of the wild and cultivated olive samples (N = 331) based on $K = 3$ subdivisions. The geographical origins as well as the putative status of the samples (cultivated or wild) are specified

cultivars were derived from an alternative domestication or diversification process, possibly involving the direct selection from local oleasters or the admixture between them and not local cultivars. In agreement with this concept, Belaj et al. (2010) suggested the possibility of admixture events gave rise to the olive cultivars in northeast but not in south Spain (Belaj et al. 2010). However, the similarity between cultivated and wild E and NE samples could be due to the feral status of our putatively wild samples. Indeed, oleasters and feral forms are sometimes hard to distinguish morphologically; moreover, in the east and northeast, wild olive populations are scarce and fragmented compared to those in the south and west.

However, presuming the wild status of the samples, our data suggest that the genetic diversity within olives has been shaped by hybridization with wild oleasters mostly in E and NE Spain. Conversely, this process has been absent or very subtle in the south and west, where wild and cultivated populations were grouped in homogeneous and distinctive genetic clusters.

10.3 The Loss of Genetic Variability: Conservation Strategies in Olive

The knowledge about the relationships between cultivars and wild olives is critically important for conservation purposes, breeding programs, the design of genome association studies, and to untangle the population history. In addition, these studies allow us to track the evolution of genetic diversity and its potential loss in crops as a consequence of domestication and the posterior intensification of growing systems. This phenomenon has not been well documented despite its crucial importance for the sustainability of agriculture and food security (van de Wouw et al. 2009).

Regardless of the primary origin of olive cultivars, our dataset provides two snapshots of olive genetic diversity in the main olive-growing regions of Spain. First, the wild olives depict the genetic diversity of the species as part of the spontaneous Mediterranean vegetation; and second, the traditional cultivars maintain the genetic diversity that has served as a foundation for the solid and extensive rainfed olive-growing system over centuries.

The wild olive populations showed an outstanding allelic variability, most of which was not present in the cultivars. This wild germplasm represent an uncharacterized source of genetic resources for breeding; moreover, as suggested by our results, the highly diverse wild olives from south Spain played a minor role in the domestication of olive. The key to combat devastating diseases with no sources of complete resistance within the cultivated olive, such as Verticillium wilt that is caused by the fungus *Verticillium dahliae* Kleb., might be provided by wild germplasm (Colella et al. 2008; Trapero et al. 2015), as observed in other perennial

crops such as pistachio (Morgan et al. 1992). For these reasons, the characterization and preservation of wild olive germplasm is of outstanding importance.

Conservation efforts should also focus on traditional olive cultivars. The intensification of olive-growing systems is triggering both the standardization of cultivars in new plantations and the development of breeding programs to search for cultivars adapted to new planting systems. For example, high-density hedgerow systems in both rainfed (>1000 olives per ha) and irrigated (>1500 olives per ha) conditions are spreading worldwide (Rallo 2014). Only a handful of cultivars fit the requirements needed for this new system. Among them, Arbequina is the cultivar of choice, which is planted worldwide. A wave of newly bred olive cultivars, a product of the crossing between cvs. Arbequina and Picual, will soon be released to complement the availability of cultivars for intensive planting systems (Rallo 2014).

This substitution process may mimic the transition between pre-cultivated forms and the current traditional cultivars in the past. However, its geographical scale is quite different. In the past, olive growing had different characteristics even between regions from small geographical areas (e.g., south and northeast Spain). Currently, the new olive-growing systems are global. The same five cultivars, Arbequina, Arbosana, Frantoio, Koroneiki, and Picual, are being used in most new olive plantations worldwide. This trend might lead to a genetic erosion process where the traditional local cultivars could be progressively substituted and finally lost unless conservation plans are implemented.

In addition, the outbreaks of epidemic diseases can seriously affect the maintenance of local cultivars. For example, a devastating disease, denoted as "Olive Quick Decline Syndrome," affected olive trees in the Apulia region of southern Italy in October 2013. This syndrome, which is generally associated with the quarantine bacterium *Xylella fastidiosa*, several fungal species of the genus *Phaeoacremonium* and *Phaemoniella*, and the moth *Zeuzera pirina* (Saponari et al. 2013), mainly killed 200–300-year-old olives—most of them local cultivars.

In this scenario, ex situ and in situ conservation efforts are required to avoid the irreversible loss of traditional cultivars. Ex situ field collections of trees have been the typical method for the conservation of olive cultivars. In 1994, the International Olive Council (IOC) promoted a Network of National Banks of Germplasm. This network also includes two international repositories, the Olive World Germplasm Banks of Córdoba (Spain) and Marrakech (Morocco). A third repository is under development in Izmir (Turkey). Despite the existence of this network, the exploration and conservation of olive genetic resources is still incomplete and requires further efforts in all the olive-growing countries. For instance, a review by FAO reported the existence of 107 collections of olive cultivars worldwide; however, even in these institutions, approximately 20 % of the accessions were labeled as "unknown" (Bartolini and Cerreti 2008). One of the main advantages of ex situ conservation is that it allows the evaluation of the cultivars for many traits in the same environment. Recent efforts have been paid to the development of core collections in olive (Haouane et al. 2011; Belaj et al. 2011; Díez et al. 2012; El Bakkali et al. 2013). These core collections, which consist of a limited

number of the accessions, were chosen to cover the genetic spectrum of the entire collection (Brown 1989). Core collections represent an efficient strategy for studying the interaction of genotypes and environments to reduce the effort in the evaluation of agronomic characters.

In situ conservation permits the coevolution of genotypes in their original environment. It appears as a valuable tool not only for the preservation of wild olive populations but also for monumental olives. The long life span of olive results in the existence of both centennial and millennial trees across the Mediterranean Basin. The study of ancient olives has been fruitful for both germplasm collection and to increase the knowledge regarding olive domestication (Erre et al. 2009; Diez et al. 2011; Cicatelli et al. 2013; Salimonti et al. 2013; Barazani et al. 2014). An international network of in situ monumental and wild olives appears to be a strategic initiative for the future of this crop (Rallo 2014).

Thus, knowledge about the local genetic variation of olive germplasm, including wild and cultivated forms, is the first and necessary step for the sustainability of olive growing. The sustainability of olive-growing systems is particularly important when considering the forecast for climate change in the Mediterranean Basin and its possible effects on olive growth (Ponti et al. 2014). More frequent extreme weather is predicted by most climate change models, along with a significant increase in the summer air temperature and water stress, mainly for Mediterranean regions (Tubiello et al. 2000). In particular, shifts in precipitation patterns will affect most European regions, with increased risks of drought; given this scenario, the consequences would be most dramatic for the Mediterranean coast of Europe (Lung et al. 2014). Under these circumstances, the evaluation of the potential adaptation of the olive cultivars to different climatic conditions is crucial. To do so, it is necessary to examine the phenological characterization of the genotypes under different climatic conditions, as well as to evaluate their tolerance to biotic and abiotic stresses. The establishment of several core collections, managed by the IOC network of Germplasm Banks composed of 23 banks, may provide an ideal opportunity to achieve this goal.

10.4 Conclusions and Prospects

The new olive-growing systems, which are more intensive and mechanically harvested, are leading to the progressive reduction in the number of traditional olive cultivars used in new plantations. This phenomenon might cause the irreparable loss of genetic variation in olive. In this context, the exploration, identification, and conservation of olive genetic resources, both cultivated and wild, is an urgent task. The phenotypical characterization of olive germplasm is crucial for identification purposes, breeding programs and to examine the impact of climate change on olive-growing systems. Wild olives represent an unexplored source of genetic variability, which also require further characterization and conservation efforts. The characterization of wild and cultivated germplasm at a regional level is

necessary for conservation purposes, as well as for olive breeding and to untangle the domestication history of this crop. Global and coordinated ex situ and in situ conservation programs should be designed to evaluate and preserve the wealthy genetic legacy present in olive germplasm.

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Chapter 11
Genetic Diversity, Genetic Erosion, Conservation of Genetic Resources, and Cultivation of Medicinal Plants

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Abstract Wild or cultivated plants used in traditional and modern medicines are categorized as medicinal plants (MPs). Out of over 70,000 MPs, 3000 are traded and 900 are cultivated. Fragmentation/loss of habitats, unsustainable harvests, excessive grazing, invasive species, pollution, and climate change are destroying genetic diversity. Regular use of MPs in modern medicines, consumer/industrial merchandises, and increasing popularity of complementary and alternate (CAM) therapies are expanding national/global trade inciting irrational wild collections beyond regeneration potential of wild populations consequently losing species and genetic diversity. Investigations on endangered species indicated frightening levels of genetic erosion and dwindling population densities/sizes below minimum viable limits. Only a small fraction of known MPs have been evaluated for their genetic diversity and genetic erosion. Morphoagronomic, biochemical and molecular marker, and enzyme studies on wild and cultivated genotypes, populations, species, and geographical regions revealed genetic diversity with varied levels of polymorphism (14–100 %), number of alleles (2–14/locus), observed (0.0–1.0) and expected (0.06–0.84) heterozygosities, Nei's gene diversity (0.12–0.36), Shannon's index (0.08–0.51), gene flow (0.22–4.69), genetic distances (0.02–0.54), and similarities (0.02–0.98). Recovery, conservation, and cultivation programs initiated by governments have slowed down genetic erosion. Cultivation helped in relieving harvest pressure on wild flora and in preserving genetic diversity of some species. Existence of large number of species, paucity of adequate research funds, loss/degradation of forests, ever increasing local/world demand, genetic resource utilization with benefit sharing, and patent conflicts are the concerns that need to be resolved for conserving genetic diversity and preventing genetic erosion.

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11.1 Introduction

Plants have been used for their curative property since antiquity. Plants possessing therapeutic activity are known as medicinal plants (MPs) or pharmaceutical botanicals or healing herbs or minor forest or underutilized or industrial crops. The earliest record of MPs use by human beings dates back to around 7000 years (Kathe 2006). A medicinal plant is defined as "a plant (wild or cultivated) used for medicinal purposes" (http://apps.who.int/medicinedocs/pdf/s4928e/s4928e.pdf. Viewed June 17, 2014) or "all higher plants that have been alleged to have medicinal properties, i.e., effects that relate to health or which have been proven to be useful as drugs by western standards or which contain constituents that are used in drugs" (Farnsworth and Soejarto 1991) or "the term medicinal as applied to a plant indicates that it contains a substance or substances which modulate beneficially the physiology of sick mammals and that has been used by humans for that purpose" (http://wwwlib.teiep.gr/images/stories/acta/Acta%20500/500_1.pdf. Viewed June 17, 2014) or "those that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to play a beneficial role in healthcare" (Srivastava et al. 1996) or "a plant which has been used for medical purposes at one time or another and which, although not necessarily a product or available for marketing is the original material of herbal medicines" (http://www.wpro.who.int/publications/docs/Guidelines_Appropriate_Use_of_Herbal_Medicines.pdf. Accessed June 17, 2014) or "useful plants for primary healthcare, as remedy for diseases and injury, plants used traditionally for foods and drinks and which are believed that they are good for health; the MPs include foods, drinks, herbs, and spices" (Bekele 2007). For the purpose of this chapter, MPs include aromatic, dye-yielding, pesticidal plants, and many spices. Plants are natural factories producing thousands of primary and secondary metabolites. The curative property of MPs is attributed to low-molecular weight secondary metabolites such as alkaloids, steroids, glycosides, phenolics, flavonoids, coumarins, saponins, stilbenoids, lactones, terpenoids, tannins, lignans, etc. accumulated in one or more parts in varying concentrations in response to stress, predation, competition, for attracting pollinators and conversion to primary metabolites. Being chemical repositories or libraries, MPs are wild-collected or cultivated for their prized phytochemicals in contrast to food, fodder, fuel, flower, fruit, foliage, fiber, timber, and other crops (Heywood 1999; Lubbe and Verpoorte 2011). The phytochemicals are biosynthesized through mevalonate, shikimate, and methyl erythritol phosphate pathways. In addition to majority (70–80 %) of citizens of developing nations, increasing number of denizens of developed countries (37 % Americans, 31 % Germans) are relying on traditional (TM), complementary, and

alternate (CAM) medicines for healthcare steadily boosting demand for MPs. The global search (bioprospection) for biologically active, therapeutically effective, stable, and safe phytochemicals has pushed them into transnational patent conflicts. Several modern medicines that treat constipation (e.g., *Cassia senna*, *Plantago psyllium*) to cancer (e.g., *Camtotheca acuminata*, *Catharanthus roseus*, *Podophyllum hexandrum*, *Prunus africana*, *Taxus* species) are made from phytochemicals. Between 1959 and 1980, 25 % of prescription medicines worth US$ 8.1 billion dispensed through USA community pharmacies had one or more MP derived biochemicals. In 1981, 121 prescription medicines containing phytochemicals of 95 MPs were used worldwide (Farnsworth and Soejarto 1991). More than 25 % of pharmaceutical medicines in use (Lubbe and Verpoorte 2011) and 26–50 % of new medicines that entered markets in recent years are plant based. Estimates indicate that tropical forests can yield 328 more plant medicines worth US$147 billion (Memdelsohn and Balick 1995). Plant-based medicines or herbal medicines or botanical drugs or phytomedicines or phytopharmaceuticals are in use in China, India, Germany (30–40 % of prescription medicines), Japan (15–20 % of prescription medicines), Ukraine (20–50 % of prescription medicines), Organization for Economic Cooperation and Development (OECD) countries, African and Asian countries (Principe 1991). Human and environmental factors namely, habitat change (habitat loss, fragmentation, degradation or conversion to other uses such as human habitation, agriculture, slash and burn cultivation, ranching, timber logging, ecotourism, mining and industry), climate change (global warming, tsunamis, erratic rainfall, forest fires, glacier melting), invasive species (intentionally or accidentally introduced native or exotic species which compete out native species and invasive pests that damage wild flora), over-harvesting (frequent wild harvests at wrong phenological stages beyond species' regeneration capacities, wasteful wild collections exceeding market needs, and destructive harvests exterminating plants), pollution (caused by human activities, agricultural chemicals, sewage, traffic, industrial effluents), overgrazing, and booming world trade (8–15 % growth per annum; Grünwald and Büttel 1996) are the driving forces of genetic erosion (depletion or loss of genetic diversity and gene pool wealth over time) and extinction of MPs. International organizations such as United Nations Environment Program (UNEP), United Nations Educational, Scientific and Cultural Organization (UNESCO), United Nations Industrial Development Organization (UNIDO), World Health Organization (WHO), International Union for Conservation of Nature and Natural Resources (IUCN), World Wide Fund for Nature (WWF), Food and Agriculture Organization (FAO), Convention on Biological Diversity (CBD), Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), Trade Record Analysis of Fauna and Flora in Commerce (TRAFFIC), MPs Specialist Group of the Species Survival Commission of IUCN, G-15 Gene Bank for Medicinal and Aromatic Plants, Asian Network on Medicinal and Aromatic Plants, Asia Pacific Information Network on Medicinal and Aromatic Plants, International Council for Medicinal and Aromatic Plants, Biodiversity International (formerly, International Plant Genetic Resources Institute), International Trade Center (ITC), etc. are providing

guidelines, directions to governments on scientific information, recovery, conservation, cultivation, sustainable utilization, quality control, and international trade in threatened MPs for protecting genetic diversity and resources.

11.2 Global Genetic Resources

Forests, wooded lands, banks of water sources, wastelands, roadsides, and agricultural lands are the natural habitats. Medicinal herbs that grow in agricultural fields are weeded out by farmers ignorant of their economic significance or due to lack of local market. Forests are the primary habitats where MPs grow as undergrowth and in open grasslands (subjected to overgrazing). The present forest area is 4.033 billion hectares or 31 % of world's land area relative to earlier 6.2 billion hectares. Primary forests (rich in native species) account for 36 % (1.4 billion hectares), naturally regenerated forests for 57 %, and planted forests for 7 % of total area. American Samoa, French Guiana, Gabon, Micronesia, Palau, Pitcairn, Seychelles, Solomon Islands, Suriname, Turks, and Caicos Islands are endowed with 79–98 % of their land area covered under forests. In Brazil, Brunei, Darussalam, French Guiana, Gabon, Micronesia, Papua New Guinea, Peru, Singapore, Suriname, and Tajikistan, 65–100 % forest cover is primary forests (FAO 2010). UNEP World Conservation Monitoring Centre (http://www.unep-wcmc.org/) has identified 17 mega-biodiversity countries that support bulk of global ecosystem, species, genetic and molecular diversity. These are: Australia, Brazil, China, Colombia, Democratic Republic of Congo, Ecuador, India, Indonesia, Madagascar, Malaysia, Mexico, Papua New Guinea, Peru, Philippines, South Africa, USA, and Venezuela.

Out of 370,000 (900,000 species including outdated names and synonyms; Paton 2009) to 422,000 estimated plants; 72,000–77,000 are MPs (Schippmann et al. 2006; Rajeswara Rao et al. 2012). With several countries inventorying, digitizing, and investigating their MPs resources this number may get revised from time to time. Bulgaria (750 MPs, 200–300 in common use), China (11,146), Ethiopia (1000, 300 often used), Finland (100), France (900), Hungary (270), India (7500–8000, 960 regularly used), Italy (1500), Jordan (363), Macedonia (700, 150 in frequent use), Malaysia (1200), Malta (458), Nepal (1950), Pakistan (1500), Philippines (850), Republic of Korea (1000), Romania (283), Serbia (400), Slovenia (400), Sri Lanka (1414, 208 commonly used), Thailand (1800), Turkey (500), USA (2564), Vietnam (1800), Yugoslavia (>700), and other countries recorded their MPs resources (Schippmann et al. 2006; Guo et al. 2009). WHO has published monographs on 118 MPs and information on MPs of Myanmar (59), Mongolia (92), South Pacific (102), Papua New Guinea (126), Republic of Korea (150), and Vietnam (200). Plant families Apiaceae, Apocynaceae, Araliaceae, Asclepiadaceae, Asteraceae, Canellaceae, Euphorbiaceae, Guttiferae, Lamiaceae, Lauraceae, Leguminosae, Menispermaceae, and Rosaceae have higher number of MPs. For most MPs information on centers of their origin, biology, genetic diversity, population sizes, distribution, trade volumes/value, and threat levels is scanty.

11.3 Assessment of Genetic Diversity

The survival, evolutionary capability, and agility of wild flora to adjust to changing ecological and environmental conditions are determined by genetic diversity developed over millennia. Genetic diversity among and within populations in a habitat is a result of natural selection, gene flow, genetic drift, inbreeding, and mutation. Genetic diversity assessment helps in designing conservation and crop improvement strategies (Sheng et al. 2004; Rahimmalek 2012). Past research recorded ethnomedical (folklore/tribal medicine) uses by ethnic/tribal communities. Concurrently, taxonomists prepared district, province, and country floras to assess species diversity, abundance, and distribution. These floras served as baseline surveys for conservation and utilization programs. Morphoagronomic, biochemical variations within and among genotypes, populations, species, and regions were subsequently studied. Researchers are currently employing enzymes and molecular markers for assessing and preserving genetic diversity, establishment of phylogenetic relations of populations or related species, identification of species and varieties (DNA fingerprinting) or discrete genetic units within species, DNA bar coding, marker-assisted selection for crop improvement, authentication of herbal materials, detecting adulteration in commercial herbal products, predicting from which wild population or geographical location a commercial sample has been sourced, estimating variation between in vitro and conventionally propagated plants or wild and cultivated populations, estimating gene flow, estimating disease susceptibility, and assessing geographical variation at genetic level (Atangana 2010; Lal et al. 2011). The literature survey indicated that not even a small fraction of known MPs have been evaluated for genetic diversity and gene pool wealth. To the best of author's knowledge no attempt has been made so far to prepare genome map of any MPs although morphotypes, chemotypes, genotypes, and ecotypes differing in morphology, physiology, categories, and contents of phytochemicals have been recorded. Polymorphism (14–100 %) is evident in the investigated species with 2–14 alleles/locus. Observed (0.0–1.0) and expected (0.06–0.84) heterozygosities, genetic distances (0.02–0.54) and similarities (0.02–0.98), Shannon's index (0.08–0.51), Nei's gene diversity (0.12–0.36), gene flow (0.22–4.69), AMOVA (Analysis of Molecular Variance), UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering, PCoA (Principal Coordinate Analysis), and PCA (Principal Component Analysis) revealed that genetic diversity is high in the existing populations (Table 11.1). Genetic variations in leaf (e.g., *Achillea* species, *Aloe vera*, *Hemidesmus indicus*, *Ocimum sanctum*), flower (e.g., *A. vera*, *C. roseus*, *Clitoria ternatea*), fruit (e.g., *Emblica officinalis*), seed (e.g., *Abrus precatorius*, *Mucuna cochinchinensis*, *Withania somnifera*), tuber (e.g., *Chlorophytum borivilianum*, *Curculigo orchioides*) and stem (e.g., *Phyllanthus amarus*, *O. sanctum*) characteristics, plant architecture (e.g., *Artemisia annua*, *Piper longum*), chemical profile (e.g., *A. annua*, *A. dracunculus*, *A. judaica*, *Atractylodes lancea*, *C. galioides*, *Hypericum triquetrifolium, P. hexandrum*, *Primula ovalifolia*), and ploidy levels (e.g., *Artemisia dracunculus*, *C. roseus*) have been reported.

Table 11.1 Genetic diversity reported in several medicinal plants from different countries employing morphological, agronomical, biochemical, and molecular markers and enzymes

Botanical name	Country	Method	Genetic diversity	Reference
Achillea bieberstenii	Iran	Morphoagronomic	25 populations from 12 provinces were clustered into 11 groups with significant variation in studied marker characters	Seyed et al. (2012)
Achillea fragrantissima	Jordan	RAPD	Five populations from five locations showed genetic diversity with similarity values ranging from 0.02 to 0.58 and were grouped into four clusters	Rawashdeh (2011a)
Achillea millefolium subsp. *millefolium/Elbursensis*	Iran	Morphology, ISSR	Accessions from various regions showed 87.3 % polymorphism, 32.6 % genetic variation among groups, 40.9 % among populations and 26.9 % within populations. Gene diversity over loci varied among regions and accessions were grouped according to regions. Morphological and molecular markers produced similar results	Gharibi et al. (2011)
Achillea santolina, A. tenuifolia	Iran	RAPD, ISSR	16 accessions of both species showed genetic diversity. Genetic similarity ranged from 60 to 86 % in *A. tenuifolia* and 40–84 % in *A. santolina* with low similarity (0.36) between them	Ebrahimi et al. (2012)
Achillea tenuifolia	Iran	ISSR	Genotypes from diverse regions exhibited 86.8 % polymorphism, clustered into three groups with 68.7 % variation among and 31.3 % within groups. Varied in morphological characters	Rahimmalek (2012)
Actaea racemosa (Black cohosh)	USA, Canada	AFLP	Principal component analysis (PCA) distinguished distant populations and pointed out genetic similarity within geographic regions	Motley et al. (2004)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Allanblackia floribunda, A. stanerana, A. gabonensis	Cameroon	Microsatellites	70 trees from four sites showed no significant difference between wild and cultivated trees with inbreeding in cultivated trees of *A. floribunda*. Seven microsatellite loci displayed polymorphism in *A. stanerana* and *A. gabonensis*	Atangana (2010)
Allium sativum (Garlic)	Different countries	Isozymes, AFLP	High heterogeneity was noticed within Central Asian gene pool	Kamenetsky et al. (2007)
Aloe vera	Iran	RAPD	10 accessions from different regions showed 70.3 % polymorphism and were grouped into two clusters	Barandozi et al. (2012)
Arnica montana	Romania	RAPD	Six genotypes from two regions were grouped into two clusters which recorded high genetic distance coefficients	Pop et al. (2008)
Artemisia annua	India	Chemical, RAPD	Eight individuals of a population exhibited chemotypic and genetic variation (96 % polymorphism, 0.79 polymorphic information content) with no similarity (0.64 dissimilarity)	Sangwan et al. (1999)
Artemisia dracunculus	USA	Biochemical	Genetic diversity varied from 22 % between and 78 % within populations. Diploid to decaploid ecotypes differed in chemical composition	Eisenman (2010)
Artemisia judaica	Egypt	RAPD	Egyptian populations showed 57.5 % polymorphism and genetic distance	Badr. et al. (2012)
Artemisia judaica	Jordan	RAPD	10 primers gave 165 polymorphic bands with 0.61–0.02 similarity index. No similarity in some samples	Rawashdeh (2011b)
Asparagus racemosus	India	RAPD	Accessions of *A. racemosus* and ornamental species showed 48.3 % intra and 51.7 % interspecies variation. Two accessions were related to unknown *A.* species	Shasany et al. (2003)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Asparagus racemosus, *A. officinalis*, *A. springeri*, *A. plumosus*, *A. densiflora myersii*	India	RAPD	Species displayed 94.5 % polymorphism with no similarity	Lal et al. (2011)
Blumea balsamifera	China	AFLP	35 plants from 5 provinces had 99.5 % polymorphism. They were grouped into four clusters with less variation within provinces	Pang et al. (2014)
Butea monosperma	India	RAPD	16 accessions from five provinces recorded 0.43 mean genetic divergence, 0.53–0.79 similarity coefficients and were grouped into four clusters	Khan et al. (2008)
Bunium persicum (Black cumin)	Iran	RAPD, AFLP	20 populations of black cumin had 75–86 % polymorphism, 0.39–0.96 similarity coefficients. Genetic distances among populations did not correlate with geographical distances	Pezhmanmehr et al. (2009)
Cassia occidentalis	India	RAPD	10 accessions from different districts had 71.2 % polymorphism, 0.54–0.73 similarity coefficients. They were grouped into two clusters	Arya et al. (2011)
Calligonum polygonoides	India	Chemical, RAPD	54 wild plants from eight locations of Thar desert showed 90.2 % polymorphism, 0.43–0.89 similarity coefficients. Plants differed in nutrient composition. Anthropogenic activity in one location led to low diversity and affected genetic composition	Vyas et al. (2012)
Carapichea/Cephaelis ipecacuanha (Ipecac)	Brazil	ISSR	50 wild clusters with 291 aerial stems showed no genetic differentiation at the cluster level	de Oliveira et al. (2010)
Catharanthus roseus	India	RAPD, ISSR	14 cultivars displayed 82 % polymorphism in 17 loci and were classified into two clusters where some cultivars were closely related	Shaw et al. (2009)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Chamomilla recutita	European countries	ISSR	Seven cultivars, eight accessions showed 85.4 % polymorphism, 0.65 mean genetic similarity. Genetic similarity was high in cultivars	Okoń and Magdziak (2011)
Changium smyrnioides	China	RAPD	Five populations recorded 69 % polymorphism with 51.2 % diversity among and 48.8 % within populations	Fu et al. (2003)
Chimonanthus praecox	China	RAPD, ISSR	72 clones from two regions showed 63.6–78.3 % polymorphism. Distribution of clones was consistent with regions. Genetic variation among clones of a region was 85.6–86.7 %	Zhao et al. (2007)
Cibotium barometz	China	SRAP	79 plants from seven populations showed 86 % polymorphism, 0.23 Nei's gene diversity index, and 0.36 Shannon's information index. Genetic diversity within and among populations was 58.9 and 41.1 %. Gene flow was low (0.72) and geographical distribution was not distinctive	You and Deng (2012)
Coleus amboinicus, C. aromaticus, C. forskohlii	India	RAPD	Three species exhibited genetic diversity	Govarthanan et al. (2014)
Commiphora wightii	India	RAPD	Accessions collected from different locations recorded 83.5 % polymorphism with 0.55–0.79 similarity coefficients	Suthar et al. (2008)
Coptis chinensis	China	ISSR	214 plants from seven wild and three cultivated populations revealed that polymorphism in wild (52.4 %) and cultivated (65.2 %) and genetic variation (10.9 %) between them was not significantly different. Cultivation did not result in genetic erosion	Shi et al. (2008)
Crocus sativus	Iran	Microsatellites	Observed and expected heterozygosities varied from 0.07 to 0.92 and 0.10 to 0.58, respectively, with 2.6 alleles/locus	Nemati et al. (2012)

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Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Cuminum cyminum	Iran, Syria, Afghanistan	ISSR, RAPD, morphoagronomic	44 accessions recorded 54.9–67.3 % polymorphism, 0.25–0.94 similarity coefficients in different analyses. Morphoagronomic and molecular markers differentiated the accessions differently	Ahmadvandi et al. (2013)
Cunila galioides	Brazil	RAPD	Three chemotypes with wide genetic variation were recognized. Two chemotypes were closely related while the third chemotype represented a different genetic pool	Fracaro et al. (2005)
Cuscuta reflexa	India	ITS nrDNA	30 populations from nine geographical locations showed high degree of diversity as they evolved in reproductive isolation. Two locations indicated genetic exchange among populations	Ali et al. (2011)
Dacydium pierrei	China	RAPD	Nine plants from Hainan province showed 33.3 % polymorphism and 0.11 genetic distance	Su et al. (1999)
Dendrobium species	India	RAPD, SSR	30 individuals of five species recorded polymorphism with 4–7 alleles per locus. SSR markers were better than RAPD markers	Chattopadhyay et al. (2012)
Dioscorea opposita	China	ISSR	28 cultivars exhibited 83 % polymorphism and were grouped into four clusters	Zhou et al. (2008)
Dracocephalum kotschyi, D. multicaule, D. polychaetum, D. surmandinum Lallemantia sp.	Iran	RAPD	17 accessions of *Dracocephalum* and 1 of *Lallemantia* displayed high genetic diversity. *D. polychaetum* and *D. surmandinum* were closely related to *D. kotschyi*. RAPD markers differentiated the species at molecular level and were useful in estimation of inter- and intraspecific variations	Sonboli et al. (2011)
Dysosma pleiantha	China	Microsatellites	38 plants from three populations recorded 2–14 alleles per locus with 0.54–0.85 heterozygosity values. Homozygosity was recorded in 10 loci	Guan et al. (2011)

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Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Echinacea angustifolia	USA	AFLP	10 populations exhibited genetic divergence with restricted gene flow along north–south climatic gradient with 60 % genetic variation within populations, 20 % among populations, and 20 % among groups	Still et al. (2005)
Echinacea species	USA	AFLP	Nine species and three varieties displayed 90 % polymorphism and were closely related. *E. purpurea*, *E. sanguinea,* and *E. simulata* were grouped in one clade and the others in the second clade	Kim et al. (2004)
Epimedium pubescens, *E. sagittatum*, *E. wushanense*	China	Isozymes	471 plants from 11 populations exhibited 69.2–84.6 % polymorphism with 2–3 alleles/locus and 0.27–0.38 heterozygosity. *E. pubescens* was closely related to *E. sagittatum*. Results indicated mixed breeding system, gene flow among populations and genetic relationship among the species	Xu et al. (2007)
Epimedium sagittatum	China	SSR	16 synthesized primer pairs transferred to 52 species showed high genetic diversity with 0.35 observed and 0.65 expected hyterozygosities and 11.9 alleles per locus	Zeng et al. (2010)
Fritillaria cirrhosa	China	AFLP	159 individuals of nine wild populations recorded 91.9 % polymorphism with 0.27 estimated heterozygosity in population and 0.37 at species levels. Genetic differentiation among populations was 0.28. Genetic diversity among some populations was high while in others it was low	Zhang et al. (2010)
Gymnema sylvestre	India	ISSR	Plants collected from 12 geographical regions recorded 85 % polymorphism	Mouna et al. (2014)

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Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Gardenia jasminoides	China	AFLP	Eight wild or cultivated populations registered 67.6 % polymorphism at species and 36.9–59.4 % in population levels. Genetic diversity within populations was 0.21–0.31. Genetic differentiation among populations was 64.8–76.6 %. Regions did not show differences. Gene flow (1.74) was consistent with mean genetic identity (0.93) among populations. Genetic and geographic distances were correlated between populations	Han et al. (2007)
Gastrodia elata	China	AFLP	27 accessions from five provinces exhibited 78 % polymorphism, genetic distance varied from 0.54 to 0.018. One province indicated high diversity and can serve as germplasm source for improvement	Guan (2013)
Ginkgo biloba	China	RAPD	Nine populations recorded 97.9 % polymorphism, expected heterozygosities of 0.24–0.36, Shannon's information index of 0.34–0.51, genetic differentiation of 0.16 and 89 % within population genetic variation. They were classified into two groups	Fan et al. (2004)
Haloxylon ammodendron	China	ISSR, RAPD	Four populations exhibited 89.7 % polymorphism and 0.33–0.37 Shannon's index. There was no genetic differentiation and variation among populations was low (10.6 %) due to high rates of gene flow	Sheg et al. (2004)
Heliotropium indicum	India	ISSR	In five wild populations 34 loci displayed 79.1 % polymorphism, 1.8 observed and 1.5 effective number of alleles, 0.43 Shannon's index, 0.29 estimated heterozygosity, 0.19–0.50 genetic distance between and 0.60–0.82 genetic similarity within populations	Kumar and Britto (2011)
Hippophae spp. (Sea buckthorn)	Different countries	Isozymes, RAPD, AFLP, ISSR, cpDNA, ITS	Genetic diversity high among populations, origins, and subspecies	Cheng et al. (2007)

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Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Hydrastis canadensis (Golden seal)	USA	RAPD	Cultivated and wild plants collected from different regions revealed genetic diversity within and among populations but not between cultivated and wild plants	Kerry (2009)
Hypericum perforatum (St. John's wort)	Croatia	RAPD	109 samples collected from eight locations recorded 0.12–0.31 Nei's gene diversity. Two populations had low and high genetic diversity. One population was polymorphic due to outcrossing. High fixation index (0.33) indicated low gene flow due to apomixes	Pilepic et al. (2008)
Launaea arborescens	China	Microsatellites	10 polymorphic (2–6 alleles/locus) and 9 monomorphic microsatellite loci recorded 0.00–0.83 observed and 0.06–0.71 expected heterozygosities	Li et al. (2012)
Lepidium sativum	India	RAPD	18 genotypes displayed polymorphism with 23–66 % genetic relatedness.	Bansal et al. (2012)
Justica adhatoda	Pakistan	PBA, chemical	Genetic diversity was high (90 %) within populations due to absence of genetic drift than among populations (10 %) though habitats were fragmented with limited number of plants. Chemical variation was higher among populations	Gilani et al. (2011)
Manilkara hexandra, Averrhoa carambola	India	RAPD	25 accessions of the species produced six highly polymorphic bands and exhibited genetic variation at amplicon level. Both the species are related indicating ancestral linkage	Goraniya et al. (2013)
Melissa officinalis (Lemon balm)	Iran, Germany, Japan	Morphoagronomic, chemical	Nine populations from Iran and one each from Germany and Japan revealed significant variation in morphoagronomic traits. Phenotypic diversity was not associated with geographical region in Iranian populations. Phenotypic variation was high among populations of different countries. German population displayed low diversity	Aharizad et al. (2012)

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Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Mentha aquatica	Iran	RAPD	51 wild populations exhibited 93.9 % polymorphism, 0.21–0.79 similarity coefficients and were grouped into 13 clusters. RAPD effectively distinguished populations, estimated genetic variation and distance	Kazemi and Hajizadeh (2012)
Mentha cervina	Iberian Peninsula	ISSR	18 populations exhibited 14.2–58.3 % polymorphic loci, 0.14–0.21 Nei's diversity, and 0.08–0.33 Shannon's index; high species diversity (98.3 %, 0.33 and 0.23, respectively) with 50 % variation within and 44 % among populations and 6 % between regions. Genetic differentiation coefficient was 0.53. Maximum number of populations needs to be conserved	Rodrigues et al. (2013)
Mucuna monosperma, M. atropurpurea, M. gigantea, M. bracteata, M. pruriens	India	AFLP	25 accessions of five species collected from seven provinces displayed high polymorphism. Genetic diversity was high in *M. pruriens* germplasm. *M. pruriens* var. *pruriens,* and var. *hirsuta* were closely related	Sathyanarayana et al. (2011)
Morinda citrifolia, M. tinctoria, M. pubescens	India	RAPD, ISSR	22 accessions collected from four regions showed polymorphism. Two accessions were closely related (0.94 similarity index) and two were distantly related (0.25–0.39 similarity index). Both markers were effective	Singh et al. (2011)
Myrtus communis	Tunisia	Isozymes RAPD	Six populations from three climatic zones disclosed high genetic diversity within populations, in populations of subhumid zone, and high differentiation among populations in relation to climatic zones and geographical distance	Messaoud et al. (2007)
Nothofagus nervosa, N. oblique, N. dombeyi	Chile	RAPD, ISSR	125 trees of three species recorded 80 and 97 % polymorphism with the two markers. The trees were grouped into three clusters	Mattioni et al. (2002)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Ocimum basilicum, O. americanum, O. polystachyon, O. viride, O. gratissimum, O. sanctum	India	RAPD, SSR, ISSR	All markers recorded 100 % polymorphism in 41–329 loci. *O. basilicum* and *O. polystachyon* registered close similarity indexes and *O. viride* and *O. americanum* least similarity index. They were divided into two clusters	Lal et al. (2012)
O. gratissimum	Kenya	AFLP	139 samples from all provinces exhibited polymorphism with central Kenyan population recording highest genetic diversity. Genetic differentiation coefficient was 0.29. 71 % of variability was within populations. More plants need to be selected from few populations for conservation	Matasyoh et al. (2011)
Oroxylum indicum	India	RAPD	Accessions collected from eight locations indicated high similarity (87 %) with 49.6 % polymorphism	Jayaram and Prasad (2008)
Panax quinquefolius	USA	Allozymes	In 21 wild populations expected heterozygosity values were higher in protected populations (0.08) than in those permitted to be harvested (0.07). Coefficient of genetic differentiation was greater in unprotected (0.49) relative to protected (0.17) populations. Juvenile plants (0.07) had lower heterozygosity relative to reproductive plants (0.08)	Sanders and Hamrick (2004)
Paris polyphylla var. chinensis	China	Microsatellites	30 plants from a natural population possessed 2–5 alleles/locus with 0.00–0.47 observed and 0.38–0.66 expected heterozygosities	Zheng et al. (2012)
Podophyllum hexandrum	India	RAPD, chemical	12 accessions displayed high degree of genetic diversity. Chemical markers identified new chemotype	Sultan et al. (2010)
Phlomis olivieri, P. anisodonta, P. bruguieri, P. persica	Iran	RAPD, biochemical	*P. olivieri* plants exhibited genetic distances. This species was closely related to *P. anisodonta* and *P. persica*. Verbacoside content varied in species and was influenced by geographical locations and growing conditions	Sarkhail et al. (2014)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Phyllanthus emblica	India	Isozymes	Four populations exhibited genetic diversity	Shaanker and Ganeshaiah (1997)
Phyllanthus emblica	India	Microsatellites	Two populations displayed polymorphism and heterozygosities of 0.36–0.76 (observed) and 0.49–0.81 (expected). Genetic resources depleted due to overexploitation and fragmentation	Pandey and Changtragoon (2012)
Podophyllum hexandrum (Indian may apple)	India	RAPD, biochemical	28 genotypes from 11 geographical regions exhibited 92.4 % polymorphism, 0.50 Shannon's information index, 0.69 mean coefficient of gene differentiation with limited gene flow (0.22). Variation among groups was 53 %, among genotypes 47 %, and within genotypes 33.8 %. Podophyllotoxin content varied in the genotypes and was positively correlated to altitude and regional ecological conditions	Alam et al. (2009)
Primula ovalifolia	China	Chemical, ISSR	Three chemotypes were recognized in five populations. Chemical and molecular markers yielded similar results	Nan et al. (2003)
Primula veris	Poland	Enzymes	Three natural (500–1200 plants) and three cultivated populations were evaluated. 1–2 loci were polymorphic with 2–3 alleles. Cultivated populations were more polymorphic. Heterozygosity was low (0.03–0.06)	Morozowska and Krzakowa (2003)
Rauvolfia serpentina	India	RAPD	Inter- and intrapopulation diversity was evident with 70 % polymorphism. Accessions were divided into two clusters	Padmalatha and Prasad (2007)
Rauvolfia tetraphylla	India	RAPD	Plants from five locations had 0.08–0.35 genetic distance between populations, 0.70–0.93 genetic identity, and Nei's gene diversity of 0.20	Mahesh et al. (2008)
Satureja bachtiarica	Iran	RAPD, ISSR	46 plants of five populations recorded 95.9–98 % polymorphism. 79 % genetic variation was within and 21 % among populations	Saidi et al. (2013)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Retama raetam	Tunisia	RAPD	Three populations collected from different habitats recorded 68 % variation within populations with significant differentiation among them. Genetic distance varied from 0.10 to 0.51 and gene flow from 0.49 to 4.69	Abdelloui et al. (2014)
Rheum officinale	China	ISSR	12 populations revealed low population (28.9 %) and high species (95.2 %) genetic diversity. Genetic variation among populations was 74.4 % with limited gene flow (0.28). Genetic and geographic distances were positively correlated signifying the role of geographic isolation in shaping the population genetic structure. Conservation should aim at preserving all existing populations	Wang et al. (2012)
Rheum tanguticum	Tibet	ITS	Mean intraspecific distance was 0.13 with rich variation. 87 clones were closely related to *R. rhabarbarum*, 10 clones to *R. officinale* and 5 clones to *Fagopyrum esculentum*. *In situ* conservation was suggested	Ma et al. (2014)
Rhodiola dumulosa	China	AFLP	1089 individuals within 35 populations revealed high genetic diversity that decreased with increasing altitude. Closely related populations occurred in close proximity with significant gene flow. Two gene pools were identified. Gene diversity ranged from 0.20 to 0.22. Gene flow of distantly distributed populations was low	Yan and Anru (2011)
Rhodiola rosea (Roseroot)	Greenland Sweden, Faroe Islands	SSR, ISSR	91 samples recorded 83.8 % polymorphism for Sweden, 94.6 % for Greenland, and 48.7 % for Faroe Islands	Kylin (2010)
Rhodiola rosea	Russia, Kazakhstan	SSR, ISSR	Four geographically distant populations registered 85.9 % polymorphism (6.25 alleles/locus) with high species genetic diversity (gene diversity = 0.33, Shannon's index = 0.48) but low population diversity. Observed and expected heterozygosities ranged from 0.4 to 1.0 and 0.47 to 0.84, respectively	György et al. (2012)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Semecarpus kurzi	India	RAPD	Plants collected from 12 areas displayed 86.8 % polymorphism and were grouped into three clusters. Genetic distances were low	Das and Mandal (2013)
Swertia chirayita	Nepal	RAPD	40 accessions from five populations registered 92.3 % polymorphism. Genetic distance varied from 33 to 68 % among populations	Shrestha et al. (2013)
Rhodiola rosea	India	RAPD, CAPS	RAPD markers produced 12 polymorphic loci in 30 genotypes from three regions while CAPS markers failed to do so. They were classified into three clusters. Genetic differentiation was considerably low with significant gene flow through seed dispersal	Soni et al. (2010)
Rhododendron species	China	RAPD	43 samples of 49 species belonging to three subgenera revealed 98 % polymorphism. Genetic similarity coefficient ranged from 0.26 to 0.91. Species of three subgenera could be distinguished with morphology and RAPD markers	Zhou et al. (2008)
Salvadora oleoides	India	Allozymes	500 plants of 11 populations exhibited high genetic diversity. Coefficient of genetic differentiation among populations was 0.02. Genetic similarities between population pairs were high (0.98)	Saini and Yadav (2013)
Salvadora persica	Egypt	AFLP, RAPD	Six wild populations of different areas exhibited 58–65 % polymorphism. Genetic distances and distances of collection sites were not correlated. Some individuals were different in spite of closeness of locations	Hegazi et al. (2011)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Salvia miltiorrhiza	China	ITS nrDNA	Seven accessions of *S. miltiorrhiza* and 28 of other taxa revealed high genetic diversity. The taxa were classified into three clusters and one clade (three accessions). *S. bowleyana*, *S. yunnanensis*, *S. cavaleriei* var. *simplicifolia* are potential gene germplasm resources for *S. miltiorrhiza*	Zhang et al. (2012)
Sargentodoxa cuneata	China	RAPD	Three populations from three altitudes registered 84.1 % total polymorphism. Decrease in altitude reduced percentage polymorphic loci and increased similarity index. Genetic variation within and among populations was 43.7 and 56.3 %, respectively. Genetic differentiation was 0.54 and gene flow was low (0.40)	Li et al. (2004)
Scutellaria baicalensis	China	cpDNA	28 wild and 22 cultivated populations recorded similar genetic variation. Genetic differentiation of cultivated (0.22) was less then wild (0.70) populations. Genetic structure of wild populations was influenced by geographical distances	Qing et al. (2010)
Taxus brevifolia (Pacific yew)	Canada	RAPD	RAPD markers that revealed polymorphic loci were developed using 39 haploid megagametophytes from a single mother tree	Göçmen et al. (1996)
Thuja sutchuenensis	China	ISSR	Seven populations recorded 76.1 % polymorphism, 0.16 gene diversity, 0.25 Shannon's index in population and 0.17, 0.30 respectively, at species levels; low genetic differentiation (0.10), high gene flow (4.41) and were clustered into two classes. Genetic and geographical distances were not correlated	Liu et al. (2013)
Tinospora cordifolia	India	SCoT	21 accessions collected from two provinces recorded 87.0 % polymorphism and were grouped into two clusters. Genetic similarity varied from 1.0 to 0.68 with two accessions having 100 % similarity	Paliwal et al. (2013)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Tylophora hirsuta, Wattakaka volubilis, Cryptolepis buchananii	Pakistan	RAPD	Intra- and interspecific diversity was low with 24.2 % polymorphism. The three species are closely related	Tariq et al. (2014)
Utrica parviflora	India	RAPD	Plants collected from different altitudes of Kumaun hills displayed 68.8 % polymorphism and were grouped into two clusters	Chirag et al. (2011)
Vaccinium macrocarpon (American cranberry)	USA	RAPD	A gradient of molecular diversity was found between central and marginal populations	Stewart and Excoffier (1996)
Vitex rotundifolia	China	ISSR	135 plants from 14 populations displayed 0.19 overall genetic diversity with 40 % variation within populations. Genetic differentiation among populations was relatively high (0.59) with limited gene flow	Hu et al. (2008)
Warburgia ugandensis	Kenya	AFLP	Trees within populations had higher genetic variation (59 %) than among populations (41 %). Genetic and geographic distances were not related among populations	Muchugi et al. (2012)
Withania somnifera	India	RAPD	Five accessions collected from a province registered 0.24–0.48 genetic distance, 0.62–0.78 genetic identity, 0.25 Nei's genetic diversity with 1.4–1.6 alleles/locus. One accession showed 83.8 % polymorphism	Dharmar and Britto (2011)
Withania somnifera	India	RAPD	Five accessions collected from a province registered 0.24–0.48 genetic distance, 0.62–0.78 genetic identity, 0.25 Nei's genetic diversity with 1.4–1.6 alleles/locus. One accession showed 83.8 % polymorphism	Dharmar and Britto (2011)

(continued)

Table 11.1 (continued)

Botanical name	Country	Method	Genetic diversity	Reference
Withania somnifera	India	RAPD	Five accessions collected from different sites of a province registered 0.24–0.48 genetic distance, 0.62–0.78 genetic identity, 0.25 Nei's genetic diversity with 1.4–1.6 alleles/locus. One accession showed 83.8 % polymorphism	Dharmar and Britto (2011)
Withania somnifera	India	Morphoagronomic, RAPD	30 genotypes collected from different provinces displayed high phenotypic variation in morphoagronomic parameters. Root yield was correlated with plant height. They recorded 87.3 % polymorphism, 0.18–0.90 genetic similarity. They could not be grouped into clusters	Khatak et al. (2013)
Withania somnifera	India	RAPD, ISSR	16 accessions collected from different locations showed 74.5 and 81.1 % polymorphism. They were grouped into two clusters. Similarity coefficient ranged from 0.42 to 0.94 (ISSR) and 0.44–0.94 (RAPD). Both markers differentiated the genotypes	Tripathi et al. (2012)
Withania somnifera, W. coagulans	India	AFLP	35 plants of *W. somnifera* and 5 of *W. coagulans* were classified into two clusters with similarity coefficient of 0.3 and high levels of polymorphism. Three morphotypes were recognized from plants sourced from different regions with low genetic variation within populations	Negi et al. (2000)
Withania somnifera, Rauvolfia serpentina	India	RAPD	Plants collected from seven locations of a province exhibited 92.3 % polymorphism in *W. somnifera* and 52.9 % in *R. serpentina*. The plants were classified into two clusters each	Sairkar et al. (2013)

RAPD Random amplification of polymorphic DNA, *AFLP* Amplified fragment length polymorphism, *RFLP* Restriction fragment length polymorphism, *SSR* Simple sequence repeat or microsatellite, *ISSR* Intersimple sequence repeat, *SCoT* Start codon targeted, *cpDNA* Cytoplasmic deoxyribonucleic acid, *ITS nrDNA* Internal transcribed spacer region of nuclear ribosomal DNA, *CAPS* Cleaved amplified polymorphic sequence, *SRAP* Sequence related amplified polymorphism, *PBA* P450-based analog

11.4 Genetic Erosion and Its Consequences

Habitat destruction, degradation, fragmentation or conversion for agriculture, ranching, horticulture, mining, ecotourism, industry, population fragmentation, commercial over-harvesting to satisfy urban and export demands, overgrazing (out of 11,146 MPs more than 3000 are facing genetic erosion in China; Guo et al. 2009), competing uses such as logging of medicinal trees for building material, fuel, paper, dyes are some of the human exerted pressures on native populations, their biology, and potential to respond to environmental shifts leading to dwindling population sizes (for many MPs, population size is directly related to genetic diversity), population densities, diminished fitness, enhanced isolation, genetic erosion, and species extinction. Population fragmentation, isolation, and decreased population densities/sizes force inbreeding within sites modifying patterns of gene exchange, pollen and seed movement between fragmented populations leading to genetic erosion. For wild-collected MPs the impact of over-harvesting depends on the part collected, biology, range, distribution, and economic value. Populations may disappear rapidly due to overcollection than from fragmentation or habitat destruction (Sanders and Hamrick 2004; Sanders et al. 2005). In crop improvement programs selecting and breeding MPs for a character under genetic control increases the frequency of specific alleles within population. Recurrent selection for that character in each breeding cycle disrupts the equilibrium among evolution forces and results in losing gene pools (Han et al. 2007). Chinese *Scutellaria baicalensis* suffered decline of wild populations during the past few decades. To sustain supplies large-scale cultivation was initiated. Cultivated populations experienced loss of 10 out of 25 identified alleles and became increasingly homozygous. Preserving wild populations therefore, is imperative (Quing et al. 2010). American ginseng (*Panax quinquefolius*) roots are extensively collected in the United States for sale as herbal panacea. Wild roots are regarded as more potent and valuable than cultivated roots. Harvest pressure declined wild populations to below minimum viable sizes accelerating species extinction (Sanders and Hamrick 2004). A simulation program on *P. quinquefolius* revealed that random harvests resulted in significant genetic erosion, especially its allelic wealth relative to initial levels. Harvesting fewer mature plants was suggested to minimize negative effects (Sanders et al. 2005). Such computer simulations based on remote sensing and ground data can help conserve critically endangered species. Demand and price are high for wild-gathered ginseng (*Panax ginseng*) roots considered stronger than cultivated roots. Illegal harvests of young plants wiped out wild populations from Asia (Behrens 2014). South Indian forests are treasure houses for costly sandalwood (*Santalum album*) and red sandalwood (*Pterocarpus santalinus*) trees. Illegal felling and smuggling wiped out large number of trees from several locations. In Brazil, leaves of *Lychnophora ericoides* are used for anti-inflammation. Human interference declined population density to 0.16 individuals/m^2 and put it at 73 % risk of genetic erosion (Almeida et al. 2012). *Ficus insipida* latex is used to treat intestinal parasites, as beer's chillproofing agent,

meat tenderizer, and for digestion. Instead of tapping the trees for latex whole trees were indiscriminately felled drastically bringing down their numbers and increasing intestinal parasites in ethnic communities of Peru. Sustainable harvests should hence take into account the relationship between MPs and health needs of indigenous people (Behrens 2014). *E. officinalis* fruits rich in vitamin-C are plucked and traded by tribal communities of India. Of late, the tribal youth are axing trees for fruits inflicting genetic erosion (Rajeswara Rao 2012). In Côte d'Ivoire vines of *Griffonia simplicifolia*, trees of *Voacanga africana* and *V. thouarsii* are chopped down to gather fruits. In Chile woody branches of *Haplopappus taeda* (aids digestion) were cut to the ground level destroyng the shrubs. Extract of devil's claw (*Harpagophytum procumbens*) aids in treating rheumatic disorders. Erratic collections severely reduced wild population size and genetic diversity in South Africa, Botswana, and Namibia. Bark of *P. africana* is employed for curing prostatic hyperplasia. Excessive and destructive stripping of 59,000–90,000 trees/annum from African mountains and highlands led to near disappearance of wild trees from Mount Oku forest in Cameroon. The leaves of bearberry (*Arctostaphylos uva-ursi*) are medicinally useful. Uprooting whole plants disturbed other plants, inhibited regrowth, created space for other plants to takeover and caused soil erosion, landslides, and death of innocent people in Pohnpei in the South Pacific. Kava kava (*Piper methysticum*) populations inhabiting these mountains were uprooted to meet increasing demand instead of harvesting branches and leaves for anxiolytic kavalactones. This led to soil erosion. Over-harvesting of *Arnica montana* wild populations created space for the growth of *Rhododendron* plants in their place. Thus, faulty harvesting methods led to genetic erosion and socioenvironmental problems with a cascading effect on the biodiversity and ecology of the region (Behrens 2014). Orchid *Nervilia fordii* is known for its febrifuge and antitussive properties. The plant produces only one leaf/year. Being an export commodity, 7–8 tons of whole bulbs were dug every year diminishing wild populations and making it an endangered species (Heywood 1999). In China *Dendrobium* is rarely found in the wild. Chinese wild *Panax notoginseng* is believed to be extinct in the wild due to overcollection (Liu et al. 2011). Fortunately, cultivated *P. notoginseng* retained reasonable level of genetic diversity (Guo et al. 2009). In India *Gnidia glauca* var. *sisparensi,* a medicinal tree used in *Ayurveda* is believed to be extinct in the wild. Recently, three trees were found in the Western Ghats after 148 years. Overexploitation, unsustainable harvests, and population fragmentation severely depleted genetic diversity of *Phyllanthus emblica* (syn. *E. officinalis*) in India (Rajeswara Rao 2012; Singh et al. 2012). Forest-dwelling communities and rural people in forest fringe areas depend on the trade of fruits for their livelihoods. Loss of genetic resources adversely impacts their income. Chinese and Asian *Blumea balsamifera* yields borneol, a widely used phytopharmaceutical. Chinese wild resources have diminished at a rapid rate during recent years limiting supplies and endangering it (Pang et al. 2014). Populations of several Chinese MPs namely, *Acanthopanax senticosus*, *Asarum heterotropoides* var. *mandshuricum*, *A. lancea*, *Bupleurum chinense*, *Cistanche deserticola*, *Dioscorea zingiberensis*, *Ephedra sinica*, *Eucommia ulmoides*,

Gastrodia elata, *Glycyrrhiza uralensis*, *Magnolia officinalis*, *Notoptetygium incisurn*, *Phellodendron chinense,* and *Swertia milensis* have declined due to over-exploitation. Several African and Indian species were rendered endangered through commercial harvesting. People living in Indian Thar desert depend on its fragile natural resources. *Calligonum polygonoides,* a perennial shrub is food for people and animals. Flower buds are effective in countering negative effects of sunstroke. Root decoction as a gargle cures sour gums. Aqueous extract is used as an antidote against poisonous effects of plants and opium. Human activities diminished populations of *C. polygonoides* at an alarming rate affecting their genetic composition and diversity in Bikaner province (Vyas et al. 2012). *Carapichea* (*Cephaelis*) *ipecacuanha* (ipecac) roots are known for their emetic, nauseant, expectorant, and diaphoretic properties. Plants are being commercially harvested in Brazil since eighteenth century. Deforestation, habitat fragmentation, and uncontrolled harvesting without replanting declined wild populations. In spite of limited cultivation in India, world demand is met through wild-gathering eroding its diversity and gene pool (de Oliveira et al. 2010). *Changium smyrnioides* is an endangered medicinal plant endemic to eastern China. Medicines of this plant quench thirst, moisten lungs, soothe the throat, and removes toxins that cause skin infections. Continuous wild collections constricted the size of natural populations and made them rare (Fu et al. 2003). Chinese *Fritillaria cirrhosa* bulbs are used as antitussive, expectorant, and hypotensive agent. Owing to its strict habitat needs, domestication and cultivation are difficult. Over-harvesting, habitat fragmentation, and overgrazing during the past decades decreased population sizes and their genetic diversity pushing it to the brink of extinction (Zhang et al. 2010). *Epimedium* species are used in traditional Chinese medicines. Commercial overexploitation relegated some of the species to endangered status (Zeng et al. 2010). Roots and leaves of Malabar nut *Justica adhatoda* (syn. *Adhatoda vasica*) are employed in treating bronchitis, asthma, fever, and jaundice in traditional medicine systems in the Indian subcontinent. The plant grows under harsh conditions in Pakistan. Over-harvesting to satisfy domestic and commercial needs and habitat loss fragmented and imperiled populations (Gilani et al. 2011). Rhizomes of *Paris polyphylla* var. *chinensis* are used in Chinese medicines for treating hemostasis, protitis, and snakebite. This plant has been on the verge of extinction due to severe deforestation, small population sizes, inbreeding and absence of alleles at some loci (Zheng et al. 2012). *Swertia chirayita* is a commercial medicinal plant of Nepal. Overexploitation to meet high trade demand depleted wild populations beyond their regeneration capacities losing genetic diversity and gene pool (Shreshta et al. 2013). Traditional healers use bark of *Warburgia ugandensis* against malaria, constipation, cough, candidiasis, and as skin cream. Overuse of the bark, the root, and indiscriminate tree felling for timber/wood over many decades wiped out populations in many African regions causing loss of genetic diversity (Muchugi et al. 2012). *Rhodiola dumulosa* population is fragmented across northern, central, and northwestern China. Two distinct gene pools were discovered, one in northern and the other in central and northwestern China with restricted gene flow among these populations. Conservation schemes should

include samples containing both the gene pools to avoid genetic erosion (Yan and Anru 2011). *A. montana* is a poisonous medicinal plant endemic to Europe and is protected by European laws. In the absence of cultivation, unauthorized, illegal wild collections and overgrazing turned it into an endangered plant in Romania (Pop et al. 2008). Red lists of IUCN and different countries and CITES appendixes cite numerous MPs with varying levels of threat to their survival. It is not known how much genetic diversity or how many gene pools have been lost. The big question is can we protect the existing genetic diversity without inflicting further genetic erosion?

11.5 Influence of Loss or Fragmentation of Habitats on Genetic Diversity and Genetic Erosion

Human interference disturbs the equilibrium of evolutionary forces of selection, gene flow, mutation, genetic drift, inbreeding affecting adaptive capacities of species. The consequences of human atrocities such as fragmentation, degradation, or destruction of forests or their conversion for other uses result in irreplaceable loss of species, genetic, and ecosystem diversity as reforestation programs concentrate on timber/wood or commercial species. An overlooked problem of habitat fragmentation is the proliferation of other species spacing out MPs. Human introduced commercial species replace native MPs quickly depleting genetic diversity. In India forest bamboo plantations and invasive weeds *Lantana camara*, *Parthenium hysterophorus*, *Hyptis suaveolens,* etc. replaced native species. Exotic blue pine *Pinus wallichiana* has edged out local white oak and medicinal herb *Lilium polyphyllum* in Shimla. Logging and timber/rubber tree plantations destroyed large tracts of Amazon rain forests. Forest destruction leads to loss of microflora and fauna adversely affecting soil fertility (loss of organic matter, nutrients, and beneficial microorganisms) consequently limiting plant germination, growth, and survival. Loss of trees and shrubs that support climbing/trailing species, parasitic plants (e.g., *C. deserticola* on *Haloxylon persicum/ammodendron*), shade-loving species, beneficial flora and fauna poses problems for the survival of these species. Exposure of denuded forests to sun light, winds, heavy rains cause moisture and organic matter losses, loss of soil productivity, and lead to soil erosion and landslides. During 2000–2010, 13 million hectares/year of forests were lost (in comparison to >16 million hectares/year during 1990–2000) due to deforestation and natural disasters out of which primary forests accounted for 4.188 million hectares/year. Even after taking into account natural regeneration, afforestation, and reforestation, the net loss was 5.211 million hectares/year (i.e., the world lost 0.13 % of existing forests/year). Forest fires and insect pests and diseases damaged 1 and 2 % of forests, respectively (FAO 2010). Though FAO (2010) stated that US$ 628 million worth medicinal and aromatic plants (MAP) were collected as part of nonwood forest products, no details were given on the species collected or loss of species. Studies in Brazil and Peru showed that nonwood forest products

yielded higher net returns/hectare than timber and were harvested with less damage to the ecosystem. Conservation International identified 34 biodiversity hot spots with high levels of species endemism (>1500 at each hot spot) and frightening levels of biodiversity depletion (70 % original habitat lost). Eight of them are in Africa, 13 in Asia Pacific, 4 in Europe and Central Asia, 5 in South America, and 4 in North and Central America (http://www.cepf.net/resources/hotspots/Pages/default.aspx. Viewed 17 June 2014). Two of them namely, Himalayas and Western Ghats are in India. Though these hot spots occupy only 2.3 % of Earth's surface, they are habitats for more than half of global endemic species, many of which are medicinally valuable. The frightening aspect is the increasing number of biodiversity hot spots (up from earlier 17) pointing to loss of species and genetic diversity. Walter and Gillett (1998) estimated that out of 49,000 plant species evaluated 34,000 species (8 % of global flora of 422,000) were threatened with extinction. Later, Bramwell (2003) enhanced it to 21 % of world flora. Based on these estimates, Schippmann et al. (2006) calculated that 21 % of 72,000 MPs, i.e., over 15,000 MPs are threatened globally. Edwards (2004) scaled down this number to 4000–10,000. In 2001, IUCN revised its criteria (version 3.1) for classifying plants into nine categories (http://www.iucnredlist.org/static/categories_criteria_3_1) such as extinct, extinct in the wild, critically endangered, endangered,

Table 11.2 Estimated number of plants, medicinal plants (MPs), and threatened MPs worldwide

Estimates	Number of species	Reference
Estimated number of plants	370,000–422,000	Schippmann et al. (2006) and Paton (2009)
Estimated number of MPs by WHO in 1970s	Over 21,000	Heywood (1999)
Estimated number of MPs	>35 000	Lewington (1993)
Estimated number of plants used ethnomedically	70 000–80 000 (>20,000 plants in NAPRALERT database)	Farnsworth and Soejarto (1991) and Heywood (1999)
Estimated number of MPs	72,000	Schippmann et al. (2006)
Estimated number of MPs	77,000	Rajeswara Rao et al. (2012)
Estimated number of MPs	80,000	Joy et al. (1998)
Estimated number of flowering plants of pharmacological value	125,000	Memdelsohn and Balick (1995)
Number of MPs threatened in 1997 (8 % of world flora)	5760–6160	Walter and Gillett (1998)
Number of MPs threatened in 2003 (21 % of global flora)	15,120–16,170	Bramwell (2003), Schippmann et al. (2006)
Number of MPs threatened in 2004	4000–10,000	Edwards (2004)
Number of MPs threatened in 2014 (2.5 % of world flora)	1800–1925	IUCN Red List of Threatened Species™ version 2014.1

WHO World Health Organization, *NAPRALERT* Natural Product Alert (http://www.napralert.org/), *IUCN* International Union for Conservation of Nature and Natural Resources

vulnerable, least concern, data deficient, and not evaluated. IUCN prepared Red List of Threatened Species™. In its version 2014.1, IUCN provided trends during the period 1996/1998–2014 (http://www.iucnredlist.org/). As per Table 3b: "Status category summary by major taxonomic group (plants)" 128 plant species are extinct, 104 are possibly extinct, 2000 are critically endangered, 3178 are endangered, 5205 are vulnerable (up to here 10,487 species or 2.5 % of global plant species are threatened + 128 are extinct), 1544 are nearly threatened, 210 at lower risk are conservation dependent, 5466 are of least concern, and for 1539 species' data are deficient (19,374 or 4.6 % of global species were comprehensively assessed). The details of threatened MPs are depicted in Table 11.2. In India 265 MPs, in Europe 150 MAP, in Croatia 17 MP, in Ukraine 202, in Estonia 16, in Finland 20 are threatened; in Malta 9 MPs are extinct and 34 are threatened; in Serbia 6 are extinct, 4 are thought to be extinct, and 24 species are critically endangered.

11.6 Effect of Wild Collections on Genetic Diversity and Genetic Erosion

Wild collection provides income and incentives for local communities for conservation and sustainable use of MPs resources. Wild collection for healthcare needs of indigenous people cause little damage to genetic diversity as the quantities collected are small. Commercial, destructive, or over-harvesting (low prices, un- or underemployment, lack of livelihood options force (majority women) collectors to mine rather than manage the resources; Lange 2006b; Schippmann et al. 2006) threaten MPs genetic diversity. Crude collection methods result in loss of yield, quality, and reduction in price. Habitat-specific, slow-growing, popular MPs with narrow geographic distribution and small population sizes are susceptible to overharvesting and are at a greater risk of genetic erosion due to demand–supply mismatch relative to fast-growing, widely distributed species with high population densities, reproductive rates, and regenerative capacities (e.g., *Peumus boldus* trees). Endemic species are particularly at a greater risk due to their restricted habitat and small population sizes. Collection pressures differ among species (trees vs. herbs, slow vs. fast growing, perennials vs. annuals, vegetatively vs. reproductively propagated MPs). Overcollection of fruits or seeds of a tree causes minimum harm, while annual herbs will be wiped out from a location if all their seeds are collected. Slow-growing trees that produce few seeds are however, susceptible to genetic erosion (Schippmann et al. 2006). Harvesting branches, leaves, flowers, fruits, and seeds do not destroy MPs. Stripping bark, cutting wood or main stem, and digging underground parts kill them causing genetic erosion, e.g., *Aconitum ferox/heterophyllum/spicatum*, *Nardostachys jatamansi*, *Neopicrorhiza scrophulariiflora*, *P. ginseng/quinquefolius*, *Saussurea costus*, *Valeriana jatamansi*, *Warburgia salutaris,* etc. Majority of MPs in trade are not cultivated and most

material is forest gathered. The unscientific harvesting practices rapidly decline wild populations and accelerate their extinction (Sanders and Hamrick 2004; Sanders et al. 2005). Duke (1997) stated that human population pressure endangers species the most "the better a medicinal plant, the more it threatens itself." Further, overplaying (herbal hype) and intentional misrepresentation (herbal hoax) of claims of herbal medicines encourage over-harvesting. Since phytochemicals are widely distributed in the plant kingdom, he felt that alternate sources can be found in nature for threatened MPs and invasive weed MPs (*Hypericum perforatum* in western US) need to be contained rather than conserved. It is estimated that 70–90 % MPs and 50–70 % of their biomass traded internationally and regionally are wild-sourced (Edwards 2004; Balunas and Kinghorn 2005; Lange 2006a). About two-thirds of MPs were wild-procured (Edwards 2004). In Europe 90 % of over 1300 MPs were wild-harvested (Balunas and Kinghorn 2005). In China 60–80 % of 700,000 tons of MPs were used in 1990s and 80 % of the species were wild-gathered, in the United States 90 % herbs were wild-sourced, and in Germany 70–90 % of 1560 species traded were wild-harvested in Africa, America, Asia, and Europe (Heywood 1999). In Hungary 30–35 % (10,000–15,000 tons dry phytomass of 120–130 MPs), Spain 50 %, Ecuador 90 %, Albania 90–100 % MPs and in Romania 11,300 tons were wild-collected. In India 77 % MPs were wild collected (12 % from temperate forests, 40 % tropical forests, and 25 % roadsides; Ved and Goraya 2008), 72 % of them in a destructive manner. The scenario is the same in other countries. In addition to regulating wild collections, certification is being insisted (FairWild Standard version 2.0 for wild-collected plants, fungi, and lichen; http://www.fairwild.org/standard) for quality control, to discourage illegal collections, to ensure fair, ethical trade practices, and for social accountability (International Fair Trade Association http://www.ifat.org; Social Accountability International http://www.sa-intl.org; Fair Trade Labeling Organization International http://www.fairtarde.net). WHO (2003) outlined strategies and techniques for small and large-scale collection to ensure long-term survival of wild populations and their habitats. WHO pointed out that collection is associated with geographical, economical, sociocultural, environmental, and business issues that varies from region to region and have to be tackled locally. WHO stressed on the quality of wild-collected material avoiding contamination by men/women and machines. The strategies were given under five subheads emphasizing on correct identification (confusion arises due to common local names for different species, e.g., Punarnava for *Boerhaavia diffusa* and *Trianthema portulacastrum*; Sankhapushpi for *C. ternatea*, *Convolvulus microphyllus,* and *Evolvulus alsinoides* in India. Computer databases and traditional herbaria help in identification and authentication), inventorying population densities of targeted MPs for exempting threatened species from collection, preparing management plans for correct collection practices (sustainable, e.g., Hambledon Herbs, UK, sourced sustainably wild-harvested *H. procumbens* from Namibia through Oxfam) to encourage regeneration of source material, best time of collection to ensure quality and quantity of active constituents, avoiding polluted areas or collection of contaminated

MPs, protecting collected material from postharvest contamination (improper drying causes fungal contamination), ensuring proper storage (avoid pest contamination, phytochemical content degradation), transport, hygiene, and safety of the personnel. Subsequently, Medicinal Plant Specialist Group (2007) of the Species Survival Commission of IUCN published international standard (version 1.0) for sustainable wild collection of MAP containing six principles and 18 criteria which are briefly discussed. 1. Maintaining wild MAP resources (three criteria: conservation status of targeted species is to be periodically evaluated and reviewed as per IUCN version 3.1; collections should be monitored based on identification, inventory, and assessment discouraging collection of threatened species, minimizing waste collections; and collection intensity should match species' regeneration capacities). 2. Preventing negative environment impacts on other wild species, habitats, and surrounding areas (two criteria: protection of sensitive taxa, their habitats and ecosystem diversity; and services). 3. Complying with laws, regulations, and agreements (two criteria: tenure collection rights to be issued to authorized collectors; local, national, and international laws on collection and management should be strictly adhered to). 4. Respecting customary rights of ethnic communities and indigenous people to utilize and manage collection sites (two criteria: access rights, traditional use, and cultural heritage of ethnic communities are to be recognized and respected; benefits accruing from the use of wild-collected MAP should be shared with these people). 5. Applying responsible management practices (four criteria: management plans are to be drawn for sustainable collection, to maintain quality and prevent biotic and abiotic contamination and to conserve habitats; the impacts of collection are to be assessed and recorded; collection activities should be transparent with stakeholder participation; collection methods, storage, transportation, etc. should be documented). 6. Applying responsible business practices to support quality, financial, and workers needs of the trade without compromising on resource sustainability (five criteria: species with no market value should not be collected and collected species should conform to quality specifications of buyers; traceability of collected material should be ensured through proper labeling and certification concerning origin, collection site, year/time of collection, etc.; financial viability of collection, conservation of species and habitats, and management of resources should be ensured; collectors and managers should be trained for sustainable collections and to comply with this standard, national, and international laws; health and safety of collectors and managers should be safeguarded with adequate compensation). Several countries have complied and passed legislations for assuring quality and stopping illegal collections. In India Girijan (Tribal) Cooperative Corporations are permitted to purchase forest products from tribal collectors and market them. In Andhra Pradesh province the Corporation is permitted to collect about 35 MPs from forests. In spite of the efforts of governments and international organizations, irrational and illegal wild collections continue threatening genetic diversity and causing genetic erosion.

11.7 Impact of National and International Trade on Genetic Diversity and Genetic Erosion

Rapid urbanization and opening up of urban markets for traditional herbs placed large demand for MPs. In the old world countries MPs are used for warding off evil spirits/enemies/jealousy/competition; for good luck, blackmagic, attracting/retaining partners; as aphrodisiacs, fish/animal poisons, dyes, etc. enhancing market requirement and value. MPs are traded within countries, across nations within a continent, and exported across the world. From collectors/cultivators the material passes through complex trade channels before it is used or exported. Hong Kong, Tokyo, New York, and Hamburg are important trading centers (Lubbe and Verpoorte 2011). It is difficult to distinguish between wild-collected and cultivated materials. Correct market data and trends are scarce (Schippmann et al. 2006) and are difficult to ascertain as MPs are traded in vast array of products. Global trade data (http://comtrade.un.org/db/default.aspx. Accessed 5 June 2014) sourced from UN Comtrade database from HS (Harmonized Commodity Description and Coding System) 1992 classification and commodity 1211 ["plants and parts of plants (including seeds and fruits), of a kind used primarily in perfumery, in pharmacy or for insecticidal, fungicidal or similar purposes, fresh or dried, whether or not cut, crushed or powdered"] are presented in Table 11.3. The annual international trade is in excess of 500,000 tons during 2008–2012 which is higher than earlier figures (1991–2003: global annual average exports were 467,000 tons valued at US$ 1.2 billion with 12 countries making up ca 80 % of exports and imports; Lange 2006b) and the value of imports and exports are consistently increasing. Data for 2013 is incomplete as data for China, Hong Kong, and other countries are not available. The number of importing countries is more than exporting countries. The value of imports has risen by 30.2 % and exports by 42.1 % between 2008 and 2012. As a result unit prices of MPs have increased substantially (more than double in some cases) in the exporting countries (Larsen 2011). Poor, unskilled, unemployed, or low-wage earning gatherers overexploit MPs to shore up their income

Table 11.3 Global imports and exports of perfumery and pharmacy plants and plant parts during 2008–2013 (UN Comtrade database for the years 2008–2013 in HS 1992 for commodity 1211)

Year	Imports		Exports		Number of importing (exporting) countries
	Quantity (000 tons)	Value (US$ million)	Quantity (000 tons)	Value (US$ million)	
2008	513.8	1966.8	524.9	1793.7	157 (139)
2009	527.6	1867.7	533.9	1782.1	155 (133)
2010	546.1	2124.1	519.9	2087.3	153 (133)
2011	527.4	2488.2	633.2	2467.7	147 (131)
2012	575.0	2560.1	547.7	2548.8	139 (121)
2013	371.4	1750.2	338.2	1497.4	81 (74)

UN United Nations, *HS* Harmonized Commodity Description and Coding System
US United States

in countries exporting unprocessed, wild-sourced MPs at cheaper prices (Lange 2006b; Schippmann et al. 2006). In terms of value Belgium, Canada, China, France, Germany, Hong Kong, India, Italy, Japan, Malaysia, Mexico, Netherlands, Republic of Korea, Singapore, the United Kingdom, and USA are the main importing countries. Belgium, Canada, Chile, China, Egypt, France, Hong Kong, Germany, India, Mexico, Morocco, Poland, Republic of Korea, Singapore, and USA are the major exporting countries. Major markets are in developed countries but bulk of botanicals is exported from developing nations as unprocessed, raw material yielding low profits. International demand is confined to few regions leading to overexploitation (Lange 2006b). Profits of exporting developing nations can be improved by exporting processed botanicals. Indian exports grew from 48,525 to 87,745 tons and US$ 106.3 to 207.8 million (95.5 % increase) during this period. Chinese exports increased from 188,249 to 227,038 tons and US$ 450.0 to 844.8 million (87.7 % enhancement). Data on local consumption for different countries are sparse. In China, 1–1.6 million tons of MPs are used in traditional Chinese medicines compared to earlier 700,000 tons (Heywood 1999; Liu et al., 2011). Germany's use in 1996 was around 40,000 tons, Bulgarian's requirement (60–70 % for exports, 30–40 % for domestic consumption) was 12,000–15,000 tons (70–80 % wild-collected, 20–30 % cultivated), Croatian 109 tons was wild-collected from 87 species in 2001, in Nepal over 15,000 tons were wild-harvested from 100 species, and in Poland 8000–10,000 tons (>50 % exported) of 200 wild MAP were collected. Indian domestic demand was pegged at 263,000 tons in 2005–2006 (Ved and Goraya 2008). In Ukraine, 1000 tons (60 % wild, 40 % cultivated) of 44 MPs are used in the domestic market (Minarchenko 2011). If the domestic consumption of all importing countries is taken into account, the total annual demand runs into several million tons of MPs. Assuming 60–70 % moisture content in the plant parts, actual wild collections are 2–3 times higher than their trade volumes as most of the raw material is traded as dry biomass. Around 3000 (others estimated 4000–6000) MPs are globally traded with larger number in national markets (Schippmann et al. 2006). A flourishing trade with consistently increasing demand has devastating consequences on wild-collected MPs, their genetic diversity, and gene pools. Some of the species traded in large volumes are: *Actaea racemosa* (*Cimicifuga racemosa*), *Allium sativum*, *Aloe ferox*, *A. vera* (*barbadensis*), *A. montana*, *Atropa belladonna*, *Carapichea* (*Cephaelis*) *ipecacuanha*, *Cassia senna*, *Centella asiatica*, *Echinacea purpurea*, *E. angustifolia*, *E. sinica*, *Ginkgo biloba*, *Glycyrrhiza glabra*, *Hippophae rhamnoides*, *Hydrastis canadensis*, *H. perforatum*, *Matricaria chamomilla* (*recutita*), *Melissa nettle*, *Oenothera biennis*, *P. africana*, *P. ginseng*, *P. quinquefolius*, *Papaver somniferum*, *Pelargonium sidoides*, *P. methysticum*, *P. psyllium*, *Sabal serrulata*, *Serenoa repens*, *Silybum marianum*, *S. chirayita*, *Tanacetum parthenium*, *Taxus wallichiana*, *T. brevifolia*, *T. chinensis*, *Ulmus rubra*, *Vaccinium macrocarpum*, *V. myrtillus*, *Valeriana officinalis*, *V. wallichii*.

MPs are exported as fresh or dried plants or parts (leaf, stem, bark, wood, bud, flower, fruit, berry, seed, root, rhizome, tuber, bulb, corm) cut into pieces, crushed, or powdered. They are used as culinary herb, powder, paste, juice, decoction/

infusion, extract/tincture, macerate, cooked or fermented, phytochemicals, and as formulations in herbal teas, health/herbal/sports drinks, TM, CAM, over-the-counter medicines, functional foods, pharmaceuticals, nutraceuticals, cosmeceuticals, medicine adjuncts, dietary supplements, aromatherapy, flavors, fragrances, herbal pesticides, etc. The economic significance of MPs products sourced from several web sites is detailed in Table 11.4. With burgeoning world population (>7 billion) consumption of these products continues to rise.

The escalating trade in regional and transnational markets acts as a driving force for over-harvesting and illegal wild collection of threatened species. TRAFFIC, the wildlife trade monitoring network (http://www.traffic.org/overview/) analyzes trade trends, patterns, and impacts on wild animals and plants to manage wildlife trade and maintain wildlife populations and ecosystems to meet human requirements. CITES regulates and monitors global trade in threatened

Table 11.4 Global economic significance of products containing medicinal plants and their derivatives

International trade	Value
International market for herbal medicines	US$ 60 billion in 2000 (WHO 2003), US$ 43 billion in OECD countries in 1985. US$ 8 billion in USA in 1980 estimated at US$ 11 billion in 1985 (Principe 1991). With 10 % growth rate the current market size is over US$ 140 billion
Global market for herbal teas (around 300 species used in USA, China)	US$ 100 million
World market for nutraceuticals	US$ 142 billion in 2011 and is expected to be US$ 205 billion by 2017
International market for cosmeceuticals	US$ 27 billion in 2010 (US market in 2004 US$ 12.4 billion)
International trade in functional foods	US$ 57 billion
Market for dietary supplements	US$ 21.3 billion in 2005
World market for aromatherapy	US$ 400 million
Natural products for animal care	US$ 1 billion in 2009
Global pharmaceutical market	US$ 965 billion in 2012 and is expected to reach US$ 1.2 trillion by 2016–2017. It takes 10–15 years and US$ 1.38 billion to develop a medicine or vaccine (http://www.ifpma.org/)
US market for energy, sports, and functional drinks	US$ 12.87 billion during 2004–2006
Chinese trade in herbal medicines, functional foods, herbal extracts, etc	US$ >40 billion in 2011 (Liu et al. 2011)
Germany's herbal market	US$ 12.7 billion in 1989
Brazil's botanical market	US$ 160 million in 2007
Western Europe's herbal trade	US$ 5 billion in 2003–04
Indian traditional medicines trade	£88 billion in 2005–06 (Ved and Goraya 2008)

WHO World Health Organization, *OECD* Organization for Economic Cooperation and Development, *USA* United States of America

species of animals and plants by educating customs officials and advising countries on banning commercial exports and imports of threatened species. CITES (http://www.cites.org/eng/disc/species.php) prepares species lists under three appendixes. Species can be added, deleted, or moved from one appendix to the other by Conference of the Parties to CBD (http://www.cbd.int/). Species threatened with extinction are listed in appendix 1. Out of 931 species and 47 subspecies of fauna and flora listed in this appendix, 301 (32.3 %) species belonging to 20 families and 4 (8.5 %) subspecies belonging to 2 families are plants. Appendix 2 lists species that may become extinct if neglected and are not protected. These species are permitted to be commercially traded provided they are legally acquired from sustainable sources. Out of 34,419 species and 11 subspecies in this appendix, 29,592 (74.4 %) species belonging to 46 families including 162 populations belong to the plant kingdom. Species whose trade is regulated by a country but require the cooperation of other nations to avert illegal trade are included in Appendix 3 on the request of that country. Out of 147 species, 13 subspecies, and 1 variety in this appendix, 12 (8.2 %) species including 2 populations and 1 variety belonging to 9 families are plants. Notwithstanding inclusion of a particular species in these appendixes, artificially grown or cultured plants or plantlets, hybrids developed by government or private agencies and cultivated species are permitted to be commercially traded. Several species of *Aloe*, *Cycas beddomei*, *Dendrobium cruentum*, *Saussurea costus* are some of the species listed in Appendix1. *Adonis vernalis*, *Aloe* species, *Aquilaria* species, *Cibotium barometz*, *Dalbergia* species, *Dionaea muscipula*, *Dioscorea deltoidea*, *Euphorbia* species, *Guaiacum* species, *Heydychium phillippinense*, *Hoodia* species, *H. canadensis*, *Nardostachys grandiflora*, *P. ginseng*, *P. quinquefolius*, *Picrorhiza kurrooa*, *P. hexandrum*, *P. africana*, *P. santalinus*, *Rauvolfia serpentina*, *Senna meridionalis*, *Swetenia* species, *Taxus* species are some of the species included in Appendix 2. *Dalbergia* species, *Gnetum montanum*, *Magnolia liliifera* var. *obovata* are some of the species shown in Appendix 3. These, IUCN and countries' red lists help in identifying MPs which need conservation, recovery, or cultivation.

11.8 Consequences of Climate Change on Genetic Diversity

Considering the major influence of environment on survival, growth, yield, and quality of MPs, climate change may impact ecosystem composition, function, population structure, dynamics and interspecific interactions. One of the consequences ascribed to climate change is the infestation of plants with virulent native or exotic species of insects and disease producing fungi, bacteria, or phytoplasma causing extensive damage to wild populations leading to loss of plants with valuable genes. The other change is the replacement of native plants with species more adapted to the modified climate and significant changes in growth, flowering, and reproductive capacities of native plants. Frequent or regular occurrence of forest

fires (natural, accidental, or deliberate) destroying local flora and fauna is attributed to rising temperatures and dry conditions. Not all MPs with valuable gene pools revive after a major fire disaster. Loss of organic matter and microbiome (rhizoshere microorganisms and microbial biomass) adversely affects subsequent growth, yield, and quality of MPs. Changes in rainfall and wind patterns, occurrence, and prevalence of drought and moisture stress induce long-lasting effects on MPs survival and distribution. Landslides, soil erosion destroy local flora eroding genetic diversity. The increase in temperatures adds competitive edge to species that thrive at higher temperatures and adversely impact growth and reproductive capacities of MPs that prefer lower temperatures. Higher temperatures influence litter decomposition and soil organic matter content (Veteläinen et al. 2007). Earthquakes, volcanic eruptions, glacier melting, floods, and tsunamis destroy vast tracts eroding genetic diversity.

11.9 Conservation of Genetic Resources and Genetic Diversity

The aims of conservation are preservation of genetic diversity and promotion of evolutionary processes. Conservation programs should be ecology-friendly and indigenous people-friendly. UNEP World Conservation Strategy defined conservation as (http://www.unep.org/geo/geo3/english/049.htm. Viewed July 20, 2014) "the management of the human use of the biosphere so that it may yield the greatest sustainable benefit to the present generations while maintaining its potential to meet the needs and aspirations of future generations." For many decades conservation of genetic resources of MPs was neglected. The impetus was given by the Chiang Mai declaration "Save the plants that saves lives" by health professionals who gathered at the WHO/IUCN/WWF international consultation on MPs conservation held at Chiang Mai, Thailand, from March 21 to 26, 1988. They called up on UN, its member states, government and nongovernment organizations for international cooperation and coordination for recognizing MPs importance in primary healthcare, their economic significance and the threat being faced by them owing to habitat loss and unsustainable harvests, and the vital inevitability of conserving to assure continuous supplies for future use. Subsequently, WHO et al. (1993) released guidelines for MPs conservation. Conservation of genetic resources requires team effort of national and international organizations such as IUCN, WWF, FAO, Botanic Garden Conservation International (BGCI, http://www.bgci.org/), UNIDO, UNESCO, etc. with the involvement of ethnic communities and indigenous people. WHO guidelines cover eight strategies: 1. To record and digitize traditional knowledge of local communities of each country on ethnobotanical uses and share the benefits arising out of commercial exploitation of such knowledge with the communities. India has digitized 220,268 medicinal formulations used in Indian systems of medicine through traditional knowledge digital library

(TKDL, www.csir.res.in) initiative that has helped the country to get some patents granted based on Indian traditional knowledge revoked. The Tropical Botanical Gardens and Research Institute (TBGRI), Thiruvananthapuram, Kerala, documented and patented the medicinal properties of *Trichopus zeylanicus* and shared the benefits arising out of commercial utilization of the traditional knowledge of *Kaani* tribal community with them. The agreement between the National Biodiversity Institute of Costa Rica and Merck for bioprospection of Costa Rica's 4 % world's biodiversity for benefit sharing from commercial products arising out of the bioprospection is another example. The problem is in recognizing ownership of genetic resources and the knowledge arising out of them. Some countries regard genetic resources as a nation's heritage and should be shared with financial compensation. Others opine them to be human heritage and should be freely shared. In both the cases the ecosystem and indigenous peoples' needs (who fear that governments and companies indulge in biopiracy) are largely ignored. Extreme arguments include bioprospection as a conservation measure to protect species from extinction. The guidelines provided by CBD on access to genetic resources and fair and equitable sharing of the benefits arising out of their utilization (Secretariat of the Convention on Biological Diversity 2002) and the 2010 Nagoya Protocol (www.cbd.int/nagoya/outcomes/) are useful in resolving this conflict. 2. To prepare countrywise databases of MPs, their distribution patterns, herbaria sheets, and identify threatened species for conservation. Remote sensing and GIS (Geographic Information System) technologies are currently used by many countries for assessing the distribution of MPs and their threat levels (Liu et al. 2011). 3. To encourage cultivation of MPs through development of high-yielding varieties, their agrotechnology, raising nurseries, and training the stakeholders. Cultivated MPs can then be used for trade. 4. To ensure sustainable wild collections, banning collection of threatened species and regulating their trade. Nepal has banned wild collection of rare species, India has banned export of wild-collected endangered species, Bulgaria prohibited wild collection of 14 species, Croatia protected 44 species from wild collection, in different regions of Italy wild-harvest of 15–174 species is prohibited, 2–51 species restricted, and 26 species regulated, in Lithuania wild-harvest of 21 threatened MPs is regulated, and in Poland 20 MPs strictly and 16 are partially protected. In India and China threatened MPs are substituted with species having the same medicinal properties (Liu et al. 2011). 5. To improve harvesting, storing, and production practices with emphasis on quality control. 6. In situ conservation of MPs and their populations in their habitats through biosphere reserves (621 biosphere reserves in 117 countries; http://www.unesco.org/new/en/natural-sciences/environment/ecological-sciences/biosphere-reserves/. Viewed July 22, 2014), nature/ecological/gene reserves, wildlife sanctuaries, national parks, sacred groves, heritage sites are collectively called protected areas (currently 200,589 terrestrial protected areas covering 14.3 % land area and 9612 marine protected areas covering 10 % marine area exist; http://www.protectedplanet.net/search; http://wdi.worldbank.org/table/3.4. Accessed July 22, 2014). In situ conservation preserves species, genetic and ecosystem diversity. Reintroduction (in situ seeding, in situ or ex situ nurseries,

alginate encapsulated microshoots, etc.) of overexploited species into their natural habitats is recommended. IUCN is advising countries to identify and earmark MPs rich forests as Medicinal Plant Conservation Areas (MPCA). Several Indian provinces earmarked MPs rich forest areas (200–500 ha each) for their protection and conservation. About 40 MPCA have been established in South India. China has established in situ conservation networks such as Tibetan Plateau for alpine MPs, XinJiang province for MPs of northwestern China, ChangBai Mountain for MPs of northeastern China, and GuangXi province for MPs of southern China (Liu et al. 2011). In Samoa four village-owned and managed rain forest reserves were established in 50,000 acres. In Belize MPs extractive reserve has been created on 6000 acres. Forest gene bank where species can exchange gene pools within and among populations and evolve is another idea mooted for in situ conservation (Shaanker and Ganeshaiah 1997). 7. Ex situ conservation to complement in situ conservation and as an insurance policy but not to replace in situ conservation. Species whose habitats have been destroyed or cannot be protected or whose populations got severely depleted or that became locally extinct should be given priority for ex situ conservation. Selected species should be carefully collected to include broad genetic base for improvement, reintroduction, and recovery without endangering wild populations. Considering that 70 % genetic diversity of a species can be retained in a sample of less than 1000 accessions, many MPs can be conserved ex situ. The problem lies in sampling due to differing growth, flowering, fruiting times; geographical distances; population sizes; ecological requirements; morphotypes and chemotypes. The advantages include easy plant propagation, reintroduction, agronomic improvement, research and education on these species. Disadvantages are inability to conserve 100 % genetic diversity, conserved species suffer genetic erosion, and are dependent on human care. Every country is recommended to establish botanic gardens equipped with field gene banks (germplasm of live trees, shrubs, vegetatively propagated species) and seed banks (stored at −20 °C) of annuals and perennials. BGCI has added ex situ conservation status of 3000 MPs to its PlantSearch database and is involved in conservation of threatened MPs in Brazil, China (through community based approach), Cameroon, Costa Rica, India, Madagascar, Mexico, Morocco, Philippines, Sri Lanka, Uganda, and other countries. It has a network of >2500 botanic gardens worldwide with 7 patron gardens for ex situ conservation of species comprising over 100,000 species, 4 million living plant collections with 6.13 million accessions and 142 million herbaria. Some of the gardens are devoted to MPs such as Monastir medicinal botanic garden of Tunisia and medicinal botanic garden of Shanghai, China. With the help of modern technology (in vitro culture, micropropagation, mycorrhization, genetic transformation, plant part substitution, etc.) it is possible to preserve pollen, embryos, embryonic axes, shoot apexes, cell suspensions, adventitious buds, DNA, etc. in cryopreservation at −196 °C (Kasagana and Karumuri 2011). Artificial seeds or alginate encapsulated microshoots produced in the laboratory are being used for reintroduction of wild-extinct or endangered species (Srivastava et al. 2009). Botanical Survey of India, CIMAP (Central Institute of Medicinal and Aromatic Plants; 2762 accessions of 418 MPs in seed gene bank, 1774 accessions

of 244 MPs in field gene bank, 264 accessions of 44 MPs in in vitro gene bank, and 1389 accessions of 53 MPs in DNA bank), TBGRI (30,000 plants, 1000 angiosperms, and 100 rare species), and National Bureau of Plant Genetic Resources (NBPGR) are maintaining herbal gardens, seed banks, and in vitro banks dedicated to MPs. China has national MPs gardens in several provinces and a national MPs seed bank in Beijing (Liu et al. 2011). Croatia (900 accessions of 180 MAP), Czech republic (973 accessions of 78 MAP), Poland (159 accessions of 13 MPs), and Slovenia (650 MAP accessions) have preserved their MPs in seed/gene banks. Israeli gene bank contains 197 in situ, 584 ex situ, and 576 seed accessions of 15 MAP and 50 seeds each of 74 MAP. 8. To seek public support and cooperation through sensitizing and educating them on the importance of conserving MPs with the help of medicinal plant gardens in hospitals, parks, colleges; guided tours to such gardens; organizing lectures and campaigns; introducing courses in student curricula, etc. Sensitizing communities that reside inside forests and forest fringe areas is especially important in conserving MPs diversity as traditional knowledge on their ethnomedical uses is fast disappearing. This is also important since forests are exploited for food, fruit, flower, foliage, fodder, fuel, fiber, wood/timber, and other economic purposes and MPs form a negligible part (FAO 2010), hence ignored. WHO, IUCN, WWF, and TRAFFIC revised these guidelines taking into account information and research, policy and legislation, conservation strategies, sustainable production, healthcare, responsible business practices, equity and awareness, training and capacity building (Kathe 2006). Biodiversity informatics that links taxonomy and distribution with environmental variables to assist MAP conservation is an evolving new science (Paton 2009).

11.10 Cultivation for Protecting Genetic Diversity

The dilemma on the choice of wild or cultivated MPs for use in medicines has been raging for a long time. Scientists opine that preference for wild species is based on local perceptions (Robbins 1998) which are based on the presumption that the percentages of pharmacologically active secondary metabolites are higher in wild-gathered MPs, e.g., roots of wild American ginseng (*P. quinquefolius*) are considered more potent than those of cultivated plants (Sanders and Hamrick 2004). Some researchers feel that traditional perceptions are not completely unfounded as wild plants grow under specific ecological conditions (that influence accumulation of phytochemicals) which are difficult to replicate in cultivated regions (Schippmann et al. 2006). Scientific investigations however, confirmed that phytochemical concentrations can be regulated in cultivated MPs (Palevitch 1991). Product quality of domesticated Chinese MPs cultivated near the regions of their wild growth was found to be better than wild populations due to better cultivation practices (Guo et al. 2009). Many international traders and companies accept cultivated MPs (Laird and Pierce 2002). MPs that are presently available in copious quantities, species with restricted habitats or that can be easily multiplied

in their native environments or trees/shrubs with long gestation periods and those for which cultivation may not confer socioeconomic, environmental, or other benefits need not be cultivated (Schippmann et al. 2006). For some MPs cultivation in forests or fringe areas is advocated. In India, in joint forest management program *vana samrakshana samithies* (forest protection councils) are formed with indigenous people who are permitted to cultivate small pockets of denuded forest areas. In China, semi-wild cultivation through natural nurseries of MPs or domestic cultivation by poor families is being practiced (Guo et al. 2009; Liu et al. 2011). Ex situ cultivation of MPs that are used in home herbal remedies or locally traded in small amounts through home herbal gardens in villages and towns, roof herbal gardens and growing them in pots in cities are becoming popular (Schippmann et al. 2006; Rao and Rajeswara Rao 2006; Guo et al. 2009). Avenue plantations with trees/shrubs and national parks dedicated to MPs are also becoming common (Liu et al. 2011). MPs which have been overexploited, whose habitats have been destroyed or degraded, that are regularly traded in large quantities with insufficient wild supplies, which are expensive, which have been extinct in the wild, that are listed in IUCN or country red lists or CITES appendixes and are banned for exports and whose genetic diversity has been eroded are ideal for cultivation. Cultivation's main thrusts are to discourage over- or destructive harvesting of wild populations thereby preserving genetic diversity in situ, preventing genetic erosion and to serve as economically viable renewable resource for quality MPs (Canter et al. 2005; Lubbe and Verpoorte 2011). Both collection and cultivation are market driven. Shi et al. (2008) demonstrated that cultivation of *Coptis chinensis* has not resulted in loss of genetic diversity. A similar finding was reported in goldenseal (*Hydrastis canadensis*) where cultivated and wild plants did not display differences in genetic diversity (Kerry 2009). Wild and cultivated *Allanblackia* trees had similar genetic diversity (Atangana 2010). Domestic cultivation of several MPs through seeds collected from wild population maintained 90 % genetic diversity. Even after 40 years of domestic cultivation, cultivated populations of *Codonopsis pilosa* retained high genetic diversity. Similar observations were recorded in *P. quinquefolius*, *P. ginseng*, *P. notoginseng,* and *Paeonia lactiflora* (Guo et al. 2009). Cultivated populations of *Primula veris* were more polymorphic than their wild relatives (Morozowska and Krzakowa 2003). Hybridization of cultivated varieties with wild populations was suggested to preserve genetic diversity. MPs can be cultivated as standalone crops (pure stands), can be integrated with agricultural, forest, or horticultural crops in intercropping, alley cropping, multistoried cropping systems, or in crop sequences (Rao and Rajeswara Rao 2006; Rajeswara Rao et al. 2012). Contract (Heywood 1999: contract cultivation of MAP by US pharmaceutical and cosmetics firms in developing countries; Lubbe and Verpoorte 2011: contract cultivation of *P. somniferum* in Tasmania and other MPs in India, Poland, South Korea by European companies) and corporate cultivation are catching up.

Cultivation requires varieties bred by traditional or modern biotechnological methods (marker assisted selection, transgenic plants) or carefully selected from wild populations to yield more biomass containing greater percentages of secondary metabolites and modern cultivation practices for these varieties under

different agroclimatic conditions. Knowledge about the existing genetic diversity greatly helps in selecting plants having maximum gene pools either for cultivation or for improvement. Table 11.5 lists some of the varieties developed in India. Systematic cultivation of MPs is becoming a profitable farming enterprise. About 900 MPs are cultivated (Schippmann et al. 2006) and more species are needed to be cultivated. In China about 250 MPs were cultivated in 330,000–460,000 ha and 700–1300 MPs are grown in botanic gardens (Akerele et al. 1991; Heywood 1999). About 400 MPs are now cultivated in China in 10 million hectares (Ran 2008; Guo et al. 2009). In Europe 130–150 MAP (>100,000 ha; Lubbe and Verpoorte 2011), in Bulgaria 20–25 % of MPs in trade, in Croatia >3000 ha, in Finland 30 herbs (<5000 ha), in Poland 60 (20,000 ha with 20,000 tons production), in Hungary 40 MAP, in Romania 52 (4000 ha), in Italy over 100 MAP (3350 ha), in Spain 14 (6000 ha grown, 100,000 ha wild-collected), in Latvia 20 MPs (300 ha), in Serbia 30 MAP (<5000 ha), in UK culinary herbs (4200 ha), and in India <50 MPs (Ved and Goraya 2008) are cultivated in >95,000 ha (Chaddha and Gupta 1995). Small-scale cultivation of many more MPs is practiced in home gardens and by herbalists. Cultivation of MPs is prevalent in both developing and developed countries (Lubbe and Verpoorte 2011). The benefits and drawbacks of wild collection *versus* cultivation are enumerated in Table 11.6. Taking cognizance of importance of cultivation, WHO (2003) has issued guidelines on Good Agricultural Practices (GAP) laying emphasis on selection of MPs, their botanical identity, site selection (avoid polluted areas), ecological, environmental, and social impact, climate, soil, use of organic and inorganic nutrients with limited use of chemicals, irrigation and drainage, plant maintenance and protection, harvesting, personnel and strict quality control measures for producing biomass free of biotic and abiotic contaminants. With preference for organically produced and labeled MPs, guidelines given by World Fair Trade Organization (http://www.wfto.com/), International Federation of Organic Agricultural Movements (http://www.ifoam.org/), Fairtrade Labeling Organizations International (http://www.fairtrade.net/), Organic Trade Association (www.ota.com/pics/documents/short%20overview%20MMS.pdf) are to be followed for easy market acceptance and higher profits from organically cultivated MPs.

In addition to simple cultivation (including organic agricultural) practices under rainfed and irrigated conditions for enhancing quality and biomass yield per unit area per unit time; micropropagation protocols (Sharma et al. 2010) for rapid multiplication, for producing disease-free plantlets, for selecting somaclonal variants in vegetatively propagated species, for enhancing secondary metabolites in shoot or root (hairy root) cultures (shake flask and bioreactor technologies, e.g., *C. roseus*); biotechnological methods to identify genes and engineer biosynthetic pathways either for better accumulation of phytochemicals or elimination of undesirable phytochemicals; plants with different ploidy levels through induced mutations, soil less culture techniques, e.g., hydroponics and cultivation under controlled conditions (polyhouses, greenhouses), etc. have yielded fruitful results (Rajeswara Rao 1999; Rajeswara Rao and Rajput 2005; Canter et al. 2005; Reddy and Rajeswara Rao 2006; Rajeswara Rao et al. 2007; Lubbe and Verpoorte 2011).

Table 11.5 Cultivated varieties of medicinal plants developed in India

Common name	Botanical name	Variety
Aloe	*Aloe vera*	Sheetal
Carry me seed	*Phyllanthus amarus*	Jeevan, Navyakrit, Kayakirti
Chamomile	*Matricaria chamomilla/recutita*	Vallari, Prashant, Sammohak, Del
Coleus	*Plectranthus/Coleus forskohlii*	Bhagya
Egyptian henbane	*Hyocyamus muticus*	NP-41, HMT-1C
Gotu kola	*Centella asiatica*	Majja Poshak, Subhodak, RK1, RK2
Guggul	*Commiphora mukul/wightii*	Marusudha
Henbane	*Hyocyamus niger*	Aela, Aekla, IC-66
Indian gooseberry	*Emblica officinalis*	Banarsi, Krishna, Balwant, Francis, Kanchan, Neelam, Mehrun, Dongri, Agra bold, Modibagh, Banarsired, Amrit, Chakaiya, Faizabad, BSR-I, BGK-1, GA-1, Anand-1,2,3
Indian snakeroot	*Rauvolfia serpentina*	RS-1
Itching/velvet bean	*Mucuna pruriens*	Ajar
Kangaroo apple	*Solanum laciniatum*	EC-113465
King of bitters	*Andrographis paniculata*	Megha
Liquorice	*Glycyrrhiza glabra*	Mishree
Long pepper	*Piper longum*	Pipali, Viswam
Medicinal yam	*Dioscorea floribunda*	FB(C)-1, Arka-upkar
Milk thistle	*Silybum marianum*	Liv, Sil-9
Opium poppy	*Papaver somniferum*	Ajay, Shweta, Shyama, Shubhra, Vivek, Sanchita, Sujata, Rakshit, Sampada, Trishna, Kirtiman, JA-16, UO-285, NRBI-3
Periwinkle	*Catharanthus roseus*	Nirmal, Dhawal, Prabal
Psyllium	*Plantago psyllium/ovata*	Mayuri, Niharika, GI-1, GI-2
Sacred basil	*Ocimum sanctum/tenuiflorum*	Ayu, Kanchan, Angana
Safed musli	*Chlorophytum borivilianum*	Oj
Satavari	*Asparagus racemosus*	Shakti
Senna	*Cassia senna/angustifolia*	Sona, ALFT-2
Sweet flag	*Acorus calamus*	Balya
Sweetleaf	*Stevia rebaudiana*	Meethi, Madhu
Sweet wormwood	*Artemisia annua*	Arogya, Suraksha, Jeevan Raksha, Asha
Tropical soda apple	*Solanum viarum*	Glaxo, IIHR 2n-11
Waterhyssop	*Baccopa monnieri*	Jagriti, Pragyashakti
Wild gooseberry	*Emblica fischeri*	Champakkad large, Krishna
Wintercherry	*Withania somnifera*	Poshita, Rakshita, NMITLI-118, Chetak, Pratap, Jawahar-20, WSR, Nagori

Table 11.6 Benefits and drawbacks of wild collection *versus* cultivation of medicinal plants (Sanders and Hamrick 2004; Schippmann et al. 2006; Lange 2006b; Qing et al. 2010; Lubbe and Verpoorte 2011; Rajeswara Rao et al. 2012)

Wild collection	Cultivation
Reduction in population sizes and densities. Pollen, seed, and gene exchanges are restricted due to habitat degradation. Faulty harvesting at wrong phonological stages limits regeneration capacity of species. Genetic diversity depletion and genetic erosion. Extinction of species and ecotypes. Slow-growing and endemic species with specific habitat requirement and limited distribution are susceptible to overcollection. Exploitation of ethnic communities by unscrupulous agents	Conservation of species by discouraging wild collections. Relieves pressure on threatened, slow-growing species. Crop improvement through research and organic certification. Possibility of domestication of exotic species. High yields of biomass and secondary metabolites through cultivation of high yielding varieties. Contract and corporate farming are feasible
Irregular/diminishing availability and supplies. Knowledge about the resource is inadequate. Over- or destructive harvesting due to common access, illegal activities, lack of management plans, low prices, community needs, etc. Wastage due to overexploitation	Sustainable availability of raw material. Organic methods of cultivation and minimum use of chemicals. Amenability for inclusion in different cropping systems and crop rotations
Collection of wrong species due to confusing common names. Possibility of admixture with related species, sometimes with poisonous species	Botanical identity and purity of the species are guaranteed
Variable quality. Possibility of contamination with biotic (insect pests or diseases) or chemical contaminants. Quality control is difficult	Uniform quality. Quality improvement and quality control are practiced. Cultivation close to polluted areas is prohibited
Difficulties in harvesting, handling, drying, storage, and transportation	Harvesting, handling, storage, transportation are regulated avoiding contamination at all stages
Pest control difficult	Pest management is easy
Seasonal employment for local communities and indigenous people	Year round employment is possible by integrating different species with existing cropping patterns of agricultural, horticultural, and forest crops
Health/accident risks to collectors	Protection to workers is ensured
Adds income to local communities with no investment. Takes care of primary healthcare needs of indigenous people. Provides incentives to protect genetic diversity and wild populations. Product is organically produced and is cheaper. For habitat-specific species or species with small market or with narrow ecological range or whose plant parts require large cultivation space or where cultivation practices are nonexistent, wild collection is preferred	Product is expensive due to investments in domestication, cultivation, and research. Land for MPs is limited. Not a beneficial production system for all MPs. Heavy dependence on cultivated MPs may rob income of local communities involved in wild collections and limit incentives to protect wild populations. Narrows genetic diversity, may lead to genetic erosion. Populations become homozygous over time. Low-yielding wild relatives are ignored.

(continued)

Table 11.6 (continued)

Wild collection	Cultivation
	Continuous cultivation of varieties on contiguous areas renders them susceptible to pests and diseases. Dependent on human care and profits. Seed exchange among farmers and movement of seeds to other environments may pose the risk of maladaptation. Gene flow between maladapted plants and native populations may alter their genetic structure
Ambiguous land rights give rise to ownership conflicts. Resource utilization, benefit sharing, and IPR issues need to be resolved. Unfair/illegal trade practices. Difficulties in traceability and labeling on source and time of collection of each batch	Cultivation is carried out on private lands or public institutions with clear land records. IPR issues may still arise when imported material is used for research and patenting. Traceability and labeling of each batch are possible. Trade practices are reasonably fair

IPR Intellectual property rights

11.11 Conclusion and Prospects

MPs have been used for their curative property since ancient times. They provide revenue and health security to ethnic indigenous communities. Information on MPs is scattered in botany, chemistry, medicine, agriculture, horticulture, forestry, religion, etc., their centers of origin and biology are largely unknown. Unlike food, fruit, flower, foliage, fodder, fiber, fuel, timber crops, MPs are wild-gathered or cultivated for phytochemicals. Plants in general (<5 % of world flora have been comprehensively assessed by IUCN) and MPs in particular received less attention relative to animals and birds. Since revenue from exports constitute a small proportion, nations tend to focus on other high-priority sectors placing less importance on MPs. Forests are the primary habitats and the lives of forest-dwelling ethnic people (illiterate, poor, unemployed with few livelihood options) are intertwined with preservation and utilization of MPs through their cultural heritage and traditional knowledge a closely guarded secret passed on by word of mouth through generations. Exploding human population, rapid urbanization, and city-dwelling modern man's foray (roads, railways, airports) into the forests with exploitation/profit motive (hydel projects, mining, industries, logging, ranching, tourism) triggered today's crisis of genetic erosion and biodiversity loss. Human interference inflicted habitat loss, habitat and population fragmentation, commercial overexploitation of wild flora (MPs are collected as part of nonwood/timber forest products), overgrazing, invasive species, pollution, climate change, and escalating national and international business have severely disturbed evolutionary processes, gene flows, adaptive and regenerative capacities and increased geographical distances leading to irreplaceable loss of genetic/species/ecosystem diversity that took thousands of years to develop, wealth of gene pools and accelerated extinction of MPs. The concomitant loss of cultural diversity and traditional knowledge is largely ignored. It is feared that much of the traditional

knowledge has been lost. Wild-gathered MPs suffered severe losses of population sizes, densities, and gene pools over the past decades. Substitution of threatened MPs with alternate species with identical curative properties or phytochemicals can relieve pressure on threatened MPs. Although recovery, conservation (in situ and ex situ), and cultivation programs have yielded some gains, they need to be further strengthened by governments and stakeholders through collaborative multi-stakeholder approach to preserve existing genetic diversity and prevent further genetic erosion, since not all MPs can be brought under cultivation immediately. This is particularly important for MPs as all reforestation programs concentrate on timber or commercial species in spite of the fact that minor-forest products' collection is more remunerative and less damaging to the ecosystem. Only a small fraction of all known MPs have been investigated for their genetic diversity and genetic erosion employing morphoagronomic, biochemical and molecular markers and enzymes. There is an urgent need to gather data on other MPs before it is too late. Conservation schemes should shun profit perspective and should include ecosystem and indigenous people's needs for their preservation and sustainability. Documentation (authenticity, traceability, accountability, legal authorization), certification (organic cultivation, quality control, social accountability; good collection, agricultural, manufacturing and business practices) labeling, and brand development are becoming increasingly important for wild-collected and cultivated MPs. Cultivation should be made profitable with easy market access. The initiatives undertaken by countries may lead to revision of number of MPs and their threat perceptions from time to time. Modern research may also place new species into MPs domain demanding regular supplies initially through wild collections. Use of modern technologies such as biotechnology, remote sensing, geographic information system, biodiversity informatics, computer simulation programs and databases will greatly help in devising recovery, conservation, and cultivation schemes. Existence of large number of species (>70,000) with inadequate research funds, loss of forests, ever increasing national and global demands, hype and hoax claims, genetic resource utilization with benefit sharing and patent conflicts are the challenges that need to be resolved for checking genetic erosion, preserving genetic diversity, cultural diversity, traditional knowledge, and genetic resources for posterity.

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Chapter 12
Genetic Diversity, Erosion, and Population Structure in Cotton Genetic Resources

Narayanan Manikanda Boopathi and Lucia Vieira Hoffmann

Abstract It is strongly believed that the wide genetic variability within the cotton (*Gossypium* spp.,) increases their chance for adaptation to changing harmful environments, and thus upsurge the likelihood of long-term survival of such unusual and important cash crop in the world. Given, the importance of cotton in the world economy and its usefulness to the human, cotton genetic resources should be conserved effectively and managed wisely, since such cotton genetic resources are used as the raw material for breeding new cultivars and act as a reservoir, and/or buffer against ecological and economic changes. However, the trend is reverse as there has been significant loss of genetic diversity during the past couple of decades, and the process of genetic erosion continues. Although, the narrow genetic diversity that exists in cotton has been noticed for more than two decades, there is little data on its amount and extent. Besides the threatening genetic base of future cotton breeding programs, erosion of cotton genetic resources could pose a severe threat to the world's natural fiber production in the long-term, since loss of genetic variation may decrease the potential for a species to persist in the face of abiotic and biotic environmental changes. Future progress in the improvement of cotton largely depends on discovery, collection, and immediate conservation of genetic resources such as wild progenitors and landraces of *Gossypium* for their effective and sustainable utilization in the cotton breeding program. This chapter describes the challenges to cotton genetic diversity, presents the strategies that are being implemented to reverse the erosion of that diversity, outlines several gaps in our knowledge, and describes strategies that must be addressed

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to make such approaches more effective. Deployment of biotechnological tools in the study and conservation of cotton genetic resources are also highlighted in this chapter. Integration of the knowledge about evolution and natural population structure of domesticated *Gossypium* species combined with emerging sequence and functional genomics information will lead to the better management of cotton germplasm resources and more efficient utilization of natural variation for cotton genetic improvement.

Keywords Cotton (*Gossypium* spp.) · Genetic diversity · Cotton germplasm conservation · Genetic erosion · Molecular markers · Pollen culture · Transgenic technology

12.1 Introduction

12.1.1 Cotton: Single Crop—Multiple Uses

Cotton is uncommon among major commercial crops since it has a huge impact in the global economic, industrial, and social sectors. Cotton's primary organ of commerce, seed-borne lint fiber, is still the most preferred natural fiber in the world and greatly diminishes the dependence on synthetic fibers that are derived from highly depleting resources of petrochemicals. It is estimated that cotton fiber with improved uniformity, durability, and strength can effectively replace synthetic fibers that need ~230 million barrels of petroleum per year in the USA alone (Holt et al. 2003). Further, cotton production has enormous economic benefits in the cotton-growing countries. It is being cultivated on about 2.5 % of arable land, approximately 150 countries are involved in cotton import and export, provides income for approximately 100 million family units and sustains the textile industry, with a worldwide aggregate influence estimated annually at more than $500 billion (Kranthi 2013). Moreover, cotton is a major economic driver in several developing countries. For example, Uzbekistan produces ~4 million tons of raw cotton per year and exports ~$900 million worth cotton fiber (Chen et al. 2007). Similarly, the cultivation alleviates poverty in West Africa, where it represents from 25 to 51 % of the exportations in Burkina Faso, Chad, Benin, and Mali (Vitale et al. 2009).

All parts of the cotton plant are useful and it has hundreds of uses. During the celebration of the International Year of Natural Fibres in 2009, the usefulness of cotton fiber received topmost attention among plant and animal fibers (http://www.naturalfibres2009.org/; accessed on 15th December, 2014). In addition to their widely known uses in clothing or apparels and home furnishings, fiber-derived products are used in plastics and in many industrial goods such as digital screens. Furthermore, cotton is an important source of feed, foodstuff, and oil. Cotton seed oil and by-products of fiber processing are also used as raw materials for biofuel production.

Each cotton fiber is a single and unusually elongated cell from epidermal layer of the ovule, with about 25,000 per seed. It has been shown that there are only a few cells in the plant kingdom that are as blown up in their size or composition as cotton fibers and some of the cotton fiber cells can reach lengths of over 6 cm or one-third the height of an *Arabidopsis* plant (Kim and Triplett 2001). Therefore, besides its economic importance, cotton fiber is an outstanding model for the study of plant cell elongation, cell wall, and cellulose biosynthesis. The fiber is composed of nearly the pure cellulose, the largest component of plant biomass. Compared to lignin, cellulose is easily convertible to biofuels (Chen et al. 2007). Therefore cotton is considered as an attractive target crop in public and private sectors. Further genetic improvements that enhance the economics of production and sustainability and fiber processing characteristics will ensure competitiveness in the market of this natural-renewable product with nonrenewable petroleum-derived synthetic fibers. It is also worth here to mention that modifications to expand the use of seed derivatives for food and feed could profoundly benefit the diets and livelihoods of millions of people in food-challenged economies. Such improvements have more economic, health and ecological, and thus societal impacts on both national and international boundaries.

12.1.2 Importance of Genetic Diversity in Cotton and Its Multiple Perspectives

The improved cotton cultivars developed during the past few decades led to spectacular increases in fiber yields. On the other hand, they also led many farmers to abandon the age-old practice of planting a mix of traditional varieties as insurance against adverse conditions. For example, in India which has largest cotton cultivation area in the world (Navarro and Hautea 2014), it can be easily predicted from the current trend in cotton cultivation that less than 20 hybrids will soon cover more than 80 % of the total cotton area, replacing thousands of different cotton cultivars that were once grown there. As a consequence, vast areas of land are now planted to a small number of high-yielding cotton hybrids, which require enormous inputs of fertilizer, pesticide, and water.

Past history of agriculture clearly demonstrated that use of small number of cultivars in the given environment has led to huge loss in agricultural production. One example of this occurred in the USA in 1970, when the uniformity of the maize crop enabled a blight to destroy almost US$1000 million of maize and reduced the yields by as much as 50 %. After spending lot of time, scientific efforts and money, resistance to the blight was finally found in the genes of an African maize variety called Mayorbella (http://www.worldbank.org/html/cgiar/25years/gene.html; accessed on 15th December, 2014). As urban development destroys habitats, and farmers abandon traditional varieties in favor of modern uniform types, the resulting loss of diversity has serious implications for long-term

fiber production. Further, because of deployment of few cultivars, cotton cultivation now is more vulnerable to attacks from pests and diseases and more prominently to climate changes such as water stress.

Thus, during the past few decades it is increasingly believed that meeting the natural fiber needs of the world's growing population depends, to a large extent, on the conservation and use of the world's remaining cotton genetic resources (Boopathi et al. 2014). The conservation and use of genetic resources is as old as agriculture itself. For over thousands of years farmers have conserved seeds for future planting, domesticated wild cotton species and selected, and bred thousands of different cotton cultivars to suit their specific needs and conditions. Several centuries ago, the tetraploid cultivated cotton species *Gossypium hirsutum* and *G. barbadense* have been independently domesticated in Mexico and Peru (Campbell et al. 2010).

Yet much of this cotton genetic diversity has now been lost. As stated above, of the several thousand cotton cultivars used in the past for fiber production, only hundreds are cultivated today in the world. The most significant loss of cotton diversity had taken place in recent decades due to the introduction of new, high-yielding cotton cultivars that began on a large-scale in the late 1950s and 1960s. In addition, cotton production is increasingly market-oriented, making farmers less inclined to select for crop characteristics that once were important for local customs and culture. All the studies that were intended to estimate the genetic diversity exist in the currently cultivating cotton cultivars and the germplasm accessions used in cotton breeding program evidently revealed that there was a very narrow genetic diversity in the investigated materials (Lacape et al. 2007 and references therein; Boopathi et al. 2008; Thiyagu et al. 2011; Ravikesavan et al. 2014).

International recognition of the importance of cotton genetic diversity and the increasing threat of genetic erosion grew significantly and the scientific principles, which underlie strategies and methodologies for collecting, conserving, evaluating, and documenting cotton genetic resources were comprehensively addressed recently (Abdurakhmonov 2014). As countries became aware of the danger of genetic erosion and the need for conservation, greater priority was given for collecting the cotton genetic resources in the field and establishing in situ and ex situ gene banks (see below).

If the adoption and use of improved cultivar and hybrids and other farming technology bring significant benefits to cotton farmers, why should we be concerned with the loss of landraces and the preservation of cotton genetic diversity? There are several important reasons. Genetic diversity is the elementary factor of evolution in *Gossypium* species. It is the foundation of sustainability because it provides raw material for adaptation, evolution, and survival of species and individuals, especially under changed environmental, disease, and social conditions and it will allow them to respond to the challenges of the next century (Hammer and Teklu 2008). As it is evident from the past history, many advances in modern cotton breeding have been possible because of the wide range of genetic source material provided by cotton germplasm. Therefore, the future of global fiber supply depends on the exploitation of genetic recombination and allelic diversity

that exists in the cotton germplasm resources. The considerable genetic diversity of cotton traditional varieties is the most immediately useful and economically valuable part of cotton germplasm. Subsistence cotton farmers use landraces as a key component of their cropping systems. In addition, landraces are the basic raw materials used by cotton breeders for developing modern varieties. Landraces are a complex and continually evolving collection of local cotton varieties that reflect interactions with wild species, adaptations to changing farming conditions and responses to the economic and cultural factors that shape farmer's priorities. Over the past few decades, awareness of the rich diversity of exotic or wild germplasm has also increased. This has led to a more intensive use of such germplasm resources in regional cotton breeding programs aiming to increase fiber yields in an unprecedented way.

In addition, the preservation and utilization of cotton genetic diversity is of particular importance to the more marginal, diverse agricultural environments where modern plant breeding tools and technologies have had much less success. For example, in India, >60 % cotton is cultivated under rainfed environments by resource-poor farmers (Choudhary and Laroia 2001). Farmers in these areas tend to be poorly served by public research and extension systems. These areas are often centers of diversity for many *Gossypium* species (for example, *karunganni* cotton in South Tamil Nadu, India), but increasing poverty is forcing many of these farmers to place more dependence on nonfarm sources of income, with consequent reduction in their capacity to grow and maintain the range of cotton local varieties they have been adapted to manage. More importantly, the maintenance of a wide and evolving range of regional cotton landraces is threatened by the advent of intellectual property protection for crop varieties, accelerated by the formation of the World Trade Organization. The increasing application of plant breeder's rights has several implications for cotton genetic diversity. For a new variety to be legally protected, it must be subject to very precise description, including the requirement that it be distinct, uniform, and stable (Cooke and Reeves 2003). This is a limiting element to the promotion of inherently diverse landraces or of varietal mixtures. An additional debate concerns farmer's privilege, the ability of farmers to save the seed of a variety, to exchange it with neighbors and to adapt it to their own growing conditions. These practices could be challenged by the seed companies when cotton hybrids/varieties are sold under strict legal protection. It is even possible to envisage situations, where varieties that originated in farmer's fields may be legally protected and then denied to the farmers responsible for having developed them (Hammer 2004). Hence the advent of plant variety protection lends added urgency to the search for solutions to the conservation of cotton genetic diversity.

Nevertheless there are several decisions that must be made in designing cotton germplasm conservation projects. First, because landraces are not static entities, decisions have to be taken with regard to the nature of human intervention in the selection process. Whose criteria are to be used in the selection and adaptation of new materials? Are local farmers' criteria the only ones to be applied in deciding what is conserved or should scientists' interests also play a role in determining

the direction of conservation? Since there is no successful example in this case, it is imperative to develop a large multinational collaborative project to test and develop techniques for cotton germplasm conservation. In addition, farmers generally seek germplasm from other sources to complement their own landraces. To what extent should this be allowed or encouraged in an in situ conservation project? To what extent is the objective to build a fence around an area of genetic diversity in order to protect traditional crop development processes from outside influences and to what extent is the objective closer to that of community development? In the latter instance, resources and information are provided to farming communities to empower them to make more informed decisions about the management of local varieties and the utilization of recently released cultivars in other crops (Hammer and Teklu 2008).

There are also a number of efforts under way to encourage a wider scope for farmer participation in formal plant breeding (Boopathi 2013). Possibilities include greater farmer representation in priority setting for crop breeding programs, more explicit attention to the crops and varietal characteristics of importance to these farmers, the transfer of significant aspects of plant breeding research to farmer's fields and the organization, and training of farmers to take a more active part in the variety testing and selection process. There is a growing literature on methods to encourage farmer participation (Bhargav et al. 2014). The innovations include rapid rural appraisal techniques to understand farmer varietal preferences, the organization of various types of adaptive on-farm research to test varieties under field conditions, the wider use of landraces in formal breeding programs and the establishment of mechanisms for contact between farmers and experiment station personnel. Some plant breeders see the possibility of an integrated system that incorporates the strengths of both formal and informal plant breeding techniques. However, such efforts in cotton are found to be very scarce.

The challenges of cotton genetic resource conservation also highlight the dilemma of balancing between development and conservation in the light of policy implications. The dilemma is evident in choices of conservation strategies as well as in the design of development programs. The identification of an optimum mix of development and conservation initiatives is one of the most difficult tasks which will be faced policy makers in the next decade and the necessity to develop location-specific strategies adds to the complexity of the challenge (Hammer and Teklu 2008). Much more effort is required to develop adequate analytical tools to enable policy makers to explicitly address the trade-offs and consequences of particular decisions. It also requires clear policy decisions about the appropriate mix of public, commercial, and voluntary contributions.

Commercial cotton seed multinational companies are now replacing public cotton seed operations and are also making an increasing contribution to plant breeding and variety development (Tripp and Heide 1996). In many instances, cotton seed companies could able to respond more effectively to farmers' needs than the public sector. But the commercial sector will not be likely to address the special growing conditions that are important to resource-poor farmers, nor will they be likely to play a prominent role in the conservation activities. Adequate legal

protection for commercial varieties should be balanced against the assurance of farmer's privilege to save and adapt their own seed. Therefore, new approaches to plant breeding, plant genetic resource conservation will increasingly involve farmer participation and promotion of long-term, productive collaboration between public agricultural research and organizations active at the community level.

Another challenging feature of conservation programs is their unavoidable long-term nature. Both national policy makers and external donors who wish to support conservation programs must assure that funding is available for an extended period to include the necessary research, training, and implementation. Further, good policy always depends on worthy information and this is particularly true for genetic resource conservation (Tripp and Heide 1996). Despite rapid progress, serious gaps (see below) in our knowledge are likely to constrain the informed management of plant genetic diversity for at least the next decade. The issues demand interdisciplinary collaboration among social and biological scientists. They also require increasing collaboration between researchers and the members of grassroots development initiatives. The policy must direct national research and academic institutions to give priority to collaborative research on conservation issues and should take responsibility for establishing appropriate forum, where different perspectives can be presented, debated, and synthesized.

12.2 Genetic Erosion in Cotton

Genetic erosion refers to the loss of individual genes or combinations of genes, such as those found in locally adapted landraces and wild species (Brush 1995). Genetic erosion also denotes that the normal addition and disappearance of genetic variability in a population is altered so that the net change in diversity is negative (Scarascia-Mugnozza and Perrino 2002). Thus, genetic erosion in cotton can be simply defined as the loss of variability (heterogeneity of alleles, morphotypes, and phenotypes) in *Gossypium* populations.

Several approaches have been employed to estimate the degree of genetic erosion that *Gossypium* faces in a certain region over a given time. Such strategies generally rely on any one or combination of the following methods: (i) analysis of molecular data (such as allozymes, DNA or RNA based marker analysis) (ii) comparison between the number of species/cultivars still in use by farmers at the present time to those found in previous studies (iii) using the genetic assessment model, and (iv) using a checklist of risk factors. Among them, the most widely used figures in estimating genetic erosion are indirect, i.e., the diffusion of modern crop varieties released from crop breeding programs (Hammer and Teklu 2008 and references therein).

Therefore, the obvious cause of genetic erosion is the dissemination of modern varieties from cotton improvement programs. With the advances in cotton breeding, high-quality and homogenous new cotton cultivars were quickly and

widely distributed, and suppressed the use of cotton landraces. Improved fiber yield (or yield potential) is the most important criterion for the choice of a cotton variety by a farmer. Additionally, update in the global culture is placing a range of pressures on wild areas and on traditional cotton cultivating areas and external interests (such as economic and/or political dominations) also strongly affect the regular cotton cultivation practice. The above said forces intensely change the nature of the decision-making process and the farmer is encouraged to grow high-yield varieties in monoculture using inputs of fertilizer and pesticides. Further, in many parts of the world, farmers were given several socioeconomic incentives or rewards to replace varieties that evolved within their agroecosystem with improved/introduced cotton hybrids. Efforts to localize new populations may be effective, as it was thought that *G. mustelinum* populations in Brazil were threatened to extinction (Barroso et al. 2009) until new populations were found (Alves et al. 2013; Menezes et al. 2014).

Population growth, urbanization, developmental pressures on the land resources, deforestation, changes in land use patterns, lack of recognition of current or future value of genetic resources, poor monitoring and management of genetic resources, lack of sustainable breeding programs, and natural disasters (famine, drought, flooding, typhoons) were other noteworthy factors that are contributing to abundant habitat fragmentation and destruction of the cultivated *Gossypium* and their wild relatives.

More recently, global warming and high degrees of water and air pollutions have also been recognized as auxiliary causes for the loss of diversity in cotton. For example, droughts of just a single season could result in drastic changes in cotton seed production and stocks, while successive years of drought can prompt changes in cropping patterns and the geographic distribution of cotton. Social disruptions or wars also pose a constant threat of genetic wipe-out of the promising cotton diversity. Overexploitation and introduction of invasive unfamiliar species are the supplementary minor factors contributing to the loss of genetic resources in cotton.

Similarly, genetic drift is also found to reduce cotton biodiversity (Scarascia-Mugnozza and Perrino 2002). Genetic drift is a random change in the allele frequency in cotton that occurs because gametes transmitted from one generation to the next carry only a sample of the alleles present in the parental generation. Genetic drift changes the distribution of genetic variation in two ways: (i) the decrease of variation within populations (loss of heterozygosity and eventual fixation of alleles) and (ii) the increase of differentiation among populations. Every finite population experiences genetic drift, but the effects become more pronounced as population size decreases (Falconer 1989).

Besides, the problem of genetic erosion through inappropriate maintenance of ex situ collections in cotton is also very obvious. Genetic erosion can occur at many stages in the preparation, sub-sampling, exchange, storage, and regeneration of recalcitrant cotton seed during ex situ conservation. It is also worth to highlight that loss of diversity through genetic shifts and convergent selection during regeneration is often unnoticed. In the world collection, beyond the problem of

duplication among accessions, the security of ex situ conservation as a whole is endangered. About half of all cotton gene bank accessions maintained in the world require immediate rejuvenation. However, financial problems, lack of staff, and shortage of farms largely affect such urgent action (Abdurakhmonov 2014). The long-term storage strongly reduces the metabolism and therefore highly limits cotton viability and seed vigor.

In conclusion, to reverse this unrestricted genetic erosion trends, conservation of genetic diversity is a fundamental concern in genetic improvement of cotton, as genetic variation is the raw material for evolutionary change within cotton populations. Detecting and assessing genetic erosions have been suggested as the first priority in any major effort to arrest the loss of genetic diversity and efficiently conserve the cotton germplasm. Generally, many national cotton programs have not viewed quantification of genetic erosion as a high priority, as apparent from the paucity of genetic erosion information in cotton (Gore et al. 2014). Although *Gossypium* species-specific guidelines are not available, the risk of genetic erosion can be minimized in cotton by familiarity with the biology (including breeding system, mode of reproduction, and pattern of genetic diversity) of the affected *Gossypium* species and landraces.

12.3 Cotton Diversity Assessment: Tools and Methods

Genetic diversity in cotton is conventionally analyzed using agronomically or economically important morphological traits such as growth habit, flower petal color, number of bolls, ginning percentage, seed index, fiber quality traits, scores of disease and pest resistances, and tolerance to abiotic stresses (Thiyagu et al. 2011). However, it was realized later that the variations observed in such morphological phenotypes were also influenced by environmental factors, and hence. it cannot be used to represent the diversity that is caused by genotypes alone. Due to rapid developments in enzymology and molecular biology, isozymes and molecular markers were found to be efficient tools in genetic diversity analysis in cotton in due course (Boopathi 2013). However, it should be noted that there was an uncoupling trends in genetic diversity in cotton when they were analyzed using molecular markers and morphological traits. In general, there was a greater diversity among the cotton accessions when analyzed with phenotypic traits than with the molecular markers.

However, use of molecular markers for cotton genetic diversity analysis is the choice of the researchers. There are several methods and strategies available to study the genetic diversity in cotton using molecular markers. Precise and objective estimate of genetic relationship depends on sampling strategies, use of several marker data sets, selection of genetic distance estimate strategies, clustering procedures or other multivariate methods and their influence on genetic relationship estimation etc. Thus careful combinations of these features and use of appropriate statistical programs and strategies is the key in genetic diversity data analysis

(refer Mohammadi and Prasanna 2003 for further details). In general, the data comprises numerical measurements and combinations of different types of variables. Further, pedigree data, passport data, morphological data, biochemical data, storage proteins data, and nucleus and/or chloroplast-based DNA/RNA marker data are also being used to reliably estimate the genetic relationship. Depending on the objective of the experiment, the level of resolution required, availability of resources and infrastructure facilities and operational cost, and time constraints decide the selection of data sets and each data provide a specific type of information on genetic diversity in cotton.

When we use the molecular data, genetic distance or similarity among individuals of the given germplasm is usually calculated as a quantitative measure that differentiates the two individuals at sequence or allelic frequency level. Wide range of genetic distance measurement are methods available, use of such methods are highly decided by the selection of a software tool that we employ for the analysis (Boopathi 2013). Numerous software programs are available for assessing genetic diversity, such as Arlequin, DnaSP, PowerMarker, MEGA2, PAUP, TFPGA, GDA, GENEPOP, NTSYSpc, Structure, GeneStrut, POPGENE, Maclade, PHYLIP, SITES, CLUSTALW, and MALIGN (Labate 2000). Most are freely available through the Internet. Many perform similar tasks, with the main differences being in the user interface, type of data input and output, and platform. Thus, choosing which to use depends heavily on individual preferences.

Allele mining is another recent tool that can be used to measure the genetic diversity in cotton. Allele mining refers to the identification of naturally occurring allelic variation at agronomically important genetic loci (otherwise called as genes). This can be performed using a variety of approaches including mutant screening, quantitative trait loci (QTL) and advanced backcross QTL (AB-QTL) analysis, association mapping, genome-wide survey for the signature of artificial selection, etc. (Navreet et al. 2010). The successful allele mining procedure is highly dependent on the use of diverse cotton germplasm collections, especially which are rich in wild species. This is because the majority of allelic variations at the gene(s) of interest is largely assumed to occur in the wild relatives of *Gossypium* due to the unavoidable loss of variation during the domestication process. Despite some efforts, unfortunately, entire cotton germplasm entries have not yet been efficiently characterized for their novel phenotypes due to several challenges including lack of resources for evaluating huge collections.

It is also worth to mention here the role of EcoTILLING in allele mining. A variant of "targeting induced local lesions in genomes (TILLING)," known as EcoTILLING, was developed to identify multiple types of polymorphisms in germplasm collections or breeding materials (Comai et al. 2004). EcoTILLING allows characterization of natural alleles at a specific locus across several germplasm entries in a rapid and affordable way. Recently, geographical information system (GIS)-based data collection from spatial objects and their attributes for species richness and diversity index was proposed for germplasm characterization of wild *Abelmoschus* species for the collection of diversity on wild okra (Nizar et al. 2014), even though it has not yet reported in cotton.

12.4 Genetic Diversity Analysis in Cotton: Lessons Learnt and What Has Been Neglected?

Irrespective of different methods and tools employed to measure the genetic diversity in cotton, as stated earlier, there was a narrow genetic diversity in cotton. For example, in our study we found that even the cotton cultivars that used different parents for their development in India, they were found to be closely related (which is more than 85 % within the *Gossypium* species) at molecular level (Fig. 12.1). Interestingly, the cotton accessions in the core germplasm have shown diverse response to water stress that was imposed during the flowering phase. However, they were shown to possess poor genetic diversity when analyzed with SSR markers (Fig. 12.2).

In another study, the diversity of the dooryard plants has been studied in the North Brazil by SSR markers. It is believed that *G. barbadense* can also be cultivated in dooryards, in urban areas or farms. In Brazil it is used as a medicinal plant, and the effusion of the leaves is believed to have healing properties. It may also be planted just as an ornamental plant, or more rarely it is used to make wicks or swabs. The genetic diversity of height: expected heterozygosity (the probability that two alleles chosen at random from the population are different) among the dooryard *G. barbadense* was 0.39 (Almeida et al. 2009). This may be explained by the fact that the plants are not cultivars and have not been selected for high production, although at least in North Brazil the healing properties are believed to be stronger in plants with purple leaves than on those with green leaves.

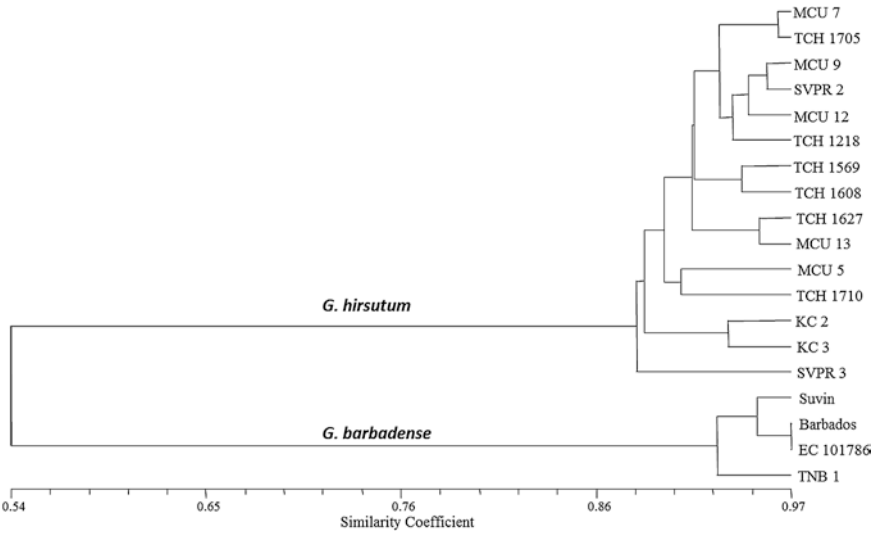

Fig. 12.1 Dendrogram of 15 *G. hirsutum* and four *G. barbadense* cultivars revealed by cluster analysis generated by similarity coefficient based on 66 SSRs

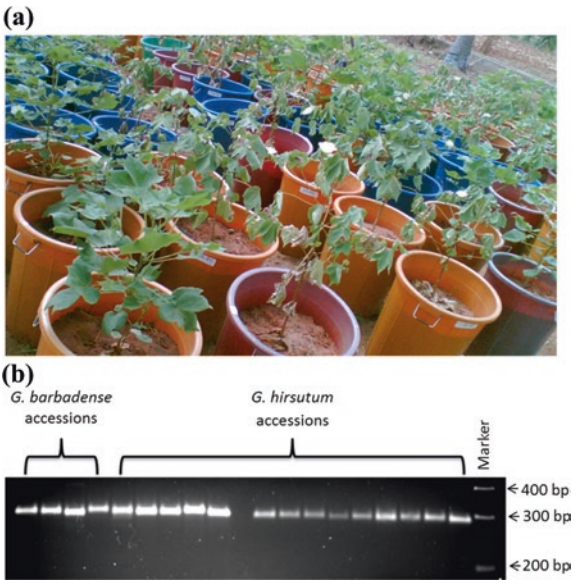

Fig. 12.2 Variation in response to water stress in *Gossypium* core collections at flowering phase (**a**). However, when the accessions were analyzed with SSR marker, CIR255, they were found to possess more or less similar genetic component (**b**)

Similarly, in the accessions of Moco cotton (*G. hirsutum* L. race *marie-galante*), a landrace reported to be selected by farmers in the Brazilian semiarid region during the second half of the nineteenth century and cultivated until around the year 1980, the expected heterozygosity was 0.52 (Menezes et al. 2010). Such a high value was due to the fact that some of the plants were cultivars and others were landraces. Hence, it can be concluded that smaller amount of genetic diversity exist in the present cultivating cotton cultivars and landraces is of serious concern.

Therefore, the lesson was that, as in other crops, "genetic uniformity will be the basis of vulnerability to epidemics and more generally, to biotic and abiotic stresses" (Scarascia-Mugnozza and Perrino 2002). Although there is undeniable evidence of the erosion of cotton genetic diversity and several innovative responses have been developed, there are important gaps in knowledge (see below) that limit our capacity to decide among the various alternatives. Some of these gaps involve our technical understanding of the nature of cotton genetic diversity, while others are concerned with our understanding of its socioeconomic implications. Appropriate measures for cotton genetic diversity still need to be developed in order to better characterize the current situation and to evaluate the changes in future.

Farmers' cotton variety classification systems are one place to start, but we do not know how these correspond to actual genetic differences. With few exceptions, there is little research that correlates the variation in conventional taxonomy with genetic differences. When moco cotton was at its peak in the 1970s, it was harvested from 1.8 million ha, and it could be classified by farmers according to its origin and morphological traits (Freire and Moreira 1991). The genetic distance measured by molecular markers is correlated to the places from where

moco cotton accessions have been collected, but not with morphological traits (Menezes et al. 2010). The capacity to evaluate genetic material in the laboratory is growing rapidly (Lacape et al. 2007; Boopathi et al. 2008; Thiyagu et al. 2011; Ravikesavan et al. 2014), but these are still expensive techniques and more robust markers and measures are required to ascertain the cotton genetic diversity.

Therefore, when better measures of cotton genetic diversity are devized they will contribute to a clearer understanding of what *Gossypium* species exactly need to be conserved. Currently, there are only very general ideas about what portion of a cotton population needs to be maintained in order to conserve particular genetic traits. This information is crucial to the efficient design of in situ conservation projects, for instance, in order to know if efforts might be limited to a few farmers' fields or should instead sample more widely from that agroecosystem.

Additional studies are also required to understand the causes of cotton genetic erosion. The cultural value placed on cotton diversity and local selection techniques are diminishing in many areas and the skills that have contributed to landrace evolution are consequently disappearing. As farmer variety selection skill is a threatened resource, there is also a need to understand how to conserve and enhance it. Although farmers deserve credit for landrace development, there is little knowledge of what the genetic consequences of their selection techniques are, or what specific effects these have on enhancing diversity. Farmers select cotton materials for practical reasons that may not always be compatible with the maintenance of genetic diversity in cotton. Very little interdisciplinary study has been devoted to understand the biological outcome of the application of indigenous technical knowledge and skills in cotton cultivar selection. Further, there is little understanding of whether attempts to improve local selection capacity should focus on individual farmers or on communities. Indeed, we know very little about how cotton varieties and their characteristics are exchanged within communities.

With the introduction of transgenic cotton cultivars/hybrids, concern has also been raised that overall genetic diversity within *Gossypium* species was decreased since transgenic cotton breeding programs concentrate on a smaller number of economically important cotton accessions (Chakravarthy et al. 2014). Though such effort has the potential and can also increase the genetic diversity, separate methodologies, and evaluation of genetic diversity with specific reference to transgenic cotton require superior attention. There is also a reason to believe that the amount of diversity is sufficient to maintain yields even in the face of most unknown pathogens that might emerge (Ronald 2014). However, the emerging combination of stresses under climate change and the opportunities for new pathogens is unprecedented. The use of *Bt* cotton is associated with the emergence of *Bt* resistance and by novel mechanisms in insect pests (Fabrick et al. 2014).

As molecular markers have shown their potential in genetic diversity analysis in cotton, other advances in biotechnology should also be used to develop and refine strategies for duplicate identification, flowering and seed propagation physiology, seed physiology for long-term storage, efficient strategies for pest and pathogen detection, germination testing procedures during long-term storage, accelerated seed aging and seed longevity, population size, and allelic frequency

changes on seed rejuvenation, genotype independent tissue culture protocols, identification of somaclonal variation in case of in vitro procedure, employment of efficient methods of cryopreservation for cotton cultivars and wild species, ethical, legal, and social guidelines for exchange of cotton germplasm resources at national and international level.

Monitoring changes in the rate of genetic erosion strictly requires directly comparable, if not identical, measures of the state of a system at several points in time. Alternatively, it is possible to measure the major agents of erosion (e.g., deterioration or destruction of habitat due to urbanization, land clearing, overgrazing, salinization, drought, climate change, etc.). However, such indirect measures are very broad and have other and possibly more profound impacts than causing loss of diversity (Brown 2008). It is also suggested that neutral or trivial changes could mask critical changes when summed over loci, genotypes, populations, or species. A temporal indicator should reveal and be most sensitive to the changes of concern and not be overwhelmed by relatively unimportant changes. For example, the loss of a few alleles at a highly polymorphic microsatellite locus is likely to be of trivial or no importance compared with the loss of disease-resistance alleles. An additional problem lies in stressing combinations of alleles: in sexual species, all multilocus genotypes are unique and ephemeral. Thus, when a claim is made that some percentage of distinct clones or genotypes have been lost from a region or a species, this is not necessarily genetic erosion. A reduction in population size and not increased recombination is the primary agent of erosion (Brown 2008).

Therefore, relevant measures of genetic erosion include some subjective assessments of the significance of any loss, based on expertise and local knowledge. The inclusion of such evaluative information in measuring erosion is desirable. The challenge is to format it in such a way that at least a tentative quantitative treatment is possible. Further research is also needed (1) on the use of GIS technology to monitor genetic diversity in cotton and to predict and minimize genetic erosion and (2) on the incorporation of the resulting information into comprehensive information systems. Additionally, it is also important to understand the nature and extent of possible threats to existing diversity on-farm and in situ. And further care must be given to the many accessions such as tree cotton species, wild species, and land races which do not receive enough attention or investment in terms of conservation research and development.

12.5 Gap Filling Strategies

Currently, there are only very general ideas about what portion of a cotton population needs to be maintained in order to conserve particular genetic traits. This information is crucial to the efficient design of in situ conservation projects in cotton. Monitoring various putative causative factors is clearly one possible approach to assess the risk of future genetic erosion within a gene pool in a given area. Once an association between genetic erosion and different causative and countervailing

factor(s) have been investigated in temporal and/or spatial comparisons, a predictive model could be constructed based on the assumptions that the association will continue into the future (Tripp and Heide 1996).

In general, solutions or mitigations for cotton genetic resources conservation have focused on ex situ conservation: seed banks, gene banks, and others. This approach allows genetic diversity to be maintained even if it is not currently represented in agricultural practice. In addition, studies on genetic research compare genetic diversity between modern and historic cultivars or progenitor wild plant species. This information helps to illuminate current or to predict future problems of genetic erosion, allowing an appropriate management response. However, in situ conservation of cotton genetic diversity could be an appropriate parallel conservation strategy, particularly for rare or endangered *Gossypium* species or those experiencing high mortality or rapid loss of habitat (Guerrant et al. 2004). Thus both ex situ and in situ methods are complementary, rather than alternative, conservation strategies (Rogers 2004).

The five tetraploid species and the cultivated diploid species *G. arboreum* and *G. herbaceum* are maintained ex situ with a considerable number of accessions, at least in eight major cotton world germplasm collections in Australia, Brazil, China, France, India, Russia, the United States, and Uzbekistan. The other 18 species of the secondary gene pool are preserved with a small number of accessions by most of these eight collections, but among the 25 species of the tertiary gene pool, five are not preserved in these banks, and two are represented by less than five accessions (Campbell et al. 2010).

Hence, what is needed to be further strengthened is the complementarity between seed conservation in gene banks (ex situ) and in ecosystems, and natural habitats (in situ). It is imperative to better manage cotton diversity in farmers' fields, develop strategies to protect, collect, and conserve its wild relatives that are under threat, support the use of a wider range of traits for cotton breeding and strengthen seed systems, especially those of locally adapted cotton cultivars. The main focus should be on strengthening the conservation and sustainable use of conserved cotton materials and the crucial linkages between them, through a combination of appropriate policies, use of scientific information, farmers' knowledge, and action.

Cotton cultivating countries need to establish or strengthen systems for monitoring genetic erosion, including easy-to-use indicators. Some examples of the proposed core indicators include number and kind of threatened and endangered species in cotton, number and kind of wild cotton relatives for in situ conservation, number and kind of protected areas for in situ conservation, number of in situ conservation sites and wild species conserved, number of species and accessions preserved ex situ, medium and long-term storage strategies, degree of genetic integrity of accessions preserved ex situ, and list of major environmental constraints to ex situ conservation. Support should be given to collecting farmers' varieties/landraces in particularly vulnerable or threatened areas, where these are not already held ex situ, so that these genetic resources can be multiplied for immediate use and conserved for future use. In some countries, the threat

of invasive alien species should also be considered, as these may contribute to genetic erosion and support should be provided to establish monitoring mechanisms at all levels. The World Information and Early Warning System (WIEWS) application for remote searching, updating, and reporting on genetic erosion (http://apps3.fao.org/wiews/wiews.jsp; accessed on 15th December, 2014) should also be strengthened with reference to cotton.

In conclusion, what can be done to improve genetic diversity in cotton while maintaining fiber yields and motivate a transition from high input high vulnerability monocultures to sustainable low input high-yield cropping systems? First, annual statistics of on-farm cotton genetic diversity should be collected, especially for the largest farms. These should be collected with relevant biotic and abiotic stress events to create a picture of performance and resilience. Second, on-farm diversity should be encouraged, perhaps by redirecting the subsidy program to support farmers transitioning to higher resilience farming practices with diverse numbers of cotton cultivars. As an example, natural brown and green fibers can be used from *G. barbadense* and *G. hirsutum* germplasm. This has dual applications: (i) production of eco-friendly colored cultivars and (ii) increasing the genetic diversity in the cotton field. Third, innovation strategies that promote long-term sustainability and yields, rather than peak quantity, should be introduced. This may require revising or inventing new intellectual property rights (IPR) instruments to maintain private sector incentives or a return to a public breeding and farm extension strategy that does not require capture of a revenue stream from licensing of IPR (Heinemann et al. 2014). It is further highlighted that addressing cotton genetic diversity with modern biotechnological tools, access and benefit sharing through appropriate IPR and biosafety guidelines, and bioethics on socioeconomic development also need additional attention.

Within the past decade the concept of biodiversity and their conservation has passed from the domain of academicians to the widespread attention of the common man. The general public and policy makers are ever more aware of the scope and seriousness of the fading of the genetic heritage. Although much of the debate focuses on animals and wild plant species, there is a growing recognition that the diversity of cultivated cotton species has vastly diminished, affecting the livelihoods of resource-poor farmers and threatening the future of fiber production and development. A number of proposals and policy initiatives are being discussed to address the problem, including preparations for a global plan of action for the conservation and use of cotton genetic resources (Abdurakhmonov 2014).

12.6 Conservation of Cotton Germplasm: Underexploited Treasure Available for Continually Reap the Benefits

Methods for cotton germplasm conservation are determined by a number of factors. One of the first factors to be deliberated when conserving cotton genetic diversity is the efficient and effective selection of the *Gossypium* genetic resources

(Fig. 12.3). Such invaluable and irreplaceable resources are (i) cultivated varieties (cultivars) in current use and newly improved varieties (ii) obsolete cultivars (iii) primitive cultivars (landraces) (iv) wild and weed species, near relatives of cultivated varieties, and (v) special genetic stocks (including elite and current breeder's lines, recombinant inbred lines, back cross progenies, doubled haploids, cytogenetic stocks, and mutants). Occasionally, genes, DNA fragments, and RNA derived from *Gossypium* are also included under the purview of genetic resources. The decision must focus that the selected accession is of sufficient importance to warrant active conservation and that the particular gene pool is not adequately conserved in the available cotton germplasm resources. While formulating strategies for such conservation, it is essential to know its areas of distribution and identify regions where both collection and conservation activities could effectively be initiated (Fig. 12.3). Such strategies should also consider any one or combination of the following: high levels of genetic diversity at the site(s), interest of the user community in the specific genetic diversity found at or believed to be found at the site, lack of previous conservation activities, and imminent threat of genetic erosion.

Hence, an ecogeographic survey is the first step in defining the most appropriate conservation strategy and *Gossypium* accession specific conservation objectives should be formulated, involving both ex situ and in situ components. The collection and analysis of ecogeographic data empower conservationists to make correct decisions on which taxa to be included in the target group, where to find these taxa, which combination of ex situ and in situ conservation to use, what sampling strategy to adapt, and where and how to store the germplasm. Since the ecogeographic data will rarely be sufficiently comprehensive to locate actual populations precisely, the preparatory element of conservation activities should be followed by field exploration (Fig. 12.3) during which the actual populations are located. For example, Central Institute for Cotton Research (CICR) at Nagpur, India has taken the initiative to collect and conserve the landraces of desi and perennial (tree types) cotton with desirable characters that are grown in the home gardens, foothills, and agricultural fields from Maharashtra, Madhya Pradesh, West Bengal, Andhra Pradesh, Mizoram, Meghalaya, Tripura, Gujarat, and Tamil Nadu. The important cotton landraces like *Ponduru, Karuganni, Commilla, Uppam,* and *Wagad* were collected from different states of India and conserved in the CICR cotton germplasm unit (Saravanan 2013). Similarly, collections and conservation from dooryard and other areas have also reported in Brazil (Menezes et al. 2014).

As discussed before, there are two primary complementary conservation strategies, ex situ and in situ and each of which includes a range of unlike techniques that can be implemented to achieve the aim of conservation of cotton genetic resources (Fig. 12.3). However, there is a great need to strengthen the conservation and sustainable use of *Gossypium* species and seed systems through a combination of appropriate policies, use of scientific information, farmers' knowledge, and action. Recently, it has become clear that the best strategy combines ex situ conservation with on-the-ground (in situ) conservation by farmers in their agroecosystems and in areas where *Gossypium* wild relatives are protected for their environmental value.

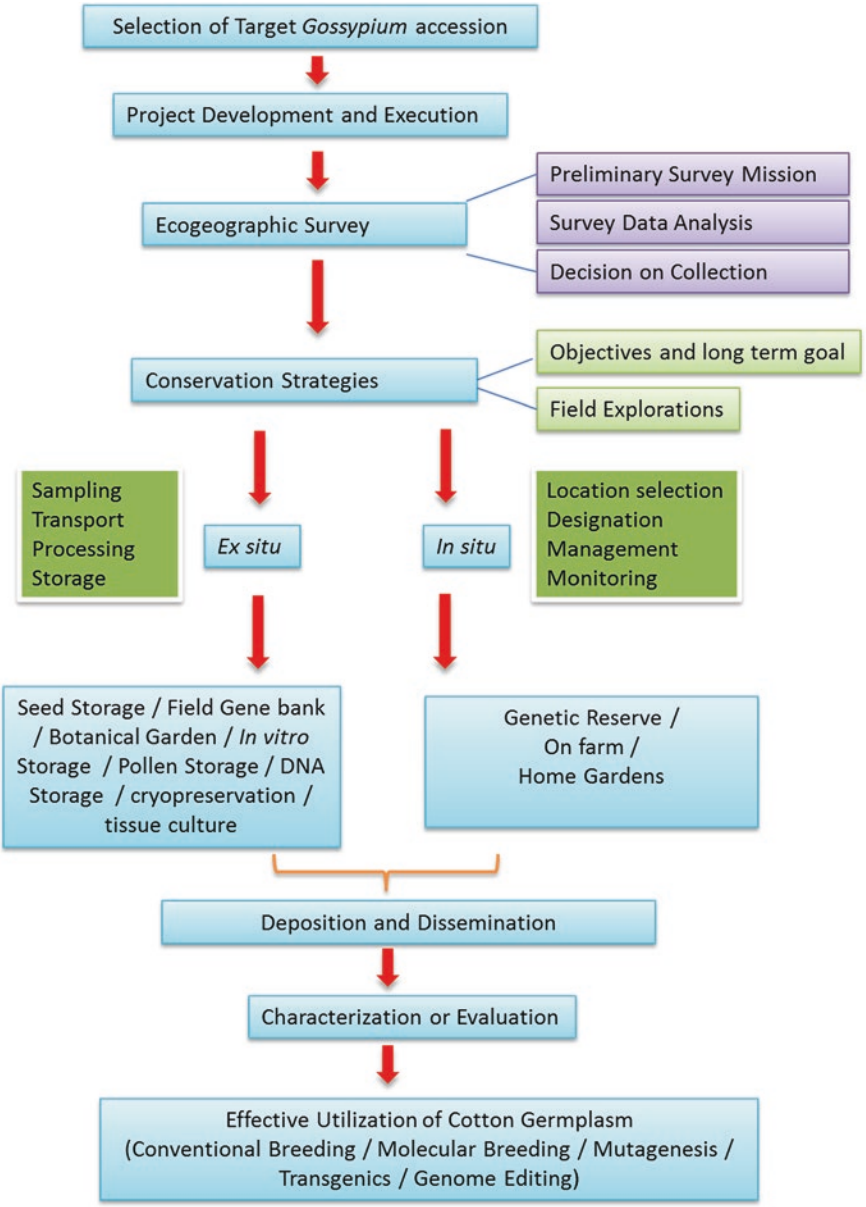

Fig. 12.3 Flow chart portraying the process of conservation of novel *Gossypium* accession and strategies for its effective utilization

In situ conservation enables cotton species to be conserved under conditions that allow them to continue to evolve. For some species, such as tree cotton species, it is the only feasible method of conservation. The main drawback

is the difficulty in characterizing and evaluating the crop's genetic resources and susceptibility to extreme weather conditions, pests, and disease (Tripp and Heide 1996). In contrast, conservation in ex situ gene banks ensures that the stored materials are readily accessible, can be well documented, characterized, and evaluated and are relatively safe from external threats. The ex situ conservation of genetic resources also allows the reintroduction of cultivars in areas where they have been lost. On the other hand, genetic adaptation and the rate of evolutionary response to selective forces of such stored cotton germplasm depend on inherent levels of genetic diversity present at the time a *Gossypium* species experiences a threat to its survival.

While such mechanisms are important, the sustainable use of plant genetic resources is equally essential because cotton genetic diversity increases options and provides insurance against future adverse conditions, such as extreme and variable environments and outbreak of new pests and diseases. The existence of variability is essential for breeding as much as for evolution and it must be present to obtain gains in selection. Therefore, conservation without use has little point. On the other hand, use without conservation means neglecting the genetic base needed by farmers and breeders in the future. To be of use, material held in cotton gene banks must be well-documented. This involves maintaining passport data, collection location, site characteristics, species, cultivar name, characterization data, recording highly heritable characteristics that can be used as a basis to distinguish one accession from another and evaluation data (documenting important traits such as yield, fiber quality parameters, phenology, growth habit, and reactions to pest, disease, and abiotic stresses).

If the material stored in cotton gene banks is to be used, it must be accessible with simple efforts. To this end, many cotton germplasm collections have established small subsets of collections, known as core collections, to facilitate research and use (Lacape et al. 2007; Boopathi et al. 2014, Tyagi et al. 2014). Development of core collections aim to include the maximum amount of diversity in a relatively small number of accessions, for example, a subset comprising 10 % of a collection is expected to contain at least 70 % of the total genetic diversity found in the whole collection. Another important method of widening the use of cotton genetic resources is through networks. Networks bring together all those with an interest in cotton genetic resources, including germplasm collectors, curators, researchers, breeders, and other users and provide a means for identifying the genetic resources within a cotton gene pool, and for taking collective action to conserve and use them.

The modern intensive cotton cultivation calls for uniformity in cotton cultivars that favors mechanization. Thus, the cotton production is limited by use of a smaller number of cotton cultivars and hybrids that possess uniform phenological traits and consequently has a narrow genetic base. Though the global cotton area has been increased during the past five decades due to advances in breeding efforts complemented with transgenic technology (Navarro and Hautea 2014), cotton fiber production is threatened by emerging problems such as sucking pest outbreaks, salinization of cotton cultivating area, unpredictable water stress, and so

on. To this end, discovery and utilization of new *Gossypium* diversity is imperative for sustainable cotton production (Boopathi et al. 2014). Similar kind of reports is also seen in other crop plants. It was estimated by FAO that about three-quarters of the genetic diversity of agricultural crops have been lost over the past century and a narrow genetic diversity exists in cultivated crop plants (FAO 2012). The natural 'genetic bottleneck' imposed by polyploid formation in cotton has been exacerbated by repeatedly crossing relatively few closely related genotypes to one another to breed new cultivars and using only a few cultivars to deploy transgenes. For example, an impending worldwide water crisis makes it important to identify adaptations that permitted wild cotton accessions to endure periodic drought and temperature extremes, restoring such valuable alleles that may have been left behind during domestication to create cultivars that produce more with less water.

Therefore, to sum-up, it is imperative to conserve genomic resources in cotton gene banks. Such resources provide valuable traits needed for meeting the challenges of adapting cotton varieties. An individual genotype with seemingly useless set of characters today may suddenly become essential tomorrow due to changing climatic conditions or outbreaks of disease. Today, we do not yet know everything about future demands for cotton cultivars. But we know the supply source and it has to be conserved with its full potential. Therefore, it is right to time to realize that let us "conserve all the cotton diversity we have."

12.7 Trends and Novel Tools in Cotton Germplasm Evaluation, Improvement, and Storage

Cotton diversity evaluation have traditionally been based on phenotypic characters evaluated on living plants managed in seed banks, field gene banks, botanic gardens or in situ reserves or based on dried plants managed in herbarium collections. In the recent past, several cotton germplasm conservation units are turning to DNA technologies to have effective conservation strategies. The DNA bank is an efficient, simple, and long-term method used in conserving genetic resource for biodiversity (Kelleher et al. 2005). Compared to traditional seed or field gene banks, DNA banks lessen the risk of exposing genetic information in natural surroundings. It only requires small sample size for storage and keeps the stable nature of DNA in cold storage. Since whole plants cannot be obtained from DNA, the stored genetic material must be introduced through genetic techniques (DNA Bank 2012). Currently, the plant taxonomy and systematic community have responded to the biodiversity crisis by defining three major challenges: (1) completing the inventory of life, (2) discovering evolutionary relationships through phylogenetic, and (3) providing information via the Internet. DNA collections can help with all three of those activities (Kelleher et al. 2005).

Therefore, DNA sequence analysis is useful in the identification and delimitation of species and higher taxa and is also set to become increasingly important via DNA taxonomy and DNA barcoding (Ronald 2014). Analysis of morphological,

chemical, and anatomical characteristics of cotton plant specimens can be used for assessment of genetic variation within and between species, but none of these can claim to offer the same potential as DNA. Genomic DNA samples represent the entire genetic component of the target organism. Therefore, together with the traditional techniques, DNA technologies offer great hope in cotton genetic resources and their diversity analysis.

A range of DNA-and RNA-based molecular markers, from restriction fragment length polymorphism (RFLP) to single nucleotide polymorphisms (SNPs) and insertion–deletion polymorphisms (InDels) and diversity array technology (DArT) are being employed in genetic diversity analysis in cotton. Boopathi (2013) tried to provide a collective depiction of relevant information about the usage of some commonly used markers in cotton and other agriculturally important crops, which help researchers to find out the frequentness and application of different markers and compare their results. Such markers may also serve as a platform and help the intellectuals for the selection and modification of their marker system in cotton diversity analysis. However, use of such markers in cotton genetic diversity analysis has both pros and cons. On the one hand, the designed markers can be well used in diversity studies and tetraploid cotton genetic mapping. On the other hand, the developmental efficiency of markers and polymorphism of designed primers are relatively low (Li et al. 2014a).

It is also equally important to increase the variation in the available cotton germplasm collections and scientists are developing new and more efficient breeding strategies that integrate genomic technologies and high throughput phenotyping to better utilize natural and induced genetic variation. Rapid developments in next generation sequencing (NGS) technologies over the past decade have opened up many new opportunities to explore the relationship between genotype and phenotype with greater resolution than ever before. As the cost of sequencing has decreased, breeders have begun to utilize NGS with increasing regularity to sequence large populations of plants, increasing the resolution of gene and QTL discovery and providing the basis for modeling complex genotype–phenotype relationships at the whole-genome level (Varshney et al. 2014). NGS technology is vitally important as a tool for characterizing cotton genetic resources globally. The vast majority of accessions found in the world's cotton gene banks are currently poorly characterized and as a result, rarely used. An international effort is essential to take advantage of the low cost and high throughput of NGS, in combination with development of appropriate database of information, large-scale phenotyping, and population development, to help characterize gene bank materials and to provide a rational basis for their utilization.

Breeders using marker-assisted selection (MAS) to introgress a favorable QTL allele from a wild or unadapted donor parent into an elite, adapted line often encounter the problem of linkage drag. The transfer of a large QTL region from a donor plant into a divergent breeding line may introduce undesirable phenotypic effects owing to the presence of linked genes in the introgressed QTL region. These linked genes often have nothing to do with the target trait but can make the new line unacceptable. NGS is vital for quickly identifying the individuals that

carry critical recombination breakpoints that break the linkage drag. Because the landraces that served as the breeding donors carried the favorable and the unfavorable alleles in coupling, it took a concentrated effort and deep sequencing within the target region on a large segregating population to identify a recombinant individual in which the linkage had been broken. In such cases, if the causal gene(s) and/or functional polymorphism(s) for the favorable and/or the deleterious trait(s) are known, the breeder can use that information to guide the selection of individuals that carry key recombination events to minimize the effect of linkage drag. Once a recombinant individual is identified, it becomes immediately useful as a donor in breeding and may serve to introduce new genetic variation into a breeding pipeline. Thus, NGS can be extremely helpful to identify the recombinants in breaking linkage drag and liberating new forms of genetic variation for use in cotton breeding. Overcoming linkage drag effects may lead to the achievement of varieties bearing *G. hirsutum* adaptability and *G. barbadense* fiber quality.

Specialized genetic stocks, such as bi-parental and multi-parent mapping populations, mutant populations, and immortalized collections of recombinant lines are being generated in cotton to facilitate mapping and gene function analysis via association studies and QTL mapping. Knowledge about the identity and map location of agriculturally important genes and QTL provides the basis for parental selection and MAS in cotton breeding (Boopathi 2013). Alternatively, genotypic and phenotypic datasets on training populations can be used to develop models to predict the breeding value of lines in an approach called genomic selection (Varshney et al. 2014). MAS and marker-assisted back-crossing (MABC) have been valuable for harnessing agriculturally and economically valuable genes and QTLs from wild or unadapted cotton genetic resources, particularly where the phenotype of a wild accession offers little or no insight about its potential value as a breeding parent. Prior to the advent of DNA markers, it was extremely cumbersome and inefficient to try to select for recombinant offspring from interspecific populations that carried the favorable wild allele(s) of interest because many unfavorable alleles that were also inherited from the wild donor typically masked the favorable phenotype. Genomics-assisted breeding has dramatically shifted the way breeders are able to work with unadapted genetic resources. The development of improved breeding lines for commercial cotton cultivation has traditionally been a time consuming and expensive task. With the deployment of genomics-assisted breeding, the generation of such lines is intended to become easier and faster. However, the major limitation is more expensive.

Genome-wide association studies (GWAS) utilize association mapping, also known as linkage disequilibrium (LD) mapping, to map QTLs by taking advantage of historic LD to identify statistically significant phenotype–genotype associations (Varshney et al. 2014). GWAS have been successfully performed in several crop plants, including maize, rice, wheat, soybean, sorghum, and foxtail millet. However, the role of GWAS in cotton has very limited information (Jia et al. 2014). In the future, it is speculated that the use of GWAS will enrich the gene pools of cotton by identifying useful variants that have only rarely been used in modern cotton genetic improvement programs.

Besides, advances in other fields of biotechnology have also generated new opportunities for cotton genetic resources conservation and utilization. Techniques like in vitro pollen culture, DNA banks, and cryopreservation have made it possible to collect and conserve genetic resources, especially of species that are difficult to conserve as seeds. Cryoconservation (storage in extreme deep freeze situations) is accomplished with liquid nitrogen at −196 °C (Hammer 2004). It is also suitable for seeds and leads to a dramatic prolongation of germination rates. It allows for an extremely long storage of many species. For in vitro maintenance cultures, it is the choice of preference because somaclonal variation can be prevented. The problem with cryoconservation is its high cost, especially for technical equipment. A constant supply of liquid nitrogen also has to be available at all times (Hammer 2004). DNA and pollen culture also contribute to ex situ conservation. Since, cotton has shown poor response to tissue culture protocol (Wilkins and Rajasekaran 2000), no single conservation technique can adequately conserve the full range of genetic diversity of a target species or gene pool. Greater biodiversity security can be obtained from the application of a range of complementary ex situ and in situ techniques, (Fig. 12.3) since one technique acts as a backup to the other techniques.

Similarly, recombinant DNA technology increased the possible use of distantly related trait carriers (sometimes completely unrelated, such as microbial and animal biological systems) as donors for the desired characteristics. However, the movement of genes across species boundaries presents many opportunities for both expected and unexpected risks. In addition to food safety issues related to cotton seed oil, other concerns involve ecological risks, such as new or increased resistance to insecticides and weed resistance to herbicides due to hybridization or excessive selection pressure, changes in the ecological competitiveness and the possible loss of genetic diversity in the transgenic areas (Ronald 2014). Transgenes conferring novel traits that enhance survival and reproduction may inadvertently disperse from cultivated plants to wild or weedy populations that lack these traits and might generate similar but unwanted effects in their weedy relatives through gene flow. There is a gain of fitness when *G. barbadense* plants are crossed to *G. hirsutum*, showed by a greater seed production of the hybrids when compared to the parents, and transgenic *G. barbadense* plants harboring *Cry1Ac* perform better than non-transgenic ones when exposed to *Alabama argillacea* or *Pectinophora gossypiella* pests. The in situ preservation of pure *G. barbadense* can be improved by reproductive isolation, not only from transgenics but also from crossings to traditional upland cotton. Reproductive isolation is not enough to in situ preservation of *G. barbadenses* in Brazil, since it is used as a medicinal or ornamental plant (Hoffmann et al. 2013). However, it is envisaged that careful deployment of transgenic technology can increase the cotton fiber yield as well as the genetic diversity.

A more recent technology, called genome editing, which makes it possible to precisely alter DNA sequences in living cells, is expected to lead to new crop varieties in the near future (Voytas and Gao 2014). In this technique, targeted double-strand DNA breaks are introduced in the genome at or near the site where a DNA

sequence modification is desired using sequence-specific nucleases. The repair of the break can be used to introduce specific DNA sequence changes, DNA deletions, or even serve as an insertion site for arrays of transgenes. Genome editing can thus be used to introduce genetic variation without transgenic technology and can even be used to recreate naturally occurring mutations into elite varieties of crops. For this reason, some scientists and farmers believe that crops generated through this technology will prove to be more socially acceptable elsewhere than those generated by genetic engineering. Genome editing has been successfully used to engineer rice for resistance to the bacterial pathogen, *Xanthomonas oryzae pv. oryzae*. Researchers created mutations in the promoter of a rice sucrose-efflux transporter gene, which is targeted by a pathogen effector (Voytas and Gao 2014). These mutations, which are mostly DNA deletions, eliminated the transcriptional induction required for pathogen virulence, rendering the plant resistant. However, the role of genome editing in cotton is yet to be demonstrated.

In general, plant breeders recognized three major gene pools based on the degree of sexual compatibility (Huynh et al. 2013). All crop species belong to a primary gene pool together with such material with which they produce completely fertile crosses through hybridisation. In contrast, all those plant groups that contain certain barriers against crossing belong to the secondary gene pool. The tertiary gene pool includes groups that can only be crossed with the help of radically new techniques. Plant breeders have traditionally emphasized closely related, well-adapted domesticated materials within the primary gene pool as sources of genetic diversity. More recently, however, recombinant DNA technology, plant transformation and genomics have led to a new quality which may be defined as a fourth gene pool or as a special case for the third gene pool. Such new tools of biotechnology allow us to bypass sexual incompatibility barriers altogether and introduce new genes into existing cultivars. It should be emphasized here that the major function of such technologies is not the creation of new cultivars but the generation of new gene combinations that can be used in cotton breeding programs.

To this end, crop multi-genotype breeding, which combines the advantages of both old and modern agricultures at the high level of productivity and sustainability, is considered as a promising strategy. The concept, necessity, principle, technical tactics, and characteristics of crop multi-genotype breeding are elucidated in detail and successful case of its application in cotton was documented (Li et al. 2014b). A multi-genotype hybrid variety, Jing-Mi 1 revealed superiority in seeded cotton and lint yields over the check variety in regional trial. Multi-genotype variety could be maintain and recover the genetic diversity in production system. Unlike a set of naturally diverse germplasm, multi-parent advanced generation intercross populations (MAGIC) population is tailor-made for breeders with a combination of useful traits derived from multiple elite breeding lines. The MAGIC populations also present opportunities for studying the interactions of genome introgressions and chromosomal recombination (Li et al. 2014b).

G. longicalyx is a diploid species of the secondary gene pool of *G. hirsutum* immune to the reniform nematode (*Rotylenchulus reniformis).* Synthetic tetraploid

triple-species hybrids have been crossed four to seven times to cultivated upland cotton, leading to the obtainment of plants bearing the resistance and indistinguishable to *G. hirsutum* plants under greenhouse conditions (Robinson et al. 2007). Similarly, advanced backcross populations were also constructed in cotton in which transgressive variation, the occurrence of progeny displaying phenotypes more extreme than either parent, was genetically dissected (Lacape et al. 2007).

12.8 Prospects for Sustainable Use of Genetic Resources in Cotton

The prime focus of the cotton breeders has now shifted to addressing problems due to climate change by developing resilient cotton cultivars. This can be achieved by effective utilization of cotton genetic resources and widening the cotton genetic diversity. Each of the three major approaches to increasing genetic diversity—mutagenesis, germplasm introgression, and transformation—have advantages and disadvantages. Interspecific germplasm introgression is particularly attractive in that it utilizes a broad germplasm base, can be targeted to one or more specific traits/genes or modulated to include thousands of genes/even entire genomes and is readily coupled to marker-assisted genome analysis and selection (Saha et al. 2006). Though, QTL mapping and MAS have potential applications in genetic improvement of cotton for higher productivity, their applications are not yet widely documented in cotton breeding program due to poor knowledge on physiological and genetic nature of fiber quality and productivity traits, low, and complex heritability of investigated traits, genotype X environment interactions, etc. (Lacape et al. 2007; Boopathi et al. 2011). Although introgression of genes across species boundaries is difficult, it is quite desirable because the gene pools of cultivated species do not contain all of the desired alleles. Alternatively, mutagenesis and transgenic technology has been proposed. However, currently they have limited applications due to several technical reasons such as nonavailability of novel genes, lack of efficient method to alter/transfer large genetic element, etc. (Wilkins and Rajasekaran 2000). Therefore, sustainable utilization of cotton genetic resources highly demands system-wide, regional, and global focussing programs with strong cooperation among stakeholders for the design, implementation, compliance, and utilization of cotton genetic diversity for breeding new cultivars.

Genetically improved seed, whether derived from conventional genetic modification or newly developed genomics technologies, must be integrated into ecologically based farming systems to maximize their impact on enhancing sustainable fiber production. For example, farmers cannot rely on seed alone to eliminate pests. For example, deployment of a ‘‘refuge strategy’’—creating refugia of crop plants that do not make *Bt* toxins—promotes the survival of susceptible insects and helps to delay the evolution of pest resistance to *Bt* crops. Whereas this approach has been successful in the US, where farmers are required to plant

refugia, failure to provide adequate refugia appears to have hastened pink bollworm resistance in India (Gujar et al. 2007). It emphasizes the need to deploy crop rotation and diversity to reduce the evolution of insect resistance. Well-funded, long-term, multinational, multidisciplinary collaborations are vital if we continue to make significant progress in developing new crop varieties to enhance cotton fiber production using cotton genetic resources.

12.9 Global Cotton Germplasm Data Management: Annotation, Curating and Dissemination

In order to realize the complete potential of cotton germplasm resources in the future breeding programs, it is essential to develop bioinformatic and database tools to assemble, analyze, and make the information useable to the cotton community. CottonDB (http://cottondb.org; accessed on 15th December, 2014) is one such comprehensive database that was established with the above said aim. Through a website interface, it provides genomic, genetic, and taxonomic information, including germplasm, markers, genetic and physical maps, trait studies, sequences, bibliographic citations. Similarly, the Cotton Portal (http://Gossypium.info; accessed on 15th December, 2014) offers the scientific community a single port of entry to participating Cotton Web resources. The Cotton Diversity Database (http://cotton.agtec.uga.edu; accessed on 15th December, 2014) provides for integrative queries relating to performance trial, phylogenetic, genetic, and comparative data and is closely integrated with comparative physical, EST and genomic sequence data, expression profiling resources and with the capacity for additional integrative queries. Cotton marker database (CMD; http://www.mainlab.clemson.edu/cmd/AboutUs.shtml; accessed on 15th December, 2014) provides centralized access to all publicly available cotton microsatellites and other markers available for genetic diversity analysis and it also contains a core set of markers that are useful for initial genetic diversity analysis in the given cotton germplasm. TropGENE-DB (http://tropgenedb.cirad.fr/en/cotton.html; accessed on 15th December, 2014) integrates a subset of published mapping data.

Besides, several project websites such as cotton functional genomics (http://cottongenomecenter.ucdavis.edu/; accessed on 15th December, 2014), cotton fiber genomics (http://www.cottongenomics.org/; accessed on 15th December, 2014), genetic and physical mapping (www.plantgenome.uga.edu; accessed on 15th December, 2014) the cotton microarray (http://cottonevolution.info/microarray; accessed on 15th December, 2014), Cotton Gene Indices (CGI) (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=cotton; accessed on 15th December, 2014) and Arizona Genomics Institute (http://www.genome.arizona.edu/; accessed on 15th December, 2014) are primarily used for disseminating genomic resources and coordinately distributed genomic resources to the cotton research community.

However, it is increasingly argued that there is a great need to expand bioinformatic infrastructure for managing, curating, and annotating the large amount

of cotton genomic information that will be generated in the near future as it is developed in other crops such as Arabidopsis Information Resource (TAIR, http://www.arabidopsis.org/; accessed on 15th December, 2014), Maize Genetics and Genomics Database (MaizeGDB, http://www.maizegdb.org/; accessed on 15th December, 2014), Soybase (http://soybase.agron.iastate.edu/; accessed on 15th December, 2014) and GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml; accessed on 15th December, 2014). Such cotton database should be able to host and manage information resources in cotton using community-accepted germplasm characterization, genome annotation, nomenclature, and gene ontology.

12.10 Concluding Remarks

A sustainable strategy to provide natural fiber security for a growing population must promote cotton genetic diversity conservation and avoid further habitat loss of natural ecosystems, since the future of cotton production depends on its genetic diversity. However, the greatest challenge facing the cotton community is not collecting and conserving the cotton genetic diversity per se but the conversion of such information to knowledge and utilizing them in routine cotton breeding program for sustainable fiber production in the coming years.

Genetic improvement of fiber production with the new sources of cotton germplasm will ensure that this natural-renewable product will be competitive with petroleum-derived synthetic fibers and reduce the environmental risks. Besides, such efforts will have several other practical ramifications that include increased water use efficiency, other abiotic and biotic stress tolerance/resistance, reduced fertilizer and pesticide requirements, expanded use as specialized fibers. Modifying cottonseed for food and feed could profoundly enhance the nutrition and livelihoods of millions of people in food-challenged economies. Countries that have rich cotton genetic diversity can take advantage of their genetic resources from locally adapted varieties and races and wild relatives of cotton to increase yields. This can be performed by applying biotechnological tools, by implementing bioprospecting activities, and by establishing partnerships with public and private sector institutions in industrial and developing countries. The strategy must also deal with issues of ethics, biosafety, and IPR in the use of new biotechnologies (Krishna et al. 2014).

We can promote cotton germplasm conservation literacy through the establishment of elementary and university curriculums that highlight the social, economic, biological, environmental, and ethical aspects of cotton germplasm conservation. We must also integrate training across scientific fields, including genetics, plant breeding, computer science, mathematics, engineering, biometrics, and bioinformatics and to evolve new forms of communication and professional organization. It is also equally important to have deep discussion with the policy makers, nongovernmental organizations and journalists by providing science-based information in more creative ways—for example, through social media and videography.

An engaged, informed public will help us to attain a sustainable cotton cultivation system derived from the available cotton genetic resources that can produce increased and quality fiber in a secure, sustainable, and equitable manner.

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