# Chapter 10
# *Ocimum basilicum* L. (Basil)


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## 10.1 Botany and Characteristics

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

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### *10.1.1 Morphological Characteristics*

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

### *10.1.2 Cytogenetics and Genome Size*

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

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

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

### *10.1.3 Volatiles and Aroma Profiles*

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

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

## 10.2 Breeding Basil for Commercial Use

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

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

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

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

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

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

### *10.2.1 Breeding Basil for Performance*

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

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

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

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

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

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

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

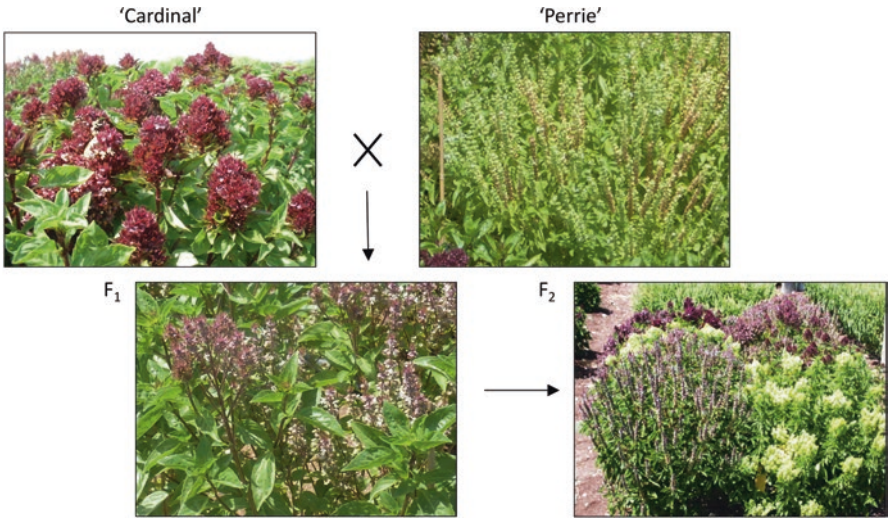


**Fig. 10.1** A cross between "Cardinal," a variety with compact inflorescence, purple bract, and calix and pink corolla, and "Perrie," a variety with a spike, green bract, and calix and white corolla. Images present parental lines, $F_1$, and several $F_2$ progeny phenotypes, demonstrating the wide-spectrum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

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

### *10.2.2 Breeding for Disease Resistance*

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

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

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

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

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

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

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

#### 10.2.2.1 Breeding for Resistance to Fusarium Wilt

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

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

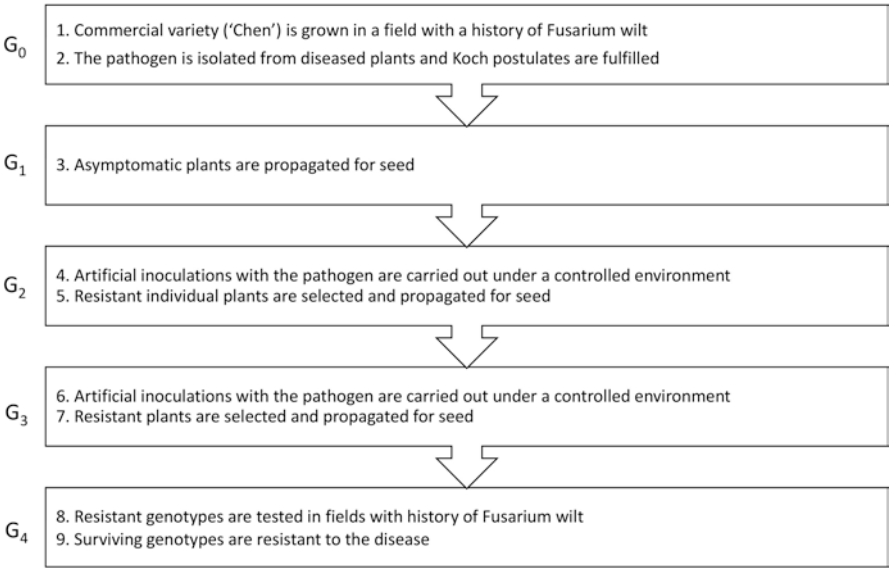


**Fig. 10.2** A schematic representation of mass selection for germplasm with resistance to sweet basil *Fusarium* wilt. $G_0$–$G_4$ represents germplasm generations in the selection process

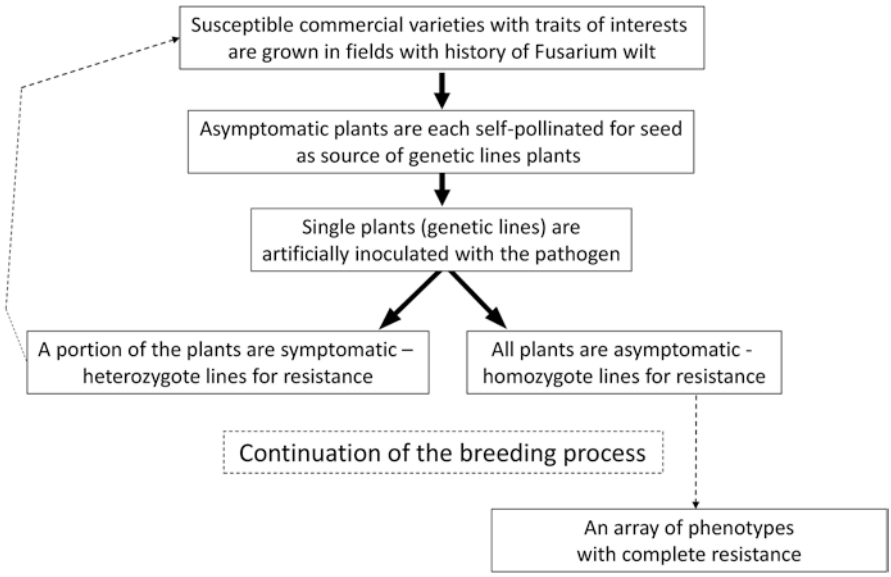


**Fig. 10.3** A schematic representation of line selection for resistance to Fusarium wilt of sweet basil. Dashed lines represent continuation of the breeding process

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

#### 10.2.2.2 Breeding for Resistance to Gray Mold

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

#### 10.2.2.3 Breeding for Resistance to White Mold

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

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

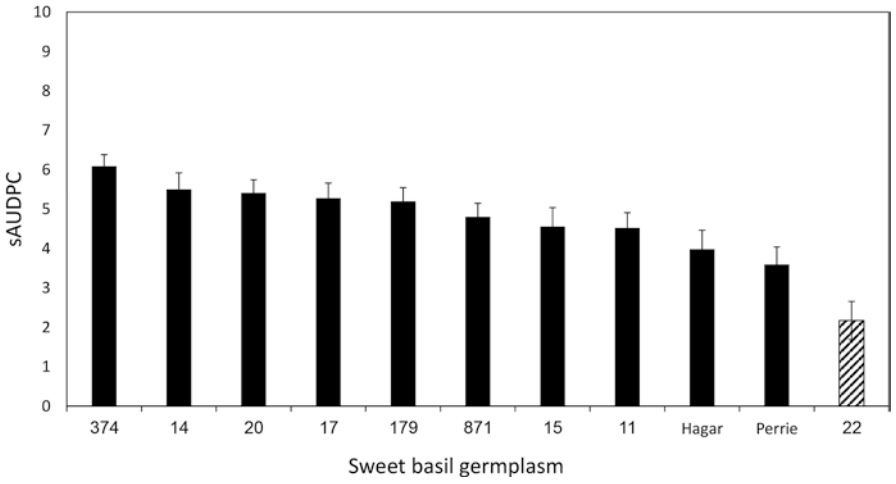


**Fig. 10.4** Standardized area under the disease progress curve (means AUDPC±SEM) scores depicting the response of two commercial sweet basil varieties and nine advanced select germplasm to infection by the white mold fungal pathogen *Sclerotinia sclerotiorum* in a trial carried out in 2011 at the Newe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

#### 10.2.2.4 Breeding for Resistance to Basil Downy Mildew

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

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

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

#### 10.2.2.5 Breeding for Resistance to Chilling Injury

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

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

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

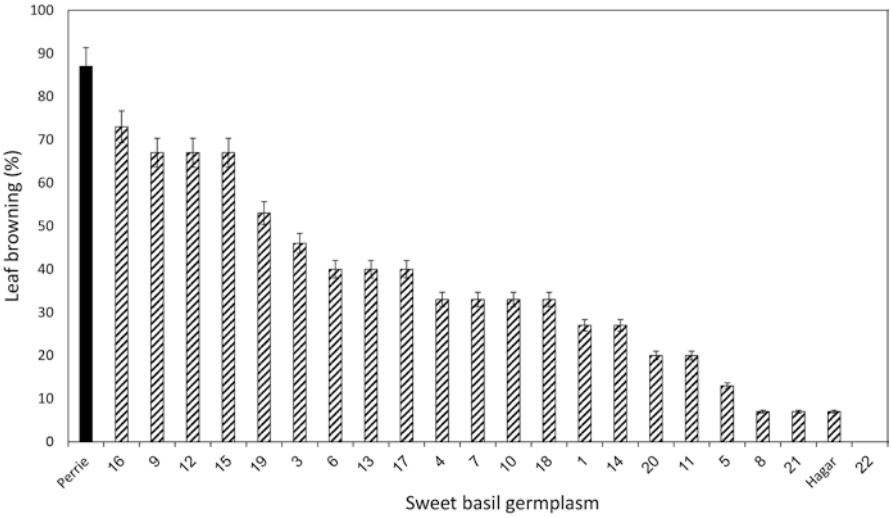


**Fig. 10.6** Leaf browning (mean % ± SEM) due to chilling injury in advanced selected germplasm depicting greater ($P \leq 0.05$) tolerance than the commercial variety "Perrie." The plants were exposed to multiple cycles of storage at 4 °C for 7 days followed by 2 days at 17 °C in the greenhouse. Among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

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

## 10.3 Breeding Basil for Flavor, Aroma, and Chemical Quality

### *10.3.1 Characteristics of Basil Chemical Quality*

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

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

### *10.3.2 Characterization of Germplasm Collections*

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

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

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

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

### *10.3.3 Experimental Crosses*

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

### *10.3.4 Biosynthesis Directs Chemical Diversity*

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

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

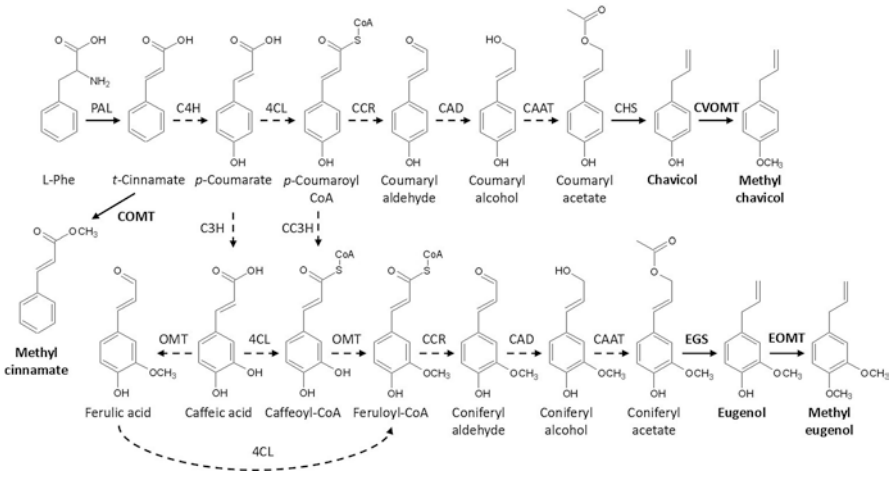


**Fig. 10.7** Proposed biosynthetic pathways for phenylpropanoid biosynthesis in basil. Solid arrows represent reactions demonstrated in basil. Bold enzyme names depict enzymes which their encoding genes where characterized from basil. Bold compound names depict volatiles found in basil essential oil.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

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

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

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

## 10.4 Crop Improvement by Transgenic Means

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

## 10.5 Summary

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

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