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Chapter 6: Safflower

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Abstract Safflower (*Carthamus tinctorius* L.) is an ancient crop with numerous past and present uses. Traditionally safflower was grown for its flowers, which were used as a fabric dye and for food coloring, flavoring, and medicinal purposes. Today, as a result of manipulation of well-characterized germplasm resources, it has become an important oil seed crop, bred for specialty niches through the development of healthier or more heat stable oil constituents, winter hardiness, and disease resistance. Molecular methodology has facilitated characterization of the world-wide diversity of safflower and identified geographical regions of similarity to assist breeders in the exploitation of available diversity. The development of molecular markers from expressed sequences should aid researchers in mapping genes of importance and reducing population size and generations required for the development of new varieties by using marker-assisted selection. Sequencing technology has established relationships among species of *Carthamus*, further aiding in the exploitation of diversity within the secondary gene pool. A coordinated, collaborative effort among safflower researchers in the development of marker-assisted characterization of global diversity would further increase the utility of available germplasm resources.

Keywords Safflower (*Carthamus tinctorius* L.) • Germplasm resources • Molecular methodology molecular markers • Sequencing technology • Global diversity • Germplasm resources

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1 Introduction

Safflower (*Carthamus tinctorius* L.) is an ancient crop with numerous past and present uses (Li and Mündel 1996). The Food and Agriculture Organization (FAO 2010) of the United Nations estimated the world safflower production at approximately 600,000 t with production in India being foremost, exceeding more than double that of any other country (Table 6.1). Traditionally safflower was grown for its flowers, which were used as a fabric dye and for food coloring, flavoring, and medicinal purposes. A brief and very interesting description of the spread of safflower throughout the ages is given by Weiss (2000). Weiss mentions references to safflower dating back almost four millenia, from florets in the tomb of Amenophis I (Scweinfurth 1887) in 1600 BC to a revenue-papyrus of Ptolemy II from around 260 BC indicating his monopoly of vegetable oils, including that of safflower (Keimer 1924). Its use as a dye is well known, but perhaps not so well known was the export of the dye from Egypt for the coloring of cheese in Italy, France, and England as early as the eighteenth century (Hasselquist 1762). Its use in Gloucestershire for coloring sausages and cheese was in such quantities as to have a purgative effect (Hanelt 1961). While synthetic dyes are now more common, the trend toward natural products may increase the value of crops such as safflower to accommodate food and textile industries.

Today, seeds are the major plant part used, resulting in a high-quality edible and industrial oil and bird feed (Knowles 1989; Bergman et al. 2007). Newer uses include specialty oil types to improve human diet (Velasco and Fernández-Maryinez 2004), biofuel (Bergman and Flynn 2009), and, because of the ease with which oleosin proteins are isolated from safflower seed (Lacey et al. 1998), production of transgenic pharmaceuticals (McPherson et al. 2004; Mündel and Bergman 2009). Singh and Nimbkar (2006) have provided an excellent review of safflower, including its history, cytogenetics, tissue culture, and breeding methodologies. At the time of their publication, however, little information was available on the molecular genetic diversity. More recently, Mündel and Bergman (2009) have published a review of safflower that covers genetic resources, major breeding achievements, crossing techniques, and new breeding technologies. This chapter discusses the present and future breeding objectives and focuses on the genetic diversity within safflower and molecular information that has become available in the past few years.

Vavilov (1951) proposed three centers of origin for safflower, which included India, Central Asia (Northwest India, Afghanistan, Tadjikistan, Uzbekistan, and Western Tian-Shan), and Abyssinia (Ethiopia and Eritrea). More important to modern plant breeding, however, may be Knowles' (1969) reference to "centers of similarity," which may be more indicative of types available for enhancement of specific traits. Furthermore, modern genetic techniques have placed some doubt on Vavilov's original proposals. The wild species of safflower native to Ethiopia has 32 pairs of chromosomes, as opposed to the 12 pairs in cultivated varieties, and thus, is not considered a center of origin as proposed by Vavilov (Knowles 1969). Ashri and Knowles (1960) included *C. tinctorius*, *Carthamus oxyacanthus*, and *Carthamus palaestinus* in their Sect. I based on chromosome number 12 and their ability to cross readily and produce fertile hybrids. *Carthamus arborescens*

Table 6.1 Annual production of safflower, the number of researchers listed, and genetic resources held by country

	Metric tons produced				
Country	2006	2007	2008	Researchers	Genetic resources (accessions)
India	228,600	240,000	225,000	24	9918
Mexico	73,536	113,334	96,413	3	1504
US	86,820	94,798	140,810	10	2484
Argentina	17,800	58,000	33,480	–	
Ethiopia	5,957	11,176	8,075	1	197
Kazakhstan	45,700	43,940	45,740	–	
China	30,000	32,000	32,500	3	7683
Tanzania	5,000	5,000	5,000	1	
Uzbekistan	3,257	3,500	3,500	–	
Canada	2,000	2,000	2,000	2	456
Australia	13,942	2,040	2,040	5	425
Iran, Islamic Republic of	500	500	500	3	
Spain	67	70	70	1	6
Russian Federation	130	40	90	–	429
Occupied Palestinian Territory	6	6	6	–	
Hungary	239	240	N/A	1	
Israel	–	–	–	2	
Morocco	–	–	–	–	
Pakistan	38	49	60	2	
Bulgaria	–	–	–	1	9
Germany	–	–	–	1	166
Romania	–	–	–	1	24
Slovenia	–	–	–	1	14
Switzerland	–	–	–	1	1
Bangladesh	–	–	–	1	
Egypt	–	–	–	5	
France	–	–	–	1	
Iraq	–	–	–	1	
Italy	–	–	–	3	
Kenya	–	–	–	1	
Korea	–	–	–	2	
Myanmar	–	–	–	2	
Nepal	–	–	–	1	
New Zealand	–	–	–	1	
Philippines	–	–	–	1	
Portugal	–	–	–		
Sudan	–	–	–	3	
Tajikistan	1,570	1,036	562		
Tekirda	–	–	–	1	
Kyrgyzstan	13,045	12,039	12,300		
Turkey	395	2,280	7,068	3	
UK	–	–	–	1	

Estimates of production are from the Food and Agriculture organization of the United Nations (http://faostat.foa.org/site/339/default.aspx). The number of researchers within country is based on Zhang and Johnson (1999) (IPGRI is now Bioversity http://www.bioversityinternational.org/)

and *Carthamus caeruleus* were also in Sect. I, but did not produce fertile hybrids. Ashri (1974) noted natural interspecific hybridization between *C. tinctorius* and *Carthamus tenuis* to occur when late planted cultivated safflower existed among the wild, unrelated wild species, but hybrids were sterile. He purports, however, that introgression probably occurred between the two species because, although the hybrids were sterile, they gave a greater mean number of bivalents than crosses of *C. tenuis* with either *C. oxyacanthus* or *C. palaestinus,* which are more closely related to cultivated safflower. Although *C. oxyacanthus* has been considered as the wild ancestor of cultivated safflower, Ashri and Knowles (1960), Garnatje et al. (2006), Bassiri (1977), and Chapman and Burke (2007) examined the phylogenetic relationships among 23 individuals of *C. tinctorius*, *C. oxyacanthus*, *C. palaestinus*, and *Carthamus gypsicola* using DNA sequence data from seven nuclear genes and found *C. palaestinus* to be more closely related to *C. tinctorius*. They thus propose *C. palaestinus*, which is native to the deserts of southern Israel and Western Iraq, as the wild progenitor of cultivated safflower. Sasanuma et al. (2008) examined 13 taxa of *Carthamus* using DNA sequence data from a nuclear gene and from an intergenic spacer region in the chloroplast. They also found *C. palaestinus* to be more closely related to *C. tinctorius* than any of the other species tasted, including *C. oxyacanthus*. Sehgal et al. (2009) using a multi-pronged DNA assay of RAPD, ribosomal DNA repeat unit length polymorphism, internal transcribed sequence (ITS) restriction fragments, and comparative sequence analysis of internal (ITS) and external (ETS) transcribed sequences, and Bowles et al. (2010) combining sequence and microsatellite data, also reached the same conclusion.

2 Breeding

Safflower, a diploid with 12 chromosome pairs (Ashri and Knowles 1960), is a predominately self-pollinating species, but has the potential for considerable outcrossing with pollen transfer by a variety of insects (Butler et al. 1966; Rudolphi et al. 2008). Moreover, the degree of outcrossing depends on genotype and environment. The thin-hulled trait has a pleitropic effect on anther dehiscence which deters pollen collectors, which prefer lines with normal hull morphology or anatomy (Rubis et al. 1966; Weiss 2000). High temperatures during pollination can reduce the time that pollinators spend collecting, which can decrease the amount of outcrossing (Ahmadi and Omidi 1997). Time to flowering is genetically controlled, but genotype and environment interact with day length, and flowering can be accelerated by high temperatures (Weiss 2000). Staggered planting of crossing blocks will ensure a timely source of pollen when stigma and pollen are ready among all desired genotypes. Cross pollination procedures are described in detail by Mündel and Bergman (2009) with excellent color images.

There are about 25 species of wild safflower divided by Ashri and Knowles (1960) into different sections based on chromosome number. Many of these are weedy, such as *C. oxyacanthus*, a noxious weed in the USA, complicating its

regeneration at the USDA Western regional Plant Introduction Station (WRPIS). Species with 12 chromosome pairs tend to cross readily. These include safflower (*C. tinctorius*), *C. persicus Desf. Ex Willd, C. oxyacanthus, and C. palaestinus*. The WRPIS has no *C. persicus Desf. Ex Willd* available, only 40 *C. oxyacanthus*, and just one *C. palaestinus*. *C. flavescens*, from areas of Turkey, Syria, and Lebanon, is entirely self-incompatible. *C. oxyacanthus*, indigenous from northwestern India to central Iraq, is a mixture of self-incompatible and self-compatible types. *C. palaestinus*, found in the desert areas of western Iraq, Jordan, and southern Israel, is a self-compatible species. Additional details concerning crossing safflower with wild *Carthamus* species can be found in Knowles (1989).

Historically, breeding objectives have included increased yield, increased or improved oil content, increased or improved protein, winter hardiness, disease and insect resistance, and the development of characteristics to facilitate hybrid production. Among the Crop Science registrations are materials representing some of the most significant advances in safflower germplasm (http://www.ars-grin.gov/cgi-bin/npgs/html/csr.pl?SAFFLOWER). The first registration was for Nebraska 10 (PI 572428) by J. Williams in 1964. It was described as an "early maturing, high-yielding variety," developed as a single selection from 852 to 895 by C.E. Classen at Alliance, Nebraska, USA in 1946. Knowles (1968) registered UC-1 (PI 572434), the first safflower with a fatty acid profile similar to olive oil; that is, 78% oleic and 15% linoleic. This was essentially the reverse of traditional, high linoleic safflower. Other notable contributors include germplasm registrations for rust, verticilium, fusarium, rhizoctonia, and phytophthora root rot resistance by C. Thomas, D. Zimmer, and L. Urie. H.H. Mündel and cooperators released three early developing cultivars and four germplasms for the Canadian Prairie. J. Bergman and cooperators have registered 13 cultivars, the most of any contributor. These include those developed for disease resistance, high oleic acid content, high linoleic content, and for bird and livestock feed. Oil and meal evaluations by Johnson et al. (1999) lead to work by Velasco and Fernández-Maryinez (2004) to register CR34 and CR-81, high alpha-tocopherol germplasm (Vitamin E). The release CR34 was derived from PI 304597 and CR81 from PI 406001.

A cooperative germplasm exchange with Li Dajue at the Beijing Botanical Garden in China in the late 1980s and early 1990s led to the first registrations of three winter hardy safflower lines, PI 651878, 651879, and 651880 (Johnson and Li 2008). These were developed by overwintering PIs 543995, 544006, and 544017 identified with overwintering capability; surviving plants were selected at Pullman, WA, over two cycles of selection.

Although unsaturated vegetable oils are considered most healthy, *trans*-fats resulting from partial hydrogenation of vegetable oils are widely considered detrimental to human health (Mozaffarian et al. 2006). The partial hydrogenation makes liquid vegetable oil solid at room temperature to increase shelf life and make vegetable fats for spreads and baking. Increased saturated fatty acid content, resulting in more viscosity, could reduce or eliminate the need for hydrogenation of vegetable oils for solidification. Hamdan et al. (2009) selected accessions based on their fatty acid profiles available in the Germplasm Resources Information Network

(GRIN: http://www.ars-grin.gov/npgs/) (Johnson et al. 1999) and developed safflower oil with high saturated fatty acid for potential applications in the food industry. Line CR-50 with high palmitic acid was developed from PI 306686 and CR-13 with high stearic acid was developed from PI 198990.

3 Disease

Mortensen et al. (1983) found both *Alternaria carthami* and *Alternaria alternata* to be problems in Montana, resulting in seed with inferior germination and seedling vigor. Patil et al. (1993) indicated diseases of safflower to be one of the most important constraints to production in both drought-prone areas and assured rainfall zones of India, with *Alternaria* spp. being the most damaging with losses recorded up to 50%. They conducted a 5-year study of 1,500 accessions from the world safflower germplasm collection under a grant from the US Department of Agriculture. They found accessions resistant to *A. carthami* Chowdhuri, *Cercospora carthami* Sund. and Ramak., *Ramularia carthami* Zaprom., *Erysiphe cichoriacearum* D.C., wilt caused by a complex of *Fusarium oxysporum* Sehl. Ex Fries and *Rhizoctonia bataticola* Bult. or *Rhizoctonia solani* Kuhn. The hybrids produced from crosses with a susceptible safflower indicated that resistance to all but the mildew from *E. cichoriacearum* D.C. was dominant. F_2 progeny were not tested because of the sheer numbers of plants involved. Singh et al. (2001) also found resistance to *F. oxysporum* to be dominant. However, F_2 progeny segregated in a ratio of 13:3, resistant to susceptible, suggesting the role of an inhibitory gene.

Urie and Knowles (1972) tested approximately 2,400 plant introductions and entries from both the USDA and commercial breeders for resistance to verticillium wilt (*Verticillium albo-atrum* Reinke and Berth.). They found 48 of those tested to have resistance. A search of GRIN of the National Plant Germplasm System (NPGS) found 33 accessions resistant to Fusarium, 30 resistant to *Verticillium*, 18 resistant to *Alternaria*, four resistant to *Sclerotinia*, and nine resistant to rust.

Thomas and Zimmer (1971) developed a safflower composite resistant to phytopthera root rot (*Phytopthera dreschsleri* Tucker) from selections from PI 250724 and PI 253538, from Portugal and Iran, respectively. Both PIs were segregating for resistance. Resistant greenhouse tested seed from homozygous-resistant plants were bulked. This accession (CSR-210) also shows high level of resistance to verticilium wilt, all known races of *F. oxysporum*, and rhizoctonia blight. Unfortunately, it is no longer available from the NPGS. Rubis (1981) developed the Arizona Wild Composite (AWC, PI 537682) by open pollinating the thin-hulled line A4138 with 12 Carthanus species (*Carthamus alexandrines, C. arborescens, Carthamus baeticus, C. caeruleus, Carthamus dentatus, Carthamus flavenscens, Carthamus glaucus, Carthamus lanatus, C. oxyacanthus, C. palaestinus, Carthamus syriacus*, and *C. tenuis*). The exact pedigree of the composite is unknown, but plant and seed characteristics indicate that most of the introgressive germplasm came from *C. flavescens* and *C. oxyacanthus*. Leaf, flower, and spine characteristics of the F_1

population also evidenced crosses to many of the other species. This accession is highly heterozygous and heterogeneous and varies in rosetteness, earliness, spininess, flower color, seed size, seed shape, seed color, hull type, hull percentage, and other characteristics. Thin-hull facilitated recurrent selection from this population with flood treatment resulted in several lines with resistance to root rot. PI 537690 exhibited 95% survival in a nursery that showed an overall 95% kill. These accessions and others developed from the AWC are available in GRIN.

Heaton and Klisiewicz (1981) developed a disease-resistant alloploid from a cross between *C. tinctorius* L. and *C. lanatus* L. The alloploid had 34 chromosomes, presumably 22 from *C. lanatus* and 12 from *C. tinctorius*, and the doubled haploid had $2n = 64$ chromosomes, the morphology of *C. lanatus*, and showed resistance to important safflower pathogens, including *Alternaria*, *Fusarium*, *Verticillium*, and bacterial blight. The alloploid is fertile and self-pollinates, but the sterility associated with non-homology of the majority of chromosomes prevents backcrossing to *C. tinctorius*. A breeding scheme effecting a translocation in an alien addition line of *C. tinctorius* needs to be achieved to introduce genes from *C. lanatus* into the cultivated *C. tinctorius*. To date, these authors could not find attempts to map genes responsible for any of the diseases affecting safflower.

4 Biofuels

Emphasis on renewable energy sources has kindled an interest in the role for oilseed crops in the production of biodiesel. A study begun in 2006 at Montana State University (Bergman and Flynn 2009) evaluated biodiesel prepared from sunflower, flax, soybean, canola, camelina, crambe, and both high-linoleic and high-oleic safflower oils. Safflower and sunflower oilseed crops produced the most gallons of oil and the most biodiesel per acre. They also had the lowest clod filter plugging point of the oilseed crops, and high-oleic safflower, along with soybean and high-erucic rapeseed biodiesel had the highest oxidative stability. Results of the study documented that safflower and sunflower grown in Eastern Montana could produce more biodiesel per acre than soybeans in the Corn Belt states.

5 Germplasm

Despite some useful breakthroughs in biotechnology allowing the tapping of tertiary gene pools (distant taxa) for genes with specific purposes, primary and secondary gene pools (same and related species, respectively) are still the most important sources of genetic variation for plant breeders. Germplasm collections worldwide provide genes for today's breeding efforts, while preserving other genes for future needs. Availability of genetic diversity is of limited use, however, without the identification and characterization of that diversity, so it can be exploited and applied in

an efficient manner. Transgressive segregation for quantitative traits, such as yield, in crop plants relies on the recombination of many different genes positively affecting that trait. Given the potential number of genetically distinct progeny from a single cross and the number of parents available for crossing, knowledge of parental characteristics and their relationship with one another is imperative. This is especially true when searching collections for useful traits such as pathogen resistance. The preservation of diversity of crop genetic resources remains as important today and for the future as it was in the past, as resources continue to be needed to meet future challenges associated with climate change, disease evolution, and the increasing needs of a growing population.

Germplasm collections remain a critical resource for development and improving safflower (*C. tinctorius* L.) cultivars and germplasm. Genetic resources are the essential raw materials needed for improving crops and for developing new, value-added uses. Safflower (*C. tinctorius* L.), with its numerous and varied uses (Li and Mündel 1996), has benefited from the diversity of genetic resources conserved and distributed by genebanks. A germplasm directory for safflower was compiled by Zhang and Johnson (1999) which documented 18 different collections in 14 countries. This publication can be found on the safflower web page (http://safflower.wsu.edu/). India reported the largest collections with nearly 10,000 total accessions held at both the National Bureau of Plant Genetic Resources in New Delhi (2,393 accessions) and the Project Coordinating Unit for Safflower in Solapur (7,525 accessions). Other significant collections are in China, Mexico, and the USA. The US safflower collection was developed starting in the late 1940s and is located at the WRPIS at Pullman, WA (http://www.ars.usda.gov/main/site_main.htm?modecode=53481500). It now includes more than 2,400 *C. tinctorius* accessions. The WRPIS is part of a national network of germplasm repositories that collectively make up the USDA-ARS NPGS. The US collection is represented by germplasm from more than 50 countries, and accessions are available to scientists worldwide. Table 6.1 lists world production by country (FAO 2010), an estimate of the safflower genetic resources held in that country (Zhang and Johnson 1999), and gives the number of researchers studying safflower.

6 Diversity

Numerous studies have been undertaken to assess the genetic diversity of global safflower germplasm. Most of these studies, prior to the 1990s were analyses of morphological and agronomic traits. The first large-scale evaluation of the world collection was initiated under a USDA PL 480 project at the Volcani Center, Beit-Dagan, Israel in 1966. Ashri (1971) evaluated nearly 2,000 lines for variation in reaction to *Erysuphe cichoracearum* D.C. (powdery mildew), *Puccinia carthami* Cda. (Safflower rust), the leaf spot diseases *R. carthami* Zaprom. and *C. carthami* Sund. and Ramak., and phyllody, which causes a reversion of florets to miniature branches with leaves and is caused by a mycoplasma. Ashri et al. found disease reactions to be associated with

geographic origin and speculated that this may be a result of selection pressure. There were also correlations with morphological characteristics.

Ashri et al. (1974) also studied variation in yield components from 903 lines from regions within the world collection. Of the three major yield components, heads per plant, seeds per head, and seed weight, heads per plant were found to be the most important and to range from an average of 14.8 in Iraq to 54 in Romania. Overall, there were significant differences in yield components of lines from different regions. However, because of mutual compensation among components, there were no significant differences among regions for yield.

Another large-scale study (Ashri et al. 1977) evaluated variation in oil content, iodine value, and their associations with morphological characters at three sites in the USA and one in Israel over a span of 12 years. More than 1,000 lines were evaluated, but not all of the lines were represented at each location. Oil content ranged from 16 to 38%, and high oil among local varieties was an indication of the progress from selection and breeding efforts. This early study showed divergence among regions for oil content, with lines from the Indian subcontinent, Iran, Afghanistan, and Egypt having the highest oil content, whereas those from Portugal, Spain, France, and Morocco the lowest. There was, however, extensive variability among local cultivars of various origins. Associations of morphological characters with oil content were evaluated to determine whether field identifiable traits could be used in breeding efforts for increased oil. Correlations differed within gene pools. The length of the outer involucral bracts (OIBs) was significantly and positively correlated with oil content in the Indian gene pool, but significantly and negatively correlated with Iranian lines. Yield per plant and yield components showed inconsistent correlation with iodine value. Correlation does not necessarily imply cause and effect and regional divergence of these characters may be a result of random association.

Regional evaluations, even on a smaller scale, are important to breeding efforts as genotype by environment interactions requires breeding for local conditions. Elfadl et al. (2010) examined 467 accessions from 11 geographical regions grown under organic farming conditions in Germany. Accessions were acquired from the USDA collection, the Vavilov Institute, and three other collections in Germany and exhibited considerable variability for all traits studied except lodging. Principal component and cluster analyses grouped accessions according to geographical regions. Accessions from the Americas, Africa, the Mediterranean, and West Central Europe formed one cluster, accessions from Central and South-Eastern Europe and Germany formed another, and those from Central Asia, South Asia, and East Asia each clustered distinctly. A study in Spain (Pascual-Villalobos and Alburquerque 1996) examined the suitability of 23 accessions for use as a dryland winter crop on the Mediterranean. They concluded that enough diversity existed among the accessions tested to provide an opportunity for selection in a breeding program for local conditions. Jaradat and Shalid (2006) examined phenotypic diversity in a subset of 591 salt-tolerant safflower accessions from the USDA collection. Their objective was to quantify phenotypic diversity among the accessions and identify salt-tolerant, high-yielding germplasm adapted to a short growing season, with a long rosette period and a high potential for biomass, seed, and dye production. They estimated 79

and 21% of the total diversity of the Middle East accessions was partitioned within and among populations, respectively, and were able to identify 87 accessions with traits adaptable to the growing conditions of Middle East.

Core collections from germplasm repositories attempt to represent the bulk of the genetic diversity in a manageable number of accessions. These cores are invaluable to breeders for initial screening for novel characteristics or disease resistance, where evaluation of the entire collection is impractical or prohibitively expensive. They can be based on geographical, morphological, and, more recently, molecular genetic diversity, or a combination of these characteristics. The USDA core collection of safflower consists of 210 accessions and represents about 10% of the total accessions held at the WRPIS (Johnson et al. 1993). An evaluation of oil and meal characteristics of 203 core and 797 non-core accessions (Johnson et al. 1999) revealed that the core was not fully representative of the non-core accessions, but they did capture a large fraction of the diversity in oil and meal factors present. The mean oil content of the non-core accessions was significantly higher ($P<0.05$) and was likely because of the presence of the numerous improved lines in the non-core accessions. The core had higher mean palmititc acid, stearic acid, and cathartic phenolic glucosides, but lower α-tocopherols and bitter phenolic glucosides. The range in oil content between the core and non-core accessions was similar. Analysis of variance of regional means resulted in highly significant F-ratios, but the variance within regions was also significantly different, which may have complicated results. The highest mean percentage oil was from accessions from the Americas, which, again, was likely due to the improved lines included in that region. This also resulted in low linoleic acid and high oleic acid means from the Americas. In some, but not all cases, oil and meal factors were differentiated between regions.

The USDA core collection was also evaluated for seven quantitative traits (Johnson et al. 2001). The results showed that for each factor measured, there was a considerable variation among accessions, indicating that the core collection was highly diverse. Comparison among regions were not significant for either OIB length or yield, but were significant for OIB width, head diameter, days to flower, plant height, and weight per seed. Accessions from SW Asia were the most distant from other regions, but S. Central Asia and East Africa grouped together.

Dwivedi et al. (2005) developed a core collection of 570 accessions based on geographic information and 12 morphological descriptors on 5,522 accessions held in India. Approximately 10% of the accessions were randomly selected from each of 25 clusters derived from the analysis of the morphological characters. Accessions from South Asia and Southeast Asia accounted for almost 80% of the accessions in the core, reflecting their predominance in the collection as a whole. The remaining accessions were from the Americas, Mediterranean, Europe, West Asia, Australia, the former USSR, and Africa. Mean comparisons and frequency distributions indicated that the variation of the entire collection had been preserved in the core subset.

The abundance of genetic variability held in world collections, and the regional divergence within them can yet be exploited to produce even more variability through recombination.

Molecular markers can be used for identifying duplicate accessions, developing and testing special groups within collections (such as core collections), estimating

and comparing diversity among countries or regions, and identifying acquisition needs and in genetic mapping. Bassiri (1977) was able to uniquely identify 14 cultivars of safflower and nine ecotypes of the wild *C. oxyacanthus* using isozyme analysis of the acid phosphatase and the cathodal peroxidase systems. Carapetian and Estilai (1997) examined 20 safflower cultivars with nine enzymatic systems. Five of the enzymes were monomorphic and four were polymorphic. Selfed progeny revealed a three-banded marker system for menadione reductase, indicating that this was a dimeric enzyme with more than one homozygous locus. Zhang (2001) characterized 89 safflower accessions from 17 countries with isozymes. Seven polymorphic loci revealing 15 polymorphic alleles classified the accessions into four major groups, but there were no clear regional associations among the groupings.

Methods using markers revealed by the polymerase chain reaction (PCR) have more recently been reported. Sehgal and Raina (2005) characterized 14 Indian safflower cultivars using RAPD, simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP). AFLP markers were found to be the most efficient system in their study, with two primer pairs sufficient to genotype the cultivars. Yang et al. (2007) examined genetic relationships among 48 safflower accessions from 32 countries using inter-simple sequence repeat (ISSR) markers. Twenty-two primers revealed 355 polymorphic bands and uniquely distinguished all accessions. Relationships were closer among accessions from the same continent.

Johnson et al. (2007) used AFLP markers to characterize 96 accessions from the USDA collection representing seven world regions (the Americas, China, East Africa, East Europe, the Mediterranean, South Central Asia, and Southwest Asia). Regions differed in all pair-wise comparisons using a bootstrap procedure comparing distances within and among populations. There was a weak but significant correlation of the AFLP matrix with a phenotypic data matrix with 16 attributes consisting of oil, meal, and growth characteristics ($r=0.12$, $P= 0.05$). This weak correspondence between molecular and phenotypic data underscores the need for both types of characterization to enhance management and utilization of germplasm.

Chapman et al. (2010) also conducted an analysis of accessions representing geographic centers of similarity using a suite of 24 microsatellite markers developed from expressed sequence tags (EST) and a pair of chloroplast markers. They analyzed 70 accessions with 4–8 accessions belonging to each of ten putative centers of similarity (Ashri 1975). The centers are (1) the Far East, (2) the Indian subcontinent, (3) Iran/Afghanistan, (4) Israel/Jordan/Iraq/Syria, (5) Turkey, (6) Egypt, (7) Sudan, (8) Kenya, (9) Ethiopia, and (10) Morocco/Spain/Portugal/France. American accessions were not included in their primary analysis, as these are considered secondary introductions. A posteriori analysis of the molecular data using the software STRUCTURE (Pritchard et al. 2000) actually placed the accessions into five well-defined groups: (1) Europe, (2) Turkey/Iran/Iraq/Afghanistan, (3) Israel/Jordan/Syria, (4) Egypt/Ethiopia, and (5) the Far East/India/Pakistan/Sudan.

Many of these accessions were also represented in the AFLP analysis of Johnson et al. (2007). Re-analysis of their data excluding the American accessions revealed strikingly similar results, but with several differences. STRUCTURE analysis using the technique of Evanno et al. (2005) placed the 80 accessions into eight likely groups (Table 6.2, Figs. 6.1 and 6.2). Afghanistan accessions formed a unique

Table 6.2 A list of the accessions associated with the eight groups designated by analysis using the software STRUCTURE. The country of origin is followed by the plant introduction (PI) number

Afghanistan	Afghan220647	Middle East	Kenya209296
	Iraq253759		Syria181866
	Afghan253908		Kazakhstan262444
	Iran380800		Iran250833
	Kuwait286199		Iran405984
	Afghan304595		Turkey251984
	Afghan268374		Italy253523
			Turkey301048
Europe	Romania209287		Turkey407624
	Bulgaria253531		India306974
	Hungary312275		Ukraine369848
	Hungary253541		RussianFed369849
	Poland253544		Israel226993
	Spain239226		Pakistan304408
	Poland311738		Africa262437
	Poland253543		
	Spain613465		
Egypt/Sudan	China506427	India	Syria386174
	India260637		India279051
	Kazakhstan314650		Kazakhstan305540
	China544041		Africa209289
	Sudan237547		Sudan237548
	Sudan237549		India283764
	Sudan305531		India248808
	China544052		India451956
	Sudan305529		India199889
	Egypt306613		India307055
	Egypt250537		Bangladesh401479
	Kenya209295		Kenya209297
	Sudan305534		Kenya209300
	Africa262438		India562638
	China514630		
	Turkey304503		
Pakistan	Pakistan259992	Ethiopia	Ethiopia193473
	Greece254976		Ethiopia262433
	Sudan271070		Ethiopia257582
	Hungary253540		Eritrea273876
	Iran406015		Syria262430
	Tajikistan369847		
	Pakistan248625	China	China543995
	Pakistan250202		China544006
	Pakistan426523		China544028
	Iran251398		China544033

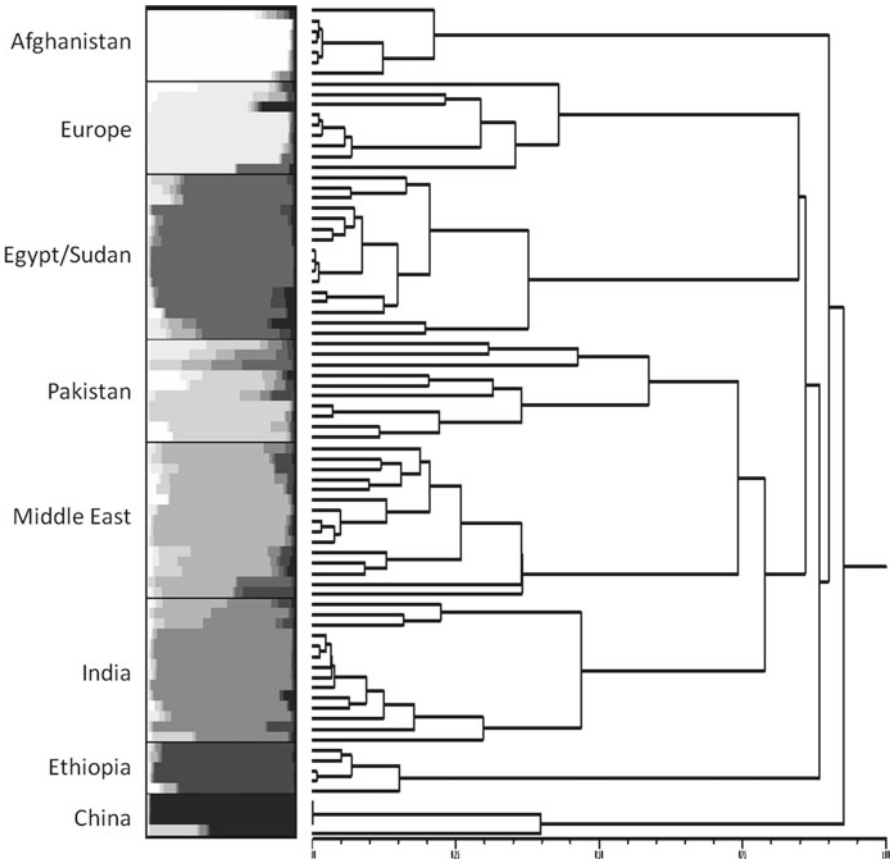

Fig. 6.1 Dendrogram of 80 safflower accessions from Johnson et al. (2007) showing eight distinct groups as evidenced by analysis using STRUCTURE. The tree was constructed based on the proportions of an individual's alleles belonging to a particular group

group, as did China and Ethiopia. Although there was some mixture, which would be inevitable given the amount of germplasm exchange that must have taken place in the past, the eight groups could be relatively distinguished as (1) Middle East, (2) Egypt/Sudan, (3) Ethiopia, (4) Afghanistan, (5) Europe, (6) India, (7) Pakistan/Iran, and (8) China.

In contrast to SSR markers, AFLP markers are biallelic and dominant. Although less informative at a locus, they allow for the efficient sampling of many loci (Powell et al. 1996; Gaudeul et al. 2004; Greene et al. 2008). Thus, AFLPs lend themselves to studies in which more loci are needed to estimate diversity because genomic heterogeneity is high (Mariette et al. 2002). Despite being dominant markers, AFLPs have shown themselves effective in discriminating among populations and correctly assigning individuals to populations, compared with SSRs (Gaudeul et al. 2004; Woodhead et al. 2005). Recently, Chapman et al. (2009)

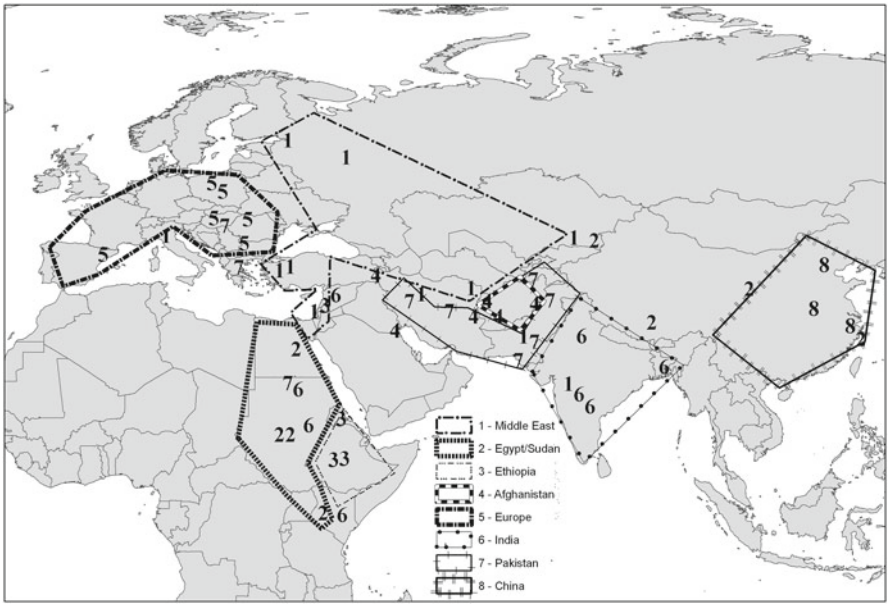

Fig. 6.2 Geographic representation of the 80 safflower accessions from Johnson et al. (2007) showing eight distinct groups as evidenced by analysis using STRUCTURE

developed a set of microsatellite primers from EST, some of which were used in their analysis described above. Microsatellites derived from ESTs have the unique characteristic of being associated with expressed genes, and may be more indicative of actual genetic differences than random markers. It is important to remember that, although random markers are effective at defining divergence, the association of markers through random drift and adaptation are separate processes (Holdregger et al. 2006). Another molecular marker with characteristics of both EST-SSRs and AFLP is the Target Region Amplification Polymorphism (TRAP) (Hu and Vick 2003). Although producing semi-random markers at multiple loci, TRAP markers can be designed to explore specific types of genes (Miklas et al. 2006). Regardless of the type of molecular marker used, more characterization of safflower with molecular markers from diverse world sources is needed to enhance germplasm management and utilization.

Although there have been numerous studies of genetic diversity in safflower using molecular markers, they share a common feature. Few, if any, studies of genetic diversity can be directly compared or compiled. One of the reasons may be due to the fact that most studies are limited to a few accessions or to accessions from a limited area of interest. As a consequence, after publication, the marker data may be lost or forgotten. Because only a small percentage of the world safflower collection is ever analyzed at a given time, and with different marker systems, or with different marker loci within a given system, collation on a world-wide scale is not possible. Kisha and Ryder (2006) make a case for organized development of common markers for diversity analyses within a given species. A subset of microsatellite

primers, AFLP primers, or designated set of other types of markers for universal use would allow data to be stored for posterity and used to generate comparisons for future marker studies. Virtual cluster analyses based on the comparison of new accessions to a complete database of accrued marker information would result in savings of both time and money. Relationship queries can be adjusted to filter data based on geographical regions, environments, latitude, etc., much as descriptor data are available through germplasm banks. Because of the somewhat imprecise nature of naming markers based on fragment size, the database would need to be curated, by a center or collaborating centers within a network responsible for a particular species. Collaborators need to define a core set of primers for each marker type, covering the genome randomly and uniformly and provide a number of "reference" accessions with defined markers so that virtual analysis could be anchored, and images defined of the expected marker pattern with monomorphic and polymorphic bands. The benefits for the conservation and use of genetic resources that can be drawn from available molecular data are almost limitless. The construction of a universal molecular database as a common platform for storage and analysis of genetic resources marker data could greatly enhance the utility of germplasm on a global scale. Its development may seem like a daunting task, but it can come to fruition by the construction of locally created databases developed through collaborative efforts among members of germplasm conservation centers and researchers.

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