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CHAPTER 6

Safflower (*Carthamus tinctorius* L.)

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CONTENTS

6.1 INTRODUCTION

Safflower (*Carthamus tinctorius* L.) — an oilseed crop — is a member of the family Compositae or Asteraceae. Carthamus is the latinized synonym of the Arabic word *quartum*, or *gurtum*, which refers to the color of the dye extracted from safflower flowers. The English name *safflower*probably evolved from various written forms of *usfar*, *affore*, *asfiore*, and *saffiore* to *safflower*. Safflower has been grown in India since time immemorial. It is mentioned as *kusumba* in ancient scriptures. Presently, in India it is most commonly known as *kardai* in Marathi and *kusum* in Hindi. In China it is known as *hong hua*.

Safflower, a multipurpose crop, has been grown for centuries in India for the orange-red dye (carthamin) extracted from its brilliantly colored flowers and for its quality oil rich in polyunsaturated fatty acids (linoleic acid, 78%). Safflower flowers are known to have many medicinal properties for curing several chronic diseases, and they are widely used in Chinese herbal preparations (Li and Mundel, 1996). The tender leaves, shoots, and thinnings of safflower are used as pot herb and salad. They are rich in vitamin A, iron, phosphorus, and calcium. Bundles of young plants are commonly sold as a green vegetable in markets in India and some neighboring countries (Nimbkar, 2002). Safflower can be grazed or stored as hay or silage. Safflower forage is palatable, and its feed value and yields are similar to or better than those for oats or alfalfa. Thus, each part of safflower has a value attached to it. Safflower has high adaptability to low moisture conditions. Therefore, its production all over the world is mainly confined to areas with scanty rainfall. *Carthamus* has 25 species, of which only *C. tinctorius* is the cultivated type, having 2n = 24 chromosomes. Though the crop has tremendous potential to be grown under varied conditions and to be exploited for various purposes, the area under safflower around the world is limited largely due to the lack of information on its crop management and product development from it.

The research and development on different aspects of safflower, despite its adaptability to varied growing conditions with very high yield potential and diversified uses of different plant parts, have not received due attention. This probably is the main reason for its status as a minor crop around the world in terms of area and production, compared to the other oilseed crops. However, interest in this crop has been rekindled in the last few years due to three major reasons:

1. A huge shortfall in oilseed production in countries having a sizable area with scanty rainfall, to which safflower is most suited.
2. The preference of consumers for healthy oil with less amounts of saturated fats, for which safflower is well known.
3. The medicinal uses of flowers in China and extraction of edible dyes from flowers have become more widely known.

The present chapter deals with distribution, production, utilization, species, genomic relationships, classification, germplasm resources, genetics, breeding, and biotechnology of safflower.

6.2 DESCRIPTION AND CROP USE

6.2.1 World Distribution and Production

Traditionally, safflower has been grown for centuries from China to the Mediterranean region and all along the Nile valley up to Ethiopia (Weiss, 1971). Presently it is grown commercially in India, the U.S., Mexico, Ethiopia, Kazakhstan, Australia, Argentina, Uzbekistan, China, and the Russian Federation. Pakistan, Spain, Turkey, Canada, Iran, and Israel also grow safflower to a limited extent. Safflower acreage and production around the world have witnessed wide fluctuations in the past. Safflower seed production in the world rose from 487,000 MT in the year 1965 to 1,007,000 MT in 1975, and subsequently it decreased to 921,000 MT in 1985 (Anonymous, 2002). Mexico was the largest producer of safflower in the world until 1980, when it occupied an area of 528,000 ha with a production above 600,000 MT in the year 1979–1980. However, the area and production of safflower in Mexico decreased significantly in later years, becoming only 10% of the area and production recorded for the year 1979–1980 (Cervantes-Martinez, 2001). Commercial production of safflower in the U.S. was started in the 1950s, and the area rapidly increased to 175,000 ha mainly in the states of California, Nebraska, Arizona, and Montana. Presently it is grown over an area of 100,000 ha (Esendal, 2001). Safflower in China is presently occupying an area ranging from 35,000 to 55,000 ha, producing 50 to 80 MT seeds annually. Xinjiang is the largest safflower producer state, which accounts for 80% of total safflower production in China. Other safflower-producing states in China are Yunnan, Sichuan, Henan, Hebei, Shandong, Jiangsu, and Zhejiang (Zhaomu and Lijie, 2001). Presently, India is the largest producer of safflower in the world, followed by the U.S., Mexico, and China. The safflower area in India in the year 2004–2005 was estimated to be 387,000 ha, with a production of 154,000 MT of seed (Anonymous, 2004–2005). In India, Maharashtra and Karnataka states account for 72 and 24% of safflower area and production, respectively. The other safflower-producing states are Andhra Pradesh, Orissa, Madhya Pradesh, Chattisgarh, and Bihar. Safflower production in India is mostly confined to rain-fed conditions during winter.

6.2.2 Utilization

Safflower in India since time immemorial has been grown apart from orange-red dye extracted from its brilliant florets, for getting high-quality edible oil rich in polyunsaturated fatty acids, which helps in reducing the cholesterol level in blood. Safflower oil is nutritionally similar to olive oil, as it contains high levels of linoleic or oleic acid. The monounsaturated fatty acid like oleic acid is also known to reduce low-density lipoprotein (LDL; bad cholesterol) without affecting high-density lipoprotein (HDL; good cholesterol) in blood (Smith, 1996). Safflower oil is highly stable, and its consistency remains the same at low temperatures, thereby making it suitable for application in frozen/chilled foods (Weiss, 1971). Safflower oil is also better suited to hydrogenation for margarine production than are soy or canola oils (Kleingarten, 1993). Safflower oil is nonallergenic, and therefore suitable in injectable medications (Smith, 1996). Safflower is considered to be ideal for cosmetics and is used in 'Macassar' hair oil and Bombay 'Sweet Oil' (Weiss, 1971). Safflower oil is a preferred for the paint and varnish industry owing to its specific properties of absence of linolenic acid, presence of high linoleic acid, low color values, low free fatty acids, low unsaponifiables, and no wax, which make the quality in paints, alkyd resins, and coatings beyond comparison (Smith, 1996). However, with the development of cheaper petroleum products and a shift to water-based paints, the use of safflower oil in the paint and varnish industry has been reduced drastically in recent times.

In India, safflower oil is also used for lighting and manufacture of soap and to waterproof leather buckets (Weiss, 1971). Additionally, it is used to prepare roghan, which is used to preserve leather and as a glass cement. Charred oil is used for healing sores and in rheumatism (Weiss, 1971). It is also used in infant foods and liquid nutrition formulations.

In addition to seed, safflower has been known and grown since ancient times for its brilliantly colored flowers, which were used to extract yellow and orange dyes for food and fabrics. With the advent of cheaper synthetic dyes like aniline, use of safflower flowers as a source of edible color gradually decreased to zero during the 20th century. However, recently interest in safflower flowers as a source of color for use in food is gaining importance owing to a recent ban on the use of synthetic colors in food in the European countries and elsewhere. The flowers are also reported to have medicinal properties to cure several chronic diseases, like hypertension, cardiovascular diseases, arthritis, spondylosis, and sterility in both men and women. Detailed information about clinical uses of safflower flowers has been given in the monograph on safflower written by Li and Mundel (1996). China produces approximately 1800 to 2600 MT of flowers annually to use them for extraction of dyes and in medicinal preparations (Zhaomu and Lijie, 2001). Flowers of nonspiny cultivar NARI-6 and nonspiny hybrid NARI-NH-1 have been reported to be rich in protein (10.4 and 12.86%), total sugars (7.36 and 11.81%), calcium (558 and 708 mg/100 g), iron (55.1 and 42.5 mg/100 g), magnesium (207 and 142 mg/100 g), and potassium (3992 and 3264 mg/100 g), respectively. All essential amino acids except tryptophan were present in safflower flowers (Singh, 2005a). A pleasant-tasting tea made with safflower flowers as its main ingredient has been developed in China (Li and Yuanzhou, 1993) and India (Singh, 2005a).

With the commercialization of flowers as herbal health tea, extraction of dyes from them, and their use for medicinal purposes, the monetary returns to farmers from both seed and flowers are expected to grow to the extent of 141% of the monetary returns presently earned from the harvesting of seed alone (Sawant et al., 2000). Therefore, commercialization of safflower flowers will make the crop most remunerative among the crops grown in the winter season. This would certainly reverse the declining trend in area and production of safflower in India.

Safflower oil meal is mainly used as animal feed. Safflower cake has the potential to be used as a human food if the bitter principles are removed (Nagaraj, 1995). Safflower cake in combination with all-purpose flour in 1:3 proportion was found to be highly suitable for manufacturing of protein-enriched biscuits with 22% protein in them (Singh and Abidi, 2005). Safflower leaves are rich in carotene, riboflavin, and vitamin C, and hence young seedlings and prunings are used as a green leafy vegetable in safflower-growing areas in India. Another important and interesting use of safflower seed has recently emerged by means of its genetic modification to produce high-value proteins as pharmaceuticals and industrial enzymes. SemBioSys — a Calgary-based (Canada) company — transforms safflower tissue genetically in order to get the proteins of interest to accumulate in the seed of the mature transgenic plant (Mundel et al., 2004). The process of transformation of safflower tissues follows the patented Stratosome™ Biologics system, which facilitates the genetic attachment of target proteins of interest to oleosin, the primary protein coating the oil-containing vesicles (oil bodies) of the seed. Such attachment permits the target protein to be purified along with the oil body fraction, which upon centrifugation floats to the surface of ground seeds/water slurry (van Rooijen et al., 1992). The purification process of the Stratosome system makes it more efficient than the other transgenic systems. The attachment of proteins to the oil bodies of safflower in the Stratosome Biologics system is expected to stabilize intracellular accumulation of foreign proteins, and also provide a useful attachment matrix and deliver benefits for end use applications.

The commercialization of genetically modified safflower will further increase the acreage and production of this crop in the world.

6.2.3 Botany

6.2.3.1 Basic Features

Safflower (*Carthamus tinctorius* L.) belongs to the family Asteraceae. Safflower plant can be described as a bushy, herbaceous annual possessing several branches, which are categorized as primary, secondary, and tertiary, with each terminating into a globular structure called capitulum (Figure 6.1).

Figure 6.1 Single plant showing primary, secondary, and tertiary heads.

Stem and branches are encompassed with leaves having numerous spines. Safflower is mainly grown under dry land conditions as an oilseed crop. It produces white, shiny, and smooth seeds (fruits) called achenes, each weighing from 0.01 to 0.1 g. They may be with or without pappus (tufts of hair present on the seed) and are four sided, having thick pericarp. Initial growth after the germination of seed is slow in safflower. During the slow growing period, called the rosette stage, several leaves are produced at the stem base. The duration of the rosette stage in safflower varies from 20 to 35 days. After this stage, the stem elongates quickly and branches profusely. The branching habit in safflower is classified as narrow, with a <30° angle to the stem, spreading with a branch angle to stem of up to 75°. Branching habit in safflower is controlled both genetically and environmentally. Appressed branching is recessive to spreading types and is controlled both digenically (Fernandez-Martinez and Knowles, 1978; Singh, 2005b) and monogenically (Deokar and Patil, 1975). Each branch produces a globular flower capitulum, which is enclosed by tightly attached bracts. Safflower has a taproot system that elongates to 2 to 3 m in soils with adequate depth. The deep root system in safflower helps to extract the water and nutrients from much deeper layers of soil, compared to other crop plants, and thus makes it an ideal plant for rain-fed cropping systems. The flowering period in safflower lasts for a month, as the capitula based on primary branches flower first, followed by flowering of the capitula based on secondaries and tertiaries. Flowering in a capitulum begins in the outermost whorl of florets and proceeds centripetally over 3 to 5 days. The flower color in safflower is broadly grouped into four classes:

1. Yellow in bloom, turning to red on drying (Y-R)
2. Yellow in bloom, turning to yellow on drying (Y-Y)
3. Orange in bloom, turning to dark red on drying (O-dark R)
4. White in bloom, turning to white on drying (W-W)

The first is the most prevalent (Figure 6.2). Safflower attains maturity in 30 to 35 days from the time when flowering ends.

6.2.3.2 Reproductive System

Safflower, a member of the family Asteraceae, has a composite type of inflorescence, with each plant producing several flowering heads commonly called capitula. Each capitulum consists of

Figure 6.2 Variability for flower colour in safflower exhibiting (from left to right) orange in bloom turning to dark red on drying (O-dark red); yellow in bloom turning to yellow on drying (Y-Y); white in bloom turning to white on drying (W-W); yellow in bloom turning to red on drying (Y-R).

Figure 6.3 Safflower capitulum having several flowers.

several flowers, with the number ranging from 20 to 250 (Figure 6.3). Flowers are enclosed by bracts in circular order. The disk flowers are attached to a flat or convex receptacle. In addition to the flowers, hairs or bristles are interspersed in between the flowers in a capitulum. A safflower flower is composed of petals that are attached to a corolla tube (Figure 6.4). The corolla tube in turn is attached at its base to an inferior ovary. Five fused anthers are attached to the corolla tube and surround the style and stigma. The corolla tube is 1.8 to 3 cm long and the five petal lobes are 6.5 to 8.5 mm long. Anther tube is 5 to 7 mm in length. The stigma, which is surrounded by five fused anthers, projects beyond the top of the anther tube by 5 to 6 mm. The inferior ovary in each

Figure 6.4 Saf o wer orets sho wing petals, stigma, anther, carolla tube, and ovary.

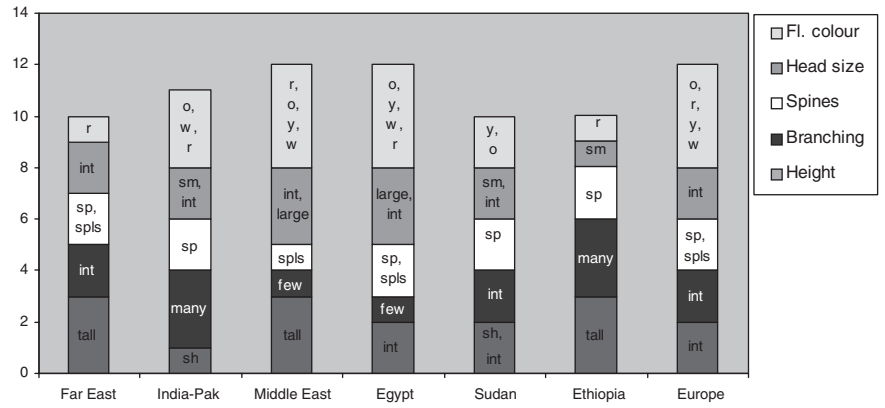
flower develops into a single-seeded fruit called an achene, which is commonly known as seed. The pollen of safflower is yellow. Pollination occurs as the style and stigma protrude out of the fused anther tube. Unpollinated stigma remains receptive for several days. Outcrossing in safflower has been reported to range from 0 to as high as 59% in different genotypes in India (Patil et al., 1987). The outcrossing in genetic male-sterile lines in safflower is reported to be 100%, as no difference in seed yield of male-sterile and male-fertile plants under open pollination was observed (Singh, 1996). Safflower pollen is transferred by insects and not by wind. The most prevalent pollinator agent in safflower is honey bees. Bees visit the safflower flowers for both pollen and nectar. Each safflower capitulum produces 15 to 60 seeds.

6.3 CENTERS OF ORIGIN

Vavilov (1951) proposed three centers of origin for cultivated safflower (*Carthamus tinctorius* L.). One in India (his center II) was based on variability and ancient culture of safflower production. A second center was proposed in Afghanistan (his center III), which was based on safflower diversity and proximity to wild species. A third center of origin, in Ethiopia (his center VI), was primarily based upon the presence of the wild safflower species in the area. The centers of safflower origin as proposed by Vavilov were reported by Kupzow (1932) in Russia after carrying out a detailed investigation of the safflower collections made in many areas. However, contrary to the above, Ashri and Knowles (1960) and Hanelt (1961) indicated the center of origin to be in the Near East. This assumption was based on the similarity of cultivated safflower to two closely related wild species: *C. flavescens* reported from Turkey, Syria, and Lebanon and *C. palaestinus* found in desert areas of western Iraq and southern Israel. Knowles (1969) described the safflower centers of cultivation as the "centers of similarity," and not as the centers of origin or diversity, as there is a conspicuous similarity between the types existing in some or most of the centers. These centers are:

1. Far East (Vavilov's center I — Chinese): China, Japan, and Korea
2. India–Pakistan (Vavilov's center II — India): India and both West and East Pakistan (East Pakistan is now Bangladesh)
3. Middle East (Vavilov's centers III and IV — Central Asiatic and Near Eastern): Afghanistan to Turkey, southern USSR to the Indian Ocean
4. Egypt (Vavilov's center V — Mediterranean): Bordering the Nile north of Aswan.
5. Sudan (the southern reach of Vavilov's center V): Bordering the Nile in northern Sudan and southern Egypt
6. Ethiopia (Vavilov's center VI — Ethiopian)
7. Europe (western portion of Vavilov's center V): Spain, Portugal, France, Italy, Romania, Morocco, and Algeria

Distinguishing characteristics of safflower from different centers of similarity are presented in order of decreasing frequency in Figure 6.5.



Abbreviations : sh = short; int = intermediate; sp = spiny; spls = spineless; sm = small; r = red; w = white; o = orange; y = yellow (modified from Knowles, 1969).

Figure 6.5 Distinguishing characteristics of saf o wer from different centers of similarity presented in order of decreasing frequency.

6.4 CYTOGENETICS

6.4.1 Genomic Relationships

6.4.1.1 Species Classification

The genus *Carthamus* consists of 25 species, distributed worldwide. Among the 25 safflower species, the cultivated safflower grown around the world is only *Carthamus tinctorius* L., containing 12 pairs of chromosomes (Patel and Narayana, 1935; Richharia and Kotval, 1940; Ashri and Knowles, 1960; Kumar et al., 1981). Safflower has four chromosome numbers, viz., 2n = 20, 24, 44, and 64 (Ashri and Knowles, 1960). Based upon the four classes of chromosome numbers, the genus was categorized into four sections and the basic chromosome numbers (x) were indicated as 10 and 12 (Ashri and Knowles, 1960), and not 8 and 12 as suggested by Darlington and Wylie (1956). These sections as per Ashri and Knowles (1960) are:

1. **Section 1 (2n = 24)**: Section 1 includes the annual species *C. tinctorius*, *C. palaestinus*, and *C. oxyacantha*. All three species cross readily and produce fertile hybrids, show high pairing between chromosomes, and are closely related (Ashri and Knowles, 1960). The possibility of natural gene transfer between *C. tinctorius* and its wild relatives *C. palaestinus* and *C. oxyacantha* appears to be very low since they are grown in different regions and seasons. *C. oxyacantha* is found from northwestern India to Iraq, and *C. palaestinus* is grown in southern Israel only. *C. tinctorius* is cultivated in much of India, in a few areas of Pakistan, over much of northern and central Iran, and in a few places in Jordan, Syria, Turkey, and Israel (Ashri and Knowles, 1960). *C. oxyacantha* is proposed to be the wild ancestor of the cultivated safflower (Bamber, 1916; Deshpande, 1952; Ashri and Knowles, 1960). The species of section 1 are described as pubescence minor or none; outer involucral bracts green, ovate to linear; inner bracts entire at apex; florets not saccate; corollas yellow, orange, red, or white; pollen grains yellow; pappus none or chaffy (Ashri and Knowles, 1960).
2. **Section II (2n = 20)**: This section represents *C. alexandrinus*, *C. glaucus*, *C. syriacus*, and *C. tenuis*. All these species are found on the eastern side of the Mediterranean Sea. All species produce blue or pink flowers, and the first three are similar morphologically. *C. glaucus* is distinct from the others, as it has a larger head and bracts that are ovate rather than linear. Other species in this group are *C. boissier* Halacsy, *C. dentatus* Vahl. (genome, A_1A_1), *C. leucocaulos* Sibth. and Sm. (genome, A_2A_2), *C. glaucus* subsp. *anatolicus* (Boiss.) Sam. subsp. *glandulosus* Han., *C. ambiguus*

Heldr., *C. nitidus* Boiss., *C. rechingeri* Davis, *C. ruber* Link., and *C. sartori* Held. Ashri and Knowles (1960) indicated that the species of sections I and II are not closely related. Artificial hybrids between species of the two sections were readily achieved, but all hybrids were sterile, as they showed very low pairing between the chromosomes of the involved species. This revealed that no exchange of genetic material between these two sections had occurred.

3. **Section III (2n = 44)**: This section consists of only one species, *C. lanatus*, with 22 pairs of chromosomes. It occurs naturally in Portugal, Spain, Morocco, Greece, and Turkey. It was initially assumed that this species is the product of hybridization of species of section I and II, followed by chromosome doubling. *C. lanatus* on crossing with species of section I showed low pairing between chromosomes, suggesting that the species of section I were not involved in the ancestry of *C. lanatus*. However, *C. lanatus* showed good pairing with the chromosomes of species of section II, indicating that some species in section II contributed 10 pairs of chromosomes to *C. lanatus* (Ashri and Knowles, 1960).
4. **Section IV (2n = 64)**: Section IV comprises two species: *C. baeticus* (Boiss and Reuter) Nyman and *C. turkestanicus* M. Popov. *C. baeticus* has 32 bivalents at MI, suggesting that it is an allohexaploid having three different genomes: one 12-chromosome genome and two separate nonhomologous genomes of 10 chromosomes. The hybrids between *C. baeticus* × *C. lanatus* showed perfect pairing of 22 chromosomes, indicating that *C. lanatus* (or a species closely related to it) is one of the ancestors of *C. baeticus* (Ashri and Knowles, 1960). *C. glaucus* with 2n = 20 chromosomes is considered the progenitor of *C. turkestanicus*. *C. baeticus* spread to the eastern Mediterranean, North Africa, and Spain. *C. turkestanicus* is found in west Asia, east to Kashmir, and in Ethiopia. It has 22 pairs of chromosomes in common with *C. baeticus* and also has white pollen, and its similarity in appearance to *C. lanatus* in Thrace suggests considerable gene exchange between the two species (Khidir and Knowles, 1970).
5. ***Carthamus* species with 2n = 22**: The only species with 11 pairs of chromosomes, viz., *C. divaricatus* (Beg and Vace.) Pamp., is found in a very limited area in Libya (Knowles, 1988). It produces yellow, purple, or white flowers with yellow pollen. It is self-incompatible and crosses readily with species possessing 10 pairs of chromosomes, but produces partially fertile hybrids. It also crosses with *C. tinctorius*, but produces sterile progeny.
6. **Other *Carthamus* species with 2n = 24**: *C. arborescens* and *C. caeruleus*, each having 12 pairs of chromosomes, possess distinct morphological characteristics and do not cross with any other *Carthamus* species (Ashri and Knowles, 1960); therefore, they were not grouped in any of the four sections. *C. arborescens* is found in southern Spain and adjoining areas of North Africa. *C. caeruleus* is well established in the Iberian Peninsula and in North Africa. Despite its occurrence around the Mediterranean Sea, it is quite different morphologically from *C. arborescens* and from other *Carthamus* species. *C. rhiphaeus* Font Quer, another species with 12 pairs of chromosomes, appears to be morphologically closely related to *C. arborescens* (Ashri and Knowles, 1960). *C. rhiphaeus* is found in a small area of northern Morocco. *C. nitidus* Boiss, which was earlier classified to have 10 pairs of chromosomes by Ashri and Knowles (1960), has been reclassified subsequently as having 12 pairs of chromosomes (Lopez-Gonzalez, 1990). This species has been grouped into a separate section due to its isolation from other species.

6.4.1.2 Reclassification of Carthamus

Lopez-Gonzalez (1990) proposed a new classification system for genus *Carthamus* that is based on anatomical characteristics, biogeographic distribution, and biosystematic information. In the new classification system genera *Carthamus* and *Carduncellus* are grouped along with two new genera, *Phonus* and *Lamottea*. The corresponding species of *Phonus*, *Lamottea*, *Carthamus*, and *Carduncellus* are *Carthamus arborescens* L., *Carthamus caeruleus* L., *Carthamus tinctorius* L., and *Carduncellus monspelienium* All. The species of *Phonus*, *Lamottea*, and *Carduncellus* are of perennial nature, and all have 24 chromosomes in them; however, the genus *Carthamus* is annual and has species with 2n = 20, 22, 24, 44, and 64 chromosomes, including various putative allopolyploid species.

The geographical distribution of the reclassified genera indicated that *Phonus* occurs in Spain, Portugal, and northern Africa; *Lamottea* is distributed in the western Mediterranean regions; *Carthamus*

is found in west and central Asia, as well as in the Mediterranean region; and *Carduncellus* is grown in the western European region of the Mediterranean, northern Africa, Egypt, and Israel/Palestine. The new genus *Carthamus* is further subdivided into three sections.

1. Section *Carthamus*, having 24 chromosomes, is composed of species *C. curdicus* Hanelt, *C. gypsicola* Ilj, *C. oxyacanthus* Bieb, *C. palaestinus* Eig., *C. persicus* Wild, and *C. tinctorius* L. The grouping of *C. nitidus* Boiss (2n = 24) along with the above species is questionable; therefore, the species is isolated from the rest of the genera to form a separate section.
2. Section *Odontagnathius* (DC.) Hanelt (including section *Lepidopappus* Hanelt) has 20 or 22 chromosomes and consists of the species *C. boissier* Halacsy, *C. dentatus* Vahl., *C. divaricatus* Beguinot and Vace. (2n = 22 chromosomes), *C. glaucus* Bieb., *C. leucocaulos* Sm., and *C. tenuis* (Boiss & Bl.) Bornm.

Section *Atractylis* Reichenb is indicated to have n = 11 chromosomes producing numerous polyploids, including the species *C. lanatus* L., *C. creticus* (*C. baeticus* (Boiss & Reuter) Nyman), and *C. turkestanicus* M. Popov.

6.4.1.3 Molecular Classification of Carthamus

Molecular classification of the genus *Carthamus* groups species into two sections (Vilatersana et al., 2005).

1. **Section *Carthamus***: This section consists of the same species as are furnished above under section *Carthamus* proposed by Lopez-Gonzalez (1990).
2. **Section *Atractylis***: This section includes sections *Atractylis*, *Lepidopappus*, and *Odontagnathius* as indicated in the classification suggested by Hanelt (1963), and it coincides with the old genus *Kentrophyllum*. The only possible point of dispute in this molecularly redefined section *Atractylis* is the inclusion of *C. nitidus* having chromosome number n = 12, as the rest of the members of the section have n = 11 and n = 10. The relationship between species with n = 10, 11, and 12, as indicated, is *a priori* disconcerting but was explained in terms of descending dysploidy (Vilatersana et al., 2000) with the exclusion of the hybridogenic basic number n = 32 from the dysploid series. Further study revealed that molecular analysis does not support the usually adopted subspecific treatment for *C. glaucus* ssp. *alexandrinus* (Hanelt, 1963), *C. glaucus* ssp. *tenuis* (Schank and Knowles, 1964), *C. lanatus* ssp. *creticus*, and *C. lanatus* ssp. *turkestanicus* (Hanelt, 1963). Instead, the study strongly favors specific treatment for them because the four purported species do not form groups together with the species to which they are subordinated. Only *C. creticus* was found to be associated with *C. lanatus*. But in this case, the subspecific treatment was already inadequate, as *C. lanatus* is one of the progenitors of the allopolyploid *C. creticus* (Khidir and Knowles, 1970), and on this basis, *C. creticus* cannot be treated as a subspecies of *C. lanatus*.

6.4.2 Classical Cytogenetics

Carthamus cytology has been extensively studied by Knowles and his coworkers in the early 1960s and 1970s. (Ashri and Knowles, 1960; Hanelt, 1963; Schank and Knowles, 1964; Harvey and Knowles, 1965; Khidir and Knowles, 1970; Estilai and Knowles, 1976; Estilai, 1977). However, most of these studies, as stated earlier, were carried out to determine chromosome numbers in different species and the extent of pairing in interspecific crosses to establish the genomic relationship among species. Attempts to assign genes to chromosomes are completely lacking in this crop, although Estilai and Knowles (1980) did identify one primary and one secondary trisomic in the progeny of an open pollinated triploid plant and used them for assigning genes to chromosomes in safflower. The identification of additional chromosomes was presumably not possible due to relatively short chromosomes of more or less equal size (Knowles and Schank, 1964). Though these aneuploids were reported to be morphologically different from diploid plants, no effort was

made to relate their morphological differences to the presence of extra chromosomes, mainly because progenies from open-pollinated plants had diverse genetic background (Knowles and Schank, 1964). Somatic chromosomes of *C. nitidus* Boiss were observed to be similar to those of *C. tinctorius*, except the sat-chromosome of *C. nitidus*, which was found to be larger. Kumar et al. (1981) reported three sat-chromosomes in *C. tinctorius*, compared to one reported by Knowles and Schank (1964). Kumar et al. (1981) carried out detailed karyotype analysis, including measurements of chromosome length and arm ratios, to identify translocation homozygotes in safflower. This study showed the identification of 10 translocation homozygotes out of 42 translation heterozygotes isolated in the M_1 generation of gamma-irradiated populations. The chromosomes involved in interchange homozygotes were 6-8 (line 58), 1-3 (line 66), 3-12 (line 123), 3-10 (line 131), 3-6 (line 153), 4-6 (line 186), 4-8 (line 197), 3-8 (lines 208 and 279), and 5-9 (line 290). Chromosome 3 (sat-chromosome) was involved in 6 of the 10 translocations, and thus the segment interchanges were not random.

The karyological characterization of individual chromosomes and the association of genes to specific chromosomes are lacking; as a result, a genetic and linkage map has not been developed in safflower.

6.4.3 Molecular Cytogenetics

Fluorescence *in situ* hybridization (FISH) is very useful in the identification of chromosomes and provides the information required for integration of genetic and physical maps, for localization of repetitive DNA sequences on the chromosomes, and to assist in functional and structural analysis of chromosomes. FISH analysis in *C. tinctorius* using ${}_{p}C_{t}k_{p}n$I-1 and ${}_{p}C_{t}k_{p}n$I-2 repeated sequences simultaneously revealed that the ${}_{p}C_{t}k_{p}n$I-1 sequence was exclusively localized at subtelomeric regions on most of the chromosomes; however, the ${}_{p}C_{t}k_{p}n$I-2 sequence was distributed on two nucleolar and one nonnucleolar chromosome pairs (Raina et al., 2005). The ${}_{p}C_{t}k_{p}nI$-2 sequence also constituted the satellite and the intervening chromosome segment between the primary and secondary constrictions in two nucleolar chromosome pairs. The $pC_{t}k_{p}n$I-2 repeated sequence, showing partial homology to the intergenic spacer (IGS) of 18S 25S ribosomal RNA genes of an Asteraceae taxon (Centaurea stoebe), and the 18S 25S rRNA gene cluster were located at independent, but juxtaposed sites in the nucleolar chromosomes (Raina et al., 2005). The application of FISH to other species will unravel the phylogenetic and evolutionary pathways in *Carthamus*.

6.5 GERMPLASM RESOURCES

In view of the large number of species in safflower, the crop has enormous diversity in the germplasm for different traits. Despite this, the exchange of genetic material between cultivated and allied species is lacking due to the sterile nature of hybrids (Ashri and Knowles, 1960). This indicates that there has been no exchange of genetic material between cultivated species and the species possessing other than 2n = 24 chromosomes. The instances of natural crossings between cultivated species *C. tinctorius* and its wild relative *C. oxyacantha* with 2n = 24 chromosomes have been observed near Isfahan in Iran and in the experimental field at the Abu Ghraib station near Baghdad in Iraq. However, the possibility of such crossing is very limited, particularly in India and Pakistan, as harvesting of *C. tinctorius* is over when *C. oxyacantha* starts to flower (Ashri and Knowles, 1960). No instances of natural crossing between *C. tinctorius* and *C. palaestinus*, the other wild relative with 2n = 24 chromosomes, has been reported, as they naturally grow in different geographic areas of the world. The introgression of genes from wild relatives to *C. tinctorius* has not received due attention in safflower because instances of such introgression are few. However, introgression of phytophthora root rot resistance gene from a wild composite of 12 species into cultivated safflower has been recorded (Rubis, 1981). Heaton (1981) suggested the use of *C. lanatus*

for improvement of disease resistance in *C. tinctorius*, as a disease resistance ability similar to that of *C. lanatus* was observed in the alloploid of *C. tinctorius* ∞ *C. lanatus*. Interspecific crosses between *C. tinctorius* and *C. oxyacantha* also showed highly resistant reactions to Alternaria leaf blight and powdery mildew, though *C. oxyacantha* was found to be susceptible to powdery mildew (Anonymous, 1976–1977). This suggests some evidence of natural crossing between *C. tinctorius* and its wild relatives.

Presently, India maintains about 7316 germplasm accessions at the Germplasm Management Unit (GMU) of the Directorate of Oilseeds Research, Hyderabad-500030 (Anonymous, 2002). The germplasm collection consists of the accessions received from safflower centers of the All India Coordinated Research Project (AICRP), local collections from traditional and nontraditional safflower-growing areas and exotic collections. The entire germplasm maintained at GMU has been characterized, and promising genotypes for economically important traits have been identified for use in the breeding programs. The U.S. maintains 2288 safflower accessions collected from more than 50 countries, at the Western Regional Plant Introduction Station (WRPIS), located at Pullman, WA, which is a part of the U.S. National Plant Germplasm System (NPGS) and the U.S. Department of Agriculture (Bradley and Johnson, 2001). The accessions from the U.S. safflower collection are distributed to scientists worldwide upon request at no charge. Data on many descriptors have been gathered on a large number of accessions in the collection and have been entered into the Germplasm Resources Information Network (GRIN), and this information is easily available to Internet users (Bradley and Johnson, 2001). Of the 2288 accessions in the U.S. safflower germplasm, a core collection of 207 accessions was developed based on country of origin and morphological data (Johnson et al., 1993). To enhance characterization and to determine diversity, the core collection was evaluated for seven quantitative factors, which indicated considerable diversity within the core collection. Correlation analysis showed the strongest association between plant height and flowering ($r = 0.62$), which was followed by association between outer involucral bract (OIB) width and OIB length ($r = 0.54$). The evaluation of accessions per their places of origin, which have been distributed in major geographical regions, namely, the Americas, Australia, China, East Africa, Europe, Japan, the Mediterranean, South-Central Asia, Southwest Asia, and Thailand, revealed significant differences among regions for all factors except OIB length and yield per plant. Among the regions, accessions from Southwest Asia were the most different from those of other regions, but those from South-Central Asia and East Africa grouped closely together. Thus, the core collection was indicated to have highly diverse germplasm, and it appears that agronomic traits could distinguish regional differences (Johnson et al., 2001). The evaluation in Mexico of 721 accessions from the world collection of safflower for *Alternaria* resistance and seed-oil content showed 84 accessions possessing tolerance to *Alternaria* and 37 accessions having good agronomic characteristics. An oil content of >32.1% was recorded, with the maximum being 42.03%. This study showed that the U.S. safflower collection can be used as a source of *Alternaria* resistance and high oil content for breeding purposes (Cervantes-Martinez et al., 2001). The Beijing Botanical Garden at the Chinese Academy of Sciences collected and evaluated safflower germplasm, including the world safflower collections, and characterized it for 33 characters (Li et al., 1993).

6.6 GERMPLASM ENHANCEMENT: CONVENTIONAL BREEDING

6.6.1 Breeding Methods

Breeding for high yield and stability has been the major thrust of safflower research in India and other safflower-growing countries of the world. Therefore, a majority of the work carried out in safflower is related to enhancement of seed yield.

Safflower falls in the category of often cross-pollinated crops, but the methods adopted to breed self-pollinated crops in general have been followed to develop cultivars in it. Crop improvement

methods used extensively to breed cultivars with improved yield and stability in safflower are described below.

6.6.1.1 Introduction and Pure Line Selection

Introduction is the simplest method of crop improvement and has been used extensively since time immemorial across the continents. Establishment of safflower as a crop in the U.S., Canada, and Argentina is a result of introductions from India, Russia, Turkey, etc., in the beginning of the 19th century (Claassen, 1981), though the introduction of varieties in a new area only occasionally results in direct release of varieties for commercial production. In general, introduced varieties require a few cycles of adaptation, followed by selection and evaluation, before they are formally released for commercial production, since the plants of an introduced cultivar show varied reaction to the changed environment. Therefore, acclimatization of the introduced cultivar is necessary before the population is subjected to selection for identifying promising selections and subsequent evaluation to release as a variety.

Selection is the most commonly used breeding method followed for cultivar development as far as safflower improvement in India is concerned. This can be realized from the fact that 17 of the 25 varieties developed so far for commercial cultivation in the country have been evolved by resorting to selection in the locals. The safflower varieties developed by using this method in India and abroad are as follows: India: N-630, Nagpur-7, N-62-8, A-300, Manjira, S-144, JSF-1, K-1, CO-1, Type-65, APRR-3, Bhima, HUS-305, Sharda, JSI-7, A-2, PBNS-12; U.S.: Nebraska-5, Nebraska-10 (N-10); Canada: Saffire (Hegde et al., 2002).

The pure line selection from local cultivars of safflower resulted in the development of several germplasm lines with many desired traits in safflower. Safflower, as indicated earlier, possesses enormous diversity for different traits of economic importance; however, the proper utilization of this diversity is lacking due to it being a rain-fed crop of minor economic importance. The availability of untapped variability for different traits in safflower is the reason that many of the safflower cultivars produced in India have been developed by pure line selection, and until today, this method is regarded as the most effective for varietal development in safflower.

6.6.1.2 Hybridization

Hybridization is practiced mainly to bring together the desirable traits of two or more varieties into one. Hybridization, in addition to generating variation for various attributes in F_2 and subsequent generations, has proved to be of great use in unraveling the genetic makeup of different traits. This has helped in formulating proper methodologies to bring out the desired improvement in different crops.

The selection of parents has an important role in determining the success of a crop improvement program. Joshi (1979) gave very useful suggestions for selecting the parents for hybridization in a self-pollinated crop, the important ones being (1) selection of the parents on the basis of their per se performance for seed yield and other desired traits, (2) consideration of extent of expression in yield components, (3) consideration of genetic diversity of the parents to bring desired genes of diverse origin together, and (4) identification of the best general combining parents as well as the best cross combination by following the diallel cross approach. Evaluation of F_1s along with their parents and check cultivars is essential in order to assess the performance of hybrids compared to parents and checks in respect to yield and other desired traits. This enables the selection of the most promising genotypes for generation advancement. The F_1s selected as per the method stated above are advanced to the F_2 generation. The selected F_2s may be grown in a large area to have a fairly large population size. This is done to get individual plants representing all possible gene combinations existing in a cross. This makes it possible to select transgressive segregates. Corresponding F_1s and standard checks may also be planted along with F_2s to generate information on

inbreeding depression. Low inbreeding depression in F_2 suggests the availability of a fair amount of additive ∞ additive components of variance that get fixed through subsequent inbreeding. The selection of promising individual plants possessing traits of economic importance is practiced in desirable crosses in F_2, and the selected plants are harvested and threshed separately for plant to progeny row evaluation. While selecting individual plants in F_2, the information generated on inheritance of yield and its components and interrelationships among them, as summarized below, may be used as a guideline for the crop improvement program.

Genetic studies on gene action in safflower exhibited the importance of nonadditive gene action for seed yield and the number of capitula per plant (Makne and Choudhary, 1980; Ramachandram and Goud, 1981, 1982b; Ranga Rao, 1983; Narkhede et al., 1992; Patil et al., 1992; Parameshwarappa et al., 1995; Ghorpade and Wandhare, 2001; Gadekar and Jambhale, 2003) and additive gene action for oil content, seed weight, and seed number (Makne et al., 1979; Deokar and Patil, 1980; Ranga Rao, 1982; Mandal, 1990). The importance of both additive and nonadditive gene actions for the above traits was revealed by Prakash and Prakash (1993) and Singh (2004). Flower yield in addition to seed yield exhibited the importance of both additive and nonadditive gene actions, with nonadditive predominant for flower yield in safflower (Singh, 2004).

For exploitation of gene action and to increase the chances of getting desirable recombinants, breeding methods such as biparental mating followed by reciprocal recurrent selection and diallel selective mating may be adopted in safflower (Ramachandram and Goud, 1981; Ranga Rao, 1982; Parameshwarappa et al., 1984; Narkhede et al., 1992). The presence of a high degree of nonadditive gene action for seed yield suggested tremendous potential for exploitation of hybrid vigor for seed and oil yields in safflower (Makne and Choudhary, 1980; Ranga Rao, 1982; Narkhede et al., 1992). Correlation studies delineated that seed yield and oil content are the most important and complex traits, with direct selection for them hampered due to the existence of large genetic–environment interaction in safflower (Ranga Rao and Ramachandram, 1979). Ineffectiveness of direct selection for yield is also reported by Nie et al. (1988). The yield components, viz., number of branches per plant, seed weight per capitulum, and 100-seed weight, contributed the most to the seed yield either directly or indirectly (Nie et al., 1988; Patil et al., 1990; Singh et al., 2004). Capitulum diameter showed positive association with seed yield (Mathur et al., 1976; Makne et al., 1979; Mandal, 1990; Singh et al., 1993; Anjani, 2000). Seed yield per plant and number of capitula per plant exhibited positive association with oil yield per plant (Prakash and Prakash, 1993). Seed weight was negatively associated with oil content (Ranga Rao et al., 1977; Mandal, 1990; Patil et al., 1990). Hull content showed significant positive association with seed weight but was negatively related to oil content (Ranga Rao et al., 1977; Sangale et al., 1982; Mandal, 1990). The magnitude of negative association between seed weight and seed number is approximately equal to that of positive correlation between seed number and oil content (Ramachandram and Goud, 1982a; Patil et al., 1990). Seed weight per capitulum, which is an outcome of seed number and seed weight, was observed to be a direct component of seed yield (Ramachandram and Goud, 1982a; Ramachandram, 1985; Ghongade et al., 1993; Nie et al., 1993; Singh et al., 2004). Thus, various investigations suggest the importance of number of capitula per plant, yield per capitulum, and seed weight for seed yield improvement in safflower.

The studies to correlate flower yield with its component traits in nonspiny hybrids indicated that flower yield was significantly and positively associated with the number of primary branches per plant, capitulum diameter, number of capitula per plant, number of flowers per capitulum, stigma length, petal area per flower, and seed yield per plant. Therefore, selection for traits showing positive association with flower yield will help in improving flower yield in safflower (Singh, 2004). Individual plant selections can be made at the maturity of the crop, as all the attributes indicated as important for improvement of seed and flower yields can be taken into consideration while selecting the individual plants.

Selection for high oil can be carried out by following the thumbnail technique (Sawant, 1989–1990). In this method, the dry seeds obtained from the main capitulum are pressed hard by

using thumbnail pressure. The seeds with high oil content in general contain thin hulls and are thus pressed easily, in contrast to seeds with thick hulls, which are not pressed at all with thumbnail pressure. Thus, the selection for high oil types can be done in the field. The segregating populations in F_2 and in subsequent generations, depending upon the trait to be improved, are handled by one of the methods illustrated below:

1. **Pedigree method**: This method has been used most frequently to improve seed yield, oil content, and other desired traits in safflower. The standard pedigree method, usually used in self-pollinated crops, is followed in safflower and is described briefly below.
   In this method, the selection of plants having desired traits is carried out in F_2 populations. About 5 to 10% plants of the F_2 population of each cross are selected, harvested, and threshed separately to raise plant-to-progeny rows in the F_3 generation. F_3 progenies may be evaluated in a replicated trial along with the standard checks for early-generation selection of the promising progenies for seed yield and desired traits. Selected progenies are advanced to F_4, F_5, and F_6 generations in subsequent years. Each generation is subjected to inter- and intraprogeny selection of promising types. The selected plants need to be selfed at every stage of the selection process, as this makes it possible to get homozygous progenies by the time they reach the F_6 generation. Uniform and homozygous progenies may be considered for yield trial at this stage, and the most promising ones of them may be further subjected to individual plant selections. The individual plant progenies are further evaluated in replicated trials to identify the most outstanding lines for multilocation evaluation. Multilocation evaluation is necessary to know their adaptability to different agroclimatic conditions before the release of the most adaptable line. Safflower cultivars developed by the following pedigree method in India and other countries, along with their years of release for commercial production, are as follows: *India*: A-1 (1969), Tara (1976), Nira (1986), Girna (1990), JSI-73 (1998), NARI-6 (2001), Phule Kusuma (2003); *U.S.*: Leed (1968), Sidwill (1977), Hartman (1980), Rehbein (1980), Oker (1984), Girard (1986), Finch (1986); *Mexico*: Sahuaripa 88 (1989), Ouiriego 88 (1989), San Jose 89 (1990); *Canada*: AC Stirling (1991), AC Sunset (1995) (Hegde et al., 2002).
2. **Bulk population method**: In the bulk method, the F_2 and following generations are harvested in bulk to grow the next generation. The major benefit of the bulk population method is that natural selection exerts strong selective pressure on bulk populations favoring the high-yielding types. As a result, poor yielders and uncompetitive types are eliminated during the process of evaluation of six to seven generations and the population becomes nearly homozygous. In the F_7 or F_8 generation the selection of promising plants carrying desirable traits is carried out. These selections are harvested and threshed separately for evaluation of individual plant progenies in replicated trials, along with standard checks, to identify the most promising ones for multilocation testing. Another advantage of the bulk method is that a breeder can handle several bulk populations simultaneously, which is not feasible in other breeding methods. It is desirable to self bulk populations of safflower, as otherwise the high rate of cross-pollination can make the bulk population method ineffective due to the presence of a large number of heterozygous plants at the end of the F_7 generation.
3. **Single-seed descent method**: This method has been used by Fernandez-Martinez and Dominguez-Gimenez (1986) in Spain to develop five safflower cultivars: Tomejil (1986), Rancho (1986), Merced (1986), Alameda (1986), and Rinconda (1986). In this method, from F_2 onwards, randomly selected single seeds from each plant are taken to increase every subsequent generation until F_5 and F_6. In F_7, a large number of individual plants are used to raise individual plant progenies. The outstanding progenies out of these are then tested for yield and other desirable attributes in a replicated trial.
4. **Backcross method**: This method has been used successfully in the U.S. to breed safflower cultivars US-10 (1959), by incorporating resistance to root rot caused by *Phytophthora drechsleri* (Thomas, 1964), and UC-1 (1966) (Knowles, 1968) and Oleic Leed (1976) (Urie et al., 1979), by transferring high oleic acid trait to both of them. This method is generally practiced with traits controlled by oligogenes. These genes are to be incorporated from a donor parent into a widely adapted variety. To incorporate a specific trait from a donor parent to the recurrent parent (widely adapted variety), a series of backcrosses are made between the hybrid and the recurrent parent. In each cycle of backcrossing, backcross progenies possessing desired characters are crossed with the recurrent

parent. Six to seven backcrosses are desirable to develop a genotype homozygous for all the genes controlling different traits in the recurrent parent and for the genes controlling the trait under transfer. Selfing of the selected plants of the last backcross generation possessing requisite traits produces homozygous progenies that are similar to the recurrent parent.

6.6.2 Hybrid Breeding

The often cross-pollinated nature of safflower, existence of high heterosis for seed and flower yield, presence of many traits of commercial importance, and presence of genetic male sterility (GMS) and cytoplasmic male sterility (CMS) systems make safflower a suitable candidate for exploitation of hybrid vigor in the crop. Reports of the existence of high heterosis for seed yield and other desired traits in safflower have attracted several workers since the 1970s to seek the simple and easy-to-use methods of commercial-scale hybrid seed production (Urie and Zimmer, 1970a; Karve et al., 1979). The identification of genetic male sterility sources in safflower (Heaton and Knowles, 1980; Joshi et al., 1983; Ramachandram and Sujatha, 1991; Singh, 1996, 1997) and development of agronomically superior genetic male-sterile lines in India have resulted in the development and release of spiny safflower hybrids DSH-129 and MKH-11 in 1997, the first nonspiny hybrid safflower NARI-NH-1 in 2001 (Singh et al., 2003a), and the spiny hybrid NARI-H-15 in 2005. These hybrids in general show a 20 to 25% increase in seed and oil yield over the national check A-1. India is the only safflower-growing country in the world to grow a hybrid safflower.

In safflower, genetic as well as cytoplasmic male sterility systems are harnessed for the development of hybrid cultivars. However, the male sterility system used for the development of safflower hybrids in India is the GMS system. The GMS systems available in safflower are of both monogenic recessive and dominant nature.

6.6.2.1 Single Recessive Genetic Male Sterility

The GMS sources in safflower controlled by single recessive genes are:

1. UC-148 and UC-149 GMS lines developed by Heaton and Knowles (1980)
2. GMS lines developed by Ramachandram and Sujatha (1991)
3. MSN and MSV male-sterile lines developed by Singh (1996)
4. DMS male-sterile lines associated with dwarfness developed by Singh (1997)

These male sterility sources segregate in a ratio of 1 male-sterile:1 male-fertile plant.

Male-sterile and male-fertile plants are identified at flowering by the presence of a pinched capitulum opening in case of male-sterile plants and a normal opening in case of male-fertile plants. In case of DMS lines, owing to a linkage between sterility and dwarfing genes, the sterile and fertile plants become obvious at 30 to 40 days after sowing. The male-sterile plants attain a height of only 5 to 10 cm at 30 to 40 days after sowing; however, the male-fertile plants attain a normal height of 20 to 25 cm. The height difference between the two types enables the roguing out of male-fertile plants at this stage, leaving behind a 100% pure stand of dwarf male-sterile plants.

6.6.2.2 Dominant Genetic Male Sterility

Joshi et al. (1983) reported a dominant gene-controlled male sterility in safflower. Identification of sterile and fertile plants as in single recessive genetic male sterility is possible in this case too at the flowering of the crop. Owing to the dominant nature of the gene imparting male sterility, the hybrids and the male-sterile line in this system segregate in a ratio of 1 MS (male-sterile) to 1 MF (male-fertile) plant. The success of hybrids based upon this source is hindered due to the occurrence of 50% MS plants in the hybrid population, which affects the yielding ability of the hybrid adversely if honey bee activity is not adequate to give 100% seed setting in the male-sterile plants.

AU: Please introduce DMS.

6.6.2.3 Cytoplasmic-Genetic Male Sterility

Cytoplasmic-genetic male sterility (CGMS) has been reported to be exploited for hybrid development in safflower (Hill, 1989). The evaluation of CMS hybrids carried out in comparison with the GMS-based hybrids in India revealed the seed yield of CMS hybrids to be only 50% that of the corresponding GMS hybrids. In addition, all the CMS-based hybrids segregated into sterile and fertile plants, thereby suggesting the lack of fertility restoration to the sterile cytoplasm (Singh et al., 2000). The commercialization of CMS-based hybrids is still awaited.

In India, too, efforts are under way to develop a CGMS system in safflower at the Nimbkar Agricultural Research Institute (NARI), Phaltan (Singh et al., 2001a), and at the Directorate of Oilseeds Research, Hyderabad. The CGMS systems at NARI are being developed by following interspecific crossing and mutagenesis with streptomycin. Both programs have resulted in development of CMS in safflower. Genotypes causing 100% restoration of fertility to the sterile cytoplasm have been identified in both cases (Singh, 2005b). Efforts are being made to develop suitable maintainer genotypes that can maintain 100% male sterility in the sterile cytoplasm.

6.6.3 Breeding for End Use

In general, safflower around the world is grown under rain-fed conditions. Therefore, the incidence of disease and pest infestation is reported to be of low severity. However, under favorable conditions they may cause considerable damage to the crop, as had happened in India during 1997 to 1998, when the entire crop of safflower in the major safflower-growing states of Maharashtra and Karnataka was completely wiped out by an outbreak of *Alternaria* (Anonymous, 1997–1998). In view of the above, the major emphasis in safflower improvement has been laid on seed yield; however, to meet the requirements of local agroclimatic conditions, cropping patterns, and market requirements, safflower improvement has also been directed to breed cultivars resistant to diseases and pests, and improved oil content and quality.

6.6.3.1 Disease Resistance

Safflower is attacked by many diseases caused by fungi, bacteria, viruses, or physiological disorders due to abiotic stresses. Patil et al. (1993) reported that safflower is recorded to be infested around the world by 57 pathogens, including 40 fungi, 2 bacteria, 14 viruses, and 1 mycoplasma. Of these, Alternaria leaf spot caused by *Alternaria carthami* and wilt caused by *Fusarium oxysporum* are the most devastating ones and can cause 13 to 49% losses and wipe out the entire crop in the region under conditions conducive to their development, as indicated above in the case of India.

Breeding safflower for disease resistance is the most economical and convenient method for controlling major diseases in safflower. Mundel and Huang (2003) described in detail how to control major diseases of safflower by breeding and using cultural practices. The genetics and the mode of inheritance of disease resistance and tolerance in safflower have not been studied for most diseases (Li and Mundel, 1996). Though germplasm lines or cultivars showing partial or full resistance to some of the major diseases have been identified, the genetics has been determined only for a few. Karve et al. (1981) showed that resistance to each of the diseases, viz., *Alternaria carthami* Chowdhari, *Cercospora carthami* Sund and Ramak, *Ramularia carthami* Zaprom, *Fusarium oxysporum* Sehl. ex. Fries, *Rhizoctonia bataticola* Bult, and *Rhizoctonia Solani* Kuhn, is imparted by single dominant genes. Study of inheritance of wilt (*Fusarium oxysporum*) resistance in safflower revealed the control of inhibitory gene action in the expression of wilt resistance in safflower (Singh et al., 2001b). The source of resistance to wilt has been identified in the local germplasm lines (Sastry and Ramachandram, 1992). Breeding for wilt resistance in safflower following backcross resulted in the development of wilt-resistant genotypes giving an increase in

seed yield to the extent of 31% over the national check A-1 (Singh et al., 2003b). Breeding safflower varieties for resistance to multiple diseases resulted in the development of germplasm line VFR-1. This line was derived from the breeding line Nebraska 4051, and it showed resistance to Verticillium wilt, Fusarium wilt and root rot, and Rhizoctonia root rot (Thomas, 1971). Australian safflower cultivar Sironaria, showing resistance to Alternaria blight and moderate resistance to Phytophthora root rot, has been developed by backcrosses (Harrigan, 1987, 1989). Safflower cultivars resistant to Alternaria blight, viz., Sidwill, Hartman, Oker, Girard, and Finch, have been successfully developed in the U.S. (Bergman and Riveland, 1983; Bergman et al., 1985, 1987, 1989a, 1989b). These cultivars have been derived from the crossing of existing cultivar AC-1 with mass-selected *Alternaria*-resistant line 87-42-3 in a disease nursery initiated in the early 1960s. Resistance to all prevalent races of root rot caused by *Phytophthora drechsleri* was incorporated into the cultivar Dart (Abel and Lorance, 1975). Mundel et al. (1985) reported the incorporation of Sclerotinia head rot (caused by *Sclerotinia sclerotiorum* (Lib.) de Bary) resistance into the first Canadian safflower cultivar Saffire by adopting mass selection.

6.6.3.2 Oil Content and Quality

Safflower varieties released for commercial production in India in general possess low oil content of 28 to 32%, except HUS-305, NARI-6, and nonspiny hybrid NARI-NH-1, each of which contain 35% oil. Of late, development of high oil-containing varieties and hybrids with in-built resistance to diseases and pests has been emphasized in the national safflower improvement program in India. Many studies have shown a negative association between hull content and oil content in safflower (Ranga Rao et al., 1977; Sangale et al., 1982; Mandal, 1990). Therefore, reduction in hull content directly increases oil content. A number of genes for different hull types in safflower have been described: partial hull (*par par*) recessive to normal hull, which is inherited independently of thin hull (*th th*) and striped hull (*stp stp*) (Urie, 1981); gray-striped hull (stp^2) (Abel and Lorance, 1975); and reduced hull (*rh rh*), with small dark blotches on the seed. Partial hull is recessive to reduced hull (Urie, 1986). However, normal hull is dominant or partly dominant to reduced hull, depending upon the normal hull genotype used in the crossing program (Urie and Zimmer, 1970b). Tremendous improvement in oil content of safflower seed has been achieved in the cultivars developed in the U.S. (Bergman et al., 1985; Rubis, 2001). Safflower cultivar Oker contains 45% oil (Bergman et al., 1985). A safflower line an having oil content as high as 55% has been reported by Rubis (2001).

The quality of any oil is determined by the fatty acid composition of the oil, and the oils rich in poly- or monounsaturated fatty acids are considered good, as they help in reducing the cholesterol level in blood. In view of the above, safflower oil is considered the best, as it contains very high amounts of polyunsaturated (linoleic acid, 70 to 75%) or monounsaturated (oleic acid, 70 to 75%) fatty acids. Safflower is reported to be the best example of a crop with variability for fatty acid composition in seed oil (Knowles, 1989). Standard safflower oil contains about 6 to 8% palmitic acid, 2 to 3% stearic acid, 16 to 20% oleic acid, and 71 to 75% linoleic acid (Velasco and Fernandez-Martinez, 2001). Variants to the above composition with increased stearic acid content (4 to 11% of the total fatty acids), intermediate oleic acid content (41 to 53%), high oleic acid content (75 to 80%), and very high linoleic acid content (87 to 89%) have been detected in the released materials (Fernandez-Martinez et al., 1993; Johnson et al., 1999). Velasco and Fernandez-Martinez (2001) reported the development of lines with modified fatty acid composition having high palmitic acid content (10.3% of the total fatty acids), medium or high stearic acid content (3.9 and 6.2%), high or very high oleic acid content (>78 and 86%), together with reduced levels of the saturated fatty acids palmitic and stearic acid (<5%), and very high linoleic acid content (>86%) combined with reduced palmitic and stearic acid content (<5%). The sources of high total tocopherol content (up to 400 mg kg^{-1} seed) and increased gamma-tocopherol content (up to 9.9% of the total tocopherols) were also identified by them.

The genetics of oleic, linoleic, stearic, and palmitic acids in seed were studied by Futehally (reported by Knowles, 1989). The genetics of fatty acids in safflower revealed that production of oleic, linoleic, and stearic acids is controlled by three independent recessive genes, *ol ol*, *li li*, and *st st*, respectively. Knowles (1968) released the first high oleic (oleic acid = 78.3%) safflower variety 'UC-1' in 1966 in the U.S., which was followed by the release of 'Oleic leed' in 1976 by him and his colleagues (Urie et al., 1979). 'Alameda' and 'Rinconada' developed by Fernandez-Martinez and Dominguez in Spain in 1986 and 'Montola 2000' and 'Montola 2001', having >80% oleic acid, developed by Bergman in the U.S. are other high oleic acid-containing cultivars released for commercial production (Li and Mundel, 1996). All other safflower varieties released for commercial production in different countries are of high linoleic type (linoleic acid = 70 to 75%). The fatty acid profile, genetic variability for fatty acids and their genetic control, suggests that fatty acid composition in safflower can be altered as required.

6.6.3.3 Insect Resistance

Aphid is the most common pest of safflower, causing up to 50% damage. Germplasm lines exhibiting a stable tolerance to aphids have been identified in safflower. Two wild species *C. flavescens* and *C. lanatus*, have been reported to be carrying genes for resistance against safflower fly (Kumar, 1993). Genetics of aphid resistance in safflower has been reported to be of both additive and nonadditive nature. However, the role of nonadditive gene action was found to be predominant (Singh and Nimbkar, 1993).

6.6.3.4 Spineless Safflower

Safflower in general is a spiny crop. However, the entire production of safflower in China is under spineless cultivars. Safflower production world over, barring China, is under spiny cultivars. Safflower production has been largely handicapped owing to its spiny nature, especially in non-traditional areas and in areas where mechanized cultivation has not yet been introduced. In India, too, safflower production is dominated by the spiny cultivars. Though spineless cultivars CO-1 and JSI-7 were available, because of their poor yielding ability, compared to spiny cultivars, they could not command a sizable safflower area. Recently, the nonspiny variety NARI-6 and nonspiny hybrid NARI-NH-1 (Singh et al., 2003a) were released, in 2001 and 2002, respectively, for all India production. The yield levels of the two cultivars are at par with their spiny counterparts, and they are reported to have better tolerance to foliar and wilt diseases than to spiny ones. Therefore, these cultivars are becoming very popular among the farmers in safflower-producing states in India.

6.6.3.5 Resistance to Abiotic Stresses

Safflower is a rain-fed crop but, in general, suffers severely due to moisture stress. Studies with regard to abiotic stresses and genetic control of tolerance to them in safflower are largely lacking.

6.7 MOLECULAR GENETIC VARIATION

The use of molecular techniques to study genetic variation in safflower has been initiated recently, and a very limited amount of information is available. Isozyme genetic markers to identify hybrid individuals from safflower populations (Carapetian and Estilai, 1997) and to study divergence in 89 accessions that originated from 17 countries have been used in safflower. The latter study revealed that materials from East Asia had the maximum estimates for both mean allele frequency and mean gene diversity. It was also indicated that the accessions from India showed high diversity; however, the accessions from Turkey were found to be closely related to those from the other

Middle East countries. The accessions of unknown origin showed more resemblance to those from India, Turkey, and Middle East than to accessions from Europe and the U.S. (Zhang, 2001).

Therandomly amplified polymorphic DNA (RAPD) technique employed to detect genetic diversity of 28 safflower genotypes, including Iranian landraces, and several wild and exotic genotypes showed that the clusters based on RAPD markers correlated fairly well with a classification scheme based on morphological traits. Thus, the RAPD method appears to have great promise for the classification of safflower germplasm, identification of safflower landraces and its wild relatives, and their subsequent use in breeding of improved cultivars (Yazdi-Samadi et al., 2001). DNA fingerprinting of Indian safflower cultivars employing RAPD, ISSR, and amplified fragment length polymorphism (AFLP) markers indicated AFLP to be the most informative in discriminating between all 14 safflower cultivars (Sehgal and Raina, 2005). The study further identified specific markers for each cultivar, and cultivar NARI-2 was reported to contain the maximum number of diagnostic markers, followed by cultivars HUS-305, Bhima, and JSI-7. These four cultivars were identified as the probable source of new and novel alleles and were expected to be of great importance in breeding new cultivars (Sehgal and Raina, 2005).

6.8 TISSUE CULTURE AND GENETIC TRANSFORMATION

6.8.1 Somatic Embryogenesis

Safflower has attracted very little attention as far as tissue culture and genetic transformation are concerned. Initial efforts in safflower were directed to develop suitable culture conditions for whole plant regeneration. It has been demonstrated that regeneration frequencies are very high, and regeneration is possible through embryogenesis and organogenesis pathways. The mode of regeneration in general is through direct or indirect organogenesis (George and Rao, 1982; Tejovathi and Anwar, 1987, 1993; Sujatha and Suganya, 1996; Nikam and Shitole, 1999; Walia et al., 2005). Direct somatic embryogenesis from cotyledonary leaves has been produced (Mandal et al., 1995). Young safflower tissues, including roots, have been found suitable for *in vitro* regeneration as evidenced by the simple media requirements, and most of the studies have used Murashige and Skoog's salts (1962) as the basal media. Studies are lacking for the enhancement of rhizogenesis in *in vitro* generated/multiplied shoots, and this has been considered a problematic area that is reducing the overall efficiency of whole plant regeneration. Survival of regenerated plants in soil has been reported to be as low as only 20% of transferred rooted plants of the var. Centennial survived in soil (Baker and Dyer, 1996). Likewise, in variety Bhima, 34% of transferred plants showed success (Nikam and Shitole, 1999). This indicates that safflower needs more intensive and systematic experimentation to develop an effective method for supporting qualitative as well as quantitative rhizogenesis to enable large-scale transplantation of *in vitro* regenerated plants to the field.

Production of haploids from anther or pollen culture, followed by chromosome doubling to produce homozygous diploids, has become an important tool to supplement the traditional breeding program in several crops. Use of haploids in development of cultivars is completely lacking in safflower. However, researchers at the Department of Genetics, Osmania University, Hyderabad, India, have developed reliable and reproducible protocols for whole plant regeneration from anther culture in safflower. Various parameters related to genotype, cold pretreatment (3 to 7°C) for 0 to 7 days — 15 days for immature flower buds, culture media (MS, Nitsch and Nitsch, Gamborg's, Chaleff's, Linsmaier and Skoog), growth regulators, growth adjuvants (abscicic acid, coconut milk, casein hydrolysate), and physiological condition of anther donor plants grown in field and in greenhouse have been evaluated for haploid production from anther culture in safflower. Cytological investigations of anther-regenerated plants revealed two ploidy levels, with haploids accounting for 64% (Prasad et al., 1991). Production of haploids could be followed by standard

techniques to double the chromosomes of haploids by using colchicine or nitrous oxide treatments to get homozygous diploids.

6.8.2 Somaclonal Variation

Somaclonal variation as a source for inducing genetic variability has been demonstrated in safflower (Seeta et al., 2000). Somaclones generated from cotyledon explants of safflower genotype Manjira showed an enormous variation for both qualitative and quantitative traits and were found to breed true. Variants for plant height, leaf shape, plant type, flower color, seed shape, seed-oil content, days to flowering, days to maturity, number of capitula per plant, and types of fatty acids were recorded.

6.8.3 Biotic Stresses

Suganya et al. (1997) highlighted the applicability of tissue culture technique to select calli exhibiting resistance to *Fusarium oxysporum* f. sp. *carthami*.

6.8.4 Abiotic Stresses

Evaluation of NaCl tolerance in safflower callus cultures by the repeated transfer of selected clones to the NaCl-rich medium enabled identification of salt-tolerant cell lines (Nikam and Shitole, 1997). Induction of high variability for qualitative and quantitative traits through tissue culture indicated that tissue culture can be suitably utilized to detect spontaneous somaclonal variants with improved tolerance to abiotic stresses.

6.8.5 Genetic Modification

Genetic engineering has become an important tool for crop improvement since it provides means of introduction of genes from diverse sources without changing the phenotype and agronomic performance of the transformed plant. *Agrobacterium tumefaciens*-mediated transformations produced transgenic safflower by using the cultivar Centennial (Ying et al., 1992). Efficient callus formation from cotyledon, stem, and leaf explants was observed. Transformation and integration of transgenes was confirmed with the application of GUS assay and DNA hybridization in kanamycin-resistant calluses and GUS assay in regenerated shoots. A protocol for transformation and regeneration of safflower has been developed (Orlikowska et al., 1995). On the formation of leafy structure, it was transferred to elongation medium containing geneticin. After, elongation shoots were detached from the explant tissue and transferred to the same medium. Only transferred shoots that remained healthy were transferred to the rooting medium.

A protocol for transformation using safflower embryo has been developed by Rohini and Rao (2000). In this method the embryo axes of germinating seeds with one of the cotyledons removed were pricked with a sterile sewing needle at the cotyledonary node and infected by gentle agitation for 10 min in a suspension of *Agrobacterium tumefaciens*. Following 24 h of co-cultivation and decontamination with cefotaxime for 1 h, they were placed on soilrite moistened with water to allow germination. Later, the seedlings were transferred to soil in pots where they grew into normal healthy plants in the greenhouse. The histochemical assay of a *uid*A gene followed by polymerase chain reaction (PCR) amplification of *uid*A and *npt*II marker genes and Southern analysis of T_0 and T_1 plant DNA indicated that the frequency of transformation was 5.3% in safflower 'A-1' and 1.3% in 'A-300'.

Matern and Kneusel (1993) attempted to introduce resistance to leaf blight caused by *Alternaria* spp. in safflower. They identified and cloned the brefeldin A-esterase gene to introduce resistance to leaf blight, but transformation of safflower with the isolated brefeldin A-esterase gene was

unsuccessful. Mundel et al. (2004) reported that a Calgary-based company, SemBioSys Genetics, Inc., genetically transformed safflower tissue to produce the modified protein of interest in the seeds. It is indicated that transgenic safflower has a fair chance of getting permission for commercial cultivation in North America, as it has relatively low acreage and no weedy relatives with whom it can cross to produce fertile hybrids. In addition, safflower has several inherent agronomic qualities such as a low tendency to weediness, low seed dormancy, and large degree of self-pollination, which translates into a system that is quite easy to confine so that target products do not mingle with food or feed and thus make it a lower-risk production platform.

6.9 POLYEMBRYONY AND APOMIXIS IN SAFFLOWER

Existence of polyembryony, like that in many plant species, has been observed in safflower (Singh et al., 2005). A safflower genotype D-149 with a tendency to produce twin seedlings at the rate of 0.6 to 10% in its different derivatives has been identified. Thirty percent of the twin seedlings were male sterile and did not show seed setting on crossing with fertile plants, as well as under open pollination, and thus were considered to have a changed ploidy level and are assumed to be haploid or triploid in nature. The haploid or triploid nature of plants from twin seedlings suggests the presence of polyembryony, since polyembryony is known to produce haploids/triploids and these in general are reported to be male sterile in nature. Efforts are under way to examine the feasibility of its utilization for cultivar development in safflower.

Apomixis is yet to be confirmed in safflower, though preliminary studies carried out at the Nimbkar Agricultural Research Institute (NARI) at Phaltan in India indicate the possibility of existence of apomixis in safflower. Cytological and breeding investigations are under way at NARI to confirm the presence of apomixis in safflower.

6.10 FUTURE DIRECTION

Information on genetic and linkage maps in safflower is completely lacking, and it needs immediate attention because this will help breeders to manipulate genes controlling different traits and to evolve new genotypes and cultivars with improved productivity and resistance to biotic and abiotic factors. The lack of homology between the chromosomes of cultivated safflower (*C. tinctorius*) with 2n = 24 and the species with other than 2n = 24 chromosomes has prevented introgression of desirable genes from the wild relatives to the cultivated types. Modern techniques like embryo rescue and other biotechnological tools may play an important role in overcoming such barriers. Development of a cytoplasmic-genetic male sterility system for hybrid breeding, a successful outcome of ongoing efforts to use polyembryony for varietal improvement, and confirmation of apomixis in safflower would argue well for further efforts to translate them into reality. Flower yield and pigment content of the flowers are the other traits that have gained economic importance recently, due to an increasing demand for safflower flowers as a source of natural food color in European and other Western countries and their use in medicines for curing several chronic diseases. No attention has been paid to improvement of these traits in safflower. The improvement in yield of flowers and pigments in flowers would certainly help in increasing total remuneration from the crop to the farmer. Genetic transformation of safflower to impart resistance to biotic and abiotic factors, in addition to development of seeds with altered fatty acid and protein profiles, is another area that has received very little attention. Conventional breeding techniques, though used for these purposes, have not been very successful. Therefore, genetic modification of safflower would be of enormous importance in improving productivity, production, and remuneration per unit area from the crop, which in turn would certainly help in increasing safflower area in the world.

REFERENCES

Abel, G.H. and D.G. Lorance. 1975. Registration of 'Dart' safflower. *Crop Sci*. 15: 100.

Anjani, K. 2000. Components of seed yield in safflower (*Carthamus tinctorius*). *Indian J. Agric. Sci*. 70: 873–875.

Anonymous. 1976–1977. *Tenth Annual Progress Report of Nimbkar Agricultural Research Institute, Phaltan*. Maharashtra, India, 244 pp.

Anonymous. 1997–1998. *Annual Progress Report*. Safflower. Directorate of Oilseeds Research, Rajendranagar, Hyderabad-500 030, India, 121 pp.

Anonymous. 2002. *Safflower Research in India*. Directorate of Oilseeds Research, Hyderabad, 96 pp.

Anonymous. 2004–2005. *Annual Progress Report*. Safflower. Directorate of Oilseeds Research, Rajendranagar, Hyderabad-500 030, India, 181 pp.

Ashri, A. and P.F. Knowles. 1960. Cytogenetics of safflower (*Carthamus* L.) species and their hybrids. *Agron. J*. 52: 11–17.

Baker, C.M. and W.E. Dyer. 1996. Improvements in rooting regenerated safflower (*Carthamus tinctorius* L.) shoots. *Plant Cell Rep*. 16: 106–110.

Bamber, C.J. 1916. *Plants of the Punjab*. Supt. Govt. Print, Lahore Punjab, 332–373.

Bergman, J.W., D.E. Baldridge, P.L. Brown, A.L. Dubbs, G.D. Kushnak, and N.R. Riveland. 1987. Registration of 'Hartman' safflower. *Crop Sci*. 27: 1090–1091.

Bergman, J.W., G. Carlson, G. Kushnak, N.R. Riveland, and G. Stallknecht. 1985. Registration of 'Oker' safflower. *Crop Sci*. 25: 1127–1128.

Bergman, J.W., G. Carlson, G. Kushnak, N.R. Riveland, G. Stallknecht, L.E. Welty, and D. Wichman. 1989a. Registration of 'Girard' safflower. *Crop Sci*. 29: 828–829.

Bergman, J.W., G. Carlson, G. Kushnak, N.R. Riveland, G. Stallknecht, L.E. Welty, and D. Wichman. 1989b. Registration of 'Finch' safflower. *Crop Sci*. 29: 829.

Bergman, J.W. and N.R. Riveland. 1983. Registration of 'Sidwill' safflower. *Crop Sci*. 23: 1012–1013.

Bradley, V.L. and R.C. Johnson. 2001. Managing the U.S. safflower collection. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 143–147.

Carapetian, J. and A. Estilai. 1997. Genetics of isozyme coding genes in safflower. In *Proceedings of the 4th International Safflower Conference: Safflower: A Multipurpose Species with Unexploited Potential and World Adaptability*, Adriatica, Editrice, Bari, Italy, June 2–7, 1997. Corleto, A. and Mundel, H.H., Eds., pp. 235–237.

Cervantes-Martinez, J.E., M. Rey-Ponce, and M. Velazquez-Cagal. 2001. Evaluation of accessions from world collection of safflower for Alternaria incidence and seed oil content. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., p. 163.

Cervantes-Martinez, J.E. 2001. Safflower production and research in Mexico: status and prospects. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., p. 282.

Claassen, C.E. 1981. Development of safflower as a commercial crop in the United States. In *Proceedings of the 1st International Safflower Conference*, Davis, CA, July 12–16, 1981., pp. 28–35.

Darlington, C.D. and A.P. Wylie. 1956. *Chromosome Atlas of Flowering Plants*. Macmillan Co., New York, p. 262.

Deokar, A.B. and F.B. Patil. 1975. Inheritance of some qualitative characters in safflower: cases of linkage. *Indian J. Hered*. 7: 31–38.

Deokar, A.B. and F.B. Patil. 1980. Combining ability analysis in a diallel cross of safflower. *J. Maharashtra Agric. Univ*. 5: 214–216.

Deshpande, R.B. 1952. Wild safflower (*Carthamus oxyacantha* Bieb.): a possible oil seed crop for the desert and arid regions. *Indian J. Genet*. 12: 10–14.

Esendal, E. 2001. Global adaptability and future potential of safflower. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. xi–xii.

Estilai, A. 1977. Interspecific hybrids between *Carthamus tinctorius* and *C. alexandrinus*. *Crop Sci*. 17: 800–802.

Estilai, A. and P.F. Knowles. 1976. Cytogenetic studies of *Carthamus divaricatus* with eleven pairs of chromosomes and its relationship to other *Carthamus* species (Compositae). *Am. J. Bot*. 63: 771–782.

Estilai, A. and P.F. Knowles. 1980. Aneuploids in safflower. *Crop Sci*. 20: 516–518.

Fernandez-Martinez, J. and J. Dominguez-Gimenez. 1986. Release of five new safflower varieties. *Sesame Safflower Newsl*. 2: 89–90.

Fernandez-Martinez, J. and P.F. Knowles. 1978. Combined effects of genes for appressed and decumbent branching in safflower. *Crop Sci*. 17: 516–517.

Fernandez-Martinez, J., M. del Rio, and A. de Haro. 1993. Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters. *Euphytica* 69: 115–122.

Gadekar, D.A. and N.D. Jambhale. 2003. Inheritance of yield and yield components in safflower (*Carthamus tinctorius* L.). *J. Oilseeds Res*. 20: 63–65.

George, L. and P.S. Rao. 1982. *In vitro* multiplication of safflower (*Carthamus tinctorius* L.) through tissue culture. *Proc. Ind. Natl. Sci. Acad*. B48: 791–794.

Ghongade, R.A., B.P. Joshi, and P.A. Navale. 1993. Correlation and path analysis of some yield components in safflower. *J. Maharashtra Agric. Univ*. 18: 240–243.

Ghorpade, P.B. and M.R. Wandhare. 2001. Application of simplified triple test cross and combining ability analysis to determine the gene action in safflower. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 79–82.

Hanelt, P. 1961. Information on *Carthamus tinctorius* L. *Die Kulturpflanze*. 9: 114–145 (in German).

Hanelt, P. 1963. Monographische Ubersicht der Gattung *Carthamus* L. (Compositae). Feddes Repertorium Specierum Novarum Regni Vegetabilis. *Bot. Taxon. Geobt*. 67: 41–180.

Harrigan, E.K.S. 1987. Safflower registration of cv. Sironaria. *Sesame Safflower Newsl*. 3: 47–49.

Harrigan, E.K.S. 1989. Review of research of safflower in Australia. In *Proceedings of the 2nd International Safflower Conference*, Hyderabad, India, January 9–13, 1989. Ranga Rao, V. and M. Ramachandram, Eds. ISOR, Directorate of Oilseeds Research, pp. 97–100.

Harvey, B.L. and P.F. Knowles. 1965. Natural and artificial alloploids with 22 pairs of chromosomes in the genus *Carthamus* L. (Compositae). *Can. J. Genet. Cytol*. 7: 126–139.

Heaton, T.C. 1981. Possibilities for the use of *Carthamus lanatus* L. in the improvement of safflower, *C. tinctorius* L. In *Proceedings of the 1st International Safflower Conference*, Davis, CA, July 12–16, 1981, pp. 70–73.

Heaton, T.C. and P.F. Knowles. 1980. Registration of UC-148 and UC-149 male sterile safflower germplasm. *Crop Sci*. 20: 554.

Hegde, D.M., V. Singh, and N. Nimbkar. 2002. Safflower. In *Genetic Improvement of Field Crops*. Singh, C.B. and D. Khare, Eds. Scientific Publishers, Jodhpur, India, pp. 199–221.

Hill, A.B. 1989. Hybrid safflower breeding. In *Proceedings of the 2nd International Safflower Conference*, Hyderabad, India, January 9–13, 1989. Ranga Rao, V. and M. Ramachandram, Eds. ISOR, Directorate of Oilseeds Research, pp. 169–170.

Johnson, R.C., J.W. Bergman, and C.R. Flynn. 1999. Oil and meal characteristics of core and non-core safflower accessions from the USDA collection. *Genet. Res. Crop Evol*. 46: 611–618.

Johnson, R.C., P.B. Ghorpade, and V.L. Bradley. 2001. Evaluation of the USDA core safflower collection for seven quantitative traits. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 149–152.

Johnson, R.C., D.M. Stout, and V.L. Bradley. 1993. The U.S. collection: a rich source of safflower germplasm. In *Proceedings of the 3rd International Safflower Conference*, Beijing, June 14–18, 1993. Li, D. and H. Yuanzhou, Eds., pp. 202–208.

Joshi, A.B. 1979. Breeding methodology for autogamous crops. *Indian J. Genet*. 39: 567–577.

Joshi, B.M., Y.S. Nerkar, and N.D. Jambhale. 1983. Induced male sterility in safflower. *J. Maharashtra Agric. Univ*. 8: 194–196.

Karve, A.D., A.K. Deshmukh, and D.V. Nagvekar. 1979. Hybrid safflower. *Abstr. Tech. Papers Int. Congr. Oilseeds Oils* 1979: 13–14.

Karve, A.D., A.K. Deshmukh, and S.M.H. Qadri. 1981. Breeding disease resistant safflower for cultivation in the Deccan peninsula of India. In *Proceedings of the 1st International Safflower Conference*, Davis, CA, July 12–16, 1981, pp. 103–107.

Khidir, M.O. and P.F. Knowles. 1970. Cytogenetic studies of *Carthamus* species (Compositae) with 32 pairs of chromosomes. II. Intersectional hybridization. *Can. J. Genet. Cytol.* 12: 90–99.

Kleingarten, L. 1993. In *Notes Safflower Conference*, Billings, MT, February 18, 1993. Mundel, H.H. and J. Braun, Eds. Lethbridge, AB, Canada, p. 5.

Knowles, P.F. 1968. Registration of 'UC-1' safflower. *Crop Sci.* 8: 641.

Knowles, P.F. 1969. Centers of plant diversity and conservation of crop germplasm: safflower. *Econ. Bot.* 23: 324–329.

Knowles, P.F. 1988. *Carthamus* Species Relationships. Lecture presented at Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences, Beijing.

Knowles, P.F. 1989. Safflower. In *Oil Crops of the World*. Downey, R.K., G. Robellen, and A. Ashri, Eds. McGraw-Hill, New York, pp. 363–374.

Knowles, P.F. and S.C. Schank. 1964. Artificial hybrids of *Carthamus nitidus* Boiss. and *C. tinctorius* L. (Compositae). *Crop Sci.* 4: 596–599.

Kumar, H. 1993. Current trends in breeding research for enhancing productivity of safflower in India. *Sesame Safflower Newsl.* 8: 70–73.

Kumar, H., R.S.N. Pillai, and R.B. Singh. 1981. Cytogenetic studies in safflower. In *Proceedings of the 1st International Safflower Conference*, Davis, CA, July 12–16, 1981, pp. 126–136.

Kupzow, A.J. 1932. The geographical variability of the species *Carthamus tinctorius* L. *Bull. Appl. Bot. Genet. Plant Breed.* 1: 99–181.

Li, D., Z. Mingde, and V. Ramanatha Rao. 1993. *Characterization and Evaluation of Safflower Germplasm*. Geological Publishing House, Beijing, 260 pp.

Li, D. and H.H. Mundel. 1996. *Safflower. Carthamus tinctorius L. Promoting the Conservation and Use of Underutilized and Neglected Crops. 7.* Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, 83 pp.

Li, D. and H. Yuanzhou. 1993. The development and exploitation of safflower tea. In *Proceedings of the 3rd International Safflower Conference*, Beijing, June 14–18, 1993. Li, D. and H. Yuanzhou, Eds., pp. 837–843.

Lopez-Gonzalez, G. 1990. Acerca de la clasificacion natural del genero *Carthamus* L., s.1. *Anales Jardin Bot. Madrid* 47: 11–34.

Makne, V.G. and V.P. Choudhari. 1980. Combining ability in safflower (*Carthamus tinctorius* L.). *J. Maharashtra Agric. Univ.* 5: 128–130.

Makne, V.G., V.D. Patil, and V.P. Choudhari. 1979. Genetic variability and character association in safflower. *Indian J. Agric. Sci.* 49: 766–768.

Mandal, A.B. 1990. Variability, correlation and path coefficient analysis of safflower (*Carthamus tinctorius* L.). *Phytobreedon* 6: 9–18.

Mandal, A.K.A., A.K. Chatterjee, and S. Datta Gupta. 1995. Direct somatic embryogenesis and plantlet regeneration from cotyledonary leaves of safflower. *Plant Cell Tiss. Org. Cul.* 43: 287–289.

Matern, U. and R.E. Kneusel. 1993. The use of recombinant DNA techniques to confer resistance to the Alternaria leaf spot disease of safflower. In *Proceedings of the 3rd International Safflower Conference*, Beijing, June 14–18, 1993. Li, D. and H. Yuanzhou, Eds., pp. 807–815.

Mathur, J.R., S.B.S. Tikka, R.K. Sharma, S.P. Singh, and S.L. Dasora. 1976. Genetic variability and path coefficient analysis of yield components in safflower. *Indian J. Hered.* 8: 1–9.

Mundel, H.H., R.E. Blackshaw, J.R. Byers, H.C. Huang, D.L. Johnson, R. Keon, J. Kubik, R. Mckenzie, B. Otto, B. Roth, and K. Stanford. 2004. *Safflower Production on the Canadian Prairies: Revisited in 2004*. Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, 37 pp.

Mundel, H.H. and H.C. Huang. 2003. Control of major diseases of safflower by breeding for resistance and using cultural practices. In *Advances in Plant Disease Management*. Huang, H.C. and S.N. Acharya, Eds. Research Signpost, Trivandrum, Kerala, India, pp. 293–310.

Mundel, H.H., H.C. Huang, L.D. Burch, and F. Kiehn, 1985. Saffire safflower. *Can. J. Plant Sci.* 65: 1079–1081.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum* 15: 473–492.

Nagaraj, G. 1995. *Quality and Utility of Oilseeds*. Directorate of Oilseeds Research, Hyderabad, India, 70 pp.

Narkhede, B.N., A.M. Patil, and A.B. Deokar. 1992. Gene action of some characters in safflower. *J. Maharashtra Agric. Univ.* 17: 4–6.

Nie, Z., F. Chen, and X.C. Shi. 1988. A study on selection indices of seed yield in safflower. *Oil Crops China* 1: 15–19.

Nie, Z., F. Chen, and X.C. Shi. 1993. Path analysis of characters related to seed yield in safflower. *Oil Crops China* 3: 26–29.

Nikam, T.D. and M.G. Shitole. 1997. Sodium chloride tolerance in (*Carthamus tinctorius* L.). a. A-1 callus cultures. In *Proceedings of the 4th International Safflower Conference: Safflower: A Multipurpose Species with Unexploited Potential and World Adaptability*, Adriatica, Editrice, Bari, Italy, June 2–7, 1997. Corleto, A. and Mundel, H.H., Eds., pp. 175–178.

Nikam, T.D. and M.G. Shitole. 1999. *In vitro* culture of safflower cv. Bhima: initiation, growth optimization and organogenesis. *Plant Cell Tiss. Org. Cult.* 55: 15–22.

Nimbkar, N. 2002. Safflower rediscovered. *Times Agric. J.* 2: 32–36.

Orlikowska, T.K., H.J. Cranston, and W.E. Dyer. 1995. Factors influencing *Agrobacterium tumefaciens*-mediated transformation and regeneration of the safflower cultivar 'Centennial'. *Plant Cell Tiss. Org. Cult.* 40: 85–91.

Parmeshwarappa, K.G., K.M. Aradhya, and K.S. Prakash. 1984. Inheritance of seed yield, hull and oil content in safflower. *SABRAO J.* 16: 129–134.

Parameshwarappa, K.G., B.T. Ninganur, V. Rudranaik, G.G. Gulaganji, U.K. Hulihalli, and N.K.B. Patil. 1995. Combining ability analysis of seed yield, percent oil and other yield attributes in safflower. *J. Oilseeds Res.* 12: 258–261.

Patel, J.S. and G.V. Narayana. 1935. Chromosome numbers in safflower. *Curr. Sci.* 4: 412.

Patil, B.R., S.G. Deshmukh, and M.P. Deshmukh. 1990. Studies on correlation and path analysis in safflower. *Ann. Plant Phy.* 4: 86–91.

Patil, M.B., Y.M. Shinde, and K.A. Attarde. 1993. Evaluation of safflower cultures for resistance to Alternaria leaf spot (*Alternaria carthami*) and management strategies. In *Proceedings of the 3rd International Safflower Conference*, Beijing, June 14–18, 1993. Li, D. and H. Yuanzhou, Eds., pp. 269–278.

Patil, P.S., A.B. Deokar, and B.K. Katule. 1987. Breeding. In *Safflower*. Patil, P.S., Ed. AICORPO (Safflower), Mahatma Phule Agricultural University, Solapur, Maharashtra, India, pp. 7–72.

Patil, P.S., A.M. Patil, and A.B. Deokar. 1992. Line X tester analysis for combining ability in safflower. *J. Maharashtra Agric. Univ.* 17: 64–66.

Prakash, K.S. and B.G. Prakash. 1993. Yield structure analysis of oil yield in safflower (*Carethamus tinctorius* L.). *Oleagineux* 48: 83–89.

Prasad, B.R., M.A. Khadeer, P. Seeta, and S.Y. Anwar. 1991. *In vitro* induction of androgenic haploids in safflower (*Carthamus tinctorius* L.). *Plant Cell Rep.* 10: 48–51.

Raina, S.N., S. Sharma, T. Sasakuma, M. Kishii, and S. Vaishnavi. 2005. Novel repeated DNA sequences in safflower (*Carthamus tinctorius* L.) (Asteraceae): cloning, sequencing and physical mapping by fluorescence *in situ* hybridization. *J. Hered.* 96: 424–429.

Ramachandram, M. 1985. Genetic improvement of oil yield in safflower: problems and prospects. *J. Oilseeds Res.* 2: 1–9.

Ramachandram, M. and J.V. Goud. 1981. Genetic analysis of seed yield, oil content and their components in safflower (*Carthamus tinctorius* L.). *Theor. Appl. Genet.* 60: 191–195.

Ramachandram, M. and J.V. Goud. 1982a. Components of seed yield in safflower (*Carthamus tinctorius* L.). *Genetica Agraria* 36: 211–222.

Ramachandram, M. and J.V. Goud. 1982b. Gene action for seed yield and its components in safflower. *Indian J. Agric. Sci.* 42: 213–220.

Ramachandram, M. and M. Sujatha. 1991. Development of genetic male sterile lines in safflower. *Indian J. Genet.* 51: 268–269.

Ranga Rao, V. 1982. Heterosis for agronomic characters in safflower. *Indian J. Genet.* 42: 364–371.

Ranga Rao, V. 1983. Combining ability for yield, percent oil and related components in safflower. *Indian J. Genet.* 43: 68–75.

Ranga Rao, V., V. Arunachalam, and M. Ramachandram. 1977. An analysis of association of components of yield and oil in safflower (*Carthamus tinctorius* L.). *Theor. Appl. Genet.* 50: 185–191.

Ranga Rao, V. and M. Ramachandram. 1979. Stability parameters for yield and its components in safflower. *Mysore J. Agric. Sci.* 13: 297–308.

Richharia, R.H. and J.P. Kotval. 1940. Chromosome numbers in safflower. *Curr. Sci.* 9: 73–74.

Rohini, V.K. and K. Sankara Rao. 2000. Embryo transformation, a practical approach for realizing transgenic plants of safflower (*Carthamus tinctorius* L.). *Ann. Bot*. 86: 1043–1049.

Rubis, D.D. 1981. Development of root-rot resistance in safflower by introgressive hybridization and thin-hull facilitated recurrent selection. In *Proceedings of the 1st International Safflower Conference*, Davis, CA, July 12–16, 1981, pp. 205–209.

Rubis, D.D. 2001. Developing new characteristics during 50 years of safflower breeding. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 109–111.

Sangale, P.B., S.D. Pagar, and S.Y. Daftardar. 1982. Note on the association of seed oil content with hull and kernel and seed sizes of safflower. *Indian J. Agric. Sci*. 52: 613–614.

Sastry, K.R. and M. Ramachandram. 1992. Differential genotypic response to progressive development of wilts of safflower. *J. Oilseeds Res*. 9: 297–305.

Sawant, A.R. 1989–1990. *Annual Progress Report of All India Coordinated Research Project on Oilseeds (Safflower)*. Jawaharlal Nehru Krishi Vishwa Vidyalaya, Indore, India, 117 pp.

Sawant, A.R., M.K. Saxena, S.L. Deshpande, and G.S. Bharaj. 2000. Cultivation of spineless safflower is profitable. In *Extended Summaries. National Seminar on "Oilseeds and Oils Research and Development Needs in the Millennium*," Hyderabad, India, February 2–4, 2000. ISOR, Directorate of Oilseeds Research, pp. 39–40.

Schank, S.C. and P.F. Knowles. 1964. Cytogenetics of hybrids of *Carthamus* L. species (Compositae) with ten pairs of chromosomes. *Am. J. Bot*. 51: 1093–1102.

Seeta, P., K. Talat, and S.Y. Anwar. 2000. Somaclonal variation: an alternative source of genetic variability in safflower. *J. Cytol. Genet*. 1: 127–135.

Sehgal, D. and S.N. Raina. 2005. Genotyping safflower (*Carthamus tinctorius*) cultivars by DNA fingerprints. *Euphytica* 146: 67–76.

Singh, R.P. and A.B. Abidi. 2005. Protein enriched biscuits from safflower (*Carthamus tinctorius* L.) cake. *Beverage Food World* 32: 46.

Singh, V. 1996. Inheritance of genetic male sterility in safflower. *Indian J. Genet*. 56: 490–494.

Singh, V. 1997. Identification of genetic linkage between male sterility and dwarfness in safflower. *Indian J. Genet*. 57: 327–332.

Singh, V. 2004. Annual Report of Ad Hoc Project on "Biometrical Investigations of Flower Yield and Its Components and Their Maximization in Safflower." Submitted to ICAR, New Delhi, 35 pp.

Singh, V. 2005a. Annual Report of Ad Hoc Project on "To Study the Usefulness of Petal from Indian Cultivars of Safflower for Developing Value Added Products of Edible Nature." Paper presented at Group Monitoring Workshop on DST, New Delhi, February 3–5, pp. 7–11.

Singh, V. 2005b. Final Report of *Ad Hoc* Project on "Identification of Early Plant Growth Male Sterility Marker in Existing GMS Systems and Search for Cytoplasmic Genetic Source of Sterility in Safflower." Submitted to ICAR, New Delhi, 61 pp.

Singh, V., M.B. Deshpande, S.V. Choudhari, and N. Nimbkar. 2004. Correlation and path coefficient analysis in safflower (*Carthamus tinctorius* L.). *Sesame Safflower Newsl.,* 19: 77–81.

Singh, V., M.B. Deshpande, M.K. Galande, S.R. Deshmukh, and N. Nimbkar. 2000. Current status of research and development in safflower hybrid in India. In *Extended Summaries. National Seminar on "Oilseeds and Oils Research and Development Needs in the Millennium*," Hyderabad, India, February 2–4, 2000. ISOR, Directorate of Oilseeds Research, p. 62.

Singh, V., M.B. Deshpande, and N. Nimbkar. 2003a. NARI-NH-1: the first non-spiny hybrid safflower released in India. *Sesame Safflower Newsl*. 18: 77–79.

Singh, V., M.B. Deshpande, and N. Nimbkar. 2005. Polyembryony in safflower and its role in crop improvement. In *Proceedings of the 6th International Safflower Conference*, Instanbul, Turkey, June 6–10, 2005. Esendal, E., Ed., pp. 14–20.

Singh, V., A.J. Dhembare, M.B. Deshpande, and N. Nimbkar. 1993. Variability and character association studies in safflower. *J. Maharashtra Agric. Univ*. 18: 483–484.

Singh, V., M.K. Galande, S.R. Deshmukh, M.B. Deshpande, and N. Nimbkar. 2001a. Identification of male sterile cytoplasm in safflower. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 123–126.

Singh, V., M.K. Galande, M.B. Deshpande, and N. Nimbkar. 2001b. Inheritance of wilt (*Fusarium oxysporum* f. sp. *carthami*) resistance in safflower. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 127–131.

Singh, V. and N. Nimbkar. 1993. Genetics of aphid resistance in safflower (*Carthamus tinctorius* L.). *Sesame Safflower Newsl*. 8: 101–106.

Singh, V., D.R. Rathod, M.B. Deshpande, S.R. Deshmukh, and N. Nimbkar. 2003b. Breeding for wilt resistance in safflower. In *Extended Summaries*. *National Seminar on "Stress Management in Oilseeds for Attaining Self-Reliance in Vegetable Oils*," Hyderabad, India, January 28–30, 2003. ISOR, Directorate of Oilseeds Research, pp. 368–370.

Smith, J.R. 1996. *Safflower*. AOCS Press, Champaign, IL, p. 624.

Suganya, A., M. Sujatha, and K.R. Sastry. 1997. *In vitro* selection for resistance to *Fusarium oxysporum* Schlecht. carthami Klisiewiez and Houston in safflower. In *Proceedings of the 4th International Safflower Conference: Safflower: A Multipurpose Species with Unexploited Potential and World Adaptability*, Adriatica, Editrice, Bari, Italy, June 2–7, 1997. Corleto, A. and Mundel, H.H., Eds., pp. 305–308.

Sujatha, M. and A. Suganya. 1996. *In vitro* organogenic comparison of different seedling tissues of safflower (*Carthamus tinctorius* L.). *Sesame Safflower Newsl*. 11: 85–90.

Tejovathi, G. and S.Y. Anwar. 1987. Plant regeneration from cotyledonary cultures of safflower (*Carthamus tinctorius* L.). In *Proceedings of the National Symposium "Plant Cell and Tissue Culture of Economically Important Plants*," Hyderabad, India. Reddy, G.M., Ed., pp. 347–354.

Tejovathi, G. and S.Y. Anwar. 1993. 2,4,5-trichlorophenoxy propionic acid induced rhizogenesis in *Carthamus tinctorius* L. *Proc*. *Ind*. *Natl*. *Sci*. *Acad*. B59: 633–636.

Thomas, C.A. 1964. Registration of 'US10' safflower. *Crop Sci*. 4: 446–447.

Thomas, C.A. 1971. Registration of 'VFR-1' safflower germplasm. *Crop Sci*. 11: 606.

Urie, A.L. 1981. Continued studies on inheritance of partial hull in safflower (*Carthamus tinctorius* L.). In *Proceedings of the 1st International Safflower Conference*, Davis, CA, July 12–16, 1981, pp. 264–271.

Urie, A.L. 1986. Inheritance of partial hull in safflower. *Crop Sci*. 26: 493–498.

Urie, A.L., W.F. Peterson, and P.F. Knowles. 1979. Registration of "Oleic Leed" safflower. *Crop Sci*. 19: 747.

Urie, A.L. and D.E. Zimmer. 1970a. Yield reduction in safflower hybrids caused by female selfs. *Crop Sci*. 10: 419–422.

Urie, A.L. and D.E. Zimmer. 1970b. Registration of reduced-hull safflower lines, reduced hull-1, -2, -3 and -4. *Crop Sci*. 10: 732.

van Rooijen, G.J., L.I. Terning, and M.M. Moloney. 1992. Nucleotide sequence of an *Arabidopsis thaliana* oleosin gene. *Plant Mol*. *Biol*. 18: 1177–1179.

Vavilov, N.I. 1951. *The Origin, Variation, Immunity and Breeding of Cultivated Plants*. Ronald Press Company, New York, 1951, 364 pp.

Velasco, L. and J. Fernandez-Martinez. 2001. Breeding for oil quality in safflower. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 133–137.

Vilatersana, R., T. Garnatje, A. Susanna, and N. Garcia-Jacas. 2005. Taxonomic problems in *Carthamus* (Asteraceae): RAPD markers and sectional classification. *Bot*. *J*. *Linn*. *Soc*. 147: 375–383.

Vilatersana, R., A. Susanna, N. Garcia-Jacas, and T. Garnatje. 2000. Karyology, generic delineation and dysploidy in the genera *Carduncellus*, *Carthamus* and *Phonus* (Asteraceae). *Bot*. *J*. *Linn*. *Soc*. 134: 425–438.

Walia, N., A. Kaur, and S.B. Babbar. 2005. *In vitro* regeneration of a high oil-yielding variety of safflower (*Carthamus tinctorius* var. HUS-305). *J*. *Plant Biochem*. *Biotech*. 14: 1–4.

Weiss, E.A. 1971. *Castor, Sesame and Safflower*. Leonard Hill Books, London, pp. 529–744.

Yazdi-Samadi, B., R. Maali Amiri, M.R. Ghannadha, and C. Abd-Mishani. 2001. Detection of DNA polymorphism in landrace populations of safflower in Iran using RAPD-PCR technique. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., p. 163.

Ying, M., W.E. Dyer, and J.W. Bergman. 1992. *Agrobacterium tumefaciens*-mediated transformation of safflower (*Carthamus tinctorius* L.) cv. 'Centennial'. *Plant Cell Rep*. 11: 581–585.

Zhang, Z. 2001. Genetic diversity and classification of safflower (*Carthamus tinctorius* L.) germplasm by isozyme techniques. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 157–162.

Zhaomu, W. and D. Lijie. 2001. Current situation and prospects of safflower products development in China. In *Proceedings of the 5th International Safflower Conference*, Williston, ND, and Sidney, MT, July 23–27, 2001. Bergman, J.W. and H.H. Mundel, Eds., pp. 315–319.