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Review article

Breeding improvements in safflower (*Carthamus tinctorius* L.): A review

Pooran Golkar*

Institute of Biotechnology and Bioengineering, Isfahan University of Technology, 8415683111, Isfahan, Iran

***Corresponding author: golkar@cc.iut.ac.ir**

Abstract

Safflower (*Carthamus tinctorius* L.) is an oil seed crop that is grown not only for its edible oil but also for its applications as animal feed and plant-made pharmaceuticals**.** Historically, biometrical genetics played a crucial role in the improvement of safflower seed yield and its components, phenologic and morphologic traits, and nutritional properties including oil, fatty (linoleic, oleic, stearic, and palmitic) acids, protein, and fiber. Breeding methods based on gene action of different traits has a significant role in releasing high-yield genotypes. In recent years, biotechnological methods have played a supplementary role in safflower breeding, but breeding programs are not being complemented adequately with molecular tools. In this review article, efforts are made to investigate and review the different genetic studies thus far conducted on the genetic control of different traits and molecular markers used for germplasm identification in safflower. The studies reviewed have left their mark on safflower improvement in recent years.

Keywords: additive, dominance, effects, genetic, oil.
Abbreviations: AFLP_Amplified Fragment Length Polymorphism; EST_Expressed sequence tag, GCA_General combining ability; GMA_generation mean analysis, GMA_Generation mean analysis, LG_Linkage group, MAS_Marker assist selection, RAPD_ Random amplified polymorphic DNA, SCA_Specific combining ability; SCAR_Sequenced characterized amplified region.

Introduction

Safflower (*Carthamus tinctorius* L.), a member of the compositae family, is an annual oilseed crop (Weiss, 2000). It is the only cultivated type of safflower that contains 2n=24 chromosomes (Singh, 2007). The plant has been traditionally grown for its flower and oilseed to produce cooking oil, fabric dyes, food coloring, and animal and bird feeds. It has also found medicinal and industrial applications such as biofuel (Dajue and Mundel, 1996). Although specially cultivated in a band from the Mediterranean Sea to the Pacific Ocean (Jaradat and Shahid, 2006) it has been traditionally grown in different countries such as India, Mexico, China, Australia, Turkey, and Iran (Singh, 2007). Iran is regarded as one of the diversity centers for safflower in the world (Knowles, 1969) where numerous types of wild and cultivated safflower genotypes are found. In recent years, its cultivation has increased because of the high demand for oilseed crops to compensate for the lack of nutritional oil (Ghaderi et al., 2011). 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 (Singh, 2007). It has remained as a neglected crop due to its low seed oil content (28-36%), spininess (in some genotypes), and vulnerability to a number of diseases and pests (Sujatha, 2008). The nutritional value of safflower oil is related to its high level of polyunsaturated oils (Weiss, 2000). Safflower oil contains about 75% linoleic acid that is essential for human nutrition (Weiss, 2000). The leaves are rich in carotene, riboflavin, and vitamin C; hence, young seedlings are used as a green leafy vegetable in India (Singh, 2007; Asqarpanah and Kazemivash, 2013).

The importance of quantitative genetics in safflower breeding

Quantitative genetics helps the inheritance of polygenic traits among related individuals (Mather and Jinks, 1982). In plant populations subjected to artificial selection, genetic parameters are required to be estimated for formulating breeding plans. Genetic designs are crucial tools for analyzing genetic parameters and gene effects which are now becoming increasingly available for a variety of plant species (Singh and Pawar, 2005). Methods differ in the magnitude of sampling variance of estimates. Different genetic designs have been carried out in safflower for estimation of genetic parameters some of which include: dialell (Ramachandram and Goud, 1981; Gupta and Singh, 1988a; Mandal and Banerjee, 1997; Golkar et al., 2012b), Generation Mean Analysis (Yermanos et al., 1967; Ehdaei and Ghaderi, 1978; Shahbazi and Saedi, 2007), line × tester (Deshmukh et al., 1998), triple test cross (Dhumale et al.,1998), and biparental mating design (Rudra-Naik et al., 2009). General combining ability (GCA) is a measure of additive gene action, whereas specific combining ability (SCA) is related to dominance (Singh and Pawar, 2005). Specific combining ability for agronomic traits is an important indicator of the potential of inbred lines for generating successful hybrid combinations (Singh and Pawar, 2005). Comparison of crosses means and combining ability of selected genotypes in the diallel design could be a good strategy for identification of superior

genotypes for hybrid production (Mather and Jinks, 1982). All crop plants offer a great scope for yield improvement. The possibilities of achieving this goal through genetic manipulation by genetic designs have been elucidated by evolving high yielding hybrids of safflower by production of different F_1 hybrids via diallel crosses (Golkar et al, 2011b). Literature review shows few efforts so far directed at the development of F_1 hybrids of safflower through exploitation of genetic variability present in exotic parents. Hybridization with marker-assisted selection could be used in formulating proper methodologies to bring out the desired improvement in different traits with dominance gene effects. In addition, selection-based breeding methods could be proposed for improving traits with additive-gene action. This article reviews the classical studies of estimation of gene action and heritability of different phenol-morphologic and agronomic traits in safflower while a brief examination is also made of the molecular improvements made in safflower. Phenological traits. Days to emergence, days to budding, days to bolling, days to flowering, and days to maturity form the sequence of developmental stages in safflower (Singh, 2007). Production of early maturing cultivars is a priority objective in many plant breeding programs. Therefore, production of early maturing genotypes could be an effective strategy for avoiding insects and disease infections. Different genetic designs including biometrical designs such as GMA and diallel have been used for the estimation of genetic parameters of phenologic traits. Golkar (2011a) reported that days to budding and days to bolling were under the genetic control of additive effects. Some phenological traits, including flowering and maturity, are the most critical stages influencing safflower yield (Weiss, 2000). Emerging and maturity dates are critical points in plant earliness. Golkar (2011a) reported the importance of both additive and dominance effects in the genetic control of earliness in safflower. The predominant role of additive gene action (Kotecha, 1979; Shahbazi and Seaidi, 2007) and the over dominance of gene action (Gupta and Singh, 1988b) have also been reportedly important in the genetic control of days to maturity.

While Golkar (2011) reported dominance gene effects involved in the genetic control of days to flowering, Gupta and Singh (1988b) reported only its partial dominance. This is while Singh et al. (2008) reported the importance of both additive and dominance gene actions for the genetic control of days to flowering. This inconsistency in results could be explained by differences in genotypes and environmental conditions used in each study.

Morphologic traits

Biometrical analyses have been carried out to evaluate gene action for morphological traits. For instance, plant height as an important morphological trait has been reported to be under the effect of additive gene action (Kotecha, 1979; Shahbazi and Saeidi, 2007; Golkar et al., 2012b). Its association with the yield component trait could be important for indirect selection via plant height index. It is reported that this morphological trait is not affected by extra-nuclear genes (Mandal and Banerjee, 1997). Thus, cyclic selection should be effective for its improvement. Stem diameter and leaf length, as two other morphological traits, have been reported to be under the effects of additive and non-additive gene action, respectively (Kotecha, 1979). Regarding number of branches per plant, Gupta and Singh (1988b) found additive gene effects as playing an important role in its genetic control. However, Narkhede and Patil (1987) claimed epistasis effects to have a significant role in controlling number of branches per plant whereas the results of Golkar et al. (2012b) indicated a non-significant effect of epistasis in this regard. Given these inconsistent findings, it seems that practical utilization of information regarding epistasis in breeding is a challenging issue that needs to be fully addressed via further biometric studies (Golkar et al., 2012b).

Number of branches per plant is an important trait in view point of its correlation with seed yield (Golkar et al., 2012b) and ornamental aspects. Branching habit in safflower is controlled both digenically and environmentally (Deokar and Patil, 1975). Apprised branching is recessive to separating types and is controlled both digenically and monogenically (Deokar and Patil, 1975). The genetic control of head diameter is under dominance gene effects (Golkar et al., 2012b). This is while Camas and Esendel (2006) reported a low broad-sense heritability for it. This finding reveals the importance of environmental effects on head diameter which is a good index for ornamental application of safflower. The additive-dominance model has also been claimed for the genetic control of node number on the main stem with GMA (Abel, 1976). Some epistatic effects have also been reported for internode distances (Abel, 1976).

Seed- related traits

Whole safflower seeds are white or creamy in color, and their typical composition is 55-65% kernel and 33-45% hull (Singh, 2007). In normal hull types, the whole seed contains 27-32% oil, 5-8% moisture, 14-15% protein, 2-7% ash, and 32-40% crude fiber (Weiss, 2000). In normal hull types The whole seeds of the plant have an oil percentage of 25-37%, but in very thin hull types, this ratio increases to 46-47%. According to Urie (1986), the pericarp (hull) of safflower seed is high in fiber whereas its embryo portion is rich in oil and protein. Reduction of the hull portion of the seed would, therefore, increase the product value. The same author claimed that partial hull is recessive to white hull. According to Ebert and Knowles (1966), striped seed and reduced pericarp are controlled by the recessive genes of *th* and *stp*, respectively. They also claimed a monogenic control for the stripped hull inheritance. Classen (1952) and Efron (1964) recognized a monogenic inheritance for the genetic control of pappus in safflower. They reported that pappus was dominant over nonpappus, but Kotecha and Zimmerman (1978b) identified a digenic inheritance for it. Regarding genetic control, Ashri and Efron (1964) concluded a dominance gene action for pappus inheritance. Moreover, pigmentation of cotyledons has been attributed to controlled monogenic inheritance. In an earlier work, Kotecha and Zimmerman (1978a) reported non-additive effects for the genetic control of seed dormancy with its heritability ranging between 33-55%. Yermanos et al. (1967) reported that the genetic control of iodine value was monogenic with infections of maternal effects. Golkar et al. (2012a) identified the genetic control of fiber to be predominated by additive gene action with influence from cytoplasmic effects while the genetic control of ash, they claimed, was dominated by dominance gene effects.

Nutritional seed traits

The protein content of safflower seeds is of a good nutritional quality. Using the genetics of safflower protein is a proper way for improving seed quality although no much information is presently available on the nature of the proteins in safflower seed. The genetic control of the protein

Table 1. Segregation and χ^2 test for spininess trait in F_2 and $F_{2:3}$ populations from 'Whiteflower-Isf' (spineless) × Mexican 22-191 (spinny) cross.

Parent / cross	Generation	Number of plants / families				χ^2	P
		Spiny	Segregating	spineless			
Whiteflower-Isf	P_1			spineless			
Mexican 22-191	P_2	Spiny					
Whiteflower-Isf'×'22-191'	F_1	40		0			
Whiteflower-Isf'×'22-191'	F_2	80	0	16	3:1	3.55	0.06
Whiteflower-Isf'×'22-191'	$F_{2:3}$	23	46	27	1:2:1	0.64	0.72

Table 2. Segregation and χ^2 test for flower color trait in F_2 and $F_{2:3}$ populations from 'Whiteflower-Isf' (white) × Mexican 22-191 (yellow) cross.

Parent /cross	Generation	Number of plants / families					χ^2	P
		Yellow	Orange	Segregating	White	Ratio		
White flower-Isf (W)	P_1	Yellow						
Mexican 22-191 (M)	P_2				White			
W × M	F_1	50						
W × M	F_2	65	15		16	9:3:4	5.40	0.067
W × M	$F_{2:3}$	4	10	11:29:17:9	16	1:1:2:4:2:2:4	9.95	0.126

Table 3. Analysis of variance for combining ability of different traits in the F_1 generations of safflower

F_1 hybrids	df	Mean square					
		Oil content (%)	Linoleic acid (%)	Oleic acid (%)	Palmitic acid (%)	Stearic acid (%)	Protein content (%)
GCA	7	47.70**	680.05**	599.6**	7.79**	72.37**	10.78*
SCA	28	9.22**	19.65**	28.23**	2.01**	7.84**	5.12**
Reciprocal	28	3.56	39.11**	22.06	0.98	8.17**	2.24
Residual	126	4.53	6.07**	6.79	0.74	3.32	2.38
GCA/SCA		5.17*	34.6**	21.23**	3.87**	3.27**	2.10
δ^2_A		1.6	27.52	24.10	0.16	2.68	0.22
δ^2_D		0.87	2.54	4.01	0.10	1.28	0.51
P.F. ¥.		0.64	0.91	0.86	0.61	0.67	0.30
h^2_b†		0.62	0.93	0.92	0.86	0.76	0.51
h^2_n ††		0.35	0.86	0.81	0.65	0.62	0.21

† and †† Abbreviations: h^2_b= Board-sense heritability, h^2_n= Narrow-sense heritability

* and ** significant at P<0.01 and P< 0.05, respectively

¥: Predictability factor: $2\sigma^2_{GCA}/(2\sigma^2_{GCA}+\sigma^2_{SCA})$

content is under additive- dominance model (Pahlavani et al., 2007; Golkar at al., 2012a). It follows, therefore, that both selection and hybridization methods could be recommended for its improvement. Oil content is a quantitative trait which is affected by genotype, environment, and genotype× environment interaction. Safflower breeding efforts should emphasize the improvement of both quality and quantity of oil (Hamdan et al., 2008). The literature on the subject reveals that both additive (Golkar et al., 2011b) and dominance (Gupta and Singh, 1988) gene effects are observed in the genetic control of seed oil yield. Pahlavani et al. (2007) reported that epistatic effects had a significant impact on the genetic control of safflower oil. Ramachandram and Goud (1981) declared that dominance alleles involved in the genetic control of safflower oil content outnumbered the recessive ones. A low value is occasionally reported for heritability affecting oil yield (e.g., Camas et al., 2006) that could be compromised by the high effect of environmental conditions on oil content.

Morphological markers

Generally speaking, safflower is a spiny crop with many sharp spines on the leaves and bracts in most genotypes (Bradley et al., 1999). Hence, one of the main goals in safflower breeding programs is to develop spineless varieties with high yield and high oil content (Golkar et al., 2010). Morphological markers are usually the visually characterized phenotypic traits such as flower color and spininess that serve usefully the purposes of plant breeders (Golkar et al., 2010). Inheritance of spininess has been studied in safflower by several researchers (Classen, 1952; Narkhede and Deokar, 1990; and Golkar et al., 2010). Nakhede and Deokar (1990) reported that spininess was dominant over spinelessness with four genes (Sa, Sb, Sc, and Sd), but Golkar et al. (2010) reported that this trait was monogenic and that the spiny trait was completely or partially dominant (Table 1). Classen (1952) reported spininess to be affected by an unknown number of modifier genes.Flower color in safflower is generally considered to be neutral in its florets (Bradley, 1999). Narkhede and Deokar (1986) and Classen (1952) identified four dominant genes (namely, Y, C, O, and R) governing the flower color in safflower at wilted stage. This is while epistatic effects have also been reported for flower color in safflower (Joglekar et al., 1956). Golkar et al., (2010), however, reported two different models of epistatic gene action for the genetic control of flower color (Table 2). Dajue and Mundel (1996) reported that some of the spiny genotypes had more seed yield than their non-spiny

counterparts. In addition, spininess and flower colour are expected to serve as valuable genetic markers for accurate determination of F_1 hybrids in hybridization programs of safflower or as morphological markers for breeding programs in MAS (Golkar et al., 2010). Pearl et al. (2014) emphasized large QTL effects for the genetic control of flower color and leaf spininess.Inheritance of certain biochemical markers have also been studied (Carapetian et al., 1994). Some isozymes (isocitrate dehydrogenase, phosphoglucomutase, malic enzyme, and triosephosphate) and monogenic inheritance have been reported for these isozymes. Izozyme gene markers are advantageous over conventional morphological gene markers because they allow seedling screening (Carapetian et al., 1994).

Seed nutritional properties

Safflower is a crop of great interest, not for its colorful petals, but because of its importance as a source of healthy vegetable oil. The crop enjoys the greatest variability of fatty acids in its oil composition (Camas and Esendal, 2006). Conventional safflower seed oil has a fatty acid content made up of palmitic acid (6-8%), stearic acid (2-3%), oleic acid (16-20%), and linoleic acid (71-75%). Given this importance, determination of the genetic control of safflower oil seed and its fatty acid composition plays an important role in breeding programs aimed at increased oil yield. While quantitative inheritance is reported for safflower oil content, non-additive gene effects have also been reported for the genetic control of oil content (Golkar et al., 2011b). Yermanos et al. (1967) reported that epistatic effects played an important role in the genetic control of oil. Both broad and narrow–sense heritabilities have also been claimed for the different fatty acids and oil content of safflower (Golkar et al., 2011b) (Table 3). Additive gene effects are reportedly important for the genetic control of linoleic acid (Hamdan et al., 2008), oleic acid (Hamdan et al, 2009b), palmitic acid and stearic acid (Hamdan et al., 2009a). Golkar et al. (2011b) reported maternal effects involved in the linoleic acid and stearic acid content of safflower. It has been demonstrated that the high oleic acid content is under the genetic control of recessive alleles (Fernandez–Martinez et al., 1993). Ladd and Knowles (1971) reported that stearic acid inheritance was monogenic.

Seed yield and yield components

Knowledge of inheritance of agronomic traits helps in planning an efficient strategy for further improvement of yield potential. Golkar et al. (2012b) implied the importance of additive gene effects on the genetic control of seed yield, but their finding is inconsistent with those of Rajab and Fried (1992), Mandal and Banerjee (1997), and Singh et al. (2008), who observed that dominance predominantly controlled seed yield. Number of capsules/plant is an important component of yield for whose genetic control dominance gene effects have been found responsible (Pahlavani et al., 2007). Deshmakh et al. (1991) carried out a line×tester analysis to find a high heterosis for capsules/plant. Shahbazi and Saeidi (2007) declared that additive×additive and dominance×dominance epistases had important roles in the genetic control of capsules/plant. Sahu and Tewari (1993) reported on the importance of additive-dominance model for its genetic control. In a study by Ramachandram and Goud (1981), the mean comparison of reciprocal effects showed that maternal effects played an important role in the inheritance of heads/plant and seed weight. Number of seeds/capsule is reportedly affected by the additive gene effects (Mandal and Banerjee, 1997; Singh et al., 2005). This implies that the selection breeding method could be applied for the improvement of seeds/capsule. Additive gene effects have also been found to play a significant role in the genetic control of seed weight (Golkar et al., 2012b).Also, the digenic model (additive-dominance) has been found to be involved in seed weight (Shahbazi and Saeidi, 2007).Based on dominance ratios (Table 3), seed yield and its components are found to be affected by the additive gene action, except for the capsules/plant (Golkar et al., 2012b). One of the most important physiologic indices for seed yield improvement is the harvest index the genetic control of which is reported to be governed by additive gene action (Golkar and Shahsavari, 2011)

Inheritance to abiotic and biotic stresses

Safflower is highly susceptible to different pathogens such as fungi, bacteria, and nematodes. A literature review reveals that little is known about disease resistance in safflower. Ghaderi et al. (2011) reported that resistance to *Pythium ultimum* was under genetic control with both simple and digenic interaction effects. Screening for resistance to a number of safflower diseases could provide useful resistant germplasm as an initial step toward potential cultivars in future. Tolerance for environmental stresses has an important role in increasing safflower yield in different eco-geographical climates. Saline and drought lands limit the growth and yield of safflower and other crops in many arid and semi-arid regions of the world. Enhancement of salinity tolerance, therefore, depends on proper identification of selection criteria. For this purpose, salinity tolerance and genetic control of tolerance indexes have been investigated in the vegetative stage of safflower (Golkar, 2011b). It has been established, for example that additive-gene effects are important for rootlet length and fresh and dry plantlet weight while dominance gene action clearly seem to affect shootlet length and leaflet number. Nakaei et al (2013) investigated the genetic control of salt tolerance in the reproductive stage. The additive model [d] has been fitted for branches/plant, seeds/capsule, and seed yield/plant under drought conditions and the simple additive–dominance model [d, h] for number of seeds/plant. Also, the dominance×dominance epistasis [l] has been added to fit the model as [d, h, l] for capsule/plant and dry weight/plant. Finally, the genetic models of [d, h, i] and [d, i] have been fitted for the genetic control of plant height and seed weight, respectively. These findings can be exploited to improve salt tolerance genotypes in safflower.

Heritability of traits

Genetic components analysis has been used by plant breeders to assist in identifying heritability of traits associated with seed yield or oil yield. Both the broad-sense and narrow-sense heritability of agronomic traits have been studied in safflower (Camas and Esendal, 2006). Discrepancies observed in the estimations of heritability of a trait are mostly because heritability is not merely a property of a trait, but also because it is related to population, environmental conditions, and method of genotype evaluation and estimation (Falconer and Mackay, 1996). High estimates of broad-sense heritability for seed yield and its components indicated that other types of genetic effects such as dominance or epistatic effects might be involved in their variation (Mather and Jinks, 1982). Yield components with high narrow-sense heritability could be used as selection criteria to improve seed yield. High narrow-sense heritability

for seeds/capsule and seed weight have been reported in many studies (Camas and Esendal, 2006; Shahbazi and Saeidi, 2007; Golkar et al., 2012b). Given the high degree of out-crossing in safflower, a high value of inbreeding depression has been reported for capsules/plant and branches per plant (Shahbazi and Seaidi, 2007).

Molecular methods in safflower improvement

Genetic diversity

Genomic studies of safflower have witnessed a greater progress than other related areas such as transcriptomics and proteomics. Molecular markers have been frequently used in safflower for genetic diversity analysis, phylogenetic studies, and marker assisted breeding. Different molecular markers including RAPD (Sehgal and Raina, 2005; Mahasi et al., 2009), AFLP (Zhang et al., 2006; Johnson et al., 2007), ISSR (Yang et al., 2007; Golkar et al., 2011c), and EST-SSR (Barati and Arzani, 2012) have been used for evaluating germplasm diversity. Chapman et al. (2009) developed a set of polymorphic EST-SSR markers as a valuable source to facilitate comparative map-based analysis in safflower. Naresh et al. (2009) reported the utility of EST-SSR markers for the assessment of genetic purity and heterozygosity in safflower hybrids. However, safflower has an enormous variability and several traits that could be genotyped via the available molecular marker systems (Sujatha, 2008).

Genomics and Marker Assisted selection

The first linkage map of safflower with three linkage groups (LG) was produced by Ravikumar et al. (2008) with RAPD primers and the complete linkage map with 12 LG groups was generated with a set of SSR and RFLP markers in safflower by Mayerhofer et al. (2009). The linkage groups (LG) ranged in size from 30.7 to 105.3 (CM), each containing 6 to 40 markers. More molecular markers would provide a foundation for fine map development in safflower. A physical map of the chloroplast genome of safflower has been constructed (Ma and Smith, 1885). According to Lulin et al. (2012), 567 nucleotide sequences and 41588 expressed sequence tags (EST_s), 162 proteins and 0 genes from *Carthamus tinctorius* L. have been deposited in the NCBI s GeneBank database until Oct of 2011. Thippeswamy et al. (2013) reported about 146 unique and novel ESTs that were related to drought responsive genes in safflower. The molecular markers of SCAR and RAPD that were linked to *Li* (the controlling gene for very high linoleic acid) and *Ms* (nuclear male sterility) (Hamdan et al., 2008) as well as Tph2 (high gamma-tocopherol) genes (Garcia-Moreno et al., 2011) were identified for MAS in safflower. In another study, the *ol* (high oleic acid content) gene was linked to the SSR marker ct365 that was mapped to the linkage group of T_3 (Hamdan et al., 2012). Kammili (2013) reported a linkage between male sterility and non-spiny marker that would be useful in the production of pure F_1 hybrid seeds. Pearl et al. (2014) have mapped 61 QTL (Quantitative Traits Loci) at different linkage groups of safflower that were related to diverse traits such as the number of heads, flower color, and fatty acids content.

Transcriptomics and Proteomics

It seems that fewer studies have been devoted to transcriptomics of safflower than to that of other oilseeds. Li et al. (2011) found at least 236 known micro RNAs (miRNA) expressed in safflower. In metabolic pathways, Lulin et al. (2012) reported four genes and new pathways that might control flavonoids and secondary metabolites synthesis in safflower by sequencing and assembling the safflower flower transcriptome and suggested that these genes encoded other anthocyanidine-related products that have not yet been identified in the flower. Two protein species with molecular masses of 34 and 40 KD associated with thioesterase activity were identified and partially sequenced by Knutzon et al. (1992). Mizukami et al. (2000) isolated a cDNA clone (CTOS1) that presumably encoded a novel protein from high oleats genotypes of safflower. Completion of cDNA libraries in the safflower genome seems to be essential for sequencing the functional and vital proteins in it.

Conclusions and future prospects

Safflower research is scattered and there is an immediate need for tapping the unexploited potential of safflower. The review of literature on safflower shows that no genetic study has been carried out on the diverse floral and physiological properties, flower yield, pigment content (carthamin, carthamidin, and luteolin), leaf and medicinal components, and antioxidant activity of safflower. There is an important gap between what is already known and what needs to be discovered because there is a growing demand for safflower flowers as a source of natural food color and as a medicine for curving chronic diseases. Medicinal use of safflower can be further studied using biotechnology toward pharmaceutical purposes. Advances in molecular farming and identification of important genes with transcriptomic studies (such as inclusion of genes in enzymatic and non-enzymatic antioxidant biosyntheses) are recommended for safflower breeding. Little is known about the type of gene action for tocopherol content (as a nutritional factor) and inheritance of physiologic-related traits (chalrophyl a, b, carotenoids) to improve stress resistance of the plant. An important gap is observed in gene action for (bacterial and fungal) disease resistance . Focusing on the genetic diversity of wild germplasms could be a good breeding strategy to transfer the suitable genes into cultivated genotypes. The inheritance of fatty acids indicate that gene transfer could be a prospective method for development of seeds with altered fatty acids in order to produce traditional lines of high linoleic and oleic acid genotypes and resistance lines to biotic and abiotic (drought, cold, and salinity) factors. Little has been done to develop mapping populations and molecular markers in safflower. It seems that information on trait mapping of safflower is lacking and further genetic studies in this connection would help breeders to locate the gene position of important agronomic and oil quality-related traits to evolve cultivars with improved productivity and resistance to biotic and abiotic stresses. Comparative mapping is expected because of the existence of ideal candidate genes in some related species of safflower. So, development of appropriate markers would support the studies on map-based cloning and MAS selection. Identification of suitable markers is proposed to identify the heterozygous maintainer in genetic male sterility (GMS) for hybrid breeding programs. No study has been seemingly conducted on the proteomics of safflower. It is, therefore, suggested that this deep gap in safflower investigations be bridged by conducting specific studies required. Development of mapping population could proceed with production of double haploid lines in future. In this connection, optimization of protocols for haploid lines production (microspore and anther culture) and efficient regeneration could be considered as good areas of research

aimed at producing new lines. Embryo rescue techniques need to be developed to overcome the cytologic and genetic barriers against introgression between cultivated safflower and wild related species. Finally, close cooperation needs to be established among research institutes involved in modern plant breeding activities, germplasm conservation, biotechnology, and bioengineering to speed safflower breeding programs.

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