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Safflower (*Carthamus tinctorius* L.): A Comprehensive Review of Its Diverse Applications and Recent Advances in Genetic Improvement

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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Abstract

Safflower (*Carthamus tinctorius* L.) is an underutilized oilseed crop with numerous past and present applications. For centuries, it was grown for its flowers, which were rich in various pigments such as hydroxysafflor yellow A, anhydrosafflor yellow B, safflor yellow A, and safflor yellow B that were used for food coloring and flavoring, fabric dyes, and medicinal purposes of Asian and European peoples. In the recent years, the need for healthy cooking oils, eco-friendly biofuel, and industrial biolubricants has increased, and safflower, with its high oleic acid content, has gained considerable attention. Safflower oil is also rich in linoleic acid that makes it suitable for human consumption. Despite its high adaptability to adverse growing conditions, high-yield potential, and diverse applications, safflower has received less research attention due to its reduced prominence in agricultural systems. Fortunately, during the past decades, the well characterization of world-wide germplasm resources of safflower has changed the situation by exploiting available intra and interspecific genetic variability to develop well-adapted high-yielding varieties. The emergence of

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modern molecular methodology and next-generation sequencing techniques have promisingly enhanced the efficiency of breeding programs leading to the discovery of novel molecular markers capable of utilizing in phylogenetic studies, marker-assisted selection, QTL mapping, linkage analysis, genome-wide association studies, and genomic selection in safflower. This article will briefly review the importance of safflower and different aspects of genetic improvement in this crop, including safflower wild relatives, interspecific hybridization, molecular markers, and omics studies.

Keywords: Safflower; neglected crop; interspecific hybridization; omics study; evolutionary bottleneck; phytoremediation.

1. Introduction

Over the past decades, oilseed crop production and productivity have gained meaningful importance to human societies. It is mainly due to a rapidly growing demand for high-quality oils used for human consumption, medicinal applications, and industrial purposes worldwide (Singh & Nimbkar, 2006). However, the industrial utilizations of vegetable oils mainly were limited to a few oilseed crop species such as cotton, oilseed rape, linseed, or castor. In recent years, global warming and climate change have also become severe issues, and thus, have led to an increased demand for the production of biofuels as an alternative clean and environmentally-friendly energy source for fossil fuels (Asokan et al., 2021). On the other hand, considering the parameters of climate change, the increasing scenario of marginal lands, and the rapidly growing demand for biofuel production has increased the future importance of particular oilseed crops (Chugh et al., 2023). Exploring plant species capable of growing under marginal conditions is an applied strategy to expand the growing area and subsequently improve the production of vegetable oils. Among neglected oilseed crops, safflower is a promising crop that is resilient enough to dry and saline conditions and, therefore, could serve as an important source of high-quality edible oil concerning consumer desires for healthier oils containing lower saturated fats. This crop also provides unique biochemical compounds with divers industrial and medicinal applications (Emongor & Emongor, 2023). However, undesirable traits such as low yielding, spiny nature, and susceptibility to several diseases have reduced its yield performance in several regions around the globe (Hassani et al., 2020).

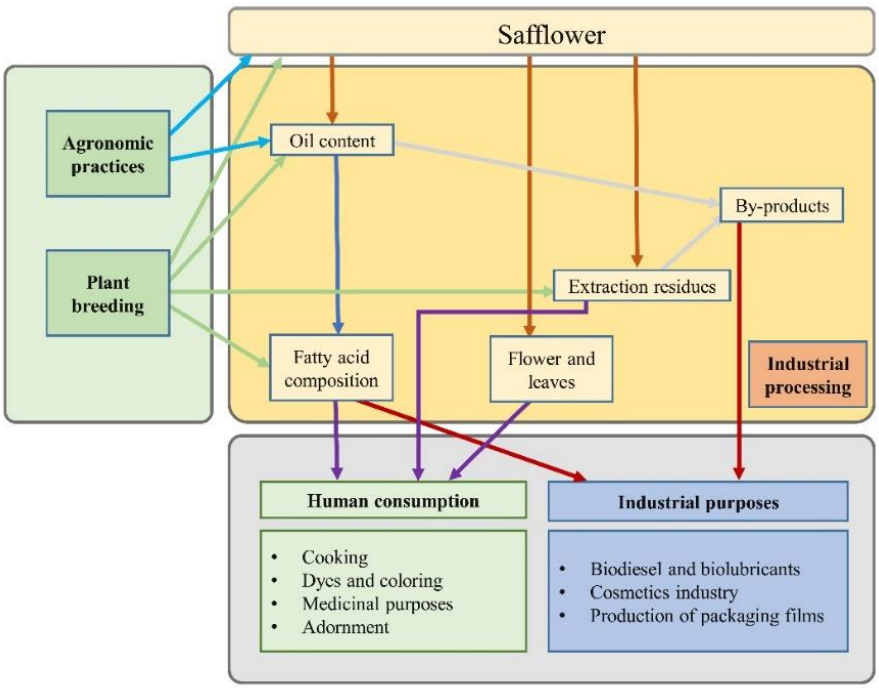

Fig. 1. A general overview of safflower utilization and the effects of agronomy and plant breeding on particular products. Modified from (Vollmann & Laimer, 2013)

Both agronomic practices and genetic improvement programs could improve safflower production. Agronomic practices such as sewing date, plant density, irrigation regime, and soil fertility as well as the plant breeding programs through developing well-adapted high-yielding varieties are the key components of the agricultural system influencing the majority of plant attributes i.e., oil content, fatty acid composition, and flower features that are of interest to the consumers (Coşge et al., 2007). In the past, most oils produced from plant resources were used for food and animal feeding purposes, whereas these oils were rarely used for industrial applications. The situation is changing due to increasing demand for biofuel, bio-lubricant feedstock, and cosmetic industries. Agronomic practices and plant breeding could improve the quantity and quality of plant production (Fig. 1) (Vollmann & Laimer, 2013). Providing better conditions for crop growth and development and breeding plant varieties with improved oil content, fatty acid composition, and nutraceutical properties could contribute to the increased market value of crops, including safflower.

2. Safflower Botany, Applications, and Breeding

2.1 Botany

Safflower (*Carthamus* tinctorius L.) is an erect, spiny, thistle-like, bushy, annual, predominantly self-compatible, day length-neutral, long-day, and diploid (2n= 2x= 24) herb. This species belongs the family Asteraceae with the haploid genome size of 1.4 GB (Sehgal & Raina, 2010). Historically, the term *Carthamus* came from the Arabic words “quartum”, or “gurtum” that refers to the natural dye obtained from safflower petals (Singh & Nimbkar, 2006). However, the common names of safflower vary depending on country, region, language, and use (Dajue & Mündel, 1996).

It is one of the humanity oldest domesticated crops not only due to its high-quality edible oil, but also due to its broad clinical applications such as amelioration of blood cholesterol levels, and treatment of osteoporosis and rheumatoid arthritis. The first safflower seed remains were found in northern Syria (dating to ca. 2500 BC) and are probably the first evidence of safflower cultivation. It is commonly believed that safflower originated from an ancestor type somewhere in the Near East, in the area demarcated by the eastern Mediterranean, Eastern Europe, the Persian Gulf, Central Asia, and Abyssinia [Ethiopia and Eritrea] (Ashri & Knowles, 1960). The domestication of safflower dates back to approximately 4000 years ago and has probably occurred in the “Fertile Crescent” somewhere between southern Israel and western Iraq. Although safflower has been considered as a “strongly domesticated” crop (Dempewolf et al., 2008), it only demonstrates moderate morphological variability from its wild progenitor(s) and has been subjected to limited breeding effort (Pearl & Burke, 2014).

Following initial domestication of safflower, its cultivation was expanded to surrounding regions throughout the Middle East, the Far East, India, and northern Africa. It was believed that the species might has been transferred to the southern Russian Republics from the Iran–Afghanistan corridor and then introduced into the Far East from Russian Republics. The cultivated safflower was originated from the Middle East, and then introduced into India–Pakistan (as a secondary center). Safflower was later transferred to Europe and other countries (Majidi & Zadhoush, 2014). Safflower was introduced into North America in the late 19th century, while its commercial production was initiated in the mid-20th century in Nebraska and Colorado and expanded to California in 1950. Although the word safflower production has fluctuated over the past decades (Fig. 2), the world's total safflower seed production was estimated at 995,776 mt/yr in 2022. Kazakhstan ranks as the first-largest safflower seed producer, accounting for 44.9% of the total production, followed by Russia and USA, with 222,619 and 74410 mt/yr, respectively. However, globally, it ranks the eighth most important oilseed following other crops including soybean, peanut, rapeseed, sunflower, sesame, linseed, and castor (FAOstat, 2024).

Compared to other major oilseed crops, safflower is recognized as “underutilized”, and sometimes a “minor” or “neglected” crop. The term underutilized species means that it has considerable genetic potential and is a part of “larger biodiversity portfolio” or genetic diversity for use, yet its genetic potential for commercial cultivation has not been fully exploited (Padulosi & Hoeschle-Zeledon, 2004). The availability of diverse assemblage of crop species including underutilized species could promisingly improve food security, therefore, the demand for the development and production of such crops tends

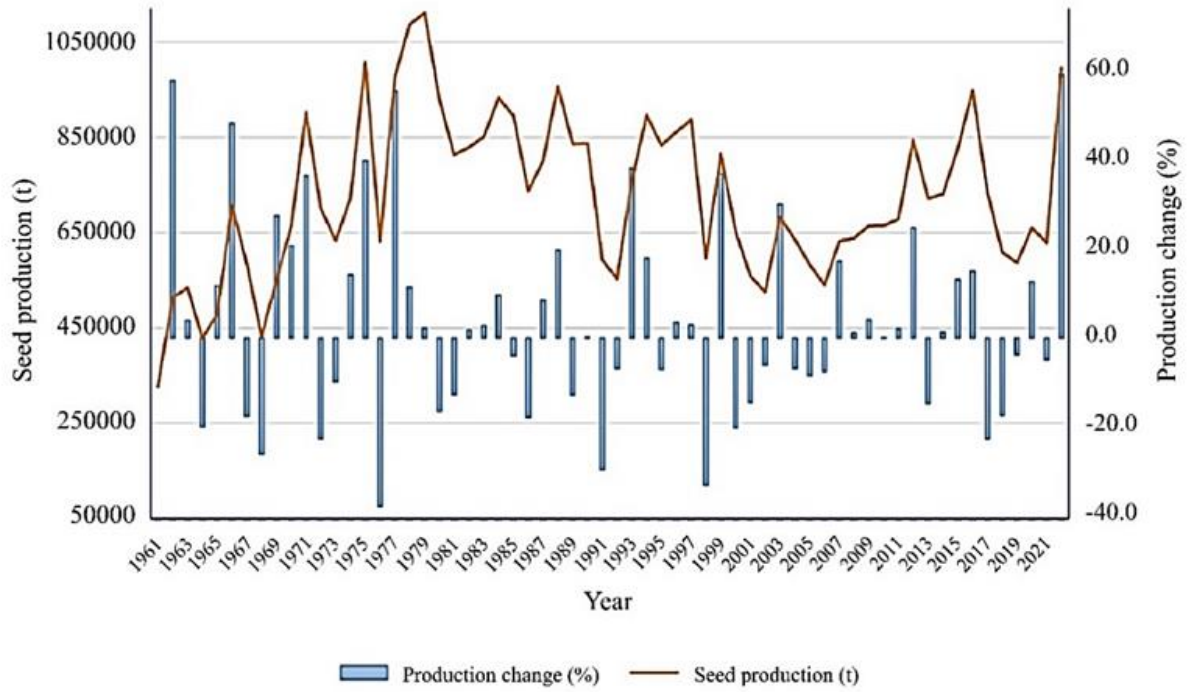

Fig. 2. Safflower seed production and production change between 1961 to 2021 (FAOstat, 2024)

to become a priority. The underutilized crops are not only well-adapted to cultivation on marginal lands, thus providing farmers with the opportunity of usage, but also offer feasible agricultural alternatives in response to global warming and climate change. Moreover, these species could potentially satisfy an increasing desire for "natural" and environmentally friendly products while providing sufficient sources of diversified income to farmers and agricultural businesses. However, in spite of having high adaptability to diverse growing conditions, high-yielding potential, and various utilization for different plant parts, safflower has received little research attention (Chapman et al., 2010).

2.2 Safflower Importance

2.2.1 Safflower as a Major Medicinal Crop

Safflower, undoubtedly, is an economically multipurpose plant that provides principal products such as vegetable, high- quality edible oil, cut flower, birdseed, and feedstock. Safflower leaves are a rich source of vitamins such as vitamins A and C, secondary metabolites including riboflavin and carotene, and minerals such as iron, phosphorus and calcium (Emongor & Emongor, 2023), thus making them suitable for fresh consumption as vegetables (Weiss, 2000). For centuries, it was locally cultivated on a limited scale for its flowers, that was served for either adorning purposes or extraction of non-toxic dyes obtained from safflower petals. Safflower petals are rich source of quinochalcones, flavonoids, polyacetylene, and alkaloids. The pharmacological applications of such chemical compounds have been extensively reviewed (Zhou et al., 2014). Safflor yellow A, Safflor yellow B, hydroxysafflor yellow A (HSYA), anhydrosafflor yellow B (AHSYB), precarthamin, carthamin, safflomin A, tinctorimine, and cartorimine are the main pigments that have been delectated in safflower petals. These natural colorants are suitable for coloring and flavoring foods, textile industries, and medicinal purposes (Emongor & Emogor, 2023). However, complex dye extraction has limited the application of such colorants to national cultures, religious ceremonies, and local customs (Wu et al., 2024). Safflower red (SR) and safflower yellow (Shafiei-Koij et al., 2020) pigments that are naturally C-c are responsible for the production of red and yellow colors, respectively. These two pigments are potentially natural compounds capable of using as dyes or medicine. As an effective constituent of safflower, SY pigment is a potential anticoagulant agent acting through prolonging the thrombin time (TT), plasma prothrombin time (PT), and

activated partial thromboplastin time (APTT), and inhibiting the ADP-induced platelet aggregation under in vivo condition (Emongor & Emongor, 2023). In China, SY and HSYA have been recently developed into novel health care medicines for treatment of cardiovascular and cerebrovascular diseases with notable clinical effects. Safflower extracts also have anti-fibrotic, neuroprotective, anti-inflammatory, and anti-mycotic effects and could play a key role in modulating the immunity system and scavenging antioxidative activities (Emongor & Emongor, 2023).

2.2.2 Safflower Seed Properties

The chemical properties of safflower seed, also called achene, are summarized in Table 1. Safflower seeds are a good source of oil and protein, which account for nearly 40 and 19% of the seed weight, respectively, and are highly important for human consumption, animal feeding, and industrial purposes. For example, oil extracted from seeds could not only be used for human nutrition, such as cooking, salad dressing, and margarine manufacturing, but also as raw materials for the cosmetics industry to produce shampoos, hair and face creams, perfumes, and body lotions due to its oily emollient and moisturizing characteristics, and biodiesel production (Emongor & Emongor, 2023). Safflower has higher oil content and an unsaturated fatty acid profile than its wild relatives. However, the quantity and quality of safflower oil may be influenced by various factors, including genotype, water stress, and seed coat color (Karami et al., 2018). Seed coat color is an important agronomic trait in *Carthamus* species naturally exhibiting diverse patterns. It varies from white in cultivated safflower and *C. palaestinus*, tan-dark brown in *C. oxyacanthus* to black in *C. glaucus* and *C. lanatus*.

2.2.3 Safflower as an Important Energy Crop

Nowadays, the fossil fuel reserves in the world are slightly decreasing, global warming and climate change are becoming serious concern, and the demand for novel and alternative sources of clean and eco-friendly energy is increasing. Biodiesel, a mixture of long-chain fatty acid alkylesters, could be produced from organic matters such as vegetable and algal oils, animal fats, and wasted oils (Asokan et al., 2021). Edible vegetable oils are accounted for over 95% of biodiesel produced globally. Safflower potential for biodiesel production has been extensively reviewed (Yesilyurt et al., 2019). Safflower oil, particularly high oleic safflower oils (HO), is a promising raw feedstock capable of converting into biodiesel and biolubricants (Nogales-Delgado et al., 2021). This is mostly due to the potential of safflower for cultivating in less-fertile lands with adverse climates like drought and salinity. Moreover, the biodiesel produced from safflower oil has unique features that meet the biodiesel production standards and thus make it a promising alternative fuel for the future.

High-linoleic (HL) safflower oil is a highly valued drying agent in varnishes and paints mainly because of its non-yellowing characteristic (Emongor & Emongor, 2023). The safflower meal, which contains 24% to 36% protein, could be used as a protein-rich supplement for animal feeding. Supplementation of sheep diets with HO or HL safflower oil has increased tissue 18:1 (trans-11) and conjugated linoleic acid (Johnson et al., 2012). It is a desirable change regarding current human dietary guidelines without any negative effects on growth performance or carcass properties (Bolte et al., 2002).

2.2.4 Phytoremediation Potential of Safflower

Safflower also has considerable tolerance to high concentrations of toxic elements. Both cultivated safflower and its wild relative, *C. oxyacanthus*, are tolerant to toxic metals such as Nickel (Ni). This plant preferentially accumulates metals including lead (Pb), cadmium (Cd), and zinc (Zn) in the belowground tissues (Ciaramella et al., 2022). The safflower bioconcentration factor (BCF= the concentration of metal(loid)s in shoot divided by the metal(loid) concentration in soil, $C_{dried\ shoot}/C_{dried\ soil}$) is > 1, while the translocation factor (TF= the concentration of metal(loid)s in shoot divided by the metal(loid) concentration in root, C_{shoot}/C_{soil}) of these species is < 1. It is noteworthy that plant species having BCF > 1 and TF < 1, or BCF < 1 and TF > 1 demonstrate well its phytostabilization potential (Rabbani et al., 2024). Therefore, safflower seems to be a promising candidate crop for phytoremediation of heavily polluted areas such as mine tailing (Thomas et al., 2022). On the other hand, both cultivated safflower and *C. oxyacanthus* are promising species to remediate heavily contaminated soils. Moreover, the relatively low concentrations of metal(loid)s in the plant aerial

parts suggest it as a good feedstock for bioenergy conversion and animal feeding. Safflower could be also used as a promising raw material for the production of eco-friendly bioplastic and composite packaging films. It is because the pulp residue or whole safflower plant, including the stem and head remaining after oil processing and harvest, are rich in cellulose that could be converted into degradable packaging films (Melikoglu et al., 2023).

2.3 Safflower Chromosome Number and DNA Content

2.3.1 Safflower Chromosome Number

The evolution of the genetic architecture in, and divergence between, species found within the genus *Carthamus* has been accompanied by considerably large-scale variation in the chromosome number (x = 10, 11, 12, 2n = 20, 22, 24, 44, 64). On the other hand, the evolution of safflower involved the core eudicot γ-triplication and whole-genome duplication events, which resulted in large-scale genomic rearrangements such as duplications, translocations, and inversions, thus indicating extensive genomic shuffling since the divergence of the dicots ancestor (Chen et al., 2023). Findings reveal that this crop might have diverged from artichokes (*Cynara cardunculus*) and sunflower (*Helianthus annuus*) nearly 30.7 and 60.5 million years ago, respectively (Dong et al., 2024). Although various authors have reported all three basic numbers (Vilatersana et al., 2000), there are still conflicting arguments regarding the original and derived basic numbers as well as the classification of the genus *Carthamus* (Vilatersana et al., 2000). The karyotype of cultivated safflower consists of seven pairs of metacentric and five pairs of submetacentric chromosomes. The majority of species within this genus, forty-four percent, are diploid with the basic chromosome number of x = 10 and x = 11. Other forty percent of the species are in diploid mode with a basic number of x = 12. Polyploid species account for only sixteen percent of the taxa carrying either the basic chromosome numbers of x =10, x =11, or x = 12. All the polyploid species carry at least two basic chromosome numbers (Agrawal et al., 2013).

Developing advanced technologies, including molecular markers and DNA sequence analysis, has opened new horizons for providing molecular evidence favoring the original chromosome number of the genus *Carthamus* (Vilatersana et al., 2000; Sasanuma et al., 2008). Furthermore, it was found that the different species within the genus *Carthamus* may carry one of three plastomes: A, B, and C. The plastome C is only found in species sharing an intermediate sequence between cytoplasms A and B. Nucleotide sequence alignments showed that the species *C. arborescens* (x = 12) carries the prototype of plastome in the genus *Carthamus*. In addition, at least five distinct genomes, namely AA (x = 10), BB (x = 12), CC (x = 12), XX (x = 10), and YY (x = 12), have also been identified (Sasanuma et al., 2008; Sehgal et al., 2009; Sehgal & Raina, 2010).

2.3.2 Genome Size Variations in Safflower

Nuclear DNA content is a key biological attribute closely related to the systematics, ecology, and distribution of plant species and has been widely used for a better understanding of the genome organization and evolution of various plant species (Murray, 2005). Studying the genome size of 17 *Carthamus* species, including diploid and polyploid species, has shown that the 2C DNA content of this taxa is generally lower than that of other plant species. It was observed that 2C values for *C. tinctorius*, *C. oxyacanthus*, and *C. palaestinus* were 2.76 pg (1 pg eq. to 978 Mbp), 2.62 pg (2,562.36 Mbp), and 2.82 pg (2,757.96 Mbp), respectively (Fig. 3). For the wild diploid and polypoid species, i.e, *C. leucocaulos* (2n =2x= 20) and *C. turkestanicus* (2n = 6x= 64), the corresponding nuclear DNA content varied from 2.26 pg (2,210.28 Mbp) to 7.46 pg (7,295.88 Mbp), respectively (Garnatje et al., 2006). Moreover, the average 2C values were significantly different between diploid compared to tetraploid and hexaploid species. Interestingly, a negative correlation between 1Cx value (2C (pg) value divided by ploidy level and an increase in ploidy level has been detected. On the other hand, as the ploidy levels increase, the 1Cx value decreases. The nuclear DNA content in allopolyploid such as *C. lanatus*, *C. lanatus* ssp. *montanus*, *C. turkestanicus* and *C. criticus* were almost equal to, or slightly less than the sum of the ancestral species (Garnatje et al., 2006).

2.4 Safflower Oil

Safflower oil has been traditionally known as a highly poly-unsaturated oil containing high linoleic acid percentage (more than 70% of the total fatty acid content) and low linolenic acid content (<1%) (Table 1). Whole safflower seed oil ranges from 16% to 65% depending on various factors including genotype,

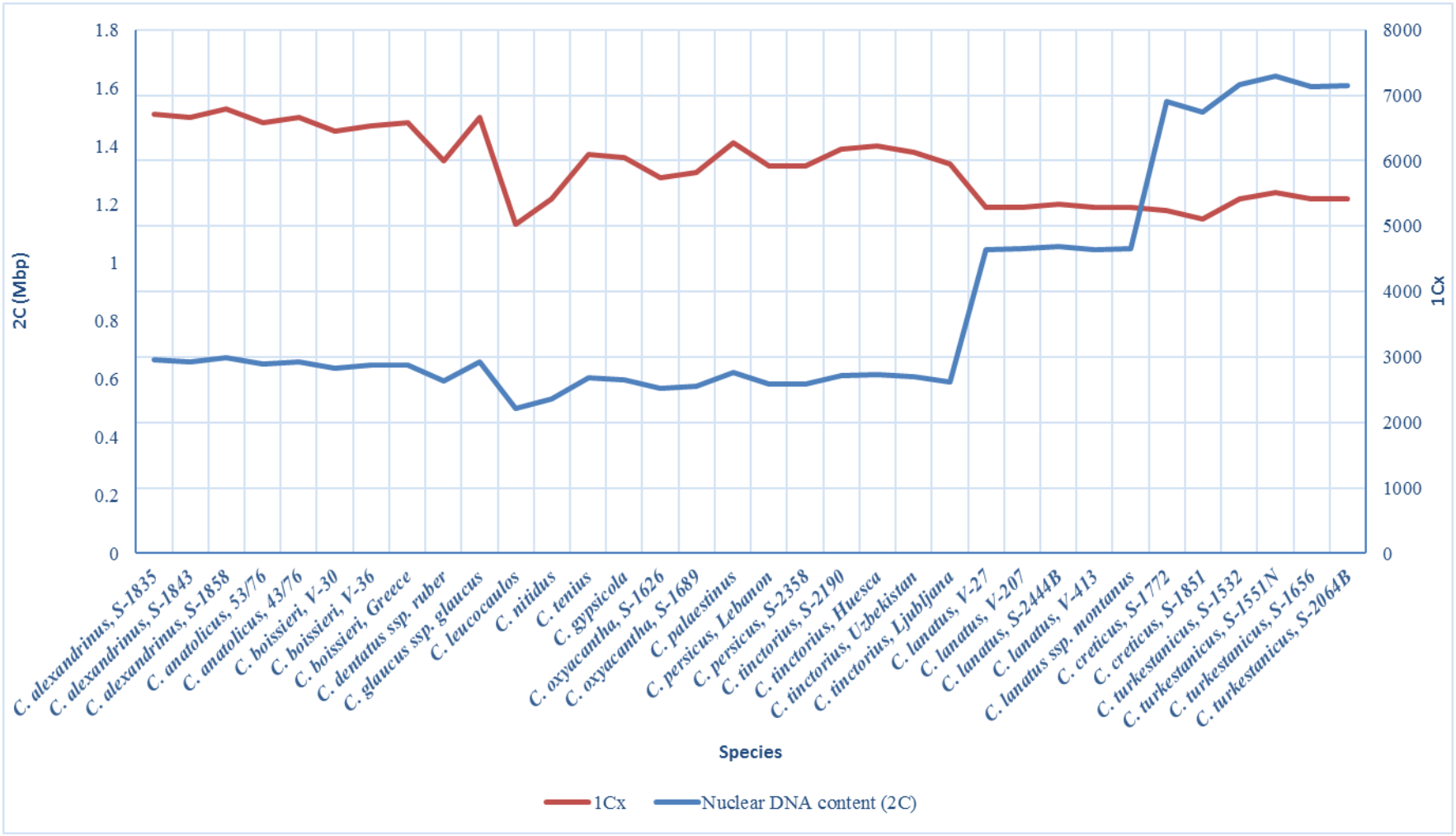

Fig. 3. The relation between nuclear DNA content and 1Cx value in the genus *Carthamus* with different ploidy level. (1Cx: monoploid genome size (2C (pg) value divided by ploidy level)). Adopted from (Garnatje et al. 2006)

environmental conditions, geographical region, growing season, and agronomic practices (Yeilaghi et al., 2012). Due to its lower saturated fatty acid content, safflower oil is assumed to be healthier than olive oil, sunflower oil, and canola oils (Dajue & Mündel, 1996). The oil content and fatty acid composition are the key properties determining the quality, nutritional characteristics, and commercial advantages of the oil for food, pharmaceutical, or industrial purposes. Compared to other common edible oil, the high level of linoleic acid makes safflower oil a premium edible oil for human consumption. Because this fatty acid has high nutritional values and potential therapeutic attributes in preventing heart disease and cancer. However, the presence of large amounts of linoleic acid make the oil highly sensitive to oxidative reactions (Al-Surmi et al., 2016).

2.4.1 Fatty Acid Composition

Safflower oil contains different fatty acids (Table 2). It includes nearly 13% and 78% of ω-3 and ω-6 fatty acids, respectively (Yildiz et al., 2022). However, wild relatives such as C. oxyacanthus have considerable oil content and fatty acid composition to cultivated safflower (Sabzalian et al., 2010).

Lipids consist of saturated fatty acids, which have no double bonds, and unsaturated fatty acids, which possess at least one double bond. Unsaturated fatty acids include monounsaturated (MUFAs) fatty acids that possess only one double bond (oleic acid (C18:1) and palmitoleic acid (C16:1)) or polyunsaturated (PUFAs) that have 2-6 double bonds (linoleic acid (C18:2), linolenic (C18:3)). Due to the lack of appropriate catalyzing enzymes, the human body is naturally unable to biosynthesize PUFAs with the first double bond on C3 (ω-3) and C6 (ω-6), that are the major essential fatty acids. Safflower oil is rich in essential fatty acids such as linoleic (LA, ω -6) and α-linolenic (ALA, ω-3) involved in various biochemical pathways. Essential fatty acids may regulate cell lipoprotein content, cell membrane fluidity, membrane enzymes and receptor interactions, eicosanoid biosynthesis, blood pressure, and mineral metabolisms. They could significantly reduce the risk of cardiovascular diseases, cancer, osteoporosis, diabetes, and other health issues. They also represent antithrombic, anti-inflammatory, antiatherogenic, hypolipidemic, and antiarrhythmic roles (Riley et al., 2022; Hernandez & Sanders, 2024).

The plant is a valuable oilseed crop that will continue to be exploited for its greater potential for extraction of high-quality edible oil from its seeds. Safflower oil is classified into two groups, one group being rich in linoleic acid (18C:2, (70%-87%)) while the other one is categorized by its high level of oleic acid (18C:1, (11%-80%)) (Hamdan et al., 2012). The high oleic safflower oil (HO, at least 70% oleic acid) is in great demand because it has optimal attributes for both human consumption and industrial purposes. Safflower oil is also classified under “specialty oil” referring to oils other than commodity oils with special dietary properties or functional attributes (Hernandez & Sanders, 2024). Because this oil combines significant hypocholesterolemic effects (Riley et al., 2022) as well as a much higher oxidative stability than oils with greater poly-unsaturation fatty acid (PUFAs) levels (Khalid et al., 2017). Linoleic acid is an important monounsaturated fatty acid (MUFAs) that has highly beneficial nutritional and therapeutic effects, such as the prevention of hyper lipemia, coronary heart disease, high blood pressure, and arteriosclerosis (Coşge et al., 2007).

2.4.2 Tocopherols

Stability of oils during long-term storage and high temperatures is a favorable characteristic of vegetable oils. It mostly depends on the intrinsic stability of fatty acids, which is negatively correlated with their unsaturation level and the presence of antioxidants in the oil (Shirvani et al., 2016). High-oleic safflower oils, HOSaO, because of its polyunsaturated nature, it is highly susceptible to oxidation, which consequently influences its shelf-life time and market value. Tocopherols, oil-soluble compounds usually known as vitamin E, naturally found in oilseeds, exhibit strong antioxidant activity in both biological systems and in the extracted oils. Tocopherols are broadly used for different purposes, from animal feeding to resins and pharmaceutical cosmetics. They are also highly valued chemical compounds in frying oils, fried snacks, and margarine industries (Fernández-Cuesta et al., 2014).

α-, β-, γ-, and δ-tocopherol are the major forms of tocopherols naturally found in safflower oil exhibiting different in vitro and in vivo antioxidant activities (Table 3). Irrespective of the oil extraction techniques, safflower oil contains α-tocopherol (46.05 to 70.93 mg/100 g), β-tocopherol (0.85 to 2.16 mg/100 g), γ- tocopherol

(from trace amount to 0.45 mg/100 g oils), and δ-tocopherol (3.06-11.50 mg/100 g) (Khalid et al., 2017). α-tocopherol exerts a high vitamin E activity and exhibits the highest antioxidant activity in vivo, but a weak *in vitro* antioxidant activity. Conversely, γ-tocopherol is the most efficient antioxidant in vitro, but its in vivo potential is weak. While β- and δ-tocopherol show intermediate properties. α-Tocopherol is the most abundant tocopherol in safflower oil, accounting for over 95% of the total tocopherols (Johnson et al., 1999). Partially replacing α-tocopherol with β-, γ-, or δ tocopherols via genetic engineering techniques could significantly improve in vitro stability of safflower oil with high oleic and high linoleic acid backgrounds (Demurin et al., 1996).

The high α -tocopherol content is associated with the activity of a γ-tocopherol methyltransferase (γ-TMT) that catalyzes the synthesis of α-tocopherol through methylation of γ -tocopherol. It was concluded that a mutation in the γ-TMT promoter sequence and an elevated activity of a corresponding promoter would probably lead to a high α -tocopherol content in safflower. However, a frameshift reading mutation in the coding sequence is responsible for SNPs between alleles of the γ-TMT gene (Tph2-γ-TMT locus) underlying the increased γ-tocopherol content in safflower and wild species *C. oxyacanthus* (Garcia-Moreno et al., 2014). Replacing high α -tocopherol with γ-tocopherol is highly preferred for industrial purposes. Although high genetic variation for tocopherol content has been reported in cultivated safflower, among wild safflower relatives, the species of *C. oxyacanthus* and *C. lanatus* subsp. *turkestanicus* have also shown high γ-tocopherol content compared to cultivated safflower (Table 3). Inheritance studies indicate that high γ-tocopherol (HGT) is controlled by partially recessive alleles at a single locus Tph1 that would facilitate the transferability of the HGT trait to the appropriate genetic backgrounds (Garcia-Moreno et al., 2014).

2.5 Safflower Breeding

2.5.1 Breeding Techniques

The major objectives of breeding programs in safflower are to develop varieties for (i) high seed yield, (Raina et al., 2005) improved oil content and fatty acid composition, and (iii) enhanced pest and disease resistance since these improvements make safflower more economic and sustainable. Safflower breeding techniques can be listed as introduction, pure-line selection, mass selection, the bulk population technique, recurrent selection, backcross breeding method, pedigree analysis, and the single-seed descent (SSD) techniques, which are the most popular methods employed in safflower breeding schemes. Among these viable methods, introduction is the easiest andthe most effective form of varietal improvement in safflower, and it has been widely used around the globe. It is required a few cycles of adaptation, selection, and evaluation prior to formal release for commercial production since they respond differently to changes in the

Table 1. Basic characteristics of safflower seed

Seed attributes	*Carthamus tinctorius*	*C. palaestinus*	*C. oxyacanthus*	*C. lanatus*	*C. glaucus*	Inter-specific hybrid line (A82) †
Palmitic acid (C16:0)	6.13	7.16	7.70	7.91	9.32	8.53
Stearic acid (C18:0)	2.60	2.91	3.29	3.36	5.53	4.34
Oleic acid (C18:1)	14.8	19.04	16.69	17.34	15.98	12.25
Linoleic acid (C18:2)	74.13	70.55	70.39	68.69	67.70	64.70
Linolenic acid (C18:3)	0.38	0.15	0.23	0.20	0.14	0.21
Cellulose (%)	20	Nd‡	Nd	Nd	Nd	Nd
Protein (%)	19	Nd	12.4	Nd	Nd	Nd
Moisture	5.7	Nd	6.3	Nd	Nd	Nd
Ash	2.2	Nd	4.2	Nd	Nd	Nd
Chlorogenic acid	3.01	3.74	3.36	3.30	3.34	2.62
Caffeic acid	7.19	5.91	6.88	6.85	8.35	7.16
p-coumaric acid	8.68	9.11	9.54	8.41	9.53	7.74
Rutin	111.99	114.53	117.95	50.24	7.23	43.67
Ferulic acid	29.80	30.41	31.24	14.88	4.49	13.3
Quercetin	14.10	11.50	11.27	3.29	3.09	5.96
Apigenin	64.88	37.09	53.96	7.88	4.37	23.63

†Progeny of the cross between C. tinctorius × C. oxyacanthus, ‡ Not determined. Adopted from (Al-Fadal & Al-Fredan, 2015; Yesilyurt et al., 2019; Karami et al., 2018)

environment (over years and locations). Consequently, before the varieties are subjected to a selection process for identifying superior genotypes and subsequent evaluations for commercial release, the introduced varieties should necessarily be acclimatized (Golkar & Karimi, 2019).

Furthermore, pure line selection, the most common technique for the development of varieties, has been widely used to develop a great number of safflower varieties, including K-1, CO-1, Type-65, A-2, Saffire, N-630, Nagpur-7, Bhima, N-62-8, A-300, Manjira, S-144, JSF-1, APRR-3, Bhima, HUS-305, Sharda, JSI-7, PBNS-12, Nebraska-5 and Nebraska-10. It is mostly because of the availability of untapped genetic variation for various traits of this crop (Singh & Nimbkar, 2006). Mass selection has been successfully employed to develop varieties with improved resistance to several serious diseases, including leaf blight produced by *Alternaria carthami* and bacterial blight caused by *Pseudomonas syringae* van Hall. The majority of released varieties in India have been developed through selection in the existing plant materials (Pushpa et al., 2023).

2.5.2 Hybridization in Safflower

Hybridization is a key step in plant genetic improvement programs, followed by the selection of promising genotypes/lines. It is not only used for deciphering the inheritance pattern of various traits, but also for introducing favorable genes/alleles controlling multiple traits into a single genetic makeup to generate genetic variations. However, influencing factors such as the selection of elite parental lines based on per se performance, parents' genetic variability bringing favorable genes/alleles of diverse origins together, and the degree of expression in yield components must be considered. For example, numerous released commercial varieties namely AC Sunset, AC Stirling, Ouiriego 88, San Jose 89, Leed, Sidwill, Hartman, Rehbein, Oker, Girard, Finch Sahuaripa 88, A-1, Tara, Nira, Phule Kusuma, Girna, JSI-73, and NARI-6 have been developed using the pedigree process. To incorporate highly inherited dominant genes/alleles conferring resistance to diseases like Phytophthora drechsleri root rot or modulating high oleic acid in safflower (Ladd & Knowles, 1971; Hamdan et al., 2009), backcrossing has been successfully employed. The technique has been successfully exploited to introduce cold tolerance and seed dormancy and into interspecific segregants with cultivated species from *C. flavescens* and *C. palaestinus*, respectively (Kotecha & Zimmerman, 1978). Backcrossing was widely used to develop wilt disease resistant interspecific hybrids carrying desirable gene from wild relatives, *C. oxyacanthus*, *C. turkestanicus*, and *C. criticus* (Anjani, 2005).

Reciprocal Recurrent Selection (RRS) could be effectively used to simultaneously improve traits negatively related to seed yield. This technique is employed to intercross phenotypically superior F_2

Table 2. Fatty acid composition of safflower oil

Fatty acid	Chemical formula	Percentage
Linoleic	C18:2	53.8%-84%
Oleic	C18:1	9.5%-91%
Palmitic	C16:0	4.9%-16.1%
Stearic	C18:0	1.7%-6.3%
Linolenic	C18:3	0%-1.5%
Palmitoleic	C16:1	0%-0.5%
Arachidic	C20:0	0%-1.9%
Behenic	C22:0	0%-0.7%
Eicosaenoic	C20:1	0%-0.2%
Lignoceric	C24:0	0%-0.8%
Lauric	C12:0	0%-0.07%
Gadoleic	C20:1	0.15%
Erucic	C22:1	C22:1, 0.20%
Margaric	C17:0	0.2%-0,03%
Margaoleic	C17:1	0%-0.01%

Adopted from (Arslan & Hacioğlu, 2018; Emongor & Emongor, 2023)

segregants helping in breaking unfavorable linkage. Intermated seeds are then grown and superior individuals are again mated after repeated selections. The intermating of the F_2 accumulate fixable components of genetic variation, breaking undesirable linkages, and thus led to increased frequency of favorable alleles/genes in the populations. It is mostly used to develop varieties with high heritable value. In safflower, Rubis and Levin (1966) enhanced stem strengths and thin hull plants' seed set through a four-cycle RRS strategy.

Modified Recurrent Introgression Population Enrichment Method (RIPE), which applies the principle of recurrent selection in self-pollinated crops could generate a large number of crosses by utilizing a parents donating different traits. This technique could significantly accelerate the recombination frequency between loci to create high potential progenies with desirable agronomic attributes and stress tolerance (Pushpa et al., 2023). The effectiveness of single plant selection (SPS), modified selected bulk (MSB), and modified bulk (MB) for *Carthamus* interspecific crosses under different water regimes have been evaluated. Findings reveal that the SPS is an efficient method for identifying promising lines under various irrigation conditions (Shafiei-Koij et al., 2020).

It is possible to generate extra genetic variability if there is not sufficient genetic variation for a particular trait in the existing genetic resources. Mutagenesis is one such approach that leads to changes in the genomic DNA sequence, which can be accomplished by exposing the seeds to physical mutagens (X-rays, gamma rays, etc.) or chemical mutagens. This breeding method in safflower is not widely employed, probably due to the availability of sufficient genetic resources and cross-compatibility of wild relatives in this genus for the potential genetic improvement of safflower (Pushpa et al., 2023). Targeting Induced Local Lesions in Genomes (TILLING) is an example of a mutagenesis approach, which exploits ethyl methanesulfonate to cause short insertion/deletion (INDELS) mutations in DNA sequence (Pushpa et al., 2023).

2.5.3 Heterosis

Heterosis is a well-known phenomenon in plant breeding that is defined as the superiority of F1 hybrids over parents. It is being widely used in the development of elite hybrids in genetic improvement programs. Several studies have reported hybrid vigor for seed yield and related attributes (Olivo et al., 2020), seed yield and oil content (Sargar et al., 2022), flowering time, plant height, yield, seed weight, percentage of empty seeds, embryo percentage, and oil percentage (Yazdi-Samadi et al., 1975) and in safflower photosynthetic traits (Anjani et al., 2022). Furthermore, the value of inbreeding depression has been estimated to be between -21.10% to 24.85% (Sargar et al., 2022). Heterosis could enhance the hybrids capacity to much more efficiently translocate photo-assimilates to sink organs. However, heterosis breeding should be aimed not only at enlarging sink size but also concomitantly improving the effectiveness of sink organs in translocating of photo-assimilates leading to genetic yield improvement in safflower (Anjani et al., 2022). Utilization of cytoplasmic-genetic male sterility (CGMS), genetic male sterility (GMS), and thermos-sensitive genetic male sterility (TGMS) in heterosis breeding in safflower could be promisingly rewarding. However, the adoption of heterosis in safflower mostly depend on factors such as the magnitude of the productivity advantage achieved, the cost/benefit ratio of using F_1 hybrids, and the efficiency of seed production, certification, and distribution companies available in the regard. DSH-129, MKH-11, and NARI-H-15 are spiny hybrids while NARI-NH-1 is a non-spiny hybrid variety released for commercial production in India. In general, these hybrid varieties demonstrate 20–25%

Table 3. Seed tocopherol content (mg/kg) and profile (% of the total tocopherols) of various species of *Carthamus*

Safflower species	Total tocopherol	α-tocopherol	β-tocopherol	γ-tocopherol
C. glaucus (GenBank seed)	192.70	97.40	2.60	0
C. lanatus (GenBank seed)	242.2	98.10	0.3	1.60
C. lanatus subsp. turkestanicus (GenBank seed)	304.30	93.90	0.80	5.20
C. oxyacanthus (seed multiplication)	405.90	97.60	1.60	0.80
C. palaestinus (GenBank seed)	337.20	98.90	1.10	0.00
C. tenuis (GenBank seed)	309.10	99.90	2.10	2.00
C. tinctorius (seed multiplication)	531.60	98.40	1.60	0.00

Adopted from (Velasco et al., 2005)

superiority in seed production and oil yield over the national check variety.

2.6 Wide Hybridization in Safflower

Crop wild relatives, CWRs, are defined as those plant taxa that are considered to bear those plant taxa that are closely related to a particular crop species and could potentially contribute to genetic improvement programs by providing desirable genes/alleles. CWRs of safflower are an important reservoir of desired alleles/genes and can sometimes be exploited not only to expand the existing genetic base, also to improve the performance of existing varieties with agronomically desirable attributes. Alien gene introgression is a powerful approach for creating novel allelic variations during plant sexual reproduction (Kopecký et al., 2010). It generally involves the transfer of favorable allelic variation between or within plant species and even between genera through wide hybridization followed by repeated backcrossing of hybrids to the parental species. It is a common phenomenon in plant breeding programs that plays a key role in reintroducing genetic variation at selective sweeps or introgression of desirable traits from CWRs into breeding materials. However, it is fundamental for both plant evolution and genetic improvement schemes accelerating accidental extensive genomic and epigenetic modifications in plant materials and, thus diversifying the plant gene pool for further selections (Rabbani & Nayak, 2023).

Analysis of genetic variability and population structure of safflower gene pools and understanding of its phylogenetic relationships with CWRs can provide new insight into the origin and subsequent evolution of safflower. It can also lead to the identification of novel sources of genetic diversity crucial for continued breeding of safflower. Although considerable genetic potential for various traits in safflower has been reported (Shafiei-Koij et al., 2019), this crops exhibits often reduced nucleotide variation mainly due to the occurrence of a population genetic bottleneck during domestication (Pearl & Burke, 2014). Safflower wild relatives have been reported to be a potential source of desirable genes capable of fueling future improvement efforts (Espanani et al., 2019b; Espanani et al., 2023), but very little attempts have been made to improve cultivated safflower employing gene introgression from wild species.

Numerous studies on crossing among the genus of *Carthamus* have been performed in the past decades; however, relatively few actual crosses have been aimed at practically improving the genetic base of safflower through interspecific hybridization. Producing viable progenies through interspecific crosses is a fundamental step usually followed by selecting superior genotypes for segregating generations. The cross-compatibility of *C. tinctorious* with other close species has been summarized in Fig. 4. It indicates that cultivated safflower can receive desirable genes through interspecific hybridization from several species. However, it is sometimes necessary to overcome hybridization barriers by employing embryo rescue and/or colchicine treatments. Safflower GP-1 also comprises the three species of *C. oxyacanthus*, *C. palaestinus*, and *C. persicus* Desf. ex Willd. (Syn. = *C. flavescens* Spreng), which possesses a similar chromosome number to C. tinctorius and demonstrates high cross-compatibility with cultivated species. They could be directly used for broadening the genetic base of cultivated safflower because of the high cross-compatibility with safflower that produces fully fertile hybrids. Conversely, these three wild relatives of cultivated safflower can contribute interspecific genetic variability to the gene pool from beyond the cultivated species. While cultivated safflower, *C. tinctorius*, is predominantly a self-compatible and self-pollination species with naturally low out-crossing habit, *C. palaestinus* is a self-compatible species, *C. oxyacanthus* demonstrates a mixture of self-compatibility and self-incompatibility; and *C. persicus* is a completely self- incompatible species. Cytogenetic studies and bio-systematic evidence confirmed that, the nuclear genomes of *C. tinctorius*, *C. oxyacanthus*, and *C. palaestinus* are genetically similar. Furthermore, inheritance studies revealed the morphological variations among these three species mainly arose from single gene differences (Ashri & Efron, 1964). Though, the two species of *C. curdicus* and *C. gypsicola* also exhibit the same chromosome number (2n=2x=24) as that of the three aforementioned species, their cross-compatibility among these species has not been attempted yet. However, it is inferred that these species are closely related and might have the potential to contribute to safflower breeding programs.

The species, *C. oxyacanthus*, *C. palaestinus*, and *C. persicus*, have comparable oil content and fatty acid composition to those of *C. tinctorius*, and are a potential source of

resistance/tolerance genes to abiotic and abiotic stresses (Majidi et al., 2011; Espanani et al., 2023). Hybridization of *C. tinctorius* with other related species may be accompanied by different ratios of success in obtaining F_1 hybrids. It varies from 0% to 68% for crosses with *C. criticus* and C. *turkestanicus*, and *C. leucocaulos*, respectively. While the cross between cultivated safflower and the three closely related species, *C. oxyacanthus*, *C. palaestinus*, and *C. persicus*, produces naturally fertile hybrids. It more likely reveals that *C. tinctorius* might be capable of hybridizing with several wild relatives if parental species are to synchronically flower (temporal sympatry) and are growing adjacent enough to one another for open pollination to transfer pollen between the plants (spatial sympatry) (Mcpherson et al., 2004).

Safflower species, including *C. oxyacanthus*, *C. palaestinus*, *C. lanatus*, *C. turkesthanicus, C*. *glaucus*, *C. lantaus*, and *C. criticus* could donate genes conferring resistance to bacterial diseases, including Fusarium wilt (Anjani et al., 2018) and *Alternaria* leaf spot (Ali et al., 2020). Among those species, *C. oxyacanthus* and *C. palaestinus* are easily crossable to cultivated safflower and the subsequent F1 hybrids are fully fertile (Ashri & Knowles, 1960). Anjani et al., (2018) successfully introgressed a single dominant gene governing resistance to Fusarium wilt from *C. oxyacanthus* and *C. palaestinus*, into a susceptible line (Nira) through wide hybridization. They also characterized eight simple-sequence-repeat (SSR) markers closely linked to resistance gene in two different segregating populations ('Nira' × *C. oxyacanthus*) and ('Nira' × *C. palaestinus*). Surprisingly, the populations recorded higher seed yields (9%–29%) than the high-yielding control variety (namely 'A1'). Their findings not only revealed that the resistance to Fusarium wilt could be introgressed from wild species to cultivated safflower, but marker assisted selection (MAS) could also be used to select superior genotypes in the early generations.

The two wild species, *C. palaestinus* and *C. lantaus*, are also known to be tolerant enough to Alternaria leaf spot (Ali et al., 2020) caused by *Alternaria carthami*. This destructive disease leads to severe yield loss up to 90% under severe disease circumstances. Anjani et al., (2019) synthesized interspecific hybrids of safflower with *C. palaestinus* and *C. lantaus*, as donor parents, to improve the tolerance of susceptible safflower lines. The ALS resistance is controlled by multiple minor alleles sharing small effects on resistance. They successfully developed six resistant and 29 moderately resistant interspecific lines resembling mainly cultivated parent (Anjani et al., 2019). Similarly, Heaton & Klisiewicz (1981) attempted to introduce alien genes conferring resistance to *A. carthami* Chowdhari, *F. oxysporum* Schlechtendal, and *Pseudomonas syringae* Van. Hall from *C. lanatus* to safflower. Sehgal and Raina (2010) also improved the resistance of safflower lines to *Alternaria* leaf blight through wide crosses of safflower and *C. oxyacanthus*.

Safflower fly (*Acanthiophilus helianthi* Rossi, Diptera, Tephritidae) is a serious pest inflicting considerable economic yield losses in safflower in terms of both seed yield and seed palatability. Unfortunately, there is lack of reliable sources of resistance/tolerance to the safflower fly within cultivated safflower lines. However, the genetic potential of safflower wild relatives, Viz *C. flavescens* WILLD. and *C. palaestinus* EIG, in donating moderate to high levels of resistance/tolerance against safflower fly has been reported (Ashri, 1971). It has also been revealed that the concomitant interactions of morphological structure (seed hardness) and seed coat color are associated with resistance against safflower fly (Karami et al., 2017).

Seed dormancy is another important trait that could significantly influence safflower productivity, particularly in areas where continuous rainfall after maturity results in seed germination in the head. Introgression of genes from *C. palaestinus* has been reported to successfully induce seed dormancy and prevent yield loss due to seed germination (Kotecha & Zimmerman, 1978). Zimmerman and Buck (1977) have also transferred the gene conferring tolerance to coldness in the early growing stage from *C. persicus* to cultivated safflower. More recently, Espanani et al., (2023) found that gene introgression from *C. palaestinus* is a key strategy to restore genes governing safflower adaptation that has been lost during evolutionary bottleneck. Recombinant inbred lines (RILs) derived from an interspecific cross between *C. tinctorius* and *C. palaestinus* demonstrated high potential for autumn planting, cold tolerance, and seed yield and oil content, indicating the possibility of gene introgression from safflower CWRs, especially *C. palaestinus* species.

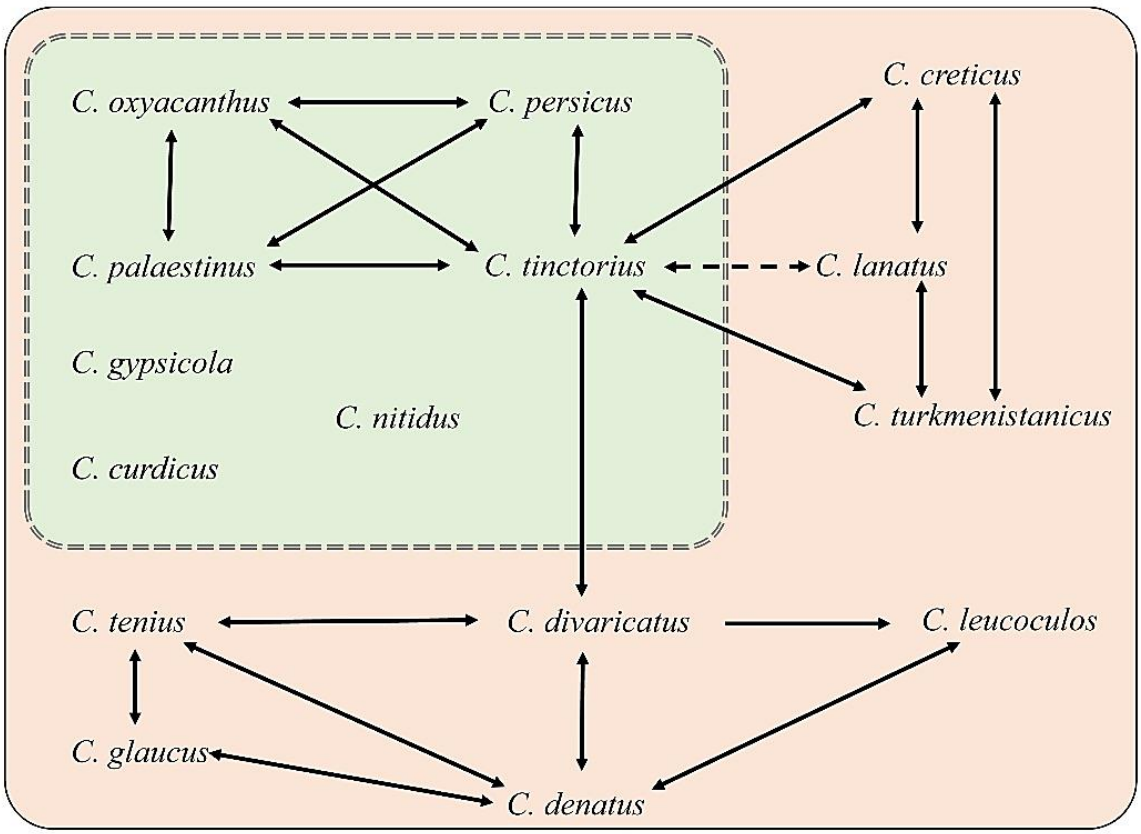

Fig. 4. Schematic representation of the artificial interspecific crosses in the genus *Carthamus* that produce fertile progeny. Solid lines point to the crosses with fertile F_1 hybrids and production of viable seeds. Dotted lines represent the occurrence of hybridization, but the F_1 hybrids could not be obtained without embryo rescue and/or colchicine treatments. Arrows illustrate the crosses direction (male → female). The broken box includes contains the species with 2n = 24. All taxa are classified in section *Carthamus* except species *C. nitidus*, which is a member of section *Atractylis*. Adopted from Mcpherson et al. (2004)

Shafiei-Koij et al., (2019) used three segregating populations of *C. tinctorius* × *C. palaestinus* (TP), *C. oxyacanthus* × *C. palaestinus*, (TO), and *C. oxyacanthus* × *C. palaestinus* (OP) to increase safflower's genetic variability for agro-morphological characteristics and fatty acid composition. Their findings showed that there was significant transgressive segregation for the fatty acid profile. For example, a positive transgressive segregation for myristic acid was observed in all to RILs. It was concluded that *C. tinctorius* and *C. oxyacanthus* probably contributed more to increasing higher values of palmitoleic acid and stearic acid, respectively. Furthermore, indirect selection for flowering and seed yield performance under water stress conditions was also efficiently used to improve seed yield performance. Interspecific hybridization in safflower is a potential technique to improve safflower oil's nutritional and pharmaceutical values. An advanced line "namely A82" resulted from the cross between *C. tinctorius* × *C. oxyacanthus* and was shown to have an appropriate ratio of polyunsaturated to saturated fatty acids (P/S) with moderate linoleic fatty acid value, making it a superior line compared to white/brown-seeded genotypes (Karami et al., 2018).

2.7 Genetic Variability and Germplasm Resources

The major objectives of safflower breeding programs are to improve seed yield productivity and other related traits, including oil content, fatty acid profiles, tocopherol content, and tolerance to biotic and abiotic stresses that are always laid on the nature and magnitude of genetic variations

available in the parental lines. Genetic variability or genetic variation refers to the differences available among the alleles of a particular gene within germplasms that might be analyzed at phenotypic (trait) or genotypic (DNA sequence) levels. Genetic variability on which artificial selection could be carried out is an important consequence of meiotic recombination during sexual reproduction that reshuffles parental genetic makeup. However, spontaneous mutations or meiotic irregularities could also result in spontaneous mutations or meiotic irregularities could also result in novel genetic variability, a highly important prerequisite for a successful breeding scheme (Rabbani & Nayak, 2023).

2.7.1 Safflower Germplasm Resources

Germplasms are important sources of desirable qualitative and quantitative genes capable of introgressing into cultivated species through hybridization. They include seeds, plants, or plant tissues useful in crop breeding efforts that are potentially genetically diverse. Assessment of genetic variation is a basic requirement for selecting genetically diverse parents. The availability of a genetically diverse panel of parents meaningfully ensures the success of the introgression of desirable gene(s) into a particular genetic background.

Assessment of genetic variations in intra and interspecific collections of safflower has been be accomplished by employing various classical and advanced tools including biochemical attributes (Bassiri, 1977), agro-morphological characters (Amini et al., 2008; Sabzalian et al., 2009; Majidi & Zadhoush, 2014; Hassani et al., 2020), and molecular markers including amplified fragment length polymorphism (AFLP) (Johnson et al., 2007; Kumar et al., 2015), RAPD (Random Amplified Polymorphic DNA) (Amini et al., 2008), sequence related amplified polymorphism (SRAP) (Mokhtari et al., 2013; Golkar & Mokhtari, 2018; Hassani et al., 2020), inter simple sequence repeat (ISSR) (Sabzalian et al., 2009; Bagmohammadi et al., 2012; Golkar et al., 2011; Yaman et al., 2014; Majidi & Zadhoush, 2014; Rahimi, 2021), inter-primer binding site (iPBS)-retrotransposon markers (Ali et al., 2019), expressed sequence tags- simple sequence repeat (EST-SSR) (Naresh et al., 2009; Derakhshan et al., 2014; Singh et al., 2021), SSR (Lee et al., 2014; Ambreen et al., 2015; Kadirvel et al., 2016; Kumari et al., 2017; Ambreen et al., 2018; Betha et al., 2019), start codon targeted (SCoT) (Golkar & Mokhtari, 2018; Rahimi, 2021), insertion/deletion (InDel) (Fan et al., 2023), conserved DNA-derived polymorphism (CDDP) and CAAT box-derived polymorphism (CBDP) (Talebi et al., 2018), and single nucleotide polymorphisms (SNP) (Cheng et al., 2024). However, findings reveal that the genetic diversity of safflower populations has narrowed during safflower domestication, and consequently, adaptation potential against threatening environments has drastically decreased. Therefore, genetic investigation of safflower collections with diverse origins would provide potentially valuable information for sustainable conservation and utilization of diversity. Additionally, a better understanding of genetic variation in germplasms could pave the way for the reliable classification of plant materials and characterization of subsets of core collections capable of being used in genetic improvement schemes (Mundel & Bergman, 2009). Safflower genetic variation has been documented in numerous investigations using a combination of morphological variation and molecular polymorphisms. Meanwhile, determining the association between phenotypic variations and molecular data is highly valuable for giving a complete picture of overall variations. To achieve this goal, molecular marker data must be balanced with morphological attribute. However, the molecular markers and phenotypic data might sometimes be weakly correlated. Without genetic linkage, genetic drift is responsible for low correlation. Conversely, the higher the correlation diversity measured by morphological attributes and variation measured by molecular markers, the easier the management of genetic resources would be (Johnson et al., 2007).

2.7.2 Molecular Markers in safflower

The emergence of molecular techniques has expedited plant breeding programs by providing new insight into the accurate and rapid evaluation of plant materials and the selection of superior genotypes. The molecular markers usually employ various DNA properties such as tandem repeats sequences, restriction sites, and/or single nucleotide mutations to identify genetic variations at the DNA level. Marker-assisted selection (MAS) is the utilization of DNA markers in plant breeding and is currently an important component of the new discipline of ‘molecular breeding’ capable of using quantitative and qualitative traits (Nair & Rabbani, 2024). MAS is an effective toolbox in

plant breeding, particularly when the target morphological/phenotypic attributes are highly laborious or expensive to measure. The application of MAS in plant breeding programs could be categorized into five major areas: marker-assisted evaluation of plant material, marker-assisted backcrossing (MABC); marker-assisted pyramiding, early generation selection, and combined MAS; however, there may be some overlap between these categories (Collard & Mackill, 2008). Despite the numerous molecular markers that have been used in safflower, their utilizations could be classified into three major categories: phylogenetic studies, genetic variation analysis, and linkage map construction/gene tagging.

Phylogenetic studies are crucial for understanding the origin, genetic relatedness, and diversification of CWRs. The phylogenetic relationships between *Carthamus* taxa have been studied based on morphological traits and cytogenetic data (Ashri & Knowles, 1960). Despite being widely acceptable, there is still lack of knowledge on the speciation level and evolutionary relatedness between the taxa within the genus *Carthamus*. Similarly, the identification of likely progenitor of *C. tinctorius* is controversial (Sasanuma et al., 2008). During the past decades, various dominant (Vilatersana et al., 2005; Sehgal et al., 2008; Sehgal et al., 2009; Mehrotra et al., 2013; Yaman et al., 2014) and co-dominant (Bowles et al., 2010; Chapman et al., 2010; Kumar et al., 2015; Shafiei-Koij et al., 2020) molecular markers have been used to classify this genus. Among these markers, genome-by-sequencing (GBS) is a next-generation sequencing-based technique that predicts single nucleotide polymorphisms (SNPs) to carry out genotyping studies. It is powerful approach allowing the discovery of genetic variations at DNA level best suited for phylogenetic studies, marker assisted selection (MAS), genome-wide association study (GWAS), developing molecular markers, linkage analysis, and genomic diversity studies for a particular trait (Vats et al., 2022), genomic prediction (GP), and genetic mapping (He et al., 2014; Bowers et al., 2016).

Different methods such as biochemical attributes (Espanani et al., 2019a; Alizadeh-Yeloojeh et al., 2020; Çulha-Erdal et al., 2021), morphological traits (Sabzalian et al., 2009; Majidi & Zadhoush, 2014; Arslan, 2018; Kumari et al., 2017; Hassani et al., 2020), cytogenetic techniques (Khidir & Knowles, 1970; Kumar, 1991; Agrawal et al., 2013; Mancia et al., 2017), and molecular markers, including RAPD, AFLP, ISSR, SSR, SCoT, iPBS-retrotransposon markers, InDel markers, and SNPs, have been used to assess the intra and interspecific genetic variability in safflower (Sabzalian et al., 2009; Kiran et al., 2017; Ali et al., 2020; Singh et al., 2022; Cheng et al., 2024). Morphological attributes have several disadvantages, such as low heritability and polymorphisms limiting their applicability. Compared to biochemical properties, morphological traits, and cytogenetic techniques, molecular markers are mostly independent of environment and growth stage, cost-effective, informative, reproducible, and easy to use, making them capable of detecting genetic diversity at the DNA level and efficiently overcoming the problems associated with morphological-based classifications (Majidi & Zadhoush, 2014). Moreover, ISSRs, AFLPs, and RAPDs are among safflower's most widely used molecular markers, because they are ideal for crop species with little genetic resources, and prior knowledge is not essential (Chugh et al., 2023). Although these markers could provide new insight into the better understanding of genetic variations within and between safflower populations, they are dominantly inherited, and their inheritance nature does not allow allelic information detection in breeding programs. SSRs and SNPs are the most valued dominant molecules that can unravel the allelic variation. These markers have several advantages, including co-dominant nature, multi-allelic inheritance pattern, wide-genome coverage, high polymorphism, high reproducibility, adaptability to automation, and effective transferability to closely wild relatives (Ambreen et al., 2015).

Characterization of loci controlling major agronomic traits accelerate marker-assisted breeding to increase crop performance. In safflower, mapping studies were mainly involved with oil content and quality (Kadirvel et al., 2020), disease resistance (Anjani et al., 2019), pest tolerance (Jegadeeswaran et al., 2021), seed yield (Ebrahimi et al., 2011), and other qualitative traits like flower color (Mayerhofer et al., 2010), and male sterility (Hamdan et al., 2008). Conventional breeding techniques generally employ highly accurate biochemical profiling methods for selecting genotypes with higher oleic content, however, they are destructive, laborious technique, and only carried out at the maturity stage (Fan et al., 2023). Liu et al., (2013) developed a perfect molecular marker for selecting HO genotypes (carrying an *olol*

mutation) through nonsense-mediated RNA decay (NMD) of CtFAD2-1. This marker was designed based on a single-nucleotide deletion in the coding sequence of CtFAD2-1, which caused premature termination of translation in the HO genotypes. It was proved to be efficiently used in MAS to manage the HO trait in safflower genetic improvement programs. Kumar et al., (2023) successfully employed the MABC scheme to incorporate the '*ol'* allele from Montola-2000 into Bhima, a popular Indian linoleic type variety. They designed a robust, non-destructive, co-dominant, and accurately predictable approach, namely Kompetitive Allele Specific PCR (KASP®) and the Amplifluor™ SNPs Genotyping System (Amplifluor®) that is based on the mutation in the CtFAD2-1 to select superior genotypes with high oleic acid content (Liu et al., 2013). Hamdan et al., (2008) characterized SCAR markers flanked two closely associated genes, Li gene, modulating the high linoleic acid content, a nuclear male sterility gene, Ms. Mayerhofer et al., (2010) mapped a dominant gene, ctfc1 gene, on the linkage group T9 that controls yellow flower color. Garcia-Moreno et al., (2011) mapped the Tph2 allele associated with high γ-tocopherol value in safflower. Hamdan et al., (2012) identified the *Ol* allele, which modulates high oleic acid content on linkage group (LG) T3 closely linked to the SSR marker ct365. Anjani et al., (2018) employed SSR markers to select genotypes carrying resistance gene to Fusarium wilt in interspecific crosses of Nira × *C. oxyacanthus* and Nira × *C. palaestinus*.

A few QTL mapping studies have been reported in safflower, which mainly involves aphid tolerance (Jegadeeswaran et al., 2021), oleic acid content (Hamdan et al., 2012), domestication-related traits including palmitic acid, oleic acid, and linoleic acid content (Pearl et al., 2014), major agronomic traits under water stress (Poodineh et al., 2021), and QTLs underlying tolerance to drought (Mirzahashemi et al., 2015). Hamdan et al., (2008) constructed a linkage map including the Li (very high linoleic acid) and Ms (male sterility) genes and five sequence-characterized amplified region (SCAR) markers closely flanked both loci at minimum distances of 15.7 cM and 3.7 cM from the Li and Ms loci, respectively. Employing SSR and RFLP markers, Mayerhofer et al., (2010) constructed a linkage map of safflower in an intraspecific F2 population of cultivated safflower and an interspecific BC1 population of *C. tinctorius* × *C. oxyacanthus*. Garcia-Moreno et al., (2011) used bulked segregant analysis with SSR and RAPD markers and constructed a Tph2 linkage map that exhibited the linkage of one SSR marker and eight RAPD markers to the Tph2 allele. Subsequently, Hamdan et al., (2012) constructed a genetic linkage map including 15 linkage groups and 116 RAPD, SSR, and SCAR markers for the CL-1 × CR-9 population.

Genome-wide association studies (GWAS) help plant breeders identify genes/alleles associated with a particular trait. This technique investigates the entire set of DNA (or the genome) of a large group of plant materials, discovering genetic polymorphisms. Singh et al., (2022) conducted a marker-trait association study to identify loci closely linked to Fusarium wilt resistance in a panel of 84 genetically diverse accessions of safflower using AFLP and SSR markers. They characterized four marker-trait associations (MTAs) tightly linked to the Fusarium Wilt resistance trait. Also, a locus, namely Locus-128, was identified as a promising MTA that facilitates MAS for FW resistance in safflower. Chen et al. (2023) performed a whole-genome study and GWAS to identify key agricultural attributes of safflower for industrial and medicinal purposes. They successfully characterized a candidate gene, HH_034464 (CtCGT1) putatively involved in biosynthesis of hydroxysafflor yellow A (HYSA). Additionally, several SNPs significantly associated with major agronomic traits including oil content, plant height, and stem diameter have been also identified. It is also proposed that regulatory mechanism, MBW-CtBB1, a novel HYSA accumulation module in safflower, might play a key regulatory role in coordinating HYSA accumulation with other responsive mechanisms through degradation by the E3 ligase CtBB1 (Hong et al., 2023).

2.8 Omics Studies in Safflower

Advances in genome sequencing techniques, specifically long-read sequencing, have led to more accurate assembly of genomes in many crops, including safflower (Bowers et al., 2016; Chen et al. 2023). Furthermore, safflower population genetics have meaningfully advanced during the last decade. Numerous safflower populations have been lately genotyped to examine the breeding footprints providing valuable insights into the molecular mechanisms modulating safflower growth and development and controlling responses to environmental stresses (Talebi et al. 2012; Derakhshan et al. 2014; Ali et al. 2019; Shafiei-Koij et al. 2019;

Hassani et al. 2020). Omics approaches, such as genomics, transcriptomics, proteomics, and metabolomics, have been used to study various aspects of safflower biology.

2.8.1 Genomics

The initial *Arabidopsis thaliana* genome assembly from Columbia (*Col*-0) reference accession was released in 2000 and followed by sequencing, assembling, and publicizing of several other plant genomes on GenBank and other genomic data repositories. However, the genome assembly of just about 0.16% of more than 300,000 land plant species has yet been released. Bowers et al. (2016) employed a low-coverage, whole-genome shotgun sequencing approach to identify millions of SNPs and to construct a high-density genetic linkage map from a bi-parental cross (*C. tinctorious* and *C. palaestinus*). They provided a draft genome assembly of cultivated safflower that covered 866 million bp (67%) of the expected ~1.4 GB safflower genome. This research provides highly valuable genomic resource to supply more detailed studies of genetic variations and breeding programs. A safflower chloroplastic (Mcpherson et al., 2004) genome sequence assembly was reported based on PacBio Sequel Platform (Wu et al., 2019). It included a total length of 152,963 bp comprising two inverted repeats (25,128 bp) separated by an extensive single-copy sequence (84,124 bp) and a small single-copy region (18,583 bp). A total of 112 genes, consisting of 79 protein-coding genes, 29 tRNA genes, and 4 rRNA genes, were also annotated. Similar findings were previously reported by Chapman et al. (2009). Wu et al. (2021) published the first high-quality genome assembly (contig N50 of 21.23 Mb) for the 12 pseudo-chromosomes of safflower employing single-molecule real-time sequencing (SMRT), Hi-C mapping approaches, and a genetic linkage map. They also provided strong molecular evidence regarding safflower's divergence and gene family expansion. It was concluded that the expansion of gene families primarily includes enriching those predicted genes involved in lipid metabolism and transport and abscisic acid signaling. Yang et al. (2023) assembled the chloroplast genomes of *C. tinctorious*, *C. persicus*, *C. lanatus*, and *C. tinctorius* × *C. persicus hybrids*. The sizes of the chloroplast genome assembly of *C. persicus*, *C. lanatus*, and interspecific hybrids of *C. tinctorius* × *C. persicus* were estimated at 153,177 bp, 152,602 bp, and 153,177 bp, respectively. The genome assembly of these *Carthamus* taxa showed to be highly conserved. Dong et al. (2024) also published the genome assembly for *C. tinctorius* variety Jihong01 achieved by incorporating Oxford Nanopore Technologies (Kadirvel et al. 2016) and *BGI-SEQ500* sequencing results. It included 1,061.1 Mb and 1,061.1 Mb and 32,379 protein-coding sequences, of which 97.71% were functionally annotated. They also unveiled the crucial roles of *FAD2* and *FAD6* in linoleic acid (LA) biosynthesis at five seed development stages that offer new insights into safflower breeding schemes for quality improvement.

2.8.2 Proteomics

Safflower proteome changes in response to drought or salinity stress or during developmental stages could expand our understanding of the mechanisms underlying safflower growth, development, and productivity. Shaki et al. (2020) studied the effects of exogenously applied salicylic acid (SA) and penconazole (PEN), as growth regulators on the protein profile of NaCl-treated safflower plants. They identified 17 salt-responsive proteins related to different metabolic pathways mainly involved in photosynthesis, ion homeostasis, and oxidative stress response, as well as carbohydrates, protein, and nitrogen metabolisms. These stress-responsive proteins may help improve safflower salt tolerance and maintain its performance under abiotic stress conditions. Çulha-Erdal et al. (2021) identified total of 72 protein spots differentially accumulated under the drought stress followed by re-watering. They were mostly involved in photosynthesis and metabolism of carbohydrates and proteins, defense responses, and energy production. Employing integrated proteomic and lipidomic profiles, Chen et al. (2022) studied safflower seeds' proteins and lipids profiles during natural seed aging. They quantified a total of 4,184 proteins and 1,193 lipids, demonstrating high variations among the different naturally aged seeds. It was concluded that the enzymes involved in glycerolipid metabolism and fatty acid degradation are responsible for degradation of oil bodies (triacylglycerols) and membrane lipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserines, phosphatidylinositol, phosphatidylglycerols), and ultimately destroy the seed structure, resulting in decreased seed vigor during natural seed aging. Moreover, a set of high complex lipophilic proteins and metabolites comprising 2179 unique compounds and 3043 peptides matching 724 differentially expressed proteins across organs and tissues during five

seed developmental stages and petal wilting of safflower has been identified (Vincent et al., 2024). It was concluded that safflower seed husks preferably featured metabolites (99%), while seed cotyledons predominantly yielded peptides (90%). The time-specific gene expression and the key roles of phenylpropanoids, flavonoids, and pigments during petal color transition and wilting has also been highlighted.

2.8.3 Transcriptomics

The study of mechanisms involved in flavonoid biosynthesis and fatty acid metabolism is pivotal for genetic manipulations in safflower for improvement of flavonoid and oil content and/or fatty acid compositions. Various molecular mechanisms regulate plant responses to drought. Cytochrome P450 (CYTP450) is among the largest oxidase families in plants playing a key role in regulating plant metabolisms, including modulation of drought stress tolerance in safflower roots. Kumar et al. (2009) identified 16 *CtCYP71A* enzyme-encoding genes in safflower. Expression studies revealed *CtCYP71A* initially up-regulated in safflower roots under PEG stress. However, its expression was ultimately downregulated with abscisic acid (ABA), gibberellic acid (GA), and salicylic acid (SA) stresses. *CtCYP71A1* mediates safflower drought tolerance through lignin accumulation in safflower roots. *CtDREB52*, a CtAP2/ERF family member, is a major transcription factor potentially regulating the UV-B-induced flavonoid biosynthesis and hydrogen peroxide accumulation in safflower. It was reported that the overexpression of *CtDREB52* could not only promote flavonoids accumulation in safflower, also induces an early flowering response and up-regulates the key genes involved in flavonoid biosynthesis. This gene operates the mechanisms by regulating the transcription levels of interacting genes involved in the flavonoid biosynthesis pathway specifically *CtMYB* and *CtF3′*H, total flavonoid and H_2O_2 contents (Yufei et al. 2024).

*CtWRKY*s are among the major transcription factors (TFs) involved in plant responses to various abiotic stresses, including drought, temperature, cold, salt, and dehydration. Wang et al. (2021) analyzed the genes related to flavonoid (C-glucosylquinochalcone) biosynthesis in safflower with different colors (white, yellow, light red, and deep red). They found that the expression of flavonoid biosynthesis-related genes decreased with increasing flower color. It was also reported that the differential expression of the *chalcone synthase* (*CHS*) gene is responsible for flavonoid variations and content in safflower with different flower colors. Three homologs to cytochrome *P450 71D9* gene (*HH_035319, HH_032689, HH_025963*) may likely contribute to C-glucosylquinochalcone biosynthesis in safflower leading to the red color appearance in safflower. Ren et al. (2022) used an integrated metabolomics and transcriptome study to elucidate the alterations in flavonoid biosynthesis in safflower flowers during color changes. A significant correlation between the expression of a *uridine diphosphate glucose glycosyltransferase* gene, *CtUGT9*, and flavonoid glycosides was detected, suggesting that *CtUGT9* may have a crucial role in flavonoid glycoside biosynthesis during color-transition in safflower. Li et al. (2021) executed the temporal transcriptome sequencing of safflower seeds at 10, 14, 18, and 22 days after flowering (DAF) to explore the molecular networks governing unsaturated fatty acids (USFAs) biosynthesis. They reported that fatty acid biosynthesis is the dominant cellular process from 10 to 14 DAF. The two genes of *stearoyl-[acyl-carrier- protein] 9-desaturase gene* (*SAD*) and *oleate desaturase* (*FAD2*–1) express temporally 10 to 14 DAF and from 14 to 18 DAF, respectively. Moreover, 13 candidate TFs were also characterized in modulating the expression level of FAD2–1 gene.

2.8.4 Metabolomics

Studying the molecular and biochemical mechanisms of drought tolerance in safflower could pave the way for genetic engineering and genetic improvement programs in safflower. Jia-Xi et al. (2019) holistically evaluated the quality of safflower samples collected from different producing regions by investigating both the primary and secondary metabolite profiles. A total of 13 primary metabolites (comprising one nucleoside, two sugars, five organic alkali/acids, and five amino acids) and 135 secondary metabolites (including 97 quinochalcone C-glycoside (QCGs) and 38 flavonoids) were characterized or tentatively identified. They suggested analyzing both the primary and secondary metabolites profiles is a powerful approach facilitating the holistic quality evaluation of safflower. Wei et al. (2020) used integrated transcriptomic and metabolomic techniques to decipher the key metabolisms, pathways, and candidate genes involved in drought tolerance in safflower. They found that

several candidate genes including *ABA2, CYP707A4, MYB62, MYB2, GolS1, NECD4, ZDS, OEE2, P5CS1, GST23, GST3, GSTL1, Cu-ZnSOD1,* and *ALDH3F1* were more likely involved with drought resistance in this crop. Additionally, three metabolites, galactitol, neoxanthin, and arbutin, were also closely associated with drought-tolerance.

3. Conclusion

Safflower is one of the most important oilseed crops, and it has numerous past and present uses. The crop can grow in marginal lands where adverse conditions such as drought, salinity, and high element concentrations are the major factors limiting crop growth, development, and productivity. Safflower is traditionally grown to extract dye from flowers used as fabrics dye, food colorant, flavoring, and medicinal purposes. The crop could also be grown for animal feeding purposes, biofuel, bioplastic, and composite packaging film production, and phytoremediation of polluted lands. Safflower seeds also contain high-quality oil rich in linoleic and α-linolenic, which are major essential fatty acids that are highly beneficial for human health by regulating blood sugar, preventing heart diseases, and reducing blood cholesterol. Despite being an important oilseed crop, the world safflower production is lower than that of some oilseed crops due to relatively lower seed yield and susceptibility to a series of biotic and abiotic stresses. Yet, little efforts have been accomplished to improve safflower productivity due to a lack of access to molecular tools that could accelerate the genetic improvement program; however, in recent years, this situation has begun to change.

The well characterization of worldwide germplasm resources, including crop wild relatives, gives the opportunity to exploit available genetic variability for developing new varieties for specialty niches through the development of healthier oil, improved shelf-life, more heat-stable oil constituents, cold tolerance, and pest and disease resistance. Promisingly, the development of next-generation sequencing techniques and modern molecular methodology are expected to aid in enhancing the efficiency of safflower breeding programs by discovering novel molecular markers such as SNPs, phylogenetic studies, marker-assisted selection (MAS), QTL mapping, linkage analysis, genome-wide association studies (GWAS), and genomic prediction (GP) in safflower. Recently, biotechnological approaches have also been employed in safflower genetic improvement programs by facilitating gene introgression from wild relatives and alien gene transfer from other living organisms. Unfortunately, researches on this crop are scattered and limited to a few research teams worldwide, and it seems complicated to achieve all genetic improvement objectives by an individual organization. There are international networks for all the major oilseed crops. However, such networks are required to be established for safflower to not only coordinate the development of biotechnological tools and molecular markers and sharing of safflower genetic resources but also facilitate the conservation and exchange of genetic resources for expediting the genetic improvement programs and making safflower a commercially viable crop.

Disclaimer (Artificial Intelligence)

The author hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this review paper.

Competing Interests

Author has declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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