

# A Review of Fatty Acids and Genetic Characterization of Safflower (*Carthamus Tinctorius* L.) Seed Oil

Lei Liu<sup>a</sup>, Ling-Liang Guan<sup>b\*</sup> and Yu-Xia Yang<sup>c</sup>

<sup>a</sup>College of Life Science & Biotechnology, Mianyang Normal University, Mianyang 621000, Sichuan Province, China

<sup>b</sup>Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, Hainan Province, China

<sup>c</sup>Sichuan Academy of Traditional Chinese Medicine Sciences, Chengdu 610041, Sichuan Province, China

\*Correspondence: Guan Ling-Liang, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, Hainan Province, China. E-mail: gllgirl23@163.com

## ABSTRACT

Safflower, *Carthamus tinctorius* L., is an annual oilseed crop that is cultivated on small plots all over the world. The seed oil content ranges from 20% to 45%; the oil is high in linoleic acid, an unsaturated fatty acid that aids in lowering the blood cholesterol level. Thus, safflower has long been used as medical plant in many countries, especially in China and India. However, for industrial purposes, it has long been neglected because of the low seed yield or oil content, until its physical role was revealed. In recent years, research works carried out in many countries mostly focused on improving the seed or oil yield. In this review, after illustrating the fatty acid composition of safflower seed oil as well as the genetic characteristics of safflower and their relationships with agronomic traits, a brief analysis of the current worldwide situation and future prospects of safflower utilization are presented.

**Key words:** Safflower, fatty acid, seed development, genetic characteristics, agronomic traits

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## INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a member of the Compositae family; it has been cultivated around the world for centuries, mainly as a source of edible oil and dyes. India, the USA, Mexico, Australia, and Ethiopia are the largest producers of safflower for oil extraction, and these countries account for 85% of the world's safflower production<sup>[1]</sup>. Other important safflower-producing countries are China, Kazakhstan, Argentina, Uzbekistan, and the Russian Federation<sup>[2]</sup>. As safflower is more drought and salt tolerant than other oil seed crops, it is especially suitable for the dry and salty areas where other oil seeds are difficult to grow<sup>[3]</sup>.

The fatty acid composition of a vegetable oil determines its best commercial uses<sup>[4]</sup>. Safflower has been used for centuries as a kind of ornamental plant, as medicinal plant, and as cosmetic material in China. Safflower seed oil with its high linoleic acid content was once largely used in the preparation of alkyd resins for paints and varnishes. Currently, in view of its reported role in reducing blood cholesterol levels, it is being utilized as premium edible oil<sup>[5-7]</sup>. It also may be a potential raw material for vegetable oil-based liquid fuel production in the near future<sup>[4]</sup>. Safflower oil, due to its high oleic acid content, has become a widely used frying oil because of its high stability and bland flavor<sup>[8]</sup>.

In this review, after illustrating the fatty acid composition of safflower seed oil as well as the genetic characteristics of safflower and their relationships with agronomic traits, a brief analysis of the current worldwide situation and future prospects of safflower utilization are presented.

## Chemical Constituent and Pharmacological Activities of Safflower

Safflower (*carthamus tinctorius* L.) is widely distributed in the Xinjiang Uygur Autonomous Region, Zhejiang, Henan, and Sichuan province of China. The flowers of this plant are used as a remedy for dysmenorrhea, wound, coronary disease, angina pectoris, and hypertension in Chinese folk medicine. Previous studies revealed that safflower contains flavonoids, serotonin, polyacetylenes, spermidines, matarsinols, sesquiterpenes, steroids, and so on. Quinone chalcones were found in safflower which occurred in few other plants. Various bioactives of Safflower had been confirmed by modern pharmacology, such as cardiovascular, nervous system, immune system, and so on, especially the cardiovascular bioactivities<sup>[9-11]</sup>. The main constituents of safflower are pigments, flavonoids, phenolic acid etc. And Safflower yellow were the effective constituents in safflower, which were extracted predominantly by water. The chromatographic technique was used to the control quality of it. More studies are needed on the quality control of safflower<sup>[12]</sup>.

## Fatty Acid Composition of Safflower Seed Oil

Oilseeds are important sources of vegetable oils. The suitability of a vegetable oil for a particular use such as in nutritional, industrial or pharmaceutical applications is determined by its fatty acid composition, which is highly variable depending on the plant species. This has encouraged

researchers to look for new sources of oil or for new fatty acid compositions in different plant species. Genetic variation in the fatty acid composition is essential for the genetic improvement of oil quality and for the development of new cultivars.

Safflower oil contains two main unsaturated fatty acids: oleic (18:1) and linoleic acid (18:2). They account for about 90% of the total fatty acids. The remaining 10% correspond to the saturated fatty acids, palmitic (16:0) and stearic acid (18:0). Standard safflower oil contains about 6–8% palmitic acid, 2–3% stearic acid, 16–20% oleic acid, and 71–75% linoleic acid<sup>[13]</sup>. However, in many other studies, the fatty acid composition of safflower seeds has shown great variability<sup>[14]</sup>. Fernández-Martínez et al<sup>[15]</sup> have reported the fatty acid compositions of 200 safflower accessions originating from 37 countries, indicating that oleic and linoleic acid have a tremendous range of variation, from 3.1% to 90.60% and from 3.9% to 88.8%, respectively. Guan et al<sup>[16]</sup> investigated the oil content and fatty acids of 21 safflower accessions from 12 countries. Their results showed that the content of oil ranged from 9.10% to 25.10%, with palmitic acid ranging from 4.04% to 7.86%, stearic acid from 1.5% to 2.75%, oleic acid from 7.9% to 32.99%, and linoleic acid from 62.7% to 83.74%. Sabzalian et al<sup>[17]</sup> reported the fatty acid compositions of three safflower species. Their results showed that the oleic acid contents in the seed oil of *C. tinctorius*, *C. oxyacantha* BIEB., and *C. lanatus* L. were in the range of 12.24–15.43%, 14.11–19.28%, and 16.70–19.77%, respectively. The corresponding ranges for linoleic acid were 71.05–76.12%, 63.90–75.43%, and 62.47–71.08%. In the past several decades, varieties with higher oil content (34–37%) and mutant types with high levels of oleic acid were developed. A safflower variety from Israel with 5–10% stearic acid, compared to 1–3% in the normal genotypes, was identified<sup>[18]</sup>. Very high levels of linoleic acid (87–89%) and very low oleic acid (3–7%) levels were found in safflower from Portugal<sup>[19]</sup>. Additionally, accessions with very high oleic acid content (>85%) have been reported<sup>[15,20]</sup>. Sabzalian et al<sup>[17]</sup> reported that the fatty acid compositions among oils from cultivated and wild species were not very different, indicating that the seed oil of the wild safflower may be suitable for human consumption and industrial purposes. The oil content was in the range of 29.20–34.00%, 20.04–30.80%, and 15.30–20.80% in *C. tinctorius*, *C. oxyacantha* BIEB., and *C. lanatus* L., respectively. The main fatty acids such as oleic, linoleic, palmitic, and stearic acid accounted for 96–99% of the total fatty acids in all species.

## Changes in the Fatty Acids of Safflower Oil during Seed Development

Safflower showed great changes in the contents of seed oil and in the fatty acid compositions during seed development. Sims et al<sup>[21]</sup> reported that, in developing safflower seeds, the oleic acid concentration increased slowly during the first 30 days after fertilization and then in some cases appeared to level off with approaching maturity. Initially, linoleic acid was present

at almost the same amount as oleic acid, but by day 20 after fertilization its concentration was three times that of oleic acid. This ratio of linoleic to oleic acid tended to increase steadily during the latter part of seed development. Hill and Knowles<sup>[22]</sup> revealed that the actual amount of palmitic acid in their safflower varieties remained at about the same level until 10 days after flowering, and then slowly increased until about 25 days after flowering, although the palmitic acid percentage in relative amounts decreased during seed development. Ichihara and Noda<sup>[23]</sup> reported that saturated fatty acids tended to accumulate at the 1- and 3-positions of the glycerol molecule, and the more highly unsaturated acids, at the 2-position. The fatty acid compositions at the 1- and 3-positions were similar in all cases investigated, but in none of the triacylglycerols (TAG) was the distribution completely symmetrical. The positional distribution of linolenic acid in TAG prepared from the immature seeds 2 days after flowering and from the leaves was unusual. Hamrouni et al<sup>[24]</sup>, working with safflower seeds found that the oil content and total lipids were increased during seed ripening; the linoleic acid content was also significantly increased, while the palmitic acid content had decreased considerably, from 13.7% to 4.0%, during seed ripening. In addition, the ripening process of the safflower seeds was also characterized by an increase in the TAG content and a decrease in the diacylglycerol (DAG), phosphatidylcholine (PC), and phosphatidic acid (PA) contents. Geçgel et al<sup>[25]</sup> reported that the moisture content declined in the 15 days from the flowering period to maturity, while the oil content increased. The rate of the palmitic acid formation decreased in both varieties in the 15 days from flowering to maturity, whereas the oleic and linoleic acid formation rates increased in the Montola-2001 and Centennial varieties, respectively. Fatty acids is absolutely necessary matter for growth of human body and also supplies him with high heat quantity. We can choose different fatty acids type of safflower oil during its seed development for different health function.

## Genetic Characteristics Related to the Fatty Acids

Safflower is one of the cultivated oil crops from which a great number of different types of oil can be developed through the combination of major genes controlling the levels of stearic, oleic, and linoleic acid, and by changing the environmental conditions<sup>[14]</sup>. In 1964, Knowles and Hill<sup>[26]</sup> reported that the major gene locus, *ol*, governs the proportions of oleic and linoleic acid, with the genotype *olol* leading to 72–80% oleic acid in the safflower seed oils, the genotype *OLOL* resulting in 72–80% linoleic acid, whereas the genotype *ol'ol'* or *OLol<sub>1</sub>* has about equal amounts (45%) of each acid. In 1989, they reported that the genotypes *OLOL* and *olol* were more stable with regard to temperature changes, in contrast to the gene *ol1*. At the highest temperature, in the genotypes *ol<sub>1</sub>ol<sub>1</sub>* and *OLol<sub>1</sub>*, the linoleic acid content was slightly decreased and oleic acid was increased by the corresponding amount. A new gene locus (*li*) – different from *st* (governing the levels of

oleic and stearic acid) or *ol* – controlling for high levels of linoleic acid was found in the genotype from Portugal (Portugal 253568) with very high levels of linoleic acid (87–89%) and very low oleic acid content (3–7%)<sup>[19]</sup>.

Hamdan et al<sup>[27]</sup> reported on the inheritance of the very high linoleic acid content in safflower and its relationship with nuclear male sterility. The results showed a linkage of five random amplified polymorphic DNA (RAPD) bands to the *Li* (controlling for the very high linoleic acid content) and *Ms* (controlling for nuclear male sterility, NMS) gene loci. The RAPD fragments were converted into sequence-characterized amplified region (SCAR) markers. A linkage map including the five SCAR markers and the *Li* and *Ms* gene loci was constructed. The SCAR markers flanked the two loci at minimum distances of 15.7 cM from the *Li* locus and 3.7 cM from the *Ms* locus.

### Relationship of Agronomic Traits and Seed/Oil Yields

The seed yield per plant and the oil content are the primary selection criteria for safflower breeding<sup>[28]</sup>. The importance of selection for a particular trait depends on the extent of direct or indirect effects of the trait on the seed/oil yield<sup>[29]</sup>. Therefore, before initiating selection in a seed/oil yield improvement program, it is necessary to know the relative importance of each of the different traits in influencing the trait of economic importance in a desired direction<sup>[30]</sup>.

Rao et al<sup>[31]</sup> reported that the capsule number, the capsule weight, and the hull percentage were the most important components in breeding for higher yield and oil content. The Spine index showed a non-significant association with yield and oil content. The yield per plant and its major components (the number of capsules and the capsule weight) revealed a negligible relationship with the oil content. Nie et al<sup>[32]</sup> worked with safflower and showed that the first branch height, the plant height, and the heads per plant were the principal components of safflower seed/oil yield. Therefore, an appropriate combination of these components would constitute the most desirable plant type, but the strong negative associations between these desirable traits were the major problem in the selection programs. Bagawan and Ravikumar<sup>[33]</sup> reported that the oil content and the seed yield are negatively correlated. Pahlavanim<sup>[34]</sup> stated that an improvement of the seed yield in safflower could decrease the oil content and the 100-seed weight due to negative association between these traits. Alizadeh and Carapetian<sup>[35]</sup> reported that the average number of seeds per head has a significant positive relationship with the grain yield. Thus, genetic gain may be achieved in the future by augmenting the number of seeds per head. Yang et al<sup>[36]</sup> reported that the number of heads per plant, the number of branches, the plant height, and the first branch height are the most important characters in safflower improvement for increasing the seed yield. Our previous research indicated that photosynthetic parameters were significantly correlated with some agronomic traits such as the 100-seed weight and the head

diameter<sup>[37]</sup>. After this, our research showed that most of the correlation coefficients between agronomic traits and fatty acids were insignificant, indicating that the fatty acid content was hardly influenced by the 100-seed weight, the plant height, and the number of seeds per plant<sup>[16]</sup>. Thus, the safflower oil quality and agronomic traits could be improved simultaneously through breeding of safflower for high photosynthetic efficiency. In addition, the results also showed significant correlation coefficients between some photosynthetic parameters and the percentage of fatty acids, which inferred that the photosynthesis rate can be used as an early selection marker in genetic improvement programs. Abd El-Lattief<sup>[38]</sup> evaluated 25 safflower genotypes for seed and oil yields in an arid environment in Upper Egypt. The results showed that the oil yield was significantly correlated with the plant height ( $r = 0.566^{**}$ ), the branches per plant ( $r = 0.591^{**}$ ), the capitula per plant ( $r = 0.625^{**}$ ), the seed weight per plant ( $r = 0.863^{**}$ ), the seed yield ( $r = 0.990^{**}$ ), and the seed oil content ( $r = 0.711^{**}$ ).

Safflower cultivars with very high levels of linoleic or oleic acid would increase the value of the oil<sup>[14]</sup>. Usually, there was an inverse relationship between oleic acid and linoleic acid<sup>[39]</sup>. Dajue<sup>[16]</sup> reported that linoleic acid is negatively correlated with all the other components, and this negative correlation is greatest with oleic acid ( $r = -0.9502$ ), followed by that of palmitic acid ( $r = -0.3247$ ). Arslan<sup>[40]</sup> positive and significant relationships between oleic acid and palmitic acid ( $r = 0.317^{**}$ ), and oil stability ( $r = 0.920^{*}$ ), while the greatest negative and significant relationship was found between oleic acid and linoleic acid ( $r = -0.999^{**}$ ). The results of our previous study also showed that linoleic acid is negatively correlated with oleic acid, but palmitic acid was positively correlated with linoleic acid. All the correlation coefficients between the content of oil and that of each fatty acid were insignificant, indicating that it should be possible to breed new safflower varieties with simultaneously high oil content and high linoleic or oleic acid content in the future<sup>[16]</sup>.

### Environment and Cultivation Techniques Affect the Safflower Agronomic Traits and the Seed/Oil Yields

Besides genetic factors, cultivation methods and environment conditions can affect the safflower growth and seed/oil yields. Arslan<sup>[41]</sup> analyzed the broad-sense heritability and variance components of the seed yield and some components of the safflower cultivars. The results showed that heritability was low for the number of primary branches per plant (76%) and the number of heads per plant (78%), indicating that these traits may be greatly affected by the environment. However, Akbar and Kamran<sup>[42]</sup> found that the safflower plant height and the number of seeds per capitulum have the highest genotypic and phenotypic variances.

To identify safflower genotypes with both high and stable yield performance across different environments, Abdulahi et al<sup>[43]</sup> evaluated 16 safflower genotypes in multi-environment trials by nonparametric methods. The nonparametric

tests of  $G \times E$  and a combined ANOVA across environments indicated the presence of both crossover and usual crossover interactions, and the genotypes varied significantly for the grain yield. And according to the RS and TOP measures, the genotype G16 (PI537598) was the best genotype with the highest yield and the widest range of adaptation. Mozaffari and Asadi<sup>[44]</sup> demonstrated significant differences among safflower mutants for plant height, capitulum diameter, number of seeds per capitulum, capitulum weight, days to 50% flowering, days to maturity, and yield, between irrigated and drought stress conditions. However, the differences in oil content and number of capitula in the two conditions were insignificant.

In 2002, Oad et al<sup>[45]</sup> determined that the inter- and intra-row spacing affects the growth as well as the seed and oil production of safflower. They found that crop maturity, plant height, number of branches, number of capitula, and seed and oil content varied significantly between different extents of inter- and intra-row spacing as well as their interactions. A safflower crop should be planted at  $45 \times 30$  cm of row and plant distance for maximum seed and oil production. Nikabadi et al<sup>[46]</sup> reported that the numbers of seeds per capitulum and the seed yields of two spring safflower cultivars (Isfaha14 and IL111) were significantly decreased as the sowing dates were delayed from March 6 to June 21.

## Prospects of Safflower Seed Oil

Safflower oil for both edible and industrial purposes will become increasingly important. In recent years, the results of nutritional studies with high-linoleic and high-oleic oils have been promising. Safflower oil, with high linoleic acid content, can be blended with other vegetable oils to nutritionally upgrade them. In a different system of utilization, the high percentage of linoleic acid in safflower oil may be exploited for lipochemical applications. Therefore, safflower oils and meals will provide versatile products that will find many applications in livestock nutrition, human nutrition, and in technical industries.

Industrial uses of safflower oil can be expanded to counteract environmental concerns raised by the exclusive use of fossil fuels. Bio-diesel and fuel additives, as well as applications, e.g., as chainsaw bar oil, may reduce the polluting effects of exhaust gases. Research in Montana, USA, is continuing to make high-oleic safflower oil more economical for use as a biofuel, by adding value to safflower meal through genetic breeding and improvement.

Nowadays, the safflower products show great potential in the world market, with their important contributions to edible oils or industrial oils as well as the highly nutritious by-products for animal feed and the petals as coloring for foods and textiles.

Moreover, development of pharmacophore point theory greatly increases the effectivity of virtual screening in the database<sup>[47-50]</sup>. It is also a valid method for the identification of a natural product in safflower later period. By weeding out inactive non-binders in silico, the numbers of compounds to

be synthesized and tested in vitro can be dramatically reduced. Meanwhile, natural products have historically represented an important source of chemical scaffolds and bioactive substructures for the medicinal chemist. Therefore, technologies related to medicine, tea, food color and textile color applications need to be developed in the international research system and the crop can thus be promoted to achieve increasing market opportunities in the world.

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## REFERENCES

1. Dwivedil SL, Upadhyaya HD, Hegde DM. Development of core collection using geographic information and morphological descriptors in safflower (*Carthamus tinctorius* L.) germplasm. *Genet Resour Crop Ev* 2005; 52(7): 821-830.
2. Dajue L, Mündel HH. Safflower. *Carthamus tinctorius* L. Promoting the conservation and use of underutilized and neglected crops. 7. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome:1996.
3. Weiss EA. Oilseed Crops (second edition). Blackwell Science, Oxford: 2000.
4. Isigigur A, Karaosmanoglu F, Aksoy HA. Characteristics of safflower seed oils of Turkish origin. *J Am Oil Chem Soc* 1995; 72(10): 1223-1225.
5. Nagaraj G. Safflower seed composition and oil quality-a review. Beijing: Third International Safflower Conference 1993:58-71.
6. Ekin Z. Resurgence of safflower (*Carthamus tinctorius* L.) utilization: a global view. *J Agron* 2005; 4(2): 83-87.
7. Emongor V. Safflower (*Carthamus tinctorius* L.) the underutilized and neglected crop: A review. *Asian J Plant Sci* 2010; 9(6): 299-306.
8. Smith JR. More than four decades of safflower development. Beijing: Third International Safflower Conference, 1993: 861-867.
9. Li F, He ZS, Ye Y. Flavonoids from *Carthamus tinctorius*. *Chin J Chem* 2002; 20(7): 699-702.
10. Kazuma K, Ekadashi T, Sato K, Takeuchi H, Matsumoto T. Quinon-chalcones and flavonoids from fresh florets in different cultivars of *Carthamus tinctorius* L. *Biosci Biotechnol Biochem* 2000; 64(8): 1588-1599.
11. Jiang JS. Studies on the chemical constituents and bioactivities of *Carthamus tinctorius* L. Beijing: Thesis of doctor degree, Chinese Academy of Medical Sciences & Peking Union Medical College, 2008.
12. Wang RJ, Yang B. Survey of Study on the Chemical Constituents and Quality Control of Flos Carthami. *Chinese Journal of Experimental Traditional Medical Formulae* 2007;13(5):65-69.
13. Velasco L, Fernandez-Martinez JM. Breeding for oil quality in safflower. Montana: Bergman JW, Mündel HH (eds) Proceedings of the 5th International Safflower Conference, 2001:133-137.
14. Knowles PF. Safflower. New York: Downey RK, Robbelen G, Ashri A (eds) Oil crops of the world, 1989:363-374.
15. Fernandez-Martinez J, Rio M, Haro A. Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters. *Euphytica* 1993;69(1): 115-122.
16. Guan LL, Wu W, Zheng YL. Seed oil and fatty acid of different safflower genotypes and their correlations with agronomic traits and photosynthetic parameters. *Philipp Agric Sci* 2008; 91 (4): 383-388.
17. Sabzalian MR, Saeidi G, Mirlohi A. Oil Content and Fatty Acid Composition in Seeds of Three Safflower Species. *J Am Oil Chem Soc* 2008; 85(8):717-721.
18. Ladd SL, Knowles PF. Inheritance of stearic acid in the seed oil of safflower (*Carthamus tinctorius* L.). *Crop Sci* 1970;10(5): 525-527.



19. Futehally S, Knowles PF. Inheritance of very high levels of linoleic acid in an introduction of safflower (*Carthamus tinctorius* L.) from Portugal. California: First International safflower conference, 1981:56-61.
20. Dajue L. Progress of safflower (*Carthamus tinctorius* L.) research and production in China. Beijing: Third International Safflower Conference, 1993:35-46.
21. Sims RA, Mc Gregor WG, Plessers AG, Mes JC. Lipid changes in maturing oil bearing plants. II. Changes in fatty acid composition of flax and safflower seed oils. *J Am Oil Chem Soc* 1961; 38(6): 276-279.
22. Hill AB, Knowles PF. Fatty Acid Composition of the Oil of Developing Seeds of Different Varieties of Safflower. *Crop Sci* 1968; 8(3): 275-277.
23. Ichihara KI, Noda M. Fatty acid composition and lipid synthesis in developing safflower seeds. *Phytochemistry* 1980; 19(1): 49-54.
24. Hamrouni I, Touati W, Dhifi W, Chahed T, Ayachi S., et al. Glycerolipid evolution during safflower seed formation and ripening. *J Food Lipids* 2004;11(4): 297-311.
25. Gecgel Ü, Demirci M, Esendal E, Tasan M. Fatty acid composition of the oil developing seeds of different varieties of safflower (*Carthamus tinctorius* L.). *J Am Oil Chem Soc* 2007;84(1): 47-54.
26. Knowles PF and Hill AB. Inheritance of fatty acid content in the seed oil of a safflower introduction from Iran. *Crop Sci* 1964(4): 406-409.
27. Hamdan YAS, Velasco L, Pérez-Vich B. Development of SCAR markers linked to male sterility and very high linoleic acid content in safflower. *Molecular Breeding* 2008; 22(3):385-393.
28. Gencer O, Sinan SN, GÜlya F. Relationships between oil yield and traits using correlation and path coefficient analysis in safflower (*Carthamus tinctorius* L.). *J Fac Agr* 1987(2): 37-43.
29. Mahasi MJ, Patha kRS, Wachira FN, Riungu TC, Kinyua MG, et al. Correlations and Path Coefficient Analysis in Exotic Safflower (*Carthamus tinctorius* L.) Genotypes Tested in the Arid and Semi Arid Lands (Asals) of Kenya (2006). *Asian j Plant Sci* 2006; 5(6): 1035-1038.
30. Singh V, Deshpande MB, Choudhari SV, Nimbkar N. Sesame and Safflower newsletter, 2004; 19: 77-81.
31. Ranga Rao V, Ramachandram M, Arunachalam V. An analysis of association of components of yield and oil in safflower (*Carthamus tinctorius* L.). *Theor appl Genet* 1977; 50(4):185-191.
32. Nie Z, Chen F, Shi XC. Path Analysis of Characters Related to Seed Yield in Safflower. *Chines journal of Oil Crop Sciences* 1993(3): 26-29.
33. Bagawan II, Ravikumar RL. Strong undesirable linkages between seed yield and oil components-a problem in safflower improvement. Montona: 5th International Safflower Conference, 2001:23-27.
34. Pahlavanim H. Some technological and morphological characteristics of safflower (*Carthamus tinctorius* L.) from Iran. *Asian J Plant Sci* 2005(4): 234-237.
35. Alizadeh K, Carapetian J. Genetic variation in a safflower germplasm grown in rainfed cold drylands. *Journal of Agronomy* 2006; 5(1): 50-52.
36. Yang YX, Wu W, Zheng YL, Huang CY, Liu RJ, et al. Correlation and path analysis on characters related to flower yield per plant of *Carthamus tinctorius*. *Chinese Traditional and Herbal Drugs* 2006; 37(1): 105-111.
37. Guan LL, Wu W, Zheng YL, Wang T. Correlation analysis between photosynthesis and yield of the leaves at different positions of safflower. *Acta Agronomica Sinica* 2007; 33(8):1352-1359.
38. Abd El-Latif EA. Evaluation of 25 safflower genotypes for seed and oil yields under arid environment in Upper Egypt. *Asian J Crop Sci* 2012; 4(2): 72-79.
39. Jianguo Y, Zhong JY, Xu Y. The research on the germplasm resources of safflower with different contents of fatty acids. Beijing: Third International Safflower Conference, 1993:14-18.
40. Arslan B. The determination of oil content and fatty acid compositions of domestic and exotic safflower (*Carthamus tinctorius* L.) genotypes and their interactions. *Journal of Agronomy* 2007; 6(3): 415-420.
41. Arslan B. Assessing of heritability and variance components of yield and some agronomic traits of different safflower (*Carthamus tinctorius* L.) cultivars. *Asian J Plant Sci* 2007; 6(3): 554-557.
42. Akbar AA, Kamran M. Relationship among yield components and selection criteria for yield improvement in safflower *Carthamus tinctorius* L. *Journal of Applied Sciences* 2006;6(13): 2853-2855.
43. Abdulahi A, Mohammadi R, Pourdad SS. Evaluation of safflower (*Carthamus* spp.) genotypes in multi-environment trials by nonparametric methods. *Asian J Plant Sci* 2007; 6(5): 827-832.
44. Mozaffari K, Asadi AA. Relationships among traits using correlation, principal components and path analysis in safflower mutants sown in irrigated and drought stress condition. *Asian J Plant Sci* 2006; 5(6): 977-983.
45. Oad FC, Samo MA, Oayyum SM, Oad NL. Inter and intra row spacing effect on the growth, seed yield and oil content of safflower *Carthamus tinctorius* L. *Asian J Plant Sci* 2002; 1(1): 18-19.
46. Nikabadi S, Soleimani A, Dehdashti SM, Yazdanibakhsh M. Effect of sowing dates on yield and yield components of spring safflower (*Carthamus tinctorius* L.) in Isfahan Region. *Pakistan Journal of Biological Sciences* 2008; 11(5): 1953-1956.
47. Liu LJ, Leung KH, Chan SH, Dik-Lung MA, Leung CH. Identification of a natural product-like STAT3 dimerization inhibitor by structure-based virtual screening. *Cell Death Dis* 2014; 5(6):96-104.
48. Chan DSH, Lee HM, Yang F, Che CM, Wong CCL, et al. Structure-Based Discovery of Natural-Product-like TNF- $\alpha$  Inhibitors. *Angew Chem Int Edit* 2010; 122(16): 2922-2926.
49. Leung CH, Chan DS, Yang H, Abagyan R, Lee SM, Zhu GY, Fong WF, Ma DL. A natural product-like inhibitor of NEDD8-activating enzyme. *Chem Commun* 2011; 47(9): 2511-2513.
50. Ma DL, Chan DS, Wei G, Zhong HJ, Yang H, Leung LT, Gullen EA, Chiu P, Cheng YC, Leung CH. Virtual screening and optimization of Type II inhibitors of JAK2 from a natural product library. *Chem Commun* 2014; 50(90): 13885-13888.