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Genetic Study of Several Seed Oil Quality Traits in Safflower

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One of the major advantages of safflower is its diversity in seed oil quality types, with contrasting fatty acid profiles ranging from about 90% linolenic acid to 90% oleic acid. Additionally, a novel seed oil type consisting of a tocopherol profile made up of 90% gamma-tocopherol instead of the standard profile of 90% alpha-tocopherol has been developed. This opens up the possibility of developing novel combinations of fatty acid and tocopherol profiles, which will result in a greater value of safflower oil for food, feed, and industrial applications. The objective of the present research was to study the inheritance of the three most relevant seed oil quality traits in safflower, i.e. high oleic acid content (>85% of the total fatty acids), high linoleic acid content (>85% of the total fatty acids), and high gamma-tocopherol content (>90% of the total tocopherols). Plants with either high oleic acid, high linoleic acid, or high gamma-tocopherol content were crossed with plants of a safflower line with standard fatty acid and tocopherol profile. The F_1, F_2, and BC_1F_1 or F_3 seed generations were analysed by the half-seed technique using gas-liquid chromatography (fatty acids) of high-performance liquid chromatography (tocopherols). In all cases, the traits were found to be controlled by single genes, which will facilitate their transference to other genetic backgrounds as well as the production of recombinants with specific combinations of fatty acid and tocopherol profiles. The results obtained are discussed in the light of future breeding strategies for developing novel safflower oil types with improved nutritional and technological properties.

Introduction

Traditionally, the concept of oil quality has been almost exclusively associated with the fatty acid composition of the oil. Recently, however, this concept has evolved and oil chemists and nutritionists are emphasizing other components of vegetable oils that influence their physical and chemical properties (Velasco and Fernández-Martínez, 2002). One of the most relevant components influencing oil quality are the tocopherols.

Tocopherols are oil soluble compounds present in oilseeds that exert an antioxidant action both *in vivo* (vitamin E activity) and *in vitro* (Kamal-Eldin and Appelqvist 1996). The tocopherols occur as a family of four derivatives named alpha-, beta-, gamma-, and delta-tocopherol, differing in their relative *in vitro* and *in vivo* antioxidant activities. Alpha-tocopherol is the most efficient antioxidant *in vivo*, but it shows a weak antioxidant potency *in vitro*. Conversely, gamma-tocopherol is the most powerful antioxidant *in vitro* but its *in vivo* activity is low. Beta- and delta-tocopherol exhibit intermediate properties (Pongracz et al. 1995). Alpha-tocopherol is the main tocopherol in safflower seeds, accounting for more than 95% of the total tocopherols (Johnson et al. 1999).

Safflower germplasm encompasses great variability for the fatty acid composition of the seed oil (Knowles, 1989). Standard safflower oil contains about 6 to 8% palmitic acid, 2 to 3% stearic acid, 16 to 20% oleic acid, and 71 to 75% linoleic acid. Variants with increased stearic acid content (4% to 11% of the total fatty acids), intermediate oleic acid content (41 to 53%), high oleic acid content (75 to 80%), and very high linoleic acid content (87 to 89%) have been identified and are currently available in released materials (Fernández-Martínez et al., 1993; Johnson et al., 1999). Additionally, sources of variation for high palmitic acid content (>10%) and very high oleic acid content (>85%) have been reported (Fernández-Martínez et al., 1993; Dajue et al., 1993).

Unlike fatty acids, very little variability for tocopherol profile has been found in safflower germplasm. Johnson et al. (1999) found practically no variability for tocopherol profile, whereas Velasco and Fernández-Martínez (2001) only identified small variation for increased gamma-tocopherol content (up to 10% of the total tocopherols). Nonetheless, we have recently identified a natural mutant of *C. oxyacanthus* which produced seeds with high gamma-tocopherol content (>90%) instead of the standard high alpha-tocopherol content

(>90%). As the mutant showed introgression of *C. tinctorius*, simultaneous selection for high gamma-tocopherol content and morphological traits produced a high gamma-tocopherol safflower line designated IASC-1 (Velasco et al., 2005).

The availability of the high gamma-tocopherol trait in safflower opens up the possibility of developing novel combinations of fatty acid and tocopherol profiles, which will produce new types of safflower oil with improved technological properties in comparison with current safflower oil types. The objective of the present research was to study the inheritance of the three most relevant seed oil quality traits in safflower, i.e. high oleic acid content (>85% of the total fatty acids), high linoleic acid content (>85% of the total fatty acids), and high gamma-tocopherol content (>90% of the total tocopherols).

Materials and Methods

The line CR-9, with high oleic acid content (>85% of the total seed oil fatty acids), was selected from the germplasm accession PI 401479 of the USDA-ARS germplasm collection (Velasco and Fernández-Martínez, 2000). The line CR-142, with high linoleic acid content (>85% of the total seed oil fatty acids), was developed from the germplasm accession PI 613459 of the USDA-ARS germplasm collection (Velasco and Fernández-Martínez, 2000). The line IASC-1, with high gamma-tocopherol content (>90% of the total seed tocopherols), was isolated from the germplasm accession PI 426425 of the USDA-ARS germplasm collection (Velasco et al., 2005). PI 560162 is a nuclear male sterile (NMS) line with a standard fatty acid profile (around 20% oleic acid and 70% linoleic acid) and a standard tocopherol profile (>90% alpha-tocopherol).

Half seeds of CR-9, CR-142, IASC-1, and PI 560162 were analysed for fatty acid (CR-9, CR-142, PI 560162) or tocopherol profile (IASC-1, PI 560162) to ensure that the plants used in the genetic studies bred true for the corresponding trait under study. NMS plants of PI 560162 were crossed with plants of CR-9, CR-142, and IASC-1. The F_1 and F_2 seed generations were analysed for fatty acid or tocopherol profile. Additionally, the BC_1F_1 to NMS seed generation was analysed for the crosses NMS x CR-9 and NMS x CR-142, whereas the F_3 seed generation was analysed for the cross NMS x IASC-1. Analysis of fatty acid and tocopherol profiles in half seeds are described in Velasco and Fernández-Martínez (2001).

Results

High oleic acid content

Seeds of the high oleic acid line CR-9 averaged 86.4% oleic acid, ranging from 84.1% to 88.0%. Seeds from the NMS line PI 560162 averaged 19.5% oleic acid, showing a range of variation from 11.7% to 29.5%. F_1 seeds from the cross NMS x CR-9 averaged 35.2%, with a range of variation between 25.2% and 55.1% (Fig. 1). The analysis of the F_2 revealed a bimodal distribution in which about one fourth of the seeds had the high oleic acid phenotype (Fig. 1), suggesting that the high oleic acid trait is determined by partially recessive alleles at a single locus. Monogenic inheritance was confirmed in the analysis of the BC_1F_1 to NMS generation, which showed the expected 1:1 segregation ratio (Fig. 1).

Knowles and Hill (1964) studied the inheritance of a safflower line with high oleic acid content (around 80%), concluding that the trait was controlled by the recessive allele *ol*. In order to preliminary determine whether CR-9 also carries the *ol* allele, plants of the cultivar 'Oleic Leed', with genotype *olol* (Urie et al., 1979), were grown under the same environment as CR-9 plants. Seeds from Oleic Leed averaged 82.2% oleic acid, with a range of variation between 79.1% and 84.0%, compared to an average of 86.4% and a range of variation from 84.1% to 88.0% for CR-9 seeds. Such differences suggested that both lines might show genetic differences for high oleic acid content. Accordingly, a comparative genetic study between both lines is being started.

High linoleic acid content

Seeds of the high linoleic acid line CR-142 averaged 88.0% linoleic acid, ranging from 85.0% to 91.0%. Seeds from the NMS line PI 560162 averaged 71.1% linoleic acid, showing a range of variation from 61.0% to 78.7%. F_1 seeds from the cross NMS x CR-142 averaged 69.6%, with a range of variation between 63.8% and 78.7% (Fig. 2). The analysis of the F_2 and BC_1F_1 to NMS seed generations did not allow the identification of discrete phenotypic classes for linoleic acid content (Fig. 2). Nevertheless, the discontinuity observed in the F_2 around 85% linolenic acid, which is the lower limit for the high linoleic acid parent class, suggested monogenic inheritance for this trait. Both the F_3 and BC_1F_1 to CR-142 seed generations are being obtained in order to confirm this.

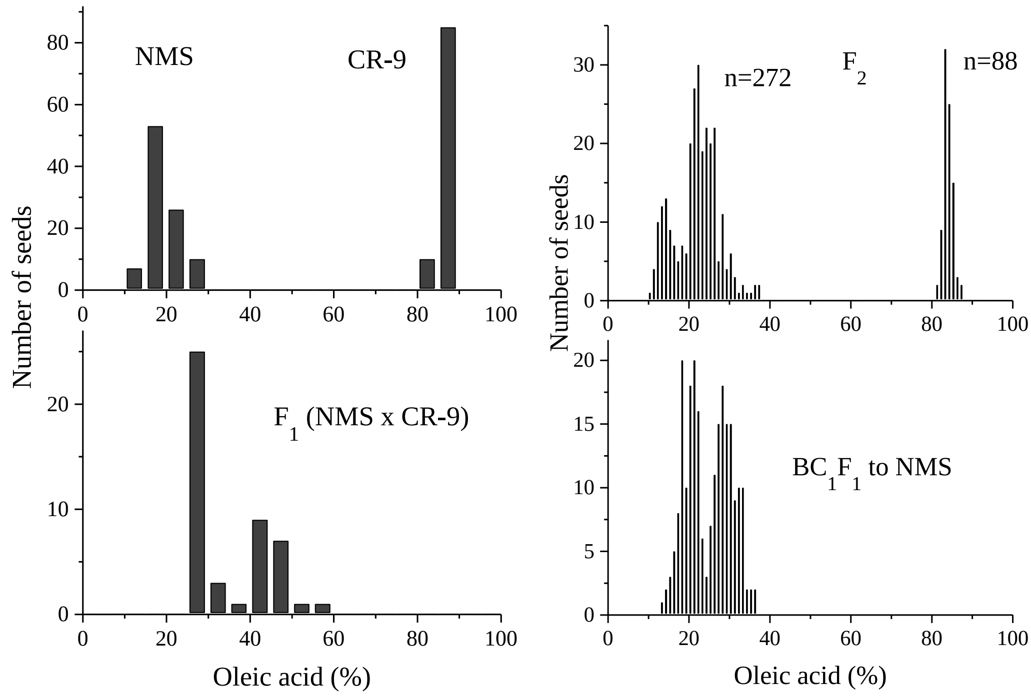

Fig. 1. Histograms of oleic acid content in seeds of the high oleic acid line CR-9, the NMS line PI 560162, and their F1, F2, and BC1F1 to NMS seed generations.

A high linoleic acid content between 85% and 90% was first observed by Futehally and Knowles (1981) in an introduction from Portugal. The authors found that the high levels of linoleic acid were controlled by a recessive alleles *li* at a single locus.

High gamma-tocopherol content

Crosses between NMS plants with standard low gamma-tocopherol content (<1%) and plants of IASC-1 with high gamma-tocopherol content (>85%) produced F_1 seeds with a gamma-tocopherol content ranging from zero to 7.8%. Gamma-tocopherol content of F_2 seeds followed a bimodal distribution, with three fourths of the seeds having a gamma-tocopherol content between zero and 8.5% and one fourth of the seeds having a high gamma-tocopherol content above 75.7% (Fig. 3), suggesting that the trait is controlled by partially recessive alleles at a single locus. The analysis of 74 $F_{2:3}$ families revealed that 19 families had a low gamma-tocopherol content, 39 families segregated for the trait, and 16 families had a uniformly high gamma-tocopherol content, which fit satisfactorily the expected 1:2:1 ratio for the segregation of a single recessive gene.

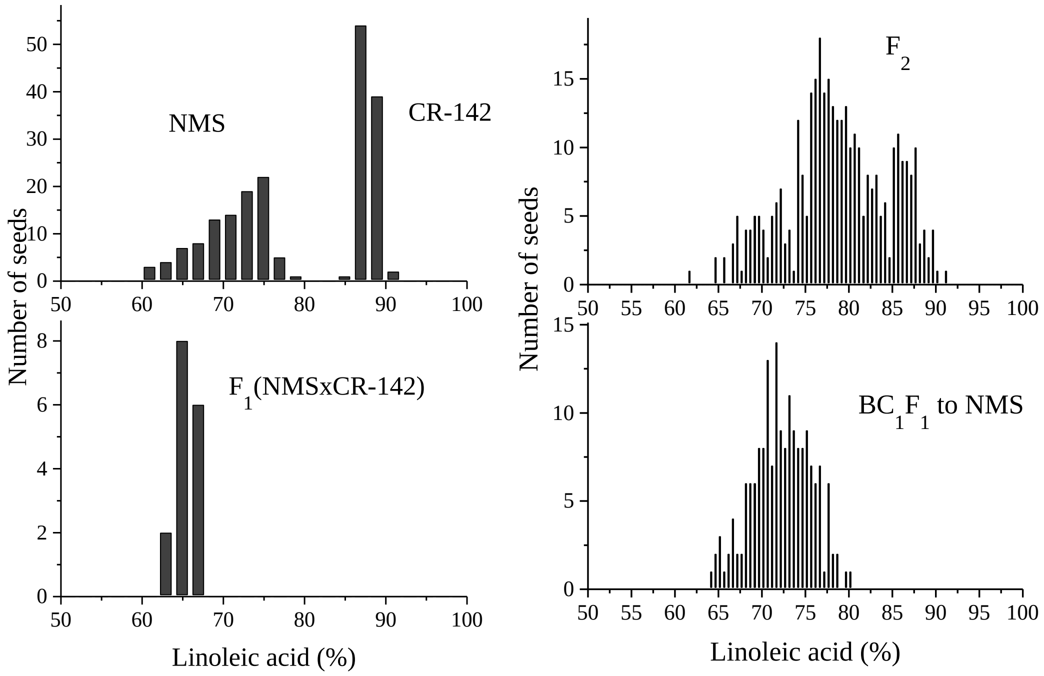

Fig. 2. Histograms of linoleic acid content in seeds of the high linoleic acid line CR-142, the NMS line PI 560162, and their F1, F2, and BC1F1 to NMS seed generations.

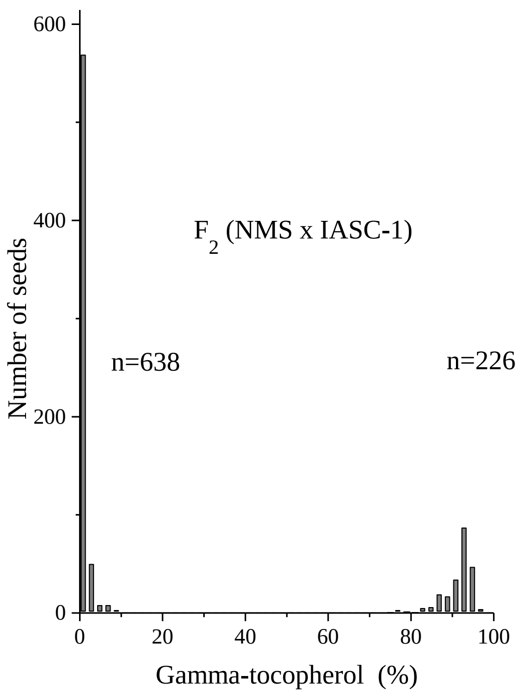

Fig. 3. Histogram of gamma-tocopherol content in F_2 seeds from the cross between the high gamma-tocopherol line IASC-1 and the NMS line PI 560162, with standard low gamma-tocopherol content.

Discussion

Current safflower varieties and hybrids are divided into two groups according to their fatty acid profiles: standard or high linoleic acid and high oleic acid. The high oleic acid type provides an oil of great value for applications demanding great oxidative stability, such as deep frying or manufacturing of biolubricants, and it is also an optimal oil for nutritive applications (Velasco and Fernández-Martínez, 2002). Even though there are other sources of high oleic acid oil, e.g. sunflower or canola, safflower possesses the main advantage of maintaining a high oleic acid content in the oil produced at a commercial scale without need for isolation, because of a lower rate of cross pollination than sunflower and canola. The high linoleic acid type provides a unique oil type that can not be obtained in any other commercial crop. High linoleic acid oil has important advantages from a nutritional point of view and it is also used for producing conjugated linoleic acid (a novel therapeutic nutrient), both *in vivo* in livestock fed with safflower (Bergman et al., 2001) as well as *in vitro* (Ma et al., 1999).

Previous studies in sunflower (*Helianthus annuus* L.) have demonstrated that the replacement of alpha-tocopherol by gamma-tocopherol, both in high oleic and high linoleic acid types, is associated with a considerable improvement of the technological properties of the oils (Demurin et al., 1996). After the development of the high gamma-tocopherol line IASC-1, two novel safflower oils, high oleic acid/high gamma-tocopherol and high linoleic acid/high gamma-tocopherol, can be produced. Genetic recombination of fatty acid and tocopherol traits will be facilitated by their monogenic inheritance. Such novel oils will probably exhibit different technological properties to their corresponding alpha-tocopherol types, which will open up new market niches for safflower oil.

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