



Inheritance of very high oleic acid content and its relationship with several morphological and physiological traits

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Abstract

Safflower oil with high oleic acid content has a great value for both food and non-food uses. The trait is mainly controlled by recessive alleles at the *O*/ locus, although the influence of modifying genes has been suggested. The objective of the present research was to study the inheritance of very high oleic acid content (>85%) and its relationship with other traits such as nuclear male sterility (NMS), flowering time, presence of spines, and corolla colour. The line CR-9, characterized by very high oleic acid content, male fertility, early flowering, absence of spines, and orange corolla, was crossed to the line CL2, characterized by conventional fatty acid profile, NMS, late flowering, presence of spines, and white corolla. The analysis of the F₂ seed and plant (F₃ seeds averaged) generation confirmed monogenic inheritance of very high oleic acid content, although the presence of modifying genes produced a reduction of oleic acid content in the high oleic acid class to minimum values of 81%, 74%, and 68% at the F₂ seed, F₂ plant, and F₃ seed level, respectively. NMS and white corolla were recessive and controlled by a single gene. The presence of spines was monogenic and dominant. Oleic acid content segregated independently from the other traits evaluated. The results confirmed the great effect of modifying genes on the expression of very high oleic acid content and revealed that the *O*/ locus is not closely linked to genes involved in the expression of the traits evaluated.

Key words: Corolla colour - flowering time - nuclear male sterility - oleic acid - spines

Introduction

Safflower oil has been traditionally characterised by a high polyunsaturation level, with linoleic acid representing more than 70% of the total fatty acids (Knowles, 1989). However, safflower germplasm producing seed oil with high oleic acid content has been identified. High oleic acid safflower was initially reported as having oleic acid content between 64 and 83% of the total fatty acids, compared to 10 to 15% in conventional safflower, which was produced by partially recessive alleles *o*/ at a single locus (Knowles and Hill, 1964). Another different allele *o*₁ at the same locus produced in homozygous condition between 35 and 50% oleic acid (Knowles and Hill, 1964). An additional genetic source of high oleic acid content (86 to 91%) in safflower was reported by Fernández-Martínez et al. (1993) in the germplasm accession PI 401472. The very high oleic acid content of this germplasm accession was observed in one environment under greenhouse conditions, where seeds of PI 401472 had around 10% more oleic acid than the cultivar UC-1, which carries the *o*/ alleles described by Knowles and Hill (1964). Apart from the major *O*/ locus, Knowles (1972) postulated the existence of modifying genes affecting high oleic acid content in safflower.

The objective of the present research was to study the inheritance of very high oleic acid content and its relationship with other traits such as nuclear male sterility (NMS), flowering time, presence of spines, and corolla colour.

Materials and Methods

The line CR-9 was developed from the USDA germplasm accession PI 401479, collected in Bangladesh within the same area as the accession PI 401472 (www.ars-grin.gov/npgs/) reported by Fernández-Martínez et al. (1993). CR-9 is characterized by very high oleic acid content, male fertility, early flowering, absence of spines, and orange corolla. CL2 is a nuclear male-sterile (NMS) line with conventional fatty acid profile, isolated from the USDA-ARS



germplasm accessions PI 560162, which in turn derived from the germplasm line UC-149 (Heaton and Knowles, 1980). CL2 is also characterized by late flowering, presence of spines, and white corolla.

Twenty-four half seeds of CL2 and CR-9 were analysed for seed oil fatty acid profile. The seeds were germinated and, after fifteen days in a growth chamber, the plants were transplanted to the field in January 2003. Before flowering, heads of all plants were covered with paper bags to ensure self-fertilization. Male-sterile heads of CL2 plants were pollinated with fresh pollen from CR-9 plants. F₁ seeds as well as seeds of the parents were analysed for fatty acid composition and the corresponding plants were grown in the field in 2004. F₁ plants were self-pollinated to obtain F₂ seeds, which were analysed for fatty acid composition. One population of 88 F₂ plants was grown in the field in 2005 to evaluate segregation for NMS, presence/absence of spines, corolla colour, days to flowering, and F₃ fatty acid profile. F₃ seeds from the male fertile F₂ plants were sown in the field in 2008 for evaluation of F₃ plants for the abovementioned traits.

The fatty acid composition of the seed oil of individual seeds was analyzed by simultaneous extraction and methylation (Garcés and Mancha, 1993) followed by gas-liquid chromatography (GLC) using a Perkin-Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA). A 2-m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Inc., Bellefonte, PA, USA) was used. The oven, injector and flame ionization detector were held at 190, 275 and 250°C, respectively.

Results and Discussion

Seeds of CL2 plants averaged $16.92 \pm 3.98\%$ (mean \pm standard deviation) oleic acid. Seeds of CR-9 plants averaged $86.36 \pm 0.85\%$. F₁ seeds from the cross CL2 x CR-9 averaged $23.74 \pm 2.70\%$ oleic acid, which was significantly higher than the average oleic acid content of CL2 ($t=9.54$, $p<0.01$), indicating partial dominance of standard over high oleic acid content. This is in agreement with a previous study of high oleic acid inheritance in safflower (Knowles and Hill, 1964). The analysis of the F₂ seed generation revealed two well defined classes for oleic acid content (Fig. 1), from 11.21 to 29.55% ($n=145$), and from 81.14 to 86.70% ($n=44$), which fitted a 3:1 ratio ($\chi^2=0.30$, $p=0.58$) corresponding to the segregation of one partially recessive gene, as previously reported by Knowles and Hill (1964).

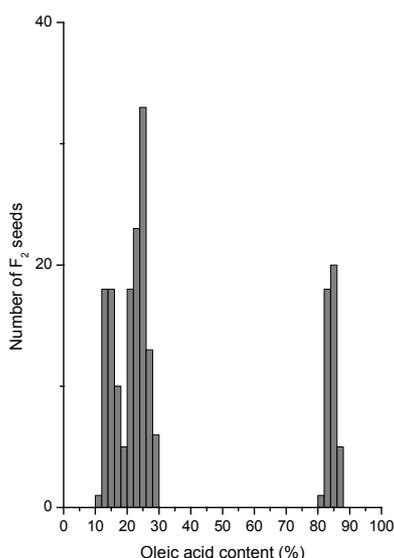


Fig. 1. Oleic acid content in F₂ seeds from the cross between the line CL2, with standard oleic acid content and CR-9, with very high oleic acid content.



Table 1 shows the segregation observed for NMS, presence/absence of spines, corolla colour, and days to flowering in the field grown F₂ populations.

Table 1. Nuclear male sterility (NMS), presence/absence of spines, corolla colour, and days to flowering (DTF) in a population of 88 F₂ plants from the cross between the safflower lines CL2 and CR-9 and in the subpopulations derived from low and high oleic acid F₂ seeds.

F ₂ oleic acid	NMS		χ ² (p)	Spines		χ ² (p)	Corolla colour		χ ² (p)	DTF
	No	Yes	3:1	Yes	No	3:1	Orange	White	3:1	
Total	60	28	2.18(0.14)	71	17	1.52(0.22)	61	27	1.52(0.22)	149.4
Low	47	23	2.30(0.13)	54	16	0.17(0.68)	50	20	0.48(0.49)	149.5
High	13	5	0.07(0.79)	17	1	3.63(0.06)	11	7	1.85(0.17)	149.2

Nuclear male sterility was controlled by a recessive gene, as previously reported by Knowles and Hill (1964). The presence of spines in this cross followed monogenic inheritance, with the presence of spines being dominant over their absence. These results are in agreement with previous reports of Deshmukh (1958) and Pahlavani et al. (2004). White corolla was recessive and controlled by a single gene, as previously reported by Joglekar and Deshmukh (1956). The observed segregation ratios in the overall population were also observed in the subpopulations derived from low and high oleic acid F₂ seeds, suggesting that genes controlling NMS, absence of spines, and white corolla are not closely linked to the *O*/locus. Similarly, days to flowering did not differ significantly in both subpopulations ($t=0.42$; $p>0.05$), indicating no close relationship between the early flowering trait and oleic acid content.

The F₃ generation from male fertile F₂ plants was phenotypically evaluated to confirm monogenic inheritance of the traits under study and to differentiate homozygotes carrying the dominant alleles from heterozygotes for future mapping of the traits through a bulked segregant analysis strategy. From the 60 male fertile F₂ plants, 31 to 33 F₂ families could be evaluated at the F₃ plant level. For NMS, 10 families showed male fertility in all the plants and 21 families segregated for the trait, which fitted the expected 1:2 (*MsMs:Msms*) ratio ($\chi^2=0.02$, $p=0.89$). Eleven families had all the plants with spines, 17 families segregated for the trait and 5 families were uniformly spineless, fitting the expected 1:2:1 ratio for a recessive gene ($\chi^2=2.20$, $p=0.33$). For corolla colour, 5 families had uniformly orange flowers, 15 families showed segregation, and 13 families had uniformly white flowers, which also fitted the expected 1:2:1 ratio ($\chi^2=4.15$, $p=0.13$).

The analysis of oleic acid content at the F₃ seed level revealed that 12 F_{2.3} families had uniformly low oleic acid content, 26 F_{2.3} families segregated for high oleic acid content, and 11 F_{2.3} families had uniformly high oleic acid content (Fig. 2A), which fitted the expected 1:2:1 (*O/Ol:Olo:olo*) ratio ($\chi^2=0.22$, $p=0.90$). However, it is noteworthy that some F₂ plants with expected *olo* genotype had a lower average oleic acid content than CR-9 plants grown in the same environment (86.2 to 88.1%), with a minimum average oleic acid content of 73.8% (Fig. 2A). Such a reduction in oleic acid content was more pronounced at the F₃ seed level. Thus, oleic acid content in F₃ seeds with high oleic acid phenotype ranged from 67.8 to 87.6%, compared to 84.5 to 88.7% in seeds of CR-9 plants grown in the same environment (Fig. 2B). Our interpretation of this observation discarded environmental effects, as the line CR-9 was stable for oleic acid content over several environments (data not shown), but focused on the possible presence of modifying genes affecting oleic acid content.

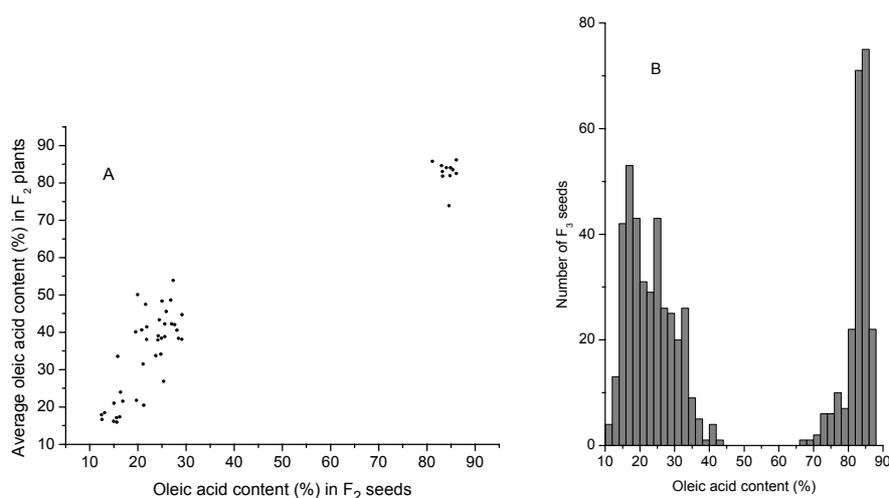


Fig. 2. (A) Oleic acid content in F₂ seeds and F₂ plants (F₃ seeds averaged) in 49 F_{2:3} families from the cross between the line CL2, with standard oleic acid content and CR-9, with very high oleic acid content. (B) Oleic acid content in the overall population of F₃ seeds.

The existence of modifying genes affecting oleic acid content has been previously suggested in safflower (Knowles, 1972) and widely demonstrated in sunflower (Urie, 1985; Pérez-Vich et al., 2002). Modifying genes were most likely present in the NMS line CL2, and produced a reduction in oleic acid content below the levels observed in the high oleic acid line CR-9. Since high oleic acid content is gaining importance in safflower oil for food and non-food uses, the occurrence of modifying genes with a negative phenotypic effect on this trait is an important factor to take into account to ensure stable expression of high oleic acid content in this crop. From the existing experience in sunflower (Pérez-Vich et al., 2002), further characterization of modifying genes and development of molecular markers linked to them will be crucial to facilitate their management in safflower breeding.

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