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INHERITANCE OF OLEIC ACID CONTENT OF F_1 SEED IN A COMPLETE DIALLEL CROSS BETWEEN SEVEN SUNFLOWER LINES

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SUMMARY

A diallel crossing design was carried out during 1999 and 2000 seasons near Montpellier under field nursery conditions. Intercrosses of seven sunflower lines were performed between genetic (ms2) or cytoplasmic male sterile (PET1) and standard versions using bagged heads. These lines contrasted for oleic acid content in oil. Four lines with high oleic acid content originated from Pervenets. Three linoleic inbred lines were added to the crossing design. Male fertile lines were selfed in the same conditions. F_1 seeds and those from selfed inbred lines were analyzed for fatty acid composition using gas chromatography. Year effect, reciprocal or maternal effects, general combining ability and specific combining ability effects were found to be significant. The level of dominance of high oleic acid content appeared related to the genetic background. We found previously that 83HR4, a linoleic line of the diallel, was bearing a suppressor allele canceling Pervenets mutation effect. In reciprocal crosses with high oleic lines, this line had a strong maternal negative effect on oleic acid content.

Key words: sunflower, fatty acids, oleic acid, diallel cross

INTRODUCTION

Mutation breeding and recurrent selection for increased oleic acid content by Soldatov (1976) followed by classical breeding have yielded impressive results and commercial hybrids averaging 80% oleic content. Xenia, i.e., alien pollen effect, is commonly observed on seed oleic acid content and isolation zones of high oleic sunflower hybrids are to be organized in order to maintain high oleic standards. Our recent studies showed that all high oleic materials studied and derived from Pervenets do not accumulate Δ-12 desaturase transcript in seeds (Kabbaj *et al*., 1996;

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Table 1: Summary of the genetic studies dealing with the oleic acid content trait in sunflower

Dominant/recessive/ incomplete dominance	Maternal effect	Number of major (M) gene with or without modifiers	References
Incomplete dominance	Not checked	1M	Fick, 1984
Dominant	No effect	1M + modifiers	Urie, 1985
Incomplete dominance	No effect	1M + modifiers	Miller *et al.,* 1987
Dominant	Not checked	1M	Schmidt *et al.*, 1989
Dominant	Not checked	3M additive + modifiers	Fernandez-Martinez *et al.*, 1989
Dominant and incomplete dominance	No effect	No conclusion	Nikolova *et al*. 1991
Dominant but also sometimes recessive	Not checked	3 hypotheses with increasing gene numbers	Demurin and Škorić, 1996
Dominant but also sometimes recessive	Not checked	1M + modifiers not clear	Dehmer and Friedt, 1998
Incomplete dominance	Not checked	2 interacting M	Fernandez *et al.*, 1999
Dominant and incomplete dominance	Maternal effect	Not addressed	Varès *et al.*, 2000
Dominant	Not checked	Major QTL (85% EV)	Perez-Vich *et al.*, 2000
Dominant	Not checked	1 M locus = Δ-12-RFLP	Lacombe *et al.* 2000
Dominant	No effect	Not addressed	Lacombe and Bervillé, 2000b
Complex some may be dominant	No effect	5 M + modifiers	Velasco *et al*., 2000
Dominant and incomplete dominance	No effect	1 Major locus with 3 alleles	Demurin *et al*., 2000
Dominant	Not checked	1 Major locus	Lacombe and Bervillé 2001
Dominant	Not checked	2 M (1 epistatic suppressor)	Lacombe *et al.*, 2001

Table 2: Oleic acid content means of self-pollinated parents and F_1 combinations

Female	Male						
	Linoleic, FT2603	Linoleic, HA89	Linoleic, 83HR4	High oleic, HA342	High oleic, HAOL9	High oleic, OA	High oleic, OC
Linoleic, FT2603	36.34	38.53	46.22	77.81	65.73	49.72	69.81
Linoleic, HA89	38.60	37.29	40.52	76.95	78.16	40.77	48.92
Linoleic, 83HR4	32.07	33.51	35.21	78.75	63.70	41.23	52.30
High oleic, HA342	72.60	77.65	78.63	74.70	84.68	83.95	82.72
High oleic, HAOL9	62.42	74.62	71.94	86.37	85.06	75.64	84.89
High oleic, OA	66.82	49.07	49.28	82.84	83.06	79.96	85.76
High oleic, OC	55.84	49.13	69.69	84.10	83.94	82.95	84.24

Lacombe and Bervillé, 2000b). Moreover, it was characterized by a specific RFLP pattern revealed by a Δ-12 desaturase cDNA used as a probe. These studies indicated a rearrangement neighboring or containing a Δ-12 desaturase seed specific gene (Hongtrakul *et al.*, 1998; Lacombe *et al.*, 2002). However, conflicting conclusions were drawn from some classic and molecular genetic results with diverse genetic backgrounds. These results were reviewed by Lacombe and Bervillé (2000a) and updated in Table 1.

Temperature effects on oleic content were reviewed by Lagravère *et al.* (2000). High temperature enhances the oleic acid content of linoleic classical cultivars but conflicting results are reported about temperature effects on oleic acid content of high oleic acid cultivars.

In the present study the direct analysis of F_1 seed issued from a complete diallel cross design was used as a mode of investigation of the inheritance of oleic acid content in seed.

MATERIAL AND METHODS

Seven parental inbred lines were used. HA89 (USA-Canada), FT2603 (INRA-Montpellier) and 83HR4 (INRA-Montpellier) are standard linoleic lines. All high oleic lines have Pervenets in their pedigree, and the corresponding RFLP characteristic of high oleic content: OA and OC were obtained from INRA (Clermont-Ferrand), HAOL9 from Spain (Cordoba), HA342 from USDA-NDSU.

PET1 cytoplasmic male sterile isogenic counterparts of all lines were used as females for crossing except the restorer line 83HR4 for which a genetic ms2 isogenic version was used as female.

Crosses and self-pollinated lines were obtained by hand pollination under paper bags in field nursery conditions at Mauguio near Montpellier by July 1999 and July 2000. F_1 and selfed seeds were harvested at maturity and dried to 5% moisture.

Gas chromatography was used for determining fatty acid content in oil obtained from 10 F_1 seeds.

Forty-two reciprocal F_1 combinations and seven self-pollinated lines were studied each year.

We used Griffing (1956) complete 7*7 diallel fixed effects, model I type 1 (parent lines, F_1 and reciprocals) with 2 repetitions.

RESULTS AND DISCUSSION

Year effect on oleic acid content was found significant but small compared with huge genotype effect (Tables 2 and 3). For this reason we analyzed years as replications in the subsequent specific diallel design analysis.

Table 3: Analysis of variance of genotype and year effect for oleic content of self-pollinated parents and F_1 combinations

Source	Degrees of freedom	Sum of squares	Mean square	F	Probability
Year	1	260.9	260.9	7.62	0.007598
Genotype	48	39221.3	817.1	23.85	0.000000
Error	62	2124.4	34.3		
Total	111	41606.6			

By using the Griffing diallel analysis, we found very highly significant general combining ability effects (P<0.001), very highly significant specific combining ability effects (P<0.001), and highly significant reciprocal effects (P<0.01), (Table 4).

Table 4: Griffing 7*7 diallel analysis model I method 1 for oleic acid content

Source	DF	Sum of squares	Mean square	F	Probability	Variance component
General combining ability	6	12965.95	2160.99	252.01	0.00	153.74
Specific combining ability	21	2405.81	114.56	13.36	0.00	60.39
Reciprocal effects	21	680.68	32.41	3.78	<0.01	11.92
Error	62	531.65	8.58			8.58

When we used a nested model of variance analysis in relation with the number of Pervenets alleles, we found very highly significant year effect (P<0.001), very highly significant (P<0.001) Pervenets effect (three classes homozygous normal, homozygous Pervenets, heterozygous Pervenets / normal) and very highly significant genotype nested in Pervenets effect (P<0.001).

We attempted to test the diallel data excluding the major effect of Pervenets mutation allele number. Each oleic acid content value was adjusted to the genotype for these classes. This was done in accordance with the analysis of diallel data means in function of categorical rearrangement situation (Table 5). Reciprocal effects were found significant (P<0.05), general combining ability effects and specific combining ability effects were found to be highly significant (P<0.01).

Table 5: Griffing 7*7 diallel analysis model I method 2 for oleic acid content with modified data (without Δ–12 desaturase Pervenets rearrangement effect)

Source	DF	Sum of squares	Mean square	F	Probability	Variance component
General combining ability	6	1189.74	198.29	11.56	<0.01	12.94
Specific combining ability	21	2286.28	108.87	6.35	<0.01	52.26
Reciprocal effects	21	680.68	32.41	1.89	<0.05	7.63
Error	62	1063.30	17.15			17.15

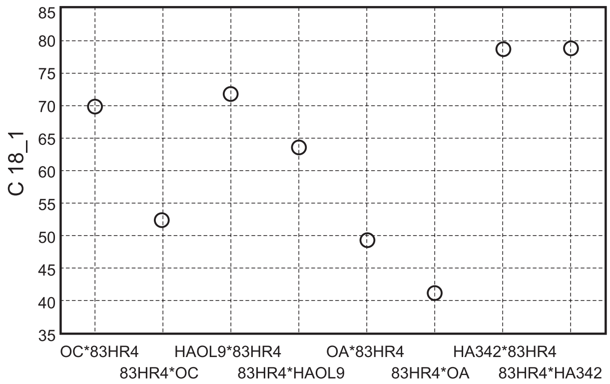

Figure 1: Reciprocal effects between 83HR4 parent and high oleic parents

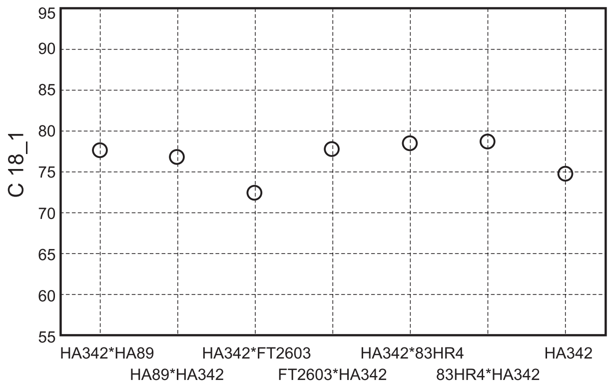

Figure 2: A case of dominant inheritance, crosses between HA342 and linoleic lines

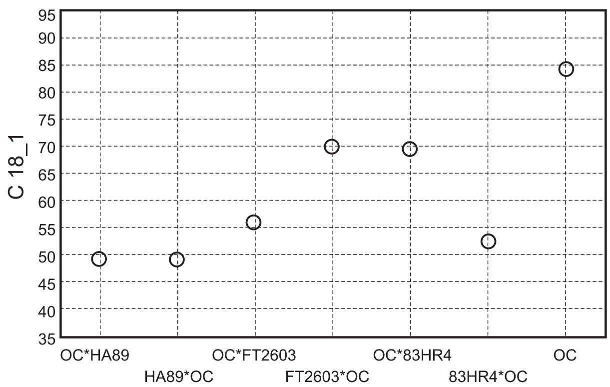

Figure 3: A case of intermediate inheritance, hybrids between OC and linoleic lines

Reciprocal effects on oleic acid content were clearly evidenced in this study and in our preliminary study based on data collected in 1999 (Varčs *et al.*, 2000). These were spectacular for crosses between linoleic 83HR4 and oleic lines (Figure 1 and Table 2). In a preceding experiment on recombinant inbred lines of a cross involving 83HR4, we found in this line an allele of suppression for Pervenets mutation effect at another locus (Lacombe *et al.*, 2001). We tried to build a physiological genetic model in order to explain this maternal negative effect on oleic content in relation to the suppressor allele effect.

Various levels of dominance were reported in different publications (Table 1). The level of dominance of high oleic appeared to be fluctuating between hybrid genotypes in the present study. Dominance may depend on genetic background since hybrids between high oleic HA342 and linoleic lines appeared high oleic (Figure 2 and Table 2), and hybrids between high oleic OC and linoleic lines appeared intermediate (Figure 3 and Table 2).

Another conclusion of this work is that some genetic variation for oleic acid content appeared in interaction with Pervenets mutation. This variation could be of interest for breeders who may find unexpected segregation in their nurseries inconsistent with one dominant Pervenets allele model.

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HERENCIA DEL CONTENIDO DE ACIDO OLEICO EN LAS SEMILLAS F_1 DENTRO DEL DIALELO COMPLETO DE SIETE LINEAS DEL GIRASOL

RESUMEN

El cruce de dialelo fue realizado durante los periodos de vegetacion de 1999 y 2000 cerca de Monpellier, en las condiciones del plantio de campo. Las versiones geneticamente masculinas esteriles (ms2) y citoplasmicamente masculinas esteriles (PET1) fueron cruzadas con las versiones standard de siete lineas del girasol por la utilizacion del metodo de cubrir las cabezas con sacos de papel. Las lineas investigadas se difereciaban segun el contenido de acido oleico. Cuatro lineas tenian el alto contenido de acido oleico, que provenia de la variedad Pervenec. Despues, tres lineas consaguineas de tipo llinoleico fueron incluidas en el esquema de cruce. Las lineas masculinas esteriles fueron autofecundadas en las mismas condiciones. Las semillas F_1 y las semillas de las lineas consaguineas autofecundadas eran analizadas por la cromatografia de gas con respecto al contenido de acidos grasos. Se constataron las influencias importantes de ano, cruce reciproco I de la madre, y de la capacidad especifica de combinacion. Nivel de dominacion del alto contenido de acido oleico parecia ser ligado con la base genetica. Hemos constatado previamente que 83HR4, la linea con alto linoleo en el dialelo, contiene el alelo supresor que anula la influencia de mutacion de Pervenec. En los cruces reciprocos con las lineas con alta oleina, esta linea mostro el efecto negativo maternal potente sobre el contenido de acido oleico.

TRANSMISSION DU CONTENU EN ACIDE OLÉIQUE DANS UNE GRAINE F_1 DANS LE CADRE D'UN DIALLÈLE COMPLET DE SEPT LIGNES DE TOURNESOL

RÉSUMÉ

Un croisement diallèle a été effectué pendant les saisons de végétation de 1999 et de 2000 dans des champs près de Montpellier. Les croisements de sept lignes de tournesol ont été effectués entre des versions génétiques (ms2) ou cytoplasmiques stériles mâles (PET1) et des versions standard au moyen de la méthode de sacs couvrant les têtes. Les lignes étudiées ont montré des différences de contenu en acide oléique. Quatre lignes ayant un niveau élevé d'acide oléique provenaient de la sorte Pervenets. Trois lignes inbred linoléiques ont alors été ajoutées au schéma de croisement. Les lignes mâles stériles ont été autofécondées dans les mêmes conditions. Le contenu en acides gras des graines F_1 et des lignes inbred autofécondées a été analysé par chromatographie en atmosphère gazeuse. L'influence de l'année, les influences réciproques ou maternelles, les aptitudes combinatoires générales et spécifiques se sont montré significatives. Le niveau de dominance en acide oléique paraît relié à la base génétique. Nous avions constaté antérieurement que 83HR4, une ligne linoléique du diallèle portait un allèle de suppression annulant l'effet de mutation Pervenets. Dans les croisements réciproques de lignes à niveau oléique élevé, cette ligne a montré un effet maternel négatif puissant sur le contenu en acide oléique.