BREEDING FOR HIGH CONTENT OF OLEIC ACID IN SUNFLOWER (*Helianthus annuus* L.) OIL

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INTRODUCTION

Sunflower oil is widely used in Spain and around the world. In Spain, where the area dedicated to this crop has surpassed one million hectares, the consumption of sunflower oil is similar to that of olive oil (Oleo, 1986). A great deal of this expansion has been due to the impact of the breeding work through the development of hybrids with increased seed yield and seed oil content. Less emphasis has been given to aspects of oil quality susceptible to be changed by genetic means as the case of fatty acid composition, main index to define the quality of vegetable oils. There are two main fatty acids, in sunflower oil, oleic (18:1) and linoleic (18:2), which together account for about 90 % of the total fatty acids, corresponding the remaining 10 % to palmitic (16:0) and stearic (18:0). In the commercial hybrids, oleic acid represents between 10 and 50 % under field conditions depending on environment, especially temperature during seed development, existing a strong inverse relationship with linoleic acid (Kinman and Earle, 1974; Fernández-Martinez et al., 1986).

As in other vegetable oils, the quality of sunflower oil is a relative concept which depends on the use of that oil (Fernández-Martinez and Alba, 1984). For example, for frying purposes, oils with a high degree of oxidative stability i.e. those with high content of oleic acid, such as olive oil, or hydrogenated vegetable oils, are prefered (Fuller et al., 1967). For soft margarine industry oils with higher level of unsaturation, higher levels of linoleic are prefered. Under the nutritional point of view researchers do not completely agree which type is more advisable although recent works reported that a diet rich in monounsaturated fatty acids reduced cholesterol in blood-plasma which is a risk factor for coronary heart disease (Greendy, 1986).

The high oleic acid sunflower variety Pervenets was developed in U.S.S.R. following a treatment of seed with dimethyl sulfate (Soldatov, 1976). Oleic acid levels in this variety averaged 75 % although individual plants ranged from 50 % to 80 % (Miller and Zimmerman, 1983) and, seed to seed variation was even higher ranging from 19 to 94 % (Urie, 1985). Selected breeding true high oleic progenies from this variety were very stable when grown under different temperature regimes with oil content of oleic higher than 83 % (Urie, 1985; Fernández-Martinez et al., 1988).

Several studies have been carried out to elucidate the inheritance of the high oleic content in germplasm derived from Pervenets. Urie (1985) concluded that the high oleic character was controlled by one single dominant gene OL with embryo genotype control. Miller et al. (1987) found a second gene modifying the oleic content of the OL gene. Fernández-Martinez et al. (1988) found segregations that indicated the presence of one, two and even three major complementary dominant genes controlling the character, depending on the genotype of the low oleic parent.

The variety Pervenets was introduced in our Department in 1980. We started a breeding programme with the aim of studying the inheritance of the high oleic trait and also incorporating it to known restorers and male sterile inbred lines in order to develop high oleic commercial hybrids.

The present paper describes the breeding programme used and the high oleic material obtained.

BREEDING MATERIAL

The original sunflower material used as high oleic source was derived from the open pollinated variety Pervenets. This material was grown in 1980 in the field at the experimental farm of the Agricultural Research Center of Córdoba (Spain). Some plants were selfed and other cross pollinated among them. Ten of these plants were analysed for fatty acid composition using bulk samples of 10 seeds. In plants with oleic acid content higher than 60 %, analyses of individual seeds were made using the half seed technique (Fernández-Martínez and Knowles, 1982) which allows growing the other half. Pollen of plants grown from seeds with levels of oleic acid higher than 85 % were used to hand pollinate emasculated capitula of RHA-274, a low oleic selfcompatible restorer line. F_1 seeds were analysed using again the half seed technique and those plants with oleic acid content higher than 85 % were selected and grown. After three generations of selfing and selection for high oleic, breeding true lines for high content of this acid were identified. One of these lines, quite self compatible and with oleic acid levels higher than 90 %, was identified as AOP-1 and used as donor parent of this character in a backcross programme to incorporate it into commercial material. The low oleic materials used as recurrent parents were the inbred lines HA-89 and cms HA-89 and RHA-271, maintainer, and its cytoplasmic male sterile and restorer lines respectively released by the USDA and widely used in breeding programmes in Spain.

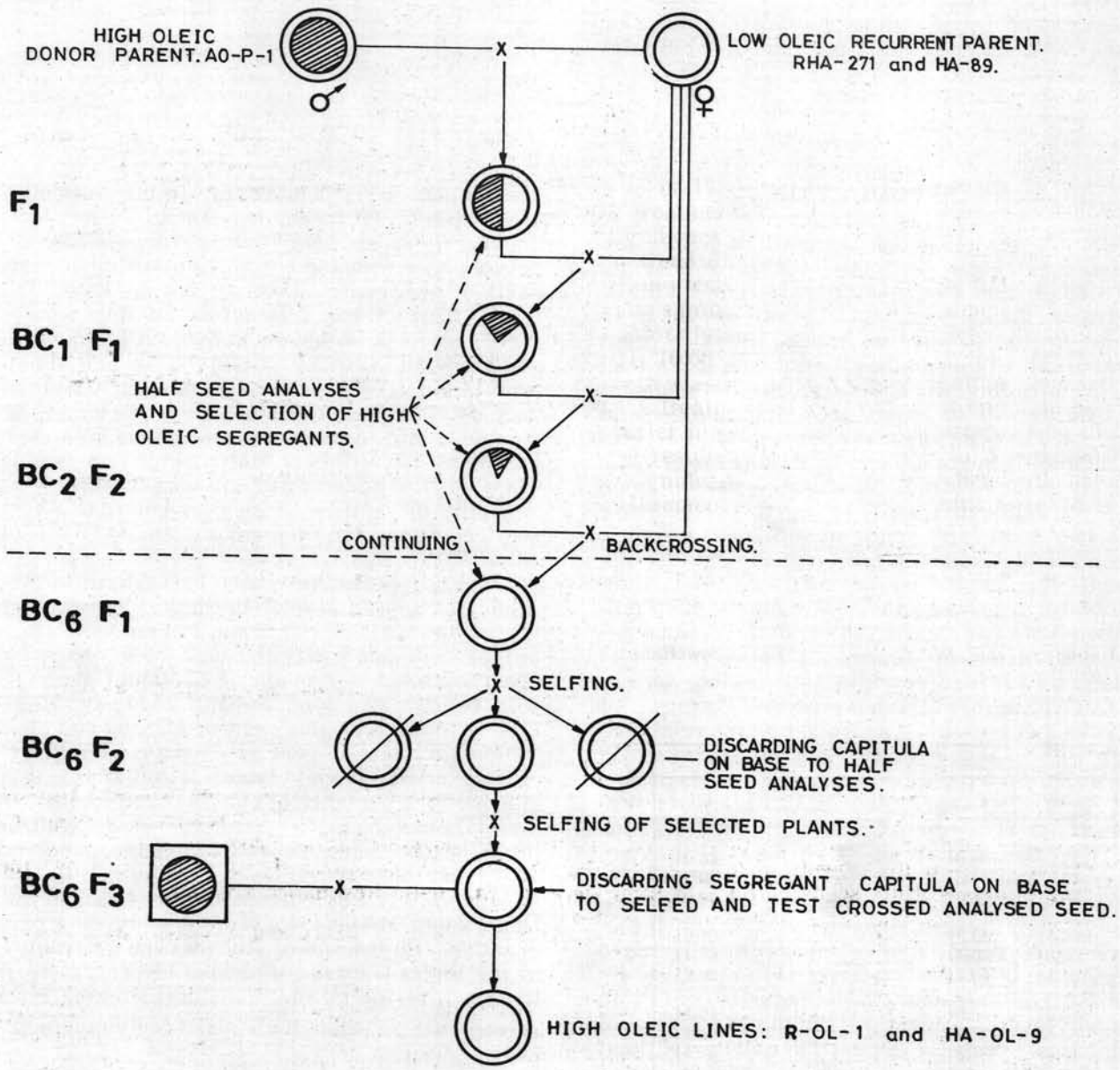

Fig. 1. — Selection scheme used for incorporating the high oleic character into the HA-89 and RHA-271 lines

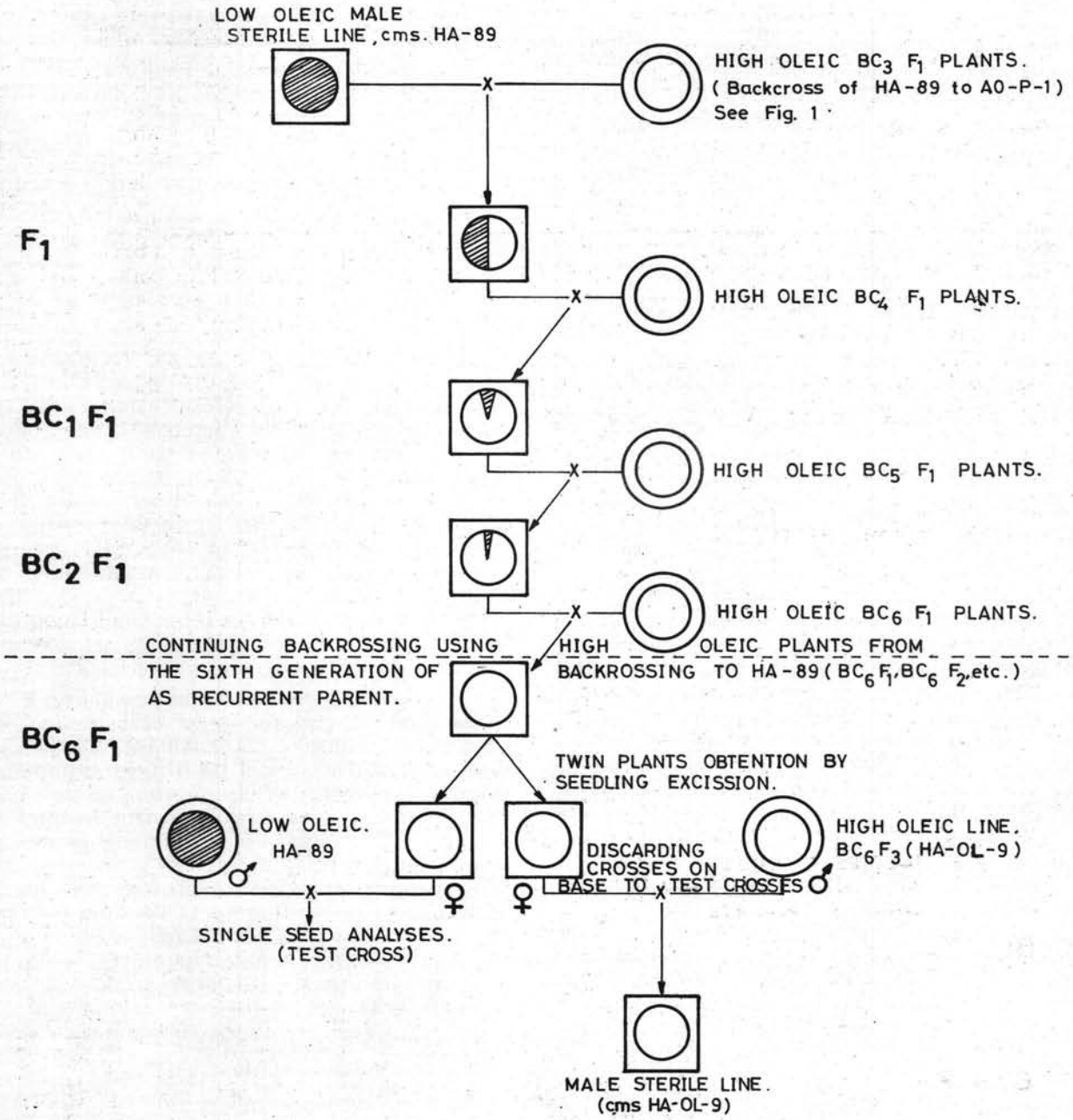

Fig. 2. — Selection scheme used in obtaining the high oleic male sterile line cms HA-OL-9

OIL ANALYSES

Fatty acid analyses were carried out on oil extracted from bulk seeds of 10 seeds or half seed analyses. Bulk seeds or the half seed were crushed and ground in a glass mortar and extracted using redistilled petroleum ether. Methyl esters of the fatty acids were used for gas chromatographic analyses. The half seeds were obtained by previously soaking the seeds for 5 minutes in sodium hypochlorite for surface sterilization followed by rinsing in water and removal of 1/3 to 1/2 of the stilar part for analysis. Methyl esters were prepared by the standard procedures and analysed using a Hewlett Packard gas chromatograph carrying a flame detector. The column was packed with 5 % diethylene glicolasuccinate on 80/100 W/An chrommosorb.

Oil content analyses were made by Nuclear Magnetic Resonance (NMR).

BREEDING METHODS

The backcross programme was started in 1982 using the low oleic lines HA-89 and RHA-271 as recurrent parents and the high oleic one AOP-1 as donor for high oleic. HA-89 and RHA-271 were emasculated and hand pollinated with pollen of the AOP-1 line. F_1 seed was analysed using the half seed technique to obtain

F_1 plants with high oleic. Pollen of these plants was used to pollinate the recurrent low oleic lines to obtain BC_1F_1 seeds (*Fig. 1*). Further backcrosses were always made using the low oleic recurrent parents as female which were emasculated prior pollinations. As the high oleic character was dominant and controlled by embryo genotype, heterozygous high oleic BC_nF_1 seeds were identified using half seed technique to obtain BC_nF_1 plants. Only BC_nF_1 plants from seeds with levels of oleic higher than 85 % were used as pollinators in the backcross programme. BC_6F_1 plants from both backcrossed parents (HA-89 and RHA-271), identified as high oleic carriers were selfed. BC_6F_2 plants were selected for high levels of oleic and selfed again and homozygous plants breeding true for the high oleic character were identified selfing and testcrossing to the low oleic parent.

The maintainer line HA-89 was converted into male sterile (petiolaris cytoplasm) by backcrossing the high oleic material to the male sterile low oleic line cms HA-89 (*Fig. 2*). This conversion to male sterility was made parallel to the incorporation of the high oleic to the maintainer HA-89 after the third backcross of the oleic character. In each backcrossing generation high oleic plants were used as male parents to pollinate low oleic male sterile female parent, at the same time that pollinated the HA-89 line in the backcrossing programme for introducing the high oleic character. Male sterile high oleic segregants were selected in each generation using the half seed technique. The final selection of male sterile plants, breeding true for high oleic genes, was made on the basis of test crosses. As the plants were male sterile an adequate number of twins BC_6F_1 plants were obtained using the method described by P a w l o w s k i (1969). One of the twin plants was test-crossed to low oleic HA-89 and the other backcrossed to HA-89 breeding true for high oleic acid content (*Fig. 2*).

RESULTS AND DISCUSSION

The original material derived from Pervenets was quite self incompatible and did not breed true for high levels of oleic acid under the environmental conditions of Córdoba (Southern Spain). The high level of variation for oleic acid content observed among seeds within the same capitulum, 30 to 94 %, confirmed the influence of the genotype of the embryo in the control of oleic acid content observed in other crosses with the same material (F e r n á n d e z-M a r t í n e z et al., 1988 and U r i e, 1985). The lines obtained using the method described above are morphologically similar to the recurrent low oleic inbred lines, RHA-271 and HA-89 and cms HA-89. The high oleic restorer line has been registered in the Spanish Catalogue as R-OL-1 and the maintainer and male sterile as HA-OL-9 and cms HA-OL-9. The mean oleic content of the high oleic lines varies from 88 to 94 % in comparison to 25 to 40 % of the isogenic low oleic lines (*Table 1*). The oleic value of R-OL-1 was slightly higher than that of HA-OL-9 and cms HA-OL-9 under the field conditions of Córdoba, the oil content being also higher. The oil content of the new lines was also higher than of its isogenic low oleic lines.

The breeding method described above, backcrossing with selection at the seed level, could be applied because of the dominant nature of the high oleic character and the strong influence of the genotype of the embryo which allowed the use of the half seed technique. Due to the high stability of the high oleic character, this method allows the obtention of several generations per year using greenhouses or growth chamber facilities. The final evaluation of the lines obtained has to be done under normal field conditions for evaluation of other plants characters. Given the dominant nature of this trait it could be incorporated to only one of the parental lines of one hybrid. However due to the strong gametophytic

Table 1

Plant and seed characteristics of high oleic parental lines R-OL-1 and HA-OL-9 in comparison with their isogenic RHA-271 and HA-89 lines

Parental line	Type of line	Days to flowering	Recessive branching	Resistance to downy mildew	Oil %	Fatty acid composition % [1]			
						Palmitic	Stearic	Oleic	Linoleic
R-OL-1	restorer	87	Yes	Yes	50.0	3.7—4.9	2.2—4.1	90.7—93.7	0.5—2.9
HA-OL-9 and cms HA-OL-9	maintainer and male sterile	79	No	No	45.2	4.1—5.6	1.9—3.4	87.6—91.5	1.1—3.8
RHA-271	restorer	86	Yes	Yes	48.1	5.0—6.5	3.5—4.4	29.8—37.6	50.9—61.3
HA-89 and cms HA-89	maintainer and male sterile	79	No	No	44.4	5.4—6.7	3.1—4.3	31.5—40.3	47.5—58.6

[1] Data from seed obtained under field conditions at Córdoba

embryo control and the genes involved (Fernández-Martínez et al., 1988) these hybrids would have lower oleic content because the seed on F_1 plants would be F_2 seed and would segregate for high, intermediate and low types. The mean oleic content would be around 70 % or even lower if these hybrids are grown without isolation. However combining only high oleic lines, homozygous for the high oleic genes, 90 % of oleic could be obtained. This material would be stable and isolation would not be necessary.

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AMÉLIORATION POUR HAUTES TENEURS EN ACIDE OLÉIQUE CHEZ LE TOURNESOL (*Helianthus annuus* L.)

Résumé

L'obtention d'hybrides de tournesol avec des hautes teneurs d'acide oléique dans l'huile pourraient ouvrir de nouveaux marchés pour cette huile. Dans ce travail on décrit la méthode d'amélioration utilisée pour l'incorporation du caractère haut oléique dans des lignées pures restauratrices et mâles stériles cytoplasmiques avec l'objectif final de développer des variétés hybrides avec ces caractéristiques. La méthode utilisée a été le back-cross et sélection de semences individuelles par le moyen de la technique de la demi-semence. La méthode décrite permet la réalisation de plusieurs générations chaque année et l'obtention des lignées avec de hautes teneurs en oléique dans une période relativement courte. On a obtenu une lignée restauratrice, R-OL-1, partant de la lignée RHA-271 et les lignées mainteneuses de cms et mâle stérile, HA-OL-9 et cms HA-OL-9, partant des lignées HA-89 et cms HA-89. Les nouvelles lignées sont similaires aux lignées originales mais avec une teneur en oléique d'environ 90 % et avec de plus grandes teneurs en huile. On discute, en fonction du contrôle génètique du caractère haut oléique, la convenance de l'utilisation de deux lignées parentales avec le caractère incorporé, dans le développement des variétés hybrides.

MEJORA DEL GIRASOL (*HELIANTHUS ANNUUS* L.) PARA ALTOS CONTENIDOS DE ACIDO OLEICO EN SU ACEITE

Resúmen

La obtención de híbridos de girasol con alto contenido de ácido oleico en su aceite puede abrir nuevos mercados a este aceite. Se describe en este trabajo el método de mejora seguido para la introducción del carácter alto oleico a líneas puras restauradoras y androestériles con el objectivo final del desarrollo de híbridos con estas características. El método utilizado ha sido el de retrocruzamiento y selección de semillas individuales mediante la técnica de media semilla. El método descrito permito la realización de varias generaciones por año y la obtención de líneas alto oleico en un periodo relativamente corto. Se ha obtenido una línea restauradora, R-OL-1, a partir de la línea RHA-271 y las líneas mantenedoras y androesteril HA-OL-9 y cms HA-OL-9 a partir de las líneas HA-89 y cms HA-89. Las nuevas líneas son similares a las originales pero con un contenido de ácido oleico de alrededor de 90 % y mayor contenido de aceite. Se discute en función del control genético del carácter alto oleico la conveniencia de la utilización de ambas líneas parentales con el carácter incorporado en el desarrollo de híbridos.