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DEVELOPMENT OF SUNFLOWER PARENTAL LINES AFTER EMS TREATMENTS OF AN INBRED LINE

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SUMMARY

Sunflower seeds of the inbred line HA-89, a well adapted and widely used line in sunflower breeding programs in Spain, were exposed to several concentrations of the chemical ethyl methanesulfonate (EMS) with the main goal of obtaining earlier variants. Increased EMS concentrations from 0.25 to 1 % adversely affected seed germination and plant vigour and increased the number of abnormal plants and the the percent of sterility in M1 populations. M1 plants were selfed eliminating those with lower fertility and seed production. Selfing of M2 plants and subsequent generations was continued up to the M6 generation. Sixty two M6 inbred lines were obtained and evaluated under field conditions. M6 lines exibited a great deal of variability in different important characters. In addition to flowering, which ranged from 76 to 93 days, plant height varied from 44 to 168 cm., oil seed content from 35 to 54 % and similar wide variations were observed in other characters in relation to HA-89. Chemically induced variation in high adapted inbred lines followed by selfing without or with a very low selection intensity may prove to be an useful method of selection specially if several generations of selfing per year are made.

INTRODUCTION

Mutations, spontaneous or induced are a major source of new genetic variability in plants. Although artificial mutagenesis can not be considered as a breeding method, is an interesting supporting technique which has been very useful in many crop species (Gottschalk and Wolf,1983). In sunflower (Helianthus annuus L.) the use of induced mutations has been limited although a remarkable success was obtained for some traits, as in the case of the high oleic acid mutant developped by Soldatov (1976) which has made possible the development of high oleic acid cultivars in this crop. Gundaev (1971) and Voskoboinik and Soldatov (1974) developped induced mutants with early ripening, thin seed hull and short plant stature. A short statured mutant with a large capitulum was obtained by Leclercq (1985) using gamma radiation. Other useful induced mutations in sunflower has been: Seed weight increase (Savin and Stepanenco, 1968), increased leaf area and plant height (Cuetkova, 1970), higher oil content (Cuetkova, 1970 and Saadat et al., 1975), improved rust resistance (Lofgren and Ramarage Urs, 1980). Sarafi (1976) obtained mutants from sunflower inbred lines with improved morphological and agronomical characteristics, heritability values for these characters were high enough to start a selection program.

Both, irradiation and chemical mutagens have been used in sunflower (Fick, 1978). Chemical mutagens induced more genic mutations with less chromosomal changes as compared to X-rays or gamma radiation (Simons, 1979). Voskoboinik and Soldatov (1974) and more recently Lofgreen and Ramarage Urs (1982) have used several kinds of chemicals and tested different means of application. In the latter work, two chemicals, ethyl metanesulfonate (EMS) and N-methyl-N'-nitro-N-Nitrosoguanidine (NTG) were tested, they conclude that the best method of application with these chemicals was infiltration.

Breeding material well adapted, but lacking some desirable characteristics is a convenient source material to be used in induced mutation programs. The objective of the work described in this paper was to induce useful mutations in the widely used sunflower inbred line HA-89 and to check the feasibility of developping parental inbred lines from the induced variants.

MATERIALS AND METHODS

Seeds of the well adapted, good combiner and widely used sunflower inbred line HA-89 were treated with four different concentrations of the chemical Ethyl metanesulfonate (EMS): 0.25, 0.50, 0.75 and 1.00 % in pH 7 phosphate buffer, each treatment was given in two times: 6 and 12 hours. EMS solutions were prepared inmediately before treatment. Seeds were transfered to the solutions which were gently shaked during the treatment time to improve mutagen infiltration. All treatments were carried out at room temperature. After treatment seeds were washed on tap water three times and them soaked on distilled water. Right after soaking, 300 seeds per each treatment were sown in the field in separated rows at the experimental farm of the

Agricultural Research Center in Cordoba (Spain). A check row of the untreated HA-89 line was seeded every five rows.

The experiment was sown in March 1980. Rows were 0.70 m apart and plants were 0.30 m within the rows. Germinations counting were made 20 days after planting. Extraneous individuals appearing during the growing period were recorded. Self pollination was assured by bagging individual capitula. M1 plants were harvested and grown as head rows in 1981 to obtain M2 plants. M3 and subsequent generations were obtained by selfing capitula of previous generation plants from 1982 to 1986 up to the M6 generation. Only selection for plant type was done in each generation, which mainly consisted in discarding plants with clear abnormalities, poor fertility, etc. Sixty two M6 lines were evaluated for morphological characters and for oil seed content and oil seed fatty acid composition. M7 lines were grown in 1987 and oil seed content and flowering time data were recorded. Oil seed content was determined using a Newport NMR Analyser. Fatty acid composition was determined by GLC as methyl esters using a Hewllett-Packard gas chromatograph.

RESULTS AND DISCUSSION

EMS concentrations were inversely related to germination rate and total germinated seeds. Besides, the longer the time of treatment the lower was the germination percentage. No germination was obtained with 1 % EMS solution. The maximum percentage of germination was 61 % and 49 % for the 0.25 % of EMS with 6 and 12 hours respectively and the lowest (11 %) for EMS at 1% for 6 hours treatment time. The 0.5 % EMS treatment showed a germination percentage of 43 % for 6 hours and 32 % for 12 hours. These results indicate that a dosis in between 0.25 and 0.50 % EMS could be used maintaining a reasonable M1 survival level. These doses are comparable to the ones reported by Lofgren and Ramaraje Urs (1982) although they use a different treatment method.

M1 plants carried beyond the seedling stage showed several differences in comparison with the control HA-89. These differences included: chlorophyll defficiencies, leave fasciation, slow vegetative growth, male and female sterility with a consistent reduction in seed yield an number of seeds per plant. In the higher doses there was a substantial number of plants with no seed at all. About 400 M2 plants were grown in 1982 from M1 seeds. Visible differences, in comparison with the control, for different morphological characters were evident. Different types of abnormalities, many of them lethals, were observed in this generation and other plants showing agronomically undesirable characters were not carried to the next generation. The remainder were selfed to obtain M3 seed. Subsequent generations up to the M6 were obtained in the same way.

Mean values and ranges for days to flowering, number of leaves, height, capitulum diameter, oil seed content and oleic acid content of M6 lines evaluated in 1986 are given in Table 1.

Table 1. Mean values and ranges for characters of sunflower M6 lines and the control, HA-89, grown in the field in Còrdoba in 1986.

	No. of Lines	Days to flower	No. of leaves	Height cm	Head diameter cm	Oil content %	Oil acid content %
				M6 lines (1)			
Mean	62	87	23	100	10.7	48	29
Range	62	76-93	16-28	44-168	6.8-17.2	35-54	19-60
				HA-89 (2)			
Mean	1	86	24	96	11.3	46	29.6

(1) The values for each line were based on observations in 5 plans.

(2) Means based on observations in 10 plants.

There was a wide range of variation for all the character evaluated in comparison with the control HA-89. It can be seen in Table 1, some lines 11 days earlier than HA-89, although the average for flowering days is later than the control. Height also varied widely (44 to 168 cm), the average height was also taller than the control. The average of all the lines for seed oil content was 48 % higher than the control (46 %), although, as we have stated before, no selection for seed oil content was made during the inbreeding process. The largest value for capitulum diameter was 17.2 cm as compared with 11.3 cm for the control.

Head diameter was positively correlated with height (Table 2), although there were some lines with short stature and large capitula. Oleic acid content showed a maximum value of 60 % in bulk seed samples analyses and a minimum of 19 % (71 % of linoleic), although these values could be influenced by the temperatures at flowering time. Single seed analyses (not shown in Table 1) gave ranges even wider (15 to 65 %).

Table 2. Correlations between plant and seed characteristics in M6 lines derived from HA-89 seeds treated with EMS.

Character	Days to Flower	No. of Leaves	Height	Head diameter
No. of Leaves	0.46**	-		
Height	-0.33*	NS	-	
Head diameter	-0.77**	NS	0.65**	-
Oil content	NS	NS	NS	NS

*, **: Significant at 5 and 1% probability levels, respectively.
NS: Non significant.

Table 2 shows the correlation between characters in the M6 lines derived from HA-89. No correlation was observed between oil content and others plant characteristics. Fick et al. (1974) reported positive correlation between oil content and plant height and days to flowering, although they pointed out that the magnitude of the correlation coefficients was not sufficient to preclude selection of early and short high oil material. The results of the present study indicate that after EMS treatments, selection can be done for any plant type and for seed oil content at the same time. The positive correlation between days to flower and leave number was expected. However, the magnitude of the coefficient,0.46, also indicates the existence of early material with high number of leaves and reciprocally. Although some of the studied characters were correlated, the results of our study show that plant types showing deviation from the original line HA-89 for several characters can be obtained. For example, dwarfness and earliness or lateness, large capitula and high seed oil content.

Progenies of M6 lines performed similarly in the M7 generation grown in 1987 (data not shown). Correlation coefficients between both generations data were high; 0.75 for seed oil content and 0.93 for days to flowering.

Most of the variants obtained in this study had been described in previous works (Voskoboinik and Soldatov, 1974; Soldatov, 1976; Leclercq, 1982 and others). However, our study bring out an array of variation for different characters, in many cases associated, which make this plant material potentially interesting for breeding purposes. At the present time research is carried out to determine the value of these lines in hybrid combinations in comparison with the original HA-89. Our results also indicate that chemically induced variation followed by selfing without or with a very low intensity selection (for example using the single descent method) may prove to be an useful method of selection, specially if it comes combined with modern techniques as "in vitro" development of embryo which allows several generations per year.

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