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Combining-ability analysis of somatic embryogenesis from epidermal layers in the sunflower (*Helianthus annuus L.*)

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Abstract Crosses were made between three cytoplasmic male-sterile and five restorer sunflower inbred lines. F_1 hybrids, including their parents, were studied for their embryogenetic ability. Sterilized seeds were germinated in culture tubes on agar-solidified basal medium. Seven days after germination, epidermal layers from hypocotyls were transferred into MS and B5 liquid-midia for 5 and 8 days respectively. Then they were transferred to MS-120 solid-medium with a high level of sucrose (120 g/l). The experimental design was complete randomized blocks with three replications. Each replication per genotype consisted of three Erlenmeyer flasks with 20–25 epidermal layers (explants). Analysis of variance indicated the presence of significant variation among genotypes for all traits studied. General combining ability (GCA) and specific combining ability (SCA) showed significant effects for the studied traits. The highest value of GCA for the number of embryogenic explants per 100 explants plated (EE/100EP) belongs to parental female line 'CMS-PET1 B9'. This inberd line also gave a high positive GCA effect for the number of embryos per ten embryogenic explants (E/10EE). Additionally, it had the highest values for EE/100EP and E/10EE (41.70 and 19.28 respectively) and should be a promising parent in crossing programmes for the enhancement of somatic embryogenesis in the sunflower. The highest values of specific combining ability (SCA) for EE/100EP and E/10EE belong to the F_1 hybrid 'CMS-PET1B9 'x' RT1B11' which has produced 53.45 embryogenic explants/100 explants and 9.67 embryos per ten embryogenic explants.

Key words Sunflower · Combining ability · Embryogenesis · Epidermal layer · Regeneration

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Introduction

Over the last 10 years, a variety of techniques for the regeneration of sunflower have been reported. Regenerated plants have been produced via organogenesis (Power 1987; Knittel et al. 1991; Pugliesi et al. 1991; Chraibi et al. 1992; Pugliesi et al. 1993; Geneviève et al. 1995; Sarrafi et al. 1996; Carola et al. 1997) and via embryogenesis (Finer 1987; Pélissier et al. 1990; Thengane et al. 1994; Zezul et al. 1995; Fambrini et al. 1996, 1997; Laparra et al. 1997).

Regeneration frequency is influenced by culture conditions, genotypes and their interaction. Prado and Bervillé (1990) and Khalid et al. (1992) reported that the induction of somatic embryos from hypocotyl sections in the sunflower occured only in a liquid medium, which enhance the contact between the tissue and the medium. In sunflower the number of embryogenic events increased with increasing sucrose concentration, while organogenesis showed the opposite trend (Geneviève et al. 1995). In order to obtain a high rate of embryo formation, it is important to maintain the explants in complete darkness until the embryos are formed (Carola et al. 1997). Estimates of genetic variation and combining ability are useful to determine the breeding value of genotypes in a breeding programme. The general combining ability (GCA) effects are important indicators of the value of inbreds in hybrid combinations. Differences in GCA effects are attributed to additive and additive x additive interactions, whereas differences in specific combining ability (SCA) are attributed to non additive genetic variance (Falconer 1972). Genetic

studies of tissuse-culture response and regeneration capacity have been conducted in numerous crop species. At present, the number of reports about the genetic control of regeneration in sunflower remain limited. Plant regeneration parameters by organogenesis have been shown to be under quantitative genetic control in the sunflower (Sarrafi et al. 1996), as in other species such as in red clover (Keyes et al. 1980), alfalfa (Hernandez-Fernandez and Christie 1989), wheat (Ghaemi and Sarrafi 1993) and rice (Quimio and Zapata 1990). Thin epidermal layers, composed of 3–6 layers of cells, are a simplified system in which different types of morphogenesis can be programmed separately and directly without a callus phase (Pélissier et al. 1990; Tran thanh van 1995). As far as we know, genetic control of somatic embryogenesis in sunflower epidermal layers has not been reported in the literature.

The main purpose of the present study was to estimate the genetic variability, as well as the GCA and SCA, of somatic-embryogenesis parameters in a crossing program between three-female and five-male inbred lines of sunflower.

Materials and methods

Three cytoplasmic male-sterile and five restorer inbred lines, as well as their 15 F_1 hybrids, were used in this study. The inbred lines and F_1 hybrids represent a high genetic diversity and were produced by INRA Montpellier, France. Before culturing, pricarps were removed and seeds were surface-sterilized in 0.01% (w/v) aqueous $HgCl_2$ solution for 10–12 min, then placed in a 5% (w/v) sodium hypochlorite solution with 0.01% (v/v) tween 80 for 20 min and rinsed three times in sterilized water. Sterile seeds were germinated in culture tubes on agar-solidified MS basal medium (Murashige and Skoog 1962) and the pH was adjusted to 5.7. Cultures were maintained at 25 $\pm$ 1 C under a light flux of 50 μE^{-2} S^{-1}, (16-h light, 8-h dark cycle).

The epidermal layers from hypocotyls were excised in 2-cm sections, 7 days after germination, and transferred to 250-ml Erlenmeyer flasks containing 100 ml of the MS basal medium (MSb) for 5 days then on B5–90, embryo-induction medium for 8 days. Cultures were maintained at 24 $\pm$ 1 C in the dark with shaking at 120 rpm.

After the period of 13 days, explants were transferred to MS-120 embryo-development medium for 15–20 days at 26 $\pm$ 1 C in the dark. Then, selected embryos were excised from explants and transferred to the same medium in order to develop secondary embryos. Ten days later, embryos were transferred to B-60 medium, in the same conditions as described above, for another 15-days period. Developed embryos were individually transferred to culture tubes on B5–30 medium to grow and to establish a functional root system. After rooting the plantlets were transferred to pots and placed in a growth chamber. All B5 media were prepared according to Pélissier et al. (1990).

The experiment was designed as a randomized complete blocks with 23 genotypes (eight inbred lines and 15 F_1 hybrids) and three replications. Each replication consisted of three Erlenmeyer flasks with 20–25 epidermal layers (explants). Remarkable differences were observed in the frequencies of somatic embryo induction among the genotypes studied. The following traits were determined for each genotype per replication:

– the number of embryogenic explants per 100 explants plated (EE/100EP),
– the number of embryos per 10 embryogenic explants (E/10EE),
– the number of plantlets per 100 explants plated (P / 100 EP).

Analyses of variance were performed and the means separated using a Newman-Keuls-test ($P = 0.05$).

Sums of squares of F_1 hybrids in factorial analysis were partitioned into female, male and female x male interaction effects. The main effect of females and males were considered as general combining ability (GCA) effects, and the female x male interaction was equivalent to specific combining ability (SCA). A fixed model was assumed and the mean squares of females, males and their interaction were tested by the error mean square of a factorial analysis of F_1 hybrids. The heritability of the two studied traits was estimated by the ratio between the additive variance (S^2_A) and the dominant variance (S^2_D) (Garretsen and Keuls 1978). Total additive variance (S^2_A) was considered to be the sum of the additive variance due to males (S^2_{Am}) and the additive variance due to females (S^2_{Af}). S^2_{Am}, S^2_{Af} and S^2_D, were calculated according to wricke and Eberhard – Weber (1986).

Results and discussions

Results of the analysis of variance indicate the existence of highly significant differences among parental genotypes and F_1 hybrids for the embryogenic traits studied (data not presented) . Mean performance concerning the

Table 1 Mean performance (x) and general combining ability (GCA) for two embryogenic parameters in inbred lines of sunflower. Means (x) followed by different letters are significantly diffrent at $P = 0.05$ level (Newman-Keuls test)

Genotype	EE/100EP[a]		E/10EE[b]	
	x	GCA	x	GCA
Female parents:				
CMS-PET1D34	7.03[c]	–8.85*	11.67[ab]	–3.7
CMS-PET1B9	41.70[a]	11.85	19.28[a]	7.45*
CMS-PET1B16	10.60[bc]	–3.00	11.67[ab]	–3.77
Male parents:				
RT1B11	16.75[b]	6.95	17.55[a]	9.17*
90HR15	7.32[c]	–8.73*	13.33[ab]	–6.71*
90HR22	7.11[c]	6.45	10.00[ab]	2.05
90HR25	37.94[a]	4.70	19.78[a]	–1.11
85B3	1.46[c]	–9.27*	3.34[c]	–3.42*

* Significant at $P = 0.05$
[a] EE/100EP: embryogenic explants per 100 explants plated
[b] E/10EE: embryos per 10 embryogenic explants

Table 2 Factorial analysis of variance for embryogenic ability in sunflower

S.O.V.	*df*	Mean square (MS)	
		EE/100EP[a]	E/10EE[b]
Total	44	206.04	88.18
Female	4	1708.56***	625.84***
Male	2	620.53***	329.11**
Female × male	8	328.81***	100.60***
Block	2	32.47[ns]	36.97[ns]
Residual	28	16.82	15.46

***: significant at $P = 0.001$, [ns]: not significant
[a] EE/100EP: embryogenic explants per 100 explants plated
[b] E/10EE: embryos per 10 embryogenic explants plated

Table 3 Mean performance (x) and specific combining ability (SCA) for two embryogenic parameters in F_1 hybrids of sunflower. Mean (x) followed by different letters are significantly different at the $P = 0.05$ level (Newman-Keuls test)

Female	EE/100EPa						E10/EEb					
	CMS-PET1 D34		CMS-PET1 B9		CMS-PET 1B16		CMS-PET1 D34		CMS-PET1 B9		CMS-PET1 B16	
Male	x	SCA	x	SCA	x	SCA	x	SCA	x	SCA	x	SCA
T1B11	7.10^{e}	–8.98*	53.45^{a}	16.68*	14.33^{d}	–7.59*	13.33^{bcd}	–5.57*	39.72^{a}	9.67*	14.72^{bc}	–4.11*
90HR15	0.01^{e}	–0.38	26.23^{bc}	5.14	1.46^{e}	–4.79	0.01^{e}	–3.02	16.76^{bc}	2.62	3.34^{de}	0.38
90HR22	15.94^{cd}	0.39	24.80^{bc}	–11.47*	32.65^{b}	11.23*	15.28^{bc}	3.5	18.44^{b}	–4.49*	12.68^{bcd}	0.97
90HR25	19.64^{cd}	5.82	25.02^{bc}	–9.49*	23.34^{bcd}	3.66	13.33^{bcd}	4.71*	12.76^{bcd}	–7.01*	10.83^{cde}	2.28
85B3	2.95^{e}	3.10	19.6^{cd}	–0.95	3.26^{e}	–2.44	6.67^{cde}	0.36	16.66^{bc}	–0.8	6.67^{cde}	0.43

* Significant at $P = 0.05\%$
[a] EE/100EP: embryogenic explant per 100 explants plated
[b] E/10EE: embryos per 10 embryogenic explants

Table 4 Mean performance (x) for the number of plantlets per 100 explants plated in some inbred lines and F_1 hybrids of sunflower

Genotype	x[a]	Genotype	x[a]
CMS-PET1 B9	3.99^{c}	CMS-PET1 × 90 HR25	5.79^{c}
RT1 B11	6.17^{c}	CMS-PET1 × 90 HR22	4.74^{c}
90HR 25	14.92^{c}	CMS-PET1B9 × RT1B11	23.49^{b}
		CMS-PET1B9 × B5B3	38.21^{a}

Means (x) followed by different letters are significantly different at the $P = 0.05$ level (Newman-Keuls test)

genetic variability of the parental inbred lines for two embryogenic traits are summarized in Table 1. Among the eight parental lines, 'CMS-PET1 B9' and '90H R25', used as female and male parents respectively, presented the highest values for the number of embryogenic explants per 100 explants plated (EE/100 EP) and the number of embryo per ten embryogenic explants (E/10EE). Conversely, the male line '85B3' gave the lowest values for these two traits.

The highest values for 'EE/100EP' were observed in F_1 hybrid 'CMS-PET1B9' x RTI B11' (35.45). This F_1 hybrid has also the highest value for the number of embryo per ten epidermal layers (39.72). Pélissier et al. (1990) and Fambrini et al. (1996) have also demonstrated that embryogenic parameters are genotype-dependent. Genetic variability for organogenesis parameters has been reported by Sarrafi et al. (1996) and Degelene et al. (1997).

Some of genotypes did not produce any plants and an analysis of variance was performed only for three parental inbred lines and four F_1 hybrids. Root formation was very poor in the majority of plantlets developed from the somatic embryos and this greatly impeded plant recovery. This phenomenon have been also observed by Fambrini et al. (1996). As far as the number of plantlets per 100 embryogenic explants plated (P/100EP) is concerned (see Table 4), the F_1 hybrid 'CMS-PET1 B9 x 85B3' had the highest value, followed by 'CMS-PET1 9 x RT1 B11' (38.21 and 23.49 respectively).

The contribution of each genotype to the progeny response was assessed by comparing the general combining ability (GCA) effects. Positive values indicate a contribution toward a large value for the studied parameters, while negative values indicate a contribution toward smaller values. Female and male general combining ability (GCA) as well as F_1 hybrid specific combining ability (SCA) variances were significant (Table 2). Three parental lines showed significant negative, and one parental line showed significant positive, GCA effects for the number of embryogenic explants per 100 explants plated (EE/100EP). The parental line with a positive GCA for this trait, 'CMS-PET1 B9' is used as female in crosses (Table 1). High 'EE/100EP' values were found in crosses in which this inbred line was employed as the parent (Table 3). CMS-PET1 B9 presented also the highest GCA value for the number of embryos per ten epidermal layers (Table 1). This genotype should be considered as the best combiner to improve embryogenic capacity in the sunflower. Some of the F_1 hybrids presented positive and significant SCA effects for the two embryogenic parameters studied. This information suggests that non-additive genetic effects are also involved in the inheritance of these crosses. The ratio of additive variance to dominant variance was 1.66 for the number of embryogenic explants per 100 explants plated (E/100EP) and 2.59 for the number of embryos per ten embryogenic explants (E/10EE), which indicate that additive effects of the genes controlling the two embryogenic traits are predominant. Sarrafi et al. (1996) have also reported a significant GCA and SCA for organogenesis parameters in sunflower. As far as we know, genetic control of somatic embryogenesis in sunflower has not been reported in the literature. This investigation indicates that significant GCA and SCA are sources of variation for both of embryogenic parameters ('EE/100EP' and 'E/10EE'), and that some of our inbred lines shoud be used in crossing programmes to improve the embryogenic capacity in sunflower.

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