Theor Appl Genet (1996) 92:225–229
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Genetic analysis of organogenesis in the cotyledons of zygotic embryos of sunflower (*Helianthus annuus* L.)

Received: 15 February 1995 / Accepted: 21 July 1995

Abstract Crosses were made between five cytoplasmic male-sterile and five restorer sunflower inbred lines. Twenty-five F_1 hybrids and their parents were studied for their organogenesis ability in a randomized block design with four replications. Each replication per genotype consisted of ten petri dishes with four explants. Regeneration medium consisted of full MS medium modified by the addition of hormones and solidified with 6 g/l agar. Statistical analysis showed that both general and specific combining abilities were significant for all of the organogenesis parameters studied, and both showed several significant positive or negative values. General combining ability values were usually higher than those of specific combining ability, indicating the importance of additive genetic control for organogenesis parameters in sunflower. Narrow-sense heritability for the number of explants producing shoots and roots was 65.8%, which suggests that organogenesis of currently inferior inbred lines in sunflower should be improved in a crossing program.

Key words Organogenesis · Sunflower · Combining abilities · *In vitro* culture · Heritability

Introduction

The ability to regenerate large numbers of plants from cultured tissues is important for the successful application of this technology to crop improvement. Over the last few years, a variety of techniques for regenerating sunflower by organogenesis (Greco et al. 1984; Power 1987; Wirtzens et al. 1988; Espinasse et al. 1989) or somatic embryogenesis (Paterson and Everett 1985; Wilcox et al. 1988; Finer 1987; Freyssinet and Freyssinet 1988; Pélissier et al. 1990; Prado and Bervillé 1990) has been reported. Plant regeneration by organogenesis from tissues of *Helianthus annuus* remains, however, problematic. The regeneration frequency and the number of shoots produced per callus are highly variable, depending mainly upon the genotype. For example, Greco et al. (1984) reported callus induction and plant regeneration from a single variety of sunflower, while Paterson and Everett (1985) described a method of regeneration limited to 1 cultivar of sunflower among the 100 tested.

Regeneration capacity is influenced by cultural conditions, genotype and their interactions. Most of the recent investigations on sunflower regeneration by direct organogenesis show a dependency on the culture conditions. Ceriani et al. (1992) used cotyledons as potential explants and under optimized conditions about 50% of the studied genotypes produced shoots and 50% to 90% of the regenerated shoots produced viable plants. Chraibi et al. (1992) transferred cultured explants from liquid to solid medium, which resulted in high regeneration efficiencies.

The genetic control of organogenesis parameters in sunflower has not yet been studied. Plant regeneration response has been shown to be under genetic control in several other crop species such as red clover (Keyes et al. 1980), alfafa (Hernandez-Fernandez and Christie 1989), winter wheat (Ou et al. 1989) and rice (Quimio and Zapata 1990). This has been of particular interest to plant breeders concerned with the utilization of in vitro techniques in the development of germ plasm. The transfer of genes controlling regeneration to elite germ plasm via crossing may be the most effective means of realizing the potential advantages of tissue culture.

The main purpose of the present study was to estimate the genetic variability and gene action of plant regeneration in a crossing program between five females and five male inbred lines of sunflower.

Communicated by G. Wenzel

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Materials and methods

Five cytoplasmic male-sterile inbred lines and five restorer inbred lines as well as their 25 F_1 hybrids were used in this study. The inbred lines and F_1 hybrids represent a high genetic diversity and were chosen by Caussade Semence Seed Company in France on the basis of their agronomic characteristics. Before culturing, seeds were surface-sterilized for 20 min in a 5% (w/v) calcium hypochlorite solution containing 0.1% Tween 20 and were washed three times in sterile distilled water. Seeds were germinated in culture tubes on hormone-free half-strength Murashige and Skoog's (MS) medium (1962), and the pH was adjusted to 5.7 before autoclaving at 120 °C for 20 min. Cultures were maintained at 25 ° ± 1 °C, under a 16-h light/8-h dark cycle with a light flux of 100 μmoles $m^{-2} s^{-1}$ (Osram L36W/36 Nature tubes).

The regeneration medium consisted of full strength MS medium supplemented with 50 m*M* KNO_3, 1 m*M* myoinositol and 500 mg l^{-1} casein hydrolysate, which is considered to be the basal medium (Chraibi et al. 1991), modified by adding 5.4 μ*M* naphthaleneacetic acid (NAA), and 4.4 μ*M* benzylaminopurine (BAP), and solidified with 6 g l^{-1} agar. Cotyledons from 2-day-old seedlings were excised and cut transversely into two pieces. The explants were transferred to the above-mentioned medium. Each petri dish, sealed with a parafilm strip to prevent desiccation, contained 4 explants. The experiment was designed as a randomized complete block with 35 genotypes (ten inbred lines and 25 F_1 hybrids) and four replications. Each replication consisted of ten petri dishes with 4 explants.

Plant regeneration by direct organogenesis was observed 4 weeks after explant culturing and the three following traits were studied:

- total number of organogenic explants per 100 explants plated (OE);
- number of organogenic explants producing roots per 100 explants plated (ORE);
- shoot production yield as the number of shoots per explant (SE).

In order to normalize the distribution, data were transformed by the arc $\sin\sqrt{\ }\times$ function before variance analysis. General combining ability (GCA) and specific combining ability (SCA) were estimated as proposed by Garretsen and Keuls (1978) and Gallais (1990). The statistical model is the following

$$Y_{ij} = \mu + \lambda_i + \lambda_j + S_{ij} + e_{ij}$$

where:
μ = general mean effect;
$\lambda_i(\lambda_j)$ = general combining ability (GCA) of the i^{th} female (j^{th}) male parent;
S_{ij} = specific combining ability (SCA) of the cross between the i^{th} female and j^{th} male parent;
e_{ij} = the environmental effect.

Narrow-sense heritabilities were calculated according to the following formula (Kempthorne 1957):

$$h^2 = 4\delta^2\text{GCA}/4\delta^2\text{GCA} + 4\delta^2\text{SCA} + \delta_r^2 + \delta_e^2$$

Results

Analysis of variance showed significant differences for all organogenesis traits. The variability of different studied traits is summarized in Table 1. This table shows that significant differences among parental genotypes were observed for all of the organogenesis parameters studied. Two inbred lines, 'CS-105' and 'CS-24', used as female parents in the diallel program gave the highest values for the number of organogenic explants per 100 explants (OE). As far as organogenic explants producing shoots and roots per 100 explants (ORE) was concerned, the inbred line 'CS-12' had the highest value, followed by 'CS-24', both used as females. The highest shoot number per explant (SE) was obtained with 'CS-105' and 'CS-121', which were used as female and male in crossing, respectively.

Several of the F_1s, however, were significantly better then their parents for one or more of the organogenesis parameters studied (Table 2). The greatest overall organogenesis response was observed in the cross, 'CS-89' × 'CS-121', which produced 90% organogenic explants (OE). Some combinations were also found to have high values for all three traits, notably the F_1 hybrid 'CS-12' × 'CS-142'.

Most of the positive and negative values of general combining abilities (GCA) were significant for all traits (Table 1). Two out of the ten parental inbred lines (CS-89 and CS-121) showed high positive GCAs for two of the three traits studied (total number of organogenic explants and average number of shoots per explant), while the inbred line 'CS-12' had a high value for the number

Table 1 Mean performance and general combining ability (GCA) of parental genotypes for organogenesis parameters in ten sunflower genotypes (*OE* total number of organogenic explants per 100 explants plated, *ORE* number of organogenic explants giving shoots and roots per 100 explants plated, *SE* average number of shoots per explant plated)

Genotypes	OE		ORE		SE	
	$\bar{X}$ [a]	GCA	$\bar{X}$	GCA	$\bar{X}$	GCA
Female parents						
CS-105	57.69 [a]	4.94*	13.28 [de]	−8.72*	5.65 [a]	−0.4
CS-89	40.55 [ab]	8.45*	31.76 [bc]	−9.11*	3.62 [ab]	1.85*
CS-24	42.55 [ab]	−3.86*	52.66 [a]	−1.36	2.17 [ab]	−0.33
CS-215	20.64 [b]	−14.67*	37.50 [ab]	9.04*	0.37 [b]	−1.57*
CS-12	27.24 [b]	9.13*	55.64 [a]	15.32*	0.32 [b]	0.54
Male parents						
CS-18	23.24 [b]	−7.92*	0.00	−20.19*	0.89 [b]	−1.29*
CS-77	29.89 [b]	−5.01*	15.86 [de]	−6.14*	0.52 [b]	−0.10
CS-142	15.00 [b]	−8.94*	49.20 [ab]	18.55*	0.32 [b]	−1.93*
CS-121	36.75 [ab]	17.70*	22.96 [cd]	−6.90*	5.47 [a]	2.45*
CS-27	37.33 [ab]	0.42	40.84 [ab]	9.52*	4.03 [ab]	0.79*

* Significant at $P = 0.05$
[a] Means ($\bar{X}$) followed by different letters are significantly different at $P = 0.05$ level (Newman-Keuls test)

Table 2 Mean performance[a] for organogenesis parameters in F_1 hybrids of ten sunflower inbred lines

♀ \ ♂	CS-18			CS-77			CS-142			CS-121			CS-27		
	OE[b]	ORE[b]	SE[b]	OE	ORE	SE	OE	ORE	SE	OE	ORE	SE	OE	ORE	SE
CS-105	34.10 cde	19.18 defg	1.57 ab	45.00 cd	29.14 cdef	1.35 ab	29.80 cde	42.95 bcde	2.64 ab	52.50 c	15.00 efg	6.75 ab	35.47 cde	21.81 defg	2.77 ab
CS-89	33.31 cde	4.87 fg	6.72 ab	37.44 cde	25.26 cdefg	6.58 ab	29.34 cde	43.84 bcde	1.58 ab	90.00 a	0.00 g	7.00 ab	45.00 cd	33.33 cdef	8.47 a
CS-24	25.35 cde	15.00 efg	2.50 ab	23.65 de	6.02 fg	4.42 ab	37.51 cde	47.88 bcd	2.65 ab	44.35 cd	30.87 cdef	6.89 ab	28.37 cde	33.11 cdef	2.48 ab
CS-215	23.15 de	29.30 cdef	0.63 b	15.00 e	41.25 bcde	3.13 ab	11.90 e	31.17 cdef	1.39 ab	36.38 cde	42.60 bcde	3.63 ab	29.84 cde	66.12 b	4.51 ab
CS-12	38.27 cde	4.19 fg	3.08 ab	43.88 cd	39.35 bcde	6.50 ab	47.73 cd	90.00 a	2.72 ab	71.15 b	40.87 bcde	8.05 a	51.47 c	55.60 bc	5.59 ab

[a] Means followed by different letters are significantly different at $P = 0.05$ level (Newman-Keuls test).
[b] For clarification of abbreviations see heading of Table 1

of organogenic explants producing shoots and roots per 100 explants (15.32). These three inbred lines were the best combiners for improving all of the organogesis parameters. In contrast 'CS-18' presented negative GCA values for the three traits analyzed.

Specific combining abilities (SCA) for F_1 hybrids (Table 3) showed several significant positive or negative values that were usually lower than those of GCA. Two F_1 hybrids, 'CS-215' × 'CS-18' and 'CS-12' × 'CS-142', exhibited a positive significant SCA for two traits (total number of organogenic explants and number of shoots per explant), while F_1 hybrid 'CS-89' × 'CS-18' was the most favorable combination for the number of shoots per explant.

Heritabilities in the narrow sense were relatively high for the number of organogenic explants producing shoots and roots (65.8%), while its value was rather low for the two other traits (27.05% for organogenic explants per 100 explants and 34.40% for the number of shoots per explant).

Discussion

Significant genetic variation for organogenesis parameters was observed in this study, which is in agreement with results previously reported by Power (1987) and Espinasse et al. (1989). In our experiment the inbred lines 'CS-12' and 'CS-27' had rather high values for all of the organogenesis parameters analyzed, whereas the poorest inbred line was 'CS-18'.

The significant values of general combining ability obtained here indicate the importance of additive genetic control for organogenesis responses in our material. Specific combining ability values in F_1 hybrids show also a high variability due to the dominance effect. The importance of additive effects in plantlet regeneration of hexaploid wheat has been suggested by Lazar et al. (1984) and in embryogenesis of tetraploid wheat by Ghaemi and Sarrafi (1993). Genetic control of organogenesis responses in sunflower has not yet been reported as far as we know.

The maximum value of GCA for different organogenesis parameters was obtained in different inbred lines. To summarize, 'CS-12' had high GCA for two out of three traits analysed (number of organogenic explants per 100 explants and number of explants giving shoots and roots per 100 explants) and 'CS-121' for the number of organogenic explants per 100 explants (OE) and the number of shoots per explant (SE). These inbred lines should respond well to organogenesis in hybrid combinations.

Although F_1 hybrids, 'CS-215' × 'CS-18' and 'CS-12' × 'CS-142' combinations should be considered as the most favorable for organogenesis responses as far as SCA is concerned. Narrow-sense heritability for the number of explants producing shoots and roots was high in our experiment (65.8%), while it had low values for the two other traits (27.05% and 34.4% for or-

Table 3 Specific combining ability (SCA) for organogenic parameters in F1 hybrids of ten sunflower inbred lines

♀ \ ♂	CS-18			CS-77			CS-142			CS-121			CS-27		
	OE[a]	ORE[a]	SE[a]	OE	ORE	SE	OE	ORE	SE	OE	ORE	SE	OE	ORE	SE
CS-105	−0.41	15.81*	−0.60	7.59*	11.72*	−2.00*	−3.68	0.83	1.11	−7.62*	−1.66	0.85	−7.38*	−11.27*	−1.47
CS-89	−4.71	1.89	2.30*	−3.49	8.22*	0.98	−7.66*	2.11	−1.95*	26.36*	−16.27*	−1.15	1.36	0.64	1.98*
CS-24	−0.36	4.27	0.27	−4.97	18.76	1.00	12.82*	−1.6	1.06	−6.98	6.85	0.93	−5.68	−7.33	−1.83*
CS-215	8.25*	43.44*	−0.36	−2.80	6.06	0.95	−1.97	−28.71*	1.04	−4.14	8.18*	−1.09	6.00	15.28*	1.44
CS-12	−0.43	−23.22*	2.27	2.27	−2.12	2.21*	10.05*	23.84*	0.26	6.83	0.16	1.22	4.24	−1.52	0.41

*Significant at $P = 0.05$

[a]For clarification of abbreviations see heading of Table 1

ganogenic explants per 100 explants and number of shoots per explant respectively).

Several authors have commented on the heritability of *in vitro* characteristics in hexaploid wheat (Bullock et al. 1982; Raquin 1982). Narrow-sense heritability for plantlet regeneration frequency by anther culture was reported by Lazar et al. (1984) to have a high value (71.8%). Further estimates of the heritability of such *in vitro* traits in other populations might, therefore, produce a more general understanding of the usefulness of genotypic variation in selection schemes.

Our overall results, based on genetic variability and general combining ability, show that organogenesis responses of currently inferior inbred lines in sunflower could be improved through genetic manipulation in a crossing program.

Acknowledgements We thank Mrs. E. Terrazoni, H. Thirad and Mr. H. Antonsanti for technical assistance during this study and Caussade Semences Seed Company for providing seed material.

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