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COMBINING ABILITY OF SUNFLOWER INBRED LINES FOR *in vitro* TRAITS

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SUMMARY

Six cytoplasmic male sterile lines used as females were crossed with four fertile lines used as males in a factorial design. The 24 F_1 hybrids obtained were evaluated *in vitro* for their organogenesis ability from cotyledonary explants. The regeneration medium was MS supplemented with 200 mg/l of glutamine, 1 mg/l of indol-3-acetic acid and 2 mg/l of kinetin. The experimental design was a complete randomized block in 2 replications. General combining ability variances were significant for some of the *in vitro* traits, while the variance due to specific combining ability was nonsignificant in all cases. Some of these positive and negative values of general combining ability were significant for the studied traits. These results suggest the importance of the additive component in the genetic control of the studied *in vitro* traits.

Key words: combining abilities, *Helianthus annuus* L., inbred lines, *in vitro* culture, organogenesis

INTRODUCTION

Biotechnology includes various methods and techniques of cellular and molecular biology which are relevant for plant breeding. Genetic progress is expected from those technologies both by saving time and increasing genetic variation (Henry *et al.*, 1994). The application of biotechnological methods to crop improvement offers the opportunity of developing new germplasm better adapted to the changing demands but a system for plant regeneration is a prerequisite for the application of such technologies. Therefore, regeneration of whole plant from tissues must be reliable and predictable. Sunflower (*Helianthus annuus* L.) has been regenerated by organogenesis or somatic embryogenesis from many tissues (Bidney and Scelonge,

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1997; Kallerhoff and Alibert, 1996; Robinson and Everett, 1990). However, regeneration by organogenesis is highly variable and is influenced by the genotype, the nature of the explant and specific medium components (Alibert *et al.*, 1994). Genotypic differences are manifested by percentage of organogenic explants, average number of shoots per regenerant explant and average number of shoots per explant plated, as reported in previous studies (Knittel *et al.*, 1991; Nestares *et al.*, 1996; Sarrafi *et al.*, 1996a and b; Berrios *et al.*, 1999). The differences observed between different cultivars during *in vitro* tissue culture with respect to regeneration result from quantitative or qualitative genetic differences (Henry *et al.*, 1994). The knowledge of the genetic basis of these differences will be useful in designing a future sunflower breeding program for tissue culturability. The objectives of this study were to analyze genetic variability and estimate combining ability of sunflower inbred lines for *in vitro* traits.

MATERIAL AND METHODS

A set of inbred lines from the Sunflower Germplasm Bank of the Estación Experimental Agropecuaria (EEA), Instituto de Tecnología Agropecuaria (INTA), Pergamino, Argentina were used in this study. Six cytoplasmic male sterile lines used as females (K-87, K-93, K119, K-127, K113, and K-97) were crossed with four fertile lines used as males (K-3232, K1989, K-1578, and K-178) in a factorial design. All inbred lines were developed at the E.E.A. I.N.T.A. Pergamino, Argentina.

The 24 F_1 hybrids were evaluated *in vitro* for their organogenesis ability. Pericarps were removed before culturing and seeds were surface sterilized in 70% ethanol for 1 min, then in 3% sodium hypochlorite followed by 3 times washing with sterile distilled water. The sterile seeds were germinated on hormone-free half strength Murashige and Skoog's medium (MS) (1962) containing 10 g/l of sucrose and solidified with 9 g/l of agar. The pH of the medium was adjusted to 6.0 and the medium was autoclaved for 20 min at 120ºC. Seeds were kept in darkness (25ºC) for 2 days and then transferred to light (25ºC and 12 h photoperiod) for another 2 days. Cotyledons were excised from the seedlings avoiding the axilary meristem. The cotyledonary explants were cultured on MS medium supplemented with 200 mg/l of glutamine, 1 mg/l of indol-3-acetic acid (IAA) and 2 mg/l of kinetin (KIN). The regeneration medium was solidified with 9 g/l of agar and the pH was adjusted to 6.0 before autoclaving. Cultures were incubated at 25±2ºC under 12 h photoperiod (35 µ mol $m^{-2}s^{-1}$) for 4 weeks. The experimental design was a complete randomized block in 2 replications. Each replication consisted of 20 explants. The following traits were studied:

Percentage of organogenic explants (PO)

PO=[number of explants giving shoots or primordia/number of explants cultured]*100.

Percentage of callusing explants (PC)

PC=[number of explants giving callus/number of explants cultured]*100.

Productivity rate (PR)

PR=[number of shoots/number of explants cultured].

Proliferation rate (FR)

FR=[number of shoots/number of explants giving shoots].

Data were subjected to an analysis of variance. The least significant difference (LSD) test was used to compare mean values of the F_1 hybrids. General combining ability (GCA) and specific combining ability (SCA) were estimated in accordance to Singh and Chaudhary (1977).

RESULTS AND DISCUSSION

The mean squares from the analysis of variance are presented in Table 1. Significant variability among crosses was observed for the *in vitro* traits percentage of organogenic explants and percentage of callusing explants. The variance due to a general combining ability of the females was significant for percentage of organogenic explants, percentage of callusing explants, and productivity rate while that of the males was significant only for percentage of callusing explants. The variance due to specific combining ability was non-significant in all cases. These results suggest that genes affecting these traits have mainly additive effects. The predominant role of additive genetic control for organogenesis-related traits observed is in accordance with previous results described by Sarrafi *et al.* (1996 a and b) in sunflower. Large proportion of the genetic variation of *in vitro* traits was demonstrated to be of the additive type in other *in vitro* culture systems. Kielly and Bowley (1997) observed that additive genetic variance was more important than non-additive genetic variance as a determinant of *in vitro* callus production in alfalfa. Ekiz and Konzak (1994) studied anther culture response in wheat and observed that most of the genetic variation for callus induction and green plant percentage was due to GCA. Additive genetic variance is also the most significant source of variation for somatic embryogenesis in red clover, black spruce, white spruce, rice, and wheat (Henry *et al.*, 1994).

Table 1: Analysis of variance for the *in vitro* traits percentage of organogenic explants (PO), percentage of callusing explants (PC), productivity rate (PR), and proliferation rate (FR)

Source	df	Mean square			
		PO	PC	PR	FR
Replication	1				
Crosses	23	416.48*	527.28*	0.05	0.64
Females (F)	5	1312.74**	999.94**	0.15*	1.58
Males (M)	3	281.81	695.02*	0.03	0.34
F x M	15	144.66	336.18	0.02	0.38
Error	23	185.94	218.52	0.04	0.61

** $p<0.01$; * $p<0.05$

Estimates of general combining ability (GCA) effects are shown in Table 2. Some of these positive and negative values of general combining ability were significant for the studied traits. The best general combiner for the organogenesis related traits was the inbred line K-87. This female parent showed high, positive and significant GCA values for percentage of organogenic explants and productivity rate. The inbred line K-113 also showed high positive GCA values for the above mentioned traits, but these values were non-significant. These two inbred lines presents potential to improve organogenesis regeneration capacity in this species. The female parent K-119 and the male one K-1989 displayed positive and significant GCA values for percentage of callusing explants. Even when plant regeneration in this species is mainly direct, there could be instances in a breeding program where large amount of callus is desired such as in the case of source of protoplast or when *in vitro* selection is conducted. In those cases the above mentioned inbred lines (K-119 and K-1989) would be useful to improve *in vitro* callus production.

Table 2: General combining ability effects for females and males for the traits percentage of organogenic explants (PO), percentage of callusing explants (PC), productivity rate (PR), and proliferation rate (FR)

Parent	PO	PC	PR	FR
Females				
K-87	13.97*	-4.75	0.18*	0.15
K-93	-11.92*	11.33	-0.09	-0.37
K-119	-18.72*	14.66*	-0.18*	-0.70*
K-127	3.98	-15.55*	-0.05	0.29
K-113	10.58	-3.68	0.15	0.47
K-97	2.11	-2.01	-0.01	0.15
Males				
K-3232	-3.98	-4.78	0.01	0.18
K-1989	-1.95	10.61*	-0.03	0.08
K-1578	-1.11	0.35	-0.05	-0.21
K-178	7.04	-6.18	0.07	-0.06

* $p < 0.05$

The F_1 hybrid K-87 x K-178 was the most favorable combination for percentage of organogenic explants, while the F_1 crosses K-93 x K-1989 and K-119 x K-1578 showed high performance for percentage of callusing explants. However, none of the hybrid combinations had positive or negative significant effect of specific combining ability for all the studied traits (Table 3).

Many agronomically elite lines regenerate poorly by organogenesis, which is a limiting factor for the application of this technology in plant breeding. The use of cross-breeding to transfer genes for regeneration ability into recalcitrant inbred lines could be an approach to improve culturability. The information on genetic variability and combining ability provided in the present study suggests the importance of the additive component in the genetic control of *in vitro* traits. Inbred lines hav-

ing a high GCA for *in vitro* reponses could be used to transfer this capacity to recalcitrant sunflower inbred lines.

Table 3: Mean values (*x*) and specific combining ability (SCA) effects of the F_1 hybrids for the traits percentage of organogenic explants (PO), percentage of callusing explants (PC), productivity rate (PR) and proliferation rate (FR)

F_1 hybrid	PO		PC		PR		FR	
	x	SCA	x	SCA	x	SCA	x	SCA
K-87 x K-3232	20.0 bcde	-12.54	27.5 bcdefg	0.86	0.32 abc	-0.07	1.41 ab	0.06
K-93 x K-3232	2.5 e	-4.14	42.5 abcde	-0.21	0.02 c	-0.12	0.50 ab	-0.32
K-119 x K-3232	2.7 e	2.92	25.8 cdefg	-20.21	0.02 c	$-7\ 10^{-3}$	0.50 ab	$6.5\ 10^{-3}$
K-127 x K-3232	26.1 bcde	3.56	5.0 g	-10.84	0.26 abc	0.09	2.00 a	0.50
K-113 x K-3232	37.5 abc	8.35	45.0 abcd	17.29	0.35 abc	-0.02	1.26 ab	-0.40
K-97 x K-3232	22.5 bcde	1.83	42.5 abcde	13.12	0.32 abc	0.11	1.50 ab	0.15
K-87 x K-1989	31.0 abcd	-3.50	48.2 abc	6.18	0.32 abc	-0.04	0.94 ab	-0.32
K-93 x K-1989	7.0 de	-1.18	65.0 a	6.89	0.07 bc	-0.03	0.50 ab	-0.23
K-119 x K-1989	2.5 e	0.63	55.0 abc	-6.44	0 c	$7\ 10^{-4}$	0 b	-0.39
K-127 x K-1989	30.0 abcde	5.42	40.0 abcdef	8.77	0.15 bc	0.02	1.5 ab	0.10
K-113 x K-1989	20.0 bcde	-11.18	35.0 abcdef	-8.11	0.30 abc	-0.03	1.99 a	0.42
K-97 x K-1989	32.5 abcd	9.80	37.5 abcdef	-7.28	0.25 abc	0.08	1.66 a	0.41
K-87 x K-1578	40.0 abc	4.59	37.5 abcdef	5.72	0.40 abc	0.06	1.10 ab	0.14
K-93 x K-1578	12.5 cde	2.98	30.0 bcdefg	-17.85	0.10 bc	0.02	0.50 ab	0.06
K-119 x K-1578	5.0 de	2.30	65.0 a	13.81	0.07 bc	0.10	0.75 ab	0.64
K-127 x K-1578	17.5 cde	-7.92	27.5 bcdefg	6.52	0.05 c	-0.05	0.50 ab	-0.61
K-113 x K-1578	27.5 abcde	-4.50	32.5 bcdefg	-0.35	0.17 abc	-0.13	1.00 ab	-0.27
K-97 x K-1578	26.1 bcde	2.56	26.6 cdefg	-7.85	0.12 bc	-0.02	1.00 ab	0.04
K-87 x K-178	55.0 a	11.45	12.5 efg	-12.76	0.50 ab	0.04	1.22 ab	0.11
K-93 x K-178	20.0 bcde	2.34	52.5 abc	11.17	0.32 abc	0.12	1.08 ab	0.49
K-119 x K-178	5.00 de	-5.85	57.5 ab	12.84	0 c	-0.09	0 b	-0.25
K-127 x K-178	32.5 abcd	-1.06	10.0 fg	-4.45	0.17 abc	-0.06	1.25 ab	$-5.4\ 10^{-3}$
K-113 x K-178	47.5 ab	7.33	17.5 defg	-8.83	0.60 a	0.17	1.68 a	0.26
K-97 x K-178	17.5 cde	-14.19	30.0 bcdefg	2.01	0.10 bc	-0.17	0.50 ab	-0.61

Mean values with the same letter are not significantly different at the 0.05 probability level (LSD test)

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APTITUD COMBINATORIA DE LÍNEAS ENDOCRIADAS DE GIRASOL PARA CARACTERES *in vitro*

RESUMEN

Se realizaron los cruzamientos entre seis líneas endocriadas androestériles y cuatro líneas endocriadas fértiles según un diseño factorial. Los 24 híbridos F_1 resultantes fueron evaluados *in vitro* por su capacidad organogénica a partir de explantos cotiledonales. El medio de cultivo fue MS suplementado con 200 mg l^{-1} de glutamina, 1 mg l^{-1} ácido indol-3-acético y 2 mg l^{-1} cinetina. El diseño estadístico fue el de bloques completamente aleatorizados con dos repeticiones. La variancia de aptitud combinatoria general resultó significativa para algunos caracteres, mientras que la variancia de aptitud combinatoria específica fue no significativa en todos los casos. Algunos de los valores positivos o negativos estimados de aptitud combinatoria general resultaron significativos. Estos resultados sugieren la importancia de las acciones génicas aditivas involucradas en el control genético de los caracteres *in vitro* estudiados.

VALEUR EN COMBINAISON DE LIGNÉES DE TOURNESOL POUR CARACTÉRES *in vitro*

RÉSUMÉ

On a fait les croisements entre six lignées androstériles et quatre lignées fertiles selon un dispositif factorial. Les 24 hybrides F_1 résultants ont été évalués *in vitro* par leur capacité organogénique à partir des explants cotylédonés. Le milieu de la culture a été MS supplémenté avec 200 mg l^{-1} glutamine, 1 mg l^{-1} acide indol-3-acétique et 2 mg l^{-1} kinetin. Le dispositif statistique a été celui de blocs complétement randomisés avec deux répétitions. La variance de l'aptitude de la combinaison générale a résulté significative pour quelques caractéres, tandis que la variance de l' aptitude de la combinaison spécifique a été pas significative dans tous les cas. Quelques-unes des valeurs positives ou négatives estimées de l' aptitude de la combinaison génerale ont donné des résultats significatifs. Ces résultats suggèrent l' importance des actions géniques additives insérées dans le contrôle génétique des caractères *in vitro* étudiés.