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 F₁ 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 Scelone,
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 F₁ 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 120°C. Seeds were kept in darkness (25°C) for 2 days and then transferred to light (25°C and 12 h photoperiod) for another 2 days. Cotyledons were excised from the seedlings avoiding the axillary meristem. The cotyledonal 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±2°C 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 F1 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. (1996a 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)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PO</td>
</tr>
<tr>
<td>Replication</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Crosses</td>
<td>23</td>
<td>416.48*</td>
</tr>
<tr>
<td>Females (F)</td>
<td>5</td>
<td>1312.74**</td>
</tr>
<tr>
<td>Males (M)</td>
<td>3</td>
<td>281.81</td>
</tr>
<tr>
<td>F x M</td>
<td>15</td>
<td>144.66</td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
<td>185.94</td>
</tr>
</tbody>
</table>

** 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)

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

* p<0.05

The F₁ hybrid K-87 x K-178 was the most favorable combination for percentage of organogenic explants, while the F₁ 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 \textit{in vitro} responses 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\textsubscript{1} hybrids for the traits percentage of organogenic explants (PO), percentage of callusing explants (PC), productivity rate (PR) and proliferation rate (FR)

<table>
<thead>
<tr>
<th>F\textsubscript{1} hybrid</th>
<th>PO</th>
<th>PC</th>
<th>PR</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x SCA</td>
<td>x SCA</td>
<td>x SCA</td>
<td>x SCA</td>
</tr>
<tr>
<td>K-87 x K-3232</td>
<td>20.0 bcde</td>
<td>-12.54</td>
<td>27.5 bcddefg</td>
<td>0.96</td>
</tr>
<tr>
<td>K-93 x K-3232</td>
<td>2.5 e</td>
<td>-4.14</td>
<td>42.5 abcd</td>
<td>-0.21</td>
</tr>
<tr>
<td>K-119 x K-3232</td>
<td>2.7 e</td>
<td>2.92</td>
<td>25.8 cdefg</td>
<td>-20.21</td>
</tr>
<tr>
<td>K-127 x K-3232</td>
<td>26.1 bode</td>
<td>3.56</td>
<td>5.0 g</td>
<td>-10.84</td>
</tr>
<tr>
<td>K-113 x K-3232</td>
<td>37.5 abcd</td>
<td>8.35</td>
<td>45.0 abcd</td>
<td>17.29</td>
</tr>
<tr>
<td>K-97 x K-3232</td>
<td>22.5 bode</td>
<td>1.83</td>
<td>42.5 abcd</td>
<td>13.12</td>
</tr>
<tr>
<td>K-87 x K-1989</td>
<td>31.0 abc</td>
<td>-3.50</td>
<td>48.2 abc</td>
<td>6.18</td>
</tr>
<tr>
<td>K-93 x K-1989</td>
<td>7.0 de</td>
<td>-1.18</td>
<td>65.0 a</td>
<td>6.89</td>
</tr>
<tr>
<td>K-119 x K-1989</td>
<td>2.5 e</td>
<td>0.63</td>
<td>55.0 abc</td>
<td>-6.44</td>
</tr>
<tr>
<td>K-127 x K-1989</td>
<td>30.0 abcd</td>
<td>5.42</td>
<td>40.0 abcddef</td>
<td>8.77</td>
</tr>
<tr>
<td>K-113 x K-1989</td>
<td>20.0 bode</td>
<td>-11.18</td>
<td>35.0 abcddef</td>
<td>-8.11</td>
</tr>
<tr>
<td>K-97 x K-1989</td>
<td>32.5 abcd</td>
<td>9.80</td>
<td>37.5 abcddef</td>
<td>-7.28</td>
</tr>
<tr>
<td>K-87 x K-1578</td>
<td>40.0 abc</td>
<td>4.59</td>
<td>37.5 abcd</td>
<td>5.72</td>
</tr>
<tr>
<td>K-93 x K-1578</td>
<td>12.5 cde</td>
<td>2.98</td>
<td>30.0 bcddefg</td>
<td>-17.85</td>
</tr>
<tr>
<td>K-119 x K-1578</td>
<td>5.0 de</td>
<td>2.30</td>
<td>65.0 a</td>
<td>13.81</td>
</tr>
<tr>
<td>K-127 x K-1578</td>
<td>17.5 cde</td>
<td>-7.92</td>
<td>27.5 bcddefg</td>
<td>6.52</td>
</tr>
<tr>
<td>K-113 x K-1578</td>
<td>27.5 abode</td>
<td>-4.50</td>
<td>32.5 abcddefg</td>
<td>-0.35</td>
</tr>
<tr>
<td>K-97 x K-1578</td>
<td>26.1 bode</td>
<td>2.56</td>
<td>26.6 cdefg</td>
<td>-7.85</td>
</tr>
<tr>
<td>K-87 x K-178</td>
<td>55.0 a</td>
<td>11.45</td>
<td>12.5 efg</td>
<td>-12.76</td>
</tr>
<tr>
<td>K-93 x K-178</td>
<td>20.0 bode</td>
<td>2.34</td>
<td>52.5 abc</td>
<td>11.17</td>
</tr>
<tr>
<td>K-119 x K-178</td>
<td>5.00 de</td>
<td>-5.18</td>
<td>57.5 abd</td>
<td>12.84</td>
</tr>
<tr>
<td>K-127 x K-178</td>
<td>32.5 abcd</td>
<td>-1.06</td>
<td>10.0 fgh</td>
<td>-4.45</td>
</tr>
<tr>
<td>K-113 x K-178</td>
<td>47.5 abcd</td>
<td>7.33</td>
<td>17.5 defg</td>
<td>-8.83</td>
</tr>
<tr>
<td>K-97 x K-178</td>
<td>17.5 cde</td>
<td>-14.19</td>
<td>30.0 bcddefg</td>
<td>2.01</td>
</tr>
</tbody>
</table>

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

ACKNOWLEDGEMENTS

The authors thank Profesora Mabel Bizet de Pratta for her assistance in translating the summary into French. The skilled technical assistance of Ing. Agr. Pedro Ludueña is gratefully acknowledged. This work was supported in part by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) grant.
APTITU

Se realizaron cruzamientos entre seis líneas endocriadas androestériles y cuatro líneas endocriadas fértiles según un diseño factorial. Los 24 híbridos F₁ 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 CARACTÈRES in vitro

RÉSUMÉ

On a fait les croisements entre six lignées androstériles et quatre lignées fertiles selon un dispositif factorial. Les 24 hybrides F₁ résultats 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 kinétine. Le dispositif statistique a été celui de blocs complètement randomisés avec deux répétitions. La variance de l’aptitude de la combinaison générale a résulté significative pour quelques caractères, 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 générale ont donné des résultats significatifs. Ces résultats suggèrent l’importance des actions géniques additives insérées dans le contrôle génétique des caractères in vitro étudiés.