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Genetic analysis of anther culture response in maize

J. F. Petolino and S. A. Thompson

United AgriSeeds Inc., P.O. Box 4011, Champaign, IL 61820, USA

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Summary. Response frequencies in maize (*Zea mays* L.) anthers cultured in vitro were examined in a diallel set of crosses among four commercial inbred lines. Significant differences among the genotypes were observed, with the crosses H99×FR16 and Pa91×FR16 displaying the highest responses. General (GCA) and specific (SCA) combining ability mean squares were calculated and determined to be highly significant. GCA effects among the parental lines were highest for FR16 and lowest for LH38. Nongenotypic, plant-to-plant differences were also found to make a significant contribution to the overall variation observed. The results from this study indicate that parents which give rise to highly responsive hybrids can be identified and that genetic improvement is possible through selection.

Key words: Androgenesis – Haploids – Plant tissue culture

Introduction

Anther culture is a means by which, theoretically, large numbers of haploid plants can be produced. Unfortunately, although rapid in some species (Bajaj 1983), progress in maize anther culture has been slow (Nitsch et al. 1982). The major problems have been the relatively low anther response frequencies and the difficulties associated with plant regeneration and chromosome doubling. Any attempt to use anther culture in commercial maize breeding will require a considerable improvement in the overall efficiency of doubled haploid seed recovery. Although reports of pollen plant production in maize date back to 1974 (Ku et al. 1978), only the Chinese claim to routinely use this technique for line development (Wu 1986).

Most reports of successful maize anther culture outside of China have involved germplasm which is not of commercial importance (Brettell et al. 1981; Ting et al. 1981; Genovesi and Collins 1982; Dieu and Beckert 1986; Pace et al. 1987). Anther culture-responsive germplasm that is of interest to commercial maize breeders has only recently been identified (Petolino and Jones 1986). Based on the significant genotype effects on anther responsiveness observed in some studies (Ku et al. 1978; Genovesi and Collins 1982; Petolino and Jones 1986; Dieu and Beckert 1986), it can be concluded that genetic factors are an important determinant of the level of response to anther culture. Thus, a genetic approach may provide a solution to some of the efficiency problems associated with maize anther culture.

The transfer of culturability to commercially important germplasm has been suggested as a means of broadening the applicability of tissue culture technique (Dieu and Beckert 1986). However, little detailed study has been done on the genetics of anther culturability in maize. Breeding for improved anther response will require a better understanding of the inheritance of this trait. The present study was designed to determine the importance of general (GCA) and specific combining ability (SCA) effects for anther culturability in a diallel mating of four inbred lines.

Materials and methods

The inbred lines used as parents in this study were H99, LH38, Pa91, and FR16. They were chosen for their known capacity for anther culture (Petolino and Jones 1986). Seed was originally obtained from Holden's Foundation Seeds, Williamsburg, USA (H99, LH38, and Pa91) and Illinois Foundation Seeds, Tolono, USA (FR16). All lines were maintained by

controlled self-pollination for 2 years prior to being used for crossing.

Donor plants were either greenhouse-grown during November to December 1985 or field-grown during July to August 1986 in Champaign, USA. Tassels with anthers containing late uninucleate-early binucleate microspores, as determined by acetocarmine squash, were removed from donor plants prior to emergence from the whorl. Tassels were then wrapped in moist paper towels, covered with aluminum foil, and maintained at 8 °C for 14 days. Before anther excision, tassels were surface sterilized for 15 min in a 0.5% sodium hypochlorite solution followed by a sterile water rinse. Only anthers from the central portion of the main tassel branch were used.

Then 60 anthers were placed in a 20 by 60 mm Petri dish containing 20 ml of medium. The medium consisted of YP basal salts (Ku et al. 1978) with the addition of 5.0 g/l activated charcoal, 500 mg/l casein hydrolysate, 0.1 mg/l 2,3,5-triiodobenzoic acid, 120 g/l sucrose, and 8.0 g/l agar (Gibco) adjusted to pH 5.8. Typically, 3–6 dishes were obtained from each tassel harvested. A total of 25,140 anthers from 108 tassels were cultured in 419 dishes.

Dishes containing freshly plated anthers were sealed with Parafilm and placed in plastic boxes covered with aluminum foil. After 1 week in the dark at 28 °C, dishes were transferred to clear boxes and grown under cool white florescent lights (60 μmol m^{-2} s^{-1}) with a 16 h photoperiod. Then 4 to 6 weeks after transfer to the lighted environment, anthers with recognizable embryo-like structures were counted. The structures were yellowish-white and globular in appearance and resembled zygotic embryos displaying varying degrees of abnormal tissue proliferation. Usually one embryo-like structure was produced per responding anther, however multiple responses were not uncommon.

Response frequency, expressed as the percentage of plated anthers that produced at least one embryo-like structure, was the variable analyzed in all instances. A square root transformation of the data did not significantly alter the relative sizes of pertinent sums of squares and was thus – not used. The HIERARCH subprogram of the MSTAT version 3.0 statistical program (Michigan State University, East Lansing, USA) was used to calculate an hierachical analysis of variance (ANOVA). Total sums of squares were partitioned among sources due to treatments (environment-cross combinations), plants within treatments, and dishes within plants.

For combining ability analysis, the plant was considered to be the experimental unit. The NONORTHO subprogram of MSTAT version 3.0 was used for this analysis. The experiment was analyzed as a completely random design. As a consequence of the unequal replication in this study, ANOVA was performed by first fitting environments, followed by crosses corrected for environments. Combining ability analysis was performed using corrected cross means according to the method of Griffing (1956; Method 4, Model I). The sums of squares due to general combining abilities (GCA) were calculated directly and those due to specific combining abilities (SCA) were obtained by subtraction.

Results and discussion

Out of 25,140 anthers plated in this study, 600 responded by producing embryo-like structures for an overall frequency of 2.4%. A distribution of response frequencies from individual plants is presented in Fig. 1. Although skewed toward the lower values, the percentage of anthers responding was as high as 18.3%. An hierarchical ANOVA of the anther response data, using individual dishes as the experimental unit, is presented in Table 1. The highly significant plants within treatments mean square is consistent with previous work (Pace et al. 1987) and suggests that non-genotypic, plant-to-plant differences in response make a substantial contribution to the overall variation observed. Thus, when screening maize germplasm for anther culture responsiveness, it is advantageous to sample several plants from a given genotype.

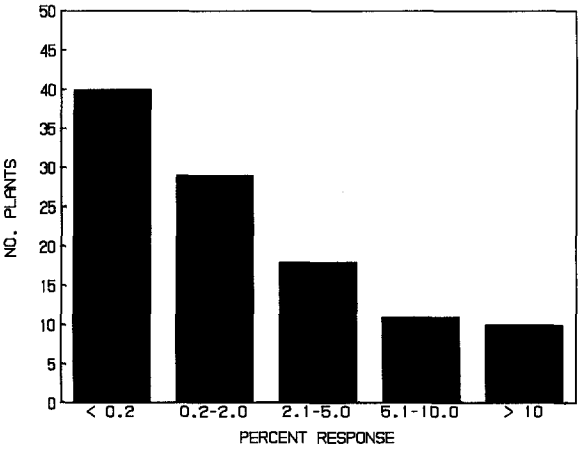

Fig. 1. Frequency distribution of anther response from individual maize plants

Table 1. Hierarchical analysis of variance of anther response in maize

Source of variation	df	Mean square
Treatments (T)	11	199.1*
Plants (P) within T	96	38.5*
Dishes within P	313	7.4

* Significant at the 0.01 level

Mean response frequencies for the six crosses ranged from 0% to 5.7% and from 0% to 6.3% for the greenhouse and field experiments, respectively (Table 2). The results were relatively consistent between the two environments with H99×FR16 and Pa91×FR16 displaying the highest responses. The response frequencies observed in the present study are in the range of those previously reported (Petolino and Jones 1986). An ANOVA for anther response combined over experiments is presented in Table 3. Highly significant effects due to crosses were observed whereas the crosses×environments interaction mean square was not significant.

The GCA mean square was highly significant, thus, the average value of a line was important in predicting

Table 2. Mean anther response from cultured anthers of crosses of maize lines

Cross	Greenhouse		Field	
	Anthers plated	% response	Anthers plated	% response
H99×LH38	1,800	0.0	2,580	0.0
H99×Pa91	1,560	0.9	1,560	1.9
H99×FR16	2,520	5.7	2,460	6.3
LH38×Pa91	2,820	0.6	1,860	2.2
LH38×FR16	2,040	0.2	2,520	1.7
Pa91×FR16	2,340	3.6	1,140	6.1
LSD (0.05)		3.5		3.2

Table 3. Analysis of variance and combining ability mean squares of anther response in maize

Source of variation	df	Mean square
Among treatments	11	71.5*
Environments (E)[a]	1	31.0 NS
Crosses (C)[b]	5	144.6*
GCA	3	177.3*
SCA	2	100.0*
C×E	5	5.4 NS
Within treatments	96	12.4

* Significant at the 0.01 level
[a] Ignoring crosses
[b] Adjusted for environments
NS: not significant at the 0.05 level

Table 4. General combining ability (GCA) effects for anther response in maize. Numbers in a column followed by the same letter are not significantly different at the 0.05 level

Inbred	Effect
FR16	+2.5 a
H99	+0.3 b
Pa91	+0.1 b
LH38	−2.5 c

the response of a given cross (Table 4). This pattern is usually a function of additive gene effects and their interactions and is typical of quantitatively inherited traits in maize. These results indicate that, within this set of inbreds, progress can be made by selecting for highly responsive lines.

SCA effects were also significant (Table 3) such that individual hybrids do deviate from the average performance of their parents. Thus, dominance or dominant types of epistasis may also play a role in the anther culture response. The results from this study suggest that, once high general responders are identified, evaluations in various hybrid combinations are needed to distinguish the most responsive specific crosses.

The use of anther culture for maize breeding is absolutely dependent on the ability to recover doubled haploid seed. The efficiency of doubled haploid production is a function of at least three components: anther response, plant regeneration, and chromosome doubling. The present study indicates that parents which give rise to highly responsive hybrids can be identified and that genetic improvement is possible through selection. However, the development of efficient procedures for plant regeneration and chromosome doubling are also required. Moreover, the technique must be available across a broad spectrum of elite germplasm.

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