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Genetic studies of corn (*Zea mays* L.) anther culture response

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Summary. Anthers of two maize (*Zea mays* L.) inbred lines, DBTS (P_1) and B73 (P_2), their F_1, F_2 and first backcross generations – $F_1 \times$ DBTS (B_1), and $F_1 \times$ B73 (B_2) – were float cultured in YP medium to study the inheritance of corn anther culturability using generation mean analysis. Significant effects of generation were observed for the three traits measured: anther response (%), frequency of embryos (%) and anther productivity. Variation among the generations was similar for anther response and frequency of embryos: no significant differences were found among the P_1, F_1, F_2 and B_1 means, but the means of P_2 and B_2 were significantly lower than those of the other generations. For anther productivity, the F_2 generation tended to have a slightly higher tendency for multiple embryo formation. A simple additive-dominance model was adequate in explaining the inheritance of anther response and frequency of embryos, but digenic epistasis (additive × dominance) was involved in the inheritance of anther productivity. Additive genetic variance was higher than non-additive genetic variance for all the traits; however, only environmental variance was significant. Narrow-sense heritability estimates were 65% and 75% for anther response and frequency of embryos, respectively. Significant inter-plant variation was observed within generations, even for the inbred line DBTS, but isozymic analysis involving five enzyme loci did not reveal any genotypic variability within the inbred lines DBTS and B73.

Key words: Corn – Anther culture – Genetic studies – Generation mean analysis

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Introduction

The potential of doubled haploids (DH) in maize (*Zea mays* L.) breeding has long been recognized (Chase 1969). The superiority of the DH scheme lies in its unique form of gamete selection (Kasperbauer et al. 1980), which permits both dominant and recessive traits to be expressed. This enhances selection for favourable traits. Furthermore, by doubling haploid genomes to obtain DH lines, additive genetic variance is doubled while dominance variance is eliminated (Choo and Kannenberg 1978).

Chase (1969) has given and elaborate account of the various methods of identifying spontaneous haploid maize from normal diploid kernels using genetic markers. One method, the indeterminate gametophyte (ig) system (Kermicle 1969), has been reported to produce haploids at a frequency of about 3%. However, at Guelph, where it is necessary to incorporate the ig gene into short-season backgrounds, this system has not been successful to date in producing haploids at an acceptable frequency. A major problem is poor penetrance of the purple plumule seed marker (Kannenberg unpublished). An attractive alternative for the production of haploids is anther culture, which has been successfully applied to over 200 species (Keller et al. 1987), or microspore culture. In maize, DH plants have been derived from cultured anthers (Ku et al. 1978; Genovesi and Collins 1982; Dieu and Beckert 1986; Petolino and Jones 1986), and from isolated microspore cultures (Coumans et al. 1989) and shed-pollen culture (Pescitelli et al. 1989).

Anther culture response is controlled by the genotype of the donor plants and non-genetic factors. Whilst studies on the non-genetic component have produced some dramatic increases in anther response, such factors are more difficult to fix than the genetic component. Changing the genetic component has the added advantage that

it can be manipulated in a desired direction (Deaton et al. 1987). As most of the anther-culture responsive maize materials have been found in non-commercial germ plasm (Genovesi and Collins 1982), Dieu and Beckert (1986) suggest that culturability be transferred from the responsive, non-commercial, exotic maize germ plasm into elite types. Furthermore, anther culture per se constitutes a selective process for increased androgenesis (Picard and DeBuyser 1977).

Petolino and Thompson (1987) studied the inheritance of anther responsiveness in elite maize germ plasm using a diallel mating design. However, they did not directly evaluate generations such as the F_2, and backcrosses, which are important in the transfer of a trait from a responsive to a non-responsive variety. The objective of our research was, therefore, to determine the inheritance of the ability of maize genotypes to produce anther-derived embryoids and plantlets using a generation mean analysis approach (Jinks and Jones 1958; Gamble 1962; Mather and Jinks 1982).

Materials and methods

Two inbred lines, DBTS (P_1) and B73 (P_2), their single cross hybrid (F_1 = B73 × DBTS), F_1 selfed (F_2), and first backcross generations, F_1 × DBTS (B_1) and F_1 × B73 (B_2), were used in these studies. Seed of all generations was kindly supplied by Dr. R. Hamilton (Agriculture Canada Experimental Farm, Ottawa). DBTS has been reported to be one of the highest responding genotypes (Pace et al 1987), while crosses involving B73 have been either non-responsive or weakly responsive (Genovesi and Collins 1982).

Anther donor plants were grown in the indoor growth facilities of the Crop Science Department, University of Guelph, 1988 and 1989. Seeds were sown in 28 litre pails (one seed per pail) containing surface as the potting medium. Plants were irrigated every other day with a solution containing fertilizer (80 g 28-14-14; 80 g 15-15-15), 40 g NH_4NO_3, 80 g $MgSo_4 \cdot 7H_2O$ and 70 ml HCl; total volume was made up to 200 l and the pH was adjusted to about 5.4–5.9) (M. Tollenaar, University of Guelph, personal communication). Light intensity was about 400 $\mu Em^{-2} s^{-1}$ for a 16/8 (light dark) photoperiod, and temperatures were about 28 °C (day) and 22 °C (night).

Tassels were harvested from donor plants just before emergence from the whorl. All leaves were removed from the tassels before they were wrapped in moistened cheese-cloth and subsequently in aluminium foil. The tassels were then cold pretreated at 10 °C for 14 days. At the end of the cold pretreatment, sections of tassels with anthers having microspores at the late-uninucleate stage were determined by squashing and staining with Azur A (Sigma, St. Louis, MO, USA). These anthers were then float cultured in liquid YP basal medium (Ku et al. 1978) and supplements (Genovesi and Collins 1982).

For each plant, 20 anthers were placed in 3 ml induction medium in 60 × 15 mm (Falcon 3002) petri dishes. Generally, between six and nine petri dishes were used for each plant, but less than six or more than nine petri dishes were used in some instances, depending on the availability of anthers with microspores at the appropriate stage. The cultures were incubated in darkness in a growth chamber at a temperature of approximately 28 °C (±2°).

After about 3 weeks, embryo-like structures (ELS) that had attained a diameter of about 1–2 mm were lifted and placed on YP regeneration medium (Petolino and Jones 1986). When plantlets had developed, they were transferred to YP regeneration medium (without sucrose of hormones). The growth chamber light intensity for the regenerating cultures was about 130 $\mu Em^{-2} s^{-1}$, and the temperature was about 28 °C (day) and 22 °C (night) for a 16/8 (light/dark) photoperiod.

Statistical analysis

Data analysis involved a nested design in which petri dishes were nested within plants, which in turn were nested in genotypes (generations). Plants were considered as replications in a mixed model with generations as fixed effects, and plants and petri dishes as random effects. For each petri dish the following variables were measured:

1) anther response, i.e. number of anthers producing at least one embryo-like structure (ELS),
2) frequency of embryos, i.e. number of ELS divided by total number of anthers cultured
3) anther productivity, i.e. number of embryo-like structures produced by responding anthers.

These variables were converted into percentages. Data analysis involved least squares measures, (SAS 1982). Means of the three traits were subjected to the following model:

$$Y_{ijk} = \mu + x_i + \beta_{ij} + E_{ijk}$$

where Y_{ijk} is the effect of the Kth petri dish in the jth plant of the ith genotype; xi is the effect of the ith genotype; β_{ij} is the effect of the jth plant in the ith genotype; E_{ijk} is the random error (or effect of the Kth petri dish within the jth plant within the ith genotype). Values analysed were the log(mean + 1) transformed scale (Steel and Torrie 1980) for each trait.

Genetic model

A generation mean analysis approach was used in the estimation of genetic parameters. The A, B, C scaling tests (Mather and Jinks 1982) were determined. The genetic component was further partitioned into six comparisons (Table 1) to determine gene effects according to the six-parameter linear model (Jinks and Jones 1958; Gamble 1962):

$$Y_{ijk} = \mu + a + d + aa + ad + dd$$

where μ = m = overall mean; a = additive gene effect; d = dominance gene effect; aa = additive × additive gene effect; ad = additive × dominance gene effect; dd = dominance + dominance gene effect.

Table 1. Coefficients for the six-parameter model (Jinks and Jones 1958; Gamble 1962)

Test	P_1	P_2	F_1	B_1	B_2	F_2
m	0	0	0	0	0	1
[a]	1/2	−1/2	0	0	0	0
[d]	−1/2	−1/2	1	2	2	−4
[aa]	0	0	0	2	2	−4
[ad]	−1/2	1/2	0	1	−1	0
[dd]	1	1	2	−4	−4	4

Table 2. Effect of generation on anther response, frequency of embryos, anther productivity and plant production

Generation	Number cultured		Number of plants responding	Anther response (%)	Frequency of embryos (%)	Anther productivity	Number of plants
	plants	anthers					
P_1	22	2,820	18	4.49	7.70	1.56	4
P_2	7	1,024	0	0.00	0.00	0.00	–
F_1	14	2,336	11	2.81	4.14	1.33	–
B_1	13	1,696	8	2.29	3.38	1.31	–
B_2	12	1,744	4	0.52	0.52	1.00	–
F_2	11	1,696	7	3.47	8.06	1.75	6

Variance components were estimated by the method of Mather and Jinks (1982) as follows:

$V_E = 0.3\,(\text{var } P_1 + \text{var } P_2 + \text{var } F_1)$

$V_A = 2\,(2 \text{ var } F_2 - \text{var } B_1 - \text{var } B_2)$

$V_D = 4\,(\text{var } F_2 - 0.5\, V_A - V_E)$;

where V = variance estimate; E = environment; A = additive; D = non-additive. Narrow-sense heritability (h^2 n.s) was estimated by Warner's (1952) method as follows:

$h^2 \text{ n.s.} = [1/2\, V_A]/[1/2\, V_A + 1/4\, V_D + V_E]$

The variance of the variance components was estimated by the method of Searle (1971): $\text{var}(V) = 2c^2\,(V)^2/df$ (where df = degrees of freedom; c = coefficient of variance estimate).

Results

Embryoids emerged from anthers within 3–4 weeks following culture initiation. Most often the responding anthers produced single embryoids; very rarely they produced multiple embryoids. Very few embryoids differentiated into plantlets. Only two genotypes, DBTS (P_1) and the F_2, differentiated into plants (Table 2) and all of these regenerated plants were phenotypically normal. However, although the tassels and ears were normal in appearance, they were highly protandrous, with the time difference between tassel and silk emergence being up to 3 weeks in most cases. Flow cytometric analysis of the leaf DNA content (Galbraith et al. 1983) of one DBTS- and three F_2-regenerated plantlets and of normal diploid DBTS and F_2 donor plants indicated that the regenerants had the same amount of DNA as the donor plants.

Anther response, frequency of embryos and anther productivity varied with generation (Table 2). The trend of variation among the generations was the same for anther response (%) and frequency of embryos (%). Analysis of the log-transformed data showed no significant ($P \geq 0.05$) difference among the P_1, F_1, F_2 and B_1 means for both traits (Table 3), but the means of the P_2 and B_2 were significantly lower than those of the other generations ($P \leq 0.05$). Anther productivity, which was a measure of the degree of multiple embryo formation (based on responding anthers), showed that the F_2 generation had a slightly higher tendency for multiple embryos than the other generations (Table 2). However, the pattern of variation among the generations for anther productivity was similar to the other two traits, except that B_2 was significantly higher than P_2 ($P \leq 0.05$). The differences among P_1, F_1, F_2 and B_1 were not significant.

Results of the A, B, C scale tests and estimates of the six genetic parameters are presented in Table 4. The scale tests were not significant for anther response and frequency of embryos (estimates greater than two times their standard error). Of the six genetic parameters, only mean effect and additive gene action were significant for anther

Table 3. Generation effect on anther response, frequency of embryos and anther productivity [log (% +1) transformed scale]

Genotype	Anther response (mean ± SE)	Frequency of embryos (mean ± SE)	Anther productivity (mean ± SE)
DBTS	1.31 ± 0.20 a	1.62 ± 0.24 a	0.92 ± 0.05 a
B73	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
F_1	1.06 ± 0.20 a	1.22 ± 0.24 a	0.84 ± 0.04 ab
B_1	0.87 ± 0.23 ab	0.99 ± 0.28 ab	0.82 ± 0.06 ab
B_2	0.30 ± 0.14 bc	0.30 ± 0.14 bc	0.69 ± 0.00 b
F_2	1.12 ± 0.29 a	1.36 ± 0.39 a	0.94 ± 0.14 a

Means within the same column followed by different letters are significant ($P < 0.05$)

Table 4. Estimates of scaling tests and the six-parameter model for anther response, frequency of embryos and anther productivity

Scaling test	Trait (estimate ± SE)		
	Anther response	Frequency of embryos	Anther productivity
A	−0.63 ± 0.52	−0.85 ± 0.63	−0.12 ± 0.14
B	−0.46 ± 0.59	−0.62 ± 0.72	0.55 ± 0.04
C	1.05 ± 1.11	1.37 ± 1.35	1.18 ± 0.55
m	1.12 ± 0.24	1.36 ± 0.29	0.94 ± 0.14
a	0.65 ± 0.17	0.81 ± 0.21	0.46 ± 0.02
d	−1.73 ± 1.19	−2.43 ± 1.45	−0.37 ± 0.56
aa	−2.14 ± 1.16	−2.84 ± 1.41	−0.74 ± 0.56
ad	−0.08 ± 0.36	−0.12 ± 0.44	−0.33 ± 0.07
dd	3.24 ± 1.70	4.31 ± 2.06	0.31 ± 0.61

Table 5. Variance components and heritability for anther response, frequency of embryos and anther productivity

Variance component	Trait (variance ± SE)		
	Anther response	Frequency of embryos	Anther productivity
V_E	0.49 ± 0.12	0.70 ± 0.17	0.05 ± 0.01
V_A	1.83 ± 1.77	4.16 ± 3.10	0.45 ± 0.30
V_D	−1.92 ± 2.12	−4.45 ± 3.51	−0.60 ± 0.31
Heritability (n.s.)	65%	75%	–

response. Hower, two of the digenic effects (aa and dd), in addition to mean and additive gene effect, were significant for frequency of embryos. On the other hand, the B and C scale tests were significant for anther productivity. The mean and additive gene effects, as well as additive × dominance epistasis, were significant for anther productivity. Dominance gene action was negative for all traits, but was not significant for any trait.

While additive genetic variance (V_A) was higher than non-additive genetic variance (V_D), with the latter negative for all traits, neither V_A nor V_D was significant (Table 5). On the other hand, environmental variance (V_E) was significant for the three traits.

Plant-to-plant variation within a generation

Plant-to-plant variation within a generation was significant for both anther response (%) and frequency of embryo production (%), even in the inbred line parent DBTS. B73, however, remained uniformly non-responsive. The variance among plants was higher in the DBTS inbred line than in the segregating generations of F_2, B_1 and B_2 for anther response. However, for frequency of embryos, inter-plant variation was higher in the F_2 generation than in all other generations, including DBTS, although the variance among DBTS plants was higher than in the other segregating generations. The variances among plants for both traits was higher in B_1 than B_2, while that of the F_1 was nearly the average of the two inbred line parents.

This plant-to-plant effect was most dramatic in the F_2 generation for frequency of embryos where a single plant (F_2 plant number 8) accounted for more than 50% of the overall F_2 mean and about 95% of the total F_2 variance (Fig. 1).

Isozymic analysis of inbred lines (DBTS and B73)

DBTS and B73 were analysed isozymically according to the method of Stuber et al. (1988) in order to investigate whether the variability in anther response and frequency of embryos among plants within supposedly "genetically uniform" generations was attributable to underlying genetic variability. The enzymes studied were acid phos-

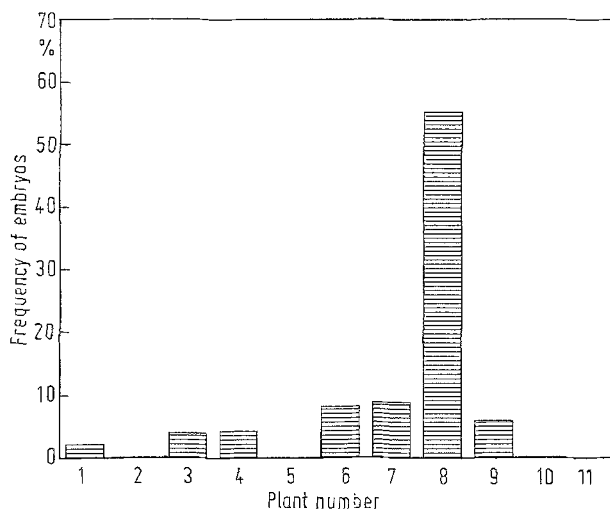

Fig. 1. Plant-to-plant effect

phatase (ACP-1), phosphohexose isomerase (PHI-1), isocitrate dehydrogenase (IDH-1, and IDH-2) and malate dehydrogenase (MDH-1). No variability or unexpected genotypes were observed among the 80 seedlings of B73 and 45 seedlings of DBTS that were analysed.

Discussion

The non-significance of the A, B, C scale tests for anther response agrees with estimates of the six-parameter model. Digenic gene action (epistasis) was not significant in either model. Thus, the simple additive-dominance model was adequate to explain the inheritance of anther response: additive gene effect was more important than dominance in controlling the trait. However, for frequency of embryos the estimates of the scale tests did not agree with results of the six-parameter test. While the scale tests were not significant, suggesting that epistasis was not important in the inheritance of this trait, the six-parameter model indicated significant additive × additive, and dominance × dominance epistasis. Disagreement between the interpretation of scale tests and other quantitative methods of detecting epistasis from generation mean analysis has been noted previously (Ketata et al. 1976). One argument against the scale tests is that not all six generations are considered together in the estimates. The presence of epistasis for frequency of embryos indicates that the estimates of the components of genetic variance (additive and non-additive genetic variances) are no longer unique, but are contaminated by epistasis (Mather and Jinks 1982; Ketata et al. 1976). It is likely that additive genetic variance has been overestimated while non-additive genetic variance has been underestimated.

The high heritability estimate for anther response suggests that relatively rapid genetic gain can be made in

transferring this trait from responsive to non-responsive germ plasm. Another indication of the relative simple inheritance of anther response is that the generation means of F_2, F_1, and B_1 were not significantly different from the mean of the high parent (DBTS). An important finding was that the backcross of the F_1 to the non-responsive parent (B73) responded to anther culture, although weakly.

The significant inter-plant variation observed here supports previous reports on significant inter-plant effects in corn anther culture (Pace et al. 1987). The inter-plant variation was very conspicuous in the F_2 generation where a single plant accounted for over 50% of the generation mean. Frequency of embryos for this plant was about 1.5 times the highest DBTS plant response and is the highest reported for corn anther culture to date. Keller and Armstrong (1983) also observed that single plant embryo yields could significantly affect the overall treatment mean. In their study they observed that a single plant of *Brassica oleracea* (cv 'Italica') increased the treatment mean by a factor of six. The *Brassica* cultivar used was a cross-pollinating species with a high level of self-incompatibility; hence the authors suggested that such inter-plant variation was due largely to genotypic differences. The influence of non-genetic factors was acknowledged, however. The F_2 generation in the present study was a segregating population; therefore, the plant-to-plant variation observed likely resulted to a large extent from genetic differences. Anther culture has been proposed as a method that involves a random sample of the gametic array. Thus, the high yielding F_2 plant was probably one of such random gametes in which was combined favourable alleles for anther culture response. Five anther-derived plants were obtained from this plant alone.

Plant-to-plant variation was observed among "genetically uniform" generations as well. The variation among DBTS plants was about twice that of the F_1 plants. The high variability among DBTS plants especially led to the suspicion that there might be some amount of residual heterozygosity in the population. However, isozymic analysis of enzyme loci suggested that both DBTS and B73 were homozygous and homogeneous, at least for the five enzyme loci surveyed. It is possible that five enzyme loci are not enough to detect variability within the cultivars. However, similar inter-plant variations were observed by Lazar et al. (1984) among donor plants from a wheat (*Triticum aestivum*) inbred line. They attributed the variation to the fact that most conventional varieties are not "pure lines", but mixtures of different lines with uniform agronomic attributes, and suggested that non-uniformity could exist especially for characteristics that the breeder did not consider when developing that variety. They proposed that selection for increased anther culturability could be practised even within an "inbred" line.

An inference from the significant inter-plant variation is that a large number of plants have to be sampled for meaningful genetic studies of anther culture response. However, the non-genetic component of anther culture response is difficult to fix. This would mean that the exact number of plants to be sampled would be difficult to specify.

Variance components are generally defined as positive, but negative variance components are rather common in research. For instance, Reeder et al. (1987) reported that about 21% of the estimates of additive × environment and dominance × environment variance components were negative. The possible causes of such negative variance estimates have been discussed (Searle 1971; Reeder et al. 1987). These include: inadequate sample sizes or experimental design, recording or computation errors, or the invalid assumption of uncorrelated identically distributed random variables. There is also an inherently small (but not necessarily zero) probability that the difference between mean squares will be negative, even though the difference of the expected values is positive. Furthermore, natural or artificial selection during the development of populations could distort the genotypic frequency and invalidate variance component estimates subsequently (Kearsey 1970).

The sample sizes within the various generations in the present study might not have been adequate. This inadequacy could have resulted in the negative estimate of non-additive genetic variance and the large standard error about the additive variance component. The theoretical sample sizes required for meaningful estimates of variance components have been addressed by Kearsey (1970) but it seems impractical to use an adequate sample in any kind of mating design in view of the large numbers required. However, inbreeding prior to crossing reduces the required sample size and also minimizes the error about the variance component estimates. Inbred line parents were included in the mating design of the present study. Thus, other factors could have contributed to the negative non-additive variance estimate. For example, the significance of the environmental component of variance probably resulted in large standard errors. Another explanation could be that the inbred line, DBTS, was not adapted to the environment used in growing the anther donor plants, and hence was unstable.

The mating design used here (generation mean approach) has the advantage that the relative importance of additive, dominance or epistastic gene effects would be valid in spite of the non-significance of the variance components, although such estimates would not be population parameters (Kearsey 1970) but specific for the genotypes evaluated. Thus, for the populations studied in the present work, additive gene action was relatively more important in the inheritance of anther response and frequency of embryos, but epistasis was involved in anther productivity.

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References

Chase SS (1969) Monoploids and monoploid-derivatives of maize (*Zea mays.* L). Bot Rev 35:117–167

Choo TM, Kannenberg LW (1978) The efficiency of using doubled haploids in a recurrent selection program in a diploid, cross-fertilized species. Can J Genet Cytol 20:505–511

Coumans MP, Sohota S, Swanson EB (1989) Plant development from isolated microspores of *Zea mays* L. Plant Cell Rep 7:618–621

Deaton WR, Metz SG, Armstrong TA, Mascia PN (1987) Genetic analysis of the anther-culture response of three spring wheat crosses. Theor Appl Genet 74: 334–338

Dieu P, Beckert M (1986) Further studies of androgenetic embryo production and plant regeneration from in vitro cultured anthers of maize (*Zea mays* L). Maydica 31:245–259

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E (1983) Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220:1049–1051

Gamble EE (1962) Gene effects in corn (*Zea mays* L). I. Separation and relative importance of gene effects for yield. Can J Plant Sci 42:339–348

Jinks JL, Jones RM (1958) Estimation of the components of heterosis. Genetics 43:223–234

Kasperbauer MJ, Buckner RC, Springer WD (1980) Haploid plants by anther-panicle culture of tall fescue. Crop Sci 20:103–107

Kearsey MJ (1970) Experimental sizes for detecting dominance variation. Heredity 25:529–542

Keller WA, Armstrong KC (1983) Production of haploids via anther culture in *Brassica oleracea* var 'italica'. Euphytica 32:151–159

Keller WA, Arnison PG, Cardy BJ (1987) Haploids from gametophytic cells – recent developments and future prospects. In: Green CE, Sommers DA, Hackert WP, Biesboer DD (eds). Plant tissue and cell culture. Allan R Liss, New York, pp 223–241

Kermicle JL (1969) Androgenesis conditioned by a mutation in maize. Science 166:1422–1424

Ketata H, Edwards LH, Smith EL (1976) Inheritance of eight agronomic characters in a winter wheat cross. Crop Sci 16:19–22

Ku MK, Cheng WC, Kuo LC, Kuan YL, An HP, Huang CH (1978) Induction factors and morpho-cytological characteristics of pollen-derived plants in maize (*Zea mays*). In: Proc Symp Plant Tissue Culture, 25–30 May, 1978. Science Press, Peking, pp 35–42

Lazar MD, Schaeffer GWS, Baenzinger PS (1984) Cultivar and cultivar × environment effects on the development of callus and popyhaploid plants from anther cultures of wheat. Theor Appl Genet 67:273–277

Mather K, Jinks JL (1982) Biometrical genetics: the study of continuous variation, 3rd en. Chapman and Hall, London

Pace GM, Reed JN, Ho LC, Fahey JW (1987) Anther culture of maize and the visualization of embryogenic microspores by fluorescent microscopy. Theor Appl Genet 73:863–869

Pescitelli SM, Mitchell JC, Jones AM, Pareddy DR, Petolino JF (1989) High frequency androgenesis from isolated microspores of maize. Plant Cell Rep 7: 673–676

Petolino JF, Jones AM (1986) Anther culture of elite genotypes of maize. Crop Sci 26:1072–1074

Petolino JF, Thompson SA (1987) Genetic analysis of anther culture response in maize. Theor Appl Genet 74:284–286

Picard E, DeBuyser J (1977) High production of embryoids in anther culture of pollen derived homozygous spring wheats. Ann Amélior Plant 27:483–488

Reeder LR Jr, Hallauer AR, Lamkey KR (1987) Estimation of genetic variability in two maize populations. J Heredity 78:372–376

SAS Institute Inc (1982) SAS user's guide: statistics. SAS Institute Inc, Cary N.C.

Searle SR (1971) Topics in variance component estimation. Biometrics 27:1–76

Steel RGD, Torrie JH (1980) Principles and procedures of statistics: a biometrical approach, 2nd edn. McGraw-Hill, New York,

Stuber CW, Wendel JF, Goodman MM, Smith JSC (1988) Techniques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L). North Carolina ARS, North Carolina State University, Technical Bulletin 286

Warner JN (1952) A method of estimating heritability. Agron J 44: 427–430