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Genetic analysis of the anther-culture response of three spring wheat crosses

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Summary. Anther-culture response was examined among three spring wheat (*Triticum aestivum* L.) cultivars to evaluate the genetic component of response and to determine whether androgenetic performance could be improved by selection. The three lines, the three possible F_1's among the three lines, their F_2's, and the backcrosses to the parents were evaluated for callus production and regeneration capacity. Significant variation was observed among the generations of the three crosses for callus formation. Genetic variation for regenerability was nonsignificant. Callus production was negatively correlated (–0.24) with regeneration capacity. The random variation in the study was too great to determine whether major-gene differences for anther-culture response exist among the three lines by examining population distributions. When the material was evaluated for quantitative gene effects, the estimates for the additive gene effects were generally greater than the estimates for the dominance gene effects for callus formation. Only the Pavon × Chris cross, however, exhibited a significant narrow sense heritability estimate for callusing response (0.94). Due to the large component of random variation and the varying selection potential among crosses for androgenetic performance, improving anther-culture response in wheat by selection could prove difficult unless the anther-culture process itself selects for response traits at the gametic level.

Key words: *Triticum aestivum* L. – Haploid – Regeneration – Heritability

Introduction

Haploids derived from microspores by anther culture have considerable potential as breeding material in crop improvement programs (Collins and Genovesi 1981). This potential is being realized in some crop species because of their excellent response in anther culture. The best example is tobacco (Chaplin and Burk 1984; Gillham et al. 1977; Miles et al. 1981), although applications have even been made in other less responsive crops such as wheat (de Buyser and Henry 1986). However, the androgenetic response of wheat and other recalcitrant crops must be improved before anther culture can be considered a generally useful tool for plant breeders.

Most of the efforts to improve the response to the anther-culture system in wheat concentrate on contributing factors most conducive to manipulation: the growth conditions of the source material (Lazar et al. 1984a); the developmental stage of the microspore (He and Ouyang 1984); pretreatments (Lazar et al. 1985; Marsolais et al. 1984); the media (de Buyser and Henry 1980; Liang et al. 1982); and the culture-conditions (Henry and de Buyser 1981; Ouyang et al. 1983).

Although improvements in the anther-culture response of wheat have been made by altering these factors, the most difficult factor to manipulate, genotype, has recently received attention. Genotype is an appealing target factor for manipulation since it is an important component in determining the observed response in wheat and can be altered in a directed fashion. Considerable variation among wheat genotypes for anther culture response has been identified (Bullock et al. 1982; Lazar et al. 1984a; Liang et al. 1982; Schaeffer et al. 1979). Examination of a diallel population among five spring wheat lines has provided

evidence that both additive and dominance genetic variation contribute to the variation observed among wheat genotypes, although the additive effects predominated (Lazar et al. 1984 b). Estimates for heritability of the response traits callus production and regeneration ability in that population ranged from 0.6–0.7.

These and similar results with other crops have led some researchers to suggest that, while improving the genetic component of androgenesis may be difficult, this approach may be the best method of improving the anther-culture response of recalcitrant species such as wheat (Lazar et al. 1984 b; Foroughi-Wehr et al. 1982). Since manipulation of simply-inherited traits is much less cumbersome and time consuming than manipulation of quantitatively-inherited traits, the task of improving the genetic component of response would be greatly simplified if important qualitative factors controlling response could be identified. Ideally, anther culture itself would impose selection for genotypes with superior anther-culture response among the gametes. Improving the genetic component of anther-culture response in this way requires no extra effort on the part of the breeder. Some evidence for gametophytic selection of this kind does exist for wheat (Picard and de Buyser 1977).

This study was undertaken to examine the basis of the genetic control of the anther-culture response among three spring wheat cultivars: Pavon, Chris, and Len. These cultivars represent the extremes of anther-culture performance in our laboratory and offer the greatest likelihood that segregation for important qualitative factors controlling anther-culture response would occur among their progeny.

Materials and methods

Three spring wheat (*Triticum aestivum* L.) cultivars were evaluated in this study. Based on previous tests, 'Pavon' responds well for callus production from anther culture, 'Chris' is a moderate responder, and 'Len' responds very poorly. The three possible crosses were made among the three lines. F_1's were then backcrossed to their respective parents and self-pollinated to produce the backcross (BC) and F_2 generations, respectively.

The plants were grown together in one growth chamber. One to two seeds were planted per 6 in. pot. The chamber was programmed for a 16 h photoperiod at 750 E and a day/night temperature regime of 17 °C/14 °C.

Tillers were collected when the top of the developing spike was level with the ligule of the penultimate leaf. This trait was verified to be correlated for each generation with the mid- to late-uninucleate stage of microspore development. One of the three primary tillers at this stage was collected from a plant of each generation on the same day. Each plant was used once only. An experimental unit consisted of one head collected from each generation on any given day. Therefore, one replicate of the experiment was collected and cultured on each of the 26 days collections were made. Tillers were pretreated for 7 days at 6 °C in the dark. The tillers were surface-sterilized by a 20 min treatment in a 0.2% sodium hypochlorite solution and two rinses in sterile distilled water. The bottom four spikelets were removed, and 72 anthers were collected from the primary florets of the spikelets in the central section of each head.

The anthers were cultured on potato-extract medium (Anonymous 1976) prepared as described by Schaeffer et al. (1979). The medium was prepared with 10% Ficoll too instead of agar, so the density of the medium in its liquid form could support the anthers. Three anthers, all from the same floret, were placed in 0.2 ml of medium in a 0.4 ml well of a 96-well micro-test plate. Anthers were cultured at 28 °C in the dark. At the end of 6 weeks, the number of anthers producing calli were counted, and the calli transferred to a medium consisting of MS basal salts supplemented with 1 mg/l napthaleneacetic acid, 1 mg/l 2,4-D, and 0.1 mg/l 2-isopentenyladenosine (Schaeffer et al. 1979). This medium induces callus proliferation and organogenic green center formation. One 60 × 15 mm Petri plate was used for the calli from one anther. Calli were cultured at 24 °C with a 12 h daylight cycle. At the end of 6 weeks, the number of calli that produced green centers (indicative of regeneration competence) were counted.

Callus production was measured as the percentage of the anthers producing calli. Regeneration capacity was measured as the percentage of calli producing green centers. All data underwent arcsin $\sqrt{}$ transformation to improve the normality of the data. The experiment was first analyzed as a randomized complete block experiment, with each day considered a block, to ascertain significant genetic variation among the 15 generations. The two traits also underwent correlation analysis. Estimates of the gene effects for a three-parameter model (mean, additive, and dominance) and scaling tests for goodness-of-fit to the model were derived for each cross from the generation means and variances using the methods of Mather and Jinks (1971). Heritability estimates for the three crosses were made by the method described by Warner (1952). F-tests and standard errors of the heritability estimates were calculated as described by Ketata et al. (1976).

Results

The generation means exhibited significant variation only for the percentage of anthers producing calli (Table 1). Differences among the generation means for percentage of calli producing green centers were non-significant. Since no significant genetic contribution to regeneration frequency was observed in this study, only genetic variation in callusing performance was examined. The correlation coefficient between the percentage of anthers producing calli and of calli producing green centers was a significant –0.24.

The distributions of the six generations of Pavon × Len for percentage of anthers producing calli exhibit the classical pattern for a trait inherited by quantitative factors (Fig. 1). The variation in the F_2 and backcross generations is continuous, not discrete as would be expected for qualitatively inherited traits. The distributions in the other crosses were similar (not shown).

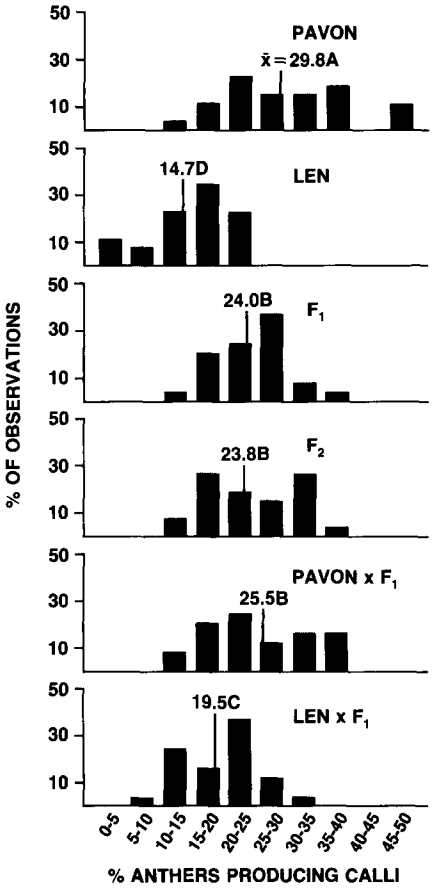

Fig. 1. Population distributions of each of the six generations from the Pavon × Len cross for the percentage of anthers producing calli (transformed to arcsin $\sqrt{}$). Means followed by different letters are significantly different a $P=0.05$ by an L.S.D. test

Table 1. Mean squares from the analyses of variance for the combined generations of the three spring wheat crosses for the two anther-culture response traits (transformed to arcsin$\sqrt{}$)

Source	df	Percentage of anthers producing calli	Percentage of calli producing green centers
Replicates	25	146.61*	622.09
Generations	41	472.88*	626.42
Error	322	41.44	449.58

* Significant difference between mean squares at $P=0.01$

Table 2. Estimates of gene effects based on generation means for each of the three spring wheat crosses for percentage of anthers producing calli (transformed to arcsin$\sqrt{}$)

Estimate	Percentage of anthers producing calli		
	Pavon × Chris	Pavon × Len	Chris × Len
Mean	25.82±0.10	21.98±0.56	18.80±0.67
Additive	3.07±0.09	7.15±0.55	4.34±0.68
Dominance	3.72±0.15	1.97±0.83	3.29±1.00

Table 3. Estimates of narrow sense heritabilities of each of the three spring wheat crosses for percentage of anthers producing calli (transformed to arcsin$\sqrt{}$)

Estimate	Percentage of anthers producing calli		
	Pavon × Chris	Pavon × Len	Chris × Len
$h^2 \pm SE$	0.94*±0.38	0.44±0.55	−0.17±0.77

* Significantly different from zero at $P=0.05$

Results from the scaling tests to evaluate the fit of the generation means to a simple additive-dominance model were all nonsignificant. These results indicate that there are no significant epistatic effects for callus production, so the data were fitted to the three-parameter model. The estimates of the mean, additive, and dominance gene effects for callusing frequency for each of the three crosses were derived by the joint scaling test (Table 2). Since gene effects were estimated for individual crosses, it is possible to observe variation for the inheritance of callusing frequency among the crosses. For Pavon × Chris, the additive and dominance gene effects were nearly equal. Additive gene effects predominated in Pavon × Len. The same was true for Chris × Len, although the predominance of the additive effects was not nearly as pronounced. All estimates of heritability were nonsignificant, except for the Pavon × Chris (Table 3). As observed in the estimates of gene effects, there was considerable variation among the crosses for the heritability estimates.

Discussion

Significant genetic variation for callus production observed in this study has been previously reported (Lazar et al. 1984a, b). However, a significant genetic contribution to regeneration frequency, which has also been reported, was not observed in this study. The different result could be attributed to several factors. By random chance, the three lines chosen for evaluation in this study could possess no variation for regeneration capacity. The difference could also be due to the callusing medium used in this study. Calli produced from the liquid form of the potato medium used in this study have exhibited almost double the regeneration frequency of calli produced on the solid medium (unpublished). Both previous reports of significant variation for regeneration frequency used the solid medium for callus induction (Lazar et al. 1984a; b). The increased regeneration capacity of the system used in this study could be minimizing or eliminating the expression of genetic

differences. Environmental variation could also have contributed to the different results for genetic variation for this anther-culture response trait.

The significant correlation (–0.24) between callusing frequency and regeneration capacity differs from previous studies in which it was nonsignificant (Lazar et al. 1984a, b). However, this correlation value is of little import. The results presented and those of Lazar et al. (1984a, b) suggest that these two response traits, which together determine overall anther-culture performance, are inherited independently. Assuming that regeneration capacity is a heritable trait, improving the overall anther-culture response would entail tandem selection for callus production and regeneration capacity. If, as our data suggest, genotype does not contribute significantly to regeneration frequency, then selection for callus production should be sufficient to improve androgenetic response.

The generation distribution patterns of the three crosses suggest that either qualitative factors do not contribute to the variation or that large random variation prevents discovery of qualitative factors. The second is more likely since previous research has shown that environment and genotype × environment interactions contribute significantly to the anther-culture response in wheat (Lazar et al. 1984a). If there are major-gene differences for androgenetic performance among the materials evaluated in this study, their expressivity is not great enough to be useful in improving wheat response. Thus, even using the extreme anther-culture response observed in this laboratory, identification of significant and easily-manipulated qualitative factors that control anther-culture response in wheat was not realized. Failure to identify major genes that control the anther-culture response indicates that improvements in response will have to be made among quantitative factors.

Previous quantitative evaluations of anther-culture response traits found that additive genetic variation contributed a much greater proportion of the total genetic variation than did dominance variation (Lazar et al. 1984b). This study confirms those results as a whole. Our heritability estimates taken as a whole also correspond with those of 0.6–0.7 previously derived for callus production in wheat (Lazar et al. 1984b). However, in this study there was considerable variation in gene effects and heritability estimates from cross to cross. Since most selection in wheat is made among progeny from a single or three-way cross and not among the progeny from a population, the observed variation from cross to cross for the quantitative inheritance of anther-culture response would complicate the selection process. A breeder would need to consider each cross' potential for producing progeny with improved androgenetic response before efficient quantitative selection for improved culturability could be made.

A large component of the quantitative variation observed in this study was due to random variation. The random effects have already been discussed with regard to the distributions observed for the generations of the three crosses. Additionally, only one of the three estimates of heritability for callus induction was significant. These results suggest that improvements in the response of wheat to the anther-culture process from selection will be difficult due to low selection efficiency and genetic gains.

There may be an alternative to direct selection for improving the genetic component of androgenetic response. Evidence suggests that gametophytic selection for genotypes with improved anther-culture response occurs in androgenesis of wheat (Picard and de Buyser 1977). If so, plant breeders could use androgenesis in their breeding programs while, at the same time, selecting for lines with improved response in culture. Gains in the androgenetic performance of wheat can also still be made through improvements in the non-genetic factors affecting anther-culture response.

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