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Nuclear genes affecting albinism in wheat (*Triticum aestivum* L.) anther culture

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Summary. Inheritance of the ability to respond in wheat anther culture was studied from 6×2 reciprocal crosses between six varieties with high and two varieties with low capacity for green plant formation and their parents, replicated in two environments. Effects of genotypes dominated embryo formation and percentages of green plants, accounting for 78.4% and 85.4% of total variation, respectively, while smaller genetic effects were indicated for regeneration. Nuclear genes could explain almost all the genotype effects in this material. Embryo formation showed heterosis over high parent for 5 of the 12 hybrids, while percentages of green plants from the hybrids were intermediate to the parents. General Combining Ability (GCA) could explain 78.8% of the variation for embryo formation among the hybrids, whereas differences in percentage of green plants were dominated by Specific Combining Ability (SCA), accounting for 67.9% of hybrid variation. A positive correlation ($r = 0.81$**) was observed between the genetic capacity for regeneration and green plant formation. Analysis of covariance indicated that effects causing GCA for green plant formation were mainly responsible for this correlation. A regression model with two parallel lines divided the six parent lines with high green plant formation into three groups with respect to their reactions with the two testers. The results are discussed with regard to possible involvement of two sets of nuclear genes affecting the percentage of green plants obtained in wheat anther culture: one set consisting of mainly additive effects affecting green plant percentage through an initial effect on regeneration ability, and another set of two or a few more major genes with dominance or epistatic effects uncorrelated with regeneration.

Key words: Wheat – Anther culture – Androgenetic responsiveness – Albinism – Genetic control

Introduction

The genetics affecting results in anther culture involve a complex trait with at least two different components: the ability of microspores to divide and produce embryos, and the ability of pollen embryos to regenerate into plantlets (Foroughi-Wehr et al. 1982). An additional problem with cereal anther culture is the high frequency of albino plants often obtained. Therefore, the frequency of albinos relative to green plants has been introduced as a third component of anther culture response in cereals (Wenzel et al. 1977; Foroughi-Wehr et al. 1982), and it has been demonstrated that this ratio is strongly affected by genotype in both wheat and barley (Andersen et al. 1987; Knudsen et al. 1989). With the recent progress in anther culture technique, reasonable numbers of embryos and plants can be obtained in many genotypes of wheat and barley. In many cases, however, the majority of plants obtained are albinos.

For embryo formation and plant regeneration in wheat anther culture, it has been shown that nuclear genes are mainly involved, resulting in both additive and non-additive genetic variation, with the additive effects predominating (Lazar et al. 1984; Deaton et al. 1987). Information on the inheritance of the capability for green plant formation has been obtained through studies of the total number of green plants obtained per cultured anther in barley (Dunwell et al. 1987; Powell 1988) and triticale (Charmet and Bernard 1984). These studies, which reflect the combined genetic effects on embryo formation, regeneration, and green plant formation, have indicated additive and non-additive gene action for green plant yield. In one case a major effect of cytoplasm has been reported (Powell 1988). A possible involvement of cytoplasm in the inheritance of albinism is interesting, because anther-derived albino plants from barley and wheat have been reported to possess altered plastids whose DNA has been changed or partly deleted (Day and Ellis 1984, 1985).

The current investigation was designed to study the inheritance of response in wheat anther culture, with special reference to the percentage of green plants obtained and with emphasis on reciprocal effects. In previous studies, six widely different varieties with high ability to produce green plants in anther culture ("green-type parents") and two varieties with low capacity for green plant formation ("albino-type parents") were selected. Analysis of anther culture response from reciprocal hybrids between these two groups of varieties and their parents are presented below.

Materials and methods

The plant material for this experiment consisted of eight widely different varieties of hexaploid wheat (*Triticum aestivum*), previously selected for extreme performance with respect to green plant formation in anther culture (Table 1): six "green-type parents" (GP1–GP6) believed to produce high percentages of green plants, and two "albino-type parents" (AP1–AP2) previously found to produce very low percentages of green plants. The 6×2 possible crosses between green- and albino-type parents were produced reciprocally, and the 24 different hybrids and their 8 parents were evaluated in anther culture in two replications, with donor plants raised in the greenhouse and in the field, respectively. Winter types were germinated and vernalized for 2 months at 1°–3 °C before planting, and 15–20 donor plants were raised from each genotype for each replication planted in early April. Field-grown plants were raised in rows, 50 cm between rows, 15 cm between plants in the row. Greenhouse-grown plants were raised in 12-cm plastic pots, 10 cm between the pots in a greenhouse with 10°–15 °C night and 15°–20 °C day temperature.

For each genotype the anthers from 20 spikes were plated as one experimental unit for each replication. The methods for selection, sterilization, and anther culture were described by Ouyang et al. (1983), except that substrate for culturing the anthers were 190-2 (Wang and Hu Han 1984), supplied with 9% sucrose, 1.5 mg/l 2,4-D, 0.5 mg/l kinetin, and without NAA. Substrates were solidified with 0.35% Gelrite (Kelco).

For the statistical analysis, the square-root transformed numbers of embryos/100 cultured anthers, numbers of plants/100 embryos, and percentages of green plants (green plants * 100/total plants) from each experimental unit were subjected to an analysis of variance with main effects from genotypes, replications, and reciprocals and interactions between genotypes and replications. Residuals were checked by graphical means. For further analysis of genotype effects, a combined error, b, consisting of residuals and genotype * environment with 31 degrees of freedom, was used for testing.

Results

From the 82,859 anthers plated during this experiment, 39,239 embryos were obtained (47.4 embryos/100 cultured anthers), from which 2,914 green plants and 16,495 albino plants were regenerated. The average result from the whole experiment, thus, was 23.4 plants per 100 cultured anthers, 15.0% of which were green.

Table 1. Plant material

	Variety	Type	Origin
"Green-type parents"			
GP 1	Krim	Winter	USSR
GP 2	Benoist	Winter	France
GP 3	Kraka	Winter	Denmark
GP 4	Kanzler	Winter	West Germany
GP 5	Ciano	Spring	Mexico
GP 6	Sleipner	Winter	Sweden
"Albino-type parents"			
AP 1	Walter	Spring	Sweden
AP 2	Curiosita	Winter	Italy

Table 2. Degrees of freedom and mean squares from analysis of variance

Source	*df*	Embryos/ 100 anthers MS	Plants/ 100 embryos MS	Percent green plants MS
Genotypes (G)	19	16.49***	1.63	10.91***
Environments (E)	1	14.32**	0.02	2.44**
Reciprocals	12	1.40	0.52	0.50
G*E	19	2.26	0.92	1.15*
Error a	12	1.03	0.82	0.33
Subdivision of genotype SS				
Within parents	7	3.94*	1.01	14.69***
Within hybrids	11	7.45***	2.10*	7.70***
Between groups	1	132.32***	0.92	19.73***
Subdivision of Hybrid SS				
GCA green parents	5	12.04***	1.95	7.55***
GCA albino parents	1	51.98***	8.10**	6.70*
SCA	5	5.41*	1.04	9.96***
Error b	31	1.79	0.88	0.83

Degrees of freedom and mean squares from analysis of variance are presented in Table 2 together with appropriate partitioning of the genotype sum of squares. Effects of the two different environments were significant for the formation of embryos and percent green plants at the 1% level. The field-grown material produced on average 53.7 embryos/100 anthers, while the greenhouse-grown material produced 41.3 embryos/100 anthers. The percentage of green plants was somewhat lower from the field-grown material (13.4%), while greenhouse-grown material on average produced 17.1% green plants.

F-tests for effects of reciprocals were nonsignificant for both embryo formation, regeneration, and percent green plants. Further partitioning of the reciprocal sum of squares into single degrees of freedom corresponding to each hybrid revealed one test significant at the 5%

level for the combination 'GP4' × 'AP2' for green plant formation. In this cross the hybrid with 'GP4' as the female produced 28.2% green plants on average, while the reciprocal cross produced 15.7% green plants. The difference, however, was the result of an exceptionally high percentage of green regenerants from one of the greenhouse-grown observations. Another reciprocal effect was found for embryo formation in the combination 'GP5' × 'AP2', in which the hybrid with 'GP5' as the female produced on average 102 embryos/100 anthers, while the reciprocal cross produced 158 embryos/100 anthers.

Genotype effects were highly significant for embryo formation and percent green plants, while they were nonsignificant for regeneration. Subdivision of the genotype sum of squares for embryo formation showed clear differences within the hybrids, a less significant difference within the parents, and a very prominent difference between parents and hybrids. The average difference between parents and hybrids was due to heterosis for embryo formation (Table 3), since the level of the hybrids was generally higher than the embryo formation from the parents. According to Duncan's test, at the 5% level, five of the six crosses with 'AP1' showed heterosis over high parent for embryo formation. For regeneration, only differences within hybrids were significant. For green plant formation both differences within parents, within hybrids, and between parents and hybrids were highly significant. Five of the six green-type parents and the two albino-type testers were well selected for the experiment, since they resulted in high and low percentages of green plants, respectively (Table 3). The remaining green-type parent 'GP3', however, was a less successful selection, since it produced no higher percentage of green plants than the two albino testers during this experiment. Hybrids were generally intermediate to their parents with respect to percentage of green plants (Table 3). The highly significant difference between parents and hybrids for percentage of green plants indicated in Table 2 arose because the five parents with high against the three parents with low percentage of green plants elevated the average percentage of green plants from the parents over that of the hybrids.

The differences within hybrids were subdivided into General Combining Ability (GCA) of the green-type parents, GCA of the albino testers, and Specific Combining Ability (SCA) for each hybrid (Table 2). For embryo formation there were highly significant differences in GCA among both types of parents and a less significant effect of SCA. For the regeneration there were significant differences in GCA between the two albino testers, and the differences in GCA among the green-type parents approached significance at the 5% level ($P = 0.08$), whereas there was no indication of SCA for this response component. Subdivision of sum of squares among hybrids for percent green plants revealed highly significant effects of GCA of the green-type parents as well as SCA, while the average effects of the albino testers were significant only at the 5% level. The variance components for percent green plants showed that SCA dominated by far the genetic variation within hybrids, accounting for 67.9% of the total variation, while GCA from green-type and albino-type parents explained only 25.0% and 7.1%, respectively.

A significant positive correlation ($r = 0.81^{**}$) was observed among the hybrids for percentage of green plants and percentage of regeneration. Analysis of covariance (Table 4) revealed that only main effects of genotypes contributed to this correlation while environments, inter-

Table 3. Average embryo formation and percentages of green plants in anther culture from wheat hybrids and their parents. Vertical lines indicate comparison with Duncan's multiple range test at the 5% level

Embryo formation		Green plant formation	
Genotype	Embryos/ 100 anthers	Genotype	Percent green plants
GP5 × AP1	126	GP6	55
GP3 × AP1	94	GP5	53
GP1 × AP1	78	GP1	47
GP2 × AP1	73	GP5 × AP2	40
GP3 × AP2	75	GP4	39
GP4 × AP1	74	GP2	32
GP5 × AP2	55	GP1 × AP1	22
AP2	53	GP4 × AP1	21
GP6 × AP2	44	GP6 × AP1	15
GP4 × AP2	43	GP6 × AP2	15
GP1 × AP2	39	GP5 × AP1	12
GP6 × AP1	36	GP2 × AP1	11
GP2 × AP2	33	GP2 × AP2	9
AP1	29	GP3 × AP1	9
GP2	24	GP3	6
GP5	19	GP4 × AP2	6
GP1	13	GP1 × AP2	4
GP4	12	AP2	2
GP3	11	GP3 × AP2	1
GP6	10	AP1	1

Table 4. Analysis of covariance between percentage of regeneration and percentage of green plants among hybrids

Source	*df*	COV	*r*
Hybrids (H)	11	3.239**	0.806
GCA	6	4.312**	0.918
SCA	5	1.952	0.607
Environments (E)	1	−0.308	–
H*E	11	−0.289	−0.266
Error	24	−0.103	−0.196
Total	47	0.631**	0.394

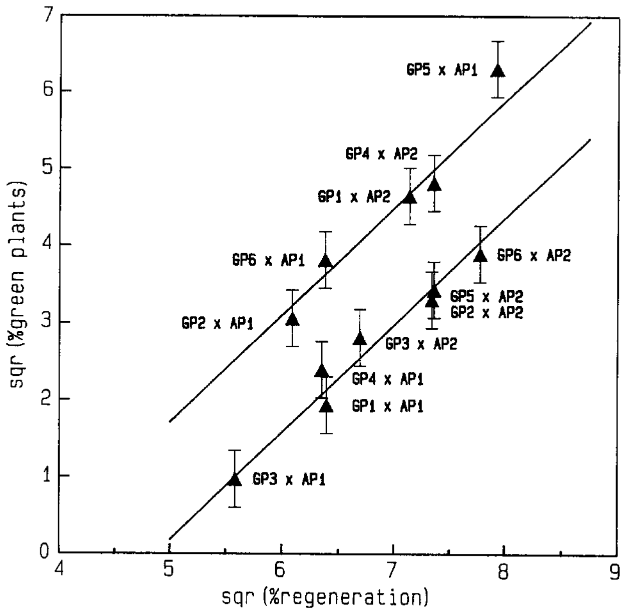

Fig. 1. The correlation between percentage of green plants and percentage of regeneration in wheat anther culture for hybrids between parents with high (GP1–GP6) and parents with low green plant percentage (AP1–AP2)

actions, and residuals were nonsignificant. Among the genotype effects, only GCA contributed significantly to this positive correlation, while covariance from SCA was nonsignificant. A regression model with two parallel lines could explain all the observed differences for green plant percentages within the hybrids (Fig. 1). The model divided the genotype effects for green plant formation among hybrids into one component correlated with regeneration percentage and one uncorrelated component. The uncorrelated component divided the six green-type parents into three groups with respect to their reaction with the two testers. One group is formed by 'GP3' which reacts low with both testers, another group is formed by 'GP1' and 'GP4' reacting low with 'AP1' and high with 'AP2', and a third group is formed by 'GP2', 'GP5', and 'GP6' reacting high with 'AP1' and low with 'AP2'.

Discussion

The majority of variation observed in the present experiment, replicated in two widely different environments, was caused by genotypes whose main effects removed 78.4% and 85.8% of the total sum of squares for embryo formation and percentage of green plants, respectively. Only an insignificant part of this variation is apparently caused by differences in cytoplasms in the plant material chosen for this investigation. This is in agreement with previous reports on wheat, barley and triticale where no reciprocal effects (Bullock et al. 1982; Henry and de Buyser 1985; Dunwell et al. 1987) or small reciprocal effects (Foroughi-Wehr et al. 1982; Lazar et al. 1984; Charmet and Bernard 1984) have been reported in some hybrid combinations for embryo formation and regeneration. The only example of a substantial reciprocal effect suggesting that the direction of a cross should be considered was reported by Powell (1988) for embryo formation and green plants/100 anthers with barley anther culture.

According to Foroughi-Wehr and Friedt (1984), the transfer of androgenetic responsiveness to wheat, barley, and probably others does not depend on the source of cytoplasm, but rather on nuclear genes. This statement has been true also in the present study with respect to green plant formation. Results conflicting with this general conclusion (Powell 1988) might be caused by differences between species or genotypes. According to Day and Ellis (1984, 1985), barley and wheat albino plants of anther culture origin have altered or deleted plastid genomes. A chromosomal type of inheritance of the genetic capacity for green plant formation could be explained by nuclear genes either affecting the frequency of DNA changes or the relative selective advantage of green to albino structures during development.

The nonreciprocal genetic effects on embryo formation and regeneration observed with wheat material selected for investigation of albinism are also in accordance with previously published results. Embryo formation in anther culture seems to be dominated by additive genes but with a significant non-additive effect in wheat (Lazar et al. 1984; Deaton et al. 1987), barley (Dunwell et al. 1987; Powell 1988), and triticale (Charmet and Bernard 1984). In addition, the observation of heterosis for embryo formation from some hybrid combinations has been reported (Lazar et al. 1984; Charmet and Bernard 1984). The relatively modest but significant genetic effect on regeneration frequencies dominated by additive genes is also in accordance with previous reports (Lazar et al. 1984; Charmet and Bernard 1984).

The estimates of GCA and SCA obtained from this 6 × 2 cross study are probably not unbiased estimates of general and specific combining ability, as would have been obtained from a diallel study. An 8 × 8 diallel including the reciprocals, however, would have required considerably more resources. The large component of SCA observed, however, indicates that major genes with strong dominance or epistasis are affecting the formation of green plants. The positive genetic correlation between regeneration frequency and percentage of green plants has not been reported previously. Some of the nuclear genes affecting green plant percentage in this wheat material are related or identical to genes affecting the ability of pollen embryos to regenerate plants. There are indications from the covariance study and the lack of SCA for regeneration that the correlated effects are mainly of an additive nature, and the grouping of the green-type parents by uncorrelated effects is a second indication of major genes affecting the green plant percentage. The three groups observed would indicate two or a few more major gene loci.

The results suggest the action of two different classes of genes affecting green plant formation in wheat anther culture: one class with mainly additive effects modifying green plant percentages through an effect on regeneration percentage, and another class of two or a few more major genes affecting the original frequency of potentially green structure. Whether such a partitioning of genotype effect on green plant formation in wheat anther culture is true will need further investigation, as do questions regarding the exact number of major genes involved and their nature with regard to dominance and epistasis.

A study of a diallel series of crosses among part or all of the wheat lines used here might provide further answers to these questions.

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