Genetic analysis of androgenetic traits in wheat (*Triticum aestivum* L.)

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Abstract

The objective of this study was to estimate genetic parameters and to investigate the type of gene action in controlling androgenesis in wheat. Two wheat cultivars of Grebe and Houtman were reciprocally crossed with two synthetic genotypes of Do1 and Pol and then a complete set of the parents, F_1, reciprocal F_1 (RF_1), F_2 and back-cross generations (BC_1 and BC_2) of each cross were used for anther culture. The ratio of responding anthers, the ratio of albino and green regenerants, and the number of embryoids per each responding anther were determined for different generations of each cross. The results showed a wide genetic variation for embryoid induction and plant regeneration among the parental lines and their progenies. The genetic model of additive-dominance effects could explain the variation among the generation means for the traits, indicating that their inheritance was relatively simple. The genetic analysis also showed predominance of additive genetic effects in genetic control of embryoid induction and green plant regeneration, implying possible improvement of these traits by selection in plant breeding programs. Maternal effects were also found for embryoid induction. The narrow-sense heritability for responding anthers, green plants, albino plants, green plants to total regenerants, and embryoids per responding anther in different crosses varied from 41% to 77%, 64% to 92%, 67% to 84%, 40% to 67% and 33% to 65%, respectively. In conclusion, it seems that the improvement of green plant regeneration in anther culture technique can be achieved by appropriate breeding and selection programs.

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Keywords: Gene effects, Heritability, Androgenesis, Wheat (*Triticum aestivum* L.)

INTRODUCTION

Androgenesis has an important role in producing doubled haploid lines in many crop species (Jain *et al.,* 1996). This technique can provide an accessible haploid system for biochemical and molecular analysis and also for *in vitro* selection (Jahne and Lorz, 1995, Barnabas *et al.,* 2001). Double haploidy serves as a useful method for producing appropriate populations to undertake genetic linkage map studies and also for combining and fixing the desirable genes of two different elite genotypes (Chaudhary *et al.,* 2003). Thus, development of androgenesis technique enhances the production of homozygote lines in breeding programs.

The efficiency of anther culture can be affected by the physiological status of donor plant anthers (Ouyang *et al.,* 1987), the developmental stage of the microspores (He and Ouyang, 1984), and by the *in vitro* culture conditions (Jing *et al.,* 1982, Patel *et al.,* 2004, Cistue *et al.,* 2006). However, genetic factors play an important role in the efficiency of anther culture and its widespread application (Deaton *et al.,* 1987; Becraft and Taylor, 1992; Barnabas *et al.,* 2001; Nestares *et al*., 2002). It has been well recognized that calli induction, regeneration, and green plantlet development which are polygenic, independently inherited traits are involved in the anther culture capability of a genotype (Agache *et al.,* 1988; Ekiz and Konzak, 1994). Picard and De Buyser (1977) also found that androgenetic ability increased in doubled haploid lines compared to their parental cultivars.

Androgenetic ability can be genetically improved in wheat (Picard and De Buyser, 1977) and genetic variation which is necessary for genetic improvement (Falconer and Mackay, 1996) has been found for wheat androgenesis (Andersen *et al*., 1987). It has been reported that the embryoid induction and plant regeneration are quantitatively inherited (Foroughi-wehr *et al.*, 1982; Agache *et al.*, 1988; Szakacs *et al.*, 1988) and both additive and non-additive genes action (Chaudhary *et al.*, 2003) and cytoplasmic effects (Sagi and Barnabas, 1989) are involved in androgenesis response of wheat.

Understanding of genetic control for androgenesis is necessary for improving its efficiency; therefore, this study was conducted to investigate the type of gene action and inheritance of wheat androgenesis and its related traits.

MATERIALS AND METHODS

Plant materials: Two wheat cultivars of Grebe (G) and Houtman (H) known to be responsive to anther culture were reciprocally crossed with each of the synthetic genotypes of Do1 (D) and Pol (P). The F_1 populations (the cultivars as female parents) were back-crossed to each of the parents to produce two sets of back-cross populations (BC_1 and BC_2). The F_2 populations were also produced by selfing of F_1 plants. A complete set of parents, F_1, reciprocal F_1 (RF_1), F_2, BC_1 and BC_2 were grown in pots filled with a soil mixture of 1:1 pine bark and coarse sand supplemented by slow release fertilizer in a greenhouse. Plants were grown at 20±1°C and 16 hours of photoperiod under natural light, supplemented by halogen quartz iodide lamps (300-340 $\mu Em^{-2}s^{-1}$ light intensity).

Anther culture: In this study, 5 plants in non-segregating (P_1, P_2, F_1 and RF_1) and 25 plants in segregating (F_2, BC_1 and BC_2) populations were used. Four spikes per plant were selected and then 40 anthers at the mid-uninucleate stage (He and Ouyang, 1984) from each spike (as a replication) were plated in the 55×15 mm disposable petri dishes containing 5 ml of semi-solid MC17 medium (Luckett *et al*., 1991). The medium was supplemented with a higher glutamine (1000 mg/L) content and a lower Fe-Na_2EDTA (32 mg/L) content, sterilized using 22 µm filter papers (Millipore Company), and then solidified with agarose (2.2 g/L, Type 1-A, Sigma Chemical Company). The dishes were sealed with parafilm and incubated in darkness at 26 ± 2°C for at least 4 weeks. The induced embryoids were transferred onto a regeneration medium consisting of MS medium with vitamins (Murashige and Skoog, 1962), supplemented with 2.5 g/L agarose (Type 1-A, Sigma Chemical Company), 1 mg/L IAA, and 1 mg/L BAP. The transferred embryoids were kept at 26 ± 2°C with 16 hours of photoperiod illumination (80-100 $\mu Es^{-1}m^{-2}$ as measured by an LI-188B Integrating Quantum Radiometer/ Photometer) provided by white fluorescent lamps for plant regeneration. After 2-3 weeks, both green and albino plants were regenerated.

Based on the cultured anthers, the ratio of responding anthers to callus induction, embryoids per responding anthers, the ratio of green and albino regenerated plants to responding anthers, and green plants per total regenerants were recorded for each of the experimental units.

Genetic Analysis: Generation mean analysis was applied to estimate genetic parameters of mid-parent [*m*], additive [*d*], dominance [*h*], and additive-dominance [*dh*] gene effects for each trait, using the joint scaling test, as described by Mather and Jinkes (1982). Transformed data (square root or Arcsin root transformation) were used for analysis to improve the precision of the estimates of the parameters. The general linear model of SAS (SAS Institute, Inc. 1989) was applied for the analysis of variance and the least significant difference test (LSD) was used to compare the generation mean values. The genetic variance components were estimated using the methods described by Mather and Jinks (1982) and the estimates of narrow-sense heritability (H^2) were calculated from Warner (1952).

RESULTS

Responding anthers to callus induction: Significant differences were observed among the generation means for the ratio of responding anthers to callus induction (Table 1, Figure 1). In all crosses, the parent P_2 had significantly higher means of responding anthers than P_1. Among the parents, H and D parents had the lowest and the highest means of responding anthers, respectively (12% *vs.* 22%). There were no significant differences between the means of F_1 and the corresponding better parent in each cross, indicating that there were no heterobeltiosis for the trait. Significant reduction of the mean in F_2 generation

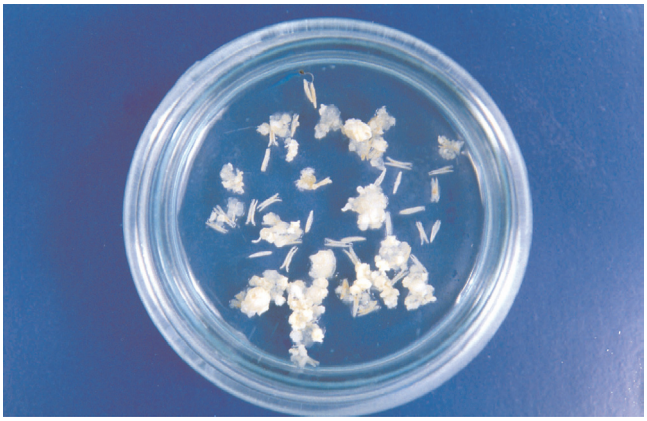

Figure 1. Responding anthers of Grebe genotype to callus induction in MC17 medium.

compared to F_1 in cross H × D indicated that there was inbreeding depression for the ratio of responding anthers to callus induction in this cross (Table 1). No significant differences between the means of F_1's and their reciprocals indicated absence of maternal effects for this trait (Table 1).

Generation mean analysis showed that the additive-dominance model could explain the variation among generation means in each cross (Table 2). The estimates of genetic parameters in all crosses showed that the magnitude of the additive [d] and dominance [h] effects were relatively consistent among the crosses (Table 2); however, only the additive effects were significant in controlling the ratio of responding anthers (Table 2).

The narrow-sense heritability for the ratio of responding anthers varied from 41% to 77%. In G×P and H×D crosses, the heritability was relatively high (Table 3). The degree of dominance for this trait was estimated as 0 to 0.86 in different crosses (Table 3).

Green and albino regenerated plants: There were significant differences among the generation means for the ratio of green and albino plants (Table 1, Figure 2). Among the parental lines, D had higher green and albino regenerated plants per responding anthers (Table 1). The significant green plant regeneration mean in F_1's compared to the better parent in G×P and H×P crosses showed heterobeltiosis for this trait. No maternal effects were observed for either green or albino regenerated plants since there were no significant differ-

Table 1. Mean of parents (P_1 and P_2), F_1, RF_1, F_2, BC_1 and BC_2 generations for different traits in four crosses of wheat.

Crosses	Generation						
	P_1	P_2	F_1	RF_1	BC_1	BC_2	F_2
				Ratio of responding anthers for callus induction			
G × D	0.13^{c*}	0.22^{ab}	0.21^{ab}	0.18^{b}	0.21^{ab}	0.24^{a}	0.24^{a}
G × P	0.13^{c}	0.21^{ab}	0.28^{a}	0.29^{a}	0.21^{ab}	0.29^{a}	0.3^{a}
H × D	0.12^{b}	0.22^{a}	0.19^{a}	0.18^{a}	0.11^{b}	0.12^{b}	0.12^{b}
H × P	0.12^{b}	0.21^{ab}	0.29^{a}	0.27^{a}	0.12^{b}	0.27^{a}	0.2^{ab}
				Green plants per responding anthers			
G × D	0.3^{ab}	0.31^{ab}	0.35^{ab}	0.44^{a}	0.24^{b}	0.29^{ab}	0.43^{a}
G × P	0.3^{b}	0.23^{c}	0.38^{a}	0.36^{a}	0.33^{ab}	0.35^{ab}	0.33^{ab}
H × D	0.006^{c}	0.31^{a}	0.006^{c}	0.006^{c}	0.006^{c}	0.02^{c}	0.067^{b}
H × P	0.006^{d}	0.23^{c}	0.3^{b}	0.33^{ab}	0.007^{d}	0.35^{a}	0.34^{a}
				Albino plants per responding anthers			
G × D	0.23^{d}	1.06^{ab}	0.80^{cd}	0.99^{ab}	0.91^{bc}	1.19^{a}	1.03^{ab}
G × P	0.23^{c}	0.62^{b}	0.6^{b}	0.72^{b}	0.71^{b}	1.01^{a}	1.04^{a}
H × D	0.73^{d}	1.06^{cd}	0.81^{d}	0.93^{cd}	1.32^{ab}	1.216^{ab}	1.105^{bc}
H × P	0.73^{c}	0.62^{d}	0.9^{b}	0.99^{b}	1.01^{ab}	1.03^{ab}	1.1^{a}
				No. of embryoids per responding anther			
G × D	1.83^{d}	2.86^{a}	2.7^{ab}	2.9^{a}	2.48^{bc}	2.76^{ab}	2.83^{a}
G × P	1.83^{c}	2.33^{bc}	2.4^{bc}	2.8^{a}	2.4^{bc}	2.7^{ab}	2.83^{a}
H × D	1.52^{c}	2.86^{a}	1.41^{c}	2.46^{b}	2.18^{b}	2.14^{b}	2.32^{b}
H × P	1.52^{d}	2.33^{a}	1.44^{d}	2.35^{a}	2.12^{bc}	2.13^{bc}	2.25^{b}

*In each row (cross), means followed by the same letter are not significantly different using the LSD test ($P \leq 0.05$).

ences between means of F_1's and their reciprocals (Table 1).

The estimated genetic parameters for both green and albino regenerated plants had relatively high variation among crosses (Table 2). Highly significant additive gene effects were observed in G×P, H×D and H×P crosses, while highly significant dominance effects were observed only in the H×D cross for green regenerated plants. However, in the G×D cross, no significant additive or dominance effects were found for this trait. Albino regenerated plants had no significant additive or dominance effects (Table 2). The estimates of gene effects for green to total regenerated plants (green + albino) showed that only the additive effects [d] were significant in all crosses (Table 2).

The narrow-sense heritability estimates for green regenerated plants were moderate to high (64% to 92%) and ranged from 67% to 84% for albino plants. The

Table 2. Estimates of genetic parameters and their standard errors for different traits in four crosses of wheat.

Genetic parameter	Crosses			
	G×D	G×P	H×D	H×P
	Ratio of responding anthers for callus induction			
m[a]	0.16±0.01**[b]	0.15±0.002**	0.17±0.009**	0.17±0.001**
[d]	-0.06±0.02*	-0.07±0.01**	-0.05±0.01**	-0.08±0.01**
[h]	0.04±0.03^{ns}	0.03±0.03^{ns}	0.015±0.01^{ns}	0.04±0.02^{ns}
[dh]	0.06±0.1^{ns}	-	-	-
[c]	0.03±0.01^{ns}	0.002±0.002^{ns}	-0.002±0.009^{ns}	0.002±0.002^{ns}
	Green plants per responding anthers			
m	0.33±0.01**	0.08±0.002**	0.075±0.001**	0.06±0.001**
[d]	0.05±0.01^{ns}	-0.05±0.001**	-0.07±0.002**	-0.07±0.002**
[h]	0.04±0.1^{ns}	-0.04±0.05^{ns}	-0.07±0.002**	-0.05±0.04^{ns}
[dh]	-	-	-	-
[c]	-0.04±0.05^{ns}	-0.001±0.001^{ns}	-0.0002±0.002^{ns}	-0.003±0.004^{ns}
	Albino plants per responding anthers			
m	0.62±0.18**	1.03±0.16**	1.17±0.17**	0.83±0.15**
[d]	-0.35±0.18^{ns}	-0.30±0.2^{ns}	-0.30±0.2^{ns}	-0.4±0.28^{ns}
[h]	0.41±0.27^{ns}	-0.28±0.24^{ns}	-0.17±0.28^{ns}	-0.2±0.24^{ns}
[dh]	-	-	-	-
[c]	0.11±0.11^{ns}	0.08±0.1^{ns}	0.02±0.14^{ns}	0.1±0.08^{ns}
	Green plants per total regenerants			
m	0.41±0.055**	0.09±0.02**	0.085±0.01**	0.22±0.03**
[d]	0.29±0.073*	0.3±0.01*	-0.1±0.019**	-0.15±0.02*
[h]	-0.04±0.08^{ns}	-0.02±0.02^{ns}	-0.07±0.02^{ns}	-0.03±0.02^{ns}
[dh]	-0.78±0.22^{ns}	-	-	-
[c]	-0.05±0.042^{ns}	-0.05±0.04^{ns}	0.019±0.013^{ns}	0.024±0.03^{ns}
	No. of embryoids per responding anther			
m	2.32±0.11**	3.02±0.2**	3.69±0.35**	2.58±0.2**
[d]	-0.8±0.14*	-0.9±0.18*	-1.50±0.32*	-1.5±0.4*
[h]	0.25±0.16^{ns}	0.28±0.2^{ns}	-1.77±0.44^{ns}	-0.35±0.28^{ns}
[dh]	1.08±0.49^{ns}	2.08±0.8^{ns}	2.69±0.74^{ns}	1.8±0.8^{ns}
[c]	0.28±0.06*	0.3±0.05*	-0.60±0.12*	-0.70±0.1*

a m: mean; d: additive effects; h: dominance effects; dh: additive × dominance effects; c: maternal effects.
b * and ** : Significant at 5% and 1% level of probability; ns: not significant.

degree of dominance, [h]/[d] in different crosses varied from 0.29 to 0.93 and from 0.35 to 0.57 for green and albino regenerated plants, respectively. The estimated heritability for the ratio of green plants to total regenerants varied from 40% to 67% and its degree of dominance ranged from 0.12 to 0.83 (Table 3).

Embryoid induction: The means of generations for embryoids per responding anthers is shown in Table 1. The parent D had the highest mean of induced embryos among the parental lines. The reciprocal F_1 had a significantly higher mean of embryo induction than the corresponding F_1 generation and its high parent in the G × P cross. In the G × P, H × D, and H × P crosses, the means of embryoids induction were significantly higher in reciprocal F_1's than in F_1 generations. The means of embryoid induction in F_2 generation was significantly higher than in F_1 generation in all cross-

Table 3. Estimates of additive (D), dominance (H) and environmental (E) variance components, narrow-sense heritability (H^2), and degree of dominance ([h]/[d]) for different traits in four crosses of wheat.

Genetic parameter	Crosses			
	G×D	G×P	H×D	H×P
Ratio of responding anthers for callus induction				
D	0.004	0.006	0.01	0.003
H	0.003	0	0	0.001
E	0.0017	0.0018	0.0015	0.0019
H^2 (%)	45	62	77	41
[h]/[d]	0.86	0	0	0.5
Green plants per responding anthers				
D	0.082	0.1	0.04	0.055
H	0.071	0.0088	0.03	0.0304
E	0.00048	0.0018	0.001	0.0024
H^2 (%)	64	92	70	67
[h]/[d]	0.93	0.29	0.86	0.74
Albino plants per responding anthers				
D	0.024	0.08	0.032	0.12
H	0.008	0.012	0.004	0.04
E	0.004	0.007	0.002	0.01
H^2 (%)	67	80	84	75
[h]/[d]	0.57	0.38	0.35	0.57
Green plants per total regenerants				
D	0.04	0.07	0.094	0.104
H	0.028	0.02	0.012	0.036
E	0.023	0.015	0.02	0.017
H^2 (%)	40	63	67	67
[h]/[d]	0.83	0.53	0.12	0.58
No. of embryoids per responding anther				
D	0.028	0.056	0.02	0.09
H	0.016	0.048	0.04	0.028
E	0.0068	0.016	0.01	0.017
H^2 (%)	56	50	33	65
[h]/[d]	0.75	0.92	1.4	0.56

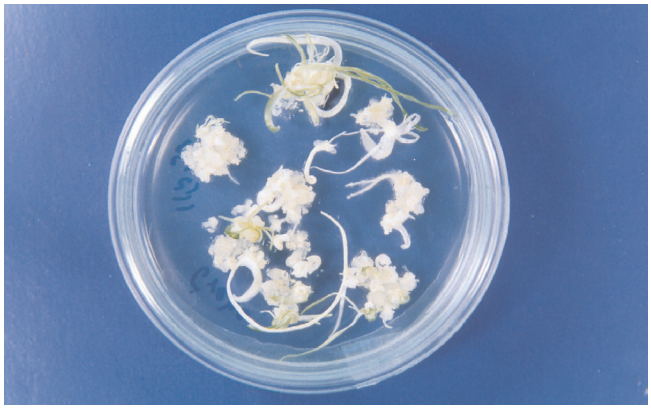

Figure 2. Green and albino regenerated plants from Grebe genotype in MC17 medium.

es, except for the G × D (Table 1). Generation mean analysis showed that additive [d] and maternal [c] effects were significant in all crosses for embryoid induction (Table 2). The narrow-sense heritability for this trait was low (33%) to moderate (65%) and its degree of dominance varied from 0.56 to 1.4 (Table 3).

DISCUSSION

Genetic analysis of quantitatively inherited traits provides useful information for breeding programs. The wide variability in androgenic response of anthers for parental lines and their progenies indicates a significant influence by the genotype on wheat anther culture and the possibility for its improvement in selection programs. These results are in agreement with those of some studies on wheat (Becraft and Taylor, 1992, Zamani *et al.* 2003) and on other cereals (Powell, 1988; Afele and Kannenberg, 1990; Lee *et al*., 2004).

Based on the results of this study, additive effects of genes were more important than dominance effects in genetic control of responding anthers (embryoid induction), embryoid production, and green regenerants, which was in agreement with the results obtained in other studies (Lazar *et al.* 1984a and Deaton *et al.* 1987). However, Chaudhary *et al.* (2003) found non-additive gene effects for embryoid induction and additive gene effects for regeneration and green plant development. The discrepancy observed in the results may be due to the genetic background of the genotypes (Lazar *et al.*, 1984b), environmental factors (Charmet and Bernard, 1984), and the spikes sampled from a plant (Petolino and Thompson, 1987).

Fitting the simple additive-dominance model to generation means indicated that the inheritance of the studied traits was relatively simple and no significant epistasis (non-allelic interactions) was involved. The genetic parameters of [d] and [h] were not significant for the ratio of albino regenerated plants to responding anthers. The parameter [d] can be small and not significant due to gene dispersion and the magnitude of the parameter [h] is affected by ambi-directionality of dominance in different loci (Kearsey and Pooni, 1996).

Significant difference between means of the F_1's and their reciprocals and also the significant genetic parameter for maternal effects for embryoid induction indicated the existence of maternal effects; therefore, selection of appropriate female parents can be important for androgenetic response. The reciprocal effects can be due to the cytoplasmic factors (Sagi and Barnabas, 1989; Ekiz and Konzak, 1991) that may affect phenotypic expression of the trait. The results indicated that the cytoplasm of parent Do1 derived from tetraploid wheat can be useful in embryoid induction. The relatively higher embryoid induction in synthetic wheat genotypes (Do1 and Pol) was not surprising since they carry the same genome of tetraploid wheat. These results were in agreement with those of De Buyser and Henry (1980), who reported that the tetraploid wheat had a higher embryoid induction level than the common bread wheat. Also, Ghaemi and Sarrafi (1994) reported that the addition of the "D" genome from *Triticum tauschii* to tetraploid wheat to produce synthetic wheat had a positive and significant effect on androgenetic response. The significantly higher mean of reciprocal F_1 for embryo induction compared to the corresponding F_1 generation and its better parent in the G×P cross indicate heterobeltiosis and the possible influence of maternal effects on the expression of heterosis in this cross.

The significant parameter of additive component [d] for the ratio of green plants to total (green + albino) regenerants in all crosses showed that the additive effects of genes were more important than dominance effects in its genetic control. Thus, this trait can be improved by selection programs. These results are in agreement with Lazar *et al.* (1984a) and Deaton *et al.* (1987) who found that this trait was very heritable and that additive effects were more important than dominance in its genetic control.

In segregating generations, some individuals had an excellent response for embryoid induction but they had a poor response for plant regeneration and *vice versa*, which imply that these traits could be independent. These results agree well with earlier findings

about wheat (Lazar *et al.* 1984a) and barley (Foroughi-Wehr *et al*., 1982). The high responses of different genotypes of F_2 compared with their parental lines for embryoid induction or plant regeneration in H×P and G×D crosses were indicative of transgressive segregation, which is useful in breeding programs.

The relatively high narrow-sense heritability for the ratio of responding anthers, embryoids per responding anthers, and the ratio of green plants in some crosses also indicated that most of the phenotypic variation for these traits was due to additive gene effects (Falconer and Mackay, 1996).

The degree of dominance which explains the ratio of dominance to additive effects of the genes in all loci was between zero to one for most of the studied traits, implying that incomplete dominance gene action was involved in their inheritance (Mather and Jinks, 1982); however, the dominance value of 1.4 for embryoids per responding anther in the H×D cross showed the overdominance gene action for this trait.

In conclusion, the existing genetic variation, simple mode of inheritance and, more importantly, gene additive effects in genetic control of androgenesis indicated that these traits can be improved by selection in breeding programs. The Do1 that is the highly responsive of the synthetic wheat genotypes to androgenesis can be used as the female parent in hybridization and selection in breeding programs.

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