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Cultivar and cultivar × environment effects on the development of callus and polyhaploid plants from anther cultures of wheat*

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Summary. Plants of three common wheat (*Triticum aestivum* L. em. Thell) cultivars and one randomly selected doubled-haploid line derived by anther culture from each of the three cultivars were each grown in three environments, a field environment, a greenhouse environment, and a growth chamber environment. Anthers containing largely miduninucleate to late uninucleate microspores were cultured and calli were induced to regenerate plants in order to assess the effects of cultivar, cultivar family (cultivar and corresponding doubled-haploid derivative), anther-donor plant environment, and cultivar × environment interaction on androgenic responses. Large differences in response were observed among cultivars as well as between cultivars and doubled-haploids. Differences between cultivar and doubled-haploid within cultivar family usually resulted from higher frequency of response in the cultivar, contrary to the hypothesis that anther culture per se constitutes a general selective device for superior androgenic responses. Also, in a second experiment, anther callusing frequency was greater in the cultivar 'Kitt' than in any of five unique doubled-haploid lines derived from 'Kitt'. Significant effects were also observed in the first experiment for the interactions of cultivar family × environment as well as doubled-haploid vs. cultivar × environment, although the effect of environment itself was less significant than these interactions.

Key words: *Triticum aestivum* L. – Doubled-haploids – Genetics – Androgenesis

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Introduction

Much recent work has established that genetic factors are of great import to in vitro responses of cultured plant tissues (Beversdorf and Bingham 1977; Deaton et al. 1982; Foroughi-Wehr et al. 1982; Izhar and Power 1977; Keyes et al. 1980). Particularly with respect to wheat, genetic improvement of culture response frequencies may prove more useful than manipulation of environmental variables (Baroncelli et al. 1978; Lazar et al. 1983; Schaeffer et al. 1979; Sears and Deckard 1982; Shimada and Makino 1975). Evidence has been provided that androgenic response frequencies in wheat are heritable, though complex, and may be transferred through crossing (Bullock et al. 1982; Picard and DeBuyser 1977; Picard et al. 1978). Some authors have even suggested that genetic improvement in wheat androgenesis may be made through successive cycles of anther culture and regeneration, proposing that such a cycle constitutes a selective device for superior response (Picard and DeBuyser 1977; Rives and Picard 1977).

In contrast to the attention given to effects of genotype and in vitro environment on wheat androgenesis, insufficient work has been done regarding the effect of the growth environment of anther-donor plants on subsequent anther cultures. This may be due in part to the general inability to maintain uniform plant growth conditions (Lee and Rawlings 1982). Similarly, few authors report genotype × environment interactions (Lazar et al. 1983).

While androgenic doubled-haploidy presents many potential advantages to wheat breeders, several key areas of research remain to be addressed before such potential may be realized, some of which will be considered here. First, we must rigorously identify cultivars with consistently high response. Second, we should determine whether anther culture per se, including subsequent plant regeneration, constitutes a selective device for high response frequency. Such selection may be possible even within an inbred cultivar which is theoretically homozygous, though not homogeneous. This is true because most conventional cultivars are not pure lines, but rather are mixtures of a

few to several hundred lines having uniform agronomic characteristics. These lines may be non-uniform for traits not considered by the breeder. Finally, we must determine to what extent our efforts at genetic improvement of androgenic response may be hampered by variation in the environment of anther-donor plants and by genotype × environment interaction.

Materials and methods

In the first experiment, denoted experiment 1, three wheat (*Triticum aestivum* L.) cultivars, Kitt, 'Centurk', and 'Downy', and one random doubled-haploid line derived by anther culture from each cultivar, designated 'Kitt DH', 'Centurk DH', and 'Downy DH', respectively, were evaluated for in vitro androgenic responses. The parent cultivars had previously been preliminarily identified as responsive in terms of both callus production frequency and plant regeneration frequency (Bullock et al. 1982; Schaeffer et al. 1979). The six lines were each grown in three environments, a growth chamber, a greenhouse, and a sandy field at Beltsville, Md, during 1980.

In a second experiment, experiment 2, the cultivar 'Kitt' and five unique doubled-haploid lines derived by anther culture from 'Kitt' were grown under greenhouse conditions. Anthers were cultured from all six sources. The five 'Kitt' doubled-haploid lines were designated KDH4, KDH6, KDH14, KDH19, and KDH22.

Anthers from each source were aseptically excised and cultured as described by Schaeffer et al. (1979). Anthers containing mid to late uninucleate microspores were cultured in 100×20 mm petri plates each containing 20 ml of an agar-solidified medium based on a potato (*Solanum tuberosum* L., cv. 'Kennebec') tuber extract (Anonymous 1976). Cultures were kept in darkness at 4 °C for 3 days and then at 26 °C for 5 days longer, followed by transfer to continuous cool white fluorescent light (1.5 $\mu E\ m^{-2}\ s^{-1}$) at 26 °C. Subsequently derived embryoids were treated for plantlet formation as described by Bullock et al. (1982).

The design of experiment 1 was that of a split-plot with three environments (E). In spite of the gross, variable nature of the environments, environment was considered a fixed effect. Cultivar family (CF), a fixed effect, was the whole plot. Comparison of cultivar to dihaploid (C vs. DH), the sub-plot, and its interactions (C vs. DH×E, C vs. DH×CF and C vs. DH×E×CF) were tested by error b. Fifteen spikes (replications) were cultured per treatment combination and the percentage of successfully cultured anthers per spike as well as the percentage of calli regenerating plants per spike were the units of measure. Callus production frequency was measured as the number of anthers producing calli divided by the total number of anthers cultured and regeneration frequency as the number of calli producing plantlets divided by the total number of calli. Experiment 2 was analyzed as a completely randomized design with ten replications (spikes) per genotype. Data from both experiments were transformed by arcsin ($X^{1/2}$) for statistical analyses.

Results

Very significant differences were observed for both callus production frequency and regeneration frequency among the three cultivar families in experiment 1 (Table 1). Also highly significant were the single degree of freedom contrast between cultivar and doubled haploid (C vs. DH) and the interaction effects, C vs. DH×CF and C vs. DH×E. In contrast to genetic factors and interactions, the effect of anther-donor plant environment was less significant for callusing frequency and nonsignificant for regeneration frequency.

With respect to callus production frequency, differences that did exist in experiment 1 within genetic lines among anther donor plant environments were largely between the greenhouse environment and the other two, but did vary even in that respect among cultivar families (Table 2). For example, the 'Downy DH' and

Table 1. Analyses of variance and mean squares for callusing frequency and regeneration frequency in experiment 1

Source	d.f.	Means squares	
		Callusing frequency[a]	Regeneration frequency[a]
Environment, E	2	0.1694*	1.025
Replications (E)	42	0.0363	0.511
Cultivar family, CF	2	0.2683***	3.021**
E×CF	4	0.1108**	1.034
Error a	84	0.0280	0.539
C vs. DH	1	0.2133***	4.149**
(C vs. DH) × E	2	0.0681**	3.260**
(C vs. DH) × CF	2	0.0906**	2.551*
(C vs. DH) × E × CF	4	0.0435*	1.066
Error b	126	0.0142	0.647

[a] Percentage data transformed using arcsin (square root) transformation prior to analysis

*, **, *** Significant effect at $\alpha = 0.05$, 0.01 and 0.001, respectively

Table 2. Initiation of callus from anthers of three wheat cultivars and one doubled-haploid line derived from each cultivar

Environment	Source cultivar	Anthers callusing, %	
		Cultivar	Doubled-haploid
Field	'Centurk'	5.0 BC	0.9 HI
	'Downy'	1.4 FGHI	1.8 EFGH
	'Kitt'	2.9 DE	0.7 HI
Greenhouse	'Centurk'	3.1 D	2.6 DE
	'Downy'	1.1 GHI	4.3 C
	'Kitt'	3.1 D	2.2 DEFG
Growth chamber	'Centurk'	6.1 B	8.7 A
	'Downy'	1.3 FGHI	2.3 DEF
	'Kitt'	1.8 EFGH	0.6 I

Entries followed by the same capital letter not significantly different by Duncan's multiple range test ($\alpha = 0.05$)

Table 3. Summary of genetic effects on callusing and regeneration in wheat anther cultures in experiment 1

Cultivar	No. of anthers	Anthers callusing (%)	Calli producing plantlets (%)
	Cultivars		
'Centurk'	2865	4.8 A	41.7 A
'Downy'	2408	1.3 D	38.3 A
'Kitt'	2661	2.6 C	30.6 BC
	Doubled-haploids		
'Centurk'	3876	3.7 B	35.8 AB
'Downy'	3829	2.8 BC	26.6 C
'Kitt'	3011	1.1 D	26.9 C

Entries within column followed by the same capital letter not significantly different by Duncan's multiple range test ($\alpha = 0.05$)

Table 4. Initiation of and regeneration from anther callus of the cultivar 'Kitt' and five doubled-haploid lines derived from 'Kitt'

Genotype	No. of anthers	Anthers callusing (%)	Calli producing plantlets (%)
'Kitt'	618	4.2 A	26.9 A
KDH 4	625	1.6 B	25.0 A
KDH 6	674	1.7 B	33.3 A
KDH 14	597	0.5 C	0.0 B
KDH 19	592	2.0 B	36.4 A
KDH 22	663	0.7 C	0.0 B

Entries within column followed by the same capital letter not significantly different by Duncan's multiple range test ($\alpha = 0.05$)

'Kitt DH' lines performed best when the plants were grown in the greenhouse, while the 'Centurk' cultivar's response was least when the anthers were produced in that environment and the productivity of 'Centurk DH' was highest when anthers were taken from the growth chamber. By contrast, relative ranking of the cultivars was consistent across environments. Ranking of the doubled-haploids across environments was also consistent with the exception of the response of 'Centurk DH' grown in the growth chamber. The relationship between the cultivars and the respective doubled-haploids within cultivar family, although significant, varied across both cultivar families and environments. Overall, 'Centurk' outperformed the other two cultivars and all of the doubled-haploids (Table 3), though the highest mean sub-treatment performance was that of 'Centurk DH' from the growth chamber environment (Table 2). Within the 'Kitt' cultivar family, the cultivar consistently had a higher callus production frequency than doubled-haploids derived from it in both experiment 1 (Table 2) and experiment 2 (Table 4).

Regeneration frequency did not differ significantly among environments (Table 1). Cultivar families in experiment 1 differed significantly, 'Centurk' and 'Centurk DH' again out-performing the other two pairs (Table 3), although many plantlets derived from 'Centurk' were albino. The ranking of both cultivars and doubled-haploids differed across environments (Table 5). Relative response frequencies of cultivar and related doubled-haploid also varied significantly among both cultivar families and environments (Tables 1 and 5). For example, regeneration rate was higher in 'Kitt' than in 'Kitt DH' when anthers were taken from the growth chamber, but when anthers were

Table 5. Regeneration from anther-derived callus of three wheat cultivars and one doubled-haploid line derived from each cultivar

Environment	Source cultivar	Calli having at least 3 embryoids (%)[a]		Calli producing plantlets (%)[a]	
		Cultivar	DH	Cultivar	DH
Field	'Centurk'	38.0 ABCDE	26.8 DEF	45.5 BC	24.2 HI
	'Downy'	43.3 ABCD	21.8 F	32.2 EFGH	15.6 J
	'Kitt'	26.8 DEF	29.2 CDEF	22.4 IJ	14.7 J
Greenhouse	'Centurk'	37.5 ABCDE	40.2 ABCD	33.3 DEFG	35.9 DEF
	'Downy'	25.0 EF	29.3 CDEF	25.0 HI	26.2 GHI
	'Kitt'	32.5 BCDE	31.5 BCDEF	27.5 GHI	38.0 CDE
Growth chamber	'Centurk'	46.3 AB	52.5 A	44.6 BC	47.5 B
	'Downy'	44.4 AB	38.0 ABCDE	55.6 A	38.0 CDE
	'Kitt'	40.4 ABCD	29.2 CDE	40.4 BCD	29.2 FGHI

[a] Entries within these headings followed by the same capital letter not significantly different by Duncan's multiple range test ($\alpha = 0.05$)

produced on greenhouse-grown plants 'Kitt DH' produced plantlets more frequently than 'Kitt'. None of the five doubled-haploid 'Kitt' lines in experiment 2 significantly exceeded the parent cultivar for regeneration frequency (Table 4). The frequencies of calli possessing varying numbers of embryoid structures were assessed in experiment 1 (Table 5). The greatest significant correlation of regeneration frequency to multiple embryoid formation existed for calli containing at least three embryoids, $R^2 = 0.88$. No significant correlation, however, existed between regeneration frequency and callusing frequency.

Discussion

All of the cultivars studied had been previously identified as relatively high responding cultivars with regard to callusing frequency (Schaeffer et al. 1979), though relatively small numbers of anthers and regenerating calli had been examined. The current study more rigorously evaluated the range of response frequencies to be expected for each cultivar with respect to both callusing frequency and regeneration frequency. In fact, optimal response frequencies for all three cultivars lie within the range of responses of relatively high responding types observed by other authors (Ouyang et al. 1973; Picard and DeBuyser 1977). Genetic factors certainly accounted for a high proportion of the observed variation in all of the in vitro characteristics examined. The effects due to cultivar were particularly pronounced. 'Centurk' clearly callused and regenerated more frequently than 'Downy' or 'Kitt'.

The current study also supports observations that cultivars have different culture, regeneration and stability characteristics. For example, over 40% of the 'Centurk' plantlets were albino, while almost all 'Downy' and 'Kitt' plantlets were green. Evidence also exist to suggest that anther culture of 'Downy' may result in high frequencies of genetically abnormal doubled-haploids (Schaeffer et al. 1983). Further, claims have been made that, in general, anther culture may induce mutations in the resultant doubled-haploid population. Clearly, although somaclonal variation induced during androgenesis may be useful in germplasm development, the aims of a wheat cultivar development program demand high frequency recovery of doubled-haploids that are agronomically true to type.

Although the environments of anther-donor plants examined were considered fixed, the variability due to environment and environmental interactions observed may be representative of the experiment to experiment variation in response frequencies that may be encountered. In order to improve response frequencies, not only the mean response, but also the range of responses must be considered. Selection schemes for increased androgenic responses should therefore be pursued over the range of environments that anther-donor plants may perceive.

The biological significance of the correlation observed between regeneration frequency and multiple-embryoid-callus frequency is uncertain. Perhaps it may result from a cross feeding effect among embryoids or a sharing of developing root systems. It is not simply related to the number of embryoids available, as the ratio of plantlets produced to embryoids available is larger when more embryoids are present. Regardless, the formation of such multiple-embryoid structures appears to be dependent upon genotype and the possibility is raised that visual selection of such structures may improve regeneration frequencies. It should be noted that the lack of observed correlation between callusing frequency and regeneration frequency lends support to the hypothesis of Foroughi-Wehr et al. (1982), suggesting that the androgenic response may be divided into at least two independent, heritable mechanisms.

The finding that cultivars usually outperformed their respective doubled-haploid lines was contrary to the hypothesis that anther culture per se is an effective general selection device for improved androgenic response (Picard and DeBuyser 1977; Rives and Picard 1977). It should be noted that cultivars used in the current study were not the same as those examined by Picard and DeBuyser (1977), which may account for the differing results. The finding of significant genotype × environment interaction may also be related in part to the superior response of doubled-haploids as compared to parent cultivars in previous reports. It should be noted that some doubled-haploids in the current study did have in vitro responses superior to the parent cultivar (e.g. 'Centurk DH') in at least one environment, suggesting that selection for higher frequencies of response should be possible. The results of this study indicated that, in general, doubled-haploids indeed differed from their parental cultivars for androgenic responses, but most of the changes were negative, particularly when 'Kitt' was the parent cultivar. The use of inbred cultivars as sources of doubled-haploids was not unreasonable, as variability for traits not considered by breeders, such as androgenic responses, may well exist in bulk composites selected only for uniform agronomic phenotype. Examination of hybrid populations for androgenic responses is underway. Selection of doubled-haploids which respond well in anther culture seems possible, but likely as a result of the existence of significant genotypic variability for these traits rather than any selective pressure imposed by the culture process.

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References

Anonymous (1976) A sharp increase in the frequency of pollen plant induction in wheat with potato medium. Acta Genet Sin 3:30–31

Baroncelli S, Buiatti M, Bennici A, Foroughi-Wehr G, Mix B, Gaul H, Tagliasacchi M, Loiero M, Giorgi B (1978) Genetic control of in vitro and in vivo growth in hexaploid wheat. 1. Behavior of ditelocentric lines. Z Pflanzenzücht 80:109–116

Beversdorf WD, Bingham ET (1977) Degrees of differentiation obtained in tissue cultures of *Glycine* species. Crop Sci 17:307–311

Bullock WP, Baenziger PS, Schaeffer GW, Bottino PJ (1982) Anther culture of wheat (*Triticum aestivum* L.) F_1's and their reciprocal crosses. Theor Appl Genet 62:155–159

Deaton WR, Legg PD, Collins GB (1982) A comparison of burley tobacco doubled-haploid lines with their source inbred cultivars. Theor Appl Genet 62:69–74

Foroughi-Wehr B, Friedt W, Wenzel G (1982) On the genetic improvement of androgenetic haploid formation in *Hordeum vulgare* L. Theor Appl Genet 62:233–239

Izhar S, Power JB (1977) Genetical studies with petunia leaf protoplasts. 1. Genetic variation to specific growth hormones and possible genetic control on stages of protoplast development in culture. Plant Sci Lett 8:375–383

Keyes GJ, Collins GB, Taylor NL (1980) Genetic variation in tissue cultures of red clover. Theor Appl Genet 58: 265–271

Lazar MD, Collins GB, Vian WE (in press) Genetic and environmental effects on the growth and differentiation of wheat somatic cell cultures. J Hered

Lee CS, Rawlings JO (1982) Design of experiments in growth chambers – uniformity trials in the North Carolina State University phytotron. Crop Sci 22:551–558

Ouyang TW, Hu H, Chuang CC, Tseng CC (1973) Induction of pollen plants from anthers of *Triticum aestivum* L. cultured in vitro. Sci Sin 16:79–95

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

Picard E, DeBuyser J, Henry Y (1978) Technique de production d'haploides de ble par culture d'antheres in vitro. Le Selectionneur Franc 26:25–37

Rives M, Picard E (1977) A case of genetic assimilation: selection through androgenesis or parthenogenesis of haploid producing systems (an hypothesis). Ann Amelior Plant 27:489–491

Schaeffer GW, Baenziger PS, Worley J (1979) Haploid plant development from anthers and in vitro embryo culture of wheat. Crop Sci 19:697–702

Schaeffer GW, Lazar MD, Baenziger PS (1983) Tissue culture of wheat. In: Ammirato PV, Evans DA, Sharp WR, Yamada Y (eds) Plant tissue culture: applications for crop improvement, vol 2. MacMillan Press, New York (in press)

Sears RG, Deckard EL (1982) Tissue culture variability in wheat: callus induction and plant regeneration. Crop Sci 22:546–550

Shimada T, Makino T (1975) In vitro culture of wheat. 3. Anther culture of the A genome aneuploids in common wheat. Theor Appl Genet 46:407–410