Theor Appl Genet (1984) 68:131–134

Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.) anther cultures*

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Received November 26, 1983
Communicated by G. Wenzel

Summary. Frequency of callus formation in wheat (*Triticum aestivum* L. em. Thell) anthers cultured in vitro and the frequency of subsequent plantlet formation from such calli were examined in a diallel population produced from five inbred spring wheat cultivars. Two of the five cultivars were believed to possess relatively high frequencies of response and the other three relatively low response frequencies, based on previous studies. General and specific combining abilities were estimated and found to be highly significant for both traits. Reciprocal effects were also estimated and were highly significant for both traits. Of the 25 entries, the largest mean callus formation frequency was observed on anthers of 'Kitt'×'Olaf', while the largest mean plantlet formation frequency was observed using anthers of the cultivar, 'Fielder'. No significant correlation was observed between the two traits. Heritability estimates in the range of 0.6–0.7 suggested, however, that both traits were highly heritable, so that rapid gain from selection for these traits should be possible. Current limitations due to genetic variation in responses therefore may not constitute a major obstacle to application of in vitro techniques by wheat breeders.

Key words: Doubled-haploids – Diallel – Tissue culture

Introduction

Genetic factors have been noted to be major contributors to in vitro growth responses of cultured plant tissues (Dunwell 1981; Foroughi-Wehr et al. 1982; Raquin 1982). This has been of particular interest to plant breeders concerned with the utilization of in vitro techniques in the development of useful germplasm.

It has been suggested that the transfer of such genes to elite germplasm via crossing may be the most effective means of realizing the potential advantages of tissue-culture-related techniques (Bullock et al. 1982; Foroughi-Wehr et al. 1982; Lazar et al. 1984; Liang et al. 1982). However, little rigorous work has been done to establish the degree of importance of additive genetic effects to in vitro traits (Deaton et al. 1982).

Anther culture is a technique for the production of haploid and doubled-haploid plants which are of great potential usefulness to plant breeders, especially for improving the efficiency of selection in relatively small populations (Griffing 1975; Scowcroft 1978). Practical applicability of doubled-haploid breeding has been adequately demonstrated in tobacco (Deaton et al. 1982), potato (Mendiburu et al. 1974) and barley (Kasha and Reinbergs 1980). However, only in tobacco are haploids routinely produced by anther culture. In wheat, as in many other crops, application of doubled-haploid breeding is currently limited by the low frequency of plantlet production per anther, especially in elite breeding lines (Bullock et al. 1982; Picard and DeBuyser 1977). Other limitations include variation in response frequencies due to the anther donor plant environment and to genotype×environment interaction (Lazar et al. 1984).

The current study was designed to estimate general and specific combining abilities, reciprocal effects and heritabilities for callus production and subsequent plantlet development in anther cultures of a wheat population.

Materials and methods

Five spring wheat cultivars, 'Chris', 'Kitt', 'Fielder', 'Olaf' and 'Waldron' were intermated in all possible combinations to form a diallel population. The five parents were chosen on the basis of previous work (Bullock et al. 1982; Lazar et al. 1984; Schaeffer et al. 1979) to represent the available range of callusing and regeneration frequencies. The 20 F_1 hybrids and five parents were grown under a combination of incandescent

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and cool white fluorescent light at an incident intensity of 250 μ Em^{-2} s^{-1} at 21 °C. Daylength was 12 h for the first 30 days of growth and 15 h thereafter. Anthers containing primarily mid to late uninucleate microspores were aseptically exised and cultured as described previously (Lazar et al. 1984).

The experiment was designed as a randomized complete block with 25 genotypes, 2 planting dates (blocks) and 8 spikes (replications) cultured per genotype and planting date. At least 1,000 anthers were cultured for each entry. Callus production frequency was measured as the number of anthers producing calli divided by the total number of anthers cultured, quantity times 100%, and regeneration frequency as the number of calli producing plantlets divided by the total number of calli, quantity times 100%. In the case of genotypes having very low callus production frequencies, additional anthers were cultured subsequently in order to allow sufficient numbers of calli for analysis of regeneration frequencies. Data were transformed by arcsin ($X^{1/2}$) prior to statistical analysis. More than one callus was often obtained from a single anther. Still, for one entry, 9,714 anthers were cultured in order to produce a sufficient callus population. General combining ability (GCA), specific combining ability (SCA) and reciprocal effects were partitioned from total genotypic variance using Griffing's (1956) model I, method 1. Narrow sense heritabilities were calculated based upon individual cultivars (h_i^2) as well as upon family means (h_f^2) according to the following formulae (Kempthorne 1957):

$$\widehat{h_i^2} = 4\sigma^2_{gca}/(4\sigma^2_{gca} + 4\sigma^2_{sca} + \sigma^2_r + \sigma^2)\,,$$

$$\widehat{h_f^2} = 4\sigma^2_{gca}/(4\sigma^2_{gca} + 4\sigma^2_{sca} + \sigma^2_r + \sigma^2/n)\,.$$

Results

Of the five parent cultivars, the greatest mean callus production response was observed in 'Chris' and 'Kitt' (Table 1), and the least in 'Olaf'. Several of the F_1's, however, produced callus more frequently than either parent. The greatest overall callusing response was observed in the cross, 'Kitt'×'Olaf', which produced calli from 8.5% of anthers cultured. Greatest mean regeneration response of all entries was observed in the cultivar 'Fielder' (Table 2). Some heterotic combinations were also found for regeneration response, most notably 'Kitt'×'Olaf' and 'Olaf'×'Chris'. For both response variables, genotypic effects were highly significant, while block effects and genotype × block interactions were nonsignificant (Table 3).

Partitioning of genotypic variability revealed that GCA, SCA and reciprocal effects were all significant at the 0.01 significance level for both callus production and plantlet production (Table 4). GCA effects were significant for both variables in all cultivars except 'Olaf' (Table 5). Several SCA effects differed significantly from zero for both traits, particularly crosses of 'Kitt' with either 'Fielder' or 'Olaf' (Table 6). No significant correlation was observed between callus production and plant regeneration from calli ($r = +0.38$). Heritabilities in the narrow sense were estimated (Table 7) for both individual cultivars and cultivar families to be in the range of 0.6–0.7 for both variables.

Discussion

This report rigorously establishes not only the existence of genetic controls over in vitro developmental traits, but also for the first time establishes estimates of quantitative inheritance parameters, including herita-

Table 1. Mean callus production frequencies (%) in anther cultures of a diallel wheat population

		Male parent				
		'Chris'	'Fielder'	'Kitt'	'Olaf'	'Waldron'
	'Chris'	4.0 BC	4.8 B	2.9 C	2.5 CD	4.0 BC
Female	'Fielder'	1.4 E	1.0 EF	0.3 F	0.9 F	0.3 F
	'Kitt'	3.3 BC	1.2 EF	2.3 CDE	8.5 A	1.5 E
Parent	'Olaf'	6.0 B	1.1 EF	2.7 CD	0.3 F	0.8 F
	'Waldron'	2.0 DE	0.4 F	1.6 DE	0.9 F	0.6 F

Entries followed by the same capital letter not significantly different by Waller-Duncan multiple range test (K ratio = 100). Percentages based upon greater than 1000 anthers cultured per entry

Table 2. Mean plantlet production frequencies (%) in anther derived callus of a diallel wheat population

		Male parent				
		'Chris'	'Fielder'	'Kitt'	'Olaf'	'Waldron'
	'Chris'	45.3 A	40.2 AB	36.5 BC	1.4 E	0.6 E
Female	'Fielder'	16.7 D	49.2 A	0.0 E	31.1 C	0.0 E
	'Kitt'	10.4 D	5.0 E	30.9 C	42.5 AB	0.9 E
Parent	'Olaf'	48.0 A	12.5 D	3.3 E	0.0 E	2.4 E
	'Waldron'	2.5 E	0.0 E	3.6 E	1.0 E	0.8 E

Entries followed by the same capital letter not significantly different by Waller-Duncan multiple range test (K ratio = 100). Percentages based upon at least 128 calli per entry

Table 3. Analyses of variance for callus production frequency and plantlet production (regeneration) frequency

		Mean squares	
		Callus production frequency	Regeneration frequency
		$\times 10^5$	
Genotype (G)	24	7 487**	172 504**
Block (B)	1	193	2 077
G×B	24	759	18 298
Error	350	983	22 823

** F-test significant at $\alpha = 0.01$

Table 4. Analyses of partitioned genotypic variance for callus production frequency and plantlet production (regeneration) frequency

Source	df	Mean squares	
		Callus production frequency	Regeneration frequency
		$\times 10^5$	
GCA	4	1 173**	19 338**
SCA	10	315**	6 297**
Reciprocals	10	631**	3 765**
Error	350	61	1 422

** F-test significant at $\alpha = 0.01$

Table 5. General combining ability (GCA) effects for callus production frequency and plantlet production (regeneration) frequency in anther cultures of a diallel wheat population

Cultivar	Callus production frequency	Regeneration frequency
'Chris'	+0.03886**	+0.20330**
'Fielder'	−0.02958**	+0.06144**
'Kitt'	+0.02789*	+0.06937**
'Olaf'	+0.00198	+0.00362
'Waldron'	−0.03920**	−0.33772**

Effects based upon $\sin^{-1}(X^{1/2})$ transformed data
*, ** t-test significant at $\alpha = 0.05$ and 0.01, respectively

bility, for two such traits. In the population under study variation due to genotype was by far the largest component of the total variability, and most of the genotypic variance was due to GCA effects, indicating that additive genetic variance was a primary contributor to the observed responses.

The importance of additive effects in anther culture responsiveness had also been suggested by a previous study (Bullock et al. 1982) in which callus production frequencies of certain F_1 hybrids were very close to the midparental values. Thus, narrow sense heritability estimates were quite high, suggesting that transfer of both callus production frequency and plantlet production frequency from superior responding lines to inferior lines could be quite rapid. It is not to be anticipated, therefore, that lack of anther culture responsiveness of many elite lines should continue to hinder application of doubled-haploid breeding in wheat. Direct application of results presented here, however, must be limited to the cultivars which were used in this study.

While many authors have commented on the likely heritability of in vitro characteristics (Bullock et al. 1982; Foroughi-Wehr et al. 1982; Keyes et al. 1980; Raquin 1982), the existence of genotypic differences in anther culture responsiveness does not necessarily imply that such genetic variance may be easily manipulated, due to genotype × environment variation (Lazar et al. 1984) as well as epistatic and dominance effects (Picard and DeBuyser 1977). Further estimates of the heritability of such traits in other populations might, therefore, produce a more general understanding of the usefulness of genotypic variation in selection schemes.

The lack of significant correlation between callus production frequency and plantlet production frequency supports the hypothesis that haploid plant production from anther culture is divisible into at least two independent, heritable traits (Foroughi-Wehr et al. 1982; Lazar et al. 1984). Indeed, 'Kitt', which produced anther callus relatively frequently, produced fewer plantlets than 'Fielder', which had a relatively low callus production frequency. Foroughi-Wehr et al. (1982) suggested that the overall production of haploids might be composed of four independent mechanisms, callus induction, callus stabilization, plantlet induction and green plant formation. While this scheme appears reasonable, the current study suggests that in our system rapid selection progress may be achieved merely by considering a two step approach. For the genotypes examined here, very few albino regenerants were observed. This may not be the case for all genotypes, however (Foroughi-Wehr et al. 1982; Lazar et al. 1984), necessitating consideration of a third step.

While the large majority of genetic variability in the current study is explainable by the action of nuclear genes, the existence of significant reciprocal effects for both characteristics studied is strongly indicative of the involvement of cytoplasmic genes as well. Significant reciprocal effects were not observed for all pairs, so the results do not conflict with the lack of observed reciprocal effects in a previous study (Bullock et al. 1982), but are in agreement with other reports which have suggested cytoplasmic effects on anther culture response (Foroughi-Wehr et al. 1982; Picard et al. 1978; Simon and Peloquin 1977).

Although the current study does not deal directly with the effects of either anther donor plant environment or culture environment on anther culture responsiveness, such subjects have been examined extensively (De Buyser and Henry 1979; Dunwell 1981; Liang et al. 1982; Lazar et al. 1983, 1984). Comparing the results of such studies with this and other work on genetic control of in vitro responses (Bullock et al. 1982; Foroughi-Wehr et al. 1982; Lazar et al. 1984) suggests that the most effective means of improving anther culture response most likely lies in the genetic improvement of currently inferior lines coupled with the alteration of environmental conditions.

Table 6. Specific combining ability (SCA) effects for callus production frequency and plantlet production (regeneration) frequency in anther cultures of a diallel wheat population

Callus production frequency				
Genotype	'Fielder'	'Kitt'	'Olaf'	'Waldron'
'Chris'	+0.02464*	+0.00992	+0.02533*	−0.01916*
'Fielder'		−0.03759**	−0.00598	+0.00360
'Kitt'			+0.07805**	−0.00061
'Olaf'				−0.01351
Regeneration frequency				
Genotype	'Fielder'	'Kitt'	'Olaf'	'Waldron'
'Chris'	+0.07023*	+0.05172	+0.03287	−0.06174*
'Fielder'		−0.22368**	+0.08345*	−0.08401*
'Kitt'			+0.16548**	−0.03960
'Olaf'				+0.00527

Effects based upon $\sin^{-1}(X^{1/2})$ transformed data
*, ** t-test significant at $\alpha = 0.05$ and 0.01, respectively

Table 7. Heritability estimates for callus production frequency and plantlet production frequency (regeneration) in anther cultures of a diallel wheat population

Estimate of heritability based upon	Callus production frequency	Regeneration frequency
Individual Cultivars ($\widehat{h_1^2}$)	0.620	0.599
Family Means ($\widehat{h_f^2}$)	0.706	0.718

Acknowledgements. Our thanks is due to Mr. W. P. Bullock and Mr. J. T. Dudley for technical assistance during this study. M. D. Lazar was supported in part by USDA competitive grant No. 59-2246-0-1-436-0.

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