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Partitioning of variation derived from tissue culture of winter wheat *

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Summary. The presence of somaclonal variation is well documented in wheat, but information is needed which identifies point(s) during the tissue culture process at which variation is most likely induced. Field experiments were designed to partition the total somaclonal variation among three potential sources: single embryo-derived calli, regenerant (R_0) plants of a common embryo-derived callus, and spike-derived lines of a common R_0 plant. Three populations of winter wheat ('TAM 105', 'Vona', and 'KS75210') totaling 72 lines were evaluated in replicated drilled plots in the R_2 and R_3 generations. The principal source of variation was influenced by parent genotype. Considering all traits, somaclonal variation in the 'TAM 105' and 'Vona' populations was predominantly attributed to tillers from the same regenerant plant. This source, as well as the original R_0 plant source, contributed to variation in the 'KS75210' population, depending on the trait measured. Embryos did not consistently provide a significant source of variation. The presence of somaclonal variation was not always associated with a downward shift in population mean compared to parental controls. Significant population increases were noted for spike density and biomass, and some lines showed significantly higher grain protein content without a yield reduction, but these responses were again genotype-specific.

Key words: *Triticum aestivum* L. – Somaclonal variation – Wheat breeding

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Introduction

Genetic variation induced by passage through tissue culture (somaclonal variation, as coined by Larkin and Skowcroft 1981) has been reported in wheat for many traits, including the presence or absence of awns, plant height, gliadin proteins, α- and β-amylases, and tolerance to aluminum. Agronomically useful variation has been reported to a lesser extent, although encouraging examples have recently emerged (Ryan et al. 1987; Lazar et al. 1988). Some initial interest in the phenomenon of somaclonal variation has shifted from its use as a source of random variability in breeding populations to reducing or possibly eliminating it during plant regeneration from transformation products (Bingham 1986). This interest shift is spurred by the growing realization that culture-derived variability has yet to contribute directly to cultivar development (Vasil 1988). However, tissue culture may still play a critical role in breeding programs by generating useful variation without outcrossing and, thus, with potentially few phenotypic changes (Maddock and Semple 1986; Larkin 1987).

Whether somaclonal variation is perceived as a novel source of genetic variability or an undesirable consequence of cell culture, further research is needed which identifies principal sources of variation during callus initiation and plantlet regeneration. Potential sources of variation might include calli from different cultured embryos of the same parent genotype, regenerant plants from the same embryo-derived callus, and progeny derived from different tillers of the same regenerant. Larkin (1987) emphasized the importance of including multiple regenerants from the same embryo-derived callus to ensure that somaclonal variation was actually culture-derived and not a result of genetic heterogeneity of the donor parent.

Table 1. Derivation of 72 somaclonal lines from three winter wheat parents

Parent	No. of embryo-derived calli	Regenerate (R_0) plants/embryo	Spike-derived lines/regenerant	Total no. of lines/genotype
'TAM 105'	2	3	3	18
'Vona'	3	2	3	18
'KS75210'	4	3	3	36

Ryan et al. (1987) showed that somaclonal wheat lines differing significantly from their parent controls were obtained from different embryos and from different regenerant plants. Their results did not indicate whether significant variants were derived from different regenerants of the same callus or whether somaclonal lines of similar derivation differed among each other. By harvesting seed of individual spikes from 70 regenerant plants, Chen et al. (1987) were able to show somaclonal variation among regenerant families and among spike-derived lines (head rows) within families. Conclusions were tentative because lines were not replicated or randomized with respect to family origin. Embryo source of each regenerant family was also unknown and, therefore, biased the estimates of among-regenerant family variance upward.

The relative magnitudes of all sources of variation resulting from embryo-derived callus culture and plantlet regeneration have not yet been systematically determined in one experiment. The primary objective of this research was to partition total somaclonal variation among three potential sources: single embryo-derived calli, regenerant plants of a common embryo-derived callus, and spike-derived lines of a common regenerant plant. Because genotype of the donor plant often influences tissue culture response (Larkin 1987), somaclonal progeny were generated from three diverse winter wheat genotypes.

Materials and methods

Donor plants

Two cultivars ('TAM 105' and 'Vona') and one experimental line of winter wheat ('KS75210') were chosen based on their adaptive value in the southern Great Plains and their relative success in tissue culture (unpublished data). All plants were grown in a greenhouse maintained between 13° and 21 °C with a 12-h photoperiod following a 6-week vernalization period at 4 °C also with a 12-h photoperiod.

Tissue culture and regeneration

Immature embryos were aseptically excised from tagged plants approximately 7–14 days after anthesis and cultured individually in tubes (25 × 150 mm). Those embryos which showed precocious germination were discarded. The callus induction medium contained the major and minor minerals of Murashige and Skoog (1962), Gamborg's B-5 vitamins (Gamborg et al. 1976), 1–2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 30 g l^{-1} sucrose, and 7 g l^{-1} agar at pH 5.8. After 4–6 weeks, calli were transferred to the same medium lacking 2,4-D to regenerate plantlets. After 2–3 weeks, plantlets were transferred to one-half strength basal medium for additional root development. Only well-rooted plantlets were transferred to soil in pots for seed production under greenhouse conditions described above.

Generation of test populations

Among the three donor parents, 51 regenerant plants from 10 embryo-derived calli were grown to maturity in 1984. Regenerant plants were designated as the R_0 generation according to the notation of Chaleff (1981). Mature spikes were labeled on each R_0 plant to maintain pedigree and threshed separately to provide a total of 280 R_1 lines. An additional 108 spikes were harvested but did not produce seed. Due to space limitations in the greenhouse, 191 R_1 lines were grown in 1985 for seed increase under conditions described above. Several R_0 plants produced abnormally low numbers of seed and had abnormal spike morphology, but their progeny were not necessarily excluded from the R_1 increase population. Each R_1 line was represented by 1–14 plants. Of those R_1 lines with 5 or more plants, 72 lines were randomly chosen for field testing (Table 1). No other selection was imposed in the sampling of lines; five plants were required to provide sufficient seed for testing in drilled plots at normal seeding rates. Populations differed in numbers of embryo-derived calli and R_0 plants sampled, depending upon availability of those sources and limits set by maintaining a balanced treatment design within populations. Test progenies were formed by bulking seeds from all plants of a line.

Experimental procedures and statistical analyses

Populations were tested separately but planted in adjacent blocks in the same field. Entries were assigned to 4-row plots (0.3 × 4.3 m) arranged in a randomized complete block design with three replications per population. The respective parent genotype was also included with the somaclonal lines. Test entries were planted in the two center rows of each plot at a seeding rate of 52 seeds m^{-1} (equivalent to ca. 56 kg ha^{-1}); seeds of the corresponding parent genotype were planted in the remaining border rows at the same rate. Tests were conducted at the Agronomy Research Station in Stillwater, Oklahoma in 1986 (R_2 generation) and 1987 (R_3 generation). Seeds harvested from R_2 plots in 1986 were used for planting in 1987.

Heading date was recorded for each plot when over 50% of the heads had completely emerged from the boot. Plant height was averaged over three determinations from each plot and measured as the distance from ground level to spike tip excluding awns. At harvest maturity, the number of seed-bearing spikes was determined from a 0.5-m section of each center row to estimate spike density. Ten random spikes were collected

Table 2. Significance levels from nested analyses of variance for grain yield and yield components in the R_2 and R_3 generations of three somaclonal populations

Parent	Source	*df*	Grain yield		Spike density		Kernels/head		Kernel weight	
			R_2	R_3	R_2	R_3	R_2	R_3	R_2	R_3
'Tam 105'	SC genotypes	17	**	**	**	*				
	Among embryos	1								
	R_0 families/embryos	4	**		**					
	Lines/R_0 families	12	**	**	*	*				
	CV (%)		8.9	6.8	11.5	12.2	13.4	8.7	12.2	6.4
'Vona'	SC genotypes	17	**		**	*		**	*	
	Among embryos	2								
	R_0 families/embryos	3	*							
	Lines/R_0 families	12	*					**		
	CV (%)		11.1	8.5	14.5	12.0	10.4	8.5	11.3	10.6
'KS75210'	SC genotypes	35		*	*		**		**	**
	Among embryos	3				**	*		**	
	R_0 families/embryos	8	**	*						
	Lines/R_0 families	24							**	**
	CV (%)		14.0	9.6	11.4	10.8	9.1	12.5	10.6	9.1

*,** *F*-test significant at $P=0.05$ and 0.01, respectively

from each section to determine kernel number per spike and 1,000-kernel weight. The two center rows were harvested at ground level, weighed for total biomass, then threshed to determine grain yield (kg ha^{-1}) and harvest index (%). Duplicate 12-g seed samples were collected from each plot for protein determinations using infrared analysis.

The analysis of variance was first combined over years for each population, but the somaclonal line × year interaction was significant for most population-trait combinations. Thus, only results from within-year analyses of variance were reported. Total variation among somaclonal lines was partitioned according to a nested balanced design into three sources: among embryo-derived calli (hereafter, embryos), R_0 families within embryos, and lines within R_0 families. All effects were considered random. Comparison of individual lines with their parental control was based on Dunnet's two-sided test with the experimentwise error rate set at $P=0.05$. This procedure prevents overestimation of the number of significant variants often generated by standard LSD tests (Chew 1976).

Results

Total somaclonal variation in grain yield was significant ($P<0.05$) in one or both generations of all populations (Table 2). Source(s) of significant yield variation were similar among populations within each generation. Grain yield was not influenced by embryo source in any population or generation. Somaclonal yield variation was instead associated with regenerant (R_0) plant source, particularly when measured in the R_2 generation. Significant variation among spike-derived lines from the same R_0 plant was detected in the 'TAM 105' and 'Vona' populations, but not in the 'KS75210' population. In general, somaclonal yield variation of 'TAM 105' was primarily attributed to lines derived from different tillers of the same regenerant plant, whereas variation of 'KS75210' was attributed to families derived from different regenerant plants of the same embryonic callus. The predominant source of variation was not so obvious for 'Vona', as genotypic variation was completely absent in the R_3 generation.

Of the three yield components, variation in spike density was most noticeable. Total variation was significant in all but one population-generation combination (Table 2). Also, patterns of spike density variation more closely resembled those noted for grain yield. Yield component variation of 'KS75210', however, was an exception. The effect of embryo source was significant in one of the two test generations for each yield component.

Total somaclonal variation in other agronomic traits was observed, albeit for different traits in different populations (Table 3). In summary, variation was significant in both test generations for biomass and heading date of 'TAM 105', biomass of 'Vona', and harvest index, heading date, plant height, and grain protein content of 'KS75210'. The among-embryo source was again unaccountable for the variation. Both R_0 families within embryos and lines within R_0 families contributed to somaclonal variation for the above-mentioned traits and populations. The predominant source, however, for any population-trait combination was not consistent between test generations.

Somaclonal variation within populations was coupled with a significant deviation in population mean from the corresponding parental value for grain yield,

Table 3. Significance levels from nested analyses of variance for several agronomic traits and grain protein content in the R_2 and R_3 generations of three somaclonal populations

Parent	Source	*df*	Biomass		Harvest index		Heading date		Plant height		Grain protein	
			R_2	R_3	R_2	R_3	R_2	R_3	R_2	R_3	R_2	R_3
'TAM 105'	SC genotypes	17	**	**	**		**	*			**	
	Among embryos	1										
	R_0 families/embryos	4	**					*				
	Lines/R_0 families	12		**	**		**				**	
	CV (%)		6.6	4.9	5.3	4.5	3.0	2.0	4.8	3.9	1.5	1.9
'Vona'	SC genotypes	17	**	*	**		**					
	Among embryos	2								*		**
	R_0 families/embryos	3	*		**							
	Lines/R_0 families	12	**	*			*				**	
	CV (%)		6.7	5.4	6.9	6.1	2.2	0.4	5.8	2.8	2.6	1.9
'KS75210'	SC genotype	35			*	**	**	**	**	**	**	**
	Among embryos	3										**
	R_0 families/embryos	8			**						**	*
	Lines/R_0 families	24					**	**		**		
	CV (%)		9.6	6.8	8.4	5.6	4.0	1.0	5.0	4.4	1.5	2.2

*,** *F*-test significant at $P=0.05$ and 0.01, respectively

Table 4. Somaclonal population means, ranges and number of significant somaclonal variants compared with their respective parents for selected traits in the R_2 and R_3 generations

Trait	Statistic	'TAM 105'		'Vona'		'KS75210'	
		R_2	R_3	R_2	R_3	R_2	R_3
Grain yield (kg ha^{-1})	Parent mean	1,222	2,200	1,228	1,925	1,348	1,429
	vs.[a]	ns	**	ns	ns	**	ns
	Population mean	1,238	2,126	1,189	1,925	1,044	1,415
	Population range	606–1,524	1,696–2,345	723–1,457	1,746–2,098	768–1,369	1,225–1,699
	No. variants > parent mean[b]	2	0	0	0	0	0
	No. variants < parent mean[b]	4	1 (1)	2	0	4	0
Spike density (no. m^{-2})	Parent mean	468	449	380	426	389	478
	vs.	ns	**	**	**	ns	ns
	Population mean	446	509	472	472	410	472
	Population range	200–525	418–599	366–568	370–569	322–491	410–537
	No. variants > parent mean	0	0	1	1	0	0
	No. variants < parent mean	2	0	0	0	0	0
Biomass (kg ha^{-1})	Parent mean	4,269	6,960	3,807	5,962	5,118	5,986
	vs.	**	**	**	**	**	**
	Population mean	4,332	6,700	3,934	6,178	4,580	5,420
	Population range	2,476–4,923	5,687–7,099	2,905–4,422	5,671–6,551	3,846–5,157	4,797–6,083
	No. variants > parent mean	0	0	0	0	0	0
	No. variants < parent mean	4	1 (1)	2	0	1	2
Heading date (days from March 31)	Parent mean	28	33	25	29	29	34
	vs.	ns	ns	ns	ns	ns	ns
	Population mean	29	33	25	29	31	34
	Population range	28–33	32–35	24–26	29	22–33	29–35
	No. variants > parent mean	4	1 (1)	0	0	1	0
	No. variants < parent mean	0	0	1	0	1	1 (1)
Grain protein	Parent man	13.2	16.4	13.0	16.3	13.3	17.9
	vs.	ns	ns	ns	ns	ns	ns
	Population mean	13.0	16.3	13.0	16.1	13.5	18.1
	Population range	12.8–13.4	16.1–16.5	12.5–13.4	15.6–16.4	13.2–13.9	17.0–19.3
	No. variants > parent mean	0	0	0	0	1	4
	No. variants < parent mean	0	0	0	0	0	0

[a] *F*-test not significant (ns) or significant at $P=0.05$ (*) or 0.01 (**) for testing H_0: Parent mean = mean of somaclonal lines; total number of somaclonal lines = 18 ('TAM 105' and 'Vona') and 36 ('KS75210')

[b] Individual line means compared against parent mean based on a two-sided Dunnet's test statistic with the experiment-wise error rate set at $P=0.05$. The number of variants common to both generations is indicated in parentheses

spike density, and biomass (Table 4). Average heading date, grain protein content, harvest index, plant height, kernels/head and kernel weight did not differ between parents and their tissue-culture derived populations. Deviations in grain yield were either nonsignificant or negative, depending on the test generation of each population. A greater number of variant lines having significantly smaller or larger values than their parents were identified in the R_2 than in the R_3 generation, with most variants having smaller yield values. Three of the four R_2 variants derived from 'TAM 105' traced to the same R_0 plant. None of those lines, however, showed a significantly negative response in the R_3 generation. Further evaluation of the consistency-in-yield performance of R_2 and R_3 lines was provided by parent-offspring regression analysis of individual line means or family means of R_0-derived lines (data not shown). Regression coefficients for individual lines were 0.06 ('Vona'), 0.12 ('TAM 105'), and 0.46 ('KS75210'). Relative magnitudes of the coefficients did not change when the regression was based on R_0-derived family means.

Despite the absence of a tissue culture-derived increase in average grain yield of any population, significant increases were noted for spike density and biomass (Table 4). Population responses were again genotype-specific. Spike density was increased in both generations of 'Vona'-derived lines, increased in only the R_3 generation of 'TAM 105'-derived lines, and was not changed in either generation of 'KS75210'-derived lines. Both generations of the 'Vona' populations showed higher biomass relative to their parent, but population means for 'KS75210' were actually lower, while population responses were opposite in sign for the R_2 and R_3 generations of 'TAM 105'. Significant variants were identified in heading date (earlier and later) and grain protein content (higher percentage) without a significant shift in population mean from their corresponding parents. Average grain yield of the four high-protein lines (R_3 of 'KS75210') was almost identical to that of their parent or entire population of somaclonal lines.

Discussion

Culture-induced variation in agronomic and quality characters of wheat has become accepted as the rule rather than exception since the initial report of Larkin and Scowcroft (1981). Limited seed supply of selfed progenies of regenerant plants, however, often precluded replicated field testing in conventional yield nursery plots. Furthermore, without a pedigree system of generating test progeny, experimental evidence is lacking which identifies phase(s) of the culture procedure in which variation is induced. In this study, testing was preceded by an additional selfing generation beyond the intial regenerant plant generation (R_0), to reduce potential bias caused by genomic instability and to provide sufficient seed for replicated drilled plots. Of the three sources of variation examined (embryos, regenerant plants within embryos, and tillers within regenerant plants), only the embryo source is subject to bias introduced by genetic heterogeneity of parents. The different embryos were sampled from different members of a potentially heterogeneous population, but clonally related regenerant plants (and, likewise, sister spike-derived lines) originated from the same embryo explant.

Somaclonal variation was demonstrated in two succeeding generations (R_2 and R_3) for all agronomic characters and grain protein content. The 'KS75210' population was variant for more traits than somaclonal populations of 'TAM 105' and 'Vona', agreeing with previous reports (Galiba et al. 1985; Maddock and Semple 1986; Ryan et al. 1987) indicating tissue culture response in wheat was genotype-dependent. The principal source of variation in somaclonal populations was also influenced by parent genotype. Considering all traits, variation in the 'TAM 105' and 'Vona' populations was predominantly attributed to tillers from the same regenerant plant. In contrast, the principal source of variation in 'KS75210' was divided between the original regenerant plant or set of tillers of the same R_0 plant, depending upon the trait measured. The original embryo-derived callus did not consistently provide a significant source of variation, although yield components and grain protein content were affected to some extent in the 'KS75210' population. Both regenerant plant and tillers within regenerant plants contributed significantly to variation in yield components measured in unreplicated head rows of R_1 lines from 'Norstar' winter wheat (Chen et al. 1987). Both sources also contributed to variation in freezing tolerance in the R_2 generations (Lazar et al. 1988). Genetic differences among plants regenerated from the same embryo-derived callus imply that variation originated during the callus culture phase. In contrast, genetic differences among lines derived from different tillers of the same regenerant plant might imply that variation arose during the plantlet regeneration phase, assuming plantlets originated from single cells. Although by observing embryoids we confirmed an earlier report of embryogenesis in wheat callus (Ozias-Akins and Vasil 1982), organogenesis might also have occurred. Thus, multicellular meristematic regions could also have resulted in chimeric R_0 plants even though cell-to-cell differences originated earlier in the culture period.

Yield potential is usually considered the most critical agronomic trait in wheat breeding programs, yet development of somaclonal variants with enhanced yield potential compared with parental controls has proven especially difficult. Yield improvement might instead result from selective pressure applied in vitro for specific stress

factors, such as herbicide (Chaleff and Ray 1984) and aluminum (Conner and Meredith 1985) resistance. Of the 72 lines evaluated for yield potential in this study, only 2 showed significant improvement in yield in the absence of culture-mediated selection. Population means were either less than or equal to their respective parent mean. But, just as Ryan et al. (1987) found no repeatable improvement in R_3 yield performance of somaclonal variants selected for high yield in the R_2 generation, we found a lack of consistency in R_3 performance of variants noted for poor yield in the R_2. This inconsistency was more likely a reflection of genotype-environment interaction than a change in genetic composition of somaclonal lines between the R_2 and R_3 generations. Inadvertent selection was most likely applied in our culture system against extremely aberrant karyotypes, because only regenerant plants with three or more tillers, each producing at least five seeds, were retained for increase and testing. This could account for significant somaclonal variation observed without a downward shift in population mean (e.g., R_2 generations of 'TAM 105' and 'Vona' and R_3 generation of 'KS75210').

Variation in plant height and seed set (an indirect indicator of sterility) has been used to partially explain somaclonal yield variation (Maddock and Semple 1986; Chen et al. 1987). Only in the 'KS75210' population was height variation significant and positively associated with yield variation (average $r=0.45$, $P\leq 0.01$). Partial sterility was apparently unrelated to yield potential, as seed set per spike was not altered in any population relative to the parental controls. Also, the large yield increase observed between the R_2 and R_3 generations of 'TAM 105' and 'Vona' was not interpreted as a result of decreased frequency of sterile plants. Parental performance increased similarly, probably reflecting improved growing conditions in 1987 versus 1986.

Grain protein content constitutes a major component in the quality evaluation of bread wheat genotypes. Somaclonal variation for this trait was significant only in the 'KS75210' population and, thus, was influenced by parent genotype. All variants showed significant improvement in protein content (up to 1.4 percentage units) compared with 'KS75210'. The majority of protein variants generated by Ryan et al. (1987) also showed significant improvement over parental controls (up to 0.9 percentage units). Although these results are encouraging, the level of improvement in both studies is within the limits of conventional breeding (Johnson et al. 1985).

In conclusion, the degree and pattern of somaclonal variation was differentially expressed among three winter wheat genotypes. If the research goal is to use tissue culture-induced variability as a supplemental source of genetic variation, then multiple sampling of progeny from individual spikes of regenerant plants is warranted. This represents little added expense to investments already allocated to embryo culture and plantlet regeneration. However, identification of true variants for agronomic traits requires testing in more than one year (generation) to account for genotype-environment interactions commonly observed for these quantitative traits. On the other hand, random variation is counter-productive when the research goal is to maximize expression of specific traits selected in vitro. Our results support the suggestion by Ryan et al. (1987) of selecting donor genotypes which minimize somaclonal variation.

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