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Somaclonal variation in some agronomic and quality characters in wheat

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Summary. A total of 256 selected lines derived from tissue culture of three hexaploid wheat cultivars were grown in a replicated hill plot experiment to examine somaclonal variation in agronomic characters. The lines were derived by single plant selection for various characters from a total of 100 regenerants. and were either SC_3 or SC_4 generation in the test. Significant variation was found in all the characters measured: height, grain number per spike, kernel weight, yield, total dry weight and harvest index. In most cases, variation could be identified which was both less than and greater than the parental controls. However, there was also an apparent effect of the parent cultivar on the total amount and direction of the variation. For two cultivars, lines could be traced back through the culture phase to individual explant embryos. Many of the original embryos contributed significant variation, and most characters showed significant variation arising from more than one embryo. In the following year, 32 lines selected from the hill plot experiment were grown in larger replicated plots and yield, harvest index and a number of grain and baking quality characters were measured. No lines selected for high yield or harvest index maintained significant improvements over their parental controls. However, significant variation was displayed for many of the quality characters examined. Significant increases in kernel weight, hardness and protein content, and a significant reduction in yellow pigmentation represented potentially useful improvements. Only unfavourable variation was seen in flour yield and in mixograph height, time and breakdown.

Key words: Somaclonal variation – Wheat breeding – Baking quality

Introduction

The phenomenon of somaclonal variation has now been widely documented but the mechanisms causing the variation have yet to be determined. Chromosomal aberrations, ranging from changes in ploidy and whole chromosome loss to translocations, have been widely reported (e.g. Ogihara 1981; Armstrong et al. 1983; Karp and Maddock 1984; Engler and Grogan 1984; Davies et al. 1986). Variants whose effects are apparently restricted to single genes have also been recorded (e.g. Miller and Hughes 1980; Chaleff and Ray 1984; Engler and Grogan 1984; Brettell et al. 1986). In the last case, analysis at the molecular level was possible and a single base pair substitution accounted for the somaclonal variant. Both dominant and co-dominant, as well as recessive variants have been isolated, so loss of gene function is not a necessary consequence of somaclonal variation.

The potential for somaclonal variation to contribute genetic variation for the improvement of plants has been widely recognized (e.g. Larkin and Scowcroft 1981; Tomes and Swanson 1982; Chaleff 1983). Chromosomal rearrangements involving translocations have potential use in the introgression of alien genes, such as has been accomplished by non-tissue culture methods (e.g. Gale et al. 1984). Single gene somaclonal variants have obvious practical application where the genes have large recognizable effects. Such variants in height (Larkin et al. 1984), seed colour (George and Rao 1983) and herbicide resistance (Chaleff and Ray 1984) have already been described, although none has yet, to our knowledge, been deployed in achieving plant breeding goals.

Genes with less recognizable effects on yield or quality are presumably just as subject to somaclonal

variation. However, there is no simple method of isolating these variants. The same difficulties are faced by plant breeders when attempting to make reliable selections in early generations of segregating material in variable environments. This requires that material be grown in the field, in well designed replicated experiments. Only a few such studies have been reported for somaclonal variation. These include experiments with barley (Friedt and Foroughi-Wehr 1983; Powell et al. 1984) and with potato (Shepard et al. 1980). Generally, significant differences were found amongst the somaclonal lines, but few lines were identified which significantly outperformed their parents in important agronomic characters.

The objective of the work reported here was to test some culture derived wheat lines in the field, evaluating performance characteristics such as harvest index and yield, and grain quality characteristics such as hectolitre weight and physical dough strength. The initial evaluation of somaclonal variation was undertaken in hill plots as these enabled a large number of lines to be efficiently examined when seed supply was low. In the location used, harvest index measured on hill plots has been shown to be highly correlated with yield in large plots (Latter and Ellison 1983). Selections from the hill plot experiment were then grown in large plots in the following year.

Materials and methods

Somaclonal lines from the hexaploid wheat (*Triticum aestivum* L.) cultivars Millewa, Warigal and Yaqui 50E, in either the SC_3 or SC_4 generation, were selected for evaluation of their field performance and grain quality characteristics. (The regenerated plant is termed the SC_1 generation, and subsequent generations of selfed progeny the SC_2, SC_3 etc. as in Larkin et al. 1984). Lines were selected to represent the range of variation seen in earlier generations in the glasshouse. Characters represented included height, maturity, awn type, kernel weight, grain colour, grain yield and tiller number. All lines were derived from single plant selections in the SC_2 (Millewa and Warigal) or SC_2 and SC_3 (Yaqui 50E). Tissue culture protocols for all three cultivars, and early generation observations on one of the cultivars, Yaqui 50E, are described in Larkin et al. (1984). Details of the numbers and origins of lines tested in the first experiment are described in Table 1.

The selected lines were evaluated in the field at Narrabri in northern New South Wales during 1983 and 1984. In 1983, 256 somaclonal lines, together with parental controls and an additional check cultivar WW15 were sown in eight 6×6 lattice design experiments with four replications. Each experiment contained 32 randomly assigned lines, the parental controls and WW15. The hill plots, which contained 20 seeds randomly distributed over an area of 15 cm × 15 cm were sown on June 23 in a square grid pattern with 1 m between plots. Three border rows surrounded the experiment.

At maturity, plant height was measured on all plots and a three ear sample collected for the estimation of grain number per spike and kernel weight. Hill plots were cut at ground level and total plant dry weight and grain yield determined for the subsequent calculation of harvest index. Analyses of variance were conducted for individual experiments; comparisons between experiments were made by weighting means according to the relative performance of the four control cultivars common to each experiment.

In 1984 16 Millewa lines and eight Yaqui 50E lines selected in 1983 on the basis of either high yield, harvest index, kernel weight or grain number per spike were further evaluated. An additional seven Millewa lines and one Yaqui 50E line were included because they represented specific contrasts for some of these characters. No Warigal lines were selected. The 32 somaclonal lines, together with the two parental controls and check cultivars Sunstar and WW15 were included in a 6×6 latice design experiment with four replications that was sown on August 10. Plots, which were 15 m long and 10 rows wide with a 0.15 m row spacing were sown at a seeding rate equivalent to 30 kg/ha, which is typical for the region.

Quadrat samples (0.5 m^2) were taken at plant maturity to estimate harvest index, the remainder of the plot area being harvested with an experimental plot harvester. Grain quality attributes were assessed only on the Millewa lines. Hectolitre weight was measured with a standard chondrometer, and grain hardness was determined as a pearling resistance by the method of Chesterfield (1971). Flour extraction was performed with a Quadrumat Junior flour mill after 100 g grain samples had been conditioned to 15% moisture content. Flour protein percentage was analysed by near infrared spectroscopy with a Technicon Infra-Alyzer 300 while variation in flour colour was assessed by determining flour reflectance at 460 nm and 560 nm with a Micromatch Colour Analyser. Physical dough strength, the height of the curve of maximim dough strength

Table 1. Description of parent cultivars and somaclonal lines in the hill plot experiment

Cultivar	Origin	No. of embryos	Periods[a] in callus culture	No. of regenerants	No. of lines	Generation
Millewa	Australia	7	1	47	78	SC_3
		3	2	12	22	SC_3
Warigal	Australia	8	1	18	38	SC_3
Yaqui 50E	Mexico	–[b]	1	15	77	SC_4
		–[b]	2	8	41	SC_4

[a] Each period is 4–6 weeks
[b] Not recorded

Table 2. Means and ranges of means of somaclonal lines compared to controls in the hill plot experiment. Data pooled from eight blocks (see "Materials and methods")

Character	Millewa			Warigal			Yaqui 50E		
	Parent	SC lines		Parent	SC lines		Parent	SC lines	
	Mean	Mean	Range	Mean	Mean	Range	Mean	Mean	Range
Height (cm)	85	83	75– 97	78	83	75– 88	68	85	55–112
Grain no./spike	58	58	44– 69	43	43	36– 48	43	48	24– 51
Kernel wt. (mg)	28	28	24– 31	29	28	22– 33	26	27	16– 35
Yield (g/hill)	130	136	83–185	122	98	37–140	58	70	19–219
Total dry weight (g/hill)	377	392	310–499	371	353	178–424	203	308	106–670
Harvest index (%)	35	35	22– 44	33	28	19– 34	28	23	6– 34

together with dough breakdown as indicated by the width of the mixograph trace after seven minutes mixing were derived from the mixograph curves.

Results

Comparisons of the 256 somaclonal lines with their parent cultivars in hill plots showed significant variation for all the agronomic characters measured. Means of all the somaclonal lines and the ranges in their means are compared to their parents in Table 2. Generally, the overall means of the somaclonal lines were little altered from the parents. However, some extreme lines could be identified with much smaller or much larger values than the parents. For example, one Yaqui 50E line had 3 times the total dry matter production of its parent. This is probably a consequence of its increased height (102 cm) compared to the Yaqui 50E parent (68 cm) which has both *Rht1* and *Rht2*, two dominant genes for reduced height.

The numbers of lines which were significantly different from their parents are given in Table 3. The pattern of variation was markedly different for each cultivar. Yaqui 50E somaclones displayed more variation than the other two cultivars and had more lines with significantly greater values than lines with significantly lower values. Millewa lines displayed less total variation but had almost equal numbers of lines significantly greater and significantly smaller than the Millewa control. In contrast, Warigal had very few lines which were significantly greater than the control, but many lines which were significantly lower.

Since some regenerants contributed more somaclonal lines to the experiment than others, the numbers of significantly different lines might not reflect the real number of regenerants carrying somaclonal variation for any one of these characters. Table 4 expresses the numbers of somaclonal lines which are significantly different from their controls, in terms of the primary regenerants from which they derived (SC_1) and also in terms of the number of subcultures experienced during culture. This partly reduces the extent of positive variation in Yaqui 50E, but otherwise maintains the same differences in pattern of variation between cultivars just described. An additional subculture period had no consistent effect on the proportions of regenerants producing lines significantly different from their parents. Millewa and Yaqui 50E together had an average of 8.2% regenerants producing such lines with one subculture, and 9.2% with two subcultures.

Table 3. Numbers of somaclonal lines significantly different from their parents in the hill plot experiment. Based on comparison using LSD (least significant difference; $P < 0.05$) within individual blocks

Character	Nos. of significantly different lines[a]					
	Millewa		Warigal		Yaqui 50E	
	<	>	<	>	<	>
Height	6	5	0	9	2	66
Grain no./spike	5	2	0	1	6	33
Kernel wt.	6	1	7	1	9	33
Yield	5	6	16	0	8	31
Total dry wt.	2	14	4	0	5	62
Harvest index	9	3	18	0	49	3

[a] Total number of somaclonal lines is 100, 38 and 118 in Millewa, Warigal and Yaqui 50E, respectively

Sister regenerants from the same cultured embryo may also be carrying the same somaclonal variation. Table 5 expresses the numbers of embryos giving rise to significantly different lines in Millewa and Warigal. Again, variation in Millewa was balanced between positive and negative effects, but in Warigal tended towards negative effects. For many of the characters measured, significantly different lines were obtained from different embryos.

In larger scale plots in the following year, lines selected for high yield and harvest index from the hill

Table 4. Numbers of regenerants contributing significantly different lines in the hill plot experiment

Character	Periods in callus culture	No. of regenerants					
		Millewa		Warigal		Yaqui 50E	
		<	>	<	>	<	>
Height	1	6 (47)[a]	3	0 (18)	9	1 (15)	11
	2	0 (12)	2			0 (8)	5
Grain no./spike	1	4	2	0	1	3	7
	2	1	0			2	6
Kernel wt	1	5	1	6	1	5	9
	2	1	0			0	6
Yield	1	5	4	12	0	6	6
	2	0	2			1	6
Total dry wt.	1	2	10	3	0	3	7
	2	0	3			0	6
Harvest index	1	7	3	12	0	12	2
	2	2	0			7	1

[a] Total number of regenerants

Table 5. Numbers of donor embryos contributing significantly different lines in the hill plot experiment

Character	No. of embryos[a]			
	Millewa		Warigal	
	<	>	<	>
Height	4	4	0	5
Grain no./spike	5	2	0	1
Kernel wt.	5	1	4	1
Yield	2	4	7	0
Total dry wt.	2	6	3	0
Harvest index	4	3	7	0

[a] Total number of embryos is 10 in Millewa and 8 in Warigal

plot experiment failed to show significant improvements over their controls (Table 6). However, correlation coefficients between the 2 years were 0.55 for yield vs yield, 0.47 for harvest index vs harvest index and 0.75 for harvest index (1983) vs yield (1984). This supports the original contention that harvest index in hill plots is a good predictor of yield in large plots (Latter and Ellison 1983), even though no individual lines showed significant increases in these characters in both years. Significant positive differences in kernel weight were maintained in both years ($r=0.39$).

Grain quality and dough strength characteristics were examined for the first time in the lines grown in the large plots. There was significant variation amongst the somaclonal lines for most characters, and for some, individual lines could be identified which were significantly different in both directions from the parent (Table 6). However, only hardness, protein content and reflectance at 460 nm showed lines with significant improvements over the Millewa parent. Eleven of the 23 lines had significantly increased protein content. To some degree this reflected the common negative correlation between protein content and yield ($r=-0.27$), but seven lines could still be identified which maintained the yield level of the parent while having significantly higher protein content. These seven lines originated from four separate embryos.

Discussion

The results of this two year study show that significant somaclonal variation can be generated for a number of agronomic and quality characters. Estimation of the level of somaclonal variation was complicated by the following: a) The lines entering the first experiment were not randomly selected. They did, however, represent the extremes in variation seen in the SC_2. b) The lines were initially tested at the SC_3 generation and could still have been segregating. This could have led to underestimation of the variation. c) Use of the LSD (least significant difference) for multiple comparisons could have led to an overestimation of the number of significant differences. Considering these qualifications, in general, the averages of all the somaclonal lines were reduced compared to the controls, but for some characters individual lines could still be identified which demonstrated significant improvements over their parents. However, characters where significant improvements could be seen were those like kernel

Table 6. Results of somaclonal lines in large plots

Character	LSD	CV %	Parent	SC lines		Nos. of lines significantly different from control	
			Mean	Mean	Range	<	>
Millewa lines ($n=23$)							
Yield (kg/ha)	262	10	2,043	1,860	1,347–2,163	6	0
Harvest index	4	7	38	36	24–39	4	0
Hectolitre wt. (kg/hl)	1.1	1	80.7	79.7	78.1–81.3	10	0
Kernel wt. (mg)	0.9	2	25.2	25.8	24.2–27.0	2	9
Hardness	0.3	4	5.0	5.0	4.7–5.6	2	3
Flour yield (%)	1.5	2	58.4	57.1	55.0–58.5	7	0
Protein (%)	0.4	2	12.3	12.6	11.8–13.2	1	11
Colour 460 nm	0.5	1	70.9	71.6	70.7–72.6	0	14
Colour 560 nm	1.4	1	84.1	84.1	84.0–84.8	0	0
Mixograph height	0.5	5	7.9	7.4	6.8–8.2	8	0
Mixograph time	0.3	7	3.6	3.3	2.8–3.6	5	0
Mixograph breakdown	0.18	18	0.82	0.69	0.42–0.82	5	0
Yaqui 50E lines ($n=9$)							
Yield (kg/ha)	262	10	2,333	1,637	1,398–1,865	9	0
Harvest index	4	7	33	29	24–36	5	0

weight and yellow pigmentation which are relatively simply inherited. No lines with repeatably improved yield, harvest index or dough mixing characters were identified. However these are the more important selection criteria in breeding. The more complex nature of their inheritance means that variation in single genes is less likely to have an impact on the final value of the character, and isolating somaclonal variants in them will be correspondingly more difficult.

The differential expression of somaclonal variation between the three cultivars suggests a genetic component to the type and amount of variation induced. Genetic variation in morphogenetic response to tissue culture has been well documented (e.g. Hanzel et al. 1984; Lazar et al. 1984), but few comparisons of genotypes have been made in the assessment of somaclonal variation. The experiments described here do not unequivocally demonstrate a genotype effect of somaclonal variation since the culture phase of Yaqui 50E preceeded that of Millewa and Warigal, and a batch effect cannot be precluded. Whether a batch or a genotype effect, the possibility of varying the degree or type of somaclonal variation has implications for its practical use. If batch effects exist, some environmental factor may be determined as responsible and could be manipulated to maximise or minimise somaclonal variation. If genotype effects exist, genotypes could be selected which similarly maximise or minimise somaclonal variation.

Length of time in culture did not affect the amount of somaclonal variation seen in the experiment described here. Accumulation of chromosomal abnormalities with increasing time in culture has been demonstrated (e.g. Orton 1980; McCoy et al. 1982; Armstrong et al. 1983) but extremely abnormal karyotypes were probably selected against in this experiment since the most infertile material did not produce enough seed to be planted in the field.

Some of the variant lines recovered, such as those for height, kernel weight or protein content may represent single gene changes in near-isogenic backgrounds. Especially where these lines showed no reduction in yield, gross chromosomal aberrations are unlikely as the cause of somaclonal variation and changes at a single locus may be postulated. These near-isogenic lines may thus provide useful material for defining the genes determining these characters, and for studying their effect on final yield. Single gene changes produced in specified genetic backgrounds may be one of the most useful applications of somaclonal variation to plant breeding: advanced breeding lines having high yield potential but single character faults such as too much yellow pigment in the flour may not have to be discarded if somaclonal variants for the character fault can be conveniently isolated.

For the characters where similar variant lines were obtained from different embryos (Table 5), different variants may be recoverable. If these represent changes at more than one locus, there is a possibility of recombining the variants to produce even more extreme variation than was initially present in the individual regenerated plants and their progeny.

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