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Heritable somaclonal variation in wheat

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Summary. Efficient tissue culture and regeneration methods were established using immature wheat embryos as explants. Genotype differences in culturability were evident, and from the ten accessions most amenable to culture, a total of 2,846 plants were regenerated. Extensive somaclonal variation for morphological and biochemical traits was observed among 142 regenerants of a Mexican breeding line, 'Yaqui 50E', and their progeny. Variant characters included height, awns, tiller number, grain colour, heading date, waxiness, glume colour, gliadin proteins and α-amylase regulation. The variant characters were heritable through two seed generations and included traits under both simple and quantitative genetic control. Segregation data suggested that mutations both from dominance to recessiveness, and from recessiveness to dominance, had occurred. Most mutations in the primary regenerants were in the heterozygous state but some were true-breeding and presumed to be homozygous. Chromosome loss or addition did not account for the variation and none of the variant phenotypes was observed in over 400 plants from the parental seed source. The distinctive parental gliadin pattern was maintained in the somaclones thus excluding seed contamination or cross-pollination as a source of the variation.

Key words: Somaclonal variation – Wheat tissue culture – Gliadin – Wheat breeding – Mutants

Abbreviations: 2,4-D = 2,4-dichlorophenoxy acetic acid; 2,4,5-T = 2,4,5-trichlorophenoxy acetic acid; IAA = indole acetic acid; BAP = 6-benzyl amino purine; ABA = abscisic acid; GA_3 = gibberellic acid; DAP = days after planting

Introduction

It is evident that genetic changes occur in plant tissue culture and that these changes are transmitted to regenerated plants and their progeny (Larkin and Scowcroft 1981; Shepard 1981; Scowcroft and Larkin 1983; Larkin and Scowcroft 1983). Here we report the occurrence of somaclonal variation in hexaploid wheat and establish its inheritance through two subsequent seed generations.

Somaclonal variation has been observed previously in cereals (McCoy et al. 1982; Schaeffer 1982; Nakamura and Keller 1982; Edallo et al. 1981). In wheat, the analysis of variation has been largely cytogenetic and restricted to the primary regenerants. Bennici and D'Amato (1978) and Lupi et al. (1981) described chromosome number mosaicism in regenerated durum wheat plants. Diplontic selection appeared to eliminate variant cells so that the pollen mother cells in the spikes of all regenerants were disomic. Yurkova et al. (1982) reported mixaploidy in primary somaclones of hexaploid wheat. These lines returned to normal ploidy only after the third or fourth generations. Ahloowalia (1982) observed that most wheat regenerants had the normal chromosome complement at anaphase I but that others showed univalents, bridges and fragments. The morphological variants described by Ahloowalia referred only to the initial regenerants and not their progeny.

Our investigation of somaclonal variation in wheat required the development of cultures which could regenerate plants efficiently. In the past, cereal tissue cultures have been initiated from mature plant tissues such as mature seed or stem pieces. Such cultures regenerated few plants, as exemplified in wheat (Shimada et al. 1969; Chin and Scott 1977; Cure and Mott 1978; O'Hara and Street 1978; Eapen and Rao 1982). Cereal cultures with more efficient plant regeneration have been obtained by the initiation of callus from immature tissues. In wheat, for example, efficient plant regeneration has been obtained from cultures of explants of the immature inflorescence (Dudits et al. 1975; Chin and Scott 1977; Gosch-Wackerle et al. 1979); immature leaf base (Ahuja et al. 1982); meso-

cotyl (Yurkova et al. 1981, 1982); and immature embryos (Shimada 1978; Shimada and Yamada 1979; Gosch-Wackerle et al. 1979; Ahloowalia 1982; Sears and Deckard 1982; Ozias-Akins and Vasil 1982). In this report we confirm that the immature embryo is an efficient explant for initiating regenerable wheat tissue cultures.

Nomenclature

The growing interest in the genetic analysis of plants derived from tissue culture has led to a need for a clear and universal designation for the regenerant and its progeny. Some confusion has arisen in the literature concerning the numeric subscripts representing successive generations. Chaleff (1981) proposed the symbol R, for the regenerant, with R_1 representing the progeny of the primary regenerated plant, and R_2, R_3 etc. the successive selfed generations. Others have used Chaleff's system, numbering the regenerant R_0 (Edallo et al. 1981; Hibberd and Green 1982; Sears and Deckard 1982), although Sibi (1976) had previously used P_0, P_1 etc. to denote the same generations in lettuce.

However, this numerical series does not correspond with accepted genetic usage where segregation is first encountered in the F_2 (hybridisation) or M_2 (mutagenesis) generations. In somaclones, a mutation present in a heterozygous condition in the primary regenerant would first segregate in the R_1 generation according to Chaleff's numbering. This discrepancy was recognised by Yurkova et al. (1982) who retained Chaleff's symbol but renumbered the series R_1 for the regenerant with R_2, R_3 etc. representing subsequent generations. To avoid confusion between duplicate numbering systems of R, and to make identification of somaclonal generations consistent with general genetic usage, we propose a change in the symbol to SC (somaclone) and the use of SC_1 for the regenerant with SC_2, SC_3 etc. representing subsequent generations. This numerical series also conforms with recently established nomenclature for dihaploids, DH, (De Paepe et al. 1981) and androgenic plants, A, (Hoffman et al. 1982). In this paper an SC_2 family is derived from selfed seed of a single SC_1 plant. An SC_3 family is derived from selfed seed of a single SC_2 plant.

Materials and methods

Embryo donor plants

Plants as sources of embryos for initiating cultures were grown in a temperature-regulated glasshouse. For most cultures, embryos were derived from spikes which were bagged at ear emergence, prior to anthesis, to prevent outcrossing. Seed for parent plants was obtained from various sources and a total of 71 genotypes were assessed, in an exploratory manner, for their ability to initiate cultures and regenerate plants. These genotypes included cultivars, breeding lines, and genetic stocks of *Triticum aestivum* and the related species, *T. dicoccoides, T. sphaerococcum, T. timopheevi* and *T. turgidum.*

Culture and regeneration

Immature caryopses were collected when embryos were 1–2 mm in length, usually about 16 days after pollination. Seed was surface sterilized by the following: 70% ethanol for 60 s; 1% Zephiran (Winthrop Labs., Sydney) in 10% ethanol for 10–15 min; 70% ethanol for 30 s; and then rinsed with two changes of sterile water. Dissection was usually made by a single cut in the seed immediately under the embryo followed by pressure with the face of the scalpel blade on the top of the seed behind the embryo. The extruded embryo was placed on initiation medium, SD1, with the embryonic axis in contact with the medium and the scutellum facing upwards. SD1 consists of the inorganic constituents of Murashige and Skoog (1962) with 2% sucrose, 150 mg/l L-asparagine, 0.5 mg/l thiamine-HCl and 1 mg/l 2,4-D (Sears and Deckard 1982). Cultures were maintained in a 27 °C room with an 8 h dark and 16 h light regime of approximately 130 $\mu E \cdot m^{-2} \cdot s^{-1}$. Cultures were transferred to fresh SD1 medium every 4–6 weeks, discarding any portions which had developed root primordia.

Shoot regeneration was initiated by transferring to a low auxin medium, MS9 (Larkin 1982) containing 0.5 mg/l IAA and 1 mg/l BAP. Individual shoots were removed to MS (Murashige and Skoog 1962) devoid of growth regulators for the induction of roots. Plantlets were transplanted to soil in individual pots and immediately placed in a misting frame in the glasshouse for one week before transferring to normal glasshouse conditions. Spikes were bagged prior to anthesis to prevent cross pollination and harvested individually from each plant.

Somaclonal variation in 'Yaqui 50E'

Four batches of 'Yaqui 50E' somaclones, described in Table 1, were regenerated. Morphological variation was evaluated in the SC_2 by planting up to 12 seed from a single head of each SC_1 plant in 13 cm diameter pots, 4 seeds/pot, in the glasshouse in May, 1982. Temperatures were maintained at 20/15 °C and the photoperiod was extended to 16 h. Parental seeds were planted as controls and located randomly amongst the SC_2 families. On every plant two or more spikes were bagged before anthesis to prevent cross-pollination. All plants were scored for various developmental and morphological characters during growth.

Heading date. Heading date was assessed at a single date, 54 DAP, by assigning the following scores to the different developmental stages: 0 (early) = base of head > 10 cm above flag leaf node, 1 = head between 5 cm and 10 cm above flag leaf node, 2 = head at least half emerged, 3 = head in boot, and 4 (late) = head below boot, or not initiated. At the same time leaves were visually rated for reduced waxiness.

Plant height, fertility, awns, glume colour and grain colour. At maturity plant height was measured and fertility of the bagged primary spike was scored. A plant was described as fully fertile if the primary and secondary florets both contained grain, partly fertile if these florets did not all contain grain, and sterile if no seed was set. Awns were classed as fully-awned, half-awned, tip-awned or awnless, and glume colour was rated white or brown. After threshing, grain colour was classed as red or white.

Morphological variation in SC_3. Progeny of selected SC_2 families were further evaluated in the field. Twenty-four SC_2 families (progeny of 24 individual SC_1 plants) were selected which encompassed the range of variation observed in the SC_2. Within each of these SC_2 families, 4 to 8 SC_3 families were chosen, and 6 or 12 seeds per SC_3 family were planted in unreplicated rows. The total of 117 rows, including 13 parental control rows (12 plants each) were randomised in 5 long rows 50 cm apart and the plot was bordered on all four sides with

Table 1. Origins of somaclone batches of 'Yaqui 50 E', and numbers of SC_1, and SC_3 plants evaluated for morphological characters

Somaclone batch	Origin	No. of culture periods on SD1	No. of			
			SC_1 plants	SC_2 individuals	SC_2 selections	SC_3 individuals
1A	[several	2	36	367	30	233
1B	[embryos (< 5)	2	23	263	22	250
1C	single embryo	2	40	387	17	180
2	unspecified sub-cultures of 1A, 1B, and 1C	3	43	482	36	295

'Yaqui 50E'. Seed were hand planted 7 cm apart in November 1982 (late spring), in Canberra. Standard cultivation practices were followed and sprinkler irrigation supplied as needed. At 38 DAP, seedling habit was scored as prostrate, intermediate or erect. At 46 DAP, heading date was scored as previously described. At maturity, fertile tiller number was counted and plant height, awns, glume colour and grain colour assessed as before.

Gliadins. The gliadin fraction of seed storage proteins was electrophoretically evaluated in the SC_2 seeds of 31 somaclones. The gliadin proteins of 3 to 10 individual seeds per family were extracted and electrophoresed essentially as described by du Cros and Wrigley (1979). Individual grains were cut transversely and the embryo half stored for later growth. The endosperm half was crushed and extracted in 1 M urea either overnight at room temperature or for 2 h at 60 °C. The samples were clarified by centrifugation and applied to the wells of 3–13% polyacrylamide gradient gels (precast by Gradient Labs Pty Ltd., Pyrmont, N.S.W. 2009, Australia). Two tracks in every gel were allocated to parental controls. The gels were pre-equilibrated with fresh sodium lactate buffer, pH 3.1, by a 1 h, 200 V prerun before sample application. The gliadins were electrophoresed for 2 h, 200 V, 55 mA (for 2 gels) then stained overnight with 25 mg Coomassie Brilliant Blue G250 in 100 ml 12% trichloracetic acid. No destaining was necessary. The resulting gels were scored for altered protein patterns.

α-amylase. SC_2 seed of 68 SC_1 plants was screened for variants affecting the production of the aleurone secretory enzyme, α-amylase, by a modification of the method described by Ho et al. (1980). Eight individual grains per family were surface sterilized and halved as described above. The endosperm half was further subdivided into one portion assayed for GA_3 induction, and one for ABA repression of α-amylase synthesis. The assay medium contained 40 mM $CaCl_2$, 20 mM sodium succinate (pH 5.2), 10 μg/ml chloramphenicol, 1% (w/v) starch (Lintner) and 0.8% (w/v) agar. Filter-sterilised GA_3 and ABA solutions were added after autoclaving to give a final concentration of 1 μM GA_3 for GA_3 induction tests, or 0.5 μM GA_3 and 100 μM ABA for ABA inhibition tests. Endosperm pieces were incubated on the surface of the medium for 36 h at 22 °C in the dark, then removed and the surface flooded with a solution of 36 mM KI and 2.4 mM I_2 in 0.05 N HCl. The diameters of the resultant white haloes were compared to parental controls. On the GA_3 medium, embryos corresponding to haloes which were less than half the diameter of the parent (14 mm) were selected as presumptive GA_3-insensitive variants. Similarly, on the GA_3 + ABA medium, which gives no response in the parent, embryos producing a clear halo > 8 mm in diameter were selected as presumptive ABA-insensitive variants. On either medium, embryos producing haloes with diameters > 20 mm were classified as super-sensitive to GA_3. Selected embryos were germinated, grown to maturity, and their progeny retested.

Cytology. Chromosome counts were made on SC_2 plants. Single plants from 7 randomly selected SC_2 families from batches 1A, 1B and 1C, and from all 43 SC_2 families from batch 2 were examined. Actively-growing root tips were incubated in water in a refrigerated water bath at 1 ± 1 °C for 24 h. The root tips were immediately fixed in freshly made ethanol : acetic acid (3 : 1) for at least 2 h, then briefly softened in 45% acetic acid. Meristematic cells were teased from the tip into a droplet of aceto-orcein and examined.

Results and discussion

Culture and regeneration

Various experiments were conducted to compare the effects of different basal media, different synthetic auxins, and different auxin and other growth regulator concentrations on the initiation of tissue cultures and

Table 2. List of *T. aestivum* accessions giving efficient regeneration

Origin	Accession	No. of	
		Regenerants	Contributing embryos
CIMMYT	'Yaqui 50 E'	604	15
	'Kalyansona'	56	4
Australia	'Bencubbin'	262	9
	'Bindawarra'	210	9
	'Gamenya'	169	12
	'Lance'	276	10
	'Millewa'	619	30
	'Warigal'	262	19
Other	'Chinese Spring'	252	18
	'D6899'[a]	136	8

[a] Ho et al. 1980

the regeneration of plants (unpublished data). The results established the culture protocol described in the materials and methods.

Following the transfer of plantlets to soil, immediate protection against desiccation was imperative. Either placing pots in a misting frame, or covering the plantlets with transparent plastic cups, for one week, was effective in allowing the plants to adapt to glasshouse conditions. Routinely, plant survival was greater than 90%.

Seventy-one genotypes have been assessed for their ability to initiate cultures and regenerate plants. There were distinct genotype differences in ability to initiate callus and regenerate plants, and Table 2 lists the accessions which consistently gave good regeneration. There was a considerable batch effect on culturability, possibly resulting from seasonal effects on the embryo donor plant, and manipulation of culture and embryo donor conditions could give acceptable cultures from genotypes which did not respond under our conditions.

The following results relate only to the first four batches of somaclones derived from 'Yaqui 50E' (Table 1), since this is our most advanced material. This CIMMYT line cultured well, giving as many as 140 regenerants from a single embryo after three months culture on SD_1, and up to 231 regenerants after eight months culture. Usually, about 20 regenerants could be readily recovered from each embryo.

Somaclonal variation in 'Yaqui 50E'

a) Fertility. The fertility of SC_1 plants was not scored but 142 of the 158 regenerated plants set some seed. The fertility of SC_2 plants was variable. Percentages of fully sterile plants ranged from 1.5% in batch 1B to 12.3% in 1A (Table 3). However, if partial sterility was also considered, batch 2 had the lowest proportion of fully fertile plants. Individual families varied in their average fertility (data not shown), ranging from one family in which 4 plants were fully fertile, 3 were partly fertile and 5 were sterile, to 40 families in which all individuals were fully fertile. The fertility of SC_3 plants was high, only 0.6% being sterile, but in most cases only fully fertile SC_2 parents had been selected.

Table 3. Fertility of *SC_2* individuals

Somaclone bach	Percentages of individual *SC_2* plants		
	Sterile	Partly sterile	Fully fertile
1A	12.3	4.9	82.8
1B	1.5	1.5	97.0
1C	8.3	9.6	82.1
2	5.2	21.4	73.4
Parental control	0	0	100.0

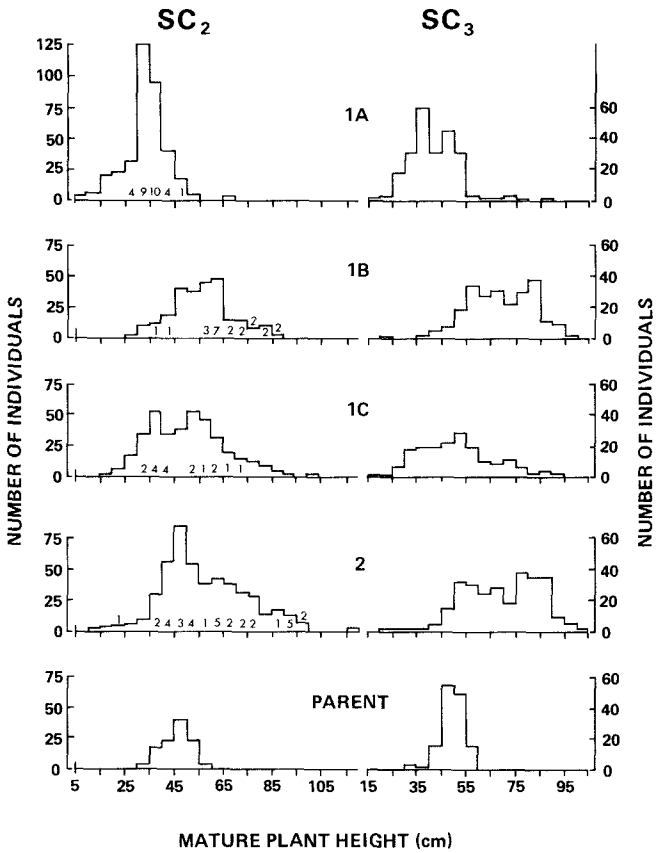

Fig. 1. Frequency distributions of mature plant height for all *SC_2* individuals grouped in four batches, and for *SC_3* progeny of selected *SC_2* plants. The numbers in the *SC_2* histograms represent the numbers of individuals from each class advanced to give the *SC_3* data in this figure. The *SC_2* data are from glasshouse-grown plants and the *SC_3* data from field-grown plants

b) Height. Variation in the mature plant height of SC_2 individuals was evident in all four somaclonal batches (Fig. 1). Fully fertile individuals ranged from 23 to 119 cm tall, compared to the parental range of 31 to 58 cm. The parental mean was 46 ± 0.5 ($SD_{\bar{x}}$) cm. Analysis of these data was complex since each of the 142 SC_2 families should be treated separately; each SC_1 plant may have had a unique genotype. Classification of SC_2 families into those segregating and those nonvariant was possible for characters with distinct phenotypic classes, such as awns, but not possible for characters such as height which are subject to environmental as well as genetic variation.

Evaluation of the SC_3 progeny of 103 selected SC_2 plants indicated a strong genetic basis for the variation in height (Fig. 1). The extremes of height in these SC_3 plants is illustrated in Fig. 2. The regression analysis of SC_3 family means on their respective SC_2 parents yielded a heritability estimate of 0.67 ± 0.043. This represents a maximum value, since complete inbreed-

Fig. 2. Selected *SC_3* plants demonstrating the full range of heights observed in the 'Yaqui 50 E' somaclone families compared to the parent plants on either end

ing decreases the estimate by a factor of 2 (Kempthorne 1957). Since the degree of inbreeding is unknown for these somaclonal lines, the true heritability should lie between 0.34 and 0.67.

In some cases, the effects of specific major genes could be tentatively identified. Since the pooled SC_2 and SC_3 data (Fig. 1) do not indicate the distribution of height within individual families, selected families are illustrated, as examples, in Fig. 3. Batch 1A contained a number of uniformly short SC_2 families. Individuals from these also gave rise to uniformly short SC_3 families, as illustrated in one case in Fig. 3. Some SC_3 families apparently segregated in the SC_3 (example for batch 1B), some produced uniformly tall offspring (example for batch 2), and others were similar to the parent (example for batch 1C).

From its parentage and stature, the parent, 'Yaqui 50E', is deduced to have two dominant genes, *Rht1* and *Rht2,* for reduced height. Variants taller than the parent may therefore reflect a change to the recessive state in *Rht1* and/or *Rht2.* Variants shorter than the parent may also reflect changes at these loci, for example, *Rht3* is allelic to *Rht1* and results in a very short phenotype (McVittie et al. 1978). However, a number of genes are known to affect height in wheat (Worland et al. 1980), and test crossing is required to identify the genes involved in the height variation induced in these somaclonal lines.

c) Heading date. Variation in heading date was observed in the SC_2 of all four somaclonal batches (Fig. 4). The earliest and latest of the five categories of heading date were open-ended and contained some extreme individuals, which are not evident in Fig. 4. Batch 1A contained a number of individuals which headed earlier than the parent, and batches 1B and 2 produced variants later than the parent. The parental control for the SC_2 generation (glasshouse grown) was

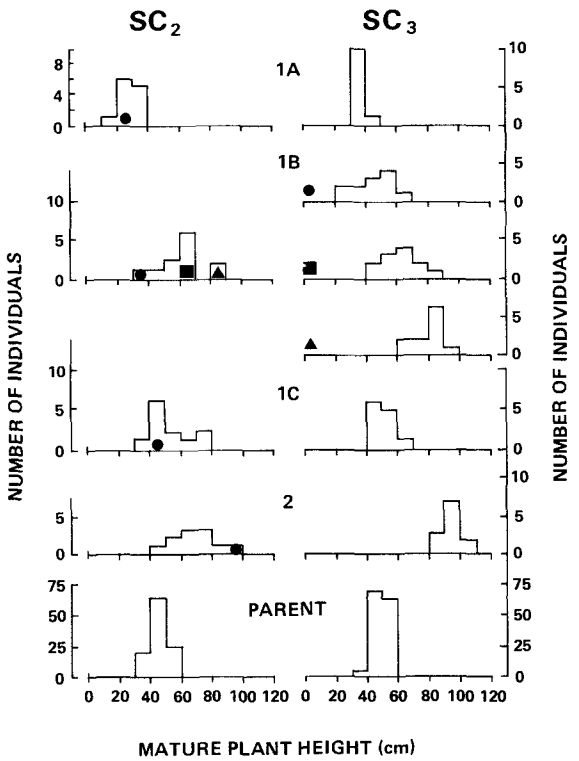

Fig. 3. Frequency distributions of mature plant height for four SC_2 families (head rows of individual primary somaclones) and for SC_3 progeny (head rows) of selected SC_2 plants. Symbols represent the height class of the individual SC_2 plants giving the SC_3 families shown

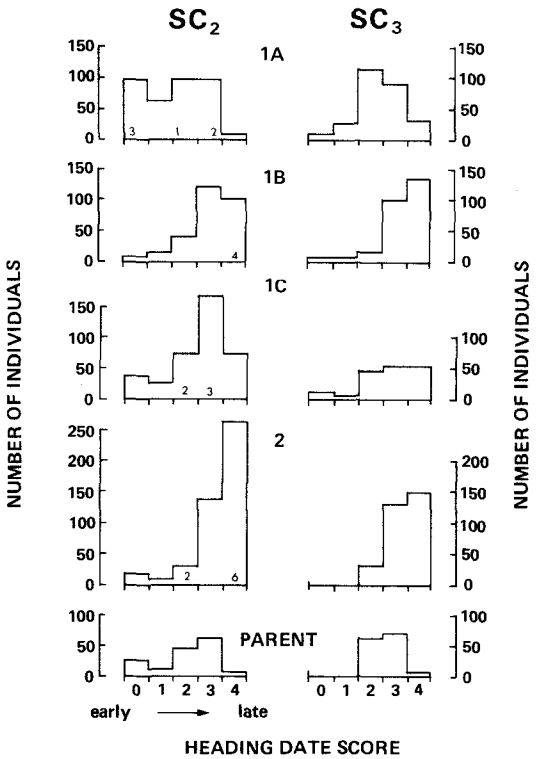

Fig. 4. Frequency distributions of heading date score for all SC_2 individuals in four somaclonal batches, and for all SC_3 progeny of selected SC_2 plants. The numbers in the SC_2 histograms indicate the distribution of the selections giving the SC_3 data

rather variable, but in the SC_3 generation (field grown) fell into only three heading date groups. However, the somaclones still maintained a wide range of variation in the SC_3: the earliest and latest individuals ranged in heading date from 41 to 72 DAP compared to 45 to 53 DAP for individual parent plants. Although heading date data were collected for individual SC_2 plants, not all plants were identified and the SC_2–SC_3 parent-offspring regression could not be calculated. However, some extremely early and extremely late SC_2 plants were identified and these generally gave early or late SC_3 progeny means respectively.

As was the case for plant height, some SC_2 families displayed considerable variation within the family, whilst others showed relatively little variation for either parental, earlier, or later heading date scores (data not shown). Again it was not possible to definitely categorise families as segregating or non-segregating, or to determine segregation ratios for possible major genes.

d) Awns. Unlike height and heading date, distinct phenotypic classes could be identified for awns. In the SC_1 generation 74 plants were tip-awned (parental), 47 plants were half-awned, and 21 plants were fully-awned. In the SC_2 and SC_3, families could be classified as segregating or non-segregating for four awn classes (Fig. 5). All half-awned plants segregated into tipped, half and fully-awned progeny. This is suggestive of heterozygosity for an incompletely dominant gene. However, many tip-awned plants segregated in both SC_2 and SC_3 which suggests that the awn inhibitor allele is completely dominant. Fifty-five out of 57 fully-awned plants gave only fully-awned progeny (a total of 536 SC_2 and SC_3 individuals). The remaining 2 fully-

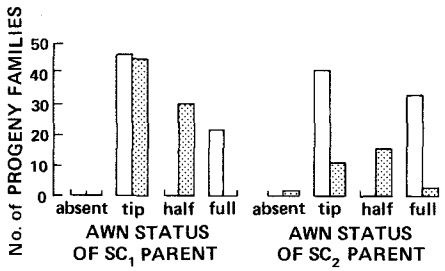

Fig. 5. Assignment of SC_2 and SC_3 progeny families into those segregating or not segregating for awns. The families are also grouped according to the awn status of the parent of each family. 'Yaqui 50 E' is tip-awned. ⊠ segregating; □ not segregating

Table 4. Segregation of characters with distinct phenotypic classes in the SC_2, tested against 3:1 ratio. Families pooled within batches

Character	Batch	No. of families	Nos. observed[a]	Deviation χ^2	P	Hetero-geneity χ^2	P
Awns	1B	22	174 : 77	4.32	0.05 – 0.025	14.87	0.90 – 0.75
	1C	35	219.5 : 130.5	28.18	< 0.005	30.09	0.75 – 0.50
	2	7	54.5 : 24.5 tipped : awned	1.52	0.25 – 0.10	7.75	0.50 – 0.25
Glume colour	2	18	127.5 : 79.5 brown : white	19.84	< 0.005	34.44	0.01 – 0.005
Grain colour	1B	6	29.5 : 27.5	16.43	< 0.005	20.81	< 0.005
	1C	29	199.5 : 72.5	0.40	0.75 – 0.50	18.48	0.95 – 0.90
	2	16	139 : 38 red : white	1.18	0.50 – 0.25	8.62	0.90 – 0.75
Wax	1C	3	5.5 : 22.5 reduced : present	0.43	0.75 – 0.50	1.16	0.75 – 0.50

[a] Sum of individual family data, including Yates corrections

awned SC_2 plants each gave 1 tipped: 11 fully-awned SC_3 progeny. These results confound a simple genetic model. Indeed the χ^2 analysis (Table 4) of pooled SC_2 families segregating tip, half and full awns revealed no fit to the one gene model. Three dominant awn inhibitors have been described in wheat together with other modifying genes (McIntosh 1973) and further testing is required to determine the status of the individual somaclonal variants.

Six awnless plants also appeared in the SC_2, in six separate somaclone families, but insufficient numbers were available to make hypotheses about these variants. Two were advanced to the SC_3: one segregated 7 awnless: 4 short-tipped, and the other had a single short-tipped progeny.

The existence of 21 fully-awned SC_1 plants whose progeny did not segregate in the SC_2 (Fig. 5) suggests that the SC_1 plants were already homozygous for the induced change(s). These apparently homozygous, fully-awned plants arose only in batch 2.

e) Glume colour. A glume colour change from white (parental) to brown was observed, but not recorded, in some SC_1 plants. In the SC_2, 111 families could be classified as parental (all white), 19 as segregating (brown or white), and 10 as non-segregating (all brown). All but one of the families in the latter two categories arose in batch 2. A χ^2 test of SC_2 families segregating in batch 2 was not consistent with a 1:3 segregation for glume colour (Table 4), despite previous reports that brown glume colour is determined by a single dominant gene (McIntosh 1973). A single SC_2 family in batch 1C segregated 11 white:1 brown. However, classification of glume colour was not always unequivocal, and conservative judgement could have inflated the white phenotypic class. In the field-grown SC_3, the brown phenotype was more distinctive. All 92 SC_3 families whose parents occurred in either white or brown non-segregating SC_2 families were true-breeding. Progeny of 12 plants from segregating families were all white if the parent was white, and were either all brown or segregating if the parent was brown. This evidence is consistent with the change from white to brown glumes being caused by a change from a recessive to a dominant allele.

f) Grain colour. As for glume colour, variation in grain colour was observed but not recorded in the SC_1 generation. The 'Yaqui 50E' parent has red grain, but some white seeded variants were noted in the SC_1. The red colour is located in the testa, which is maternal tissue (Bradbury et al. 1956), so the seed produced on an SC_1 plant reflects the genetic constitution of the SC_1 plant. In the SC_2 generation 69 families were red (parental) and not segregating, 55 were segregating, and 16 were white and not segregating. All batches except 1A contained some segregating families, while the white non-segregating families were restricted to batch 1B.

A χ^2 test of segregating SC_2 families indicated that the white grain colour variant was inherited as a single recessive gene in batches 1C and 2, but not in batch 1B (Table 4). Red grain colour may be due to one, two or three dominant genes (McIntosh 1973), and the frequency of single gene white variants in the somaclonal lines suggests that 'Yaqui 50E' has a single gene for red grain colour. In batch 1B, the pooled data fit a 9:7 ratio ($\chi^2 = 1.84$, $0.10 < P < 0.25$), but the families were

not homogeneous (heterogeneity $\chi^2 = 83.10$, $P < 0.005$), and the fit must be considered fortuitous.

In the SC_3, all families were non-segregating when derived from non-segregating SC_2 families (46 red-grained and 16 white-grained plants and their progeny). Progeny from segregating families had grain colour consistent with their parentage and a single gene model in 35 cases, but not in the remaining 7. Phenotypic classification of grain colour can be complicated by other grain characteristics, and genetic testing is required to determine the basis for the variation observed.

g) Other morphological characters. Other characters for which variation was seen in the SC_2 included spikelet density, presence of supernumerary spikelets and waxiness. Three families segregated for reduced wax, and when the data were pooled they appeared to be single gene mutants (Table 4). In the field-grown SC_3, variation in seedling habit (from erect to prostrate), auricle colour (green or red), and fertile tiller number was also observed. Family mean tiller number varied from 2.8 ± 0.19 ($n = 12$) to 11.1 ± 1.34 ($n = 11$). Although family rows were unreplicated, parental control plants in 13 random rows averaged 6.7 ± 0.18 ($n = 141$) tillers. Grain yield from individual plants was not measured but ranged from 0.3 to 15.4 g/plant on a family row basis. Grain size and shape also appeared to vary, but no measurement of these has been attempted.

h) Gliadins. The 290 SC_2 grain from the 31 families examined averaged 3.0 gliadin changes per individual. In contrast, 85 individual grains of our accession of 'Yaqui 50E' had identical gliadin patterns. Changes in the SC_2 of the somaclones included deletion of specific protein bands, appearance of new bands and changes in intensity relative to the parent (Fig. 6 a, Tables 5 and 6). Within each of 10 families so far tested, band changes in the SC_2 were inherited in the SC_3 (data not shown), and in three families inheritance to the SC_4 generation has been verified (Fig. 6 b).

The nature and positions of the band changes, and their segregation within the 31 families, are shown in Tables 5 and 6. Within a family, any specific band change was either uniform across all individuals, or was segregating. However, for different bands a family could contain both segregating and non-segregating changes simultaneously. The within-family sample size was too small to calculate χ^2 ratios for segregation of individual bands. Comparison across families shows that the frequency of changes varied for different bands. This ranged from no changes in parental band positions 5, 45, 60 and 65, to 58% of all SC_2 families carrying a change in band 50. Families also varied in the number of different bands containing changes, from 0 bands altered in family 1A-25, to 12 in 1C-63. In some cases a pair of band changes may merely reflect a single simultaneous loss and addition due to altered mobility, as might be illustrated with bands 80 and 82 in family 1B-93. Batch effects were also evident. For example, all batch 2 families contained the new band 43, and most families displayed a change in parental band 50 and new band 87. In batches 1A, 1B and 1C only one family contained new band 43, but all members of 1B had new band 83.

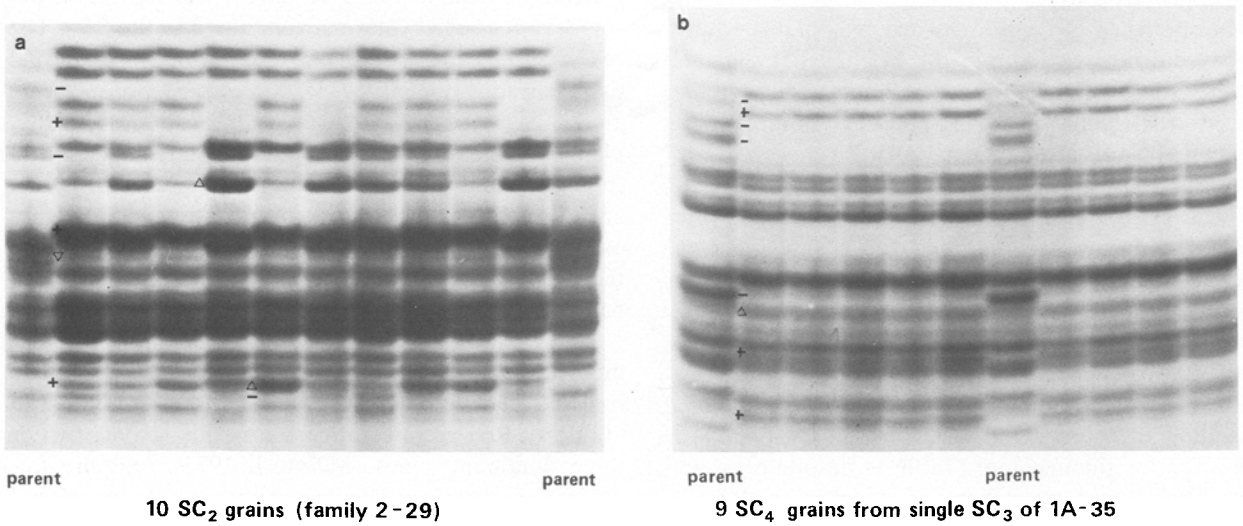

Fig. 6. **a** A gradient polyacrylamide electrophoretic separation of gliadins from 10 SC_2 seed of a single somaclone. Two 'Yaqui 50 E' parental tracks are also shown. Positions of some changes are marked. **b** A gliadin separation of SC_4 seed from an SC_3 individual showing the near homogeneity of the specific gliadin alterations as exemplified by the marked positions

Tables 5 and 6. Summaries of the positions and types of changes to the gliadins observed in SC_2 seed of 'Yaqui 50 E' somaclone families. The parental bands are numbered and new bands given unique numbers as indicated. The symbols used are: +, for a new band; –, for a deleted bands; △, for a significant increased intensity of an existing band; ▽, for a significant decreased intensity of an existing band. The numbers following each symbol indicate the number of grain showing that change

Batch	1A						1B								1C						
Family	2	14	19	20	25	35	78	81	84	86	90	93	94	98	49	55	62	63	64	65	
No. seed	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
BAND No. PARENT / NEW																					Parent Bands
mean changes per individual	1.5	0.4	3.1	0.9	0	1.4	3.2	3.5	5.0	5.3	6.4	6.1	3.3	2.8	2.8	3.0	4.9	2.3	4.2	2.5	
batch mean per individual	1.2						4.5								3.3						

Batch	2										
Family	1	2	3	4	5	6	7	8	9	10	29
No. seed	12	6	14	14	9	7	5	3	5	5	10
Band No. Parent / New											Parent Bands
mean changes per individual	2.9	4.8	2.1	1.6	1.4	1.3	1.2	2.0	1.6	1.8	7.0
batch mean per individual	2.6										

The genetic interpretation of gliadin band changes is complicated. Besides the problem of simultaneous loss and addition, two dimensional separations have shown that some of the bands in a one dimensional separation are in fact more than one protein (Wrigley 1970; Mecham et al. 1978). A decrease in intensity may be due to the deletion of one protein of a pair which normally migrates to the same position. Interpreting the segregation of intensity changes is also complicated by the fact that gliadins are synthesised in the triploid endosperm by codominant genes (Mecham et al. 1978). Thus there are four possible phenotypes with 0, 1, 2 or 3 doses of an altered gene. Despite these complications many of the gliadin bands are known to behave as simply inherited units coded by codominant genes (Mecham et al. 1978). The behaviour of the deletions and additions observed in these somaclone families is consistent with this simple genetic model.

The gliadin patterns of these somaclones and their parent also serve as internal controls to detect whether seed contamination or outcrossing to other cultivars could account for the variation observed. The patterns of gliadin proteins from different wheat cultivars are sufficiently distinctive and reproducible, such that they are used in cultivar identification (Wrigley and Shepherd 1974; Jones et al. 1982). The 'Yaqui 50E' pattern is highly dissimilar to that of other cultivars grown in our glasshouse, yet all of the somaclones derived from it maintained the same, or broadly similar pattern. Since gliadin genes behave codominantly (Mecham et al. 1978), an outcross to another cultivar would readily be identified by the complex additive gliadin pattern.

i) α-amylase. Among 68 SC_2 families assayed, eight segregated for insensitivity to GA_3-induced α-amylase synthesis, or for insensitivity to ABA repression of GA_3-induced α-amylase synthesis. The progeny of four GA_3-insensitive SC_2 variants segregated parental and GA_3-insensitive types, and the progeny of one segregated both GA_3-insensitive and GA_3-supersensitive types. Among the three ABA-insensitive SC_2 variants, one gave all ABA-insensitive progeny, one segregated parental and GA_3-insensitive types, and one segregated parental, ABA-insensitive and GA_3-supersensitive types.

j) Cytogenetics. All 50 SC_2 families examined for chromosome number in root mitoses had the normal count of $2n=42$. These families included many which were variable for a number of the parameters analysed, and some which were segregating for sterility or reduced fertility. However, no gross abnormalities were observed in the size, morphology or number of chromosomes. These results do not exclude the possibility of abnormalities which might have been apparent in mitoses in SC_1 plants, or in meioses in SC_1 or SC_2 plants. However, the extensive range of variation observed in fully fertile SC_2 and SC_3 families was not explained by gross cytogenetic abnormalities such as aneuploidy.

k) Concurrence of variation in all characters. The results described demonstrate a high frequency of variation in many of the individual characters measured. When a number of characters were considered together, the somaclone lines also differed in the number and composition of variants they contained. To illustrate these points, SC_2 data for a few selected somaclones are presented in Table 7. Some somaclones were relatively invariant, e.g. 2-43, yet others like 1B-78 and 2-29 were variant for many characters simultaneously. SC_3 progeny testing of 1B-78 and 2-29 confirmed independent assortment of these combinations of variants (data not shown). No association between particular groups of variant characters could be distinguished, except that shorter families tended to be earlier (batch 1A), and taller families later (e.g. 1B-78, 2-8, 2-29). This may reflect a physiological rather than a genetic relationship. The diversity of the combinations of variant traits implies that the mechanism producing this variation operated at many loci independently.

The disparate frequencies of variants at different loci within a batch of somaclones also suggests that genetic changes occurred at various times during the culture phase. Within batch 1C, which was known to originate from a single embryo, some changes were particularly widespread (e.g. new gliadin band 83, awns), and others were relatively infrequent (e.g. new gliadin band 68, reduced wax). Presumably the widespread changes occurred early in the culture when fewer cells were present, so that after cell proliferation these changes were present in a large proportion of cells and of plants regenerated from them. Changes which occurred later in culture would be present in a smaller proportion of cells at the time of regeneration and thus appear in a smaller proportion of regenerated plants. Alternatively, certain loci may be more highly mutable than others.

The occurrence of non-segregating and segregating variant characters also appeared to be independent. A single somaclone family, e.g. 1B-91, could be segregating for height, awn and α-amylase variants, not segregating for grain colour differences, and invariant (parental) for heading date score and glume colour. Alternatively, any character examined alone displayed all classes of variation: invariant, segregating, and non-segregating in different families. This apparently random occurrence of homozygosity suggests that a mechanism generating homozygosity in the SC_1 also affects different loci independently.

Table 7. Association of variation in the SC_2 progeny of 16 selected somaclones

Batch	Family	Height $\bar{x} \pm SD_{\bar{x}}$	Heading date score $\bar{x} \pm SD_{\bar{x}}$	Awns	Glume colour	Grain colour	Gliadins 40 bands	α-amylase GA_3 or ABA
		cm	0 = early 4 = late					
Parent		46± 0.5[a]	1.9±1.2	p[b]	p	p	p	p
1A	8	36±12	2.0±0.6	vs	p	p	–	p
	19	39± 2	0.3±0.5	p	p	p	vs/vns	vs
	25	34± 4	0.1±0.5	p	p	p	p	vs
	35	32± 8	3.0±0.7	p	p	p	vs	p
1B	78	62±13	4.0±0	vs	p	vs	vs/vns	p
	88	52± 9	3.0±0.6	vs	p	vs	–	vs
	89	57±15	3.5±0.7	vs	p	vns	–	p
	91	63±11	3.1±0.3	vs	p	vns	–	vs
1C	48	63±24	2.8±1.0	vs	p	vs	–	–
	49	51±14	1.7±1.7	p	p	p	vs	p
	63	43±14	3.0±0.7	vs	p	vs	vs	P
	69	54±15	3.4±0.5	vs	p	p	–	vns
2	7	66±14	3.6±0.5	vns	vns	p	vs	–
	8	64±18	4.0±0	vns	vs	p	vs/vns	–
	29	83±17	4.0±0	vs	vs	p	vs/vns	–
	43	44± 3	2.2±0.9	p	p	p	–	–

[a] For morphological characters, $n = 144$ for parent, $n = 10$ to 12 for somaclone families
[a] Abbreviations: *p* = parental phenotype; *vs* = variant segregating; *vns* = variant not segregating; – = not tested

No sectoring was seen for characters such as awn type or glume colour in individual SC_1 plants. Therefore, there was no evidence that the shoots derived from culture were chimaeras. SC_2 progeny were obtained from single heads of SC_1 plants and again no chimaeras were observed.

Concluding discussion

These results demonstrate that heritable somaclonal variation was generated in wheat. Progeny analysis of plants regenerated from tissue cultures of 'Yaqui 50E' revealed the following features:

1. Variation was manifested for both morphological and biochemical traits.

2. Variation was found not only for traits under simple genetic control (e.g. gliadins, grain colour) but also for quantitatively inherited characters (e.g. height, heading date).

3. A single somaclone could be variant for a number of traits which appeared to assort independently in preliminary progeny analysis.

4. Both heterozygous and homozygous mutants were recovered in the primary regenerant. A single somaclone could contain both states at different loci.

5. Chromosome loss or addition was not evident as the primary cause of the variation.

6. Gliadin analyses excluded seed contamination or cross-pollination as the source of variation.

7. The variation did not appear among seed progeny of the parent. None of the variant phenotypes has been observed among more than 400 plants grown from the original seed source or its progeny.

8. Genetic testing is required to ascertain whether the variant phenotypes observed were due to alterations in the principal structural genes mapped for these characters. However, the following observations are relevant:

(a) The mutations affected characters for which major genes are known to be located on all seven homoeologous chromosome groups.

(b) The mutants observed were the result of genetic changes from dominance to recessiveness (awns, grain colour), and from recessiveness to dominance or co-dominance (glume colour, gliadins).

These observations suggest that somaclonal variation may encompass two mechanisms, one operating to generate a mutant gene and the other in some cases operating to make the mutant homozygous. Molecular mechanisms which might be responsible for generating somaclonal variation have been discussed previously (Larkin and Scowcroft 1981; Scowcroft and Larkin 1983).

The mobilization of transposable elements provides one explanation for the multiple, unliked genetic changes which have occurred in these wheat somaclones. Mobile genetic elements are known to occur in

yeast, *Drosophila*, animal cell cultures and maize (Shapiro and Cordell 1982), and can be directly responsible for mutational change. The consequences of DNA transposition can include new patterns of gene regulation, inactivation or reactivation of gene function, and genome rearrangements such as deletions, gene fusions, inversions and gene transpositions. Changes in the intensity of a gliadin band may, for example, result from an alteration in the regulation of one of the gliadin genes caused by the insertion of a mobile element in the regulatory sequences.

Non-segregating, non-parental variant families were also frequently observed. This phenomenon could result from cycles of monosomy/disomy or trisomy/disomy. A change induced in a chromosome while monosomic would become homozygous when that chromosome returned to disomy. Alternatively, a change induced in one chromosome of a disomic cell may become homozygous if that chromosome doubles (trisomy) and then returns to disomy losing the wild type chromosome. Such changes in chromosome number have been documented for cultured plant cells (Bayliss 1980).

Alternatively, homozygosity of mutant genes in primary regenerants could result from non-reciprocal transfer of genetic information between repeated DNA sequences. This phenomenon of gene conversion (Holliday 1964) is known to occur within chromosomes, between homologous chromosomes (Klein and Petes 1981) and between repeated genes on non-homologous chromosomes during mitosis (Mikus and Petes 1982). The latter authors suggest that the probability of gene-conversion is likely to be proportional to the number of times a nucleotide sequence is repeated. In hexaploid wheat three distinct genomes are present, non-coding sequences are extensively repeated and many genes are known to be organised in multi-gene families e.g. gliadins. Such sequence repetition could provide the opportunity for mitotic gene conversion to produce the homozygosity of some mutant genes in the primary somaclones of wheat.

A number of the varying characters described in this report are of agronomic interest: height and tiller number can affect yield; Australian wheat farmers prefer awnless cultivars during drought; the Australian milling industry demands white grained wheats; particular gliadin proteins can affect flour quality; and waxless mutants may confer resistance to certain insects (Bingham, personal communication). While the variants described here may not include any that are not already available to wheat breeding programmes, the application of this culture technique to adapted varieties, in conjunction with appropriate screening and testing, may provide useful variation to plant breeders.

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