Theor Appl Genet (1986) 71:637–643

Variation at the *Nor* loci in triticale derived from tissue culture

R. I. S. Brettell, M. A. Pallotta, J. P. Gustafson[1] and R. Appels

Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, ACT 2601, Australia

[1] Curtis Hall, University of Missouri, Columbia, MO 65211, USA

Received July 24, 1985; Accepted September 12, 1985
Communicated by G. Mechelke

Summary. Plants derived from tissue cultures of six triticale genotypes were the subject of an analysis for changes in the rRNA genes located at the site of nucleolar organizer regions (the *Nor* loci) on chromosomes 1B, 6B and 1R. In addition whole plant phenotypes and the chromosomal constitutions of their progenies were examined for alterations. Following treatment of DNA with the restriction endonuclease TaqI, it was possible to assign electrophoretic bands representing rDNA spacer sequences to each of the chromosomes known to carry a major *Nor* locus. In general, the rRNA genes were found to be stable except in one family where a marked reduction in the number of rDNA units was observed. This reduction in 1R rDNA spacer sequences was heritable and correlated with reduced C-banding at the position of Nor-R1 on chromosome 1R. The change was clearly a consequence of tissue culture since six other plants regenerated from the same culture, and the original parent, did not carry the alteration.

Key words: Tissue culture – Triticale – Ribosomal DNA – Somaclonal variation

Introduction

Tissue cultures with the potential to regenerate plants have been established in a wide range of cereal species and from several different types of explant.

Cultures have been derived from immature embryos (Green and Phillips 1975; Nakamura and Keller 1982a), immature inflorescences (Dudits et al. 1975; Brettell et al. 1980; Dale et al. 1981; Nakamura and Keller 1982b) and very young leaves (Wernicke and Brettell 1980; Wernicke et al. 1981; Lu and Vasil 1981). Heritable phenotypic variation has been observed among regenerated plants of maize (*Zea mays* L.) (Edallo et al. 1981; McCoy and Phillips 1982) and wheat (*Triticum aestivum* L. em Thell.) (Larkin et al. 1984), however, the precise genetic basis of this somaclonal variation has yet to be established (Scowcroft and Larkin 1983). In wheat, stable variants were found among the progeny of regenerants of the line 'Yaqui 50E' for a number of morphological and biochemical characters (Larkin et al. 1984). In addition, karyotypic alterations have been identified in tissue culture regenerants of wheat (Karp and Maddock 1984), triticale (× *Triticosecale* Wittmack) (Armstrong et al. 1983; Lapitan et al. 1984) and oats (*Avena sativa* L.) (McCoy et al. 1982). To date cereal tissue culture derivatives have not been analysed for changes in their nucleolus organizer regions (the *Nor* loci). The *Nor* locus consists of tandem arrays of repeated rRNA genes which have been shown, in flax (*Linum usitatissimum* L.), to undergo quantitative changes in response to environmental stress (Cullis 1979). In the present study we examined changes at the *Nor* loci with the aim of distinguishing between karyotypic changes and alterations in the DNA at the locus.

The *Nor* loci in hexaploid triticale are principally associated with three chromosomes: 1B and 6B of wheat and 1R in rye (*Secale cereale* L.) (reviewed in Appels 1982). The DNA from the 6B and 1R *Nor* loci has been cloned and characterised (Gerlach and Bedbrook 1979; Appels and Dvorák 1982; Appels and Moran 1984). The wheat DNA clone pTA250.4 contains a 2.7 kb TaqI fragment encompassing most of the rDNA spacer region and this sequence, as well as derivatives from it, has been used as a probe to assay the high levels of spacer length variation in this region among members of the *Triticeae* (Appels and Dvorák 1982; Dvorák and Appels 1982).

We demonstrate in the present study that variation at the *Nor* loci is sufficient to allow the 1B, 6B and 1R loci of two triticales to be identified at the DNA level. These loci were thus chosen as a focus for examining variation in hexaploid triticale following proliferation of tissues in culture.

Materials and methods

Establishment and maintenance of tissue cultures

Cultures were established from immature embryos of six hexaploid triticale genotypes according to a procedure devel-

oped for wheat (Larkin et al. 1984). Seed was kindly supplied by Professor C. Driscoll (Adelaide). The cultivars 'Venus', 'Coorong' and 'Currency' and the advanced breeding lines designated X77-366-6, X77-378-15 and T701 were examined. Immature caryopses were collected from bagged inflorescences when the embryos were 1–2 mm in length, approximately 14 days after anthesis, and surface sterilised by treatment with 1% Zephiran (Winthrop Labs, Sydney) in 10% ethanol for 10 min followed by four rinses in sterile distilled water. The embryos were excised and placed, scutellum upwards, on a modified Murashige and Skoog (1962) medium designated SD1, containing 2% sucrose, 150 mg/l L-asparagine, 0.5 mg/l thiamine-HCl and 1 mg/l 2,4-dichloro-phenoxyacetic acid (Sears and Deckard 1982). Cultures were maintained in 90 mm petri dishes at 24–27 °C with an 8 h dark/16 h light (40 $\mu E \cdot m^{-2} \cdot s^{-1}$) cycle. Cultures were transferred to fresh medium every 4–8 weeks, with care being taken to discard portions of tissue which had developed root primordia.

2 Regeneration of plants

Shoot growth was initiated by transferring cultures to a low auxin medium, MS9 (Larkin 1982) containing 0.5 mg/l indole acetic acid and 1 mg/l 6-benzyl amino purine. After four to six weeks, individual shoots were separated from the cultures and placed on a basal medium (Murashige and Skoog 1962) containing no growth regulators. The resulting plantlets were placed in 60 mm peat pots, containing a light soil mixture, on a misting bench for ten days before transplantation to larger pots in the glasshouse. Inflorescences of the regenerant plants were routinely bagged prior to anthesis to prevent cross-pollination.

3 Nomenclature

We have followed Larkin et al. (1984) in designating the regenerant plant SC_1, with SC_2, SC_3 etc. representing subsequent, selfed, generations. An SC_2 family thus derives from seed obtained from a single self-pollinated SC_1 plant. For designation of the rye chromosomes, the nomenclature of Sybenga (1983) was used.

4 DNA extraction and analysis

Two tillers from approximately one-month-old plants were taken so as not to destroy the plant, and used for isolating DNA following the procedure detailed by Appels and Moran (1984). Generally, 0.5–1 mg of DNA was recovered and frozen as a stock solution of approximately 1 mg/ml in TE buffer (0.001 M EDTA, 0.01 M Tris-HCl, pH 8). Samples for analysis (10 µg) were incubated with the restriction enzyme TaqI in a standard buffer (6 mM $MgCl_2$, 6 mM Tris-HCl pH 8, 70 mM NaCl, 10 mM β-mercaptoethanol, 100 µg/ml BSA) at 65 °C for 1 h. The TaqI restriction endonuclease was prepared from 50 g aliquots of *Thermus aquaticus* using the procedures described by Greene et al. (1978). Digested samples were electrophoresed in 1% agarose gels containing TAE buffer (0.02 M Na acetate, 0.002 M EDTA, 0.04 M Tris-HCl pH 8.4) so that the bromophenol blue marker migrated 18 cm. The segment of the gel from the origin to 12 cm was set up to transfer the DNA to Gene-screen (NEN) following manufacturers instructions. The filter was then baked for 2 h at 80 °C under vacuum, prehybridized in PH buffer (3×SSC, 50% formamide, 0.1% SDS, 0.1% polyvinylpyrrolidone, 0.1% BSA (bovine serum albumin), 0.1% ficoll, 0.01 M Tris-HCl pH 8, 0.001 M EDTA) for 2–3 h at room temperature. SSC is 0.15 M NaCl, 0.015 M sodium citrate. Hybridization of the filters was carried out in PH buffer containing 0.01 µg/ml ^{32}P-labelled pTA250.4 insert DNA (specific activity ca. 10^7 cpm/µg) at 37 °C (unless otherwise stated) for approximately 10 h; this solution was heated to 95 °C for 5 min before use. Following hybridization the filters were washed at room temperature in 2×SSC, 0.1% SDS which was initially at 65 °C for approximately 5 h with 3 changes of the wash.

Leaf and stem material from plants at approximately the 8 leaf stage was processed specifically to examine the possibility of alteration in hybridization pattern related to the age of the tissue used as the source of DNA: No such effect was found; if loss of nuclear DNA occurs in older leaf cells, as has been suggested in rye (Hesemann and Schröder 1982), it does not seem to affect the results reported in this paper.

5 Cytological analysis

Roots were harvested from germinated seeds and chilled for 25 h at 4 °C in water before fixing in 1 : 3, acetic acid : ethanol. Root tips were macerated and squashed in 45% acetic acid.

Identification of individual chromosomes was aided by C-banding and N-banding techniques. C-banding was based on a procedure supplied by Dr. A. Mujeeb-Kazi (CIMMYT, Mexico) and was carried out as follows. Cover slips were removed by freezing in liquid nitrogen and the slides were immersed in absolute alcohol for 30 min and then dried. Slides were then treated with a saturated barium hydroxide solution for 4 min at 35 °C, rinsed with distilled water and placed in 2×SSC at 60 °C for 1 h before staining with Giemsa. N-banding was carried out following the modified procedure described by Endo and Gill (1984).

For the slides prepared in Missouri, C-banding was carried out according to the procedure of Lukaszewski and Gustafson (1983). A comparison of the two methods of C-banding showed the same patterns for the bands studied. The data were therefore combined.

6 Evaluation of SC2 and SC3 families in field plots

Plants were established in randomised replicated rows of 12 plants each, with parental controls. Seeds were hand planted 8 cm apart in November/December (late spring/early summer) 1983 and 1984 in Canberra and sprinkler irrigation supplied as needed. Individual plants were scored for heading date (the date at which the first spike had fully emerged from the boot), number of tillers, plant height at maturity, the presence or absence of hairy neck and the presence or absence of the waxy leaf character.

Results

1 Analysis of variation at the Nor loci

To assign the major bands observed in TaqI digests to particular chromosomes, the samples which had been hybridized, under standard conditions, to the wheat rDNA spacer probe were subjected to increasing temperatures. This procedure was expected to identify the band(s) originating from hybridization to the 1R sequences of rye because hydrogen-bonding between the wheat probe and rye 1R rDNA is not as stable to heat (i.e. has a lower melting point) as for the wheat-wheat hybrid (Appels and Dvorak 1982). Figure 1A shows that in both 'Currency' and T701 the same band (ca 2.2 kb in length) is identifiable as the rye 1R rDNA spacer band by this procedure.

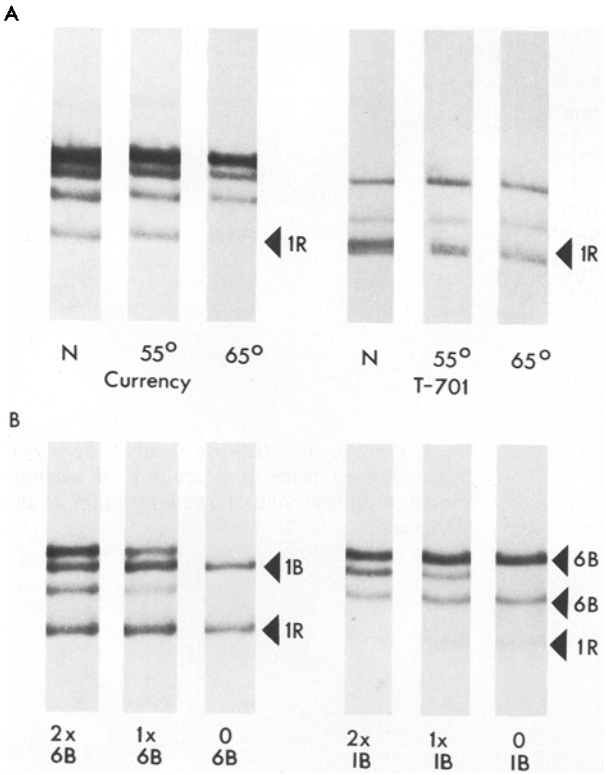

Fig. 1. A Assignment of the 1R band. Replicate filters with Taq1 digests of the triticales 'Currency' and 'T701' hybridized to ^{32}P pTA250.4 probe at 37 °C (standard condition) were subjected to the temperatures indicated, in PH buffer. The lane marked N was hybridized and washed under standard conditions. The indicated band preferentially loses its radioactivity and is thus designated as originating from 1R. **B** Assignment of the 1B and 6B bands. The derivatives of the embryo 'Currency'-5g formed families which segregated different numbers of either chromosome 1B or 6B. Filters with Taq1 DNA digests were hybridised to ^{32}P pTA250.4 at 37 °C. Chromosome identifications were carried out by N-banding and allowed the assignments shown

To assign the remaining major bands, use was made of the fact that among 'Currency' derivatives there were some individuals monosomic for either 1B or 6B. The selfed progeny segregated nulli-1B, or nulli-6B, individuals which allowed unambiguous assignments of bands belonging to the respective loci (Fig. 1 B). Chromosome 1B contributes a single band while chromosome 6B contributes two bands, a situation analogous to that found in the wheat variety 'Chinese Spring'.

A total of 192 SC_1 plants derived from 26 cultures (including 6 different triticales) were analysed for changes at the *Nor* loci as well as whole plant phenotypes. Overall there were few obvious alterations in electrophoretic banding pattern of the rDNA spacer region among the plants examined. No evidence was found for changes in the length of the rDNA spacer regions on chromosomes 1B, 6B and 1R.

The one major alteration not accounted for by monosomy or nullisomy was a significant loss of rRNA genes at the *Nor-R1* locus of chromosome 1R among the progeny of the SC_1 plant 'Currency-5g1*1'. This was seen most clearly in the SC_3 generation (lineage given in Fig. 2). Figure 3 shows five progeny of the SC_2 plant 5g1*1.1 which all featured a significant reduction (approximately 80%) in amount of 1R rDNA spacer sequences. The history of this family is as follows. The SC_1 plant 'Currency-5g1*1' was distinctive in having a large number (21) of sterile tillers and only a single fertile tiller. At the DNA level the *Nor* loci in this plant showed a pattern typical of individuals monosomic for chromosome 6B as well as a reduction in intensity of the 1R band (Fig. 3). Seeds obtained from the single fertile tiller were used for further analysis. C-banding of the resulting SC_2 plants revealed segregation for a marked reduction in the amount of staining at the position of *Nor-R1* on chromosome 1R (Fig. 4). Of fifteen individuals examined, all had two 1R chromosomes with normal size and relative arm length. Five were scored as homozygous for the reduced C-band, six as heterozygous and four as carrying C-bands of normal intensity.

Analysis of the progeny of the SC_2 plant 5g1*1.1, using N-banding, indicated that it was monosomic for chromosome 1B as well as being homozygous for the reduction at the *Nor-R1* locus. Chromosome and DNA analyses were consistent in showing the reduction in sequences at the *Nor-R1* locus as well as segregation for chromosome 1B. No new rDNA bands could be identified in this family and it would appear that a real reduction in the amount of rDNA at the *Nor-R1* locus had occurred. The SC_2 plant 5g1*1.2 was monosomic for chromosome 6B (chromosome and DNA analyses were consistent) and the SC_3 progeny segregated for the number of 6B chromosomes present, but did not show the reduction in sequences at the *Nor-R1* locus.

Smaller changes in amount of rDNA spacer sequences were also observed in some families. These included a slight increase in the relative intensity of hybridisation associated with chromosome 1B in derivative of 'Currency-5g1*2', however, analysis of SC_3 progenies could not establish statistically significant alterations from the parent.

2 Overview of the cytogenetic, morphological and other variation in the triticale somaclones

The technique of C-banding mitotic chromosomes was employed to assess the variation in the rye heterochromatic regions for somaclones obtained from four genotypes (see Table 1). One hundred and sixty-five plants

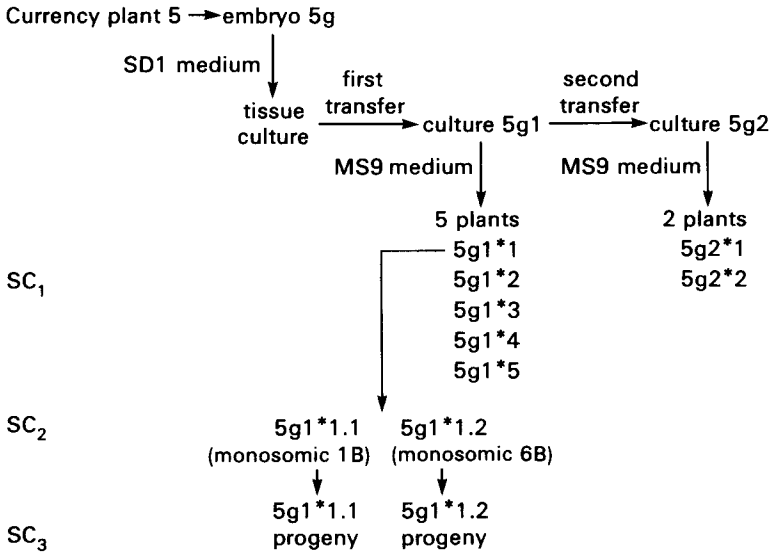

Fig. 2. Lineage of a triticale family. The soma-clones derived from tissue culture of a single immature embryo and showed variation at the *Nor* locus

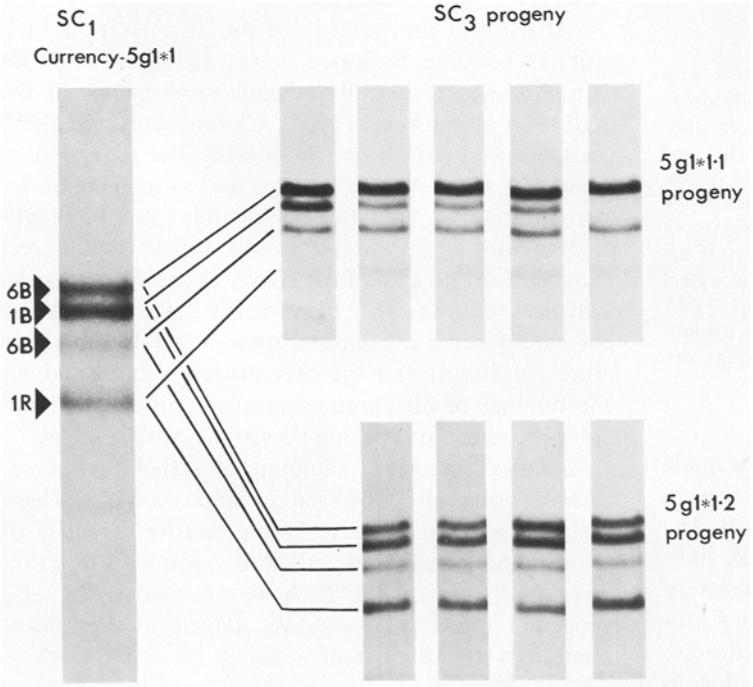

Fig. 3. Analysis of SC_3 progeny from 'Currency-5g1*1'. Filters with Taq1 digests of leaf DNA were hybridized to ^{32}P pTA250.4 probe at 37 °C. The figure shows two SC_3 families derived from the SC_1 plant 'Currency-5g1*1' where each lane represents DNA from an individual plant

of 50 SC_2 families from 'Currency-5' were analysed. C-band variation was found in the *Nor-R1* locus of three plants in three separate families, including the family 5g1* previously described. Only 6 families showed a reduction in the terminal heterochromatin band on 1RL, and 3 of those were heterozygous. Three families out of one regenerant showed a reduction in the terminal heterochromatin band on 7RL. All other bands in every family were normal in appearance. Thirty-six plants from 12 families derived from 'Coorong-2' were analysed. One family was heterozygous for a reduction of the *Nor* region on 1R. The same family showed some slight reduction of the terminal heterochromatin bands on 3RL and 5RS.

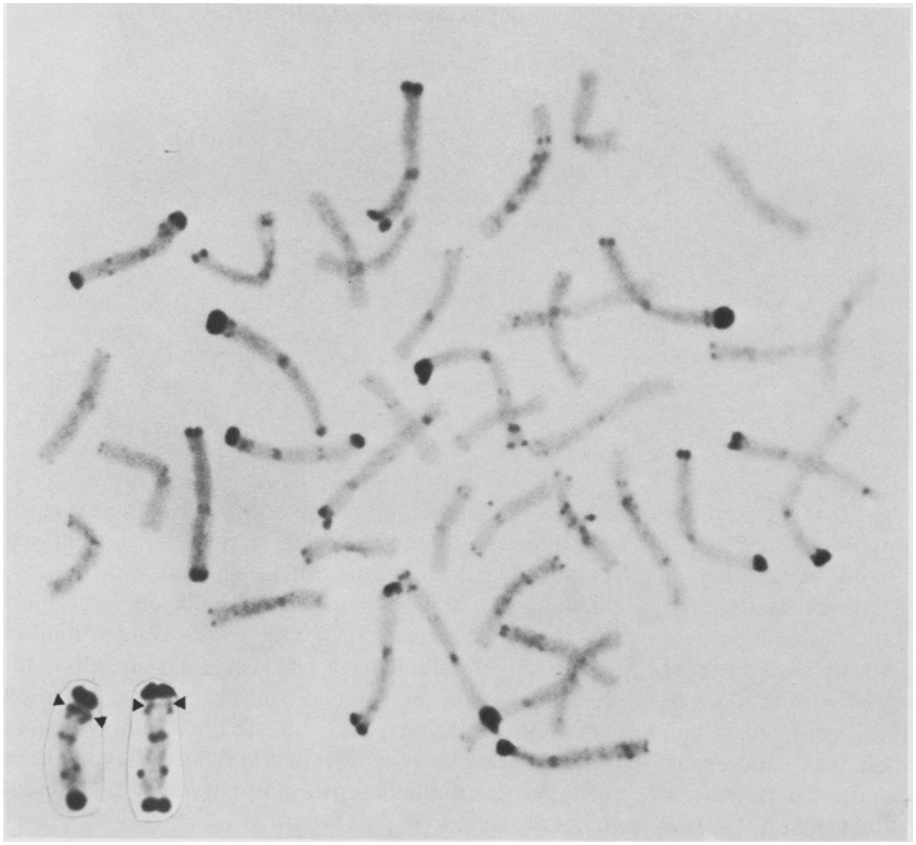

Fig. 4. C-banding of 'Currency-5g1*1.1.1'. The cut-out chromosomes compare the 1R chromosomes in 'Currency-5g1*1.1.1' (*right hand side*) with a "normal" 1R (*left hand side*). The *arrow* indicates the *Nor* C-band. The cut-out chromosomes were C-banded using the University of Missouri technique

Table 1. Analysis of triticale SC_2 families at mitosis for changes in rye terminal heterochromatin

Variety	No. of cultures analysed	No. of SC_2 families examined	No. of plants	No. of families showing variation of terminal or *Nor* heterochromatin block relative to the parent											
				1RS	Nor	1RL	2RS	2RL	3RS	3RL	4RS	5RS	6RS	7RS	7RL
Parent: 'Currency.5'	–	–	2												
Culture: 'Currency.5'	8	50	165	–	3	6	1	–	–	–	–	–	–	–	3
Parent 'Coorong'	–	–	12												
Culture: 'Coorong'	3	9	27	–	1	–	–	–	–	1	–	2	–	–	–
Parent: 'T701.4.2'	–	–	6												
Culture: 'T701.4.2.'	4	17	39	–	1	–	–	–	–	–	–	1	–	–	–
Parent: 'Venus.1'	–	–	6												
Culture: 'Venus.1'	4	6	36	–	–	–	–	–	–	–	–	–	–	–	–
Total	19	82	267	–	5	6	1	–	–	1	–	3	–	–	3
% families showing variation				–	6%	7%	1%	–	–	1%	–	4%	–	–	4%

The amount of variation observed in rye telomeric heterochromatin appeared to vary between triticale varieties (no variation in families from 'Venus' compared to 26% of the families from 'Currency'). The numbers of families examined however are small and this needs further investigation. It was clear that variation in heterochromatin bands did not occur at random throughout the genome. The majority of the reductions involved only two terminal heterochromatin bands (i.e. 1RL and 7RL).

A representative sample of 58 SC_2 families from four genotypes ('Currency', 'Coorong', T701 and

Table 2. Analysis of variance of 'Currency' SC_3 families and parental controls grown in the field 1984/5 for height and days to heading

	df	MS		F	
		Height	Heading date	Height	Heading date
Genotype	29	171	34	4.7**	7.6**
Between somaclones	(25)	173	30	4.8**	6.7**
Between parents	(3)	20	75	0.6	17.0**
Somaclones vs. parents	(1)	591	9	16.3**	2.0
Reps	1	322	103	8.9**	23.0**
Error	288	36	5		

** Significant at the 1% level

X77-378-15) were planted out in the field in December 1983 to assess the extent of morphological variation. Owing to a paucity of plants surviving to maturity, it was not possible to make useful statistical analyses of the data for height and heading date. A single waxless plant was found in a family in which a number of plants had reduced fertility associated with the loss of several chromosomes (chromosome counts from the root-tips of five sib plants indicated that between 37 and 40 chromosomes were present). There was a natural infection by a race of stem rust (*Puccinia graminis*) identified by Dr. N. H. Luig as 34-2,12 which was virulent on 'Coorong' and gave hypersensitive flecks on the other three genotypes. Comparison with the parents indicated that no change in rust reaction was observed among 146 SC_2 plants of 'Coorong' and 406 SC_2 plants of the other three genotypes.

In the following season we selected 28 SC_3 families of 'Currency', including some that had shown variation at the DNA level, for further study in the field. An analysis of variance was made on the data for height and heading date (Table 2). There were significant differences in height between somaclone families, whereas no significant differences were found for the mean heights within the parental controls. Significant differences were also observed for heading date of the somaclones, although in this case the parents showed as much variation. In addition, one family was found to be segregating for the hairy neck character.

Discussion

Passage through tissue culture was found to result in occasional chromosome loss. However, the C-banding analysis revealed that little chromosome structural variation occurred under the conditions of culture used in this study. This is in contrast to the observations of Armstrong et al. (1983) where a relatively high frequency of changes, involving both rye and wheat chromosomes, was found in plants regenerated from six-month-old cultures of the triticale cultivar Welsh.

Variation was seen in a few somaclones for morphological characters including leaf waxiness, mature plant height and hairy neck. The high variation for heading date in the parental controls makes comparison with the somaclones difficult. The parental variation might be ascribed to the parental lines having been collected from plants grown and harvested at different times of the year.

In general the rRNA genes loci were stable, when the chromosomes carrying these loci were present, and in only one family could a quantitative change in the number of rDNA units at the *Nor-R1* locus be established. The quantitative change arose during tissue culture and was present in a primary regenerant, 'Currency-5gl*1'. At the DNA level the plant was apparently monosomic for chromosome 6B (Fig. 3) but also showed a somewhat reduced amount of hybridisation in the major 1R band, when assessed relative to band 1B, which would be consistent with heterozygosity for the alteration at the *Nor-R1* locus. The SC_2 progeny showed segregation for a 6B monosome as well as for a reduced C-band at the position of *Nor-R1* on chromosome 1R. The appearance of a 1B monosome in one of the SC_2 individuals could be due to asynapsis at meiosis in the SC_1 parent in a way analogous to that described in the phenomenon of univalent-shift (Person 1956). Chromosome 1B was previously observed to be the most unstable chromosome in the hexaploid triticale cultivar 'Rosner' (Shigenaga et al. 1971).

The reduction in the 1R rDNA spacer sequences was a consequence of tissue culture since neither the parent nor six other plants regenerated from the same embryo culture carried the alteration. This alteration at the *Nor-R1* locus is heritable as judged by the analysis of SC_2 and SC_3 progenies. Further work will, however, be necessary to confirm the stability of the apparent

reduction in ribosomal gene copy number on chromosome 1R. The process of rapid fixation of variants or quantitative changes, if it occurs, is an event of low frequency in rye or wheat since experiments examining F_2 segregants, from crosses where the *Nor* loci were distinguishable, did not provide any evidence for the phenomenon (Lawrence and Appels 1986).

Quantitative changes at the rDNA locus have been reported following environmental stress in flax (Cullis 1979) and tissue culture in potato (*Solanum tuberosum* L.) (Landsmann and Uhrig 1985). The degree of chromosome alteration or loss in these systems is not clear. It is possible that culture of triticale tissues similarly imposes a stress which leads to the observed genomic changes.

Acknowledgements. The authors thank L. Moran, M. Jeppesen and L. McIntyre for technical assistance, and Drs. P. Larkin, S. A. Ryan and W. R. Scowcroft for their help and encouragement during the course of this work. Part of the C-band analysis was carried out in Columbia, Missouri and the authors are grateful to K. Robertson for preparing slides. Support for this research was provided by the Rural Credits Development Fund.

References

Appels R (1982) The molecular cytology of wheat-rye hybrids. Int Rev Cytol 80:93–132

Appels R, Dvorák J (1982) The wheat ribosomal DNA spacer region: its structure and variation in populations and among species. Theor Appl Genet 63:337–348

Appels R, Moran LB (1984) Molecular analysis of alien chromatin introduced into wheat. In: Gustafson JP (ed) 16th Stadler Genet Symp. University of Missouri, Columbia Mo, pp 529–557

Armstrong KC, Nakamura C, Keller WA (1983) Karyotype instability in tissue culture regenerants of triticale (× *Triticosecale* Wittmack) cv 'Welsh' from 6-month-old callus cultures. Z Pflanzenzücht 91:233–245

Brettell RIS, Wernicke W, Thomas E (1980) Embryogenesis from cultured immature inflorescences of *Sorghum bicolor.* Protoplasma 104:141–148

Cullis CA (1979) Quantitative variation of ribosomal RNA genes in flax genotrophs. Heredity 42:237–246

Dale PJ, Thomas E, Brettell RIS, Wernicke W (1981) Embryogenesis from cultured immature inflorescences of *Lolium multiforum.* Plant Cell Tissue Organ Culture 1:47–55

Dudits D, Nemet G, Haydu Z (1975) Study of callus growth and organ formation in wheat (*Triticum aestivum*) tissue cultures. Can J Bot 53:957–963

Dvorák J, Appels R (1982) Chromosome and nucleotide sequence differentiation in genomes of polyploid *Triticum* species. Theor Appl Genet 63:349–360

Edallo S, Zucchinali C, Perenzin M, Salamini F (1981) Chromosomal variation and frequency of spontaneous mutation associated with in vitro culture and plant regeneration in maize. Maydica 26:39–56

Endo TR, Gill BS (1984) Somatic karyotype, heterochromatin distribution, and nature of chromosome differentiation in common wheat, *Triticum aestivum* L. em Thell. Chromosoma 89:361–369

Gerlach WL, Bedbrook JR (1979) Cloning and characterisation of ribosomal DNA from wheat and barley. Nucleic Acids Res 7:1869–1885

Green CE, Phillips RL (1975) Plant regeneration from tissue cultures of maize. Crop Sci 15:417–421

Greene PJ, Heyneker HL, Bolivar F, Rodriguez RL, Betlach MC, Covarrubias AA, Backman K, Russel DJ, Tait R, Boyer HW (1978) A general method for the purification of restriction enzymes. Nucleic Acids Res 5:2373–2380

Hesemann CU, Schröder G (1982) Loss of nuclear DNA in leaves of rye. Theor Appl Genet 62:325–328

Karp A, Maddock SE (1984) Chromosome variation in wheat plants regenerated from cultured immature embryos. Theor Appl Genet 67:249–255

Landsmann J, Uhrig H (1985) Somaclonal variation in *Solanum tuberosum* detected at the molecular level. Theor Appl Genet 71:500–505

Lapitan NLV, Sears RG, Gill BS (1984) Translocations and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture. Theor Appl Genet 68: 547–554

Larkin PJ (1982) Sugarcane tissue and protoplast culture. Plant Cell Tissue Organ Culture 1:149–164

Larkin PJ, Ryan SA, Brettell RIS, Scowcroft WR (1984) Heritable somaclonal variation in wheat. Theor Appl Genet 67:443–455

Lawrence GJ, Appels R (1986) Mapping the nucleolus organizer region, seed protein loci and isozyme loci on chromosome 1R in rye. Theor Appl Genet (in press)

Lu C, Vasil IK (1981) Somatic embryogenesis and plant regeneration from leaf tissues of *Panicum maximum* Jacq. Theor Appl Genet 59:275–280

Lukaszewski AJ, Gustafson JP (1983) Translocations and modifications of chromosomes in triticale × wheat hybrids. Theor Appl Genet 64:239–248

McCoy TJ, Phillips RL (1982) Chromosome stability in maize (*Zea mays*) tissue cultures and sectoring in some regenerated plants. Can J Genet Cytol 24:559–565

McCoy TJ, Phillips RL, Rines HW (1982) Cytogenetic analysis of plants regenerated from oat (*Avena sativa*) tissue cultures; high frequency of partial chromosome loss. Can J Genet Cytol 24:37–50

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nakumura C, Keller WA (1982a) Callus proliferation and plant regeneration from immature embryos of hexaploid triticale. Z Pflanzenzücht 88:137–160

Nakamura C, Keller WA (1982b) Plant regeneration from inflorescence cultures of hexaploid triticale. Plant Sci Lett 24:275–280

Person C (1956) Some aspects of monosomic wheat breeding. Can J Bot 34:60–70

Scowcroft WR, Larkin PJ (1983) Somaclonal variation and genetic improvement of crop plants. In: Better crops for food (Ciba Foundation Symposium 97). Pitman Books, London, pp 177–193

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

Shigenaga S, Larter EN, McGinnis RC (1971) Identification of chromosomes contributing to aneuploidy in hexaploid triticale, cultivar 'Rosner'. Can J Genet Cytol 13:592–596

Sybenga J (1983) Rye chromosome nomenclature and homoeology relationships. Z Pflanzenzücht 90:297–304

Wernicke W, Brettell R (1980) Somatic embryogenesis from *Sorghum bicolor* leaves. Nature 287:138–139

Wernicke W, Brettell R, Wakizuka T, Potrykus I (1981) Adventitious embryoid and root formation from rice leaves. Z Pflanzenphysiol 103:361–365