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Somaclonal variation in wheat: genetic and cytogenetic characterisation of alcohol dehydrogenase 1 mutants *

P. A. Davies, M. A. Pallotta, S. A. Ryan, W. R. Scowcroft and P. J. Larkin

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

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Summary. The progeny of 551 regenerants of the hexaploid wheat cultivar 'Millewa' were analysed for somaclonal mutants at the three *Adh-1* loci in hexaploid wheat. Seventeen regenerants gave rise to progeny having altered ADH1 zymograms. Progeny with altered zymograms in 13 of these regenerants were aneuploid. The remaining 4 regenerants gave rise to euploid progeny with altered ADH1 zymograms. The genetics of three of these somaclonal mutants is described in detail. These regenerants were interpreted to possess a 4Aα isochromosome, a 3BS/4Aα translocation and a 7BS/4Aα translocation, respectively.

Key words: Somaclonal variation – Alcohol dehydrogenase – Cytogenetics – Tissue culture

Introduction

Somaclonal variation refers to increased genetic variation seen in plants regenerated from tissue culture (Larkin and Scowcroft 1981). The genetic changes that have been observed include aneuploidy, chromosomal translocations, inversions and deletions, increased recombination frequency, gene amplification and deamplification, activation of transposable elements, point mutations, cytoplasmic genome rearrangements and changes in ploidy level (Larkin and Scowcroft 1983; Orton 1983, 1984; Evans et al. 1984; Scowcroft 1985; Larkin et al. 1985). To date, the somaclonal mutants recovered from tissue cultures of hexaploid bread wheat include chromosomal structural rearrangements (Ahloowalia 1982; Karp and Maddock 1984) and alterations in the expression of single genes (Larkin et al. 1984; Cooper et al. 1985; Maddock et al. 1985).

This analysis was designed to isolate somaclonal mutants at any of the genes which synthesise the enzyme alcohol dehydrogenase 1 (ADH; E.C. 1.1.1.1) in the absence of anaerobic induction in hexaploid wheat. This enzyme is expressed in endosperm tissue and is coded for by three genes, *Adh-A1*, *Adh-B1* and *Adh-D1* (Hart 1970). A simple electrophoretic assay allowed identification of null mutants, or mutants giving rise to altered enzyme activity or electrophoretic mobility, at each of the *Adh-1* loci. The somaclonal mutants were cytogenetically analysed.

Materials and methods

ADH1 genetics

ADH1 isozymes are dimers formed by random association of α, β and δ monomers coded for by the genes *Adh-A1*, *Adh-B1* and *Adh-D1*, respectively (Hart 1970). Random dimerisation results in the production of six functional isozymes. However, some dimers co-migrate under electrophoresis (Hart 1970) so that only three active zones are distinguishable (Fig. 1). In wild type individuals, the fastest band is approximately one quarter the intensity of the two slower bands. By using group 4 nullisomic-tetrasomic genetic stocks, Hart (1970) deduced that the fastest band is composed of $\alpha\alpha$ homodimers and the middle and slowest bands are composed of $\alpha\beta+\alpha\delta$ and $\beta\beta+\delta\delta+\beta\delta$ dimers, respectively.

The model described by Hart (1970) was used to predict the zymogram phenotype of endosperm tissue that is either homozygous or heterozygous for a null mutation at one of the three *Adh-1* loci. This model relies on the assumptions that monomeric components are produced in equal quantities by each gene, that dimerisation occurs randomly, and that the different dimers possess equal enzymatic activity.

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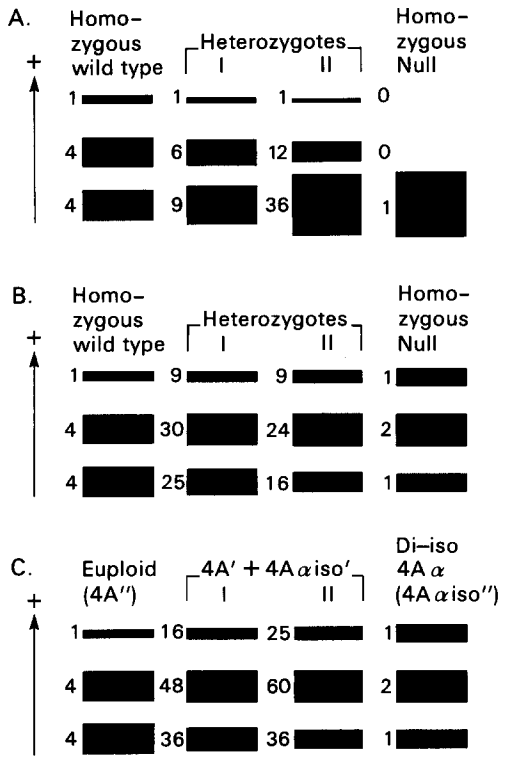

Fig. 1 A–C. Possible ADH1 zymogram phenotypes for triploid endosperm tissue of progeny of an individual heterozygous for **A** an null *Adh-A1* mutation, **B** a null *Adh-B1* or *Adh-D1* mutation. The null allele or deleted gene may be transmitted through either the pollen (I) or egg (II). The fastest band is composed of $\alpha\alpha$ homodimers, the middle band $\alpha\beta+\alpha\delta$ heterodimers and the slowest band $\beta\beta$ and $\delta\delta$ homodimers plus $\beta\delta$ heterodimers. The numbers refer to the relative intensity of the bands for each genotype, which is also conveyed diagramatically by the thickness of the bands. **C** illustrates possible ADH1 zymogram phenotypes for triploid endosperm tissue of individuals possessing various combinations of chromosome 4A and isochromosome 4Aα. The 4Aα isochromosome may be transmitted through either the pollen (I) or egg (II)

Following these assumptions, a seed homozygous for wild type alleles at all three *Adh-1* loci would have an equal proportion of each of the α, β and δ monomers. Random association of the monomers produces the following expected trinomial distribution of the six possible dimers: $(1/3\ \alpha+1/3\ \beta+1/3\ \delta)^2=1/9\ \alpha\alpha+1/9\ \beta\beta+1/9\ \delta\delta+2/9\ \alpha\beta+2/9\ \alpha\delta+2/9\ \beta\delta$. When the dimers are pooled into classes of coincident electrophoretic mobility the ratio of bands is $1\ (\alpha\alpha) : 4\ (\alpha\beta+\alpha\delta) : 4\ (\beta\beta+\delta\delta+\beta\delta)$.

Since triploid endosperm tissue was used for electrophoresis, seed heterozygous for a mutation could have either of two genotypes. These genotypes depend on whether the mutant allele is transmitted through the pollen or egg. For example, if an egg containing a wild type *Adh-A1* allele was fertilised by pollen having a null *Adh-A1* allele, the embryo would contain one wild type and one null *Adh-A1* allele, but the endosperm would have one null *Adh-A1* and two wild type *Adh-A1* alleles. Therefore, the overall frequency of α monomers in the endosperm would be two-thirds that of the wild type endosperm. Random association of the monomers would result in the following expected trinomial distribution of the six possible dimers: $(2/8\ \alpha+3/8\ \beta+3/8\ \delta)^2=4/64\ \alpha\alpha+9/64\ \beta\beta+9/64\ \delta\delta+12/64\ \alpha\beta+12/64\ \alpha\delta+18/64\ \beta\delta$. When the dimers are pooled into classes of coincident electrophoretic mobility the ratio of bands is $1\ (\alpha\alpha) : 6\ (\alpha\beta+\alpha\delta) : 9\ (\beta\beta+\delta\delta+\beta\delta)$.

If, on the other hand, the heterozygote was formed by *Adh-A1* wild type pollen and a null *Adh-A1* egg, the overall frequency of α monomers would be one-third of the wild type frequency. Random association of the monomers would produce the following expected trinomial distribution of the six possible dimers: $(1/7\ \alpha+3/7\ \beta+3/7\ \delta)$, which may be expanded and collated to produce the following ratio of bands, $1\ (\alpha\alpha) : 12\ (\alpha\beta+\alpha\delta) : 36\ (\beta\beta+\delta\delta+\beta\delta)$.

The ratios calculated by this method for homozygous or heterozygous null mutations at the *Adh-A1*, *Adh-B1*, and *Adh-D1* loci are illustrated in Figs. 1 A and 1 B.

Tissue culture and somaclone nomenclature

Thirty immature embryos of the wheat cultivar 'Millewa' were cultured according to the protocol of Larkin et al. (1984). Regenerated plants were self-pollinated and prevented from cross-pollination by bagging spikes. The seed produced by the regenerants was then used for analysis of somaclonal variation at the *Adh-1* loci.

We have adopted the nomenclature proposed by Larkin et al. (1984) where the regenerant is designated as the SC_1 generation and subsequent generations produced by self-fertilisation as the SC_2, SC_3 etc.

Electrophoresis

At least five mature SC_2 seed from each regenerant were electrophoretically assayed for ADH. Seeds were cut into two halfs and endosperm distal to the embryo was used for electrophoresis. The half seed containing the embryo was retained for subsequent germination. For initial screening of SC_2 seed, endosperm was macerated in 45 µl of an aqueous 1 mg/ml dithiothreitol (DTT) solution and allowed to stand for 15 min. Ten microlitres of liquid was then withdrawn from the sample and loaded onto a Titan III cellulose acetate plate, using an applicator designed for use with this type of gel (Helena Laboratories, Beaumont, Texas, USA). The samples were electrophoresed at 300 V for 30 min in 0.1 M TRIS buffer containing 0.2 M glycine at pH 8.2. Immediately after electrophoresis, the gel was stained with 3 ml of a solution containing 5% ethanol, 1.5 mM nicotinamide-adenine dinucleotide (NAD), 160 µM phenazine methosulphate (PMS), 480 µM 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 0.1 M TRIS-HCl buffer, pH 8.0, adapted from the protocol of Hart (1969). The staining reaction was stopped with the addition of a 7% acetic acid solution.

Presumptive ADH mutants were also subjected to polyacrylamide gel electrophoresis for refined resolution. The stacking gel contained 3.6% acrylamide, 0.9% N,N′-Methylenebisacrylamide, 0.09% (v/v) Tetramethylethylenediamine (TEMED), 0.05% (w/v) ammonium persulfate (APS) and 0.07 M TRIS-HCl buffer, pH 6.9. The separating gel contained 6.7% acrylamide, 0.3% N,N′-Methylenebisacrylamide, 0.03% (v/v) TEMED, 0.06% (w/v) APS and 0.2 M TRIS-HCl buffer, pH 8.9. Endosperm was macerated in a solution containing 0.1 M Sorensen's buffer, 0.1 M 2-mercaptoethanol, 6.5 mM DTT and 20% sucrose and then centrifuged at 10,000 g for 5 min. The supernatant was electrophoresed for 3.5 h at 300 V in 0.02 M TRIS buffer containing 0.04 M glycine at pH 8.8. The gel was stained with 50 ml of a solution containing 1% ethanol, 300 µM NAD, 100 µM MTT, 325 µM PMS and 0.1 M TRIS-HCl buffer at pH 8.0.

Cytology

The embryo halves of seed from variant families were germinated on moist filter paper. Root tips of 10 to 15 mm length were collected for mitotic chromosome analysis. Mitotic chromosome preparations from selected seedlings were N-banded by the method of Endo and Gill (1984). Some plants were further analysed by examining meiotic metaphase-I of pollen mother cells by phase contrast microscopy. Meiotic chromosome preparations from selected plants were N-banded by the method of Jewell (1979).

Gibberellic acid (GA) insensitivity

Seedlings were tested for GA insensitivity by the method of Gale and Gregory (1977) with the following modifications (R. A. Richards, personal communication). Up to 5 seeds were sown in plastic tubes of 8 cm length and 4 cm diameter. The tubes contained a 50/50 mix of perlite and vermiculite and were initially saturated with aqueous solution of 10 μM GA_3. Pots were watered with this solution until seedling emergence, after which they were watered with a half strength Hoaglands solution containing 10 μM GA_3. When the second leaf was fully expanded, the distance between the point of attachment of the seed and the second node was measured.

Mature plant height analysis

Each plant was grown in a 13 cm diameter pot in a greenhouse maintained at 22/16 °C under natural lighting. Eight plants of each genotype were arranged in a fully randomised design on a single bench and plant height was measured at maturity.

Results

The progeny (SC_2) of 551 regenerants (SC_1) derived from 30 immature embryos were analysed electrophoretically. The progeny of 17 of the 551 regenerants included individuals that differed from the wild type ADH zymogram. Using zymogram data alone, the variants in each of these 17 families could be interpreted as being homozygous or heterozygous null mutants for one or other of *Adh-A1, Adh-B1* or *Adh-D1* (Fig. 1). There were no variants detected with an altered electrophoretic mobility.

Both the variant and wild type segregants from variant families were germinated and mitotic root tip chromosomes analysed. In 13 families, all individuals having a variant isozyme phenotype were aneuploid for one of the chromosomes of homoeologous group 4, thereby conferring a heterozygous null mutant phenotype. Although regenerants were not studied cytologically, chromosome segregation in SC_2 plants suggested that regenerants giving rise to these families may have had the following genotypes: (1) One regenerant was monosomic for chromosome 4A; (2) One regenerant was monosomic for chromosome 4D; (3) One regenerant possessed one copy of chromosome 4A plus a 4Aβ isochromosome; (4) One regenerant was double monosomic for chromosomes 4A and 7B; (5) One regenerant gave rise to frequent aneuploids for chromosomes 4A and 4D. This regenerant may have been aneuploid for these chromosomes or may have possessed translocations which gave rise to aneuploidy; (6) Eight regenerants gave rise to a low frequency (ca. 1/25) of monosomic 4A progeny. These monosomics may have resulted from a simple spontaneous meiotic non-disjunction of 4A. This occasional loss of 4A has been observed in the progeny of some non-cultured Millewa plants at a frequency of approximately 1/70.

In one family, the regenerant gave rise to a low frequency of progeny (2/20) with a variant 1 : 12 : 36 ADH zymogram. Variant progeny of one of the two variant SC_2's was analysed by N-banding meiotic metaphase preparations and found to have an open quadrivalent involving chromosome 4A and possibly 7B. Although this mutant line has not been comprehensively analysed, it is thought that it involves a translocation and deletion of an *Adh-A1* gene in a similar manner to the SV5 mutant described below.

This report describes in detail the genetic changes that were observed in the progeny of the remaining three variant regenerants whose variant progeny could not be explained by simple aneuploidy. The progeny of these 3 regenerants generally had 42 chromosomes but mutant ADH-1 phenotypes.

1 Isochromosome 4Aα

One of the regenerants gave rise to progeny with a 1 : 4 : 4 phenotype as well as some progeny with 1 : 12 : 36 and some with 1 : 6 : 9 ADH phenotypes which are characteristic of individuals heterozygous for a null *Adh-A1* mutation (Fig. 1 A). Other progeny from this same regenerant had zymograms similar to individuals heterozygous or homozygous for null *Adh-B1* or *Adh-D1* mutations (Fig. 1 B).

Analysis of N-banded mitotic metaphase chromosomes revealed that, in general, segregants possessing the 1 : 4 : 4 phenotype were euploid and segregants possessing the 1 : 12 : 36 and 1 : 6 : 9 phenotypes were monosomic for chromosome 4A. However, the segregants possessing zymograms characteristic of *Adh-B1* and *Adh-D1* null mutations were not monosomic for 4B or 4D. Mitotic N-banding analysis indicated that these segregants possessed one or two isochromosomes of 4Aα (4Aα iso), the chromosome arm on which the *Adh-A1* gene is located.

Theoretically, a di-iso 4Aα genotype would have the same ADH1 zymogram as nullisomic 4B or 4D (Fig. 1 B, C), since an increase in the overall proportion of α monomers should have the same effect on isozyme ratios as a decrease in the proportion of β or δ monomers. This was in fact observed, since di-iso 4Aα produced an ADH1 zymogram which is the same as that of nullisomic 4B and 4D endosperm.

In addition, it was interesting to note that plants possessing a 4Aα isochromosome(s) had a considerable reduction (ca. 50%) in mature plant height. The variety 'Millewa' is a semidwarf (Whan 1979), and our experiments (data not shown) with a spontaneous monosomic 4A 'Millewa' line have shown that the semidwarfing gene is located on chromosome 4A. It is probable that this gene is *Rht1*, a semidominant semidwarfing gene located on chromosome 4Aα (Gale and Marshall 1976; McVittie et al. 1978). It is therefore likely that plants possessing the isochromosomes have extra doses of the *Rht1* gene and are consequently reduced in mature plant height.

The data suggest that this variant regenerant may have possessed one copy of chromosome 4A and one copy of isochromosome 4Aα. The progeny of this regenerant include plants with one or other or both 4A and 4Aα iso. Monosomic 4A progeny are also observed, presumably because the identical chromosome arms of 4Aα preferentially paired with each other at meiosis to form an iso ring and were subsequently omitted from gametes.

2 Translocation resulting in duplication of Adh-A1, Rht1 and Gai1 loci (SV1 mutant)

a) ADH1 zymograms and gibberellic acid (GA) response. SC_2 seedlings from this mutant line were originally assayed for GA insensitivity and segregated for response to exogenous GA application. Some of the segregants had a GA response and height similar to uncultured Millewa controls, while other segregants were more insensitive to GA and of reduced mature plant height. Much of the mutant SC_2 seed was used in the GA tests so SC_3 seed was used to assay for variation at the *Adh-1* loci.

Some SC_3 families possessed only individuals with a wild type 1 : 4 : 4 *Adh-1* zymogram. Other families segregated for wild type 1 : 4 : 4, variant 1 : 2 : 1 individuals, and variant individuals with an intermediate phenotype similar to the theoretical 9 : 24 : 16 or 16 : 48 : 36 ratios of Fig. 1 B, C, respectively. The 1 : 4 : 4 and 1 : 2 : 1 segregants were true breeding. Segregants with the intermediate phenotype produced wild type as well as variant offspring.

When the ADH zymogram data and GA data were combined, it was observed that all plants homozygous for the wild type 1 : 4 : 4 ADH pattern had the same GA response and mature plant height as 'Millewa' controls. However, plants homozygous for the variant 1 : 2 : 1 ADH phenotype gave rise to offspring with a significantly reduced response to GA (Fig. 2). These plants also had significantly reduced mature plant height (Fig. 3 A). Furthermore, all plants which segregated for ADH pattern also segregated for response to GA and mature plant height.

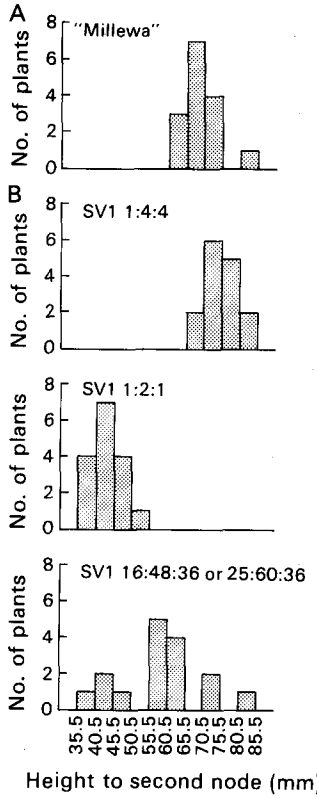

Fig. 2. **A** Response of seedlings of the cultivar 'Millewa' to 10 µM GA_3, measured as the distance from the point of attachment of the seed to the second leaf node when the second leaf is fully expanded. **B** GA_3 response of seedling progeny of different SV1 segregants. Each graph indicates the response of progeny of a single plant representing the genotype, although the progeny of many plants were tested

b) Cytogenetics. Plants derived from the SV1 regenerant had the euploid chromosome complement. Plants having the wild type ADH zymogram formed 21 bivalents at meiosis. On the other hand, all plants with the variant 1 : 2 : 1 ADH zymogram formed 19 bivalents plus a regular ring quadrivalent at meiosis. N-banding indicated that the quadrivalent always included two 4A chromosomes plus two other banded chromosomes which were probably 7B (Fig. 4 A). Furthermore, the β arms of the 4A's always paired with each other and the α arms always paired with what appears by N-banding to be the short arm of 7B, 7BS.

Plants with the 1 : 2 : 1 ADH zymogram only rarely produced aneuploid offspring. Since the ring quadrivalent was passed from one SC_3 plant to all 8 of its SC_4 progeny examined at meiosis, and since the 1 : 2 : 1 variant phenotype did not segregate, it must contain a homozygous non-reciprocal translocation, rather than a heterozygous reciprocal translocation.

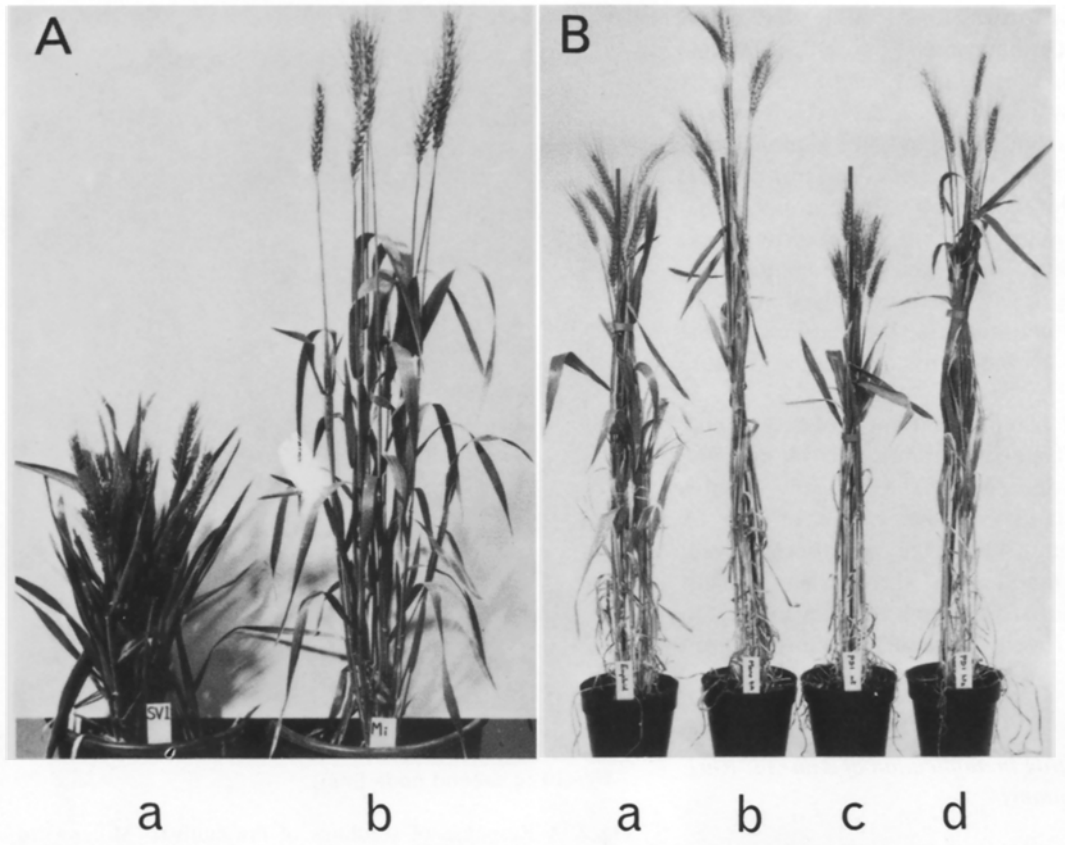

Fig. 3. **A** Representative segregants of the SV1 mutant. (*a*) Segregant having 1:2:1 ADH zymogram phenotype; height = 38 cm. (*b*) Segregant having 1:4:4 wild type ADH phenotype; height = 82 cm. **B** Representative segregants of 'Millewa' monosomic 4A compared to representative SV5 segregants: (*a*) Euploid 'Millewa'; ADH 1:4:4; height 87 cm; (*b*) 'Millewa' monosomic 4A; ADH 1:12:36; height 108 cm; (*c*) SV5 segregant; 1:4:4 ADH phenotype; height 78 cm; (*d*) SV5 segregant; 1:12:36 ADH phenotype; height 104 cm

To test the hypothesis that the α arm of chromosome 4A was involved in the translocation, reciprocal crosses were made between plants homozygous for the 1 : 2 : 1 ADH phenotype and a ditelo 4Aα genetic stock of the wheat cultivar 'Chinese Spring' which was kindly provided by K. W. Shepherd. Although the F_1 embryos were genetically identical, the ADH-1 zymogram differed in accordance with the direction of the cross because triploid endosperm tissue was used for the ADH zymogram assay (Fig. 5 A). Meiotic analysis of F_1's demonstrated that the telocentric was frequently part of a heteromorphic trivalent (Fig. 6 A) which clearly involved two 7B chromosomes, leaving chromosome 4A as a monosome. This indicates that a segment of chromatin from 4Aα has been translocated to the non-homologous chromosome arm, 7BS. If the translocation had been in the opposite direction, that is, if a segment of 7BS had been translocated to the α arm of 4A, then the 4Aα telocentric would only rarely, if ever be involved in a heteromorphic trivalent. This conclusion, which is based on cytogenetic evidence, provides the following rationale for understanding the SV1 mutant zymogram, the GA insensitivity and the mature plant height phenotypes. The non-reciprocal translocation of a segment of 4Aα to 7BS resulted in a duplication of some 4Aα genes on chromosome 7BS. Included among the duplicated genes are *Adh-A1* and both *Rht1* and *Gai1* which are either two very closely linked genes or the same gene on chromosome 4Aα (Gale and Marshall 1973, 1976; McVittie et al. 1978). Duplication of *Adh-A1* results in a 1 : 2 : 1 zymogram, as observed for di-isochromosome 4Aα. Duplication of *Rht1* and *Gai1* results in accentuated height reduction and increased insensitivity to exogenous application of GA.

To summarise, the SV1 mutant is a translocation mutant in which a section of chromatin from 4Aα has been translocated to another chromosome, probably 7BS. The translocated segment carries the *Adh-A1, Gai1* and *Rht1* loci. Although the translocation event may have occurred as a reciprocal exchange in culture, plants carrying the reciprocal translocation chromosome have not been detected in progeny of the SV1 regenerant.

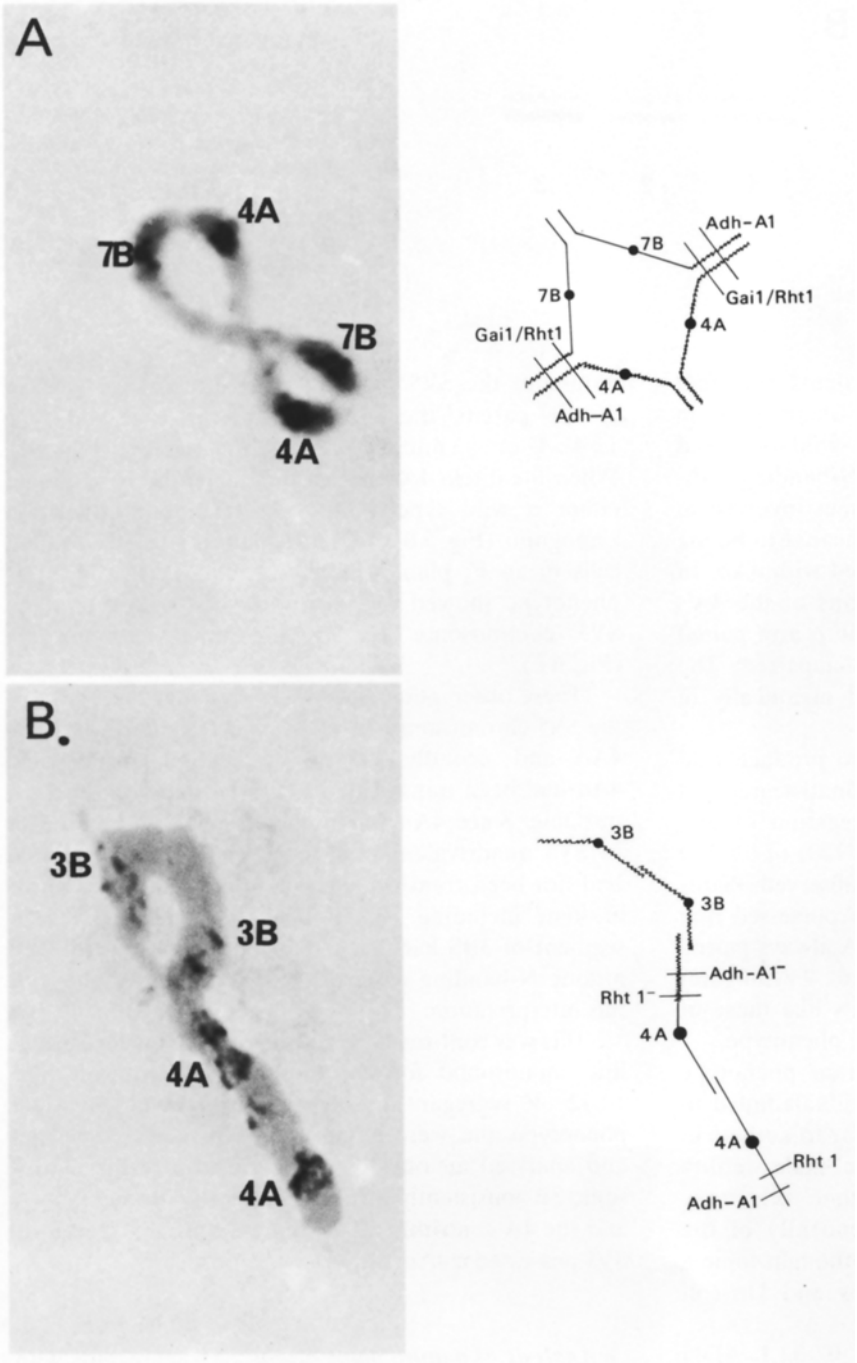

Fig. 4. **A** *Photograph:* ring quadrivalent in SV1 mutant having a 1:2:1 ADH zymogram; *Drawing:* schematic representation of quadrivalent. Gene order extrapolated from data of Suseelan et al. (1982) for tetraploid wheat. **B** *Photograph:* open quadrivalent in SV5 mutant having 1:12:36 ADH zymogram phenotype; *Drawing:* Schematic representation of quadrivalent

3 Translocation resulting in deletion of Adh-1 and Rht1 loci (SV5 mutant)

a) ADH1 zymograms and mitotic chromosome analysis. From this regenerant, 36 SC_2 seeds were electrophoretically analysed. Twenty-two had a 1 : 4 : 4 wild type phenotype, 12 had a 1 : 12 : 36 phenotype and 2 had a 1 : 6 : 9 phenotype. Of these, 11 of the 1 : 4 : 4 types, 10 of the 1 : 12 : 36 types and both 1 : 6 : 9 individuals were examined cytologically at mitotic metaphase and all had 42 chromosomes. N-banding indicated that the plants having a wild type ADH zymogram had two 4A chromosomes that were identical to 'Millewa' wild type. However, the 1 : 12 : 36 individuals had one wild type 4A and one 4A with a slight modification in N-banding pattern on the α arm (Fig. 7).

b) Meiotic chromosomal analysis. Analysis of N-banded meiotic metaphase preparations indicated that the

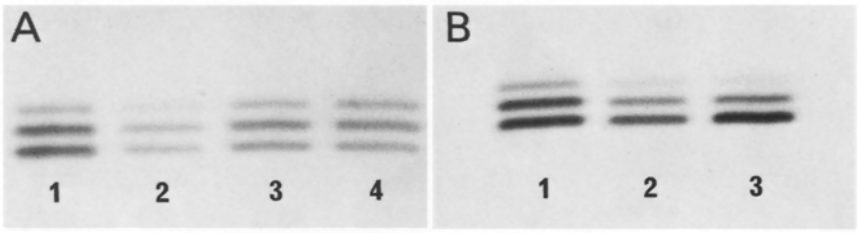

Fig. 5 A, B. ADH1 zymograms. **A** *1* 'Millewa' wild type, 1:4:4 theoretical ratio; *2* F_1 (Ditelo 4Aα×SV1 (1:2:1)), 4:12:9 theoretical ratio; *3* F_1 (SV1 (1:2:1)×Ditelo 4Aα), 25:60:36 theoretical ratio; *4* SV1, 1:2:1 theoretical ratio. **B** *1* 'Millewa' wild type, 1:4:4 theoretical ratio; *2* F_1 (Ditelo 4Aα×SV5 (1:12:36)), 1:6:9 theoretical ratio; *3* F_1 (SV5 (1:12:36)×Ditelo 4Aα), 1:12:36 theoretical ratio

1 : 4 : 4 segregants possessed 21 bivalents but that 1 : 12 : 36 segregants possessed 19 bivalents plus an open quadrivalent involving both the wild type and modified 4A chromosomes (Fig. 4 B). N-banding indicated that the other pair of chromosomes involved in the quadrivalent was 3B and that it appeared to be the short arm of 3B, 3BS, that always paired with 4Aα. In all these open quadrivalents, the β arms of the 4A's were always paired with each other, one α arm paired with 3BS and one α arm remained unpaired. The 1 : 6 : 9 segregants were not examined meiotically in this generation.

The 1 : 4 : 4 individuals bred true to produce wild type SC_3 progeny. The 1 : 12 : 36 individuals segregated in a similar way to the original segregation of SC_2 seeds, except that a low frequency (12/120) of 0 : 0 : 1 ADH zymogram phenotypes were also observed. Plants having a 0 : 0 : 1 zymogram phenotype possessed ring quadrivalents in which the β arms of 4A always paired with each other. The plants having a 1 : 6 : 9 zymogram phenotype possessed open quadrivalents like those of SC_2 and SC_3 segregants with a 1 : 12 : 36 phenotype.

The plants with a 0 : 0 : 1 zymogram phenotype were sterile. The anthers of these individuals failed to dehisce and were morphologically similar to anthers in plants homozygous for the 'Cornerstone' male sterility mutant, *mslc* (Driscoll 1977). 'Cornerstone' is a γ-ray induced mutation which maps independently of the centromere on chromosome 4Aα and is thought to be a terminal chromosomal deletion (Barlow and Driscoll 1981).

Since the SV5 segregants with a 0 : 0 : 1 ADH phenotype were sterile, SV5 plants with a 1 : 12 : 36 zymogram were used for crossing to a genetic stock to determine the nature of the translocation that resulted in quadrivalent formation. It was known that the α arm of 4A was involved in the translocation because the α arm always paired with the non-homologous chromosome in quadrivalents. Therefore reciprocal crosses were made between 'Chinese Spring' ditelo 4Aα and an SV5 1 : 12 : 36 segregant.

In each reciprocal cross, two types of F_1 ADH phenotype were observed because of the heterozygous nature of the SV5 parent. When ditelo 4Aα was the female parent, the F_1 seed had either a wild type 1 : 4 : 4 or a mutant 1 : 6 : 9 zymogram (Fig. 5 B). When the ditelo 4Aα parent was male, the F_1 seed had either a wild type 1 : 4 : 4 or a mutant 1 : 12 : 36 zymogram (Fig. 5 B). Meiotic analysis of 68 N-banded cells in an F_1 plant with the mutant 1 : 12 : 36 ADH phenotype showed no pairing of 4Aα with either the SV5 chromosome 4A or any other chromosome (Fig. 6 B).

These observations indicate firstly that the α arm of the SV5 chromosome 4A was very different to wild type 4Aα, and secondly that no segment of chromosome 4Aα had been translocated to a non-homologous chromosome. Since 4Aα was involved in pairing with 3BS in SV5 quadrivalents and since chromatin from 4Aα had not been translocated to a non-homologous chromosome including 3BS, it must be concluded that a segment of 3BS had been translocated onto 4Aα. The mitotic N-banding patterns (Fig. 7) are consistent with this interpretation.

This was confirmed by pollinating a 'Chinese Spring' line monosomic for chromosome 3B with an SV5 1 : 12 : 36 segregant. F_1's which had a 1 : 12 : 36 ADH phenotype and were monosomic for 3B were selected and analysed meiotically. The short arm of the monosome 3B consistently formed trivalents with the SV5 4A and the 4A contributed by 'Chinese Spring'. Therefore, SV5 possessed a 4Aα/3BS translocation.

c) Analysis of mature plant height. SV5 segregants with a 1 : 12 : 36 or 1 : 6 : 9 ADH zymogram were taller than segregants with a wild type 1 : 4 : 4 phenotype (Fig. 3 B). The data in Table 1 summarise the results of an experiment to compare the mature plant height of SV5 segregants with the height of 'Millewa' euploid and monosomic 4A plants. Clearly, the height of 'Millewa' euploid plants is the same as that of SV5 segregants with a 1 : 4 : 4 ADH phenotype. However, in the same way that monosomic 4A plants are taller than 'Millewa' euploids, the SV5 1 : 12 : 36 segregants are significantly taller than the wild type 1 : 4 : 4 segregants.

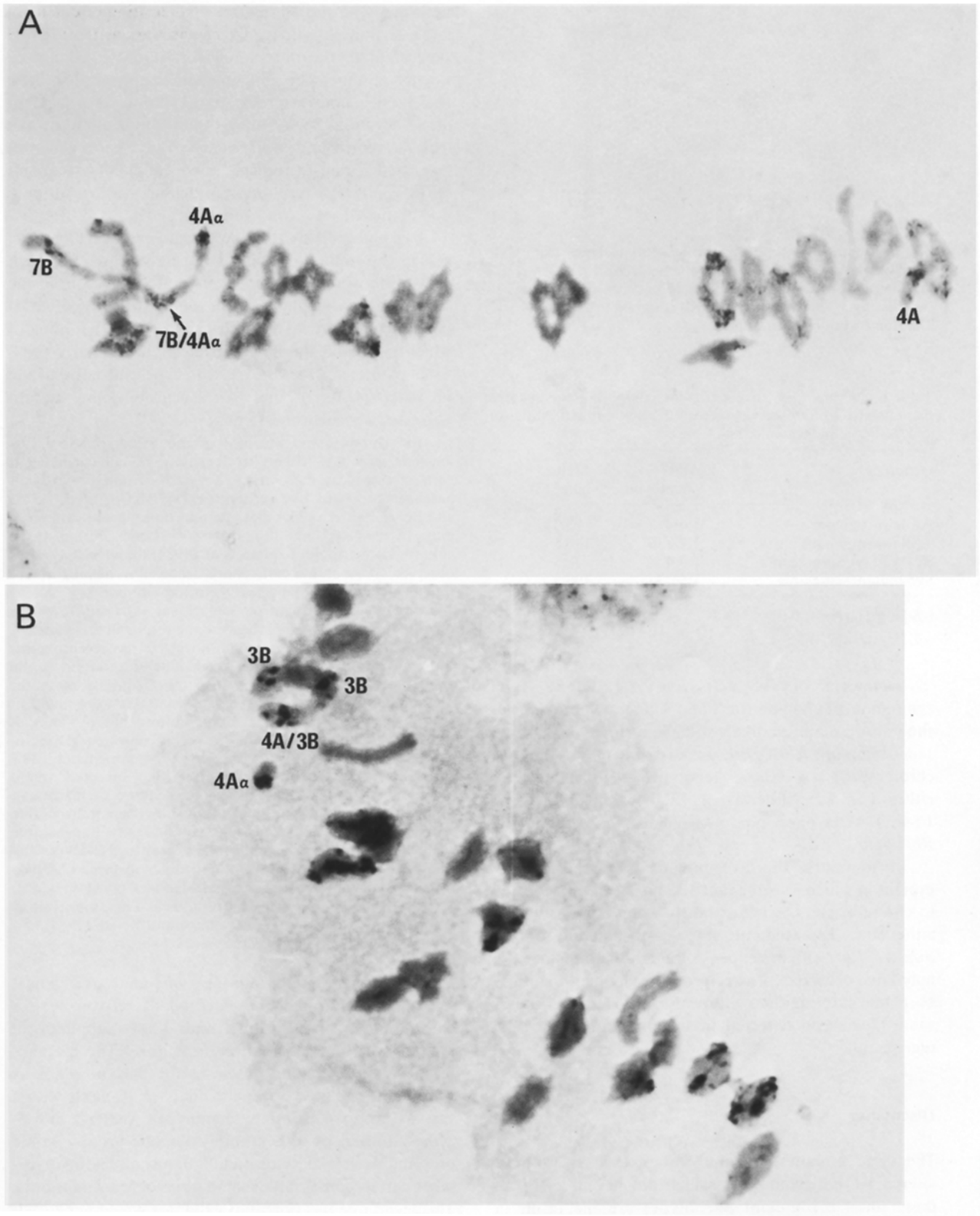

Fig. 6. **A** Heteromorphic trivalent in F_1 plant produced by a 'Chinese Spring' ditelo 4Aα × SV1 (ADH = 1:2:1) cross. **B** No pairing of 4Aα telocentric was observed in F_1 plant produced by a SV5 (ADH = 1:12:36) × 'Chinese Spring' ditelo 4Aα cross

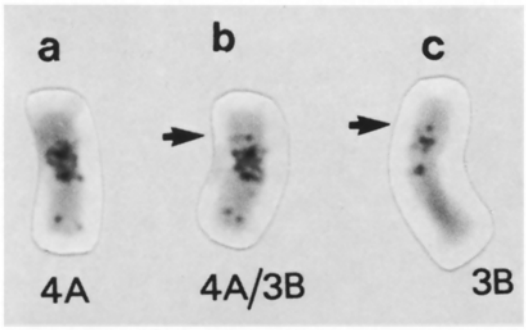

Fig. 7. N-banded mitotic metaphase chromosomes from SV5 plant having a 1:12:36 ADH phenotype. *a* Wild type 4A chromosome; *b* 4A having 4Aα/3BS translocation; *c* Wild type 3B chromosome. *Arrows* indicate bands common to 3BS and 4Aα/3BS translocation chromosome

Table 1. Summary of an experiment comparing the mature plant heights of SV5 segregants with those of euploid 'Millewa' and 'Millewa' monosomic for 4A

Genotype	ADH phenotype	Mature plant height $\bar{x}$
'Millewa' euploid	1:4:4	85.0
'Millewa' mono 4A	1:12:36	110.5
SV5 wild type segregants	1:4:4	78.5
SV5 mutant segretants	1:12:36	100.5

L.S.D. ± 8.6 ($P = < 0.01$)

Segregants of SV5 that have a 1 : 12 : 36 ADH zymogram phenotype (or 1 : 6 : 9 phenotype, data not shown) are therefore likely to have a single dose of the semi-dominant *Rht1* gene, as do plants monosomic for chromosome 4A. These genotypes were taller than either euploid 'Millewa' or SV5 segregants with a 1 : 4 : 4 ADH phenotype which had two doses of the *Rht1* gene.

In summary, the SV5 mutant is a translocation mutant in which a segment of 3BS has been translocated to chromosome 4Aα. This translocation has resulted in either the displacement or loss of function of the *Adh-A1, Rht1* and *Mslc* loci. Although the translocation may have occurred as a reciprocal exchange in culture, no plants carrying the reciprocal translocation chromosome have been detected in the progeny of the SV5 regenerant.

Discussion

The three mutant lines analysed in this report have altered ADH1 zymograms which are heritable. In all three lines, the mutant phenotypes are the result of modifications which have been described by cytogenetic analysis, and all involve duplication or deletion of large sections of chromatin. The arguments presented suggest that one mutant regenerant possessed a 4Aα isochromosome. The SV1 regenerant appeared to have possessed a non-reciprocal translocation of a segment of 4Aα onto 7BS resulting in a duplication of the *Adh-A1, Rht1* and *Gai1* genes. The SV5 regenerant possessed a non-reciprocal translocation of a segment of 3BS onto 4Aα resulting in a deletion or loss of function of the *Adh-A1, Rht1* and *Mslc* genes.

It is highly likely that the genetic events which gave rise to the mutants occurred during culture. For each mutant, most of the sister regenerants derived from the same embryo explant displayed a wild type ADH phenotype. There were 65 sister plants to SV1, none of which possessed the SV1 translocation. Similarly the 7 sister plants of SV5 were all wild type and none of the 11 sister plants of the 4Aα isochromosome mutant possessed an isochromosome.

The chromosomes involved in the SV1 and SV5 translocations were 4A, 7B and 3B. It may be no coincidence that each of these chromosomes is heavily N-banded because it has been proposed by Sacristan (1971); McCoy et al. (1982) and Benzion et al. (1985) that late replicating heterochromatin may be involved in chromosome breakage during tissue culture. Furthermore, Lapitan et al. (1984) found that 12 of 13 observed chromosome breakpoints occurred in heterochromatin in ten culture derived wheat × rye amphiploids.

Other recent research on somaclonal variation in cereals provides interesting comparisons to the results in this paper. A somaclonal ADH isozyme mutant in maize has recently been shown to result from a single nucleotide substitution in the *Adh-1* locus (Brettell et al. 1986a). This contrasts with the present study where no ADH point mutations were found in 551 regenerants. In triticale, tissue culture has produced a somaclonal mutant in which the highly repeated rDNA of chromosome 1R has been reduced by approximately 80% (Brettell et al. 1986b). Peschke (see Benzion et al. 1985) provides tentative evidence that tissue culture of maize can result in activation of silent Activator (Ac) transposable elements. The present study did not find any transposable element activity or deamplification events. However, the cytogenetic alterations reported are similar to those observed by Ahloowalia (1982) and Karp and Maddock (1984) in wheat, McCoy et al. (1982) in oat, Armstrong et al. (1983) in triticale, Ahloowalia (1983) in triploid ryegrass, and Fedak (1984) and Lapitan et al. (1984) in *Triticum* × *Secale* hybrids.

Finally it is apparent that isozyme analysis of wheat somaclones is an efficient method of recovering isochromosomes, translocation lines and aneuploids in any cultivar amenable to tissue culture. The chromosomal rearrangements observed for chromosome 4A were identified by cytogenetic analysis of plants with a variant group 4 isozyme phenotype. Analysis of isozymes located on other homoeologous groups would presumably reveal cytogenetic abnormalities involving other chromosomes in this same series of 551 somaclones. This technique has potential value for wheat geneticists who require translocation and deletion stocks for

mapping genes to chromosomes. It may also be useful for generating aneuploid stocks in a wide variety of cultivars.

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