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Heritable somaclonal variation in wild barley (*Hordeum spontaneum*)

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Summary. Progenies of *H. spontaneum* plants regenerated from immature embryo derived calli were analysed for somaclonal variation on the following traits: (1) organization of the intergenic spacer of the rRNA genes; (2) B and C hordein pattern on SDS-PAGE; (3) genomic organization of the B and C hordein coding sequences; (4) mitochondrial DNA organization assayed by hybridization of Southern blots of total DNA with mitochondrial coding genes; (5) cytology. One out of twelve progeny plants was characterized as variant for two traits: (a) a loss of 1.8 and 2.5 kb Taq I intergenic rDNA spacer fragments and (b) a variant pattern of hordeins on 1-D SDS-PAGE. No numerical or structural chromosome variation was detected among the control plants therefore it is assumed that the variation was caused by the in vitro culture and transmitted, through sexual reproduction, to the analysed progeny.

Key words: *Hordeum spontaneum* – Ribosomal DNA – Intergenic spacer – Hordeins – Mitochondrial DNA – Heritable somaclonal variation

Introduction

The term somaclonal variation was suggested by Larkin and Scowcroft (1981) to describe the phenomenon of variability occurring in plants regenerated from tissue culture. The occurrence and frequency of somaclonal variation is still a debated issue, ranging from plant uniformity to high frequency of variants (Hanna et al. 1984; Sun et al. 1983; Larkin et al. 1984).

Evidence for somaclonal variation in the nuclear and organellar genomes has been documented in several species. A thousand-fold increase in the gene conversion event was measured in tobacco plants regenerated from tissue culture (Dulieu and Barbier 1982); protoplast derived potato variants were revealed to be deficient in the number of rRNA genes (Landsmann and Uhrig 1985) and a heritable reduction in the number of rRNA units was observed in regenerated Triticale, flax and wheat plants (Brettell et al. 1986; Cullis 1985; Breiman et al. 1986).

DNA alterations within mitochondrial genomes were detected in regenerated maize and potato plants (McNay et al. 1984; Kemble et al. 1982; Kemble and Shepard 1984).

Immature embryos and anther-derived calli provide most suitable systems for plant regeneration of diploid and haploid barley plants respectively (Orton 1979; Foroughi Wehr et al. 1982). Plant regeneration from immature embryo-derived calli was reported in several barley species: *Hordeum vulgare*, *H. spontaneum*, *H. bulbosum*, *H. jubatum* (Orton 1980; Breiman 1986). The ability of the immature embryo-derived calli to regenerate plants was found to be genotype dependent in *H. vulgare* (Hanzel et al. 1985; Goldstein and Kronstad 1986). Chromosomal variability has been described in regenerated *H. vulgare*, *H. jubatum* and their interspecific hybrid (Orton 1980). However, information on heritable somaclonal variation in barley is very scarce (Deambrogio and Dale 1979).

In the present study we have evaluated the organization of the rDNA intergenic spacer, the hordein SDS-PAGE pattern, the genomic organization of hordein genes and the mitochondrial DNA arrangement as markers for nuclear and mitochondrial heritable somaclonal variation. We have also checked the cytology of a sample of progeny from the regenerated plants.

Ribosomal DNA spacer length variation

Many studies have been carried out on the organization of the rRNA genes of higher plants (Flavell 1985). The genes for 18S, 5.8S and 26S cytoplasmic ribosomal RNAs in eukaryotes are organized in tandem arrays in the nucleolar organiser region (NOR) on one or several chromosomes. Each unit consists of one structural gene for each ribosomal RNA species and an intergenic spacer. The spacer region shows extensive sequence

divergence among cultivars and species, while the coding sequences are relatively conserved. Length variation is the result of a variable number of repeated units within the spacer region (Appels and Dvorak 1982; Saghai Maroof et al. 1984).

Since the NOR loci are variable among barley cultivars or accessions, but uniform within each accession, we have analysed these loci for evaluating somaclonal variation.

Evaluation of B and C hordeins

Several traits of agronomic importance, including grain storage proteins, have demonstrated heritable somaclonal variation (Larkin et al. 1984; Maddock et al. 1985; Cooper et al. 1986). In barley there are two major groups of prolamine storage proteins, one sulphur rich (the B hordein) and one sulphur poor (the C hordein), which together account for 25% of the alcohol soluble fraction.

The B and C hordeins are encoded by separate genetic loci, *Hor 2* and *Hor 1*, respectively, (Jensen et al. 1980; Shewry et al. 1980). Previous studies have indicated that each group is composed of at least 10 closely related polypeptides (Faulks et al. 1981; Shewry et al. 1981). The B hordein polypeptides are encoded by at least two sub-families of mRNA and about 11–13 genes (Kreis et al. 1983). About 20–30 C hordein genes per haploid genome were estimated (Shewry et al. 1985).

Since extensive polymorphism of both hordein gene organization and polypeptide pattern on SDS-PAGE were detected (Faulks et al. 1981; Kreis et al. 1983; Shewry et al. 1985), we have examined these two parameters on both the proteins and the DNA extracted from grains of regenerated plants.

Mitochondrial DNA

Increase in genetic variability can be observed in the cytoplasmic organelle genome of cells that have been in tissue culture (Hanson 1984). These changes are limited to alterations in the mitochondrial genome, as no changes in the chloroplast genome have been reported except in albino wheat and barley plants regenerated from anther culture (Day and Ellis 1984, 1985). In maize, the genetic reversion to fertility of the T cytoplasm and its resistance to a fungal toxin (*Helminthosporium maydis* T toxin) occurs in plants regenerated from tissue culture (Brettell and Thomas 1980; Umbeck and Gengenbach 1983) although it has never been observed in breeding populations.

In the present study the organization of mtDNA in progenies of regenerated barley plants was analysed by Southern blotting of the restricted total DNA and hybridization with specific mitochondrial probes.

Cytology

Structural and numerical chromosome changes have been observed amongst plants regenerated from cultured immature embryos of wheat (Karp and Maddock 1984), oats (McCoy et al. 1982), maize (McCoy and Phillips 1982), triticale (Jordan and Larter 1985) and triploid rye grass (Ahloowalia 1983). Chromosome variation appears to be most prevalent in regeneration from polyploids, and no chromosomal changes were observed in regenerants from cultured immature embryos of diploid *Pennisetum americanum* (Swedlund and Vasil 1985).

In this present study we have assessed the cytological status of the regenerated *H. spontaneum* plants by screening a sample of their progenies at meiosis.

Materials and methods

Plant material

Plant regeneration of *H. spontaneum* has been previously described (Breiman 1985). Callus cultures were initiated from isolated immature embryos of *H. spontaneum* on MS (Murashige and Skoog 1962) media supplemented with 2 mg/l 2,4-D. Shoot regeneration occurred upon the transfer of tissue to media containing 1 mg/l IAA and 1 mg/l zeatin with a photoperiod of 16/8 h light/dark. Regenerated shoots were rooted on MS (Murashige and Skoog 1962) medium without hormones. The plants were transferred to "Jiffy" turf pots, kept for one week under plastic covers and subsequently planted into 5 l pots in the greenhouse.

Self fertilization of the regenerated plants (SC1) lead to seeds from which SC2 plants were obtained. A subsequent cycle lead to SC3 plants. SC2 and SC3, as well as control plants from regular seeds of the respective accessions, were used for the molecular analyses. The accessions were supplied by Prof. E. Nevo, Institute of Evolution, University of Haifa, Israel. Fifty regenerants obtained from immature derived calli were found to be phenotypically similar (Breiman 1985). The progenies (SC2) of 12 selfed regenerants were chosen for further molecular studies.

The nomenclature of the accessions and their geographical distributions is according to Nevo et al. (1979):

233* = individual 57 in population 25;
300* = ind. 24 pop. 10; 307* = ind. 15 pop. 24;
350* = ind. 18 pop. 8; 228* = ind. 49 pop. 10.

DNA extraction

Total DNA was prepared according to Appels and Dvorak (1982) with minor modifications. Seedlings were germinated aseptically for about 10 days. Ten to 20 seedlings having a total fresh weight of about 1 g were crushed to fine powder with a pestle and mortar in liquid nitrogen in the presence of a small quantity of acid-washed sand. The powder was mixed with 5 ml of DNA isolation buffer (0.1 M NaCl; 0.1 M Tris-HCl, pH 8.5; 0.05 M EDTA; 0.2% SDS; 0.1 mg/ml proteinase K). The homogenate was extracted twice with phenol-chloroform (1:1, v/v) and once with chloroform-isoamyl alcohol (24:1) and the aqueous phase was ethanol precipitated in the presence of 0.3 M sodium acetate. The DNA was redissolved in 0.5 ml of TE buffer (0.01 M Tris-HCl pH 8.0; 0.001 M EDTA) and incubated for 2 h at 37 °C with 100 µg/ml RNAse A and 60 units/ml RNAse T1. The aqueous phase was re-extracted with phenol-chloroform (1:1, v/v) and chloroform as previously mentioned. The DNA was ethanol precipitated in the presence of 0.3 M sodium-acetate and the precipitate was vacuum dried and resuspended in 100 µl TE buffer.

Restriction endonuclease digestion, blotting and hybridization

One microgram of the extracted DNA was digested with TaqI, separated on a 1% agarose gel and transferred to a nitrocellulose filter (Southern 1975). Each filter was baked for 2 h at 80 °C and incubated in the prehybridization solution:

* Our code number

6×Denhart , 4×SSC, 0.1% SDS and 100 µg/mg sonicated salmon sperm DNA – 1×Denhart is 2% Ficoll, 0.2% polyvinylpyrollidone, and 0.2% bovine serum albumin – 1×SCC is 0.15 M NaCl, 0.015 M sodium citrate.

The prehybridization solution was replaced with the hybridization solution containing the same components and P^{32} labelled plasmids or cosmids.

Hybridization of Southern blots with plasmids of the hordein and mitochondrial coding genes required higher amounts of DNA. Therefore, 10 µg of each DNA preparation was digested with EcoRI, Hind III or BamHI, separated on 0.8% agarose gel and transferred to a nylon membrane (Gene Screen Plus NEN Research Products).

When membranes were to be used for rehybridization, the probes were removed by washing the membranes in 100 ml 0.4 M NaOH for 30 min at 42 °C, followed by a 30 min 42 °C wash in 0.1×SSC, 0.1% SDS, 0.2 M Tris-HCl, pH 7.5.

Recombinant DNA clones

The plasmid pHV 294 contains a 9kb EcoRI fragment which includes the 18S, 26S and the spacer sequences of barley rRNA inserted into the pAC 184 vector (Gerlach and Bedbrook 1979). (The pHV 294 plasmid was a present from Dr. R.B. Flavell, Plant Breeding Institute, Trumpington, Cambridge, UK). The plasmids pB7 (977 bp) and pB11 (884 bp) are cDNA clones of the B-hordein cloned into the pUC8 Hind III site (Forde et al. 1984). The pcP387 plasmid represents a C hordein clone obtained from a cDNA library (Forde et al. 1984). (The hordein clones were a present from Dr. P.R. Shewry, Biochemistry Dept. Rothamsted Experimental Station, Harpenden, Herts, UK).

The cosmid clone 4A7 represents contiguous 40 kb mtDNA fragments (which include the 18S and 26S mitochondrial rRNA genes) from a *Triticum aestivum* mtDNA library cloned in the pHC79 cosmid vector (unpublished results), according to Hohn and Collins (1980) and Lonsdale et al. (1984). The L4C is a M13 clone of 900 bp, Hind III-Pst I fragment of *T. aestivum* mitochondrial cytochrome oxidase subunit I, including the 240 bp of the flanking region upstream of the 660 bp 5′ terminus of the coding sequence and referred to as the 5′ wheat COX I clone (a present from Dr. L. Bonen, Dept. of Biochemistry, Ottawa, Canada).

The inserts of the hordein clones pB7, pcP387, the plasmid pHV294 and the cosmids were P^{32} labelled by nick translation (Rigby et al. 1977) to a specific activity of $1–5 \times 10^8$ dpm/µg.

Hordein extraction and separation

Single seeds were crushed to powder by mortar and pestle. Total hordein were extracted for 1 h at 60 °C by the addition of 200 µl 55% (v/v) propan-2-ol (Shewry et al. 1978). After centrifugation the meal was re-extracted and the combined supernatant was brought to dryness by evaporation at 40 °C. Proteins were reduced by the addition of 200 µl Tris nitrate buffer pH 7.5 containing 8 M urea and 1% (v/v) 2-mercaptoethanol at 4 °C overnight. The samples were S-pyridylethylated by the addition of 3 µl 4-vinylpyridine, according to Friedman et al. (1970). The samples were sonicated for 30 min in a sonicator bath, incubated for 4 h at room temperature (Shewry et al. 1978) and acidified with glacial acetic acid. After an overnight dialysis against 1% SDS, the samples were dissolved in 8 M urea containing 10% SDS. Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 10% acrylamide, 0.15% bisacrylamide modified from Laemmli (1970) and Forde et al. (1981).

Cytology

For meiotic studies, 5 seeds were picked at random from SC1 plants from each of the five groups (228, 233, 300, 307, 350), germinated and grown to heading. Inflorescences were collected at a stage when the individual heads were approximately 4–5 cm in length. The heads were stripped of leaves, fixed in Carnoys solution (6:3:1, alcohol:chloroform:glacial acetic acid) and mordanted with two drops of ferric chloride solution, for at least four weeks. Pollen mother cells were squashed in aceto-carmine. All stages of meiosis were examined for evidence of aberrations.

Results

Analysis of variation at the NOR loci

Total DNA was extracted from seedlings originating from seeds of four *H. spontaneum* accessions. The DNA was digested with Taq I or EcoRI-Bam HI, fractionated by electrophoresis on 1% agarose gel, transferred to nitrocellulse (Southern 1975) and hybridized with the P^{32} labelled pHV294 plasmid, in order to assess the rDNA spacer length polymorphism within and among

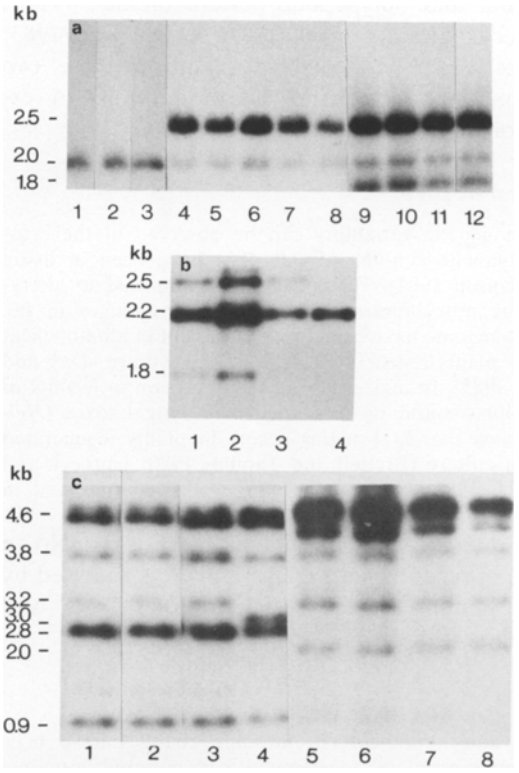

Fig. 1 a–c. Southern blot hybridization analysis of total genomic DNA from control plants and progenies of regenerated (SC2) plants of *H. spontaneum* L. digested with Taq 1 (**a, b**) or EcoRI-Bam HI (**c**) and hybridized to pHV294. Size indicators in Kbp. **a** Lanes 1–3 accession 300, 3 = control; lanes 4–8 accession 307, 8 = control; lanes 9–12 accession 350, 12 = control. **b** Lanes 1–4 accession 233, 1 = control. **c** Lanes 1–4 accession 233, 1 = control; lanes 5–8 accession 350, 5 = control

these accessions. The Taq I endonuclease was chosen since it cleaves the barley rDNA to small fragments, with the exception of the variable intergenic spacer region where Taq I sites are rare, therefore producing long Taq I fragments characteristic to the spacer length.

The EcoRI-Bam HI endonucleases cleave the intergenic spacer and the ribosomal coding genes into fragments of variable and fixed length fragments respectively (Gerlach and Bedbrook 1979). The hybrididization pattern revealed distinct combinations in spacer length of the rRNA genes for each accession (Fig. 1) but no within variation could be detected (data not shown). Taq I fragments of 2.0 Kb, 2.0 and 2.5 Kb, 1.8, 2.0, 2.5 Kb, or 1.8, 2.2, 2.5 Kb were present in accessions 300, 307, 350 and 233, respectively. To reveal any diversity in progenies of immature imbryo derived plants (SC2), DNA was extracted, digested with Taq I or EcoRI-Bam HI and the Southern blots hybridized with the radiolabelled pHV294 plasmid. The spacer length of Taq I fragments of the SC2 plants was similar in size to the donor plants in three out of the four accessions tested (Fig. 1 a, lanes 1–12).

A major alteration in the Taq I and EcoRI-Bam HI hybridization pattern of the intergenic rDNA spacer with the P^{32} radiolabelled pHV294 was observed in one out of three SC2 plants of accession 233. This plant has lost the 1.8 and 2.5 Kb Taq I spacer fragments (Fig. 1 b, lane 4) which could not be detected even after prolonged exposure.

The EcoRI-Bam HI digestion of barley results in a wide range of fragments hybridizing with the pHV294. The complexity of the pattern obtained is due to the methylation of cytosine residues in some of the rRNA repeats (Gerlach and Bedbrook 1979). The EcoRI-Bam HI pattern of two accessions is demonstrated in Fig. 1 c. The 3.8 kb and 0.9 kb fragments were present in all the plants, whereas the other fragments were unique for each accession. The DNA extracted from seedlings of the 233-SC2-(4) plant (Fig. 1 c, lane 4) demonstrated a variant pattern after digestion with EcoRI-Bam HI. The 3.2 kb fragment disappeared and a shorter fragment of 3.0 kb was detected. The plant 233 (4) was demonstrated to be a variant in the rRNA gene intergenic spacer by digestion of the DNA with Taq I and EcoRI-Bam HI endonucleases (Fig. 1 b, c). The variation in length is probably due to variation in the number of 115 bp repeats because these repeats occupy most of the DNA between the Taq I sites (Appels and Dvorak 1982; Saghai Maroof et al. 1984).

Evaluation of variation in the B and C hordeins

Hordeins were extracted from single seeds of 12 individual SC2 plants and a similar number of control plants. The control plants did not exhibit variation within each accession (data not shown), however, differences in the number and mobilities of the B and C hordeins can be detected among the accessions (Fig. 2).

The B and C hordein SDS-PAGE pattern of 12 SC2 plants belonging to three of the accessions tested was similar to the respective donor plant (Fig. 2 a, b).

In one accession, a variant pattern was revealed (Fig. 2 c, lane 4). The variant 233 (4) was observed to vary in number and intensity of bands migrating in the B and C hordein region. One of the possible causes of variation in the polypeptide pattern may be a different genomic organization of the hordein coding genes. To analyse this possibility, Southern blots of total DNA digested with restriction endonucleases were hybridized with radiolabelled plasmid clones containing the cDNA of the hordein genes. Polymorphism in the organization of the hordein coding sequences among accessions but not among SC2 plants belonging to a single accession could be observed after hybridization of the EcoRI digest of genomic DNA's with the radiolabelled pB7 (a B hordein clone) (Fig. 3 a). No variation in the organization of B or C hordein genes among SC2 plants was revealed by hybridization of a Southern blot of EcoRI I or Hind III digest with the radiolabelled pB11 and pcP387 recombinant plasmids (cDNA clones of B and C hordeins, respectively) (Fig. 3 a, b). However, a quantitative difference in the 3.54 and 3.89 kb bands was revealed in the DNA extracted from the 233-SC2-(4) plant (Fig. 3 b, lane 3).

The different migration pattern of the DNA extracted from the plant SC2-300-2 observed in Fig. 3 b,

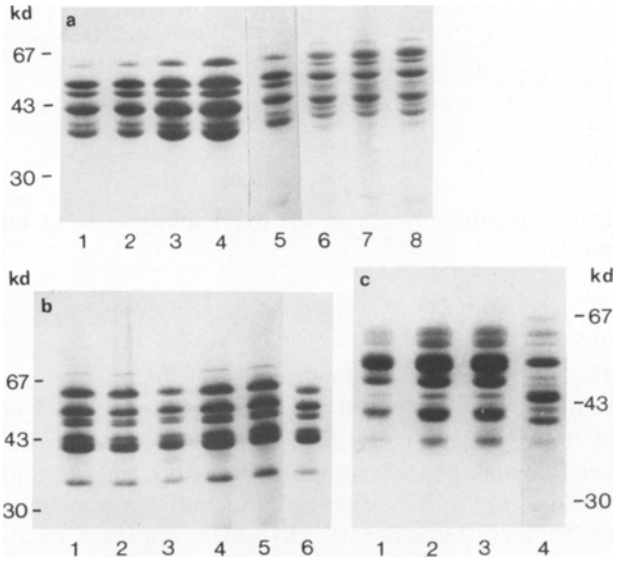

Fig. 2a–c. SDS-PAGE of B and C hordein fraction extracted from single seeds of control and progenies (SC2) plants of *H. spontaneum* L. **a** Lanes 1–5 accession 307, 1 = control; lanes 6–8 accession 300, 6 = control. **b** Lanes 1–6 accession 350, 1–3 = control. **c** Lanes 1–4 accession 233, 1 = control

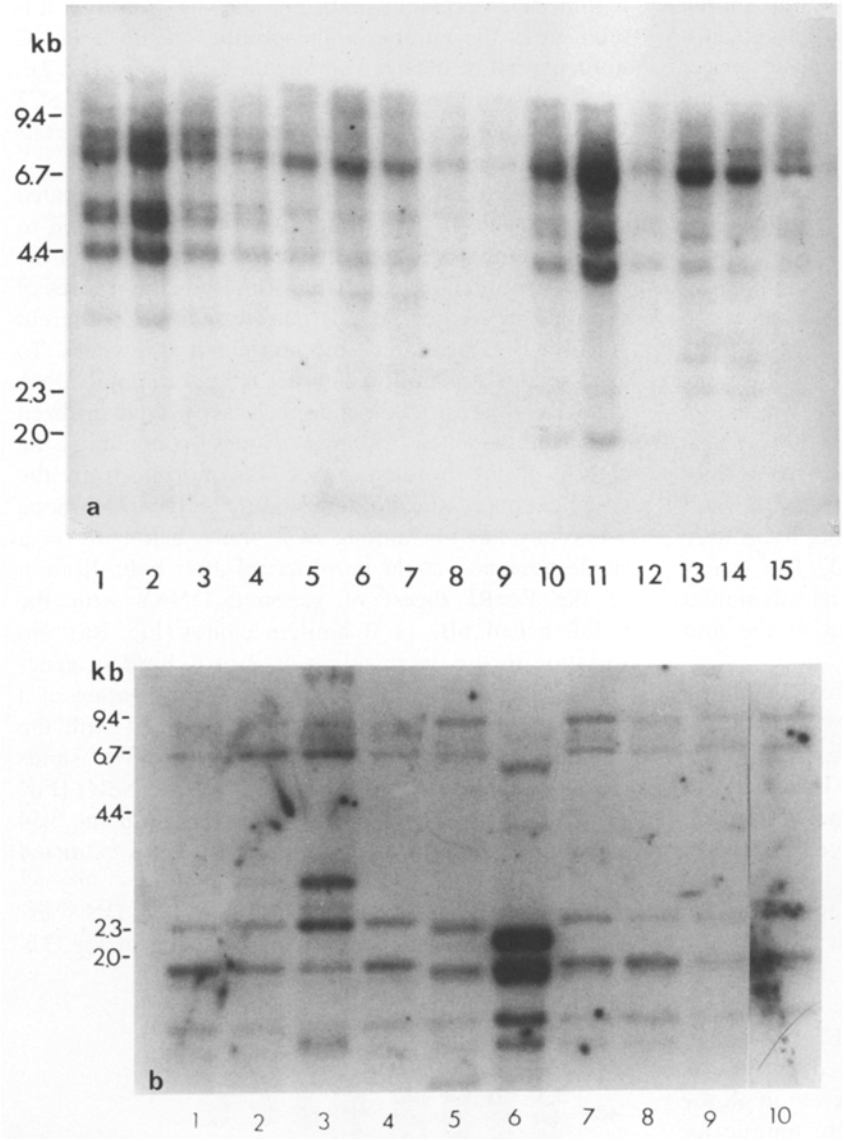

Fig. 3. **a** Hybridization of a B hordein cDNA clone (pB7) to EcoRI restriction digest of DNA extracted from control plants and progenic of regenerated *H. spontaneum* plants: lanes 1–4 accession 307, 1 = control; lanes 5–7 accession 300, 5 = control; lanes 8–11 accession 233, 8 = control; lanes 12–15 accession 350, 12 = control. **b** Hybridization of a C hordein cDNA clone (pCP387) to Hind III restriction digests of DNA extracted from control plants and progenies of regenerated *H. spontaneum* plants: lanes 1–3 accession 233, 1 = control; lanes 4–6 accession 300, 1 = control; lanes 7–8 accession 307, 1 = control; lanes 9–10 accession 350, 1 = control

lane 6, is probably due to the overloading of DNA on this lane.

Mitochondrial DNA organization in the progenies of barley regenerated plants (Sc2)

The mitochondrial coding sequences among cereals exhibit a high degree of sequence homology (Boer et al. 1985; Bonen et al. 1984). Therefore, we have used heterologous mitochondrial genes from maize and wheat as probes for detecting barley mtDNA sequences on Southern blots of total DNA. This approach facilitated the screening of the regenerated barley plants, without the need to purify mitochondrial DNA.

Probing EcoRI restriction digests of total DNA extracted from etiolated barley seedlings (SC2 generation) with the -L4C- clone (wheat mitochondrial 5′ COX I fragment) revealed an identical pattern of hybridization (a 9 kb fragment) among all the accessions, and no variation could be detected among the progenies of the regenerated plants (Fig. 4 b).

A cosmid from a wheat mitochondrial DNA cosmid library was used as a probe for assessing the organization of the barley mitochondrial DNA (Fig. 4 a). The 4A7 clone has been characterized and shown to include the 18S and 26S rRNA coding genes in a contiguous 40 Kb fragment of wheat mtDNA (unpublished results). The 12 fragments of barley DNA hybridizing with the cosmid clone were identical in all the regenerated plants. Cosmids representing other regions of the wheat mitochondrial DNA exhibited similar hybridization pattern in all the SC2 plants tested (data not shown).

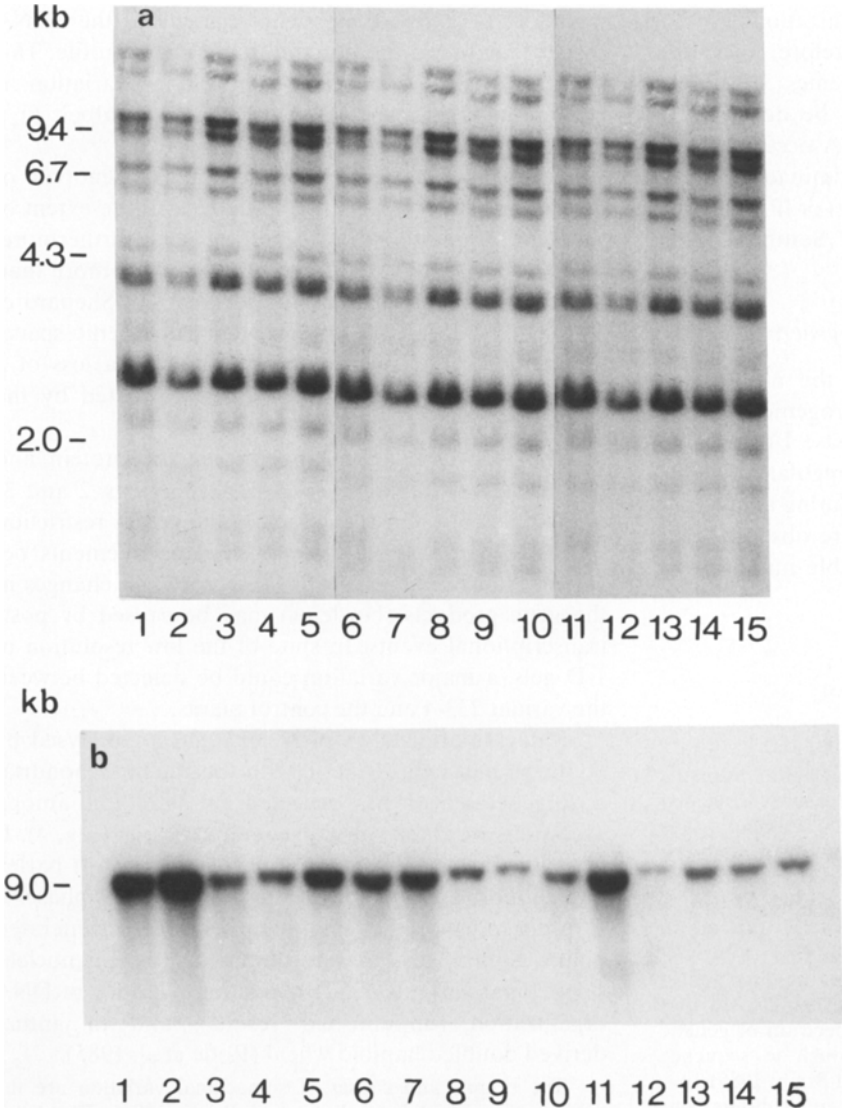

Fig. 4a, b. Autoradiograms of Southern blots of EcoRI digest of DNA, from control plants and progenies of regenerated *H. spontaneum*, hybridized with P^{32} labelled (**a**) 4A7 wheat mitochondrial cosmid containing the 18S and 26S rRNA genes; (**b**) L4C a recombinant plasmid of 5′ COX I wheat mitochondrial gene: lanes 1–4 accession 307, 1 = control; lanes 5–7 accession 300, 5 = control; lanes 8–11 accession 233, 8 = control; lanes 12–15 accession 350, 12 = control

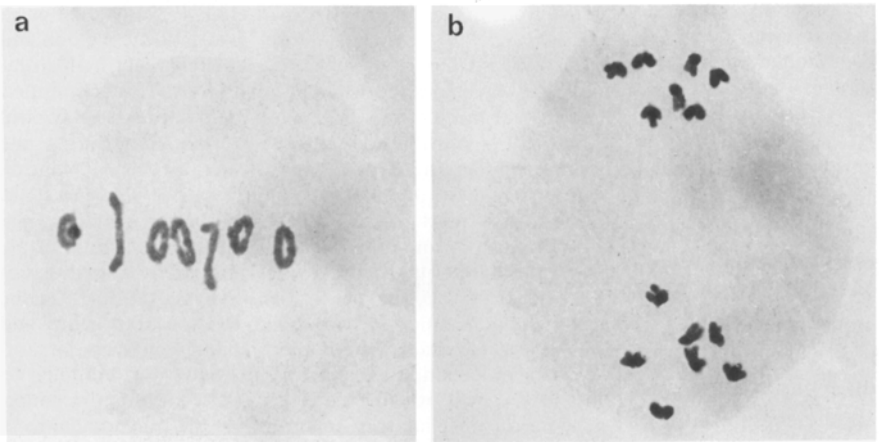

Fig. 5a, b. Regular meiosis in SC1 progenies (SC2): (**a**) metaphase I showing 7 normal bivalents (**b**) anaphase I showing regular segregation of 7 chromosomes to each of the poles

These results suggest that the organization of wild barley mtDNA is very similar and therefore, no variation among the accessions or progenies of plants regenerated from tissue culture could be detected. If variation in the organization of mtDNA occurs during the tissue culture process it is either eliminated during the sexual cycle (therefore not heritable) or if it persists, it is undetectable by hybridization of Southern blots with wheat mitochondrial DNA clones.

Meiotic studies on the progenies of the regenerated plants

No variation was detected in either the number or structure of chromosomes in the progenies of the *H. spontaneum* regenerants. At metaphase I of meiosis the 14 chromosomes were paired in 7 regular bivalents (Fig. 5 a) and segregation was normal during anaphase I (Fig. 5 b). No bridges or fragments were observed and tetrad formation was free of (appreciable numbers of) micronuclei.

Discussion

The ability to enhance the level of genetic variability in plants regenerated from tissue culture has been extensively discussed (Larkin and Scowcroft 1981; Scowcroft 1985). Although it is recognized that some of the variation observed amongst somaclones is not stable, the inheritance of variant traits produced through tissue culture has been documented in a few species (Sun et al. 1983; Scowcroft 1985; Larkin et al. 1984; Umbeck and Gengenbach 1983). Moreover, following numerous reports of extensive phenotypic variation amongst regenerated plants, a number of studies have indicated that much of this variation is due to a whole spectrum of genetic changes, from, chromosome variation through to sequence amplification and point mutations (Karp and Bright 1985).

The degree of somaclonal variation observed differs considerably from one report to another. In general, more variation is found in the regeneration of polyploid species compared with diploids. For example, in the cereals more variation was observed in wheat (Karp and Maddock 1984; Larkin et al. 1984), oats (McCoy et al. 1982) and triticale (Jordan and Larter 1985) than in maize (McCoy and Phillips 1982) and pearl millet (Swedlund and Vasil 1985). Other factors affecting the degree of somaclonal variation include genotype, tissue culture source, media components and the regeneration system. Protoplast regeneration in particular is associated with considerable variability (Karp and Bright 1985).

Hordeum spontaneum is diploid and would therefore be expected to be relatively intolerant of genotypic variability. As such, no obvious phenotypic differences were observed among fifty regenerants from immature embryo-derived callus (Breiman 1985). Similarly, in this study, we found no gross chromosomal changes amongst 25 progeny plants screened. However, the evaluation of nuclear and mitochondrial variation in a small sample (12) of the progenies of the regenerated plants has revealed one plant (233-4) demonstrating variation in two independent characters: the rDNA spacer length and the hordein SDS-PAGE profile. This result shows that heritable somaclonal variation is present in regenerants of *H. spontaneum*, albeit at a subtle level.

The results may also indicate that the genotype of the donor plant has a significant effect on the extent of variation generated during culture and furthermore, that a somaclonal variant can be affected in more than one trait (McCoy 1982; Brettell et al. 1986; Shepard et al. 1980). The deletion of two rRNA intergenic spacer fragments (Fig. 1 b) may be explained by a loss of a chromosomal fragment which was undetected by the cytological studies.

The lack of relationship between the protein and the restriction fragment polymorphism (Figs. 2 and 3) observed is not surprising, since changes in restriction fragment pattern may arise from rearrangements occurring outside the encoding sequences and changes in the gene products (hordeins) may be caused by post-transcriptional events. In spite of the low resolution of 1-D gels, a major variation could be detected between the variant 233-4 and the control plants.

Mitochondrial DNA of *H. spontaneum* analysed by Southern blot hybridization with specific mitochondrial coding sequences has revealed no variation among accessions or among the regenerated plants (Fig. 4). It is assumed that if rearrangement does occur in barley mitochondrial DNA during the tissue culture phase, it is rarely transferred to the progenies of the regenerated plants. Similar results were obtained in wheat nuclear somaclonal variants (SC2) plants analysed for mtDNA organization (unpublished results), and in anther derived doubled haploid wheat (Rode et al. 1985).

The origins and causes of somaclonal variation are the subject of much debate (Karp and Bright 1985). The possibility of a common mechanism causing variation was raised recently by Walbot and Cullis (1985) as a result of a comparison between flax plants regenerated from culture with flax genotrophs.

Since extensive variation in the repetitive fraction of the genome occurs in natural populations and in plants regenerated from tissue culture (Breiman et al. 1987; Brettell et al. 1986; Flavell 1985; Saghai-Maroof et al. 1984; Walbot and Cullis 1985) it has been suggested that relatively high instability may be associated with repeated DNA. This assumption implies that mechanisms have evolved to suppress or control variation. A possible destabilization occurring during the undifferentiated callus phase may lead to a loss of control, causing a higher mutation rate in the repeated sequences and the release of mechanism of instability such as amplification, translocation, chromosome breakage and transposition. In the present study, heritable somaclonal variation was analysed at a molecular level. The use of sensitive assays, such as the genomic organization of ribosomal RNA coding genes and their intergenic spacer, the hordein coding genes, the organization of mitochondrial DNA and the pattern of hordeins on SDS-PAGE, will provide a deeper insight towards the understanding of mechanisms involved in the phenomenon of somaclonal variation.

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