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Somaclonal variation in *Solanum tuberosum* detected at the molecular level

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Summary. The many reports of phenotypic variation among plants regenerated from tissue culture suggest underlying alterations at the DNA level. This hypothesis was tested with protoplast-derived *Solanum tuberosum* plants. Random potato-DNA clones were used to probe the genome of individual plants at specific sites. Two out of twelve plants were shown to be variant by Southern-hybridisation with one of the tester-clones. As this clone turned out to represent 25S-rDNA, both somaclonal variants can be regarded as mutants deficient in ribosomal RNA-genes.

Key words: Somaclonal variation – Potato – Tissue culture – Ribosomal RNA genes – Repetitive DNA

Introduction

Plant tissue culture has been regarded as a means of propagating cells in a rapid and fairly stable clonal fashion. However, many phenotypic variants appear among the regenerated plants. This has been termed somaclonal variation and is reported for many species; for review see Larkin and Scowcroft (1981) and Orton (1983). The phenomenon has attracted much interest because a high frequency of variants will bias stable plant propagation on the one hand, but on the other might be regarded as a novel source for crop improvement.

The many reports of somaclonal variation suggest that changes at the DNA level cause these phenotypic alterations. Gross genome and chromosome mutations such as polyploidy, aneuploidy and chromosome loss have been shown in some cases (Orton 1980; Karl et al. 1982; Browers and Orton 1982; Jacobsen et al. 1983). To find out whether more subtle DNA rearrangements also take place, we used the Southern-hybridisation technique to test the genome of protoplast-derived individual *Solanum tuberosum* plants at specific sites. As hybridisation probes, random fragments of potato DNA were cloned into cosmid pJL34 and plasmids pUR2 and pUR250. The clones were characterised as being of unique, repetitive or plastid origin. Most of the tester-clones used contained repetitive plant DNA, because the potato genome may consist of about 80% repetitive DNA as may be inferred from tobacco (Zimmermann and Goldberg 1977) and parsley (Kiper and Herzfeld 1978).

Two out of a set of 12 regenerated plants where shown to be variant by Southern-hybridisation with the repetitive clone 432, which turned out to be homologous to 25S-rRNA.

Materials and methods

Plants

Solanum tuberosum protoplasts were isolated from the tetraploid line HH258 and the diploid line H78.2022.14 and fused as described earlier (Wenzel 1980; Uhrig 1981; Hein et al. 1983). After induction of calli, shoots were developed into plants, and all of these showed a phenotype and isozyme pattern identical to HH258 (H. Uhrig, unpublished) and therefore most likely represented pure derivatives of line HH258. From each of 12 regenerated plants five tubers were taken and grown as a second vegetative generation. Slight variations in shape and color were found among the tubers of the somaclones as well as one case of more rapid shoot induction (plant F80.2541). Three tuber-derivatives were aneuploid, plant F80.2528/3a was a chimera with 46 and 47 chromosomes, F80.2541/2d was chimeric with 48 and 96 chromosomes and plant F80.2536/1b was diploid with 24 chromosomes. For the molecular analysis DNA was prepared from 36 individual plants (nos. F80.25) of the second vegetative generation representing 12 original somaclones. HH258 and H78.2022.14 were always taken as controls. In addition, an independent set

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of eight *S. tuberosum* plants (line 82.2401) was regenerated from leaf calli. Two of these were aneuploid.

Plant DNA preparation

Between 3 and 30 g leaf material was powdered under liquid nitrogen in a Waring Blender and incubated 20 min at 60 °C in 10 mM Tris-HCl pH 7.6, 50 mM EDTA, 100 mM NaCl, 1% N-laurylsarcosine, 10 mM β-ME, 100 mM DIECA (diethyldithiocarbamic acid). After phenolisation and isopropanol precipitation, the redissolved DNA was treated with RNAse (0.1 mg/ml) and with Proteinase K (0.1 mg/ml). Final centrifugation in CsCl-gradient (R.I. = 1,392) and dialyses yielded about 12 µg DNA/g plant material ready for restriction enzyme digestion.

Bacteria and plasmids

Plasmids were grown in *E. coli* HB101 or 79-02 (Rüther 1982). Plasmid DNA was prepared according to Birnboim and Doly (1979). Packaging extracts were prepared as described previously (Sheperd et al. 1982).

Cloning of potato DNA

Total EcoRI or BamHI digests of *Solanum tuberosum* DNA were used for cloning into the respective sites of plasmids pJL34 (Fig. 1), pUR2 and pUR250 (Rüther 1982). PJL34 was used as a cosmid. According to its size of 15 kb, pJL34 may be packaged as monomer and multimers and thus turned out to contain 3 to 23 kb of insert DNA.

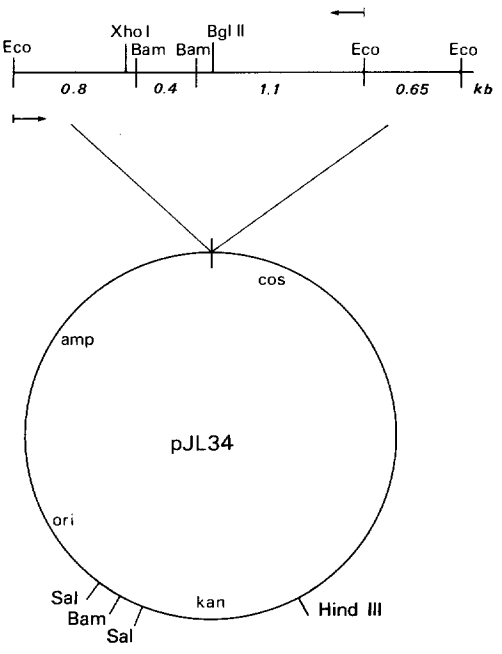

Fig. 1. Restriction map of potato DNA fragments of clone 4324, inserted into cosmid pJL34. The EcoRI-BamHI (ori-fragment of pJL34 is derived from pBR328 (Covarrubias et al. 1981), the BamHI-HindIII Kan-fragment from Tn5, the HindIII-EcoRI cos-fragment from pDG128/9-1 (Sommer et al. 1981). *Arrows* indicate sequenced ends of the 2.3 kb potato DNA insert fragment

DNA and RNA hybridisation

Restriction digests of plant DNA were run on horizontal 0.6% agarose TAE and TBE gels. Radioactive labeling of plasmid and plant DNA was achieved by nicktranslation with DNA-Polymerase I. Southern hybridisation (Southern 1975) to restricted plant DNA, Northern hybridisation to plant rRNA and colony hybridisation were done according to standard procedures (Maniatis et al. 1982). DNA sequencing was done according to Maxam and Gilbert (1977).

Results

Cloning of unique and repetitive potato DNA fragments

To detect DNA rearrangements in the genome of the somaclonal plants, a set of homologous hybridisation probes was created (see "Materials and methods"). Twenty-six tester-clones representing 167 kb potato DNA were selected for the molecular analysis. Most of the clones contained several small insert-fragments, presumably from different chromosomal locations, due to complete restriction-digestion of plant DNA used. Thirty percent of the insert DNA was characterised as being of unique, 47% as repetitive and 23% as of plastid origin by hybridisation with radioactive total genomic or chloroplast DNA (Fig. 2).

Genomic hybridisation

Southern hybridisation with these specific probes to restriction digests of individual plant DNAs will reveal deletions, inversions, translocations, duplications and amplifications at the tested sites if the fragments fall within the analysed molecular weight range (0.5 to 15 kb). To test the feasibility of the system seven different *Solanum* species and five commercial *S. tuberosum* varieties were analysed (data not shown). In most cases differences in signal positions and relative intensities could be detected between those genotypes. Repetitive probes revealed more variation than any other type of probe. For each individual tester sequence only a subset of restriction enzymes resulted in a well distributed separation of hybridising bands. Smear signals, high molecular weight bands and accumulations of signals at specific, nearly unique positions were not very suitable for distinguishing small variations.

Somaclonal variation

A set of eight *S. tuberosum* plants regenerated from leaf calli and an independent set of 36 propagules were chosen for the molecular analysis. The 36 plants had been grown from tubers, taken from 12 protoplast-derived potato plants. While the calli cloned plants did not show any differences in Southern hybridisation patterns (data not shown), variation could be found with the protoclones.

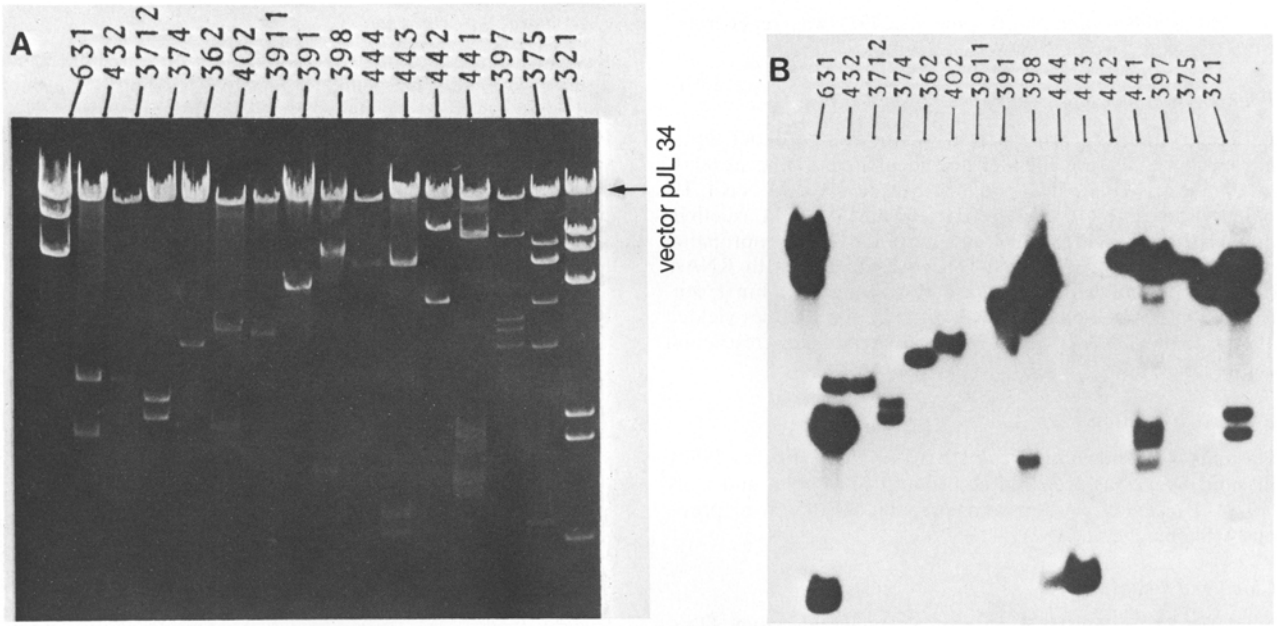

Fig. 2. Characterisation of genomic potato DNA test clones. **A** Restriction pattern of clones containing EcoRI-inserts; **B** Hybridisation with nicktranslated total genomic potato DNA. Only fragments being repetitive in the genome light up

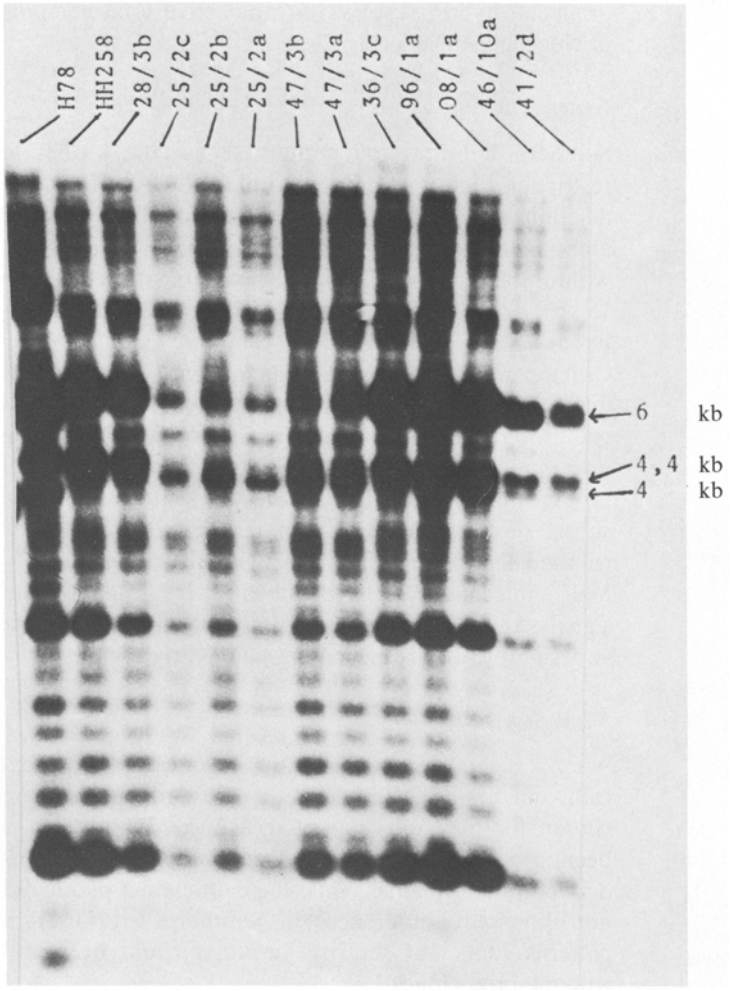

Fig. 3. Hybridisation of clone 4324 to BamHI digests of plant DNA from *S. tuberosum* somaclones. End-numbers of plants F80.25 are indicated. *Arrows* indicate positions of reduced signal intensity in autoradiography with plants 25/2 and 47/3. Compare relative intensities of the 4.4 kb and 4 kb regions. Note that less DNA has been used from plants 46/10 and 41/2 but bands in question are still more intense than in plants 25/2

The repetitive test clone 432 showed about 30 prominent bands in BamHI Southerns. With plants F80.2525/2a–e and F80.2547/3a–b, a reduction of signal intensities at specific molecular weight positions could be detected in comparison to the parent HH258 and to all of the other regenerants tested. To clarify the interpretation, parts of the tester probe were subcloned. Genomic hybridisations with subclone 4324 (Fig. 3) again showed a large reduction of signal intensities at the 6 kb and 4.4 kb positions with the same plants. All individuals within each of the two groups of plants had identical variant patterns, although there was a slight but significant difference in pattern between the two groups. With plants F80.2547/3a–b reduction was largest in the 6 kb region. With plants F80.2525/2a–e

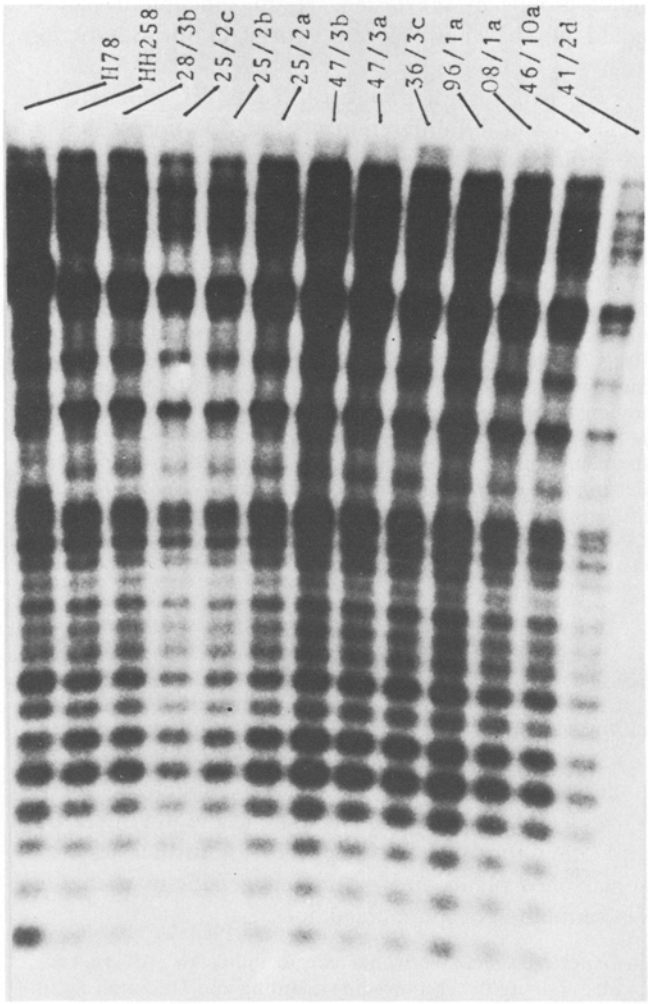

Fig. 4. Hybridisation of clone 4323 to BamHI digests of plant DNA from *S. tuberosum* somaclones. This is a parallel experiment to Fig. 3 but a repetitive probe has been used which does not show variation among the plants. A comparable distribution of signal pattern is essential as a control of the amount and quality of loaded plant DNA

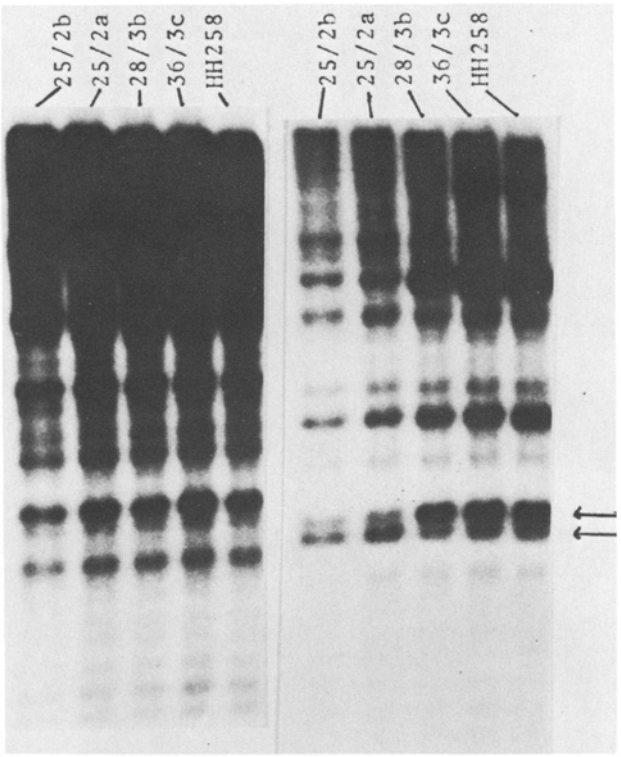

Fig. 5. Hybridisation of clones 4324 (right) and 4323 (left) to Bgl II digests of somaclones. Changes of relative signal intensities are indicated

an even larger deamplification was apparent at this position and in addition the 4.4 kb bands were totally deleted (Fig. 3).

Bgl II digests revealed a change of relative signal intensities slightly below the 4 kb region (Fig. 5). EcoRI Southerns showed quite an obvious reduction at the only 2.3 kb position, but no change for the lower ladder signals (Fig. 6). XhoI, XbaI, ClaI, BclI and HindIII digests of plant DNA led to mainly one hybridisation signal at high molecular weight ranges with clone 4324 and these were not sufficiently descriminatory.

The optical impression does not give a precise calculation of the quantitative differences. Therefore the Cerenkov counts retained on the NC-filter after autoradiography were measured. The cutout 6 kb region of each individual Bam-Southern lane was compared to the rest of the signals. This showed that plants F80.2525 and F80.2547 had lost about 69–74% of the homologous fragments in question.

Analyses of the test clone 4324

Clone 4324 (Fig. 1) contains two EcoRI potato DNA insert fragments, a 2.3 kb fragment which is homologous to the variant bands in the genomic Southerns, and a

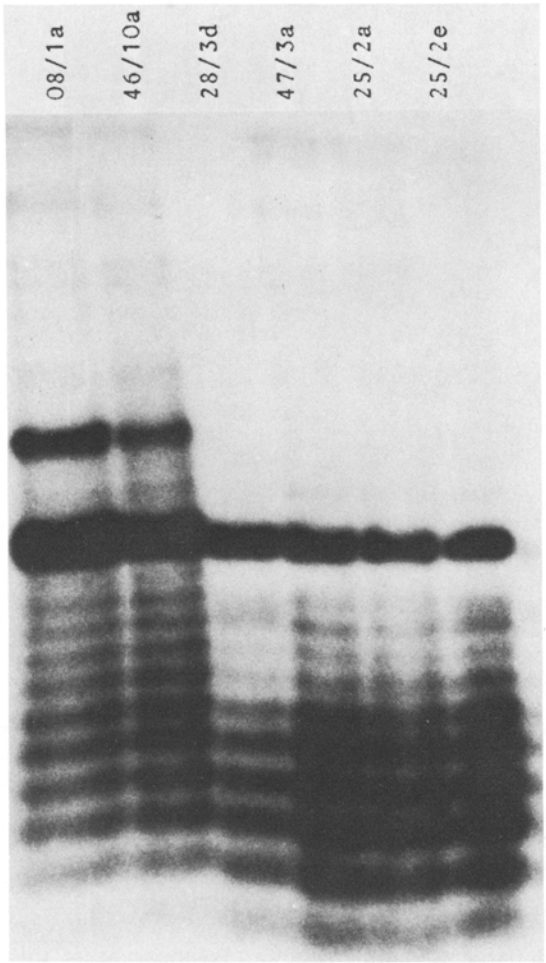

Fig. 6. Hybridisation of clone 4324 to EcoRI digests of somaclones. The ladder signals may be used as an indication of the amount of DNA loaded (see "Analyses of the test clone"). Although less DNA from plants F80.25-08, 46 and 28 has been used the 2.3 kb signal is stronger (plants 08 and 46) or equivalent (plant 28) to signals from plants 25 and 47. Partial bands show up with plants 08 and 46

0.65 kb fragment which is responsible for the ladder type signals in Eco Southerns.

Northern blotting of the 2.3 kb potato DNA fragment to plant rRNA and determination of about 200 bp DNA sequences from both ends of this fragment (Landsmann, in preparation) revealed a strong homology to 25S nuclear ribosomal RNA. The coding strand of this potato rDNA is rich in purines. The extent of nucleotide homology to the large rRNA subunits from *E. coli* and maize chloroplasts is 57% (Edwards and Kössel 1984), from yeast 78% (Georgiev et al. 1981), from *Xenopus* 80% (Ware et al. 1983), and rat 82% (Chan et al. 1983).

Discussion

Genomic DNA clones were used to search for DNA rearrangements in somaclonal potato plants. In Southern-blot analyses with a probe consisting of the central part of 25S rDNA, variation could be detected among tuber-derivatives of protocloned plants. Two groups of derivatives out of 12 showed a 70% reduction in 25S ribosomal RNA-genes, but the two groups were affected slightly differently. The alteration was stable via one cycle of tuber propagation: all propagated plants in a group retained exactly the same set of homologous fragments. Phenotypically no effect on vigor and morphology could be observed in these plants, so the reduction of rDNA did not seem to have reached a critical level for normal vegetative growth. Although there was some chromosome instability observed in other groups of the tested set, the aneuploid plants could not be shown to be variant in the Southern-analyses.

The two variant groups of plants originated from single shoots of different calli, no additional shoots from the same calli were available. Therefore it cannot be decided when the mutational events had taken place, during protoplast regeneration or during ontogeny of the parent plant.

Ribosomal RNA-genes are multicopy DNA sequences which are known to be subject to amplification processes in insects and animal oocytes. Deamplification of rDNA under stress has been reported in flax (Cullis and Chareton 1981). Similarly tissue culture stress (e.g. artificial hormone concentrations) might influence the protoplast genome. Effects may not be restricted to rDNA. It might be that the genes most frequently affected are those which are under developmental deletional or amplificational control as is known for the multicopy immunoglobulins in vertebrates, and for repetitive DNA in humans (Shmookler-Reis et al. 1983), ascaris (Müller et al. 1982), copepoda (Beermann 1977), ciliates (Dawson et al. 1984) and tobacco (Durante et al. 1983). Deletion and suppression of amplification would be the simplest mechanisms to be considered although many different ways of creating genetic changes may actually be involved.

This is the first reported characterization of somaclonal variation at the molecular level. Our success with a relatively small sample of plants and discoveries of hypervariable repeated sequences (Shepherd et al. 1984; Jeffreys et al. 1985) recommend the use of rDNA and other repetitive DNA probes for further tests of somacloned plants and possibly as genetic markers for discrimination of commercial lines.

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