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Variation in Organisation and Copy Number of Ribosomal RNA Genes in *Petunia hybrida* Somaclones

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Abstract. The copy number of genes encoding 5S ribosomal RNA has been found to be constant in *Petunia hybrida* plants regenerated from protoplast and leaf disc-derived callus cultures. However, in one somaclone a heritable change in the length of the major 5S rDNA repeat has arisen. Despite the constant copy number of 5S rRNA genes, that of the 18S–25S rRNA genes is found to very by at least ten-fold. The relevance of these findings to ribosomal RNA gene variability and to somaclonal variation is discussed.

Ribosomal RNAs (rRNAs) are encoded by two sets of genes in higher eukaryotes. The 18S–25S genes (18S–25S rDNA) encode the 18S RNA of the small ribosomal subunit and the 5.8S and 25S RNAs of the large subunit. These RNAs are processed from a 45S precursor RNA molecule, transcribed from tandem arrays of genes. The copy number of such genes can reach many thousands in plants (Rogers and Bendich 1987). The 5S rRNA of the large ribosomal subunit is also synthesised from multiple tandemly arranged genes (5S rDNA), but these are not linked to the 18S–25S genes (Gerlach and Dyer 1980). The mechanisms by which gene copy numbers are controlled, and rRNA amounts regulated, are not understood.

Several reports have recently described changes in the organisation and copy number of the 18S–25S rDNA sequences in cultured plant cells and plants regenerated from them. Landsmann and Uhrig (1985) have shown that two potato plants regenerated from protoplasts contained 70 % less 18S–25S rDNA than the parent plant. Brettell *et al.* (1986) have demonstrated a loss of 18S–25S rDNA from the 1R locus of triticale plants regenerated from callus culture. Breiman *et al.* (1987) have reported the loss of two particular length variants of the 18S–25S genes in one plant regenerated from callus of *Hordeum spontaneum*, and Rode *et al.* (1987) have detected new length variants in these genes in wheat plants derived from anther culture. In contrast to these observations, changes in the organisation and copy

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number of 5S rDNA sequences in regenerated plants have not been reported to our knowledge.

Studies of changes to rDNA sequences resulting from the cell culture process may enable us not only to investigate mechanisms which control the replication and expression of rDNA but also may help provide information about the types of processes which contribute to somaclonal variation (Lee and Phillips 1988). Here we report variation in the organisation and copy number of rDNA sequences in *Petunia hybrida* plants regenerated from cultured cells.

MATERIAL AND METHODS

Petunia hybrida cv. Rose of Heaven was obtained commercially. This is a long established diploid cultivar (2n = 14) which has been inbred over many generations. Callus cultures were established either from leaf mesophyll protoplasts or from leaf discs. Conditions for cell culture, regeneration and chromosome counts have been described elsewhere (Lewis-Smith *et al.*, 1990).

DNA was isolated from expanding leaves as described previously (Anderson *et al.* 1990). DNA was digested with restriction endonucleases according to manufacturers specifications, separated by electrophoresis in a 0.8 % (w/v) agarose gel containing TBE buffer (90 mM Tris-borate, pH 8.3, 0.25 mM ethylenediaminetetraacetic acid) and transferred to Genescreen-plus (DuPont) for hybridisation (Anderson *et al.* 1990). The 5S rDNA probe used was the 410 base pair (bp) *Bam*HT fragment from pTA794 (Gerlach and Dyer 1980). The 18S–25S rDNA probe was the 9 kbp *Eco*RI fragment from pTA71 (Gerlach and Bedbrook 1979). Probes were labelled with α-[^{32}P]dCTP by the random primer method (Feinberg and Vogelstein 1984).

RESULTS AND DISCUSSION

Plants have been regenerated from cultured callus tissue and protoplasts of *Petunia hybrida* cv. Rose of Heaven, among which genetic variation has been observed (Lewis-Smith *et al.* 1990). Since variation in rDNA sequences has been reported previously in regenerated plants, we investigated these sequences in the *Petunia* somaclones. The plants examined include twelve regenerated from callus cultures and eight regenerated from callus derived from a single protoplast. The origins of these plants and their chromosome numbers are summarised in Table 1.

Equal amounts of DNA isolated from leaves of regenerated plants were digested to completion with restriction endonuclease *Alu*I, which cleaves once within most *Petunia* 5S rRNA genes (Pental *et al.* 1986). DNAs were then fractionated by agarose gel electrophoresis, transferred to 'Genescreen' and hybridised with a wheat 5S rDNA probe. The copy number and organisation of these genes was found to be

TABLE 1

Chromosome numbers of regenerated plants used in this study

Plants regenerated from callus		Plants regenerated from protoplast	
Plants	Chromosome number	Plant	Chromosome number
CRA	28	Theta 2	28
CRB	14	Theta 4	28
CRC	28	Theta 6	28
CRD	28	Theta 8	28
CRE	16	Theta 11	28
		Theta 12	29
CRI	14	Theta 13	28
CR2	28	Theta 14	28
CR3	28		
CR4	14		
CR5	28		
CR6	28		
CR7	27		

Plants in group CRA–CRE were obtained from discs from five different plants. Plants in group CR1–CR7 were obtained from discs from a single plant (ROH4). Theta plants are derived from a single protoplast.

similar in nearly all plants examined (Fig. 1a, b, c). However two plants, CR1 and CR4, did show an unusual organisation of 5S rRNA genes (Fig. 1c). The unusual pattern of hybridisation in these regenerants was further investigated by removing the 5S rDNA probe from the Genescreen filter whose autoradiograph is shown in Fig. 1, and rehybridising with a probe (pCAS13) for a dispersed repeat of unknown function in *Petunia* (Shepherd *et al.* 1990). The resultant hybridisation (result not shown) gave a similar pattern for each plant, indicating that the *Alu*I digest was complete, and that the DNA samples had fractionated normally.

The organisation of 5S genes in CR1 was subsequently compared with that in CR2 by digestion with *Alu*I again, and with *Rsa*I and *Taq*I. The result with *Taq*I showed (Fig. 2a) that the 5S genes in CR2 have a repeat length of about 500 bp, while the majority in CR1 have a repeat length of about 470 bp. A higher proportion of the shorter 5S genes lack an *Alu*I site, but a greater proportion contain an *Rsa*I site, relative to the longer 5S genes. Progeny of CR1 and CR2 obtained following self fertilisation were next analysed, which showed the unusual 5S rDNA complement of CR1 to be heritable (Fig. 2b).

The unusual 5S rDNA length variant found in CR1 and CR4 could have been present at low copy number in the parent plant, and been amplified in the cells giving

rise to these regenerants. A process of deletion of some 5S RNA genes followed by amplification of others is thought to bring about the appearance of new length variants (Scoles *et al.* 1988). However, our results are also consistent with the hypothesis that the longer 5S rDNA genes were converted into the shorter ones. Whatever the mechanism which gave rise to the shorter genes, it was probably a single event which occurred early in callus formation, from which both CR1 and CR4 plants were regenerated.

Equal amounts of DNA isolated from leaves of regenerated plants were next digested to completion with *Hind* III, which cleaves once within the *Petunia* 18S–25S rDNA repeat (Waldron *et al.* 1983). DNAs were then fractionated and hybridised with the complete wheat 18S–25S rDNA sequence. The copy numbers found in callus regenerants CR1 – CR7 vary up to ten-fold (Fig. 3a). Examination of other somaclones shows further variations in copy number of these genes (Fig. 3b). For example the copy number in CRA is about six-fold higher than in CRB. In all cases which we have examined, the tetraploids have a higher copy number than diploids (Fig. 3). This higher copy number of 18S–25S rRNA genes in tetraploids relative to diploids might result from random variation (Rogers and Bendich 1987), or might suggest that copy number is controlled by gene dosage. This question might be resolved by generating more plants differing in ploidy, using other methods. It is clear from our results however, that changes in copy number of 18S–25S genes are not accompanied bysimilar changes in the copy number of 5S genes. Both sets of genes are apparently present in excess in the genome (Rogers and Bendich 1987, Scoles 1988), and may vary in amount independently of one another.

We do not know if the variation observed in the copy number and organisation of rDNA in the *Petunia* somaclones described here, was generated during the cell culture phase or was present in the original leaf cells. Neither do we know if these differences have an effect on plant phenotype. However, they may be indicative of other genetic changes which do contribute to somaclonal variation, and they clearly demonstrate that cultured cells and regenerated plants are a valuable source of material which will help us to understand more about the behaviour of ribosomal RNA genes in plants.

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REFERENCES

Anderson, S., Lewis-Smith, A. C., Smith, S. M.: Methylation of ribosomal RNA genes in *Petunia hybrida* plants, callus cultures and regenerated shoots. – Plant Cell Rep. **8** : 554–557, 1990.

Breiman, A., Rotem-Abarbanell, D., D., Karp, A., Shaskin, H.: Heritable somaclonal variation in wild barley (*Hordeum spontaneum*). – Theor. appl. Genet. **74** : 104–112, 1987.

Brettell, R. I. S., Pallotta, M. A., Gustafson, J. P., Appels, R.: Variation at the *Nor* loci in triticale derived from tissue culture. – Theor. appl. Genet. **71** : 637–643, 1986.

Feinberg, A. P., Vogelstein, B.: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activities. – Anal. Biochem. **137** : 266–267, 1984.

Gerlach, W. L., Bedbrook, J. R.: Cloning and characterisation of ribosomal RNA genes from wheat and barley. – Nucl. Acids Res. **7** : 1869–1885, 1979.

Gerlach, W. L., Dyer, T. A.: Sequence organisation of the repeating units in the nucleus of wheat which contains 5S rRNA genes. – Nucl. Acids Res. **8** : 4851–4865, 1980.

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

Lee, M., Phillips, R. L.: The chromosomal basis of somaclonal variation. – Annu. Rev. Plant Physiol. mol. Biol. **39** : 413–437, 1988.

Lewis-Smith, A. C., Chamberlain, M., Smith, S. M.: Genetic and chromosomal variation in *Petunia hybrida* plants regenerated from protoplast and callus cultures. – Biol. Plant. **4** : 247–255, 1990.

Pental, D. Hamill, J. D., Pirrie, A., Cocking, E. C.: Somatic hybridisation of *Nicotiana tabacum* and *Petunia hybrida*. – Mol. gen. Genet. **202** : 342–347, 1986.

Rode, A., Hartmann, C., Benslimane, A., Picard, E., Quetier, F.: Gametoclonal variation detected in the nuclear ribosomal DNA from doubled haploid lines of a spring wheat (*Triticum aestivum* L., cv. Cesar). – Theor. appl. Genet. **74** : 31–37, 1987.

Rogers, S. O., Bendich, A. J.: Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. – Plant mol. Biol. **9** : 509–520, 1987.

Scoles, G. J., Gill, B. S., Xin, Z.-Y., Clarke, B. C., Mc Intyre, C. L., Chapman, C., Appels, R.: Frequent duplication and deletion events in the 5S RNA genes and the associated spacer regions of the *Triticeae*. – Plant Syst. Evol. **160** : 105–112, 1988.

Shepherd, A. L., Anderson, S., Smith, S. M.: Species-specific repeated DNA sequences from *Petunia* –Plant Sci. **67** : 57–62, 1990.

Waldron, J., Dunsmuir, P., Bedbrook, J.: Characterisation of the rDNA repeats in the Michell *Petunia* genome. – Plant mol. Biol. **2** : 57–65, 1983.

Figs. 1 to 3 at the end of the issue