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DNA MUTAGENESIS IN *PANAX GINSENG* CELL CULTURES

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At the present time, it is well documented that plant tissue culture induces a number of mutations and chromosome rearrangements termed “somaclonal variations”. However, little is known about the nature and the molecular mechanisms of the tissue culture-induced mutagenesis and the effects of long-term subculturing on the rate and specific features of the mutagenesis. The aim of the present study was to investigate and compare DNA mutagenesis in different genes of *Panax ginseng* callus cultures of different age. It has previously been shown that the nucleotide sequences of the *Agrobacterium rhizogenes rolC* locus and the selective marker *nptII* developed mutations during long-term cultivation of transgenic cell cultures of *P. ginseng*. In the present work, we analyzed nucleotide sequences of selected plant gene families in a 2-year-old and 20-year-old *P. ginseng* 1c cell culture and in leaves of cultivated *P. ginseng* plants. We analysed sequence variability between the *Actin* genes, which are a family of house-keeping genes; the phenylalanine ammonia-lyase (*PAL*) and dammarenediol synthase (*DDS*) genes, which actively participate in the biosynthesis of ginsenosides; and the somatic embryogenesis receptor kinase (*SERK*) genes, which control plant development. The frequency of point mutations in the *Actin*, *PAL*, *DDS*, and *SERK* genes in the 2-year-old callus culture was markedly higher than that in cultivated plants but lower than that in the 20-year-old callus culture of *P. ginseng*. Most of the mutations in the 2- and 20-year-old *P. ginseng* calli were A↔G and T↔C transitions. The number of nonsynonymous mutations was higher in the 2- and 20-year-old callus cultures than the number of nonsynonymous mutations in the cultivated plants of *P. ginseng*. Interestingly, the total number of N→G or N→C substitutions in the analyzed genes was 1.6 times higher than the total number of N→A or N→T substitutions. Using methylation-sensitive DNA fragmentation assay, we showed that the level of methylcytosine was higher in the DNA of the 20-year-old *P. ginseng* calli that than that in the DNA of the 2-year-old calli. Taken together, the data obtained demonstrate that both 2- and 20-year-old subculturing of *P. ginseng* tissues *in vitro* increased the number of point mutations, the diversity of mutation types, and the number of potential DNA methylation sites in the analyzed gene regions. It is possible that these mutation processes is the main reason underlying the decline in the vigor and regenerability of *P. ginseng* tissue culture over time.