Abstract

*Freesia hybrida* is an important worldwide cut flower, especially in America and Europe. For efficient regeneration of this flower from young inflorescence and rachillae in tetraploid, we developed a simple in vitro micropropagation protocol. Explants of *Freesia hybrida* can regenerate plantlets through somatic embryogenesis via two kinds of pathways, that is, directly from the epidermal cells or indirectly from an embryonic callus, depending on the exogenous plant growth regulators (PGRs) used in the culture media. In direct embryogenesis, when the explants were cultured on Murashige and Skoog (MS) medium supplemented with 11.43 μM indole acetic acid (IAA) and 4.44 μM 6-benzylaminopurine (6-BA), the induction rate was 84% for young inflorescence and 100% for rachillae. After the multishoots were subcultured on the rooting MS medium containing 1.08 μM α-naphthalene acetic acid (NAA), the rooting rate was close to 100%. In indirect embryogenesis, embryonic calluses were formed when the culture medium contained 22.22 μM 6-BA and 4.52 μM 2,4-dichlorophenoxy acetic acid (2,4-D), and the induction rate was 92.4% for young inflorescence and 100% for rachillae. After the embryonic calluses were transferred to the medium supplemented with 11.43 μM IAA and 13.33 μM 6-BA, they could develop into plantlets with roots. In assessing the two regeneration pathways in terms of genetic and epigenetic fidelity of the regenerants, two kinds of molecular markers [amplified fragment length polymorphism (AFLP) and methylation-sensitive amplified polymorphism (MSAP)] were employed. The AFLP analysis used 20 primer pairs that yielded 916 scorable bands among the donor plant and 11 regenerants from direct embryogenesis, of which 8 (0.87%) were polymorphic. The regenerants from indirect embryogenesis had 1075 clear bands of which 3 (0.27%) were polymorphic scorable bands from 18 primer pairs. Moreover, the variant band patterns included two types, that is, loss-of-original and gain-of-novel bands. MSAP analysis revealed that tissue culturing of the flower induced DNA cytosine methylation alterations in both CG and CNG levels and patterns compared with the donor plant. The variation rate was 1.1 and 1.3% for the direct and indirect embryogenesis pathways, respectively. The findings show that tissue culture of flowering plants is a form of stress which can induce some heritable epigenetic variations and should be considered in future long-term genotype preservation programs of *Freesia hybrida*.