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Molecular analysis of genetic stability in micropropagated apple rootstock MM106

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Abstract

Random amplified polymorphic DNA (RAPD) markers were used to assess the genetic stability of 10 micropropagated plants regenerated through axillary buds of clonal apple (*Malus pumila* Mill.) rootstock MM106. Eleven random decamer primers were successfully used to analyse genomic DNA from mother plants and in vitro plant material. A total of 129 scorable fragments were amplified with an average of 11.73 bands per primer. Among them, 99 were monomorphic and 30 were polymorphic with 23.2% polymorphism. Among these 30, 12 were found monomorphic across seven plants and parent. Three plants could be regarded as off-types. Our results show that RAPD markers could be used to detect the genetic similarities and dissimilarities in micropropagated material.

Keywords: Apple; Micropropagated plants; Genetic stability; RAPD markers; Somaclonal variation

1. Introduction

Several breeding programmes for apple rootstocks have produced new apple rootstock clones. Among them, MM106 has many attributes, i.e. good induction of cropping, resistance to woolly apple aphid and intermediate vigor.

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In apple, in vitro culture is considered to be the most effective method for mass clonal multiplication. True to type clonal fidelity is one of the most important pre-requisite in the micropropagation of apple rootstocks. The occurrence of somaclonal variation is a potential drawback when the propagation of an elite tree is intended, where clonal fidelity is required to maintain the advantages of desired elite genotypes. Tissue culture has been used to propagate apple clones (Zimmerman, 1983) and evidence exists that this technique can induce genetic change (Hartman and Kester, 1983) possibly without major changes in phenotype. Micropropagated plants from the cultures of preformed structures, such as shoot tips and axillary buds, and from the tissues of hardwood shoot cuttings have been reported to maintain clonal fidelity (Wang and Charles, 1991; Ostry et al., 1994), but there is still a possibility that in vitro cultured plantlets exhibit somaclonal variation (Rani et al., 1995). This variation is often heritable and, therefore, unwanted in clonal propagation (Brieman et al., 1987). Thus, screening the micropropagated plants at an early stage is essential to reduce the chances for inclusion of variable genotypes.

There are limited reports available to assess the genetic stability of tree species. For example, the genetic stability of in vitro propagated pines has been very sparsely studied through isozyme and RAPD markers (Ishii et al., 1987; Goto et al., 1998). Isabel et al. (1993) evaluated the genetic integrity of somatic embryogenesis-derived populations of *Picea mariana* (Mill). Rani et al. (1995) reported the usefulness of random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoides* Marsh. Damasco et al. (1996) detected the dwarf off-types in micropropagated Cavendish bananas (*Musa acuminata)* using RAPD. Recently developed microsatellite DNA markers have been employed to determine the clonal fidelity of micropropagated plantlets of trembling aspen (*P. tremaloides*) (Rahman and Rajora, 2001). Rani et al. (2001) studied RAPD fingerprinting diagnostics for genetic integrity of enhanced axillary branching-derived plants of 10 forest tree species. Devarumath et al. (2002) considered RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* spp. *assamica* (Assam-India type).

The detection of off-types among micropropagated plants, especially of trees, by morphological observations and karyotype analysis of metaphase chromosomes has several limitations such as extensive evaluation time needed for assessment. Isozymes provide limited number of informative markers and are affected by environmental variations. On the other hand, DNA markers are an attractive means for detecting somaclonal variations since they are more informative and are not developmentally affected. Restriction fragment length polymorphism (RFLP), though has been used for screening of tissue culture derived plants (Valles et al., 1993), is laborious, usually involves radioactivity and not suited for routine application of tissue culture systems. RAPD requires only small amounts of starting DNA, does not require prior DNA sequence information, nor involves radioactivity (Williams et al., 1990; Welsh and McClelland, 1990) and data can be generated faster with less labour than other methods like RFLP and microsatellites. After going through the literature, it was found that micropropagation in apple rootstocks has been achieved successfully through axillary buds and meristems, but very little information is available to assess the genetic stability. It is, therefore, intended to