Molecular Markers in Micropropagation, Tissue Culture and In Vitro Plant Research

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ABSTRACT

Molecular marker technologies have become a powerful tool in crop improvement through their use in germplasm characterization, linkage mapping and molecular breeding. In vitro plant research has become one of the areas of extensive molecular marker application, as they can be used to monitor the somaclonal variation, verify the genetic fidelity of micropropagated plants and to identify genotypes with desired response to in vitro culture conditions. The objective of this paper is to summarize literature concerning application of various molecular markers for genetic fidelity assessment of in vitro cultured plants.

Keywords: cryopreservation, genetic stability, in vitro, micropropagation, molecular markers, somaclonal variation, tissue culture

Abbreviations: AFLP, amplified fragment length polymorphism; IRAP, inter-retrotransposon amplified polymorphism; ISSR, inter-simple sequence repeats; MAS, marker-assisted selection; MSAP, methylation sensitive amplified polymorphism; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; REMAP, retrotransposon-microsatellite amplified polymorphism; RFLP, restriction fragment length polymorphism; S-SAP, sequence-specific amplification polymorphism; SSR, simple sequence repeats; TCL, thin cell layer

INTRODUCTION

Molecular markers are specific DNA fragments that can be detected within the whole genome by means of an adequate technique. Several classes of molecular markers, such as RFLP, RAPD, AFLP, SSR, ISSR, have been developed to date, and used for numerous purposes. Recently, in vitro plant research has become one of the areas of extensive molecular marker application.

In vitro regeneration and micropropagation form the primary core of many, if not most horticultural plant and agricultural crop studies. A number of well defined problems in physiological, epigenetic and genetic quality are associated with the culture of plant cell, tissue and organs in vitro, namely, absence or loss of organogenetic potential (recalcitrance), hyperhydration (vitrification) and somaclonal variation, complex phenomena that are genotype and environment dependent and which affect the practical application of plant tissue culture in plant propagation and genetic manipulation.

In order to ensure the successful use of in vitro technology either for breeding programs or market distribution molecular markers are used to monitor the somaclonal variation, verify the genetic fidelity of micropropagated plants.
and to identify genotypes with desired response to \textit{in vitro} culture conditions.

This review investigates how various molecular markers have been used for detection of somaclonal variation in several (but not exhaustively) \textit{in vitro} micropropagation systems, but does not extend towards the stability of transgenes in genetically-modified plants. Application of molecular markers in tissue culture response studies, including identification of QTLs governing the reaction of different explant types to \textit{in vitro} conditions and use of MAS in identification of genotypes with a desired response has been recently reviewed elsewhere (Bolibok and Rakocz-Trojanowska 2006).

**MOLECULAR MARKERS FOR THE ANALYSES OF IN VITRO CULTURED PLANTS**

The main aim of clonal propagation is to retain the genetic integrity of the propagated plants. Somaclonal variation, although to a certain extent overcame by TCLs, a system that has allowed for the standardization of clonal micropropagation of numerous monocotyledonous and dicotyledonous species, allowing for mass-production of morphologically and genetically stable and uniform plants (Teixeira da Silva and Nhut 2003; Nhut et al. 2006), remains a factor affecting practical application of micropropagation. Various molecular techniques are used to check genetic stability and lack of somaclonal variation in tissue culture derived plants, however easy to use, cheap and requiring no previous sequence information RAPD and ISSR markers clearly prevail in the published studies dealing with the subject. Nevertheless, technically more demanding AFLPs and sequence specific, highly polymorphic SSRs are also used relatively frequently.

As some of the somaclonal variation is of epigenetic nature it cannot be detected with conventional structural molecular markers. In those cases markers such as methylation sensitive amplified polymorphism (MSAP) (Jaligot et al. 2003; Hao et al. 2004), methylation-sensitive AFLPs (Jaligot et al. 2002), or a gene expression approach (Moricillo et al. 2006) need to be used. Moreover, tissue culture has been shown to induce transposition of transposable elements of plants (Hirochika et al. 1996; Courtial et al. 2001), and their insertion at novel sites results in somaclonal variation (Wessler et al. 1995). Hence, using retrotransposon-based molecular markers (S-SPAN, IRAF, REMAP) in studies of \textit{in vitro} cultured plant seems an interesting option and recently IRAF markers were successfully used for the analysis of banana somaclones (Asif and Othman 2005). Application of RFLP technology with appropriately chosen probes gives the possibility to assess the genetic fidelity of micropropagated plants with respect to their nuclear, mitochondrial and chloroplast genomes (Abe et al. 2002; Devaramath et al. 2002). Generally, simultaneous application of different DNA fingerprinting techniques is advisable in tissue culture derived plants as it allows for detection of variation in diverse components of the genome (Asif and Othman 2005).

In the following sections we summarize literature concerning application of molecular markers for genetic fidelity assessment of \textit{in vitro} cultured plants.

**GENETIC FIDELITY OF MICROPROPAGATED PLANTS**

**Ornamental plants**

If tissue culture is being employed for commercial production of ornamental plants it is important to achieve a rapid and easy method to detect somaclonal variation, not only to make sure that the characteristics of the variety propagated have not been changed, but also, in some cases to use it as a source of variation that may be employed or obtaining new varieties or forms. Somaclonal variation could be quantified in tissue-cultured \textit{Phalaenopsis} (Chen et al. 1998), \textit{Feteronia} (Cassels et al. 1997), \textit{Petunia} (Cerny et al. 1996), \textit{Saintpaulia} (Paek and Hahn 1999) and \textit{Cymbidium} hybrids (Teixeira da Silva and Tanaka 2006; Teixeira da Silva et al. 2006) using RAPD markers. Asiatic \textit{Lilium} \textit{in vitro}-induced bulblets showed zero RAPD variation (Varshney et al. 2001). RAPD has also proved to be an efficient tool for assessment of genetic stability in the tissue culture of \textit{Chrysanthemum} (Martin et al. 2002). A more comprehensive analysis of somaclonal variation in ornamental plant tissue culture and its assessment has been covered extensively elsewhere (Teixeira da Silva 2006) and will not be covered here.

**Trees**

Molecular markers are especially valuable for efficient tree germplasm management due to the high cost of space and maintenance of plants in most clonally-propagated tree species. Moreover out-crossing of many tree species resulting in high levels of polymorphism, thus consequent duplications or mislabeling are avoided. Identifying variants during the early stages of plant development is essential to avoid propagation of mutant plants in species with extensive growing periods.

**Tropical and sub-tropical fruit trees**

The analysis of somaclonal variation in micropropagated banana, which is widespread, has been recently reviewed (Sahijram et al. 2003). RAPDs and SCARs allowed for the successful detection of dwarf off-types in micropropagated cavendish (\textit{Musa} spp. AAA; Umali et al. 2002) and bananas (Ramage et al. 2004). \textit{In vitro}-micropropagated somaclonal variants could be identified using RAPD profiling of \textit{Musa} (Grajal-Martín et al. 1998). No genetic variation was detected in analyses of micropropagated banana plantlets obtained from different cytokinin-treatments. In total 625 reproducible monomorphous bands were obtained with 50 RAPD and 12 ISSR primers (Venkatachalam et al. 2006). Micropropagated pineapple (\textit{Ananas comosus}) was shown to be genetically stable using RAPDs and isozymes (Feurer et al. 2004). Similarly, no polymorphic marker was found among 669 RAPD and 1307 AFLP markers used for the analysis of micropropagated chestnut rose (\textit{Rosa roxburghii} Tratt; Weng and Deng 2005).

**Temperate fruit trees**

Modgil et al. (2005) showed the genetic fidelity of adventitious bud, clonally propagated apple (\textit{Malus pumila}) using RAPD and ISSR, while RAPDs were also used to confirm the genetic stability of leaf-derived apple clones (Virscek-Marn et al. 1999), peach somatic embryos (Hashmi et al. 1997) or lemon (\textit{Citrus limon}) protoplast regenerants (Deng et al. 1995). Hao et al. (2004) found that RAPDs were insufficiently sensitive to detect small changes in the DNA of grapefruit callus cultures, but DNA methylation changes could be detected by methylation sensitive amplified polymorphism (MSAP). Eight \textit{Rubus} species could be regenerated on 55 different media, and resulting plants’ genetic stability was confirmed by RAPDs. The paternity of embryo culture-derived cherry plants was confirmed with RAPD markers (Hormaza 1999). Repeatable somaclonal variation could only be detected when both RAPD and SSR were used in micropropagated kiwi (Palombi and Damiano 2002).

**Nut trees and palms**

The clonal identity of 4-year long \textit{in vitro}-propagated chestnut (\textit{Castanea sativa}) trees could be confirmed using 40 arbitrary 10-mer primers, with genetic variants, differing from each other and from the original mother plants (Carvalho et al. 2004). In hazelnut RAPD analysis revealed no somaclonal variation between donor plants from which \textit{in vitro} cultures were initiated and micropropagated plants (6-
year cultures), and no somaclonal variation was detected among in vitro-propagated plants; polymorphism (15.6%) was detected between the parent plant and its in vitro-propagated progenies, however (Nas et al. 2004). Somaclonal variants, which were shown to be age dependent in date palm, were also suitably detected by RAPD analysis in oil palm, Elaeis guineensis (Saker et al. 2000). However, the occurrence of somaclonal variants (ca 5%) among populations of somatic embryogenic cultures of black spruce (Picea mariana) and loblolly pine (Pinus taeda); Tang 2002) or Norway spruce (Picea abies) (Fourré et al. 1993), loblolly pine (Pinus taeda; Tang 2002) or Norway spruce (Picea abies) (Fourré et al. 1993) in vitro-propagated plants; polymorphism (15.6%) in somatic embryos maintained haploidy over long periods (Bueno et al. 2002). Genetic variation (0.0045%) in in vitro-propagated cedars (Cedrus libani) could be rapidly detected using 11 arbitrary 10-mer RAPD primers (Piola et al. 1999) while in vitro-cultured C. libani showed reduced methylation as compared to adults (Renau-Morata et al. 2005). Long-term cultures (>10 years) of micropropagated shoots of Japanese black pine (Pinus thunbergii) showed no RAPD variation (Goto et al. 1998) nor was there any somaclonal variation in somatic embryogenic cultures of black spruce (Picea mariana; Israel et al. 1993), loblolly pine (Pinus taeda; Tang 2002) or Norway spruce (Fourné et al. 1997). Bands or pools of RAPD bands among clones originating from three different sources of sweet cherry (Prunus avium) revealed the occurrence of genomic variation, though at a relatively low level (10.62% polymorphic bands; Guo et al. 2006). Six of 48 RAPD primers tested were able to detect somaclonal variation in embryos derived from six Norway spruce (Picea abies) (Fourré et al. 1993), loblolly pine (Pinus taeda; Tang 2002) or Norway spruce (Picea abies) (Fourré et al. 1993). Restriction fragment length polymorphisms (RFLPs) were detected in the mitochondrial, plastid and nuclear genomes in the Festuca pratensis plants of 23 micropropagated plants of a single Populus deltoides clone, eleven 10-mer RAPD primers were used, of which 5 distinguished a total of 13 polymorphisms commonly across 6 micropropagated plants while the remainder of the amplification products were monomorphic across the micropropagated plants (Rani et al. 1995).

**Forest and timber trees**

Molecular markers in forest breeding and biotechnology have been used to elucidate genome mapping, organization and evolution of forest tree species, provided insight into the maternal and paternal mode of organelle inheritance in angiosperm and gymnosperm trees, especially since repetitive DNA sequences are much more frequent than in the genomes of higher plants (Ahuja 2001), and are also widely used for assessing somaclonal variation. SSRs were used to confirm the genetic fidelity of micropropagated trembling aspen (Rahman and Rajora 2001), and of 10 forest tree species (Rani et al. 2001). Two of the economically most important and widely planted Eucalyptus species, E. camaldulensis and E. tereticornis, showed uniform tissue culture (axillary branch-derived plantlets) and monomorphic fragments generated from 12 arbitrary 10-mer primers (Rani and Raina 1998). Using 17 RAPD primers Tripathi et al. (2006) found a small variation (0.01 and 0.02) in two groups of micropropagated Eucalyptus plants. Melia azedarach, an important timber species also with therapeutic and insecticidal application, displayed many polymorphisms when in vitro-propagated clones were RAPD fingerprinted, the intrACLone heterogeneity being attributed to chimerism (Olmos et al. 2002). Genetic variation (0.0045%) in in vitro-propagated cedar (Cedrus libani) could be rapidly detected using 11 arbitrary 10-mer RAPD primers (Piola et al. 1999) while in vitro-cultured C. libani showed reduced methylation as compared to adults (Renau-Morata et al. 2005). Long-term cultures (>10 years) of micropropagated shoots of Japanese black pine (Pinus thunbergii) showed no RAPD variation (Goto et al. 1998) nor was there any somaclonal variation in somatic embryogenic cultures of black spruce (Picea mariana; Israel et al. 1993), loblolly pine (Pinus taeda; Tang 2002) or Norway spruce (Fourné et al. 1997). Bands or pools of RAPD bands among clones originating from three different sources of sweet cherry (Prunus avium) revealed the occurrence of genomic variation, though at a relatively low level (10.62% polymorphic bands; Guo et al. 2006). Six of 48 RAPD primers tested were able to detect somaclonal variation in embryos derived from six Norway spruce (Picea abies) (Fourré et al. 1993), loblolly pine (Pinus taeda; Tang 2002) or Norway spruce (Picea abies) (Fourré et al. 1993). Restriction fragment length polymorphisms (RFLPs) were detected in the mitochondrial, plastid and nuclear genomes in the Festuca pratensis plants of 23 micropropagated plants of a single Populus deltoides clone, eleven 10-mer RAPD primers were used, of which 5 distinguished a total of 13 polymorphisms commonly across 6 micropropagated plants while the remainder of the amplification products were monomorphic across the micropropagated plants (Rani et al. 1995).

**Endangered trees**

Genetic integrity of introduced rare and endangered species is vital, especially when these are derived from in vitro mass propagation. About 97% homology could be detected between the mother plants and micropropagated plants of Syzygium travancoricum, a rare and endangered tree (Anand 2003).

**Grain, cereal and forage crops, and grasses (Poaceae)**

Analytical methods have been developed and applied to identify and quantify cereal species in food products and also to fingerprint and identify grain at the genotype and variety levels since the composition of cereal-based foods is a key factor in determining the quality and safety of the final product, while the reliable identification of cereal species and cultivars is essential for the handling, marketing and processing of grain, and for the protection of plant breeders’ rights (Terzi et al. 2005).

Yang et al. (1999) reported more than 50 polymorphic bands using 4 DIG-labelled RAPD primers in regenerated rice plants obtained from callus cultures, while an increase in polymorphism from 12% to 17% and to 26% in 1, 3 and 6 month-old cultures, respectively, could be detected within a single line (Kim et al. 2003). Godwin et al. (1997) analyzed 8 rice somaclones generated from mature seed-derived callus cultures using RAPDs, and all somaclonal families differed significantly from the original material, indicating genomic alterations in all families, as did Phong et al. (2001) in desiccation-tolerant-derived plants, but Aifza et al. (2001) found limited androclonal variation in rice anther cultures. Differences in some somaclonal variants derived from a landrace rice variety, Indrayani, were shown to be high yielding and resistant to multiple diseases, and could be differentiated on the basis of SSRs (Chowdari et al. 1998). Alteration in chloroplast DNA (ctDNA) in long-term cultures of calli derived from rice seeds were investigated using a BamHI-1 fragment of rice ctDNA as a probe. Forty eight callus cultures were analyzed for five repetitive sequences (T3E-1 and T3E-2) were found to have large deletions (76 kb and 63 kb, respectively) in the chloroplast genome, while none of the same callus lines cultured for 6 months showed any ctDNA alteration (Abe et al. 2002).

RAPD analysis was performed to assess DNA variation among rye (Secale cereale) plants regenerated from immature embryos and inferences. From the studied plants, 40% showed changes in somaclonal variants derived from one culture, and 56 months, two mutations per plant was quite high, ranging from 1 up to 12 (Li-ncero et al. 2000). Similarly, Rakoczy-Trojanowska (2002) found a relatively high proportion of immature embryos derived rye R1 plants with changed RAPD profiles. In Triticum (and also tobacco) protoplast cultures and subsequent regenerants, somaclonal variation was detectable by RAPDs (Brown et al. 1993). No restriction fragment length polymorphisms (RFLPs) were detected in the mitochondrial, plastid and nuclear genomes in the Festuca pratensis plants.
regenerated from suspension cultures (Vallès et al. 1993).

Somaclonal variation could be detected using RAPD analysis in tissue-cultured Lolium derived from cell suspension-derived protoplasts (Wang et al. 1993). The microspore origin of anther-culture-derived plants of flax (Linum usitatissimum) was determined using ISSR and RAPD markers (Chen et al. 1998). Somaclonal variation in axillary branching propagation and indirect somatic embryogenesis in Eclipta prostrata was estimated by RAPD fingerprinting (Piccioni et al. 1997), while SSR fingerprinting revealed a reduction in the number of copies of a highly repetitive DNA sequence (Pluher et al. 2001).

**Medicinal and aromatic plants**

Monitoring genetic fidelity in medicinal plants with the help of molecular markers allows for the formulation of drugs free from batch-to-batch variations (e.g. in garlic, Ipik et al. 2003), for effective ex situ conservation programs. An overview of the regeneration of medicinal plants by direct and indirect organogenesis and by somatic embryogenesis from various types of explants is presented elsewhere (Rout et al. 2000), as is their use with other biotechnological approaches to improve medicinal plants through somaclonal variation, and molecular markers to assess its level.

Jamrosa, a hybrid Cymbopogon, is an essential oil-yielding plant containing geraniol, used as a substitute for palmarosa (C. martini), and commercially used in perfumery, pharmaceuticals, soaps and cosmetic industries, and reclamation of waste land; Callus-derived Cymbopogon somaclones, despite gross differences in RAPD banding patterns displayed uniformity in essential oil yield and quality (Nayak et al. 2003). RAPDs and SSRs proved genetic stability in micropropagated Achillea, comprising ten taxa of the medicinally important A. millefolium group and six related species (Wallner et al. 1996). Varietal specific RAPD markers could be detected in tissue-cultured Piper longum, a dioecious medicinal herb (Philip et al. 2000). Digitalis obscura plants produce cardenolides, secondary metabolites of major interest to the pharmaceutical industry. RAPDs provide a simple and effective assay for screening elite cultivars and confirm their genetic stability over long-term cultures (Gavidia et al. 1996). Similar RAPD controls for in vitro-propagated Epipedium (Nakai et al. 1996) resulted in standardization of plantlet selection and greater scrutiny of medicinal plants. Guo et al. (2006) found genomic instabilities in phenotypically normal regenerated plants of Codonopsis lanceolata Benth. et Hook. f. using ISSR and RAPD markers, which confirmed 7.7% to 24.9% of the bands, respectively, were polymorphic. Five arbitrary RAPD primers used to analyze the genetic fidelity of Chlorophytum arundinaceum Baker plants regenerated through shoot bud differentiation displayed the same banding profile within all the micropropagated plants and in vivo explant donor (Lattoo et al. 2006). The cytological and molecular analysis complemented and compared well and showed no genomic alterations in Chlorophytum. Baker, whereas RAPD banding patterns differed within medicinal herb regenerated through shoot bud differentiation.

The capacity to detect genetic mono- or polymorphisms in callus-induced plants of garlic (Allium sativum; Al-Zahim et al. 1999), Panax notoginseng somatic embryos (Shoyama et al. 1997), and turmeric (Salvi et al. 2001) allows for elimination of genetic variants for clonal micropropagation, or exploitation of somaclonal variation for the selection of new varieties with novel features, such as increased secondary metabolite yields, but in the case of garlic, morphogenic characters and RAPD banding could not be correlated. In the case of American ginseng, P. quinquefolium, long term suspension cultures showed different RAPD banding patterns to young seedling-derived somatic embryos (Punj et al. 2004). Gametoclonal variation was detected by novel RAPD bands in onion genotypes (Bohanec et al. 1995). Tylophora indica, a medicinal plant with multifarious uses, once in vitro-propagated by 2,4-D- and kinetin-induced callus, produces morphonic amplification products when 20 arbitrary nucleotide primers are used, indicating the genetic homogeneity and true-to-type nature of regenerants (Jayanthi and Mandal 2001). Devarumath et al. (2002) considered RAPD and ISSR 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). Out of 20 RAPD primers used by Mondal and Chand (2002) to assess genetic instability among micropropagated tea [Camellia sinensis (L.) O. Kuntze] cultivar ‘T-78’, 11 generated polymorphism in four out of 17 micropropagated plants and one mother plant, which otherwise were morphologically indistinguishable. Polymorphism among those four plants showed an identical banding pattern suggesting the occurrence of a single mutation. Similar results were obtained by Singh et al. (2004) in vegetatively propagated clones. SSRs have been adapted as the standard for cocoa (Theobroma cacao) genetic diversity and fingerprinting analysis (Saunders et al. 2005), and have been used to detect and quantify in vitro-culture induced chimerism (Lopez et al. 2005). Somaclonal variability in hop (Humulus lupulus) in vitro meristem cultures and clones (Patzak 2003) was assessed by RAPDs and ISSRs. Somaclones of in vitro-cultured leaf segment-derived shoots of Lycium chinense, commercially important as a medicinal plant, and as a beverage in Asia, could be detected by RAPDs (Ahn et al. 2004) Mango ginger, Curcuma amanda, an Indian medicinal plant showed new polymorphic RAPD bands, i.e. variation, in in vitro rhizome-derived, callus-induced shoots (Prakash et al. 2004). RAPD bands generated using twenty 10-mer primers used in RAPD were monomorphic in tissue cultured clonal plants of Plumbago zeylanica, important for the alkaloid, plumbagin (Rout and Das 2002). In Angelica, a local Japanese medicinal plant, RFLP could not distinguish different in vitro plants, whereas RAPDs could (Watanabe et al. 1998).

**Other fruit and vegetable crops**

ISSR analysis was effective to eliminate somaclonal variants in in vitro leaf-derived horseradish plants (Rostiana et al. 1999). Somaclonal variation was detectable (0.05%) in tissue-cultured sugarbeet (Beta vulgaris) using RAPDs (Munthali et al. 1996). In vitro propagated cauliflower (Brassica oleracea) somatic embryos did not show any variation in ISSR banding (Leroy et al. 2000b). Numerous molecular markers are used for checking somaclonal variation in S. melongena (eggplant) somatic embryogenesis (Kanthajaj and Golegaonkar 2004). Despite the presence of dwarf phenotypes, 157 RAPD markers could not detect intraclonal variation in 77 asparagus plants regenerated from embryogenic lines (Raimondi et al. 2001). Taylor et al. (1995) reported that RAPDs detected gross genetic changes occurring in sugarcane tissues subjected to prolonged (2 years) in vitro culture. Regeneration in sunflower (Helianthus annuus) based on successive excision of the apical and axillary shoots originating from pre-existing meristems resulted in no somaclonal variation, as confirmed by AFLP analyses (Hewezi et al. 2003).

No polymorphisms could be detected through RAPD analysis in tomato calli or progeny of regenerants, despite morphological differences (Rus-Kortekaas et al. 1994), nor using RAPD or ISSR markers (Martins et al. 2004).

**GENETIC FIDELITY OF PLANTS AFTER CRYOPRESERVATION**

**Fruit trees**

Long-term stored, cryopreserved by vitrification ‘Gala’ apple (Liu et al. 2004), Amorphophallus (Zhang et al. 2001) or Prunus (Helliott et al. 2002) shoot tips showed no RAPD polymorphisms, confirming isozyme analyses, showing that RAPDs are useful for discerning variation in this useful
germplasm conservation technique. On the other hand Hao et al. (2001) reported that cryopreservation of apple, despite no differences at the cytological and AFLP levels, produced new demethylation sites. No differences were found between the DNA patterns obtained using RAPD from plantlets regenerated from control and plantlets regenerated directly from cryopreserved shoot-tips of four grape (Vitis vinifera) cultivars and one kiwi (Actinidia delicosa) cultivar (Zhai et al. 2003).

Forest and timber trees

No reproducible variation of the RAPD profiles generated by ten 10-mer primers was observed in cryopreserved tissues of hybrid aspen (Populus tremula L. × Populus tremuloides Michx.), an economically important woody plant and widely used model system of forest scientists (Jokipi et al. 2004). High levels of polymorphism were shown in RAPD analysis of cryopreserved apical meristem-tips of Melia azedarach (Scoccio et al. 2004). de Verno et al. (1999) found large differences in RAPD banding following cryopreservation (2-12 months) of P. glauca somatic embryos. In other studies with larch (Larix X euroleposis), black spruce (Picea mariana; Klimaszewska et al. 1992), Norway spruce (Picea abies; Norgaard et al. 1993), Douglas fir (Pseudotsuga menziesii; Gupta et al. 1995), Carribean pine (Pinus caribaea; Lainé et al. 1992), Scott’s pine (Pinus silvestris; Håggman et al. 1998) and maritime pine (Pinus pinaster; Berceche and Pâques 1995) no genetic changes were found when RAPD analysis was conducted on cryopreserved regenerants.

Medicinal and aromatic plants

Plants derived from cryopreserved shoot tips of Dioscorea floribunda, a medicinal species of yam, were genetically stable, with only 1 polymorphic band being obtained from over 5,000 RAPD bands (Ahuja et al. 2002).

Tuberous crops

Embryogenic tissues of Dioscorea bulbifera (yam) were cryopreserved using encapsulation-dehydration, and the genetic stability of plants regenerated from cryopreserved embryogenic tissues was assessed using RAPDs: 10 primers produced 62 clear reproducible DNA fragment profiles and the amplification products were monomorphic for all the plantlets except one; a total of 4960 DNA fragments were obtained from this study showing no variation in RAPD profiles (Dixit et al. 2003).

FUTURE PERSPECTIVES

As the agricultural and horticultural sectors, especially the floricultural sector, move towards a greater efficiency in production, higher stringency in quality control, and greater demand by the consumer, there is a need to accompany the advances in transgenic and molecular biotechnology with an equal focus on tissue culture. More and more plants produced clonally by micropropagation are becoming the source of material for field plantations, which creates a constantly growing demand for sensitive, efficient and reliable tools for analysis of the somaclonal variation, or the elimination of genetic stability of the somaclonal variation. Molecular markers are those tools.

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