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A REVIEW

Molecular markers : An important tool to assess genetic fidelity in tissue culture grown long-term cultures of economically important fruit plants

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INTRODUCTION

India with its wide diversity in climate and soil is bestowed with a variety of ecosystems. It produces a large number of fruits commercially in various agroclimatic zones. India is the second largest producer of fruits with a production of 49 million tons and contributes 10 per cent share in global food production. India occupies first place in production of mango, banana, litchi, papaya, pomegranate and sapota (Anonymous, 2007). It has higher national average productivity in banana and sapota compared to world average productivity. India accounts for an area of 4.96 million hectare under fruit crops with a production of 49.29 million ton. Among states, Maharashtra ranks first in area and production of fruits and contributes 27 per cent and 21.5 per cent, respectively (Anonymous, 2007).

Fruits occupy an important place in human diet as they provide a wide variety of nutrients essential for good health and happiness. Fruits contain carbohydrates (source of energy), minerals, dietary fibres, vitamins and some enzymes which are necessary not only for proper body functions but also for providing protection against diseases (Singh, 1969).

The fruit plants are propagated both by sexual (seeds) and asexual (vegetative) methods. In sexual method the plants are raised from seeds producing seedlings. In asexual or vegetative propagation of plants, a vegetative part (leaf, stem or root) is placed in such an environment that it develops into a new plant.

Conventionally, fruit plants are generally propagated by the vegetative or asexual methods. They involve no change in genetic makeup of the new plant. All the characteristics of the parent plant are reproduced in the daughter plants due to exact duplication of chromosomes during cell division. Thus, the plants are true-to type in growth, ripening, yield and fruit quality.

The conventional methods of propagation are generally slow, labour intensive and requiring large number of propagules. Besides this, vegetatively propagated crops are often infested by pests and diseases which cause severe production loss.

Most of the fruit plants are woody perennials and take several years to bear. Therefore, propagation of the fruit trees using conventional methods is tedious and cumbersome. Thus, a different strategy has to be adopted to increase the production of plants to cater to the requirements of burgeoning human population. Modern methods of biotechnology can be very useful to achieve the objectives which cannot be realized by the conventional methods.

Plant tissue culture, an essential component of biotechnology has become a major tool in agriculture, horticulture and forestry. It has a great potential for rapid multiplication of elite genotypes on a large-scale in a comparatively short time (Rao, 1993 and Jain, 1997). This technology is mainly of benefit for those plants which have long maturation periods, low seed viability and self–incompatibility, or those that are difficult-to-multiply by conventional means (Trigiano *et al*., 1992). Other advantages of micropropagation are- *in vitro* cloning of plants can be continued all year round and so become independent of the season and it also provides novel approach for genetic manipulation (Prakash and Pierik, 1992).

Micropropagation can be rewarding only if complete genetic fidelity of micropropagules is maintained. Genetic fidelity is the maintenance of genetic constitution of a particular clone throughout its growth span (Chatterjee and Prakash, 1996). Periodic monitoring of the degree of genetic stability of *in vitro* conserved plants is of utmost importance for commercial utilization of true-to-type plants of the desired genotype (Mohanty *et al*., 2011). The assessment of the genetic integrity of *in vitro* grown regenerants in regular intervals can significantly reduce or eliminate the chance of occurrence of somaclonal variation (Larkin and Scowcroft, 1981) at early or late phase of culture. Many factors are known to be associated with the occurrence of somaclonal variation which affect genetic fidelity of tissue culture plantlets, particularly when they are maintained for prolonged duration. These factors include genotype, age of donar plant, explants type (Haisel *et al*., 2001; Peredo *et al*., 2008), plant growth regulators in the culture medium (Bairu *et al*., 2006) and number of subcultures (Chatterjee and Prakash, 1996; Gangopadhyay *et al*., 2003). Skirvin *et al*. (1994) stated that the level of genetic variation that should be expected in *in vitro* culture is about 1-3 per cent.

It is therefore, considered important to evaluate the

Table 1 : Assessment of genetic fidelity in long-term cultures of important fruit plants using various molecular marker systems

Plant species	Marker system	Stability/ variability	Reference
Actinidia deliciosa	RAPD	S	Palombi and Damiano, 2002
	ISSR	V	Palombi and Damiano, 2002
	AFLP	V	Prado *et al*., 2007
Aegle marmelos	RAPD and ISSR	S	Mishra *et al*., 2008
Ananas comosus	RAPD	V	Feuser *et al*., 2003
			Santos *et al*., 2008
Citrus limon	RAPD	S	Orbovic *et al*., 2008
C. cinensis	RAPD	S	Hao and Deng, 2002
Cucumber	RAPD	V	Elmeer *et al*., 2009
Feronia limonia	RAPD and ISSR	S	Joshi, 2011
Malus pumila	RAPD	V	Modgil *et al*., 2005
Musa acuminate var. *Najanagudu*	RAPD and ISSR	S	Venkatachalam *et al*., 2007
Musa spp.	RAPD and ISSR	V	Ray *et al*., 2006
Psidium guajava	ISSR	S	Liu and Yang, 2012
P. guajava	SSR and ISSR	S	Rai *et al*., 2012
P. dulcia	RAPD and ISSR	S	Martins *et al*., 2004
Phoenix dactylifera	AFLP	V	Saker *et al*., 2006
Rubus spp	RAPD	S	Gajdosova *et al*., 2006
	AFLP and SSR	S	Castillo, 2007
Vanilla planifolia	RAPD and ISSR	S	Sreedhar *et al*., 2007
Vitis vinifera	RAPD	S	Yang *et al*., 2008

plants obtained from different methods of tissue culture for occurrence of abnormal plants, if any, before the protocol is adopted for potential commercial applications. When plant tissue is passaged through *in vitro* culture, many of the regenerated plantlets appear to be no longer copies of their donor genotype. If the analysis is carried out during various culture passages it would establish genetic variation, if any, very early in the culture system so that one can suitably modify the micropropagation protocol to avoid the variations.

A range of markers based on morphological, cytological, biochemical and molecular traits has been recommended to evaluate the tissue culture plants for genetic stability and clonal fidelity (Rani and Raina, 2002).

In recent years, DNA based molecular markers have become popular for easy and precise detection and better understanding of somaclonal variation. These markers provide valuable data to assess the genetic homogeneity and true-to-type nature of micropropagated plants (Rai *et al*., 2012). Nowadays, DNA based markers are being preferred over others to test the genetic stability in tissue culture derived plants.These markers have acted as versatile tools and have found their own position in various fields like taxonomy, plant breeding and genetic engineering etc. (Joshi, 2011). They offer numerous advantages over conventional phenotype based alternatives as they being stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell. They are not confounded by the environment, pleotrophic and epistatic effects (Agarwal *et al*., 2008). DNA based molecular markers are more stable and ubiquitous to most of the living organisms (Johan *et al*., 2011) and have become an important tool to check the genetic uniformity and true-to-type nature of the micropropagated plants (Kumar *et al*., 2011).

Molecular markers suitable for generating DNA profiles have proved to be an effective tool in assessing the genetic stability of regenerated plants. A wide variety of PCR based markers are random amplified polymorphic DNA (RAPD; Williams *et al*., 1993), amplified fragment length polymorphism (AFLP; Vos *et al*., 1995), Inter simple sequence repeats (SSR; Ziettiewicz *et al*., 1994), restriction fragment length polymorphism (RFLP: Bostein *et al*., 1993) and simple sequence repeats (SSRs: Litt and Lutty, 1989) have been used for assessment of genetic stability of regenerated plantlets. The choice of molecular marker based technique depends on its simplicity and reproducibility (Chandrika *et al*., 2008). Among all the available molecular markers, PCR based RAPDs and ISSRs have been the most commonly used techniques for the assessment of genetic fidelity in micropropagated plants because of their simplicity and cost effectiveness. There are several reports where molecular markers such as RAPD, AFLP, ISSR and SSR are used for the assessment of genetic fidelity in long term cultures of fruit plants (Table 1).

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