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A REVIEW

Role of cytological markers for evaluation of genetic integrity of *in vitro* regenerated plants

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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 and Peredo *et al*., 2008), plant growth regulators in the culture medium (Bairu *et al*., 2006) and number of subcultures (Chatterjee and Prakash, 1996 and 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.

Phenotypic variability among cell and tissue culture-derived regenerants may be attributed to epigenetic, genetic, and chromosomal changes induced by the culture conditions (Evans and Reed, 1981; Sibi, 1984; Evans *et al*., 1984; D'Amato, 1985; Karp, 1986; Vasil, 1988; Stelly, 1989; Wersuhn, 1989; Oono, 1991 and Skirvin, 2000). The culture-induced variants have been termed "calliclones" (Skirvin and Janick, 1976 and Skirvin, 1978), "protoclones" (Shepard *et al.*, 1980), and a widely used term "somaclones" (Larkin and Scowcroft, 1981).

The frequency of somaclonal variation is at a higher rate (upto 10% per cycle of regeneration) than chemical- or radiation-induced mutation.This makes somaclonal variation a viable alternative to mutagenesis and a valuable tool for a plant breeder to introduce variation into breeding programs (Skirvin, 2000). Epigenetic variations are due to the results of culture stress and these variations are not transmitted from generation to generation. Thus, these changes are acquired traits and are not genetically controlled.

The genetic variations are induced during culture due to single nuclear gene mutations. The mutants exhibit Mendelian inheritance. A large number of plant species have been regenerated from cell and tissue cultures

carrying somaclonal variation; the nature of mutation has been elucidated in only a few cases.

A majority of morphological variants observed among the regenerated plants are due to numerical (aneuploidy, polyploidy) and structural (deletions, duplications, interchanges, inversions) chromosome changes induced during the culture. Generally, a high frequency of regenerants from diploid species carries normal chromosome complements. On the other hand, regenerants from polyploid species such as sugarcane, wheat, oat, triticale, potato, and tobacco have a comparatively higher frequency of plants with aberrant chromosome numbers. This is due to the fact that polyploidy species can tolerate, to a greater extent than true diploid species, aneuploidy, because of the buffering capacity of the polyploid condition.

Despite many potential uses claimed for somaclonal variation, and substantial efforts by scores of individuals, the fact remains that thus far, there is not a single example of any significantly important new variety of any major crop species developed as a result of somaclonal variation (Vasil, 1990).

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). Among them cytological markers have proved to be useful and reliable markers in breeding and genetic studies of plant species due to consistency in results obtained from them.

Cytological investigations involving chromosome analysis have been considered useful not only in characterization of germplasm but also for the evaluation of genetic integrity of *in vitro* regenerated plants (Singh and Srivastava, 2004). For all such studies, cytological characters including chromosome number and karyotype analysis have been reported as reliable guides (Davis and Heywood, 1963; Moore, 1968; Stace, 1980 and Soliman, 2002). Das *et al.* (1995) and Stace (2000) have considered determination of chromosome number and karyotype analysis as a primary requirement for assessing the genomic status of any plant species. While analyzing karyotype, chromosome morphology is usually studied on the basis of the position of the primary constriction or centromere (Levan *et al.*, 1964 and Adhikary, 1974). The differences and similarities in the karyotype are regarded as parameters of variations, as well as distances or closeness of affinities (Sharma and Sharma, 1999). The mechanisms and pathways of alterations in chromosome complement are also reflected in the karyotype, which provides an index of variability.

Cytological evaluation in terms of karyotype, pairing behaviour of chromosomes and their segregational pattern have been conducted for the assessment of genetic stability in micropropagated plants of *Aconitum balfourii* (Pandey *et al.*, 2004), *Foeniculum vulgare* (Bennici *et al.*, 2004), *Chlorophytum arundinaceum* (Lattoo *et al.*, 2006), *Curcuma longa* (Panda *et al*., 2007) and *Phoenix dactylifera* (Abdalla and El-Kawy, 2010). On the basis of chromosome number and morphology cytogenetic stability have also been observed in long term cultures of *Wrightia tomentosa* (Khan, 2010) and *Achras sapota* (Chittora, 2012).

Chromosome instability in tissue culture is a very common phenomenon, which is induced by media components, culture age, explants tissue and even plant genotype (Peschke and Phillips, 1992). There have been many reports of aneuploidy induced by tissue culture (Karp and Maddock, 1984; Swedlung and Vasil, 1985; Evans and Sharp, 1986; Lee and Philips, 1988 and Karp, 1991). Such unbalanced conditions are often associated with propagation techniques involving callogenesis or cell culture (Karp *et al*., 1982). Aneuploidy phenomena have also been observed in *Triticum aestivum* regenerated by direct organogenesis (Karp and Maddock, 1984). Chromosomal abnormalities and aneuploidy generated by tissue culture have often been noted in polyploid species (Lee and Philips, 1988 and Karp, 1991).

Although, chromosomal analysis is a very common parameter for evaluation of fidelity, but its application in a number of cases has proved limiting on account of small chromosome size (e.g. tree species), their high number (Varshney *et al.*, 2001) and difficulty in obtaining metaphase cells required for such analyses. In addition, karyological analysis cannot reveal alternation in specific genes or small chromosomal rearrangements (Isabel *et al.*, 1993).

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