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Exploitation of somaclonal variations in improvement of fruit crops - A review

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Abstract

Clonal propagation through micropropagation is hailed as a revolutionary technology as it can be achieved in a short time and space with limited number of plant propagules. Recent studies have shown that cell or tissue cultures undergo frequent genetic changes. Variants selected in tissue cultures have been referred to "somaclonal variation". Though, genetic variations may be considered obstructive and worthless from the point of clonal fidelity, it opens a window of opportunity for increased genetic variability relatively rapidly and without applying a sophisticated technology, which may itself have numerous applications in plant breeding and genetic improvements. The recovery of novel variants can be enhanced by applying suitable *in vitro* selection pressure. Tissue culture induced somaclonal variation in fruit crops is similar to variations induced with chemical and physical mutagens, which proffers an opportunity to unearth natural variability for their potential utilization in crop improvement.

Key Words: *In vitro* propagation, genetic variation, Somaclones, crop improvement

Introduction

Vegetative propagation is primarily used to produce progeny plants, which is identical in genotype to a single source mother plant. The biological process of producing identical plants is referred as "cloning", while the resulting population of plants, derived through cloning, is termed as a "clone". The *in vivo* clonal propagation of fruit crops is often cumbersome, expensive and even unsuccessful. Alternatively, tissue culture methods or micropropagation can be employed as a means of vegetative propagation for clonal multiplication. Clonal propagation through micropropagation can be achieved in a short time and space (Razdan, 2003). The uniformity of individual plants within a clone population is a major advantage of clonal cultivars in commercial production. However, it is well known now that genetic variations occur in undifferentiated cells, isolated protoplasts, calli, tissues and morphological traits of regenerated plants. Recent advances have revealed that cell or tissue cultures undergo frequent genetic changes (polyploidy, aneuploidy, chromosomal breakage, deletion, translocation, gene amplifications and mutations) and that these are also expressed at biochemical or molecular levels. Variants selected in tissue cultures have been referred to "somaclonal variation". Variation of any kind, in particular, genetic variations may be considered obstructive and worthless; since, such variations may lead to loss of genetic fidelity and as a result, trouncing of desirable characteristics of *in vitro* raised plants. However, plant cell and tissue cultures provide increased genetic variability relatively rapidly and without applying a sophisticated technology, which may itself have numerous applications in plant breeding and genetic improvements. The recovery of novel variants can be enhanced by applying suitable *in vitro* selection pressure (Jain, 2001).

Genetic variation is a vital element of any traditional crop breeding programme. In general, a typical crop improvement cycle in fruit crops requires a minimum of 10-15 years in order to complete various stages of crop improvement such as germplasm manipulations, genotype selection and stabilization, variety testing, variety multiplication, intellectual protection and crop production stages. Plant tissue culture is an enabling technology from which many novel tools have been derived to help out plant breeders (Karp, 1991). Tissue culture induced somaclonal variation in fruit crops is similar to variations induced with chemical and physical mutagens (Jain, 2001), which proffers an opportunity to unearth natural variability for their potential utilization in crop improvement.

Like any other technology, *in vitro* induced somaclonal variation has its own intrinsic advantages and disadvantages, which have been indicated in Table 1.

Somaclonal variation has been most successful in crops with limited genetic systems (e.g., apomicts, vegetative reproducers) and/or narrow genetic bases. In ornamental plants, for instance, the exploitation of *in vitro*-generated variability has become part of the routine breeding practice of many commercial enterprises. In

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addition, somaclonal variation can become a part of plant breeding provided they are heritable and genetically stable. Only a limited numbers of promising varieties so far had been released using somaclonal variations. This is perhaps due to the lack of interaction between plant breeders and tissue culture scientists, and non-predictability of somaclones (Jain, 2001).

Molecular basis of somaclonal variations

Several bases of somaclonal variation have been proposed by various researchers, which comprise changes in chromosome number (Mujib *et al.*, 2007), point mutations (D'Amato, 1985), somatic crossing over and sister chromatid exchange (Duncan, 1997), chromosome breakage and rearrangement (Czene and Harms-Ringdahl, 1995), somatic gene rearrangement, DNA amplification (Karp, 1995), changes in organelle DNA (Cassells and Curry, 2001), insertion or excision of transposable elements (Gupta, 1998), DNA methylation (Guo *et al.*, 2007), epigenetic variation (Kaeppler *et al.*, 2000; Guo *et al.*, 2006) and segregation of pre-existing chimeral tissue (Brar and Jain, 1998; Vazquez, 2001).

Sources of variations detected in plant tissue culture

Different factors such as explant/explant source (Sahijram *et al.*, 2003), mode of regeneration (Shen *et al.*, 2007), length of culture period and number of subculture cycles (Kuznetsova *et al.*, 2006; Mohanty *et al.*, 2008), culture environment (Chawla, 2002; Siragusa *et al.*, 2007) and genotype & ploidy (Hossain *et al.*, 2003; Thieme and Griess, 2005) affect the frequency of development of somaclones under *in vitro* conditions.

Recovery of somaclonal variants

Though the somaclonal variants are noted at several occasion during micropropagation, their frequency from the point of fetching new variations for breeding purpose is usually low. The recovery of variants can be improved by promoting the factors which are responsible for the development of somaclonal variations such as use of callus and cell suspension culture for several cycles and regeneration of large number of plants from long-term stored cultures. In addition, plant genotype is a major factor, which determines the type and frequency of somaclonal variation. The efficiency of recovering variants *in vitro* can further be enhanced by putting selection pressure through screening of desirable traits, e.g. *in vitro* selection for tolerance against abiotic and biotic stresses. This attains more significance in view of the fact that the selection of desirable traits takes several years and many generations under field conditions. *In vitro* selection can shorten considerably the time for the selection of desirable traits under *in vitro* selection pressure with minimal environmental interaction, and can complement field selection (Jain, 2001).

The recovery of somaclones can be further increased by combining micropropagation technique with *in vitro* induced mutagenesis. Kuksova *et al.* (1997) suggested that somaclonal variation and mutagens can be combined to enhance the frequency of induced mutations.

Table 1. Advantages and disadvantages of somaclonal variations

Advantages	Disadvantage
• Cheaper than other methods of genetic manipulation. • Tissue culture systems are available for many plant species. • Not necessary to have identified the genetic basis of the trait, or indeed, in the case of transformation, to have isolated and cloned it. • Novel variants have been reported among somaclones. • Variation may be generated from different locations of the genome than those, which are accessible to conventional and mutation breeding. • No possibility of obtaining chimeric expression if somaclones are raised through cell culture.	• Inability to predict the outcome as they are random and lack reproducibility. • The variations are usually negative. • Positive changes are also altered in negative ways, sometimes. • There are chances that the changes are not novel. • The changes may not be stable after selfing or crossing. • No *in vitro* selection methods exist for complicated traits such as yield, solids, sweetness, texture or shelf life

Table 2. In vitro selection of desirable traits and development of some commercially exploited varieties through somaclonal variations in different fruit crops

S. No.	Horticultural Crop	Characteristic of somaclone	Reference
1.	Apple (*Malus domestica* Borkh.)	Resistance to *Erwinia amylovora*	Chevreau *et al.* (1998)
2.	Apple rootstocks M 26 and MM 106 (*Malus pumila* Mill.)	Resistance to *Phytophthora cactorum*	Rosati *et al.* (1990)
3.	Banana (*Musa acuminata* L.)	Semi-dwarf and resistant to *Fusarium* wilt TC1-229	Tang *et al.* (2000)
		Var. CIEN-BTA-03, resistant to yellow Sigatoka	Gim□nez *et al.* (2001)
		Larger bunch size var. TC2-425; Resistant to *Fusarium oxysporum* f. sp. cubense (Foc) race 4; bunch 40% heavier Formosana	Hwang (2002)
		Var. CUDBT-B1, reduced height and early flowering	Martin *et al.* (2006)
4.	Blackberry	Thornless var. Lincoln Logan	Hall *et al.* (1986)

5.	*Citrus* spp.	Resistant to *Phoma tracheiphila*	Deng *et al.* (1995)
		Salinity tolerance	Ben-Hayyim and Goffer (1989)
6.	Grapevine (*Vitis vinifera* L.)	Resistant to *Botrytis cinerea* and *Plasmopara viticola*	Kuksova *et al.* (1997)
7.	Mango (*Mangifera indica* L.)	Resistant to *Colletotrichum gleosporiensis*	Litz *et al.* (1991)
8.	Peach (*Prunus persica* L.)	Resistant to root-knot nematode (*Meloidogyne incognita* Kofoid and White)	Hashmi *et al.* (1995)
		Resistant to bacterial canker (*Pseudomonas syringae* pv. *syringae*)	Hammerschlag (2000)
9.	Pear (*Pyrus* sp.)	Resistant to *Erwinia amylovora*	Viseur (1990)
10.	Pineapple (*Ananas comosus* L., Merr.)	Spineless variant	Jaya *et al.* (2002)
		Cvs. P3R5 and Dwarf, variation in fruit colour, growth habit, fruit size and length of plant generation cycle	Perez *et al.* (2009)
		Improved size, shape, appearance, starch content and starch yield	Thieme and Griess (2005)
11.	Quince A (*Cydonia oblonga*)	High soil pH	Dolcet-Sanjuan *et al.* (1992); Marino *et al.* (2000)
12.	Strawberry (*Fragaria* sp.)	Resistant to *Fusarium oxysporum f. sp. fragariae*	Toyoda *et al.* (1991)
		Resistant to *Alternaria alternate*	Takahashi (1993)
		Resistant to *Phytophthora cactorum*	Battistini and Rosati (1991)

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