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Tissue Culture Induced Variability in some Horticultural Important Ornamentals: Chromosomal and Molecular Basis-A Review

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Abstract: Ornamentals are important groups of plants in plant kingdom. Conventionally, these groups of plants propagate vegetatively. During the last few decades plant tissue culture method has been used successfully for commercial production of a wide range of economic and elite plants including ornamentals. Although the *in vitro* technology assures true-to-type clones, tissue culture-induced variations are also common in plants. In this present review, the authors discussed various observed changes in tissue-culture regenerated plants. The changes have been categorized into three different types such as chimerical, temporary or physiological and somaclonal variation, both heritable and non-heritable in nature. The causes of variation, detection mode and related mechanism that contribute variation have been discussed in ornamentals. The communication also highlighted karyotypic alterations, sequence change, DNA methylation and the involvement of transposable elements with respect to cultural variation. The creation and rearrangement of new chimera, *in vitro* separation of chimera and identification of superior somaclonal variants may offer new varieties to growers for commercial plantations.

Key words: Chimera, ornamentals, somaclonal variation, tissue culture induced variation

INTRODUCTION

Ornamental is an important groups of plants in plant kingdom. These groups of plants not only add aesthetic values it also contributes in forming a billion dollar business (Rajagopalan, 2000). The group can be divided into three different types: (a) Cut flowers plants-produce cut flowers such as roses, carnations, gerbera, orchids etc. (b) Foliage plants-cultivated only for their foliage beauty e.g., *Philodendron, Monstera*, *Calathea*, *Peperomia*, *Difienbachia* etc. and (c) Bulbous plants-produce bulbs with or without flowers, often used as a pot plant e.g., *Lilies amaryllis*, *Begonia Zephyranthes*, *Freesia*, *Hippeastrum* etc. Conventionally, these groups of plants propagate vegetatively which is a slow process. Since early 1970s, plant cell culture method has been practised as an alternative method of propagation and over 150 ornamental genera are being propagated through tissue culture world wide (Rout *et al.*, 2006). The technique has several other advantages including the creation of novel genetic variation (Table 1) like plant's morphology and leaf shape, pigmentation in leaf and flower and in physiological attributes like long vase-life. The genetic variability isconsidered to be the raw material for evolution and is very important in groups like ornamentals where propagation is mostly vegetative. These variations (phenotypic and DNA alterations) are both genetic and epigeneticin nature (Kaeppler *et al.*, 2000). The *in vitro* methodologies enhance variation frequency, increase utilization possibility (Gavazzi *et al.*, 1987) and improved cell lines/plants were also obtained (Hammerschlag *et al.*, 1995; Duncan, 1996; Brar and Jain, 1998; Veilleux and Johnson, 1998). In *in vitro* culture, the addition of mutagens (physical and chemical) are also used in order to create new cell lines and plants with improved traits were developed (Mehta and Angra, 2000; Predieri, 2001; Emaldia *et al.*, 2004; Jain, 2006; Wang *et al.*, 2007). In this present communication, various types of variations observed in plant cell culture have been discussed; the nature, the possible causes, the ways of detection and other mechanisms influence variations have also been described in ornamentals.

Chimeral variation: A chimeric plant is an individual where the same tissue contains more than one genotypic constitution. In meristem, two histogenic (outer and inner)

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Table 1: Tissue culture-induced variation in ornamentals, the cause of changes and studies of detection

Plant	Possible cause of observed changes	Mode of detection	Citation
Alstroemeria spp.	X-ray exposure	Molecular marker analysis (RAPD)	Anastassopoulos and Keil (1996)
Anubia scongensis N.E. Brown	Gamma irradiation	Phenotypic morphological characters	Tangpong *et al.* (2009)
Asparagu sofficinalis L.	Prolonged sub-culturing	Cytogenetic and molecular (RAPD) analysis	Raimondi *et al.* (2001)
Asparagus officinalis L.	Old callus and use of 2,4-D	Phenotypic and morphological characters like fruit, seed setting, pollen viability; cytogenetic analysis (meiotic behaviour, ploidy status) and molecular study (AFLP profile)	Pontaroli and Camadro (2005)
Begonia x *elatior*	*In vitro* culturing conditions, explant source of donor	Phenotypic morphological traits	Jain (1993, 1997)
Saintpaulia ionantha L.	*In vitro* conditions and explants source of mother stock	Morphological traits	Jain (1993, 1997)
Begonia x *hiemalis* (Fotsch.)	Nitrosomethylurea (NMU), a chemical mutagen	Phenotypic analysis (qualitative and quantitative); use of marker like RAPD	Bouman and de Klerk (2011)
Chrysanthemum spp.	Mother plant's genotype	Molecular marker (RAPD) analysis	Minano *et al.* (2009)
Chrysanthemum morifiolium Ramat	Gamma rays	Morphological parameters	Lamseejan *et al.* (2000)
Caladium spp.	Use of high level of PGR like auxin	Leaf colour variation morphological characters; Molecular marker analysis (ISSR, RAPD)	Ahmed *et al.* (2004)
Caladium bicolour cv. Bleeding heart	*In vitro* culturing conditions, callus	Biochemical and chromosomal analysis	Mujib *et al.* (2008)
Codonopsis lanceolata Benth.et Hoof.f	*In vitro* culturing conditions	Morphological attributes; Molecular marker analysis (ISSR, RAPD, MSAP)	Guo *et al.* (2006, 2007)
Dianthus spp.	X-ray exposure	By using imaging technology	Cassells *et al.* (1993)
Dieffenb achia spp.	*In vitro* culturing conditions, explant source of donor	Morphological attributes	Shen *et al.* (2007)
Drosera anglica, *Dbinata*	*In vitro* culturing conditions	Molecular marker analysis (RAPD)	Kawiak and Lojkowska (2004)
Freesia spp.	*In vitro* culturing conditions	Use of molecular marker analysis (AFLP, MSAP)	Gao *et al.* (2010)
Gauralindheineri	Colchicine and trifluralin as chemical mutagen	Morphological characters	Pietsch and Anderson (2007)
Gentiana pannonica Scop.	*In vitro* culturing conditions	DNA content (flow cytometry); molecular analysis (HPLS-RP, met AFLP)	Fiuk *et al.* (2010)
Gerbera jamesoni Bolus	Explant source of donor	Molecular marker (ISSR)	Bhatia *et al.* (2009)
Heliconiabihai L.	Old aging callus	Morphological characters	Rodrigues (2008)
Hippeastrum hybridum	*In vitro* culturing conditions and/or pre-existing variation	Chromosomal analysis through root tip squash preparation	Mujib *et al.* (2007)
Hypericum perforatum L.	*In vitro* culturing conditions	Estimation of DNA by flow cytometry, chromosomal study	Brutovska *et al.* (1998)
Ledebouria graminifolia	*In vitro* culturing conditions	Phenotypic traits	Shushu *et al.* (2009)
Limonium perezii Hubbard	Separation of chimera during culture	Morphological parameters study	Kunitake *et al.* (1995)
Pelargonium graveolens	*In vitro* culturing conditions	Morphology of plant's trait	Saxena *et al.* (2000)
Petunia hybrida	*In vitro* culturing conditions, explant source of donor	Molecular analysis of rDNA (18S-25S) sequences	Anderson *et al.* (1991)
Phalaenopsis spp.	Tissue culture	Morphological characters; Biochemical study (isozymes); Molecular marker analysis like RADP	Chen *et al.* (1998)
Phalaeno psishsiang Fei	*In vitro* culturing conditions	Molecular analysis (cDNA-AFLP)	Hsu *et al.* (2008)
Philodendron erubescens	Nodal callus	Morphological traits	Mujib and Jana (1995)
Rheum rhaponticum L.	*In vitro* culturing conditions	Morphological and anatomical features	Zhao *et al.* (2005)
Robiniaambigua var. idahoensis	*In vitro* culturing conditions	DNA molecular marker (ISSR)	Guo *et al.* (2006)
Robinia pseudoacacia	Explant of donor	Molecular marker (RAPD)	Kanwar and Bindiya (2003)
Sinningi aspeciosa	Use of PGRs (NAA, 2,3,5-Triiodobenzoic acid-TIBA)	Plant morphological characters; Physiological analysis	Xu *et al.* (2009)
Stylosanthes guianensis (Aubl.) *Sw*	*In vitro* culturing conditions	Morphological traits; Physiological analysis	Rao *et al.* (1992)
Syngonium podophyllum Schott	*In vitro* culturing conditions	Molecular marker analysis (AFLP)	Chen *et al.* (2006)
Tricyrtishirta	Old callus and prolonged subculturing	Morphological traits and chromosomal analysis	Nakano *et al.* (2006)
Zephyranthus roseus	Callus	Molecular marker (RAPD)	Gangopadhyay *et al.* (2010)

layers are present. Change of inner or outer layer produces periclinal chimera and in sectorial chimera, a part of inner or outer histogenic layer is changed. The two forms of chimeras are very common in nature and are

found in several plants like *Dianthus, Hemerocallis*, *Malus*, *Acalypha*, *Caladium, Philodendron*, *Pelargonium, Rhododendron* and many of them are asexually propagated (Pogany and Lineberger, 2006). The periclinal chimeras remain stable during asexual propagation while tissue culture based propagation separates the chimeras in a number of plants like grapevine (Skene and Barlass, 1983), blackberry (McPheeters and Skirvin, 1983; Kunitake *et al*., 1995) reported chimera formation in statice (*Limoniumperezii)* in which protoclone from leaf segments of meristem derived plants showed abnormal flowers with poor pollen fertility. It was also observed that genetic fidelity depends on explants source and the selection of younger tissue is also important as it shows stability during culture. Different explant sources from the same mother stock cause variation (Kunitake *et al*., 1995) suggesting the importance of genetic uniformity of mother plant, requiredin establishing culture. The variants created by chimeralseparation is easily detected by the loss or gain of morphological and biochemical attributes (Mujib and Jana, 1995), distinguishable from the normal seedlings (Table 2). In others it was not detected phenotypically although changes were observed at different levels (Preil, 1986).

Pre-existing chromosomal variation, another type of variation is alsonoticed in non-chimeric plants that is not always separated during culture. In *Poa*, for example, no change in ploidy was reportedduring culture although the plant tissue composed of diploid meristematic and endopolyploid cells (Wu and Jampates, 1986).

Physiological or temporary variation: Variation of *in vitro* raised plant's morphology and performance was observed to be temporary in strawberry, potato, lily, apple etc. The regenerated plants showed morphological (leaf, phyllotaxy, spine and branch numbers), physiological and biochemical (altered flowering, seed-set, sex determination) and cytogenetical alterations (Zimmerman, 1986; Preil, 1986). This changed morphology and behaviour was noted to be due to rejuvenation, caused by the use of various added Plant Growth Regulators (PGRs) in medium. The PGRs commonly used for promoting juvenile phase are GA, BAP, Kinetin and ABA (not yet established). The mechanism of improved rejuvenation is not well elucidated, however. Similarly, variable *ex vitro* performance of tissue cultured plants was reported to be influenced by *in vitro* use of PGRs, in which plants received PGRs' signals early during development but with strong memory the plants demonstrate apparent physiological phenotypic alteration on outdoor conditions (Evans *et al*., 1986; Detrez *et al*., 1989), those changes oftenare temporary and non-heritable, later regain normal morphology and vigour with time (Swartz *et al*., 1981; Smith and Bhaskaran, 1988; Varga *et al*., 1988).

Somaclonal variation: Somaclonal variation is defined asquantitative and qualitative (phenotypic and genetic) alterations occurs among the clonally-propagated somaclones (Larkin and Scowcroft, 1981). It is however, rather difficult to demonstrate heritability in all plants as several of them are propagating asexually, some show sexual incompatibilities, seedlessness, polyploidy and long generation cycles (Skirvin *et al*., 1994). Currently, somaclonal variation is extended to all forms of tissue culture variations that includes protoclonal, gametoclonal and mericlonal variation (Karp, 1994; Chen *et al*., 1998; Kaeppler *et al*., 2000).

Several studies suggested that *in vitro* omutagenesis may add genetic variability and can be utilized in widening genetic bases of economically important plants (Rasheed *et al*., 2003; Orbovic *et al*., 2008). The cultural condition itselfis considered to be mutagenic and plantlets derived from callus, suspension and protoplasts often show genotypic and phenotypic changes (Bouharmont, 1994; Roy and Mandal, 2005; Orbovic *et al*., 2008). It has been identified as potential new tool for raising new varieties in different cultivated plants like sweet potato (Thieme and Griess, 2005; Wang *et al*., 2007), millet (Baer *et al*., 2007) and others.

Origin and sources

Karyotypic alteration: A number of mechanisms have been proposed forthe induction of somaclonal variation

Table 2: Shoot regeneration along with variants in *Philodendron erubescens* cv. Pink prince. MS medium was amended with various combinations of BAP and NAA

PGR (mg L^{-1})					
BAP	NAA	CH	No of shoots callus mass	No of variants callus $mass^{-1}$	Frequency
1.0	-	-	10.2 ± 1.83^{c}	2.6 ± 0.48^{d}	25.49^{e}
0.5	-	-	16.6 ± 2.65^{a}	3.8 ± 0.74^{b}	22.89^{d}
0.5	-	100	16.8 ± 2.40^{a}	4.4 ± 0.80^{a}	26.19^{b}
Liquid MS medium (without agar)					
0.5	0.1	-	13.6 ± 2.65^{b}	3.4 ± 1.01^{c}	25.19^{e}
0.5	0.2	-	13.2 ± 2.13^{b}	3.6 ± 1.20^{b}	27.27^{a}

Values are means and standard deviation. Means with common letters within a column are not significantly different at $p\leq0.05$ according to DMRT

Table 3: Regenerated shoots with some variants in *Caladium bicolor*. MS medium was amended with various combinations of NAA and BAP

PGR (mg L^{-1})				
BAP	NAA	No of shoots culture^{-1}	No of variants callus mass^{-1}	Frequency
0.5	-	13.25±1.75^{a}	0.80±0.83^{b}	6.03^{b}
1.0	-	11.75±2.50^{b}	0.60±0.89^{c}	5.10^{c}
0.5	0.5	3.75±1.75^{d}	0.20±0.44^{e}	5.33^{c}
1.0	0.5	12.25±1.70^{b}	1.20±0.83^{a}	9.79^{a}
2.0	0.5	6.0±2.090^{c}	0.40±0.89^{d}	6.66^{b}
4.0	0.5	1.25±0.75^{e}	0.0^{f}	0.0^{d}

Values are means and standard deviation. Means with common letters within a column are not significantly different at $p \leq 0.05$ according to DMRT

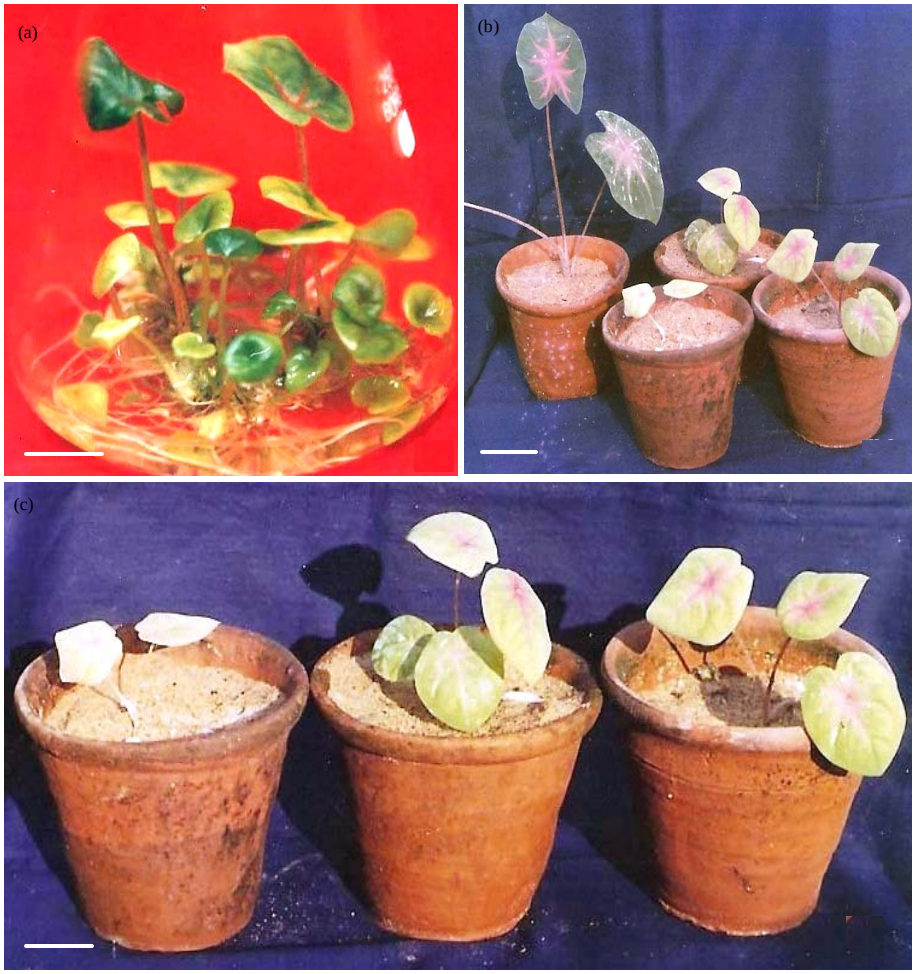

Fig. 1(a-c): Regenerated normal *Caladium* plant with variants (a) Regenerated *Caladium* plant grown in liquid MS amended with BAP (1.0 mg L^{-1}) + NAA (0.5 mg L^{-1}) and (b, c) Transplanted normal plant with variants of *Caladium* at two different stages, grown in outdoor condition (Bar a 2 mm; b, c = 1.0 cm)

of which karyotypic alteration is most important. Structural abnormalities and ploidy number changes have been the most prevalent changes among the tissue culture derived regenerants (Hao and Deng, 2002; Mujib *et al*., 2007, 2008) earlier reported 5-10% (Table 3) 'off-type' *Caladium* variants (Fig. 1) which exhibited noticeable chromosomal anomalies (Fig. 2) incytological preparations. Among rearrangement, translocations are the most common changes observed along with deletion and inversion. Other studies (Hang and Bregitzer, 1993; Kaeppler *et al*., 2000) suggested that frequent chromosomes breakage across the centromere and heterochromatin regions led to different types of cytogenetic abnormalities. It was also observed that

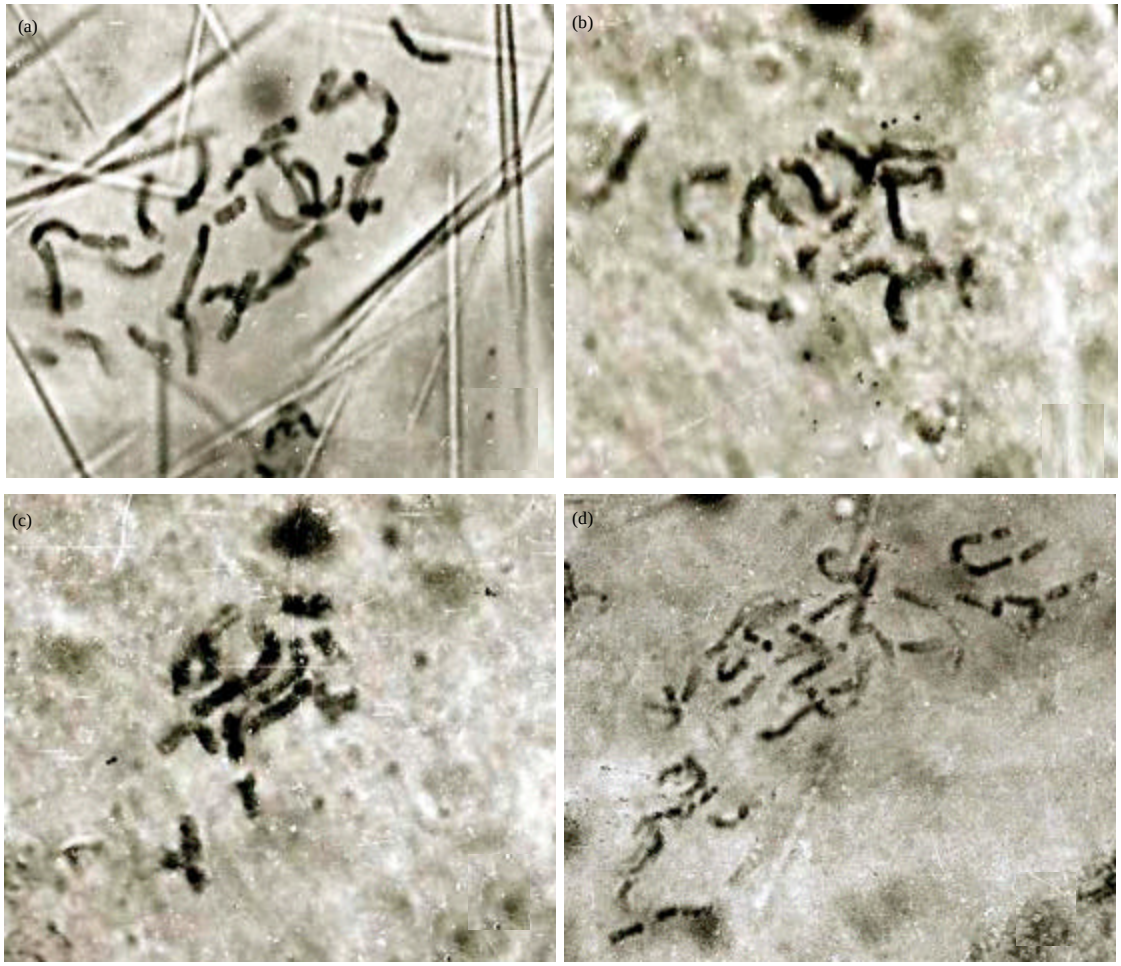

Fig. 2(a-d): Cytology of the regenerated plant (a) Regenerated root tips showing 2n = 28 chromosomes, (b-d) Metaphase plates showing altered chromosomal numbers [magnification (a-d): x 500 μ]

mitotic cell cycle disturbances cause chromosome rearrangement in tissue culture induced variations. The cell cycle has distinct four stages, namely G_1, S, G_2 and M, each has a 'species and cell' specific duration. Any irregularity that affects the programmed cell cycle duration, particularly chromosome replication stage at S, late replicating heterochromatin and post-chromosome replication repair event may induce chromosome aberration (Lee and Phillips, 1988). Karp (1994) reported errors at stage M (during microtubule synthesis, spindle formation and orientation, chromatid segregation) in protoplast culture that produced anomalies in chromosome number and structure. Early report also suggested mitotic crossing over and sister chromatid exchange induced chromosome rearrangements (Larkin and Scowcroft, 1981).

The disorganized tissues such as single cell, suspension and callus are important tissue source, causing more off-type variations than the organized tissues (Rani and Raina, 2000; Sivanesan, 2007), although there are contrasting opinion and several exceptions like in banana whereshoot tip, a composed and organized tissue exhibited somaclonal variation (Israeli *et al*., 1991). Sahijram *et al.* (2003) suggested the use of undifferentiated tissue like pericycle, cambium and pro-cambium are far more stable in culture and reduces the risk of causing variation. The mechanism of ploidy number changes is due to failure of mitotic apparatus (mitotic asynchrony), endo-polyploidy and polyteny (D'Amato, 1977), the irregularities are often marked by the appearance of bridges, laggards, bi, multi, or micro-nucleate conditions. These mitotic irregularities are

induced by PGRs like 2, 4-D and 2, 4, 5-T commonly used during *in vitro* culture and causing somaclonal variation in plants (Vidal and de Garcia, 2000; Martin *et al*., 2006; Jin *et al*., 2008). These synthetic auxin induces genetic variation and polyploidy by affecting normal DNA and post DNA replication mechanisms (Bouman and de Klerk, 2001; Ahmed *et al*., 2004; Mohanty *et al*., 2008).

The influence of cytokinin on the induction of somaclonal variation is, not however, that clear. The high concentration of BAP in the medium was reported to cause genetic variability in a number of studied plants of economic importance (Oono, 1985; Gimenez *et al*., 2001). The response is different and contrasting in other reports where high level of cytokinin failed to induce any somaclonal variation in investigated plant species (Reuveni *et al*., 1993; Gimenez *et al*., 2001). A number of biologically-active compounds like antibiotics, alkaloids, ethylene alcohol, DMSO, EDTA, Diphenylurea derivatives are either added or evolved during culture and these arereported to cause somaclonal variation in various plants byalterering DNA synthesis (Gecheff, 1989; Roels *et al*., 2005; Siragusa *et al*., 2007). Several other lines of research have shown that the observed somaclonal variations may also arise if the cultured tissues and sources are old and maintained for a long time with or without sub culturing (Stimart, 1986; Binarova and Dolzel, 1988; Reuven and Israel, 1990). The increasing number of passages and their duration enhanced the frequency of variation in culture (Reuven and Israel, 1990; Bairu *et al*., 2006). Rodrigues *et al.* (1998) noted somaclonal variation in *Musa* sp. from fifth passages onwards and the frequency of variation gradually increased with extra number of passages. In a number of other studied plants, tissues like multiple shoot and shoot tips showed genetic stability even though the cultures were kept *in vitro* for months to few years (Bennici *et al*., 2004; Smykal *et al*., 2007) suggesting the importance of selection of tissue source as starting material.

The frequency of somaclonal variation is influenced by inherent genotype of the mother plant (Popescu *et al*., 1997; Mehta and Angra, 2000; Martin *et al*., 2006). In banana cv. "New Guinea Cavendish" a higher level of *in vitro* genetic instability was observed than the other studied cv. "Willium" banana (Damasco *et al*., 1998). The same genotype-dependent genetic instability was also noted in *Coffea arabica* (Etienne and Bertrand, 2003).The plants with higher ploidy number hardly bother about the loss or gain of one or more number of chromosomes since the polyploids is added with extra set of chromosomes. In *Chrysanthemum*, the same normal morphology, growth and development was noticed in plants that had 1-3 extra chromosomes to its basic set (Dejong and Custers, 1986).

Sequence variation: Individual base pair changes is also not uncommon in nucleic acid, produced plants of altered morphology and nature (Groose and Bingham, 1984; Larkin *et al*., 1984). Although the incidence of sequence change and altered protein has been very limited, analyses of specific mutants indicate sequence change particularly A-T transversion in tissue culture-derived mutants (Brettel *et al*., 1986; Dennis *et al*., 1987). Different electrophoresis profiles of variant proteins detected this sequence change in nucleic acid. Sequence change has been detected successfully by using different molecular and DNA markers like Restriction Fragment Length Polymorphism, RFLP. RFLP detects somaclonal variation early in different tissues and has been utilized in various plant species. In oil-palm, Jaligot *et al.* (2000) used RFLP markers for differentiating altered embryogeniccalli from normal ones. Random Amplified Polymorphic DNA (RAPD) technique is also exploited in discrimination of tissue culture-induced variation from normal tissues in peach (Hashmi *et al*., 1997), orchids (Chen *et al*., 1998) and in bananas (Bairu *et al*., 2006). Amplified Fragment Length Polymorphism (AFLP) analysis is similarly used to study tissue culture induced somaclonal variation in several plant species (Sanchez-Teyer *et al*., 2003; Chuang *et al*., 2009). Microsatellite marker, also known as Simple Sequence Repeats (SSRs), Short Tandem Repeats (STRs), Sequence-Tagged Microsatellite Sites (STMS) and Simple Sequence Length Polymorphisms (SSLP) havealso been used to detect genetic fidelity in several studied plants (Ray *et al*., 2006; Welter *et al*., 2007).

Variation in DNA methylation: DNA methylation variation has been postulated for several tissue culture-induced alterations such as quantitative change, chromosomes breakage and other alterations during *in vitro* culture process (Kaeppler *et al*., 2000). Changes of methylation pattern of specific sites were noted and reported in different plant genera like rice, maize, banana and grapevine (Brown *et al*., 1991; Peraza-Echeverria *et al*., 2001; Guo *et al*., 2007; Baranek *et al*., 2010). Simultaneously, global methylation level was also reported to vary in response to different cultural and chimeral conditions (Arnholdt-Schmitt, 1995; Jaligot *et al*., 2000; James *et al*., 2004). Both kinds of analyses suggest DNA methylation variation is almost a general rule during the process of *in vitro* culture.

Activation of transposable element: Transposable elements are genetic elements that can move, make genetic rearrangements and cause mutation. Somaclonal variation involving such change has also been suggested when Kaeppler *et al.* (2000) reported the activation of quiescent transposable elements during culture process.

As a result and after heterochromatin modification a large-scale cytogenetic instabilities (which are unique during somaclonal variation) occur and phenotypic variation was noted throughthe modulation of genes. The observation also suggests that the DNA methylations are highly variable and DNA modifications are far more common in tissue culture raised plants compared to seed grown plants (Kaeppler *et al*., 2000). Similar observation was earlier noticed in other systems in which genomic shock produced from chromosome breakage activated transposable elements (McClintock, 1984). Earlier research analysis (Peschke and Phillips, 1991) showed similar incidences andcaused enhanced Ac and Spm/en transposable element activity in maize culture. Gao *et al.* (2009) noted somaclonal variation as a result of insertions of transposons in rice cultivar during *in vitro* regeneration time. Studies on transposons and quiescent retro-transposon in plant genome indicated that the epigenetic fragment that are silent in other time is activated during *in vitro* culture process and was responsible for somaclonal variation (Barret *et al*., 2006; Pietsch and Anderson, 2007). The insertion of transposon and retrotransposon into genome or with enhanced transcription activity however, did not result in altered phenotype.

Somaclonal variants can also be detected conventionally by studying morphological, physiological and biochemical parameters. Among the morphological characters, regenerant's stature, phyllotaxy, leaf morphology, leaf variegation, stomata number and size, pigmentation etc., are used to differentiate variants from normal plants (Israeli *et al.,* 1991; Zaid and Al Kaabi, 2003). However, it has long been known that phenotypic or morphological traits often additionally controlled by environment and environment and genomic interaction, therefore the changes need to be investigated more thoroughly and for at least extra couple of progenies *ex vitro*. Similar concern was earlier raised by previous workers while working on plants in which changes of genome constitution did not alter plant's phenotype (Jarret and Gawel, 1995; Mandal *et al*., 2001).

Physiological and biochemical analysis of early stages of plant tissues are conducted to detect and differentiate variants in populations as it does not require regenerated *ex vitro* plants always it also detects the variants quickly. Peyvandi *et al.* (2009) reported PGR and light dependent detection of variant somaclones from normal plants. Gibberellic acid sensitivity or in sensitivity in response to gibberellic acid treatment was obtained in plant species like banana (Graebe, 1987). Damasco *et al.* (1997) noted dwarf off-types, tolerant to low temperature and light compared to normal banana somaclones. Similarly, the synthesis and accumulation of photosynthetic pigments like chlorophyll, carotenoids and anthocyanins were used as biochemical markers in differentiating tissue culture induced variants from normal plants (Shah *et al*., 2003; Wang *et al*., 2007).

CONCLUSION

Several kinds of variations have been noticed in tissue culture propagated plants. Those variations which are genetic in nature, are induced by biochemical compounds and stress condition during culture. Altered phenotype may also arise by *in vitro* separation of pre-existing chimera. The variation occurs from dedifferentiated cells at the time of organogenesis and embryogenesis which utilizes single or multiple cells and at the same time *in vitro* cultural condition stimulates in regulating expression of pre-existing variations. Many of the changes are, however, temporary and sometimes mimic morphological and physiological variations, while a few types are non-expressive. Most of those variations are deleterious and a major problem to nurserymen and orchard people. Plant breeders, biotechnologists and other scientific communities are, however, very optimistic about the potential uses of variation in improving plant quality.

ABBREVIATIONS

2, 4-D: 2, 4-Dichlorophenoxyacetic acid
BA: 6-Benzyladenine
ISSR: Inter-simple sequence repeat
MS: Murashige and skoog
NAA: α-Naphthalene acetic acid
PGR: Plant growth regulator
RAPD: Random amplified polymorphic DNA
RFLP: Restriction fragment length polymorphism

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