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Somaclonal variations in horticultural crops

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Abstract
Tissue culture an *in vitro* technique of plant propagation has made it possible to develop a full plant from minute plant parts (explant) in a very short span of time. It is skilled process and require those people who are fully aware about this technical process. To assure stability and uniformity in this process is of immense significance. Sometimes this stable process of *in vitro* culture gets disturbed thus variations occur, which are called as somaclonal variations. Gene mutation or changes in epigenetic markings are the causes of modest somaclonal variations. These somaclonal variations can create some serious threats to the stability of this *in vitro* cloning. These variations inhibit the formation of true to type plant material. On the contrary, these somaclonal variations has become a hotspot for breeders as genetic variability is obtained rapidly without using Hi-tech and sophisticated technologies in horticultural crops. Both dominant and recessive mutations can lead to somaclonal variations. It has been seen that most of the Somaclonal variations are either useless or of limited use, but these variations are easy to detect than those of conventional mutagenesis. In this paper, we focused on reasons behind these variations and detection methods along with examples of horticultural crops and their applications has been reviewed.

Keywords: *In vitro*, genetic, epigenetic, horticultural, subculture, somaclonal variations

Introduction
When the plants are regenerated through *in vitro* culture the original expectations are the uniformity of the regenerated plants as the expectations was based on the fact that the entire process depends on the mitosis which is considered as the conservative mode of cell multiplication (Ryan *et al.*, 1987) [68]. As early as 1949, it was noticed that the meristems of tobacco plants infested with tobacco mosaic virus were free from virus by Limmasset and Cornuet. In 1952, successful experiment was conducted on Dahlia mosaic virus through *in vitro* culture of meristems by Morel and Martin (Atkinson and Mathews, 1970). [8] These studies lead to open new doors for breeders and researchers and gain their attention. The introduction of the term "Somaclonal Variation" is to describe the occurrence of genetic variation in plants which are being regenerated through *in vitro* culture or any sort of cell culture (Larkin and Scowcroft, 1981) [40]. It wasn't expected that plant tissue cultures can be useful source of variation in crop improvement (Karp, 1994) [35]. For those crops which possess polygenic attributes, it's essential to understand the value of beneficial potential of induced somaclonal variations, plant breeders through new cultivar generation by making the use of genetic manipulation that could exhibit increased yield, quality and disease resistance abilities (Nacheva *et al.*, 2014) [49]. Somaclonal variations have unpredictable nature, they can be genetic i.e., heritable in nature or epigenetic i.e., non-heritable in nature (Jain, 2001) [28]. But it is not possible to demonstrate heritable nature of these somaclonal variations because of polyploidy, seedlessness, long generation cycles or due to complex sexual incompatibilities (Bairu *et al.*, 2010) [11]. It can be difficult or almost impossible to explain the heritable nature of somaclonal variations in the plants possessing these characters (Skirin *et al.*, 1994). With the result of these somaclonal variations, some successful cultivars have been developed such as dwarf bananas, some potato cultivars, navel orange and also some resistant rootstocks which are frequently cultivated or used in order to get rid of from biotic and abiotic stresses (Ahloowalia, 1986) [2]. In this review, we have mostly focused on the origin and source of somaclonal variations, detection methods along with some examples and their implications in horticultural crops.

Origin and Source
Somaclonal variations are the minute changes but have wide range of effects and the effect can be positive or negative. There are numerous reasons behind the origin and causes of somaclonal variations and relates to plant tissue culture's interior and exterior characteristics (Karp, 1991) [33], like ageing of the callus and PGR's (Nehra *et al*, 1992) [50], light, temperature, agitation rate of culture media and osmolarity (Gould, 1984) [24].

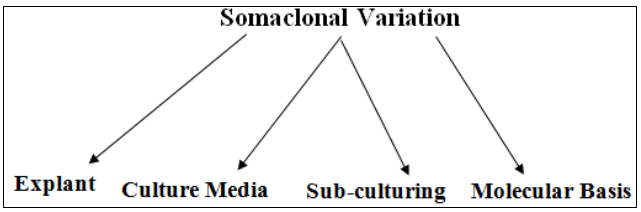

Fig 1: Sources of Somaclonal variation

Explant as a source of somaclonal variations
There may be changes in the frequency and type of somaclonal variation when regeneration is achieved from various tissue sources. (Sahijram *et al.*, 2003) [69]. As donar plant used as an explant in *invitro* cultures already exhibit somatic mutations sometimes, such as the existence of chimaera in explants, can also cause somaclonal variation (Karp, 1994) [35]. Non-embryogenic calluses produce more secondary metabolites, such as alkaloids, terpenoids, and phenolics, than embryogenic calluses under the same culture method (Rahmawati *et al.*, 2014) [61]. Furthermore, various phytochemical substances react

to the same media in different ways (Karalija and Paric, 2011) [32]. It has been seen that the explants taken from shoot tips and axillary buds with pre-existing meristems may yield some minor variations than those explants which have been taken from highly sophisticated or differentiated plant tissues such as stems, roots and leaves (Sharma *et al.,* 2007) [72]. But there exist some rare outliers also, as in case of banana where highly structured tissues such as shoot-tips induce more diversity than that of somatic embryogenesis, probably due to chimaera dissociation. (Israeli *et al.,* 1996) [26]. As a result, the frequency and form of somaclonal variation can be influenced by the tissue source (Chuang *et al.,* 2009) [15].

Culture media as a source of somaclonal variations:

It isn't still completely understood that how a single plant genotype can produce different phenotypic characters under similar *in vitro* culture conditions (Krishna *et al*., 2016) [39]. There exist some factors (external) like light, temperature, PGR's (plant growth regulators) and agitation rate of culture media which have an eminent effect on plant cell cycle *in vivo*, signifying that the improper cell cycle management *in vitro* is among the major causes which lead to somaclonal variance (Nwauzoma and Jaja 2013) [54]. Many researchers have shown that *in vitro* culture, alone or in combination with mutagenesis caused by physiochemical and biological agents, can be used to generate plants with higher genetic differences and mutants as a source of new commercial cultivars (Orbovic *et al*., 2008) [56]. Plants regenerated from organ cultures, protoplasts calli, and somatic embryogenesis occasionally show phenotypic and DNA variations, indicating that *in vitro* culture conditions can be mutagenic. (Orbovic *et al*., 2008) [56]. Within a single plant regenerated using different *in vitro* procedures, the degree of diversity varies (Biswas *et al*., 2009) [13].

PGRs play an important role in plant propagation in the plant tissue culture experiment and are thought to be one of the principal reasons of somaclonal differences in micropropagated plants (Košir *et al*., 2004) [37]. Numerous plant growth regulators, including 2, 4-dichlorophenoxy acetic acid (2, 4-D), naphthalene acetic acid (NAA) and 6 benzylamino purine (BAP), as well as synthetic phenylurea derivatives (4-CPPU, PBU, and 2, 3-MDPU), meta-Topolin (mT) have been blamed for genetic variation (Doležal *et al.,* 2007; Sun *et al.,* 2013; Sales and Butardo, 2014) [18, 78, 70]. According to reports, synthetic PGRs utilised during micropropagation cause stress in the culture environment by releasing cytotoxic by-products that cause cellular controls to be lost (Kaeppler *et al.,* 1998; Martins *et al*., 2004) [31, 44]. The experimental data demonstrated that clonal variability is rising in BA (6-benzyladenine) derived plants in both first and second regenerations, which could be related to BA's persistent toxicity and poor translocation rate. Despite being subjected to identical levels of *in vitro* stress, the incidence of clonal variability in mT-derived plants was substantially lower (Bhattacharyya *et al*., 2017) [12]. High levels of oxidative stress, which contribute to DNA damage and microsatellite instability in micro-propagated plantlets, could be the cause of such a phenomenon (Jackson *et al.,* 1998) [27]. Longer cultivation in 2, 4-D containing media causes callus cells to have larger DNA ploidy levels (Silva and Carvalho, 2014) [75]. In comparison to normal inflorescences, mantled inflorescences were found in oil palm (*Elaeis guineensis* Jacq.) contained higher amounts of cytokinins like isopentenyladenine 9-glucoside and lower levels of trans-zeatin 9-glucoside, dihydrozeatin riboside, and dihydrozeatin riboside 50-monophosphate (Ooi *et al*., 2013) [55].

Sub-culturing and number of cultures as a source of somaclonal variations

It has been revealed that the extent of somaclonal variations is increased by repeating the subculturing process and the duration of these subcultures, particularly in case of callus cultures and cell suspension. (Reuveni and Israeli, 1990; Bairu *et al*., 2006) [66, 11]. With increasing sub-culturing of micro-propagated bananas, it was found that in the rate of occurrence of variations was increased (Bairu *et al*., 2006) [11]. The higher the somaclonal variation, the longer a culture is kept *in vitro* (Jevremovic *et al.,* 2012; Sun *et al*., 2013) [78]. A statistical model was introduced with the number of multiplication cycles as the major parameter for forecasting the theoretical mutation rate. The model yielded two key conclusions: first that a variant rate rise is an exponential function of the number of multiplication cycles, and second, that variable off-type percentages can be expected after a given number of multiplication cycles (Cote *et al.,* 2001). Although the statistical technique is valuable for a better understanding of common experimental aspects in tissue culture, the model's application is limited due to the complexity of biological systems, as the authors agree. (Bairu *et al.,* 2011) [9]

Table 1: Comparison of genetic variations in all three consecutive regenerations of *Ansellia africana* as revealed by SCoT markers

Regeneration	SPAR method	Total no of bands amplified	Avg bands	Total no of PB	PP
1st	mT pathway	56	3.5	3	5.35
	BAP pathway	62	3.8	4	4.45
2nd	mT pathway	72	4.5	5	6.94
	BAP pathway	56	3.5	4	7.14
3rd	Acclimatized	70	4.37	5	7.14

*PP = percentage polymorphism; PB= polymorphic bands. (Bhattacharyya *et al*., 2017) [12]

Molecular basis as source of Somaclonal variations

Plant regeneration via indirect somatic embryogenesis include cell dedifferentiation (callus formation) and cell regeneration (regeneration), during which somaclonal alterations, including both genetic and epigenetic changes occur (Arencibia *et al.,* 1999) [7]. The uncontrolled proliferation of cultured tissue in explant callus development allows for genetic alteration and variances in the regenerated plants (Reisch *et al.,* 1981) [65]. Several explanations have been presented to explain somaclonal variation, which include changes in chromosome number (Leva *et al.,* 2012) [41], sister chromatid exchange and somatic crossing over (Bairu *et al*., 2011) [9], changes in organelle DNA (Bartoszewski *et al.,* 2007) [11] chromosome breakage and rearrangement (Alvarez *et al.,* 2010) [4], DNA amplification and somatic gene rearrangement (Tiwari *et al*., 2013), point mutations (Ngezahayo *et al*., 2007) [51], segregation of preexisting chimeral tissue (Ravindra *et al.,* 2012; Nwauzoma and Jaja 2013) [64, 54], DNA methylation (Linacero *et al*., 2011), epigenetic variation (Smulders and de Klerk, 2011) [77], RNA interference and histone modifications (Miguel and Marum, 2011) [45].

Table 2: Examples of plants with incidence of somaclonal variation, explant/ source, source of variation, detection methods and their desirability.

S. No	Crop	Scientific Name	Explant/Source	Source of Variation	Detection method	Desirability	Reference
1.	Apple	*Malus pumila* Mill.	Axillary buds	Number of subcultures	RAPD	No	Modgil *et al.* (2005) [46]
2.	Almond	*Prunus dulcis*	Axillary branching	Callus culture	RAPD and ISSR	No	Martins *et al.* (2004) [44]
3.	Dessert Banana	*Musa acuminate* L.	Shoot Apexes of suckers	Genotype, number of subcultures	RAPD	Yes/No	Sheidai *et al.* (2008) [74]
4.	Date Palm	*Phoenix dactylifera* L.	Shoot tips	Callus culture, Subculturing	RAPD-PCR	No	Al-Khateeb *et al.* (2019) [3]
5.	Garden asparagus	*Asparagus officinalis* L.	Lateral buds	Callus culture, 2,4-D, Duration in culture	Morphology, chromosome count	No	Raimondi *et al.* (2001) [62]
6.	Garden asparagus	*Asparagus officinalis* L.	Lateral buds	Callus culture, 2,4-D, duration of culture	Morphology, chromosome count, AFLP	Yes/No	Pontaroli and Camadro. (2005) [60]
7.	Ginger lime	*Citrus assamensis*	stem, leaf and root	Callus culture	Scanning Electron Microscope (SEM)	No	Yaacob *et al.* (2014) [82]
8.	Gladiolus	*Gladiolus grandifloras* L.	Cormel sprout	Callus culture, BAP	RAPD and ISSR	Yes/No	Noor-un-Nisa *et al.* (2012) [53]
9.	Pea	*Pisum sativum* L.	Cotyledonary internodes	Callus culture	RAPD and IRAP	No	Horáček *et al.* (2012)
10.	Pineapple	*Ananus comosus* L. Merr.	Base of the crown	Callus culture, colchicine treatment	Morphology, isozyme patterns, biochemical tests	No	Mujib, (2005) [48]
11.	Strawberry	*(Fragaria sp.)*	Terminal buds	Callus culture, BAP	Morphology, RAPD	Yes	Biswas *et al.* (2009) [13]

Detection of somaclonal variations

It is critical to detect somaclonal variation in order to choose somaclones that have all of the desirable characteristics of the parent cultivar plus the trait that it lacks. Phenotypic, cytological, biochemical, and molecular investigations provide an overview of horticulture crop improvement by examining the extent and type of somaclonal variations. These aid in the identification of beneficial variations with useful agronomic features, such as tolerance to biotic and abiotic stresses (Manchanda *et al*., 2018) [43]. Despite the occurrence of genetic mutations, *in vitro* produced plants are frequently phenotypically normal, making visual inspection inadequate to detect somaclonal abnormalities (Devi *et al.,* 2014). But various methods and techniques have been successfully utilized for the detection of these somaclonal variations and each of these methods has its own set of advantages and disadvantages, which are briefly mentioned below:

Morphological/Phenotypic markers

Morphological markers can be very useful tool in the detection of somaclonal variations (Nhut *et al*., 2013) [52]. For example, in case of banana (Musa spp. cv. Grand Naine), it was seen that different somaclonal variants show varying morphological characters which can be used as a differentiating agent (Abdellatif *et al*., 2012) [1]. Morphological criteria such as cane height, leaf morphology, bud form, and sugar concentration can easily detect somaclonal differences in case of sugarcane (Rastogi *et al*., 2015) [63]. Sometimes minor morphological differences can be noticed between control plants and *in vitro* cultured plants, therefore to check out the morphological variations, *in vitro* cultured plants should be first grown for field trails (Leva *et al*., 2012) [41]. Successful evaluation of somaclonal variations through morphological markers was done in some other horticultural crops such as chrysanthemum (*Dendranthema grandiflora* Kitam.) (Kengkarj *et al*., 2008) [36], olive (*Olea europaea* subsp. *europaea*) (Farahani *et al*., 2011) [21], potato (*Solanum tuberosum* L.) (Rietveld *et al*., 1991; Thieme and Griess., 2005) [67, 79] etc. Detection through this system has got some advantages like laboratory facilitation is not necessary, it can be used as a preliminary detection tool and some disadvantages like environmental effect is common and its time-consuming process.

Cytological markers

Ploidy, or the number and shape of chromosomes, is direct and convincing indication of a high possibility of variation in an organism's genetic arrangement (Al-Zahim *et al*. 1999) [5]. Changes in chromosomes, DNA and RNA are frequently used for the detection of somaclonal variations (Fiuk *et al*. 2010) [22]. This approach is useful for regular large-scale ploidy level studies but chromosome counting may be time consuming. Out of 20 'tapestry' caladium variants identified, five variants gained one, two or three chromosomes while as four variants lost one chromosome when compared to wildtype (Cao and Deng., 2020) [14]. This method was used to detect somaclonal variations in various horticultural crops such as potato (*Solanum tuberosum* L.) (Sharma *et al*., 2007) [72], Brasilero bananas (*Musa acuminata* L.) (Gime´nez *et al*., 2001).

Biochemical/Physiological markers

Plant responses to physiological stimuli like light and hormones can be used to distinguish among normal and mutant somaclones (Peyvandi *et al*. 2009) [58]. Physiological responses for finding variations are faster than morphological detection and can be performed at juvenile stages, reducing the potential for economic loss. There are different isozymes and their work is specific. One or more bands are visible depending on the number of loci, their state of homozygosity or heterozygosity, and the specific isozymes utilised, and the polymorphism of the bands exposes variation. There was a time when isozymes were frequently used for the detection of somaclonal variations (Weising *et al*. 2005) [81] however, researchers no longer use this methodology to find variations, instead opting for more sensitive methods. Because this approach is sensitive to ontogenic changes and other environmental conditions, it should be used with caution and there is limited number of isozymes.

Molecular markers

Molecular examination of DNA variants is sensitive, allowing for the discovery of alterations that aren't visible at the

morphological level. Furthermore, unlike morphological and physiological approaches that evaluate regenerated or adult plant response, molecular techniques allow for the discovery of variations in the callus or juvenile phases (Anil *et al*., 2018) [6] Because comprehensive monitoring of multiple developmental phases on the plants is insufficient to discover somaclonal variation, detecting somaclonal changes by morphological and physiological features can be quite time consuming (Palombi and Damiano, 2002). Single primer amplification reactions (SPAR) have been identified as a useful approach for detecting somaclonal differences in plantlets (Devi *et al*., 2014). The most common markers employed for this purpose are random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), direct amplification of minisatellite DNA region (DAMD), AFLP (Amplified Fragment Length Polymorphisms) and MSAP (Methylation-sensitive amplified polymorphism). These markers are widely used because to their ease of use and consistency of results (Mahto *et al*., 2018; Sai Sudha *et al.*, 2019; James *et al*., 2004) [29]. The use of these markers helps in identification of pest-tolerant variants as well as the evaluation of medicinal plant genetic diversity (Thilaga *et al.*, 2017; Bharti *et al.*, 2018). The use of a single DNA marker to measure somaclonal variation is insufficient because genetic variability can go undetected because point mutations occur outside of the priming locations (Lakshmanan *et al*., 2007). For a better assessment of genetic variation, multiple DNA amplification techniques are proposed so that diverse sections of the genome can be investigated (Palombi and Damiano, 2002). Various horticultural crops have been examined for somaclonal variations using molecular marker approach, such as Banana by using RAPD, AFLP and MSAP (Muhammad and Othman., 2005; James *et al*., 2004) [47, 29], Strawberry through RAPD (Biswas *et al*., 2009) [13], Coffee with the help of AFLP **(**Sanchez-Teyer *et al*., 2003) [71] etc.

Table 3: Advantages and Disadvantages of Somaclonal variations

	Advantages	Disadvantages
1.	It is less expensive than other genetic alteration technologies and does not necessitate ‘containment' processes. (Krishna *et al*., 2013) [38]	One of the primary drawbacks of somaclonal variation is that, despite the identification of elements that influence a certain plant species' variation response, the output of a somaclonal programme cannot be predicted because it is random and lacks reproducibility. (Karp, 1992) [34]
2.	It is not necessary to have recognized the trait's genetic origin, or even to have isolated and cloned it in the case of transformation.	In several horticultural crops, numerous approaches for selecting somaclones resistant to numerous biotic and abiotic challenges have been developed; however, there are no *in vitro* selection procedures for sophisticated attributes such as texture, soluble solids, yield, sweetness, or shelf life (Evans, 1989) [19].
3.	If somaclones are raised through cell culture, there is no way to get chimeric expression (Evans, 1989) [19].	Somaclonal modifications have only been used in a small number of promising variants so far. This could be due to a lack of communication among plant breeders and tissue culture experts, as well as the unpredictable nature of somaclones (Jain, 2001) [28].
4.	There are more plant species available in tissue culture systems than can be managed via somatic hybridization and transformation at this time.	Despite the fact that somaclonal variation has produced new varieties, improved variants have not been selected in many cases because (1) the variations were all negative; (2) Changes that were positive were also modified in negative ways; (3) The modifications were not novel; or (4) After selfing or crossing, the modifications were not stable. (Karp, 1992) [34].
5.	Somaclones have been found to have novel variations and research suggests that tissue culture can alter the incidence and distribution of genetic recombination events. This means that variation can arise from areas in the genome that aren't accessible to conventional and mutant breeding techniques. (Karp, 1992) [34].	The somaclonal technique generates random variation, with both undesirable and desirable variants recovered. As a result, screening the progeny of a large number of regenerated plants is required to assure the return of desirable variations. (Krishna *et al*., 2016) [39]
6.	Somaclonal diversity has been most successful in crops with limited genetic systems (e.g., apomicts, vegetative reproducers) and/or narrow genetic bases. In the case of ornamental plants, for example, the use of *in vitro* generated diversity has become standard practice in many commercial breeding operations.	The variance is based on the cultivar, and the frequency of change varies. (Karp, 1994) [35]

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