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Somoclones and Somaclonal Variants: A Review

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ABSTRACT

The maintenance of top genotypes that have been selected for their superior features and clonal proliferation require high level of genetic homogeneity among the regenerated plants. Plant tissue culture may nevertheless result in the production of genetic diversity or somaclonal variants due to gene mutation or changes made to epigenetic markers. Modest somaclonal variation can develop during *in vitro* cloning and can harm germplasm preservation. Numerous techniques have been used to assess the genetic fidelity of the *in vitro* generated progenies, including morpho-physiological, biochemical, cytological and DNA-based molecular markers approaches.

Keywords Micro-propagation, Somaclones, Oxidative stress, Epignetic variation.

INTRODUCTION

For horticultural crops, methods for tissue culture plant growth provide an alternative to vegetative crop propagation (Krishna *et al*. 2016). Clonal propagation *via.* tissue culture, also known as micro-propagation, is possible in a constrained space of time (Eftekhari *et al*. 2012). The uniformity of individual plants within a clone population is a key advantage of clonal cultivars in commercial production (Krishna and Singh 2013). However, *in vitro*-produced plants do show genetic variances in their undifferentiated cells, separated protoplasts, calli, tissues and morphological characteristics (Currais *et al*. 2013). Larkin and Scowkraft coined the words "somaclonal variation" and "plant variants obtained from any form of cell or tissue cultures" in 1981. In a number of vegetable crops, such as tomato, cucumber, watermelon, plants produced using micro-propagation are currently preferred over plants propagated through conventional methods. On the other hand, plant cell and tissue cultures create more genetic diversity more quickly and without the need for sophisticated technologies. Furthermore, somaclonal variations require less time and space for *in vitro* screening of desirable traits than cross seedlings of perennial crops, which require a lot of time and space. The recovery of such distinctive variations can be facilitated by using the appropriate *in vitro* selection pressure, which may have a variety

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of uses in plant breeding and genetic development (Krishna *et al*. 2016).

Plant tissue culture variations and their sources

Despite being a valuable method for clonal replication, tissue culture frequently results in regenerants that have a wide range of somaclonal variations. Most of these somaclonal variations are caused by freshly formed mutations brought on by the tissue growth process (Sato *et al*. 2011b). The causes of mutations in tissue culture have been connected to various stressors, including wounding, exposure to sterilants during sterilisation, incomplete tissue (protoplasts as an extreme example), media component imbalances (such as a high concentration of plant growth regulators (auxin and cytokinins), sugar from the nutrient medium as a replacement for photosynthesis in the leaves), lighting conditions and the disrupted relationship between high humidity and transpiration (Sato *et al*. 2011b, Smulders and de Klerk 2011). The damage caused by oxidative stress on plant tissues during *in vitro* culture may be the cause or connected to a large portion of the diversity shown in micro-propagated plants (Nivas and D'Souza 2014). In reaction to oxidative stress, pro-oxidants or reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl, peroxyl and alkoxyl radicals are increased. These ROS may alter DNA's hyper- and hypomethylation, change the number of chromosomes from polyploid to aneuploid, break chromosome strands, cause chromosome rearrangements and cause DNA base deletions and substitutions. They all have the potential to alter plant cells cultured *in vitro*. Somaclonal variation and induced mutation exhibit a similar spectrum of genetic variety because they both result in a wide range of qualitatively similar DNA modifications (Krishna *et al*. 2016).

Explant/explant source

The frequency and kind of somaclonal variation may vary when various tissue sources are used for regeneration. Explants with pre-existing meristems, such as shoot tips and axillary buds, often do not produce as many variations as highly differentiated tissues like roots, leaves and stems (Krishna *et al*. 2016). In general, adventitious shoot regeneration (also known as shoot organogenesis) occurs from atypical points of origin directly or indirectly through a callus stage, such as from leaves, petioles, shoot internodes, root segments, anthers, hypocotyls, cotyledons. The older and/or more specialised the tissue used for regeneration, the higher the chances that variation will be recovered in the regenerated plants (Table 1) (Pijut *et al*. 2012). An illustration of how somaclonal variation may develop from somatic mutations already present in the donor plant is the appearance of chimaera in explants (Krishna *et al*. 2016).

Approach to regeneration

Explants are subjected to oxidative stress during both the start of the culture and the subsequent subculture, which may cause mutations (Krishna *et al*. 2016). It is obvious that stressful processes like protoplast culture and callus development exist (Smulders and de Klerk 2011). The tissue culture method determines the extent of this stress. Therefore, cultures that undergo a callus phase are the ones that potentially

Table 1. Somaclonal variations and how they are impacted by the explants used.

Sl. No.	Crop species	Explants/explants source	Presence or absence of somaclonal variations (+/-)	References
1.	Brinjal (*Solanum melongena*)	Hypocotyl	-	Mallaya and Ravishankar (2013)
		Callus induction on leaves, nodes and intermodal explants	+	Naseer and Mahmood (2014)
2.	Patchouli (*Pogostemon patchouli*)	Callus induction on internodal and leaf explants	+	Ravindra *et al*. (2012)
3.	Potato (*Solanum tuberosum*)	Callus cultures of stem explant	+	Krishna *et al*. 2016

encourage a higher mutation rate, but the generation of plants by axillary branching does not typically result in the production of variations (Zayova *et al*. 2010). According to research (Saravanan *et al*. 2011) there is higher chromosome variability in the callus phase than in adventitious shoots, which suggests a loss of competence in the more severely disrupted genomes. The various degrees of perturbation that the cells experience may help to explain this. In the first instance, cells divide according to a pattern that is typical of a developing plant. Contrarily, callus development denotes a time of dedifferentiation followed by unchecked cell divisions (Krishna *et al*. 2016). A protoplast preparation, for instance, in which the breakdown of the cell wall mirrors the infectious process of some viruses, is one example of how some types of tissue culture imitate, in some ways, other stressful environments. Therefore, depending on the approach employed, different types and levels of stress are applied to cultivated cells. Contrary to popular perception, genetic variety could be shown in plants that spontaneously grew from explants instead of needing to develop an unstructured callus (Bhojwani and Dantu 2013). Somatic embryogenesis is occasionally chosen as the primary mechanism for producing propagules for regeneration under *in vitro* circumstances. Regeneration by embryogenesis has been said to have a better likelihood of producing genetically homogeneous plants than organogenic differentiation. This is the case because DNA methylation levels are lower in the early stages of somatic embryogenesis than they are in the later stages. *In vitro* cultures of numerous vegetable crops, including potato (Krishna *et al*. 2016), tamarillo (Currais *et al*. 2013, Krishna *et al*. 2016) and brinjal have been reported to vary (Naseer and Mahmood 2014).

Effect of the number of subculture cycles and the length of the culture period

The amount of somaclonal variation increases with the length of time a culture is kept *in vitro* (Jevremovic *et al*. 2012, Sun *et al*. 2013). With increasing callus age, it has been discovered that more variant karyotypes accumulate, which generally increases the likelihood of variant plants being produced during subsequent subcultures (Zayova *et al*. 2010). Additionally, the quick growth of a tissue during micro-propagation may compromise its genetic integrity.

Culture media

It is known that external factors including growth regulators, temperature, light, osmolarity and culture medium agitation rate have a significant impact on the cell cycle *in vivo* in plants, indicating that insufficient control of the cell cycle *in vitro* is one of the causes of somaclonal variance (Nwauzoma and Jaja 2013). It is assumed that tissue culture disrupts the normal cell cycle controls that stop cell division before DNA replication is finished, leading to chromosomal breakage. *In vitro* aberrations are caused by chromosome breakage and its aftereffects (deletions, duplications, inversions and translocations) (Krishna *et al*. 2016). Plant growth regulators can increase the rate of multiplication and induce adventitious shoots, which can both directly and indirectly alter the rate of somaclonal variation (Gao *et al*. 2010). It is possible that some plant growth regulators (PGRs) could behave as mutagens when used in certain quantities, in conjunction with other growth regulators, or in combination with specific components of a culture media. Genetic diversity has frequently been attributed to a number of growth regulators, including 2, 4-dichlorophenoxyacetic acid (2, 4-D), naphthalene acetic acid (NAA), BAP (6-benzylaminopurine) and synthetic phenylurea derivatives (4-CPPU, PBU and 2, 3-MDPU) (Sun *et al*. 2013, Sales and Butardo 2014, Krishna *et al*. 2016). Long-term cultivation in 2, 4-D-containing media causes callus cells to have larger DNA ploidy levels (da Silva and Carvalho 2014). Inositol and indole-3-acetic acid (IAA) in the growing medium caused DNA rearrangements and methylation alterations in carrot (*Daucus carota*) callus cultures, according to Krishna *et al*. 2016. Matsuda *et al*. (2014) found that adding PGRs (0.5 ppm BA and 0.1 ppm NAA) to the media inoculated with African violet leaf/leaf segment explants significantly boosted the percentage of somaclonal variants. In proliferating cultures of carrot root explants, kinetin has been found to cause significant hypo methylation of DNA within two weeks, while auxins, including NAA, have the reverse effect and promote hyper methylation (Krishna *et al*. 2016). Additionally, there is proof that during tissue culture, genes involved in chromatin remodelling and histone

methylation exhibit variable expression, which disrupts the methylation process in an unspecific way and induces hypo- and hyper methylation patterns in DNA. This is stable and transmissible to plants that are grown from these cultures (Shearman *et al*. 2013). Variations *in vitro* depend on both the concentration and the ratio of the various growth regulators.

Ploidy and genotype

Despite appearing to be primarily dependent on plant growth regulators and the culture medium, genotype-specific *in vitro* morphogenesis is nevertheless a factor (Eftekhari *et al*. 2012). Plant genotype is likely the most significant predictor of variation among the elements affecting somaclonal variation (Nwauzoma and Jaja 2013).

Identification of tissue culture variation

Somaclonal variation refers to the combination of genetic and epigenetic changes that are related to in vitro propagation and may have phenotypic effects. Therefore, phenotypic, cytological, biochemical, genetic and epigenetic changes that are displayed at a variety of levels, including somaclonal variation, are distinguished by their complexity (Krishna *et al*. 2016). Somaclones should therefore be detected using an approach based on their appearances. For the detection and characterization of somaclonal variants, a wide range of methods are available that are essentially based on the variations in morphological features (Perez *et al*. 2011, Nhut *et al*. 2013), Biochemical (Kar *et al*. 2014), molecular DNA markers (Pathak and Dhawan 2012, Hossain *et al*. 2013, Bello-Bello *et al*. 2014), cytogenetic examination to determine numerical and structural variation in the chromosomes, (Clarindo *et al*. 2012, Currais *et al*. 2013, Abreu *et al*. 2014), or their combinations (Horacek *et al*. 2013, Stanisic *et al*. 2015). According to the variations, each instrument has specific benefits and restrictions that

Table 2. Through somaclonal diversity in many vegetable crops, *in vitro* selection of desired features and creation of some economically exploited cultivars were accomplished.

Sl. No.	Vegetable crops	Characteristic of somaclone	References
1.	Brinjal (*Solanum melongena* L.)	Stress-tolerant somaclone selection	Krishna *et al*. (2016)
2.	Capsicum (*Capsicum annuum* L.)	Yellow fruited var Bell sweet	
3.	Carrot (*Daucus carota* L.)	Resistance to leaf spot (*Alternaria dauci*)	
		Resistant to drought	Rabiei *et al*. (2011)
4.	Celery (*Apium graveolens* L.)	Fusarium resistant var UC-TC	Krishna *et al*. (2016)
		Multiple-resistant (insect resistance against *Spodoptera exigua* and disease resistance against Fusarium yellow) somaclones K-26, K-108 and K-128	
5.	Chili pepper (*Capsicum annuum* L.)	Early flowering and increase of yield components	Hossain *et al*. (2003)
6.	Garlic (*Allium sativum* L.)	Consistently higher bulb yield than the parental clone	Krishna *et al*. (2016)
		Resistance against the pathogenic fungi 'Sclerotium cepivorum'	Zhang *et al*. (2012)
7.	Patchouli (*Pogostemon patchouli*)	Higher herb yield and essential oil content	Ravindra *et al*. (2012)
8.	Pea (*Pisum sativum* L.)	Resistance to *Fusarium solani*	Horacek *et al*. (2013)
9.	Potato (*Solanum tuberosum* L.)	Non-browning var. White Baron	Krishna *et al*. (2016)
		Somaclones for heat tolerance	
		Somaclones IBP-10, IBP-27 and IBP-30, derived from cultivar Desiree, showed higher resistance to *Alternaria solani* and *Streptomyces scabiei*	
		Improved size, shape, appearance, starch content and starch yield	
		Superior processing attributes than cv 'Russet Burbank	Nassar *et al*. (2011)

Table 2. Continued.

Sl. No.	Vegetable crops	Characteristic of somaclone	References
		High-yielding genotype SVP 53	Hoque and Morshad (2014)
		Increased phytonutrient and antioxidant components over cv 'Russet Burbank'	Nassar *et al*. (2014)
10.	Sweet potato (*Ipomea batatas* L. Lam.)	Tolerant to salinity	Anwar *et al*. (2010)
11.	Tomato (*Lycopersicon esculentum* L.)	High solid contents var DNAP9	Krishna *et al*. (2016)

determine whether it should be used on a small or big scale (Table 2).

Somaclonal variation's molecular underpinnings

It is still not fully understood how a single plant genotype can produce a range of phenotypic traits under the same *in vitro* growing circumstances. Chromosome number changes (Leva *et al*. 2012), point mutations (Krishna *et al*. 2016), somatic crossing over and sister chromatid exchange (Bairu *et al*. 2011), chromosome breakage and rearrangement (Alvarez *et al*. 2010), somatic gene rearrangement and DNA amplification are a few of the bases for somaclonal variation that have been proposed (Tiwari *et al*. 2013), changes in organelle DNA (Krishna *et al*. 2016), DNA methylation (Linacero *et al*. 2011), epigenetic variation (Smulders and de Klerk 2011), histone modifications and RNA interference (Miguel and Marum 2011), segregation of pre-existing chimeral tissue (Ravindra *et al*. 2012, Nwauzoma and Jaja 2013) and insertion or excision of transposable elements (Sato *et al*. 2011b). In particular, transposable elements are one of the causes of genetic rearrangements in *in-vitro* culture (Sato *et al*. 2011a). According to reports, tissue culture activates transposable silent elements, causing somaclonal variants. Transposable elements and retrotransposons that are inserted into plant genomes can behave as insertional mutagens, whereas retro transposons that are widely activated can cause a wide spectrum of chromosomal rearrangements (Krishna *et al*. 2016). These rearrangements can then result in gene degeneration, aneuploidy and additional transposon insertions (Smulders and de Klerk 2011). However, there are still many unexplained features of the mechanisms that cause somaclonal differences. Therefore, it is necessary to sequence the entire genome of the affected crop in order to investigate the genome-wide change. The entire genomes of individual plants can now be sequenced thanks to next-generation sequencing technologies (Miyao *et al*. 2012). For high-throughput functional genomic research, a new generation of sequencing technologies from Illumina/Solexa, ABI/SOLiD, 454/Roche and Helicos has created previously unheard-of prospects (Metzker 2010).

Somaclonal differences with regard to crop improvement

Every traditional crop breeding program must include genetic variety. Crop improvement typically takes 10–15 years to complete and involves manipulating the germplasm, selecting and stabilising genotypes, testing and increasing varieties, proprietary protection, and crop production stages. An enabling technology for the creation of numerous innovative tools to help plant breeders is plant tissue culture (Mathur 2013). According to Krishna *et al*. (2016), tissue culture-induced somaclonal variation is similar to variations brought about by chemical and physical mutagens. It provides an opportunity to identify natural variability for potential use in crop development. *In vitro* produced somaclonal variation, like any other technology, offers advantages and disadvantages that are like the two sides of the same coin.

Advantages

The benefits include: (1) it is less expensive than other genetic modification techniques and doesn't call for "containment" operations. (2) More plant species can be grown in tissue culture systems than can currently be done by somatic hybridization and transformation. (3) It is not necessary to have isolated and cloned the gene responsible for the trait, or even to have determined its genetic origin in the case of transformation. (4) Novel variations have been identified among

somaclones and data suggests that passage through tissue culture can change the frequency and distribution of genetic recombination events. This suggests that diversity may arise from genomic regions other than those that may be accessed through traditional and mutational breeding (5) when somaclones are produced through cell culture, chimeric expression cannot be produced. Crops with constrained genetic bases and/or limited genetic systems, such as apomicts and vegetative reproducers, have shown the most success with somaclonal variation (Krishna *et al*. 2016).

Disadvantages

One of the major drawbacks of somaclonal variation is that, despite the identification of factors influencing the variation response of a certain plant species, it is still impossible to anticipate the results of a somaclonal program since it is unpredictable and unreliable. Furthermore, the majority of R_1 segregate because the majority of genetic alterations are caused by point mutations or chromosome rearrangements. As a result, it is essentially impossible to choose people with advances in the R_1 generation for quantitative qualities like yield. Unfortunately, there are no *in vitro* selection procedures for complex qualities like yield, soluble solids, sweetness, texture, or shelf life, despite approaches for selecting somaclones resistant to diverse biotic and abiotic stressors having been developed in many horticultural crops. Plant breeding can incorporate somaclonal diversity if it is heritable and genetically stable. Somaclonal variants have only been used to create a small number of potential kinds. This may be brought on by the lack of communication between plant breeders and tissue culture researchers as well as the unpredictable nature of somaclones. Additionally, even though somaclonal variation has created new varieties, in many instances improved variants have not been chosen because (1) all variations were negative, (2) positive changes were also altered negatively, (3) the changes were not novel, or (4) the changes were not stable after selfing or crossing (Krishna *et al*. 2016).

Obtaining somaclonal variations

By encouraging the variables that lead to the emergence of somaclonal variations, such as protoplast culture (Kothari *et al*. 2010), using callus and cell suspension culture for multiple cycles and regenerating a large number of plants from long-term cultures, the recovery of variants can be improved (Barakat and El-Sammak 2011). Through somaclones with beneficial features for agronomic or industrial use, indirect organogenesis is a significant method of recovering genetic variety. On addition, plant genotype plays a significant role in crop type and somaclonal variation, compared to many other commercial horticulture crops. However, for somaclonal variation to be useful, the frequency must be high enough to pick desired features and the chosen lines must function effectively in a variety of contexts (Krishna *et al*. 2016). Applying selection pressure through the screening of desirable features, such as *in vitro* selection for tolerance against abiotic and biotic stressors, can further improve the effectiveness of recovering variations *in vitro* (Barakat and El-Sammak 2011). Given that in field conditions, the selection of desirable features takes several years and generations, this becomes greater significance. Field selection can be complemented by *in vitro* selection, which can significantly reduce the time required for the selection of desirable features under *in vitro* selection pressure with minimal environmental interaction (Krishna *et al*. 2016). Combining *in vitro* induced mutagenesis with micro-propagation can improve the recovery of somaclones (Afrasiab and Iqbal 2010). In order to induce somaclones in potato, Iuliana and Cerasela (2014) proposed exposing *in vitro*-raised plants to ultraviolet radiations (UV-C).

Somaclonal variants are used

It is widely acknowledged that clonally grown plants frequently exhibit somaclonal variants that result from special tissue culture environments. These variations can be used to good effect as a source of novel diversity in horticulture crops. To reap the rewards of such variants, however, appropriate technologies for resistant clone discovery, evaluation, identification and enhancement need be developed. Breeders can produce plants that are tolerant to biotic or abiotic stress, such as drought, high salinity, high or low soil pH and disease tolerance, through crop improvement through somaclonal variation (Krishna *et al*. 2016).

CONCLUSION

Given that maintaining genetic integrity in the regenerated plants is necessary for the economic viability of micro-propagation technology, various methodologies have been used to determine the genetic integrity of the *in vitro* produced progenies. These technologies are now very helpful for accurately and quickly identifying variations. Nevertheless, in order to maintain the success of fidelity tests connected to the creation of clonal plants, the morphological and cytological assays should continue to be the main and most important assays. Tissue culture-induced variants, which on the one hand constitute a serious threat to the genomic integrity of regenerated plants, nevertheless give plant breeders tools for improvement, especially for crops with a limited genetic foundation, such as self-pollinated and vegetatively propagated crops.

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