SOMACLONAL VARIANTS IN ORNAMENTALS

ABSTRACT

Somaclonal variation, where genetic and phenotypic diversity arises among plants regenerated from tissue culture, has significant implications for ornamental horticulture. This variation, induced during *in vitro* propagation, presents an innovative and cost-effective method for developing new ornamental varieties with desirable traits such as unique color, fragrance, flower shape, and increased resistance to diseases and environmental stressors. Somaclonal variation mechanisms include chromosomal rearrangements, DNA methylation, transposable element activation, and somatic mutations. Although somaclonal variation can sometimes result in undesired traits, careful selection and screening can enhance ornamental plant breeding, leading to the commercialization of novel and improved varieties. Advances in molecular markers and genomic tools allow for early detection and management of beneficial somaclonal variants, improving their stability and reproducibility. Thus, somaclonal variation serves as a vital resource for genetic diversity and innovation in ornamental breeding, contributing to the aesthetic, economic, and ecological value of ornamental plants in landscaping and the floriculture industry.

Keywords: Somaclonal variation, Ploidy, Markers, Tissue culture

INTRODUCTION

a sunrise industry, floriculture in India has been given a lot of importance due to its diverse uses like fulfilling the aesthetic, social, and economic aspects of day-to-day life. It helps create employment opportunities for rural people and increases foreign exchange. Floriculture contributed 270 billion rupees to the Indian economy in 2020, the highest grossing value of the output recorded in the last few years across the country (APEDA, 2020). Floriculture crops are genetically diverse and include ornamental plants widely used as bedding and garden plants, cut flowers, cut greens, foliage, and potted plants. The value of ornamental plants is attributed to their aesthetic appearance, which is manifested through their growth habits, foliage shapes, patterns of leaf variegation and texture, flower shapes, and colors (Chen *et al.,* 2003).

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The majority of commercial floriculture crops are genetically heterozygous as only a few species are self-pollinated. Breeding methods conventionally followed for maximizing heterozygosity in floriculture crops include polyploidy, either natural or induced (van Tuyl *et al.,* 2002) and interspecific hybridization (Henny and Chen, 2003). Additionally, variegated plants comprise about one-third of floriculture crops,

whose genetic heterozygosity is maintained through vegetative means such as cuttings (stems, shoots, leaves, and roots), divisions, grafting, and layering. Propagation of planting materials in floriculture is a promising commercial venture. Beyond conventional methods, tissue culture or micropropagation— cultivating plant cells or tissues *in vitro* under controlled conditions—enables mass production of uniform, healthy plantlets in a short time. The first floriculture crop propagated *via* tissue culture was the orchid *Cymbidium* Swartz (Morel and Wetmore, 1951), which was later followed commercially foliage plants (Miller and Murashige, 1976).

Tissue culture has become a reliable and efficient propagation method for many foliage crops since 1980. A form of genetic instability occurs in tissue culture that has been termed 'somaclonal variation' (SV), the term first introduced by Larkin and Scowcroft, to designate the arrival of genetic variation in regenerated plants through *in vitro* culture. Somaclonal variation was first reported in sugarcane plants derived from cell culture in 1969 by researchers at the Hawaiian Sugar Planters Association Experiment Station. It can be defined as the variations among the somatic cell cultures, tissues, regenerated plants, or their progenies which are derived either from pre-existing or by variation induced during the cell culture process (Teob, 1989). Compared to spontaneous mutations of bud sports, somaclonal variations usually occur at much higher frequencies.

Tissue culture propagation differs from traditional vegetative propagation by allowing cells from small pieces of leaves, stems, shoots, or roots, referred to as explants to differentiate *in vitro* and develop into plantlets. The use of such diverse tissues which are genetically heterozygous facilitates a greater chance of uncovering mutated cells. As a consequence, many somaclonal variants could be regenerated during tissue culture propagation (Chen and Henny, 2019). Now, tissue culture propagation has become a major method of producing disease-free plantlets for commercial production of many floriculture crops, particularly orchids, ferns, and foliage plants. In 2020-2021, India's exports of tissue culture plants stood at US$17.17 million, with the Netherlands accounting for around 50% of the shipments (DGCIS, 2021-2022). It is estimated that more than 100 million *Phalaenopsis* orchids and at least 350 million foliage plantlets are propagated worldwide annually.

Somaclonal variation, widely observed in floriculture crops, may originate from preexisting genetic differences in cultured explants or be induced during tissue culture. The occurrence of somaclonal variants in floriculture crops is significantly higher than in natural sports (Karp, 1994; Schum and Preil, 1998). Reported variations include changes in plant size and form, increased branching, reduced internode length leading to dwarf and bushy growth, altered leaf shapes and variegation, and modified flower shapes and colors. Enhanced resistance to biotic and abiotic stresses has also been documented. Screening tissue culture populations for these variants is crucial for developing new cultivars. Somaclonal variation plays a

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vital role in ornamental plant breeding, contributing to nearly 80 new cultivars in eight aroid genera (*Aglaonema, Alocasia, Anthurium, Calathea, Dieffenbachia, Philodendron, Spathiphyllum*, and *Syngonium*) (Chen *et al.,* 2003; Henny *et al.,* 2003; Chen and Henny, 2008).

In ornamental foliage like *Caladium*, somaclonal variations exhibit distinct morphological changes in leaf shape, apex, base, margin, lamina color, spotting, veins, and petiole attachment and color (Chu and Yazawa, 2001; Ahmed *et al.,* 2004; Thepsithar *et al.,* 2009). *Syngonium podophyllum* exemplifies somaclonal variant selection for cultivar development, with 22 cultivars traced back to the original 'White Butterfly' clone, each stable enough to be recognized as a named cultivar. Somaclonal variants from University of Florida's Mid-Florida Research and Education Centre in Apopka include *Anthurium* 'Orange Hot' from *A*. 'Red Hot' *Aglaonema* 'Diamond Bay' from a mutation of *A*. 'Silver Bay,' and *Aglaonema* 'Emerald Bay,' a mutation from *A*.'Golden Bay' (Henny and Chen, 2008).

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CAUSES OF SOMACLONAL VARIATIONS

Somaclonal variation is caused either by change in the genes themselves (genetic variation) or by a change in gene expression (epigenetic variation) induced during tissue culture. Broadly three reasons can be attributed to the occurrence of this phenomenon such as physiological; genetic and biochemical. Physiological change is caused by the exposure of culture to the plant growth regulators used and the culture media conditions. The genetic reason involves actual physical changes in DNA sequence that are lasting and heritable. These changes occur due to different types of mutation (point, deletion, insertion, frameshift), transposable element activation, gene amplification, and karyotypic changes. Point mutation occurs when one base is substituted for another. In deletion mutation, there is the loss of DNA sequence or a specific part of the chromosome, while in insertion mutation a section of DNA is gained. Frameshift mutation happens when an insertion or deletion of extra base pairs causes the three base pair code shifts.

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Commented [D12]: Frameshift mutations occur when the insertion or deletion of extra base pairs causes a shift in the reading frame of the DNA, altering the sequence of codons.

Transposable element activation is induced during tissue culture, when a transposable gene may be activated leading to somaclonal variation. In this process, a section of DNA can clip itself out of its place on a chromosome and move to another location. When it moves, it leaves behind a few base pairs that can interfere with the coding region of a gene, resulting in partial or full loss of the gene's function. In addition, when it inserts itself into a new site of the chromosome, it can land within the coding region of the gene, resulting in loss of function. When such genetic alteration *via*. somatic transposition event occurs early in development, it may give rise to a branch in which a whole layer of the meristem is mutant, often referred to as a periclinal chimera. Examples of chimeras, which arose by transposition, have been

described in ornamentals such as snapdragon, morning glory, and petunia (Doodeman and Bianchi 1985, Carpenter and Coen 1995).

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The transposable elements probably account for a relatively small proportion of tissue culture-induced variation (Kaeppler and Philips, 1993). Matsuda *et al.,* (2014) found high somaclonal variation (46.6% and 56.5%) in African Violet (*Saintpaulia*) from low initial mutation rates (3.6% and 1.4%), likely due to VGs1 transposition during regeneration. Preexisting mutated cells on stamens constituted only 1.4%, but the percentage of somaclonal variations was over 50%, indicating that somaclonal variations associated with VGs1 transposition occurred during the shoot regeneration step. Considering that shoots were regenerated from single cells, the observation that multiple chimeric plants regenerated indicates the occurrence of mutation during shoot regeneration. It may be stated conclusively that some exogenous factors activate VGs1, resulting in somaclonal variation.

Generation of multiple copies of a gene in response to environmental stress which can occur under *in vitro* culture conditions can lead to the process of gene amplification. Changes in the number or structure of entire chromosomes during cell division can cause significant genetic alterations in the genome leading to karyotypic changes, an often-occurring phenomenon in tissue culture (LoSchiavo *et al.,* 1989). These changes can manifest as variations in ploidy or aneuploidy, which are of different types. Aneuploidy occurs when a cell loses or gains one or more chromosomes (eg: Monosomy: loss of a single chromosome, Trisomy: gain of an additional chromosome). Monosomic plants may exhibit variations due to the expression of recessive traits that were previously masked, as only one chromosome remains, potentially exposing hidden recessive alleles. Polyploidy involves the gain of entire sets of chromosomes, resulting in cells with multiple sets (eg. Haploid: a single set of chromosomes (half the normal complement); Diploid: Two sets of chromosomes (the normal complement), Triploid, Tetraploid, etc.) giving rise to cells with three, four, or more sets of chromosomes. Polyploidy can lead to reduced fertility and significant morphological changes. In some cases, these changes are beneficial; for example, many tetraploid flowering plants have larger flowers and fruits (Sarmah *et al.,* 2017). Karyotypic changes can also lead to biochemical alterations such as a lack of photosynthetic ability due to disruptions in carbon metabolism, changes in the biosynthesis pathways, such as starch synthesis *via* the carotenoid pathway, alterations in nitrogen metabolism, and development of antibiotic resistance. These changes can profoundly affect the plant's physiology and morphology, potentially offering advantages or disadvantages depending on the context.

GENETIC BASIS OF SOMACLONAL VARIATION

Advanced techniques in molecular genetics have paved the way to explore the complex genetic mechanisms leading to somaclonal variations during tissue culture. Changes at the chromosomal level, variation of DNA sequences including activation of transposable elements, and epigenetic effects have been proven to be the underlying mechanisms of somaclonal variation.

Chromosomal instability leading to karyotype changes is the major factor for the somaclonal mechanism. Changes in chromosome number are reported to be common among regenerated floriculture crops, which include *Anthurium*, *Begonia*, *Cyclamen*, *Dendranthema*, *Dendrobium*, *Doritaenopsis*, *Euphorbia*, *Fuchsia*, *Haworthia*, *Hemerocallis*, *Kalanchoe*, *Musa* spp., *Petunia*, *Pelargonium*, and *Saintpaulia*. Increasing ploidy levels, however, appear to be common among regenerated floriculture crops. Ornamental *Musa* spp. having showy flowers and long-lasting foliage, have been grown as potted flowers or foliage plants. Shepherd and Dos Santos (1996) reported that *Musa* plants regenerated *in vitro* with N6-benzyladenine (BA) showed a higher percentage of mitotic abnormalities than plants propagated through traditional means.

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Endopolyploidy is reported in more than 90% of angiosperms (Joubes and Chevalier 2000) and is common in *in vitro* induced polyploids. This phenomenon of genome variation is caused by endoreduplication, where mitotic DNA replication in somatic cells is not followed by cell division and is reported in orchids like *Doritaenopsis* (Mishiba *et al.* 2001), *Dendrobium* (Jones and Kuehnle 1998), *Spathoglottis* (Yang and Loh 2004), and *Vanda* (Lim and Loh 2003), as well as flowering plants of *Portulaca* (Mishiba and Mii 2000) and *Rododendron*. Due to the totipotency of plant cells, tissue culture of an explant harboring cells with endopolyploidy could result in regenerated plants having increased ploidy levels.

Cao *et al*. (2014) regenerated twenty-four somaclonal variants *in vitro* from *Caladium* 'Red Flash', of which seven variants were reported to have similar DNA contents to the wild type. Two variants had the same chromosome number as the wild type but had 4.5–5.6 % more nuclear DNA content. The 12 variants contained 1.1–5.4 % less nuclear DNA, indicating loss of one chromosome (2n = 2x - 1 = 29, monosomics). Two variants that contained 5.4–9.2 % less nuclear DNA lost two chromosomes, which could be either nullisomics (2n = 2x - 2 = 28) or double monosomics (2x - 1 - 1 = 28). One variant was reported to have chromosome number, 2n = 58. In another study on the characterization of four cultivars of *Caladium* for morphological, cytological, and molecular changes, seven variants had genome duplication with an average nuclear DNA content of 18.76 pg·2C−1. The tetraploid variant of the cultivar 'White Wing' had eight more chromosomes (2n = 4x = 68) than the other tetraploids and exactly double that of its wild type. All other diploids

had the expected 2n = 2x = 30 chromosomes and tetraploids recorded 2n = 4x = 60 (Parrish *et al*., 2023).

Somaclonal variation in floriculture involves genetic changes in plants regenerated from tissue culture, such as single nucleotide mutations, deletions, insertions, or transposable element activity. Techniques like gene sequencing, protein electrophoresis, and PCR are used to detect these variations. Isozyme analysis is also employed to study changes in gene products during *in vitro* culture (Chen and Henny, 2019).

Chen *et al.,* (1998) investigated somaclones of *Phalaenopsis* 'Blume' with deformed flowers. Comparing isozyme profiles of parental plants and somaclones using 11 isozymes, they found notable differences in aspartate aminotransferase (AAT) and phosphoglucomutase (PGM). Parental plants showed three AAT bands, while somaclones had two. For PGM, parental plants had three to four bands, whereas somaclones had two or three. These findings indicated that somaclones may have repressed genes that were active in the parental plants. Regular isozyme analysis can thus help detect and manage somaclonal variation early in propagation.

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Epigenetics contributes to somaclonal variation by altering phenotype without changing the gene coding sequence or promoter region (Rapp and Wendel, 2005). Of the three epigenetic mechanisms such as DNA methylation, histone modifications, and effects of miRNAs and siRNAs, only DNA methylation has been reported among *in vitro* regenerated floriculture crops. The addition of a methyl group to cytosine affects gene expression and transposable elements without altering the DNA sequence (Wolffe and Matzke, 1999; Martienssen and Colot, 2001). Methylation-sensitive amplified fragment polymorphism (MSAP) is commonly used to detect these changes. DNA methylation is inheritable. Mutant *of Linaria vulgaris*, described by Linnaeus over 250 years ago, shows a change in flower symmetry from bilateral to radial. This mutant has extensive methylation of the L-cyc gene, which controls flower symmetry, leading to its transcriptional silencing. This heritable methylation modification correlates with the mutant phenotype (Cubas *et al.,* 1999).

FACTORS INFLUENCING THE SOMACLONAL VARIATION

Regeneration systems are mainly responsible for somaclonal variation. The highest genetic stability during micropropagation occurs in micro propagules from preformed structures (shoot tips, nodal explants, adventitiously derived shoots), followed by somatic embryogenesis, with the least stability in organogenesis from callus, cell, and protoplast cultures. *In vitro,* cultures can induce somaclonal variation due to the dedifferentiation of cells. Commercially somatic embryogenesis and enhanced axillary branching are extensively used routes (Vilasini and Latipah, 2000) to produce the greatest number of plantlets quickly. Besides, bioreactors are

effective for the large-scale production of somatic embryos (Ammirato and Styer, 1985) and their delivery through encapsulation into artificial seeds (Martin *et al.,* 2006; Trader Brian *et al.,* 2006).

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Enhanced axillary branching is a simple and rapid propagation method wherein the apical dominance is eliminated to achieve the derepression and multiplication of shoots (Debergh and Read, 1990). These approaches generally produce genetically uniform truetotype plants, as the organized meristems are immune to genetic changes (Shepherd and Dos Santos, 1996; Vidal and De Garcia, 2000; Vilasini and Latipah, 2000) and hence reduced chances for the generation of somaclonal variants. However, the growth of mutant cells in embryogenic cultures can induce variability in the tissues (Jain, 2001).

Zhao *et al.,* (2012) identified three variants from somatic embryo regeneration in *Epipremnum aureum* 'Marble Queen': (a) Green leaves (like the progenitor 'Jade') (b) Variegated plants (like 'Marble Queen') (c) Whitish plants frequencies differed between leaf and petiole explants. The frequencies of green, variegated, and whitish plants produced from leaf explants were 98.6, 1.0, and 0.3%, and from petiole explants were 94.9, 3.2, and 1.9%, respectively. Among the regenerated variegated plants, most showed the 'Marble Queen' morphology. Other variants had brighter variegation than the stock plants, green leaves on one side and variegated leaves on the other side of the vine and third type had leaves that were variegated on one side and solid green on the other side of the mid-rib, and each such variegated leaf alternated with a solid green leaf along the length of the vine. The phenotypes were stable after the plants were grown in the shaded greenhouse for three months. Mean internode length and leaf area were similar, except for the second variant which had significantly shorter internode and smaller leaf size. Both the stock plant as well as the variants exhibited similar histograms with identical peaks for flow cytometry analysis indicating uniform ploidy status. Analysis of simple sequence repeats (SSR) amplified from 14 pairs of universal primers pairs (NTCP9, NTCP40, CCMP2, and CCMP5) of chloroplast genome showed no variation, suggesting that the difference in leaf variegation among the eight types of plants may be largely attributed to nuclear genes. The majority of regenerated plants had solid green leaves, which could be due to genetic reversion. The high percentage of green plants could also be attributed to the outperformance of green plants in survival and growth compared to variegated or whitish plants during their *in vitro* culture process.

Explant type affects the genetic fidelity of regenerated tissues. Using many explants from a single donor plant increases the variation due to pre-existing mutations (Larkin and Scowcroft, 1981). Differentiated tissues (leaves, stems, roots) show more variation than meristematic tissues (cambium, pericycle) (Shenoy and Vasil, 1992; Kunitake *et al.,* 1995). Matsuda *et al.*, (2014) reported a significant

increase in the somaclonal variation percentage (26.4%) in cut-leaf laminas of 'Saintpaulia' when compared to uncut-leaf lamina. The percentage of somaclonal variation was higher with the culture of cut leaves on the medium than with uncut leaves. Meanwhile, *Saintpaulia* has been reported to be one of the genera most sensitive to wounding stress such as leaf-cutting. Cutting leaves or local wounding of *Saintpaulia* plants induces a "hypersensitive state" in the whole plant, which lasts for at least 20 minutes (Yang *et al*., 2006).

Matsuda *et al*., 2014 also evaluated the effect of *ex vitro*- and *in vitro*-cultured mother plants in inducing somaclonal variation in *Saintpaulina* 'Thamires'. The percentages of somaclonal variation from *in vitro* and *ex vitro* mother plants did not depend on the growth conditions of mother plants; the mutation percentages of regenerated plants were 9.2% and 8.5%, respectively.

Abu–Qaoud *et al.,* (2010), observed *in vitro* regeneration and somaclonal variation in *Petunia hybrida* from leaf explants with different levels of naphthalene acetic acid (NAA) and benzyl adenine (BA). The regenerated shoots were grown *ex vitro* for analysis of somaclonal variation and lateral buds of selected pink-coloured petunia plants were cultured on MS basal media supplied with 30 mgl^{-1} gentamycin sulfate and 30 mgl^{-1} Benlate. After shoots grew, leaf sections were then taken from shoots and again cultured onto shoot regeneration medium (MS medium supplemented with 2 mgl^{-1} BA). The regenerated adventitious shoots were first cultured in MS medium without a growth regulator and then rooted, acclimatized plants were transferred to the greenhouse for evaluation. The appearance of plantlets showed variation in terms of leaf shape (elliptic and orbicular) and flower colors (light pink, purple, and violet).

High or unbalanced PGR concentrations in culture media induce somaclonal variation (Thorpe, 1987). Auxins increase DNA methylation, causing genetic variation (Lutz, 1985 Griesbach*.,* 1988). In *Torenia* flowers, white or light purple flower-coloured variants were obtained from the purple parent plant by reducing Fe micronutrient in the MS (Murashige and Skoog) culture media. The flower induction was highest in modified Murashige and Skoog (MS) medium containing ½ strength of macro elements, microelements, organic additives, and full Fe when compared to MS medium containing ½ strength of macronutrients, micronutrients, full Fe, and full organic additives. The flower color was stable in two new *Torenia* varieties through three generations *ex vitro*. The results showed a wide range of somaclonal variation in flower colors; early flowering occurred in MS medium containing ½ strength of macro elements, microelements, Fe, and full strength of organic additives (Nhut *et al*., 2013). Adding PGRs like 2,4-D and BA increases variation, especially in cut explants (Matsuda *et al.,* 2014).

Rapid tissue multiplication and extended culture duration increase somaclonal variation, which correlates with the number and length of subcultures (Reuveni and Israeli, 1990; Sahijram *et al.,* 2003; Bairu *et al.,*2006; Sharma, *et al.,* 2007).

In vitro culture conditions can induce mutagenic processes, and different genomes respond differently to stress-induced variations, indicating a genotypic component to somaclonal variation (Kaeppler and Phillips, 1993). Genetic stability varies among species due to differences in genetic makeup, particularly in repetitive DNA sequences (Lee and Phillips, 1988). Kengkarj *et al.,* (2008) obtained chrysanthemum (*Dendranthema grandiflora* Kitam.) somaclones from seven commercial cultivars through the petal segments culture. The major variants within the same cultivar found were only color and inflorescence shape deviation, whereby leaf and stem characters remained unchanged. The major variation (mainly in the 'Biarizte' and 'Yellow Biarizte') was found in ray floret shape which was tubular, flat, or spoon type. Petal color and shape varied greatly in five cultivars which had red or purple petals. The two cultivars that had either white or cream petals showed no variation. Furthermore, the variations were also found in floret diameter, number of ray florets, fresh flower weight, number of inflorescences, plant height, and fresh leaf weight and leaf area. The rate of variations was cultivar-dependent and occurred more frequently in the cultivars with flower color not detected, as the pigment accumulation was different in each petal color.

IDENTIFICATION OF SOMACLONAL VARIATION

Somaclonal variation in tissue culture arises from factors like culture medium, genetic makeup, regeneration pathways, and subculture duration. These variations may not be immediately visible; some appear only after transferring plants from culture to soil, where phenotypic evaluation is crucial.

1. Morphological Evaluation: Somaclonal variants can be detected easily by morphological characteristics, such as plant height, leaf morphology, and abnormal pigmentation (Israeli *et al.,* 1991). The physical characteristics such as plant form (heights, widths, internode length, leaf numbers, leaf size), foliar variegation patterns, leaf colors, flower color if any, and petiole color are compared between the variants and parental plants. Only those variants that show distinct differences in one or more characteristics from parental plants are then selected and maintained for further evaluation.

Aglaonema ‘Diamond Bay’ was a mutation found among a population of tissue-cultured *Aglaonema* ‘Silver Bay’ plants, while *Aglaonema* ‘Emerald Bay· was discovered within a group of tissue-cultured *Aglaonema* ‘Golden Bay·. The original ‘Diamond Bay· mutation lacked one of the foliar variegation patterns normally present in ‘Silver Bay·, whereas ‘Emerald Bay’ lacked the yellow background coloration present in normal ‘Golden Bay’ leaves and petioles. Mature *Aglaonema* ‘Diamond

Bay' leaves are lanceolate, 10–12 cm wide and 28–30 cm long. Leaf margins are entire and the lamina on either side of the midrib tends to be of slightly unequal widths, resulting in some curving of the blade towards the narrower side, while the leaf center exhibits grey-green. *Aglaonema* 'Emerald Bay· leaves are lanceolate 10–11 cm wide and 30– 33 cm long. The leaf margin is entire and the lamina on either side of the midrib tends to be of slightly unequal widths, resulting in a slight curving of the blade towards the narrower side. Leaves exhibit a central grey-green area that extends out from each side of the midrib to cover slightly over half of the total leaf surface. Leaf margins are green and intersect the lighter greygreen colored central area along irregular borders that often overlap to create small distinct patches of a third color that is a darker grey-green (Henny *et al.,* 2003)

Commented [D17]: **Aglaonema 'Emerald Bay'** leaves are also lanceolate, measuring 10–11 cm in width and 30–33 cm in length. The leaf margins are entire, and similar to 'Diamond Bay', the lamina on either side of the midrib is slightly unequal, causing a subtle curving of the blade towards the narrower side. The leaves feature a central grey-green area that extends from each side of the midrib, covering slightly more than half of the total leaf surface. The leaf margins are green and intersect with the lighter grey-green central area along irregular borders. These borders often overlap, creating small distinct patches of a third, darker grey-green color (Henny et al., 2003).

In *Dieffenbachia,* a popular interior scaping plant, three somaclonal cultivars 'Carina', 'Rebecca', and 'Sarah' were selected from 'Camille'. Plant form and leaf shape between the three somaclonal cultivars and their parental plant are almost identical, but foliar variegation varies. 'Camille' has bright to pale yellow leaves with broad dark green margins. 'Carina' has a similar variegation pattern to 'Camille' except for several internal flecks of dark green. Leaves of 'Rebecca' have narrow green margins and more internal green flecks. 'Sarah' has much narrower green margins and extensive green blotches extending from the margin to the inner ivory panel. The three somaclonal cultivars along with their parent are popular as indoor plants (Chen *et al.,* 2004).

In a study on plant regeneration *via* direct somatic embryogenesis from leaf and petiole explants of *Epipremnum aureum* 'Marble Queen', Zhao *et al*., (2012) evaluated *in vitro* regenerated plantlets based on morphology. They identified three types of plants: those with completely green leaves, resembling the commercial cultivar 'Jade'; variegated plants, similar to the 'Marble Queen' type; and whitish plants. Among the regenerated variegated plants, most showed the 'Marble Queen' morphology. Some showed much brighter variegation contrast than the stock plants. Other plants produced green leaves on one side of the vine and variegated leaves like 'Marble Queen' on the other side of the vine. Still, other regenerated plants had leaves that were variegated on one side of the mid-rib and solid green on the other side of the mid-rib, and each such variegated leaf alternated with a solid green leaf along the length of the vine. The phenotypes were stable after the plants were grown in the shaded greenhouse for three months.

Parrish *et al*., (2023) morphologically characterized eight variants from four cultivars ('Freida Hemple', 'White Wing', 'White Christmas', and 'White Queen') of *Caladium* × *hortulanum*. 'White Wing' is identified by its white leaf background with white veins and a green margin, 'Freida Hemple' has a red background with red veins and a green margin, 'White Christmas' has a white leaf background with green veins and a white margin, and 'White Queen' has a white background with red veins and

white margin. 'White Wing' somaclonal variant, WW-1 was nearly identical to 'White Wing' but had slightly thicker green margins than the wild type. 'Freida Hemple' variants were very similar as well in leaf color with each of the variants matching the wild type with red backgrounds, red veins, and green margins. The three variants were differentiated by the thickness of the green margin around the edge of the leaf with FH-3 having the smallest margins followed by FH-2 and FH-4. 'White Christmas' somaclonal variant WC-2 and 'White Christmas' were nearly identical in color with a white background and green veins and WC-16 had more prominent green veins. WC7 had different coloring with a green background with white interveinal segments and green veins. The somaclonal variant of cultivar 'White Queen' WQ-1 was easily differentiated from its wild type having a green leaf background colour with red veins and a red patch of colour around the petiole attachment.

2. Morphological, cytological, and biochemical markers:

Morphological markers usually are used to identify species, genera, and families in germplasm collections. Chromosomal alteration and ploidy changes are highlighted by cytogenetic analysis, including chromosome counting and/or flow cytometry. Cytometry has been used to identify the particular characteristics of somaclonal variation (Cao *et al*., 2016).

Proteins and isozymes have been used widely as markers for identifying cultivars and characterizing somaclonal variation. Isozyme analysis has been used to assess genetic fidelity in plants regenerated through organogenesis, and somatic embryogenesis.

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In general, the use of one type of molecular marker to assess the stability of *in vitro* propagated plants may be insufficient. Recently, several authors used multiple molecular marker types to study somaclonal variation in regenerants of several plant species (Leva *et al.,* 2012). Molecular markers generally used include Random Amplified Polymorphic DNA (RAPD) – Polymerase Chain Reaction (PCR), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), and Sequence-related amplified polymorphism (SRAP). The SRAP technique is simple and easy to perform and preferentially amplifies open reading frames (ORFs) or ORFs-related sequences. It is easy to use polymerase chain reaction (PCR) technique and requires no plant genetic information (Guo *et al.,* 2009). SRAP is one of the techniques that is effective for cultivar identification and DNA fingerprinting (Mokhtari *et al.,* 2013).

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Kengkarj *et al*., (2008) used RAPD markers for analysis of somaclones regenerated from petal segments of seven commercial cultivars of chrysanthemum. The morphological differences of the tested somaclones were shown to be highly correlated using RAPD pattern analysis. All the somaclones could be singly separated at 90% similarity. The higher level of variability of RAPD patterns in

chrysanthemum rendered these RAPD fragments as good candidates for somaclonal and cultivar identification. RAPD analysis of somaclonal variants of *Phalaenopsis* revealed that 78 out of 100 primers gave 1 to 10 distinct bands per primer resulting in a total of 1116 bands. Four sets of RAPD profiles from the study demonstrated heterozygosity among somaclones. Polymorphic and reproducible RAPD profiles were obtained from 38 primers (Chen *et al.,* 1998).

In a study to analyze the genetic differences of 19 cultivars selected from somaclonal variants of *Syngonium podophyllum* Schott, along with their parents as well as seven additional *Syngonium* species and six other aroids, amplified fragment length polymorphism (AFLP) markers generated by 12 primer sets were used (Chen *et al*., 2006). Among the 19 somaclonal cultivars, 'Pink Allusion' was selected from 'White Butterfly'. The tissue culture of 'Pink Allusion' through organogenesis resulted in the development of 13 additional cultivars. Selfpollination of 'Pink Allusion' obtained a cultivar, 'Regina Red Allusion', and tissue culture propagation of 'Regina Red Allusion' led to the release of five other cultivars. The 12 primer sets generated a total of 1,583 scorable fragments from all accessions, of which 1,284 were polymorphic (81.9%). The percentages of polymorphic fragments within the 'White Butterfly' and 'Regina Red Allusion' groups, however, were only 1.2% and 0.4%, respectively. Seven out of the 15 cultivars from the 'White Butterfly' group and three out of six from the 'Regina Red Allusion' group were clearly distinguished by AFLP analysis as unique fragments were associated with respective cultivars. The unsuccessful attempt to distinguish the remaining eight cultivars from the 'White Butterfly' group and three from the 'Regina Red Allusion' group might be caused by DNA methylation and/or some rare mutations (Chen *et al.,* 2006).

Commented [D20]: may be attributed to DNA methylation

3. **Simple Sequence Repeats (SSR):** SSR is another tool used to detect somaclonal variation. SSRs are short sequences of not more than six base pairs that repeat themselves, sometimes up to 100 times or more, at various sites in a genome. The repeats mutate frequently, so they exhibit many polymorphisms and work well as molecular markers. Forty samples of *Caladium* plants, including the wild type, 24 somaclonal variants, and 15 randomly selected, normal-looking regenerated plants, were analyzed with 20 SSR markers. All markers produced one or two reproducible, scorable bands on 6.5 % polyacrylamide gels. One SSR marker (CaM1) detected loss of one allele in seven somaclonal variants; another marker (CaM103) revealed loss of one allele in one variant (M75) and change of a different allele (size change) in another variant (M36). Seven of the eight somaclonal variants showing an SSR marker allele loss were aneuploids that had lost one or two chromosomes. If the SSR markers (CaM1 and CaM103) were located on these chromosomes, the marker alleles would be lost along with the chromosome, revealing that markers CaM1 and CaM103 are located on two different caladium chromosomes, and their allele was

lost in six variants (M14, M23, M68, M187, M199, and M203); two variants (M23 and M36) exhibited pink leaves and were distinct from the wildtype.

4. **Sequence-related amplified polymorphism (SRAP**) technique is simple and easy to perform and preferentially amplifies open reading frames (ORFs) or ORFs-related sequences (Li and Quiros, 2001). It is easy to use with polymerase chain reaction (PCR) technique and requires no plant genetic information (Guo *et al.,* 2009). This is one of the techniques effective for cultivar identification and DNA fingerprinting (Mokhtari *et al*., 2013). Kaewkam *et al*., (2022) used the SRAP technique for the detection of the DNA polymorphism between the mother plant and the variant lines obtained through *in vitro* culture in *Dendranthema morifolium* cultivar 'Polaris Pink'. Ten primer combinations were selected for evaluating the difference in DNA profiling of the selected control and variant plants during second field cultivation. The results showed one specific PCR fragment of approximately 850 bp produced by the primer pair Me1/Em10 on the control line (pink flower), which was absent in the variant line (light purple flower).

CONCLUSION

Somaclonal variation offers significant potential for crop improvement by inducing genetic variation. It is particularly effective in asexually propagated crops and self-fertilizing species. To select desirable somaclones, large plant populations are needed. Somaclonal variation can create new genotypes with minimal changes to the original genome and mimics induced mutations. Molecular markers like RAPD, RFLP, and AFLP are ideal for identifying genetic and epigenetic variations. Additionally, this technique can be combined with *in vitro* mutagenesis, and genetically stable somaclones can be used in plant breeding, making it a costeffective approach, especially for developing countries. *In vitro* selection accelerates the development of disease resistance, mineral tolerance, and abiotic stress resistance. Field evaluation of *in vitro*-selected variants is essential to confirm the stability of desirable traits.

Genetic variability serves as a crucial source for crop improvement, allowing for the emergence of elite traits in regenerants that are often unachievable through conventional breeding. Somaclonal variation plays a significant role in developing disease-resistant genotypes, such as resistance in sugarcane to eye spot disease (*Helminthosporium sacchari*) and Fiji virus disease, achieved by regenerating plants from susceptible clones. Additionally, somaclonal variation can produce plants resistant to abiotic stresses (cold, drought, acidic, or alkaline soil) which have potential implications in the context of climate change. Somatic genome exchange

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also facilitates the introgression of alien genomes, contributing to the widening of the germplasm pool.

Commented [D22]: Somatic genome exchange also facilitates the introgression of foreign genomes, contributing to the expansion of the germplasm pool.

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