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Review Article

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Somaclonal Variation and its' Application in Ornamentals Plants

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ABSTRACT

Somaclonal variation can be termed as the appearance of variation among the clonally achieved somatic cells. The variations among the somatic cells are of phenotypic as well genotypic in nature. Most of these variations are the results of significant changes in cytoplasmic or nuclear genetic elements such as aberrations in chromosomal number, morphology. Some of the most important factors responsible for this phenomenon in plant system are regeneration system, type of tissue, ex-plant source, media components and culture cycle duration. Specially, the in vitro cultured plants form variants in stressful culture conditions. This phenomenon is highly undesirables when the target is to achieve identical true to type plants. But it can be a boon for producing desirable mutant genotypes which may prove to be economically more viable than a normal plant type. Somaclonal variations has been utilized in several ornamental plants such as Chrysanthemum, Coreopsis, Petunia, Phalaenopsis, Gladiolus etc. Even though, somaclonal variations sound promising but due to unpredictability and uncontrolled nature variations and unstable inheritance in the plant progenies have ceased this technology into narrower level of application.

Key words*: *In Vitro, Somaclonal Variation, Ornamentals Plants, Plant Tissue Culture.

INTRODUCTION

Larkin and Scowcroft[27], first introduced the term 'somaclonal variation' (SV) to designate the arrival of genetic variation in regenerated plants through *in vitro* tissue culture. 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. But, the term has been adopted widely in various senses, especially in practical discussion[47].

Somaclonal variation was first reported in sugarcane plant derived from cell culture in 1969 by the researchers at the Hawaiian Sugar Planters Association Experiment Station[15,16,17].

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They reported the existence of significant variation in chromosome number, morphology and enzymatic variation among the regenerated plants of the same cell culture. Apart from that, some of the plants also showed heavier tillering, slower growth rate and increased erectness. Ahloowalia[3,4,5] found a wide variation in morphology (albino, altered leaf-shape, and vigour) and chromosomal changes (polyploidy, aneuploidy, structural re-arrangements) in callus culture regenerated ryegrass plants. Shepard *et al*[42]., also reported an extensive variation among protoplast-derived potato plants. This phenomenon (variation in tissue culture-derived plants) was termed as 'somaclonal variation' by Larkin and Scowcroft[27] in 1981. Later on, it was revealed that significant alterations in the cytoplasmic and nuclear genetic elements are responsible for these phenotypic variations which are epigenetic in nature[33]. The uncontrolled occurrence of somaclonal variation could ruin valuable genetic stocks maintained under *in vitro* conditions and make them useless in plant improvement and that is why *in vitro* culture technologies were almost banned from gene banks. Perhaps this is the major reason that somaclonal variation has not been incorporated routinely in plant breeding programmes. However, a limited number of somaclonal variants have been released as cultivars in a few crops. Skirvin and Janick[45] brilliantly pointed out the remarkable potential of somaclonal variation for application in improvement of horticultural plants.

The recovery of somaclonal variation can be enhanced by:

1. Several cycles of callus and cell suspension culture,
2. Large numbers of plant regeneration from long-term cultures,
3. Screening of candidate plants and their subsequent clones for favourable traits,
4. Evaluation of selected somaclones genetic stability for subsequent generations and
5. Multiplication of genetically stable somaclones for developing new cultivars[9].

Mechanism of Somaclonal Variation

- ❖ Genetic (heritable variation):
- ➢ Change in ploidy level: e.g. euploidy (one or more full sets of chromosome) and aneuploidy (presence of chromosome number which is different than a multiple of basis chromosome number).Mainly caused by failure of spindle formation, lagging of chromosome at anaphase and fusion of spindles in multinucleate cells.
- ➢ Change in chromosome structure:
- ▪ Deletion: Loss of a segment of chromosome.
- ▪ Duplication: These are obtained due to addition of a part of a chromosome. If duplication is present only on one of the two homologous chromosomes, at meiosis, cytological observation characteristics of deficiency will be obtained.

Duplication of a chromosome segment, may be brought about by addition at any of the following positions-

a) In adjacent region
b) At a displaced position of a same arm
c) On the different arm of the same chromosome
d) On a different chromosome.

- ▪ Inversion: It is produced when there are two breaks in a chromosome and the intercalary segments reunites in reverse order i.e. the segment rotates at 180^0. The inversion can be of two types:

a) Paracentric inversion: Absence of centromere in the inverted segment.
b) Pericentric inversion: Presence of centromere in the inverted segment.

- ▪ Translocation: It include all types of unilateral or bilateral transfer of chromosome segments from one chromosome to another. The reciprocal translocation or segmental interchanges have evolutionary significance where mutual exchange of chromosome segments between two pairs of non-homologous chromosomes occur.

- ❖ Epigenetic (non-heritable) variations:
- ➢ Variation generated during tissue culture
- ➢ Caused by temporary phenotypic changes

- Occur at low frequency

Causes of Somacholnal Variations

Somaclonal variation is caused either by a change in the gene themselves (genetic variation) or by a change in gene expression (epigenetic variation) induced during tissue culture.

GENETIC VARIATION: It involves actual physical changes in DNA sequence that is lasting and heritable. They are mainly-

- Mutation: Mutation may occur when one base is substituted for another which is point mutation. When a section of DNA is lost (deletion mutation) or gained (insertion mutation), or when an insertion or deletion of extra base pairs causes the three base pair code shift, resulting in a frameshift mutation.
- Transposable element activation: Transposable element activation is another type of variation induced by tissue culture. Transposable gene may be activated in tissue culture and lead to somaclonal variation, was first proposed by Ahloowalia & Sherington[2]. It is a section of DNA that 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 gene's function. In addition, when it inserts itself into a new site of chromosome, it can land within the coding region of gene, resulting in loss of function of that gene. Groose & Bingham[14] identified an unstable flower colour mutation, which acted like a transposable element-induced mutation. However, the implication of transposable elements is as yet to be proved. Kaeppler *et al*[21]., suggested that transposable elements probably account for a relatively small proportion of tissue culture-induced variation.
- Gene amplification: It is another type of genetic change that can happen when plants are cultured *in vitro*. Gene amplification term is used to describe the production of multiple copies of a gene in response to environmental challenge.
- Karyotypic changes: Karyotypic changes are major genetic changes in the genome that can occur in tissue culture when cell division results in changes in the number of structure of entire chromosome[28]. Changes in ploidy are one type of Karyotypic change. A cell that loses or gains one or more chromosome is called aneuploid. Common types of aneuploidy include loss of one chromosome (monosomy) or gain of one chromosome (trisomy). A monosomic plant can show variation through unmasking of recessive traits, that is, if the one remaining chromosome has recessive alleles that were previously hidden, these recessive traits will be expressed.

Polyploidy is another type of Karyotypic change. Polyploidy occurs when an entire set of chromosome in a cell are gained. Ploidy is referred to by the number of sets of chromosome in a cell: haploid (half number of complement of cell i.e., an entire set has been lost), diploid (the normal complement of chromosome i.e., two sets), triploid, tetraploid etc. Changes in ploidy can lead to loss of fertility and gross morphological changes. This can be useful or not- for example: many tetraploid flowering plants have larger flower and fruits.

EPIGENETIC VARIATION:

Epigenetic variation involves changes in gene expression such as gene activation or gene silencing. Because epigenetic variation does not permanently alter the sequence of base pairs in plant's DNA, it is potentially reversible. Epigenetic variation is usually not considered to be heritable, though methylation induced changes in some plants have remained stable through several cycles of pollination.

Most investigation of epigenetic variation has focused on methylation of genes. During methylation, a methyl group attaches to cytosine base after the DNA is replicated. The methyl group protrudes from the DNA helix and binds to proteins that then act to

wind the DNA into heterochromatin. Recall that heterochromatin is DNA that is tightly packed together and euchromatin is DNA that is unwound for replication. Because heterochromatin does not present its DNA strands for transcription, methylated genes are silenced.

Methylation can persist through DNA replication and be passed on through both mitosis and meiosis. Sometimes methyl groups are added to DNA in tissue culture, but more frequently they are lost. Auxins in the tissue culture medium increase methylation, whereas cytokinins seem to have no effect.

Factors Influence the Somaclonal Variation

There are wide array of the factors which are involved in inducing somaclonal variation during *in vitro* culture. Some of the most important factors are the regeneration system, type of tissue, ex-plant source, media components and culture cycle duration[36].

REGENERARTION SYSTEM:

Regeneration systems are mainly responsible for somaclonal variation. In terms of genetic stability regeneration system can be ranked from high to low, as follows: micropropagation by preformed structures like shoot tips, nodal explants, adventitiously derived shoots; somatic embryogenesis; and organogenesis from callus, cell and protoplast cultures[46]. Cellular organization is an important factor of plant growth. But in *invitro* condition, cellular control losses to establish disorganised growth and facilitates somaclonal variation[22,44]. The commercial micro propagation systems extensively use somatic embryogenesis and enhanced axillary branching[54] to produce the greatest number of plantlets in a short time. They alsouse bioreactors for large-scale production of somatic embryos[6] and their delivery through encapsulation into artificial seeds[30,50,51]. Enhanced axillary branching has become a very important method on account of the simplicity of the approach and rapid propagation rate which involves the abolition of apical dominance to achieve the de-repression and multiplication of shoots[13,57]. These approaches generally produce genetically uniform true-to-type plants, because the organised meristems are immune to genetic changes[41,53,54]. However, growth of mutant cells in embryogenic cultures can occur as well, which can induce variability in the cultures[19].

EXPLANT SOURCE: The explant source largely influences genetic fidelity[25] and nature of somaclonal variation[11,23]. The use of meristematic tissues, such as the cambium, pericycle, and procambium as explant reduce the possibility of variation[39]. However, highly differentiated tissues, such as leaves, stems and roots generally produce more variants, probably due to the callus-phase, than explants that have pre-existing meristems[40]. Furthermore, preparation of many explants from only one donor plant increases the possibility of variation in cultures[26]. Pre-existing somatic mutations in donor plant tissues can also induce somaclonal variation[22] and first round of somaclones yield higher variability than second generation, hence can be eliminated or stabilised.

MEDIUM COMPONENT: Unbalanced and high hormonal concentrations in culture media can be powerful agents for inducing variation[48]. Synthetic compounds used as growth regulators also have been linked with somaclonal variation[31,55]. Auxins increase genetic variation by increasing the DNA methylation rate in cultures of unorganised calli or cell suspensions[29]. In addition, somaclonal variation can also be induced by rapid disorganised growth[22].

DURATION AND NUMBER OF CULTURE CYCLES: The rapid multiplication of tissue and long term cultures affect genetic stability and somaclonal variation[18,35]. It has been reported that the number of subcultures and their duration are proportional to the frequency of somaclonal variation in cell suspensions and callus cultures[7,37,38]. On the basis of multiplication cycle number, a statistical model has already been proposed for predicting the theoretical mutation rate in somaclones[12]. However, due to the complexity of biological systems, the model lacks considerable level of applications.

EFFECT OF GENOTYPE: The genotype component prove to be a vital factor for induction of somaclonal variation. Since, *in vitro* culture conditions impart different level of stress to plant cells[20,43] and different genomes respond differently to the stress-induced variation which finally may initiate highly mutagenic.

Detection and Isolation of Somaclonal Variation

Somaclonal variation can be difficult to detect. All the somaclonal variation is not obvious to the eye. Gross physical changes, such as leaf colour may be detectable *in vitro*, but most somaclonal variation won't be visible until the explants are removed from tissue culture and grown in soil.

The simplest way to detect somaclonal variation is to transfer the plants to soil and monitor them for phenotypic differences. Such an approach is useful for nurseries that micro propagate herbaceous plants and plants with short juvenile period. However plants with a longer juvenile period, such as tree, do not lend themselves to this approach. Particularly with plants such as fruits trees, the grower may have to wait 5-10 years or longer to detect changes such as flavour, yield or vigour. This is a considerable expenditure of time and money, and it can be devastating to a grower to invest this much money in a crop only to be disappointment when it does not produce as expected.

Molecular Techniques Used To Detect the Somaclonal Variation

- Random Amplified Polymorphic DNA (RAPD) – Polymerase Chain Reaction (PCR): RAPD analysis is one such technique that has been used in past, but now PCR is used with random primers i.e. random sequence base pairs, usually ten. The primers hybridize to DNA extracted from young plant tissues and during a DNA amplification step; these primers produce short fragments of DNA. The fragments are separated by electrophoresis on an agarose gel. If the resulting pattern shows difference (polymorphisms), this indicates somaclonal variation.
- Restriction Fragment Length Polymorphism (RFLP): RFLP is another technique used to detect the somaclonal variation. Here DNA is extracted from young leaves of regenerated or micro propagated plants and subjected to restriction enzyme digestion, which cleaves DNA at specific base pair sequences. The resulting fragments are separated by electrophoresis. All identical plants will have same banding pattern on the gel. Any polymorphisms among the bands represent somaclonal variation.
- Amplified Fragment Length Polymorphism (AFLP): Here restriction enzymes are used to cleave DNA; adaptors are ligated to the cut end of the DNA. The primers used with AFLPs have the same adaptors. When the primers anneal to the DNA fragments, any primers that correspond to the adaptor and the attach portion of the plant's DNA sequence will amplify. AFLPs are considered more sensitive than RFLP.
- Methylation Sensitive Amplified Polymorphism (MSAP): MSAP are similar to RFLPs except that in place of one of the restriction enzymes, a pair of isoenzymes is used. One enzyme of this pair will cut at a specific site on DNA even if the DNA is methylated, and the other enzyme will not. This produces a polymorphism that allows detecting whether it is site of methylated or not.
- Simple Sequence Repeats (SSR): SSR is another tool used to detect somaclonal variation. SSR are short sequence 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 marker.

Molecular technique can detect polymorphisms with great sensitivity. However, it is important to realize that molecular markers are not genes. While markers many indicate differences among segments of DNA, they may not actually be

within a gene or even very close to one. If the molecular markers are not closely linked to the coding region of gene, they may indicate polymorphisms in plants in which no phonotypical difference appears.

Advantages and Disadvantages of Somaclonal Variation:

ADAVANTAGES:

- Highly frequent and stable variations in somaclones.
- Novel mutations.
- Flexible to be applied in different types of cells, i.e. vegetatively or sexually or asexually propagated plants.
- Somaclonal variations are less time consuming than mutation breeding.
- Increased secondary metabolites production.
- Selections for resistance to herbicides, high salt concentration, various toxins and mineral toxicity are efficient.

DISADVANTAGES:

- Appearance of undesirable traits such as reduced fertility, growth rate etc.
- Unstable variation in progenies
- Cultivar dependent variation
- Appearance of random and wild variants are common in selected clones.

Somaclonal Variation in Improvement of Ornamental Plants

- **Chrysanthemum:** It is one of the most important commercial cut flower of the world and is the second largest flower in demand after rose, in global trade.

Vilasini and Latipah[56] reported somaclonal variation in *Chrysanthemum morifolium* generated through petal cultures. They experimented with the ray florets of five varieties [*viz*. Weldon (white), Impala (off white), Pink Impala (white with pinkish tinge), Daymark (white) and White Palaver (white)] which was cultured *in vitro* and regenerated. Even though, the regenerated plants expressed significant variation in flowering and vegetative growth but the floral variation was limited. Variation was demonstrated when a yellow coloured floret was successfully isolated and regenerated to exhibit a solid mutant.

Table 1: Floral and vegetative characteristics forming the somaclonal variation in *Chrysanthemum* varieties

Variety	Characteristic of floral variants
Weldon	Reduction in plant height at flowering, flower disc reduced by 80%, diameter of flower reduced, plant architecture changed to cascading type.
Impala	Overall reduction in plant height, single yellow petal which was isolated and cultured to produce all yellow flowers
Daymark	Reduction in flower size and number of florets
White Palaver	Increase in length of flower pedicel, reduction in plant height in flowering

Source: Vilasini and Latipah[56]

From the above table it is clear that the percentage of regenerants that exhibited any form of variation was low when compared to the control plants but there was a morphological reduction in size. Most of the regenerants in general produced inflorescence that was reduced in size and number of petals per inflorescence. This was also accompanied by reduction in plant height.

Coreopsis: The coreopsis genus belongs to *Asteraceae* family and includes both annual and perennial species. Perennial coreopsis taxa consistently rank in the top five herbaceous perennial in the wholesale value[8].

Trader *et al*[52]., studied somaclonal variation in leaf regenerated coreopsis. They used Murashige and Skoog (MS) basal medium grown true leaf explants of perennial *C. grandiflora* (A. Grey) Sherff 'Domino' and 'Sunray 'seedlings. Two seedlings (designated as E_2 and H_2) survived in *invitro* which regenerated about 175 plants. Somatic clones were screened by visible differences in flower orientation and overall appearance. The

divisionally propagated and transplanted selected soma clones along with E_2 and H_2 expressed significant variation for desirable (more petals per flower, grater flowering, shorter plants), undesirable (less flowering, smaller flower) and neutral (narrower leaves, taller plants) traits.

Table 2: Somaclones(n=15) selected in the green house among 175 1st generation tissue cultured derived plants regenerated from leaf explants of two seedlings (E2 and H2) of *C. grandiflora* 'Domino' and the characters for which they were selected

Somaclonal selection	Distinguishing characters
E2-1	Compact habit, small flowers
E2-2	Uniformity of flower peduncle length, flat corolla
E2-6	Separation of ray flower, stellate corolla
E2-26	Small flowers, tiered flowering
E2-27	Floriferous, synchronous blooming
E2-37	Flat, open flowers
E2-47	Small flowers, double ray flowers
E2-65	Compact, prostrate, small flowers
H2-4	Mutant, upright ray flowers
H2-33	Incised ray petals
H2-42	Cup shaped flower, pale red centre
H2-43	Short, compact habit
H2-45	Many ray flowers
H2-48	Many ray flowers
H2-49	Flat large ray flowers

Source: Trader *et al*[52].,

Petunia: *Petunia hybrida* is an ornamental plant species of tremendous economic potential[34]. It is greatly diversified and available in a range of colours[10].

Abu–Qauad *et al*[1]., studied *Petunia hybrid* for *in vitro* regeneration and somaclonal variation. They observed the shoot multiplication, regeneration from *P. hybrid* leaf e*xplants* with different levels naphthalene acetic acid (NAA) and benzyl adenine (BA) in in *invitro* condition. The regenerated shoots were again 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 mg l−1 gentamycin sulfate and 30 mg l−1 Benlate. After shoots grew, leaf sections were then taken from shoots and again cultured onto shoot regeneration medium (MS medium supplemented with 2 mg l−1 BA). The regenerated adventitious shoots were first cultured in MS medium without 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 colours (light pink, purple and violet).

Table 3: Number of new leaf and flower forms of petunia plant developed *in vitro*

Leaf shape				
Original(ovate)	orbicular		elliptic	total
43	11		6	60
Flower colour				
Original (pink)	Dark pink	violet	purple	total
27	8	3	2	40

Source: Abu –Qauad[1]

Phalaenopsis: *Phalaenopsis* 'Moth orchids' are considered as the most beautiful flowers of world along with its high economic value for pot plant and cut flower production. This genus is widely distributed in Southeast Asia with a few species extending from Sikkim, Taiwan to Australia and the Pacific[49].

Khoddamzadeh *et al*[24]., detected somaclonal variation of micropropagated *P. bellina* (Rchb. f.) Christenson using random amplified polymorphic DNA (RAPD) analysis. They used protocorm like bodies (PLB) of *P. bellina* which were induced from leaf segments. They proliferated the PLBs using ½ strength MS media with two subcultures at three months intervals and studied somaclonal variation through twelve decamer RAPD analysis. They found18 polymorphic bands with eight markers for all the treatments where the primer P 16 produced the highest number of bands (29), while primer OPU 10 produced the lowest number (15).There were negligible variations between the MP and the PLBs produced after 3 months of induction. The induced PLBs were then again subcultured for six months for proliferation and this resulted in about 17% dissimilarity with MP.

Gladiolus: Gladiolus is one of the most popular cut flowers which are grown all over the world for their gorgeous spikes and corm production.

Memon *et al*[32]., studied commercially important three varieties of gladiolus *viz.* Peter Pears, Trader horn and White Friendship cormels to assess the percentage of clonal fidelity with each other and with mother cormels using RAPD and Inter Simple Sequence Repeat (ISSR) markers. They used cormel sprout as an explant for regeneration under *in vitro* conditions. They reported similarity tendencies of RAPD among *in vitro* propagated cormels as80% to 90% in Peter Pears, 80% to 95% in Trader horn and 88% to 95% in White Friendship. However, the similarity tendencies between i*n vitro* propagated and mother cormels were 86% in Traderhorn, 92% in White Friendship and 83% in Peter Pears. In comparison, ISSR primers produced higher percentage of similarity matrix than RAPD. The ISSR cluster analysis for genetic similarity between mother and *in vitro* propagated cormels revealed a variation of 85% in Peter Pears, 90% in Trader horn and 96% in White Friendship. The genetic differences among *in vitro* propagated cormels ranged from 82 to 100% in Peter Pears, 88 to 100% in Traderhorn and 94 to 100% in White Friendship.

Conclusion and Future Prospects

Somaclonal variation has a vast potential for inducing genetic variation, but there is an urgent need to incorporate this technology in crop improvement. A wide range of somaclonal variation can be created in asexually propagated crops and seed propagated self-fertilizing species. To select desirable somaclones, it is essential to produce large population of plants. Somaclonal variation can be effectively applied to produce new genotypes with a very little level of change in the original genome. As a source of variation, somaclonal variation mimics induced mutations. Molecular markers such as RAPD, RFLP, and AFLP are ideal tool to identify genetic and epigenetic somaclones. Somaclonal variation can be combined with *in vitro* mutagenesis. Genetically stable somaclones can be used for plant breeding. This is a cost-effective approach and developing countries can adopt this technology at low cost. Moreover, *in vitro* selection will save time to develop disease resistance, mineral tolerance and abiotic stress. Ultimately, *in vitro* screened variants with desirable characteristics should be field evaluated to affirm persistency of the selected traits genetic stability.

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