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Somaclonal Variation- A Technique to Crop Improvement in Agriculture

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Tissue culture experiments involve growing plant tissues under a series of specific laboratory conditions different from their normal growth conditions in nature in an objective to eventually propagate and conserve them or for ultimate genetic transformation. When the explant is implanted in vitro, a specific tissue culture protocol for each species employed must be optimised or designed. In addition, changes in physiology, anatomy, and metabolism must be made in order for them to develop and reproduce in culture. Explants can be any form of plant cell or organ used in tissue culture, including embryos, spores, roots, leaves, and protoplast. Under the conditions given by the researcher, tissues from the explant undergo a cycle of dedifferentiation referred to as (callus production), followed by proliferation for a number of generations (callus proliferation), culminating in plant regeneration (Larkin and Scowcroft 1981). The regenerated plant obtained after tissue culture is known as a "somaclone," while regenerated plants obtained from stems and protoplasts are known as "calliclones" and "protoclones" respectively (Chawla 2000).

During tissue culture, conservation of the genetic integrity of the mother plant is a desirable component, but regeneration by tissue culture has been reported to yield plants which exhibit unexpected and undesired phenotypic anomalies and variation (Karp 1994). In vitro conditions include controlled media components, low light levels, and, most crucially, high humidity, all of which produce physiological and developmental abnormalities in the regenerated plantlets. In reality, somaclonal variation refers to culture-induced anomalies or variation that eventually becomes genetic and is passed down through the generations of clonal progenies (Chawla 2000).

Applications of Somaclonal Variation in Agriculture

Faced with the imminent crisis of global warming and shrinking arable soil, we must ensure food supply in the modern period. Without employing transgenics, somatic embryogenesis can be used to create plants with certain variable features compared to the starting material. Somaclonal variation might be viewed as a benefit because it allows for the development of new genotypes, which have been used in agriculture for the following purposes:

1. **Generating New Agronomic Variants with Advantageous Traits**

New breeding lines with variances of significant agronomic properties have emerged as a result of somaclonal variation. Somaclonal variants were selected for: high oil yield in Cymbopogon winterianus (Dey et al. 2015), higher flowering and fruiting ability in strawberry (Biswas *et al.* 2009), high sucrose yielding sugarcane varieties (Rastogi *et al.* 2015).

2. **Generation of Disease Resistance Varieties:** Induced somaclonal variation has been used to develop disease resistance in many crop cultivars. Somaclonal variants from

different crops with increase disease resistance include sugarcane which were resistant to eyespot and red rot disease (Rastogi et al. 2015), wheat resistant to white blotch disease (Arun *et al.* 2003).

3. **Abiotic Resistance Biochemical Variants**

Biochemical variations can be created, which can then be utilised to study plant metabolic pathways. Wheat somaclonal variations with frost and freezing tolerance have been discovered, and they have been linked to proline synthesis (Dorffling and Melz 1997). Somaclonal variation has also successfully yielded plants that display salt and herbicide resistance in a number of crop species, such as drought and salt resistance in sugarcane (Rastogi *et al. 2*015) and drought tolerance rice (Rastogi *et al.* 2015).

Genetic Basis of Somaclonal Variation

During regener- ation of plants using tissue culture, there is normally differentiation and redifferentiation of cells leading to qualitative and quantitative genomic changes. Somaclonal variation can be traced back to changes which have occurred at the genome level and include:

1. **Activation of transposable elements:** Transposable elements have been connected to somaclonal variation and were first discovered in Nicotiana tabacum species, causing coloured patches. Transposable elements have been found to be activated in maize and tobacco tissues grown in vitro. In vitro genomic rearrangements, as well as the insertion and removal of transposable elements, have been documented (Sato *et al.* 2011).
2. **Single gene mutation:** Single gene mutations in the genome of crop species such as wheat, maize, and rice (Edallo *et al.* 1981) have been related to somaclonal variants in tomato.
3. **Karyotype changes:** Variant plants with altered chromosome numbers inducing ploidy level changes namely polyploidy which includes chromosomal abnormal- ity have been observed (Leva *et al.* 2012).
4. **Plastid genetic change:** In somaclones, plastid genetic alterations such as deletion, addition, and intramolecular recombinations have occurred.
5. **Methylation:** Methylation in single-copy sequences, methylation of the genome as well as histone modifications have been reported in tissue culture (Miguel and Marum 2011).
6. **Changes in chromosome structure:** Chromosomal breakage and rearrangement, centric and acentric fragments, aberrant chromosome organisation, ring chromosomes, and micronuclei production are all examples of chromosome structural changes.

Evaluation Methods of Somaclonal Variation

1. Morphological Assessment of Somaclonal Variation

Morphological evaluation of somaclonal variation has long been utilized to identify "variants," sometimes known as "off types," in regenerated plantlets. Plant size, leaf shape, and pigmentation are the most common characteristics employed in the evaluation. Banana dwarf off-types and excessive vegetative growth in palms are examples of somaclonal variants.

2. Protein Markers Used to Assess Somaclonal Variation

Isozymes are various molecular forms of proteins that actively influence biochemical activities within cells. Isozymes fingerprints are commonly formed and can be used to identify variations between the genomes of parental and regenerated plants. Esterase (EST), endopeptidase, alcohol dehydrogenase, peroxidase, and polyphenol oxidase are some of the enzymes that are often tested.

3. Cytogenetic Analysis for the Determination of Somaclonal Variation

Chromosomal abnormalities can take place in tissue culture plantlets and could be the causal factors of somaclonal variations. During the callus induction and cell suspension phases of

citrus and oil palm, aneuploidy (an aberrant number of chromosomes) has been seen. Fluorescence in situ hybridization, flow cytometry, and microscopic examination can all be used to detect differences in chromosome number across cells in cultures. Flow cytometry is a technique that uses a fluorochrome specific to DNA found in entire cells, protoplasts, nuclei, or chromosomes to calculate the DNA content analysis.

4. Diagnosis of Somaclonal Variation Using RFLP

One technique for genome analysis is restriction fragment length polymorphism (RFLP), which includes digesting genomic DNA using restriction endonucleases enzymes, then separating the digested products on an agarose gel to get DNA profiles. The DNA profiles of the true to type regenerated plantlet will differ from the somaclonal variant in terms of the number and size of the bands.

5. Somaclonal Variation Detection Using Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) is a PCR-based technology that uses randomly generated primers to selectively amplify segments. These primers are roughly 10 nucleotides long and can bind to complementary sites in the genome, followed by amplification of the defined regions. Because of the low input cost and minimal technology required, RAPD is one of the most often used markers for assessing somaclonal variation. The absence of particular bands in regenerated plantlets implies chromosomal rearrangement during tissue culture, and this accounts for somaclonal variance. RAPDs have actually been used in the assessment of somaclonal variation in sugarcane.

6. Detect the presence of Somaclonal Variation Using Simple Sequence Repeat (SSR)

SSR markers, also known as microsatellite markers, are short DNA sequence motifs of a few base pairs that are repeated tandemly within the eukaryotic genome. The amount of repeats in each genome varies, and these can be amplified using particular primers and the polymerase chain reaction (PCR). SSRs are highly reproducible and codominant, making them excellent for quick and easy genotyping. Following amplification, various somaclones will have varied numbers of these repeat units, resulting in significant degrees of polymorphism.

7. Identification of Somaclonal Variation Using the AFLP Technique

The AFLP approach involves restriction enzyme digestion followed by PCR amplification of the fragments recovered, allowing for the creation of fragment length polymorphisms. The AFLP technique depends on the use of complementary primers to known adaptor sequences to selectively amplify restriction-digested segments, resulting in more repeatable DNA profiles. The AFLP approach is a more appropriate, sensitive, and repeatable marker system.

8. Identification of Somaclonal Variation Using Transposon-Based Marker Systems

Transposable elements are DNA segments that have the ability to replicate themselves and insert into new genomic loci during transposition. When transposable elements insert themselves into genes, normal gene function is inhibited, and gene expression is affected. Rice and maize have both shown transposable element induction. Transposable elements have been recognised as dynamic and abundant for the development of markers such as sequence-specific amplified polymorphism (SSAP), inter-retrotransposon-amplified polymorphism (IRAP), and retrotransposon-based insertion polymorphisms (RBIP) since the advent of genome sequencing projects.

9. Next-Generation Sequencing (NGS) for the Determination of Somaclonal Variation

Plant tissue culture regeneration is stressful, & it can result in genetic and epigenetic alterations that contribute to phenotypic variability. To confirm magnitude of chromosomal change that happens during tissue culture for induction of somaclonal variation, NGS-based analyses were performed in Arabidopsis and rice. In Arabidopsis regenerated plantlets, a higher genome-wide DNA sequence mutation rate was detected, with base substitution as the most significant genomic change, but no discernible change in transposable element

reactivation. Single nucleotide polymorphisms (SNPs), nucleotide alterations (including insertions & deletions), & transpositions have all been discovered in rice.

10. MicroRNA Involvement in Tissue Culture

MicroRNA, often known as miRNA, is a gene regulator identified in Caenorhabditis elegans. These miRNAs are noncoding RNAs with a length of 19–24 nucleotides that have been found to have critical regulatory roles in a variety of biological processes and pathways. Plant defence, particularly stress responses, plant development, hormone communication, and seed germination, have all been linked to miRNAs. Differential microRNA production (namely Mir169a and miR390) has been seen in strawberry plantlets regenerated via tissue culture, and these differences have been linked to phenotypic variances. Many plants' regulatory roles in somatic embryogenesis, as well as their engagement in gene regulation and epigenetic processes, have been highlighted.

Conclusion

Regenerating plants by tissue culture may be focused on maintaining the genetic integrity and the production of true to types but at times somaclonal variation may be beneficial to improve the gene pool of a population with a narrow genetic base. Various methods for detecting somaclonal variation have been reviewed, and molecular methods for assessing variant genomes allow for early and simple selection during a breeding experiment. These methods can be employed separately, but they're best when used together to determine genetic fidelity.

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