Chapter-7

Modern Plant Breeding Technology: Somaclonal Variation, rDNA Technology and Cryopreservation

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ABSTRACT

Utilizing cutting-edge instruments and techniques, modern plant breeding technology increases crop resilience, quality, and output. Plant genomes can be precisely altered through the use of molecular markers, genomic selection, genetic engineering, and genome editing methods like CRISPR/Cas9. Improved crop varieties can be developed more quickly thanks to these technologies, which make it easier for breeders to find and incorporate advantageous genes. Furthermore, the quick reproduction of elite lines and the preservation of genetic resources are facilitated by tissue culture and micropropagation methods. When combined, contemporary plant breeding technologies provide long-term answers to the problems of global food security and climate change adaptation.

KEYWORDS: Somaclonal variation, hybrid, crops, cryopreservation, rDNA, Plant Biotechnology.

INTRODUCTION

Numerous horticultural species can now be regenerated in vitro thanks to developments in tissue culture techniques, and a variety of crops can now be multiplied on a commercial scale using micropropagation processes. A high level of genetic homogeneity among the regenerated plants is necessary for clonal proliferation and the maintenance of elite genotypes that were chosen for their exceptional traits. However, genetic variability (somaclonal variations) may be produced by plant tissue culture due to gene mutation or modifications in epigenetic markers. A disadvantage of both germplasm preservation and in vitro cloning is the presence of minor somaclonal variation. As a result, ensuring the genetic homogeneity of plants grown in vitro at an early stage is crucial. The genetic integrity of the progenies produced in vitro has been determined using a variety of techniques, including morpho-physiological, biochemical, cytological, and DNA-based molecular marker approaches. Somaclonal variation can pose a considerable obstacle in any micropropagation endeavor where the production of true-to-type plant material is highly sought after. However, in horticultural crops that are either hard to breed or have a limited genetic foundation, somaclonal variation has given breeders a new and alternative option for obtaining genetic variability reasonably quickly and without complex technology.

Somaclonal variation in plant biotechnology denotes the genetic alterations that arise in plants produced from tissue culture, a method often employed for micropropagation and genetic modification. These variations result from the artificial conditions of in vitro culture, including hormonal imbalances, extended culture durations, and the dedifferentiation and redifferentiation of plant cells. Somaclonal variation, though it can be seen as a barrier to the creation of genetically uniform clones, is also an effective resource for enhancing crops. It offers a natural way to achieve genetic diversity without resorting to

traditional breeding methods or genetic engineering. Somaclonal variation also refers to the genetic variation observed among plants that have been produced through plant tissue culture techniques, such as callus culture, somatic embryogenesis, or organogenesis. This variation can occur spontaneously as a result of cellular stress, mutations, chromosomal rearrangements, or epigenetic changes during the in vitro culture process. Somaclonal variation can be both beneficial and detrimental: while it may lead to undesirable traits that affect plant quality or uniformity, it also serves as a valuable source of novel genetic diversity for plant breeding and crop improvement. Screening somaclonal variants for favorable characteristics like higher yield, stress tolerance, or disease resistance can be performed by biotechnologists, making it a valuable approach for creating improved plant varieties.

Despite its potential benefits, somaclonal variation can have several negative impacts, particularly in commercial plant propagation where genetic uniformity is essential. Unintended genetic or epigenetic changes can lead to undesirable traits such as reduced vigor, abnormal growth patterns, lower yield, or altered flowering and fruiting characteristics. These variations can compromise the quality and consistency of plant products, making them unsuitable for large-scale agriculture or horticulture. In some cases, somaclonal variation may also result in increased susceptibility to diseases or environmental stress. As a result, careful screening and quality control are necessary in tissue culture programs to detect and eliminate undesirable variants, ensuring the production of genetically stable and high-quality plant material.

HISTORY

Somaclonal variation has its origins in the early advancements of plant tissue culture in the middle of the 20th century. Even though the regeneration of plants from cultured cells was first shown to be possible in the 1950s, it wasn't until

the late 1970s and early 1980s that researchers started to systematically observe and record genetic variability among regenerated plants. Larkin and Scowcroft introduced the term "somaclonal variation" in 1981 to refer to the heritable variation observed in plants regenerated from somatic (non-reproductive) cells in culture. This finding called into question the prior belief that tissue culture would invariably yield genetically identical clones, paving the way for advancements in plant biotechnology and crop enhancement.

TYPES OF VARIATION

Somaclonal variation is categorized into two primary types: genetic variations and epigenetic variations. Genetic variations refer to alterations in the DNA sequence, including point mutations, chromosomal rearrangements, deletions, insertions, or polyploidy. These changes are stable and can be inherited, frequently resulting in lasting modifications to plant traits. In contrast, DNA sequences are not changed by epigenetic variations; rather, they influence gene expression via mechanisms like histone modification or DNA methylation. These alterations can be reversed and are not always transmitted to the next generation. Both forms of somaclonal variation can affect plant morphology, physiology, and development, making them significant factors in plant tissue culture and crop enhancement initiatives.

FACTORS INDUCING VARIATION

Numerous stressors have been identified as the causes of tissue culture mutations, such as wounding, exposure to sterilants during sterilization, incomplete tissue (protoplasts are an extreme example), media component imbalances like high concentrations of plant growth regulators (auxin and cytokinins), sugar from the nutrient medium replacing photosynthesis in the leaves, lighting conditions, and the disturbed relationship between high humidity and transpiration.

A significant portion of the variability observed in micropropagated plants could be caused by or connected to oxidative stress damage to plant tissues that occurs during in vitro culture. Oxidative stress leads to an increase in pro-oxidants or reactive oxygen species (ROS), including superoxide, hydrogen peroxide, hydroxyl, peroxyl, and alkoxyl radicals. These ROS may contribute to altered hyper- and hypo-methylation of DNA, changes in chromosome number ranging from polyploidy to aneuploidy, chromosome strand breakage, rearrangements of chromosomes, and deletions and substitutions of DNA bases, which in turn can result in mutations in plant cells *in vitro*.

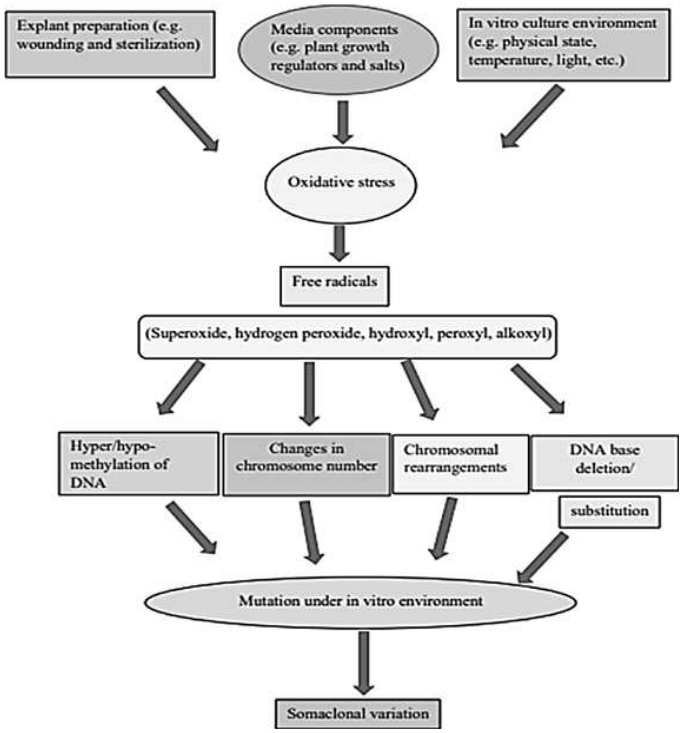

Figure 1. Graphical presentation of various stages in Somaclonal variation.

a. Variation from mother explants

Somaclonal variation may arise from somatic mutations present in the tissues of the donor plant. Somatic embryos obtained from the first regeneration round can be subjected to a further in vitro regeneration round to check for pre-existing somaclonal variation. Tissues exhibiting pre-existing variation are expected to produce greater variability in the first somaclonal generation compared to the second. Consequently, the variation in the second round can be reduced or stabilized.

b. Phytohormones in culture medium

Polyploidy may be induced by auxins and cytokinins being present in unbalanced concentrations, while cells exhibit normal ploidy when growth regulators are present at low levels or not at all. Moreover, a rapid and disorganized growth process can lead to somaclonal variation. Suboptimal and supraoptimal levels of growth regulators, especially synthetic compounds, have been linked to somaclonal variation. When auxins are added to cultures of unorganised calli or cell suspensions, the rate of DNA methylation increases, thereby enhancing genetic variation. Likewise, in strawberry callus cultures, the synthetic auxin 2,4-Dichlorophenoxyacetic acid (2,4-D) is frequently linked to genetic irregularities like polyploidy and enhanced DNA synthesis, potentially leading to endoreduplication.

c. Frequency of culture cycles

With a greater number and longer duration of subcultures, somaclonal variation occurs more often, especially in callus cultures and cell suspensions. In addition, the swift amplification of tissues or cultures developed over time may impact genetic stability, resulting in somaclonal variation. A statistical model has been suggested for estimating the theoretical mutation rate, mainly based on the number of multiplication cycles. Nevertheless, the model's applicability is restricted because of the intricacy of biological systems.

ROLE IN CROP IMPROVEMENT

Somaclonal variation denotes the genetic diversity seen in plants that have been regenerated from somatic cells cultured in vitro. This variation is caused by mutations, chromosomal rearrangements, or epigenetic changes that happen during the tissue culture process. Somaclonal variation is a useful resource in crop enhancement for creating new characteristics, including disease resistance, stress tolerance, and yield improvement. Somaclonal variation can introduce new traits without crossbreeding, unlike traditional breeding that relies on existing genetic diversity. This renders it particularly beneficial for crops with narrow genetic bases or in situations where conventional breeding poses difficulties. Somaclonal variation plays a role in creating enhanced crop varieties that possess desirable agronomic characteristics.

Somaclonal variation is particularly important in banana improvement, as bananas are mostly propagated vegetatively and have limited genetic diversity. Through in vitro tissue culture techniques like micropropagation, somaclonal variation can lead to the emergence of new traits, offering a valuable source of genetic variability. This variation has been effectively used to develop banana plants with improved resistance to major diseases such as Fusarium wilt (Panama disease) and Black Sigatoka, as well as enhanced tolerance to abiotic stresses like drought and salinity. In addition, somaclonal variation can result in improved yield, fruit quality, and shelf life. Since conventional breeding in banana is difficult due to sterility and parthenocarpy in many commercial varieties, somaclonal variation provides an efficient alternative for generating and selecting beneficial traits, thereby supporting the development of more resilient and productive banana cultivars.

Through tissue culture techniques, such as callus induction and plant regeneration, variations arise at the genetic and epigenetic levels, which can lead to desirable traits in rice plants.

These include improved tolerance to abiotic stresses like drought, salinity, and cold, as well as enhanced resistance to pests and diseases such as bacterial blight and blast. In addition, somaclonal variation has been used to develop rice lines with better yield, grain quality, and maturity traits. Since rice has a relatively narrow genetic base, somaclonal variation offers an alternative approach to conventional breeding by creating new traits that may not be found in natural populations.

RECOMBINANT DNA (RDNA) TECHNOLOGY

Recombinant DNA (rDNA) technology represents a potent method in genetic engineering, which entails merging DNA from diverse origins to produce novel genetic combinations. With this method, scientists can isolate, alter, and add specific genes to an organism's genome, making it possible for desired traits to manifest. In the agricultural sector, rDNA technology has been employed to create genetically modified (GM) crops that feature enhanced traits like resistance to pests, tolerance for herbicides, improved nutritional value, and extended shelf life. In the field of medicine, it allows for the manufacturing of crucial drugs such as insulin, human growth hormone, and vaccines. The procedure usually employs restriction enzymes for DNA cutting, ligases for the joining of DNA fragments, and vectors like plasmids to insert the recombinant DNA into host cells. By offering accurate and effective methods for altering genetic material for research, industrial, agricultural, and medical purposes, rDNA technology has transformed the field of biotechnology.

IMPORTANT MILESTONES

- **1960s:** Werner Arber, Hamilton Smith, and Daniel Nathans identified restriction enzymes, which cut DNA at specific sequences, paving the way for manipulating DNA.
- **Early 1970s:** Paul Berg demonstrated the feasibility of

splicing and recombining genetic material, creating the first recombinant DNA molecules in vitro.

- **1973:** Stanley Cohen and Herbert Boyer, building on Berg's work, successfully inserted recombined genes into bacterial cells, demonstrating that engineered DNA could be replicated and expressed in foreign cells.
- **1974:** Stanford University applied for a patent on recombinant DNA technology, marking a significant step in its commercialization.
- **1970s-1980s:** Restriction enzymes and reverse transcriptase were discovered, enabling scientists to generate recombinant DNA molecules and leading to advancements in medicine, agriculture, and industry.
- **1982:** The first genetically modified crop plant, an antibiotic-resistant tobacco plant, was produced.
- **1986:** The first field trials of genetically engineered plants occurred in France and the US, with tobacco plants engineered for herbicide resistance.
- **1990s:** The "Gene Revolution" era began, with rDNA technology leading to improved crop yield, economic benefits for farmers and consumers, and reduced environmental impacts of agriculture.

PRINCIPLE OF RDNA TECHNOLOGY

Recombinant DNA (rDNA) technology operates on the principle of cutting and splicing together DNA from various sources in order to forge a new genetic combination that possesses specified characteristics. The initial step involves identifying and isolating a gene of interest from the donor organism. The gene is then placed into a suitable vector (i.e. plasmid) which serves as a carrier to deliver the gene into a host organism. To cut the donor DNA and vector at predetermined locations, restriction enzymes are utilized, while DNA ligase is employed to link the resulting fragments. The recombinant DNA is introduced into the host cell, where it

can replicate and express the desired gene. This enables the creation of genetically modified organisms (GMOs) that possess particular advantageous characteristics. rDNA technology is based on precision, which allows scientists to manipulate genetic material at the molecular level for use in agriculture, medicine, and industry.

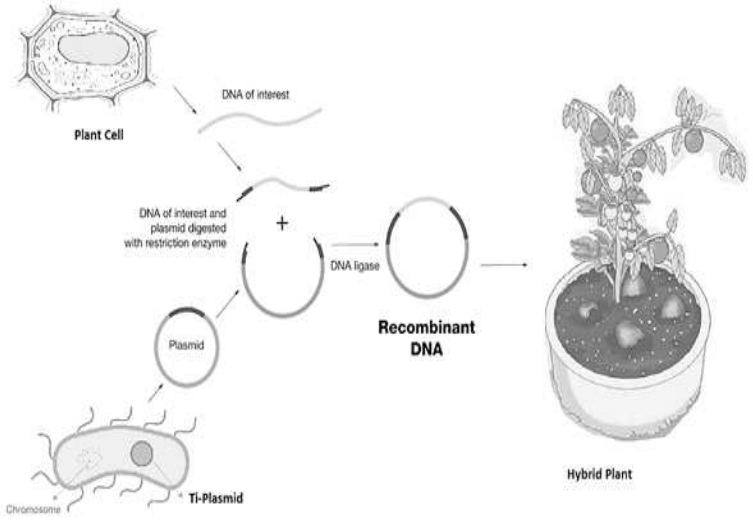

Figure 2. Production of hybrid plants by using rDNA technology.

APPLICATION

Recombinant DNA (rDNA) technology has transformed plant biotechnology, providing many new methods for crop development and enhancement. A major application is the development of genetically modified (GM) plants with improved characteristics, such as pest, disease, and herbicide resistance, which results in lower pesticide application and better yields. It is also utilized to create crops with enhanced nutritional value, like Golden Rice, which is fortified with vitamin A to address malnutrition. Through rDNA technology,

it is possible to add genes that confer drought and salinity tolerance, aiding plants in enduring difficult environmental conditions. Moreover, it aids in the creation of plant-produced pharmaceutical compounds (molecular farming) and fosters research by generating model organisms with reporter or marker genes.

CRYOPRESERVATION

The process of cryopreservation serves to maintain biological specimens like cells, tissues, embryos, or seeds at extremely low temperatures—usually in liquid nitrogen at -196°C. At such temperatures, all metabolic and biochemical processes cease, enabling genetic material to be stored over the long term with minimal loss of viability or function. In the field of plant conservation, cryopreservation is commonly employed, particularly for safeguarding the genetic resources of species that are rare, threatened, or of commercial significance. In agriculture, it is essential for the upkeep of germplasm collections, the provision of a consistent supply of superior plant varieties, and the facilitation of breeding initiatives. The procedure requires meticulous preparation, such as employing cryoprotectants to avert ice crystal formation that can harm cells. In summary, cryopreservation is an effective means in biotechnology and conservation for the secure long-term storage and retrieval of important genetic resources.

It involves freezing plant materials such as seeds, embryos, meristems, or somatic cells at ultra-low temperatures, typically in liquid nitrogen at -196°C, where all cellular activities are suspended. This method ensures the preservation of plant germplasm without genetic alteration over extended periods. In plant biotechnology, cryopreservation supports the conservation of endangered species, the maintenance of elite breeding lines, and the secure storage of genetically modified or tissue-cultured plants. It is especially useful for species with recalcitrant seeds or those that do not produce viable seeds. By

enabling the safe and cost-effective storage of valuable plant material, cryopreservation plays a crucial role in biodiversity conservation, crop improvement programs, and sustainable agricultural development.

APPLICATION

Cryopreservation guarantees a steady provision of elite and genetically diverse germplasm for breeding programs by preserving the viability and genetic stability of stored material. It is especially useful for conserving rare, endangered, or clonally propagated species that cannot be preserved through standard methods. Tissues that have been cryopreserved can be regenerated into complete plants for the purpose of reintroducing beneficial characteristics, including disease resistance, stress tolerance, or enhanced yield. Moreover, it aids biotechnological methods such as genetic transformation and somaclonal variation by offering a reliable backup of important lines. Therefore, for genetic conservation and breeding purposes, as well as for the sustainable development of enhanced crop varieties, cryopreservation is a vital resource.

REFERENCE

- *Dorani, E., Dehghanian, Z., Gougerdchi, V., Hamedpour-Darabi, M. (2024). Application of Somaclonal Variation in Crop Improvements. In: Kumar, N. (eds) Plant Mutagenesis. Sustainable Landscape Planning and Natural Resources Management. Springer, Cham. https://doi.org/10.1007/978-3-031-50729-8_8*
- *Krishna, H., Alizadeh, M., Singh, D., et al. (2016). Somaclonal variations and their applications in horticultural crops improvement.* 3 Biotech, *6:54. DOI 10.1007/s13205-016-0389-7*
- *Ferreira, M.d.S., Rocha, A.d.J., Nascimento, F.d.S., Oliveira, W.D.d.S., Soares, J.M.d.S., Rebouças, T.A., Morais Lino, L.S., Haddad, F., Ferreira, C.F., Santos-Serejo, J.A.d., et al. (2023). The Role of Somaclonal Variation in Plant Genetic Improvement:*

A *Systematic Review.* Agronomy, *13*, 730. *https:// doi.org/ 10.3390/agronomy13030730*

- *Duta-Cornescu, G., Constantin, N., Pojoga, D.-M., Nicuta, D., Simon-Gruita, A. (2023). Somaclonal Variation–Advantage or Disadvantage in Micropropagation of the Medicinal Plants.* Int. J. Mol. Sci., *24, 838. https://doi.org/10.3390/ijms24010838*
- *Efferth, T. (2019). Biotechnology applications of plant callus cultures.* Engineering, *5, 50–59.*
- *Alfalahi, A.O., Hussein, Z.T., Sadder, A.K.M.T., Qasem, J.R., Al-Khayri, J.M., Jain, S.M., Almehemdi, A.F. (2022). Epigenetic variation as a new plant breeding tool: A review. J. King Saud Univ. Sci., 34, 102302.*