SOMACLONAL VARIATION IN PLANTS

Somaclonal variation in plants: causes and detection methods

Somaclonal variation is defined as variation originating in cell and tissue cultures. Presently, the term somaclonal variantion is universally used for all forms of tissue culture derived variants, however, other names such as protoclonal, gametoclonal and mericlonal variation are often used to describe variants from protoplast, anther and meristem cultures, respectively. Some scientists added another aspect to the definition and require thatsomaclonal variation be heritable through a sexual cycle. Unfortunately, it is not always possible to demonstrate heritability because of complex sexual incompatibilities, seedlessness, polyploidy or long generation cycles. Therefore, explaining the heritable nature of somaclonal variation for these types of plants could be difficult and almost impossible. Since the first observation and report of somaclonal variation by Braun (1959), it has been and remains one of the major problems of many tissue cultured plants. The growth of plant cells in vitro and their regeneration into whole plants is an asexual process, involving only mitotic division of the cell and theoretically, it should not cause any variation. Ideally, clonal multiplication of genetically uniform plants is the expectation The occurrence of uncontrolled and random spontaneous variation during the culture process is, therefore, an unexpected and mostly undesired phenomenon. Contrary to these negative effects however, its usefulness in crop improvement through creation of novel variants are also well documented. Induced somaclonal variation can be used for genetic manipulation of crops with polygenic traits. It can also be an importanttool for plant breeding via generation of new varieties that could exhibit disease resistance and improvement in quality as well as better yield. Hence it is important to acknowledge the useful potentials of somaclonal variation.

Origin and sources of somaclonal variation

Spontaneous heritable variation was known to plant growers before the science of genetics was established and the art of plant breeding practiced. The commencement of the domestication of plants coincided with the occurrence of ‘‘sports’’, ‘‘bolters’’, ‘‘off-types’’ and ‘‘freaks’’ in vegetatively propagated plants such as sugarcane, potato and banana. Some of the successful cultivars based on spontaneous mutation such as the naval orange, dwarf bananas, coloured and striped sugarcane as well as several potato cultivars are comparable to somaclonal variants and are frequently cultivated. In contrast to spontaneous mutations in vivo, in vitro generated variations seem to occur more frequently, and are detected easily because variants canbe readily spotted in a limited space and within a short time. The exposure of unprotected genetic material to chemicals in the medium and survival of the resulting variants in a non-selective environment increases the mutation rate several fold over that in glasshouse or field grown plant populations. Even if the rate of mutagenesis are the same in cell and tissue cultures as in field grown plants, the sheer number of occurrences in a cell population (106 after 20 cell divisions) would make accumulation of mutants far greater than in field grown plants. Therefore, somaclonal variants can be detected more frequently in cell cultures than mutations in field grown populations. The

in vitro culture of plant material can induce or reveal variation between cells, tissues and organs thereby creating variation within cultures, or among the somaclones. Some, or all, of the somaclones may be physically different from the stock plants from which the culture was derived. Variability of this kind, which usually occurs spontaneously and is largely uncontrolled or directed, can be of two different kinds viz changes caused by cells having undergone persistent genetic change and those caused by temporary changes to cells or tissues, which are either genetically or environmentally induced. Generally though, somaclonal variation in vitro can be the result of individuals exhibiting one or more of the following changes; physical and morphological changes in undifferentiated callus; differences in the ability to organize and form organs in vitro; changes manifested among differentiated plants; and chromosomal changes. Somaclonal variants may differ from the source plant permanently or temporarily. Temporary changes result from epigenetic or physiological effects and are nonheritable and reversible. However, permanent variants referred to somaclonal variants are heritable and often represent an expression of pre-existing variation in the source plant or are due to the de novo variation via an undetermined genetic mechanism(s). As a result, the causes of somaclonal variation are not always well understood and have not been fully elucidated. Although it has been studied extensively, the causes remain largely theoretical or unknown. Generally, variation in tissue culture could either be pre-existing or tissue culture induced. The literature to date indicates that this variation could range from a specific trait to the whole plant genome. For instance, Gengenbach and Umpeck (1982) demonstrated that somaclonal variation is not limited to nuclear DNA by revealing mitochondrially controlled male sterility using restriction enzyme analysis of isolated mitochondrial DNA.

Pre-existing variation

Heritable cellular variation could result from mutations, epigenetic changes, or a combination of both mechanisms. The distinction between the two mechanisms is an important one because genetic mutations are essentially irreversible and are likely to persist in the progeny of regenerated plants, whereas epigenetic changes are not transmitted by sexual reproduction. Use of chimeric plants, variation in ploidy level, tissue culture induced chromosome aberrations and rearrangement, mechanisms regulating the cell cycle, activation of cryptic transposable elements are some of the factors thought to induce pre-existing variations and are briefly discussed below.

Use of chimeras

Chimeras are a source of pre-existing variation in vitro. The arrangement of the genetically different tissues within the plant meristem affects chimera stability. For instance, McPheeters and Skirvin (1983) reported that nearly half of the tissue obtained from tissue cultured chimeral ‘Thornless Evergreen’ blackberry were dwarfs and pure ‘Thornless Evergreen’. Protoclone with abnormal flowers and low pollen fertility regenerated from leaf segments of meristem-derived plants of statice was linked to chimera formation. Similarly, Krikorian et al. (1993) demonstrated that genetic fidelity largely depends on explant source by regenerating a stable plantain variant

called ‘Superplantano’ from spontaneously produced useful variant chimeric cv. ‘Maricongo’. So often, when more than one explant is taken from one plant, it could cause variation. These can best explain the importance of the inherent genetic composition and genome uniformity of the mother plant that is used as starting material for tissue culture. Therefore, it is imperative to assess the entire plant for genetic uniformity before using it for tissue culture, a practice seldom done in most tissue culture experiments.

Chromosome aberration and rearrangements

Thorough characterization and classification of tissue culture induced chromosome aberrations have led to a better understanding of somaclonal variation. Variation in chromosome number and structure has been observed among tissue cultured somaclones. Detailed studies have indicated that structural chromosome changes mostaccurately reflect the frequency and extent of karyotypic changes. In tissue cultured cells, the predominant type of aberration is the result of changes in chromosome structure. Therefore events leading to chromosome breakage, and in some instances subsequent exchange or reunion of fragments, appear to be of fundamental importance. Late replicating heterochromatin and nucleotide pool imbalance are two possible origins of chromosome rearrangement in tissue culture. The former involves the mitotic cell cycle of higher organisms. This cell cycle consists of four phases, G1 (gap), S (synthesis of DNA), G2 (gap), M (mitosis consisting of prophase, metaphase, anaphase, and telophase), each with a species specific and cell type specific duration. Any perturbation affecting the synchrony between chromosome replication during S phase and cell division would likely result in chromosome aberration. Because heterochromatic regions replicate later than euchromatic segments, their integrity may be particularly vulnerable to fluctuations in the cycle.

Transposable elements activation

Transposable elements are mobile DNA sequences in a genome that can induce gene mutations and contribute to genome rearrangements. Transposons account for signifi- cant portions of most plant genomes and were first discovered in maize culture by McClintock (1950). Activation of cryptic transposable elements is another source of chromosome based somaclonal variation. Chromosome breakage is a means for initiating activity of maize transposable elements. The discovery of activation of maize transposable elements in tissue culture suggested a possible relationship between somaclonal variation and mobile elements. Genetic evidence also suggests that certain unstable mutants may be explained by transposable elements and the tissue culture environment probably provides a conducive environment for DNA sequence transposition. For instance, the induction of callus followed by subsequent shooting and rooting would disrupt normal cell function and may activate transposable elements, stress-induced enzymes or other products. Gao et al. (2009) observed that the new insertions of transposons in a rice cultivar regenerated through tissue culture were responsible for somaclonal variation. Therefore, it has been suggested that transpositional events such as activation of transposable elements and the putative silencing of genes and a high frequency of methylation pattern variation of single-copy sequences play a major role in somaclonal variation. However,

the extent of that role and the mechanism of the process have not been elucidated and is poorly understood. Generally, to test for pre-existing somaclonal variation, somaclones may be subjected to another round of in vitro regeneration. Clones with pre-existing variation should yield more variability in the first generation than in the second and thereafter variation should be eliminated or stabilized. Subsequent variation is more likely to be tissue culture derived.

Tissue culture induced variation

During in vitro culture, the propagation methods, genotype, nature of tissue used as starting material, type and concentration of growth regulators, number as well as the duration of subcultures are some of the factors that determine the frequency of variation. The effects of some of these mentioned factors on the occurrence of somaclonal variations are discussed below.

In vitro propagation method used

The presence of a disorganized growth phase in tissue culture is considered as one of the factors that cause somaclonal variation. In vitro growth conditions can be extremely stressful on plant cells, and may instigate highly mutagenic processes. Cellular organisation is also important in terms of describing the origin and cause of somaclonal variation. Tissue culture involves disorganised growth at various levels, ranging from those systems which least disturb cellular organization such as meristem tip culture to systems such as protoplasts and non-meristem explant cultures where regeneration is achieved through the formation of adventitious shoots after a phase of disorganised callus or cell suspension culture. Systems subject to instability and disorganised growth demonstrated that cellular organization is a critical feature and that somaclonal variation is related to disorganised growth. Generally, the more the organizational structure of the plant is broken down, the greater the chance of mutations occurring. Although the direct formation of plant structures from cultured plant tissue, without any intermediate callus phase, minimizes the chance of instability, the stabilizing influence of the meristem is usually lost when plants are grown in culture.

Types of tissue or starting material used

Highly differentiated tissues such as roots, leaves, and stems generally produce more variants than explants from axillary buds and shoot tips which have pre-existing meristems. There are however, some exceptions where more organized tissues like shoot-tips cause more variation compared to somatic embryogenesis as reported in bananas, possibly due todissociation of chimeras. The use of undifferentiated tissue such as the pericycle, procambium and cambium as starting material for tissue culture reduces the chance of variation. Gross changes in the genome including endo-polyploidy, polyteny and amplification or diminution of DNA sequences could also occur during somatic differentiation in normal plant growth and development. Tissue source therefore can affect the frequency and nature of somaclonal variation. The processes of de-differentiation and re-differentiation may involve both qualitative and quantitative changes in the genome and different DNA sequences may be amplified or deleted during these changes in the state of the cell that is related to the original tissue source and regeneration system. Somaclonal variation, therefore, can arise from somatic mutations already present in the donor plant.

Type and concentration of applied plant growth regulators (PGR)

Optimal concentration and precise ratios of auxins and cytokinins is essential for efficient micropropagation. The primary events, controlled by exogenously applied plant growth regulators (PGRs), that trigger morphogenesis via cell-cycle disturbance might induce variability. PGRs also preferentially increase the rate of division in cells already genetically abnormal. The genetic composition of a cell population can therefore, be influenced by the relative levels of both auxins and cytokinins. Cells of normal ploidy are often seen to be at an advantage in media where these chemicals are present in low concentrations or totally absent. Evidence for direct mutagenic action of growth regulators is somewhat inconclusive and most evidence points to a more indirect effect through stimulation of rapid disorganised growth. The presence of a relatively high concentration (15 mg l-1) of BA was implicated in the increase in chromosome number in a somaclonal variant derived from the banana cultivar 'Williams'. High levels of BA (30 mg l-1) also greatly increased the genetic variability of rice callus cultures compared to that found in cultures incubated with 2 mg l-1 BA. Diphenylurea derivatives were implicated in incidence of somaclonal variation in bananas, calamondin and soybean. Auxins used during cultures of unorganised calli or cell suspension were found to increase genetic variation by increasing the rate of DNA-methylation. Likewise, the synthetic auxin 2,4-D that is frequently used in callus and cell cultures, is often associated with genetic abnormalities such as polyploidy and the stimulation of DNA synthesis that may result in endoreduplication. The possibility of unbalanced concentrations of auxins and cytokinins inducing polyploidy was also highlighted. Induction of callus using 2,4-D at high concentration has been implicated as cause of somaclonal variation in strawberry, soybean and cotton. Hence, sub- and supra-optimal levels of PGRs, synthetic hormones to a greater extent, in the culture media have been linked with somaclonal variation. In view of these contradictory reports, the role of type and concentration of PGRs particularly cytokinins on incidence of somaclonal variation in different plant species remains a subject for debate and warrants further stringent experiments.

Number and duration of subcultures

Increasing the number of subculture and their duration enhances the rate of somaclonal variations, especially cell suspension and callus cultures. During micropropagation, a high rate of proliferation is achieved in relatively shorter periods and leads to more frequent subculturing. Two main conclusions derived that a variant rate increase can be expected as an exponential function of the number of multiplication cycles and secondly, after a given number of multiplication cycles, variable off-type percentages can be expected.

Effect of stress and genotype

Stress during tissue culture can also induce somaclonal variation. Different genomes however, respond differently to this stress-caused variation indicating that somaclonal variation has genotypic components. The differences in stability are related to differences in genetic make-up whereby some components of the plant genome make them unstable during the culture

process. This could be better explained by the repetitive DNA sequences, which can differ in quality and quantity between plant species. Inherent instability of a cultivar was a major factor that influenced dwarf off-type production in banana tissue culture.

Methods of detecting somaclonal variants

High rates of somaclonal variation during micropropagation of many plants remain a major problem, especially in large-scale commercial operations. Early detection and elimination of variants is therefore essential to reduce the losses to growers. Efficient detection of variants can also be used to spot variants with useful agronomic traits. Somaclonal variants can be detected using various techniques which are broadly categorized as morphological, physiological/biochemical and molecular detection techniques. Each of these techniques has their peculiar strengths and limitations which are briefly discussed below

1. Morphological detection
2. Molecular detection
3. Cytological methods
4. Proteins and isozymes

The term somaclonal variation is now universally accepted to represent heritable variations arising in tissue culture. There however, remains some concern in the universal use of the term somaclonal variation especially in polysomatic and chimeric plants. Nevertheless, the causes of somaclonal variations are generally categorized as induced and preexisting. Visible pre-existing variations such as found in chimeric tissues could theoretically be cultured separately and later manifest themselves phenotypically in somaclones. These may not necessarily represent variations arising during tissue culture. The term should therefore, be restricted to variations that were not visible to the naked eye during the culture initiation stage. In discussing somaclonal variation, both the negative and positive effects need to be treated in parallel. This is due to the potential induced variation has in crop improvement as much as it is essential to detect and eliminate variants at early stages to minimize loss. Somaclonal variation can be detected using a wide range of techniques having their own strengths and limitations. The choice of detection method therefore, depends on the task at hand. Generally, though molecular techniques enable detection of variants in juvenile stages using nucleic acids as opposed to morphological and physiological methods where adult plant response is measured.

Germplasm conservation and cryopreservation.

Germplasm broadly refers to the hereditary material (total content of genes) transmitted to the offspring through germ cells. Germplasm provides the raw material for the breeder to develop various crops. Thus, conservation of germplasm assumes significance in all breeding programmes.

As the primitive man learnt about the utility of plants for food and shelter, he cultivated the habit of saving selected seeds or vegetative propagules from one season to the next one. In other words, this may be regarded as primitive but conventional germplasm preservation and management, which is highly valuable in breeding programmes.

The very objective of germplasm conservation (or storage) is to preserve the genetic diversity of a particular plant or genetic stock for its use at any time in future. In recent years, many new plant species with desired and improved characteristics have started replacing the primitive and conventionally used agricultural plants. It is important to conserve the endangered plants or else some of the valuable genetic traits present in the primitive plants may be lost.

A global body namely International Board of Plant Genetic Resources (IBPGR) has been established for germplasm conservation. Its main objective is to provide necessary support for collection, conservation and utilization of plant genetic resources throughout the world.

There are two approaches for germplasm conservation of plant genetic materials:

1. In-situ conservation
2. Ex-situ conservation

Ex-Situ Conservation:

Ex-situ conservation is the chief method for the preservation of germplasm obtained from cultivated and wild plant materials. The genetic materials in the form of seeds or from in vitro cultures (plant cells, tissues or organs) can be preserved as gene banks for long term storage under suitable conditions. For successful establishment of gene banks, adequate knowledge of genetic structure of plant populations, and the techniques involved in sampling, regeneration, maintenance of gene pools etc. are essential.

Germplasm conservation in the form of seeds:

Usually, seeds are the most common and convenient materials to conserve plant germplasm. This is because many plants are propagated through seeds, and seeds occupy relatively small space. Further, seeds can be easily transported to various places.

There are however, certain limitations in the conservation of seeds:

i. Viability of seeds is reduced or lost with passage of time.
ii. Seeds are susceptible to insect or pathogen attack, often leading to their destruction.
iii. This approach is exclusively confined to seed propagating plants, and therefore it is of no use for vegetatively propagated plants e.g. potato, Ipomoea, Dioscorea.
iv. It is difficult to maintain clones through seed conservation.
Certain seeds are heterogeneous and therefore, are not suitable for true genotype maintenance.

In vitro methods for germplasm conservation:

In vitro methods employing shoots, meristems and embryos are ideally suited for the conservation of germplasm of vegetatively propagated plants. The plants with recalcitrant seeds and genetically engineered materials can also be preserved by this in vitro approach.

There are several advantages associated with in vitro germplasm conservation:

i. Large quantities of materials can be preserved in small space.
ii. The germplasm preserved can be maintained in an environment, free from pathogens.
iii. It can be protected against the nature’s hazards.
iv. From the germplasm stock, large number of plants can be obtained whenever needed.

v. Obstacles for their transport through national and international borders are minimal (since the germplasm is maintained under aspectic conditions).

There are mainly three approaches for the in vitro conservation of germplasm:

1. Cryopreservation (freeze-preservation)
2. Cold storage
3. Low-pressure and low-oxygen storage

Cryopreservation:

Cryopreservation (Greek, krayos-frost) literally means preservation in the frozen state. The principle involved in cryopreservation is to bring the plant cell and tissue cultures to a zero metabolism or non-dividing state by reducing the temperature in the presence of cryoprotectants.

Cryopreservation broadly means the storage of germplasm at very low temperatures:

i. Over solid carbon dioxide (at -79°C)
ii. Low temperature deep freezers (at -80°C)
iii. In vapour phase nitrogen (at -150°C)
iv. In liquid nitrogen (at -196°C)

Among these, the most commonly used cryopreservation is by employing liquid nitrogen. At the temperature of liquid nitrogen (-196°C), the cells stay in a completely inactive state and thus can be conserved for long periods. In fact, cryopreservation has been successfully applied for germplasm conservation of a wide range of plant species e.g. rice, wheat, peanut, cassava, sugarcane, strawberry, coconut. Several plants can be regenerated from cells, meristems and embryos stored in cryopreservation.

Mechanism of Cryopreservation:

The technique of freeze preservation is based on the transfer of water present in the cells from a liquid to a solid state. Due to the presence of salts and organic molecules in the cells, the cell water requires much more lower temperature to freeze (even up to -68°C) compared to the freezing point of pure water (around 0°C). When stored at low temperature, the metabolic processes and biological deteriorations in the cells/tissues almost come to a standstill.

Precautions/Limitations for Successful Cryopreservation:

Good technical and theoretical knowledge of living plant cells and as well as cryopreservation technique are essential.

Other precautions (the limitations that should be overcome) for successful cryopreservation are listed below:

i. Formation ice crystals inside the cells should be prevented as they cause injury to the organelles and the cell.
ii. High intracellular concentration of solutes may also damage cells.
iii. Sometimes, certain solutes from the cell may leak out during freezing.
iv. Cryoprotectants also affect the viability of cells.
v. The physiological status of the plant material is also important.

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