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Somaclonal, Protoclonal and Gametoclonal Variations

1. Introduction

Plants generally exhibit cytogenetic and genetic variations which help the plant breeders in crop improvement. When such variants arise through the cell and tissue culture process using any plant portion as an explant material, variations arising are termed as **somaclonal variations** . Variants obtained using callus cultures are referred as "**Calliclones**" (Skirvin, 1978) while variants obtained using protoplast cultures are known as "**Protoclones**" (Shepard et al. 1980). Larkin and Scowcroft (1981) proposed a general term 'Somaclonal variation' to describe genetic variation in plants regenerated from any form of cell cultures. Accordingly, the plants derived from cell and tissue cultures are termed as '**somaclones**', and the plants displaying variation as 'somaclonal variants'. Another term suggested by Evans et al. (1984) as '**gametoclonal variation**' for those variations arising in cell cultures of gametic origin like, in pollen and microspores cultures, to distinguish them from somatic cell derived regenerants. However, generally the term somaclonal variation is used for genetic variability present among all kinds of cell/plants obtained from cell cultures *in vitro*. Plants regenerated from tissue and cell cultures show heritable variation for both qualitative and quantitative traits. Several useful somaclonal variants have been obtained in large number of plant species such as, potato, sugarcane, banana, tomato etc. Chaleff (1981) labeled plants regenerated from tissue cultures as R_0 generation and their successive sexual generations as R_1, R_2 and so on.

The basic cause of these variations may be attributed to changes in karyotype (chromosome number and structure), chromosome rearrangements, somatic crossing over, sister chromatid exchange, DNA amplification and deletion, transposable elements and DNA methylation. Somaclonal variation can be characterized based on morphological, biochemical (isozymes) and DNA markers such as, Random Amplified Polymorphic DNA (RAPDs), Restriction Fragment Length Polymorphism (RFLPs) and Inter-Simple Sequence Repeats (ISSR).

The variations could also arise in tissue culture due to physiological changes induced by the culture conditions. Such variations are temporary and are caused by **epigenetic changes**. These are non-heritable variations and disappear when the culture conditions are removed.

There are different approaches (steps) to create somaclonal variations, which include:

- i. Growth of callus or cell suspension cultures for several cycles.

ii. Regeneration of a large number of plants from such long term cultures.

iii. Screening for desirable traits in the regenerated plants and their progenies. For example, *in vitro* selection to select agronomically desirable somaclones for tolerance to various biotic and abiotic stresses, herbicides, high salt concentration and extremes of temperature.

iv. Testing of selected variants in subsequent generations for desirable traits.

v. Multiplication of stable variants to develop new breeding lines.

To be of commercial use, a somaclonal variant must fulfill certain basic requirements:

- i. It must involve useful characters.

ii. It should be superior to the parents in the character(s) in which improvement is sought.

iii. The improved character(s) must be combined with all other desirable characters of the parent, and

iv. The variations must be inherited stably through successive generations by chosen means of propagation.

2. Origin of Somaclonal variation

The somaclonal variations observed in plants regenerated from cultured cells are derived from two sources: (i) some of the variations could be revelation of the inherent cellular heterogeneity of the explant, and (ii) culture conditions may bring about new genetic changes.

2.1. Pre-existing variability

Plant development in general involves change in nuclear DNA, such as change in chromosome number, structure (Bennici and D'Amato, 1990). Cells of plant apical meristems like, root-tips and shoot-tips are uniformly diploid in their genome due to DNA synthesis immediately followed by karyokinesis and cytokinesis (normal cell cycle). However, the derivatives of these meristematic cells do not divide by normal mitosis but may undergo DNA duplication and endoreduplication. The varying degree of endoreduplication results in somatic cells with 4C, 8C or higher DNA content or may result in polysomaty. Usually these genetic changes are not noticed as these cells do not divide. However, under culture conditions these cells may divide and undergo redifferentiation and express this change in their genome content as an inheritable character within the whole plant. Another type of pre-existing chromosomal variability which is rarely observed in hybrid plants is **aneusomaty**. In such plants the apical meristems and, consequently, the mature tissues comprise a mosaic of cells with varying number of aneuploid chromosome numbers. This condition is transferred or enhanced in callus cultures derived from such tissues.

2.2. In vitro induced variability

Under the stressful culture conditions, the plant cells undergo genetic and epigenetic changes. This could happen even in the explants from non-polysomatic species. Generally less variations are found in plants than the callus because in mixed population of cells with different ploidy, euploid cells tend to be more regenerative than aneuploid cells. Several factors affect the type and frequency of somaclonal variations, explant source, genotype, culture conditions and age of the culture.

i. Culture medium

Culture media constituents, particularly certain growth regulators, BAP, NAA, 2,4-D, induce mutations in the cultured cells. Sunderland (1977) reported that *Haplopappus* cells in 2,4-D containing medium is converted from entirely diploid state to a entirely tetraploid state within few months. Torrey (1965) observed that in the cultures of pea root segments on a medium with 2,4-D as the sole hormone, only diploid cells divide but when the medium contained Kinetin and yeast extract in addition to 2,4-D, the tetraploid cells were selectively induced to divide. Most of the literature suggests that growth regulators influence somaclonal variation during the culture phase by affecting cell division, degree of disorganized growth and selective proliferation of specific cell types.

ii. Growth pattern and regeneration mode

In vitro growth may occur from meristem cultures, which may form callus (undifferentiated mass of cells) or direct shoot formation. Callus is further differentiated into organized structures by organogenesis or somatic embryogenesis. The departure from organized growth is a key element in somaclonal variation. In general, longer the duration of callus and cell suspension in culture phase, the greater the chances of generating somaclonal variation. These somaclonal variation can also occur in embryogenic cultures, if they are kept for a long time in cultures, depending upon the plant species.

iii. Length of culture and the subculture frequency

The age of callus and cell suspension in culture has a marked effect on the frequency of somaclonal variation which increased with the increase in the duration of in vitro cultures. The reduction or even total loss of regeneration ability is a general phenomenon observed in many long-term callus or cell culture lines. The level of DNA polymorphism has been found to be increased with length of time in culture. Although the chloroplast genome is generally considered to be more highly conserved and stable than nuclear and mitochondrial genomes, the prolonged culture resulted even in the deletion of parts of the chloroplast genome. These deletions were associated with changes in plastid morphology. The age affects the frequency of mutation which is primarily due to sequential accumulation of mutations over time than an increased mutation rate in old cultures. The frequent subcultures of callus and cell suspension favors stability in ploidy and chromosomal constitution compared to extended subculture intervals.

iv. Physical factors

Chemical composition of the media to a great extent influences cytological behavior of cultured cells. Besides, the physical conditions, such as temperature and nature of media (liquid or semi-solid) also influences the rate of mutation.

v. Ploidy and genotype

Genotype of the parent plant is a major determinant of variability in cultures. It influences somaclonal variation irrespective of regeneration mode. Somaclonal variation resulting from changes in chromosome number and rearrangements is easier to recover in regenerants of polyploids than diploids and haploids, since polyploidy can tolerate more gross genomic alterations as compared with diploids and haploids, particularly when such changes are deleterious. On the other hand, gene mutations or point mutations could be better expressed in haploids and diploids. Generally, ploidy levels lower than the usual ploidy level of the respective species are proved to be more or less unstable. Sacristan (1971)

compared the cytological changes in long-term cultures of haploid and diploid strains and described that diploidization in haploid tissues was more common than the occurrence of tetraploids in diploid lines.

3. Mechanisms leading to genetic variations

3.1. Changes in ploidy level: The most frequently observed chromosomal abnormality in the cultured cells and the regenerated plants is the occurrence of the change in ploidy. Such ploidy changes are (i) euploidy, which is the increase in the chromosome number in simple multiples of the basic chromosome number (2n, 3n, 4n, 5n, etc.), and (ii) aneuploidy where the chromosome constitution is not simple multiples of the basic chromosome number.

Formation of a restitution nucleus due to the failure of spindle formation and chromosome lagging at anaphase and the fusion of spindles during synchronous divisions in multinucleate cells are common sources of the occurrence of euploid cells of even series (4,8,16 and so on) in tissue culture. While the odd euploid series in tissue culture arise through nuclear fusion or genome segregation during polyploidy mitosis. The haploid cells found in diploid calli may due to somatic pairing and reduction.

There is strong selection system exist in tissue culture which plays critical role in establishing dominant karyotype or model chromosome number. In mixed cultures of diploid and tetraploid cell lines of carrot (both lines show identical growth rates in monoculture), the frequency of tetraploid cells gradually increased, and the cultures attained a tetraploid mode. The regeneration process itself acts as a screen to eliminate a portion of varying karyotypes, generally, a strong selection exist in favour of diploids or at least euploids than aneuploids during plant regeneration from callus and suspension cultures.

3.2. Changes in chromosome structure: Structural changes in chromosome usually refer to the loss or gain of chromosomal segments which generally results into an altered karyotype but the chromosome number remains the same. In *Haplopappus gracilis* occurrence of accentric fragments, deleted chromosomes, dicentric chromosomes was frequently observed (Singh and Harvey, 1975a). Structural changes in chromosomes originate from breakage during the various stages of the cell cycle. The dicentric chromosomes can bring about continuing variation by initiating a breakage fusion bridge (BFB) cycle. The culture conditions may cause delay in DNA synthesis in the heterochromatin region of the chromosomes until mitosis, resulting in the formation of non-replicated heterochromatin bridges and breakage at anaphase.

3.3. Gene mutations and amplification: Several somalones due to dominant or recessive single or multiple gene mutations have been described by many workers. Recessive mutations may not express in the R_0 generation of somaclones but can be detected in their self progeny. Many distinct gene mutations in several tomato plants regenerated from leaf callus have been well characterized and mapped to specific loci on the chromosomes.

It was suggested by Brown in 1981 that if a gene cannot modulate its expression, it is likely to undergo the gene amplification but if the right selection agent is available. Goldsborough et al. (1990) selected *Nicotiana tabacum* cell lines resistant to normally lethal concentrations of glyphosate and found that the level of enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), which is inhibited by increase level of glyphosate, was elevated. Selected cells maintained high levels of

mRNA for EPSPS even in the absence of the herbicide which is due to the amplification of at least two genes encoding the enzyme. This implies that selection resulted in stable genetic modification.

3.4. Variation in extranuclear genes: The genetic material of cytoplasmic organelles may also undergo changes under in vitro conditions. Mostly changes in mitochondrial DNA are observed and these changes in mt-DNA at molecular level can be very well observed while performing its RFLP restriction pattern. Hartman et al (1989) demonstrated significant changes in mt-DNA of the wheat plants obtained by the regeneration, the extent of change was determined by the length of the tissue culture period. The non-embryogenic cultures of wheat suffered about 8kb fragment loss of mt-DNA found in embryogenic cells.

4. Analysis of Somaclonal variants

Most useful somaclones are those which carry almost all of the good parental characters as well as incorporate within it certain desirable characters which were lacking in its parents. It becomes extremely important to select variants as early as possible, with minimal exposure of cells to tissue culture environment. With prolonged culture gross abnormalities may appear. The variants are generally assessed at the phenotypic level, and in over 50% cases it is based on R_0 plants. However, this approach of screening R_0 plants would the screening of only homozygous or dominant traits. The recessive mutations in heterozygous regenerants can be recognized only in the segregating R_1 and R_2 progenies. It is, therefore, important that the variants should be assessed in the sexual progenies of the in vitro regenerated plants so that their heritability is established.

The degree of variation of a plant can be determined by estimating the standard deviation for a particular quantitative trait. It is usually determined as the percentage of plants showing aberrations for one or more defined characteristics, such as plant height, time of flowering, fertility, flower and fruit color. The effect of environment on the phenotype of plant can also be detected using biochemical characterization mostly involving protein electrophoresis. These above mentioned methods can be very well used for the assessment of phenotypic variations but the variation or change at genome level cannot be monitored. In order to detect the variation at DNA level, use of certain molecular markers is encouraged. RFLP appears to be a better technique as it helps in identifying slight changes and also in studying plants grown in different environments.

5. Applications of Somaclonal Variations

i. Variability generated at the genetic level proves to be a source of crop improvement which can be greatly beneficial to plant breeders.

ii. Distinctive mutations may sometimes give rise to elite characters in the regenerants which cannot be achieved by conventional methods of breeding.

iii. Disease resistant genotypes of various plants can be attained. Resistance was first reported in sugarcane for eye spot disease (*Heliminthosporium sacchari*) and Fiji virus disease by regenerating plants from callus of susceptible clones.

iv. Plants with characteristic resistance to abiotic stress (cold, draught, acidic or alkaline soil) can be obtained as somaclones.

v. Somatic genome exchange may give rise to regenerants where a part of alien genome can be introgressed thereby leading to germplasm widening.

6. Limitations of Somaclonal variations

i. Poor plant regeneration from long-term cultures of various cell lines.

ii. Regeneration being limited to specific genotypes which may not be of much interest to breeders.

iii. Some somaclones have undesirable features, such as aneuploidy, sterility etc.

iv. Unpredictable variations that are often generated are of no use.

v. Variations attained may not always be stably integrated.

vi. Variants attained may not always be novel. In majority of cases improved variants are not even selected for breeding programs.

7. Gametoclonal variation

The variation observed among plants regenerated from gametic cell cultures is termed as gametoclonal variation. The concept of gametoclonal variation evolved from that of somaclonal variation. Both somaclonal and gametoclonal variations were detected in cultured cells and regenerated plants for morphological, biochemical characteristics, and chromosome number and structure.

The life cycle of higher plants comprises a sporophytic (2n) and a gametophytic (n) generation. For genetic reasons, it is necessary to distinguish between plants regenerated from somatic (2n, somaclones) and gametic tissues (n, gametoclones), and also between somaclonal and gametoclonal variation.

i. Unlike somatic cells, which in theory should be homogeneous within any single plant, the gametes are products of meiosis, governed by Mendel's laws of segregation and independent assortment.

ii. Direct expression of both dominant and recessive mutations in the regenerated plants is a distinct feature of gametoclonal variation. Since plants derived from gametic cells are usually haploid but to retain the fertility chromosome number should be doubled. Chromosome number can be doubled by treating haploid plants with colchicines which is a frequently used mutagen to induce diploidization.

iii. Clone is defined as a population of a cell or organisms derived from a single cell or common ancestor by mitosis. The concept of somaclonal variation is built on this definition. Variation is induced either during plant development or in vitro cell cultures, in the mitotic process.

On the other hand, the term ‘gametoclonal variation' can be described as the variation among derivatives of gametic cells in culture, or sexual progeny of plants regenerated from gametic cells in culture. These variants are obtained from both meiotic and mitotic divisions.

There are four distinct sources of variations when referring to gametoclonal variation:

- i. New genetic variations induced by the cell culture procedures.

  ii. Variations resulting from segregation and independent assortment.

  iii. New variation at the haploid level induced by the chromosome doubling, and

  iv. New variation induced at the diploid level, resulting in heterozygosity.