Somaclonal variation and crop improvement

Larkin and Scowcroft (1981) proposed the term somaclone to describe the plants originating from any type of tissue culture. Genetic variation found to occur between somaclones in plant tissue cultures was called somaclonal variation. This variation includes aneuploids, sterile plants and morphological variants, sometimes involving traits of economic importance in case of crop plants. The usefulness of variation was first demonstrated through the recovery of disease resistant plants in potato (resistance against late blight and early blight) and sugarcane (resistance against eye-spot disease, Fiji disease and downy mildew)

Genetic variation - mutations or other changes in the DNA of the tissue those are heritable. This is only transmitted to the next generation and is thus important for crop improvement. Therefore it is necessary to study the transmission of variation to sexual progeny to facilitate the estimation of its utility for improvement of a sexually propagated crop. In several crops R0, R1 and R2 progenies were analyzed for genetic analyses and 3:1 segregation leading to the isolation of true breeding variants was observed.

Epigenetic variation - non-heritable phenotypic variation. Epigenetic changes can be temporary and are ultimately reversible. However, they may also persist through the life of the regenerated plant.

Physiological variation- temporary in response to stimulus and disappear when it is removed.

Causes for variation

Changes of mother plant origin

Chimeral - rearrangement of tissue layers. Many horticultural plants are periclinal chimeras, that is, the genetic composition of each concentric cell layer (LI, LII, LIII) in the tunica of the meristematic tissues is different. These layers can be rearranged during rapid cellular proliferation. Therefore, regenerated plants may contain a different chimeral composition or may no longer be chimera at all. Cell variation also occurs if callus is initiated from explants containing differentiated and matured tissues that have specialized function.

Explant derived variation

The most stable cultures are obtained from meristematic tissue of a mature plant or tissues of a very young organ of meristematic nature. Polyploid cells can give more variability than diploids

Genetic changes arising in culture

Ploidy changes

Three phenomena that occur during mitosis lead to most changes in ploidy:

- Endomitosis (sister chromatids separate within the nuclear membrane, but there is no spindle formation nor cytoplasmic division)
- Endoreduplication (chromosomes at interphase undergo extra duplications)
- Spindle fusion (giving binucleate or multinucleate cells).

Gross structural rearrangements appear to be a major cause of somaclonal variation. These involve large segments of chromosomes and so may affect several genes at a time.

- Deletions (genes missing, for example 1,2,3,4 now 1,2,4)
- Inversions (gene order altered, for example 1,2,3,4 now 1,3,2,4)
- Duplications (1,2,3,4 now 1,2,2,3,4)
- Translocations (whole chromosomal segments moved to a new location, for example 1,2,3,4 now 1,2,3,4,A,B,C)

Transposable elements are segments of DNA that are mobile and can insert into coding regions of genes, typically resulting in a lack of expression of the gene. The culture environment may make the transposable elements more likely to excise and move.

Point mutations (the change of a single DNA base), if they take place within a coding region of a gene and result in the alteration of an amino acid, can lead to somaclonal variation. Point mutations are often spontaneous and are more difficult to detect. Note that they result in single gene changes

Structural changes in the DNA sequence

Chromosomal rearrangements, point mutations, or transposition of transposable elements can occur during culture. These changes can occur spontaneously or can be induced with chemicals or radiation

DNA methylation: Most of the mutational events occasioned by tissue culture are directly or indirectly related to alterations in the state of DNA methylation. A decrease in methylation correlates with increased gene activity

Lack of nucleic acid precursors: Shortage of the precursor necessary for rapid nucleic acid biosynthesis, which occurs in many tissue cultures

Growth regulators: One of the triggers of polyploidy *in vitro* is growth regulators; both kinetin and 2,4-D have been implicated.

Composition of culture medium: The level of KNO_3 influences the albino plants from wheat cultures. Level of organic N_2, chelating agents and other micro nutrients are other factors.

Culture conditions: Temperature, method of culture

Effect of the genotype

Effects of the culture process itself (lengthy culture periods, growth and other aspects of the culture medium may also affect the ploidy of the cultured cells. Medium that places cells under nutrient limitation will favor the development of "abnormal" cells. Chromosomal alterations, like ploidy changes, increase with increased lengths of culture. In mixed populations of cells with different ploidy, diploid cells retain their organogenic potential better than polyploid and aneuploid cells (probably due to an enhanced ability to form meristems).

One common alteration seen in plants produced through tissue culture is *rejuvenation,* especially in woody species. Rejuvenation may lead to changes in morphology, earlier flowering, improved adventitious root formation, and/or increased vigour.

Isolation of somaclonal variants

Mutants for several traits can be far more easily isolated from cell cultures than from whole plant populations. This is because a large number of cells, say 10^6-10^9, can be easily and effectively screened for mutant traits. Screening of as many plants would be very difficult,

ordinarily impossible. Mutants can be effectively selected for disease resistance, improvement of nutritional quality, adaptation of plants to stress conditions, e.g., saline soils, low temperature, toxic metals (e.g., aluminium), resistance to herbicides and to increase the biosynthesis of plant products used for medicinal or industrial purposes. The various approaches to the isolation of somaclonal variants can be grouped into two broad categories: (i) screening and (ii) cell selection.

1. Screening

It involves the observation of a large number of cells or regenerated plants for the detection of variant individuals. This approach is the only feasible technique for the isolation of mutants for yield and yield traits. In general, R1 progeny (progeny of regenerated, Ro, plants) are scored for the identification of variant plants, and their R2 progeny lines are evaluated for confirmation. Screening has been profitably and widely employed for the isolation of cell clones that produce higher quantities of certain biochemicals. Computer based automated cell sorting devices have also been used to screen as many as 1000-2000 cells/second from which desirable variant cells were automatically separated.

2. Cell selection

In the cell selection approach, a suitable selection pressure is applied which permits the preferential survival/growth of variant cells only. Some examples of cell selection are, selection of cells resistant to various toxins, herbicides, high salt concentration etc. When the selection pressure allows only the mutant cells to survive or divide, it is called positive selection. On the other hand, in the case of negative selection, the wild type cells divide normally and therefore are killed by a counter selection agent, e.g., 5 BUdR or arsenate. The mutant cells are unable to divide as a result of which they escape the counter selection agent. These cells are subsequently rescued by removal of the counter selection agent. Negative selection approach is utilized for the isolation of auxotrophic mutants.

The positive selection approach may be further subdivided into four categories: (i) direct selection, (ii) rescue method, (iii) stepwise selection and (iv) double selection.

In direct selection, the cells resistant to the selection pressure survive and divide to form colonies; the wild type cells are killed by the selection agent. This is the most common

selection method. It is used for the isolation of cells resistant to toxins (produced by pathogens), herbicides, elevated salt concentration, antibiotics, amino acid analogues etc.

In the rescue method, the wild type cells are killed by the selection agent, while the variant cells remain alive but, usually, do not divide due to the unfavourable environment. The selection agent is then removed to recover the variant cells. This approach has been used to recover low temperature and aluminium resistant variant cells.

The selection pressure, e.g., salt concentration, may be gradually increased from a relatively low level to the cytotoxic level. The resistant clones isolated at each stage are subjected to the higher selection pressure. Such a selection approach is called stepwise selection. It may often favour gene amplification (which is unstable) or mutations in the organelle DNA.

In some cases, it may be feasible to select for survival and/or growth on one hand and some other feature reflecting resistance to the selection pressure on the other; this is called double selection. An example of double selection is provided by the selection for resistance to the antibiotic streptomycin, which inhibits chlorophyll development in cultured cells. The selection was based on cell survival and colony formation in the presence of streptomycin (one feature) as well as for the development of green colour in these colonies (second feature; only green colonies were selected). This approach has been used for the selection of cells resistant to the herbicide amitrole, 2, 4-D, tobacco mosaic virus (TMV) and aluminium.

Selection of somaclonal variants on subjecting the cells to selection pressure

Selection	**Selection of cells in the presence of**
Resistance to herbicide	Herbicide
Resistance to salt	Sodium chloride / Aluminium
Resistance to drought	PEG / Mannitol
Resistance to frost	Hydroxy proline resistant lines
Resistance to pathogens	Pathotoxin / Culture filtrate

Crop improvement through somaclonal variation for desirable characters

Crop	Characters modified
Sugarcane	Diseases (eye spot, fiji virus, downy miledew, leaf scald)
Potato	Tuber shape, maturity date, plant morphology, photperiod, leaf colour, vigour, height, skin colour, Resistance to early and late blight
Rice	Plant height, heading date, seed fertility, grain number and weight
Wheat	Plant and ear morphology, awns, grain weight and yield, gliadin proteins,amylase
Maize	T toxin resistance, male fertility, mt DNA
Medicago sativa	Multifoliate leaves, elongated petioles, growth, branch, no.of plants, dry matter yield.
Tomato	Leaf morphology, branching habit, fruit colour, pedicel, male fertility, growth
Avena sativa	Plant height, heading date, awns
Hordeum spp	Plant height and tillering
Lolium hybrids	Leaf size, flower, vigour, survival

Characterization of variants

Somaclonal variants isolated through cell selection are often unstable. The frequency of stable variants may range from 8-62%, perhaps depending on the species and the selection agent. Many selected clones fail to exhibit their resistance during further screening or selection. Obviously these clones are susceptible and were misclassified as resistant, called as **escapes**. Several clones lose their resistance to the selection agent after a period of growth in the absence of selection pressure. Such clones are called unstable variants and may result from changes in gene expression and from gene amplification (increase in the number of copies of a gene per genome of the organism in comparison to that naturally present). Some variant phenotypes are quite stable during the cell culture phase, but they disappear when plants are regenerated from the variant cultures, or when the regenerated plants reproduce sexually, in case they are expressed in the regenerated plants. Such changes are known as **epigenetic changes** and are attributed to stable changes in gene expression e.g., hormone habituation of cell cultures and, possibly, cold resistance in *Nicotiana sylvestris*.

The remaining variants which stably express the variant phenotypes during the cell culture as well the regenerated plant phases, and exhibit the transmission of these phenotypes through the sexual reproduction cycle are called mutants. Only this category of variants would find an application in crop improvement. These may represent true gene mutations or some other types of changes. Usually, expected mendelian ratios are obtained in the RI progenies. But sometimes aberrant segregation ratios are encountered in RI possibly due to the chimeric nature of Ro plants, the involvement of some cytological anomalies like aneuploidy, deletions etc., gene dosage effects etc.

Achievements

Over a dozen varieties have been developed through the exploitation of somaclonal variation. 'Ono' variety of sugarcane is a Fiji disease resistant somaclone of the susceptible cultivar 'Pindar'. It was identified by screening of plants regenerated from unselected calli. 'Ono' also shows yield advantage over 'Pindar' and has been cultivated to a limited extent in Fiji. A sweet potato cultivar 'Scarlet' was selected from shoot-tipculture-derived clones. 'Scarlet' is comparable to the parent cultivar in yield and disease resistance, but shows darker and more stable skin colour, which is a desirable quality trait. A geranium variety called 'Velvet Rose' is a somaclone of 'Rober's Lemon Rose'. The new variety has twice the chromosome number of the parent variety. An alfalfa variety called 'Sigma' is a polycross of selected somaclones.

In India, so far somaclonal variation is the only biotechnological approach to give a commercial variety. A somaclonal variant of Citronella java, a medicinal plant, has been released as 'Bio-13' for commercial cultivation by CIMAP (Central Institute for Medicinal and Aromatic Plants), Lucknow. Bio-13 yields 37% more oil and 39% more citronellol than the control varieties. A somaclonal variant of the B. juncea variety 'Varuna' has been released for commercial cultivation as 'Pusa Jai Kisan'. The new variety has bolder seeds and some yield advantage over the parent variety Varuna.

Advantages

- Somaclonal variations occur in rather high frequencies, which is a great advantage over conventional mutagenesis.

- Some ‘new’ alleles or even ‘new’ mutations may be isolated which were not available in the germplasm or through mutagenesis, e.g., joint less pedicel mutant in tomato.
- Use of somaclonal variation may reduce by two years the time required for the release of new variety as compared to mutation breeding. This is because somaclonal variations are usually free from undesirable features like sterility, while induced mutations are generally associated with such defects, which necessitate one or two backcrosses with the parent variety.
- A very effective selection can be practised at the cell level for several traits, e.g., disease resistance etc. This approach effectively selects few desirable cells from among millions with relatively small effort, time, cost and space requirements.
- This is the only approach for the isolation of biochemical mutants, especially auxotrophic mutants, in plants.

Limitations

- The technique is applicable only to those species of cell cultures which regenerate complete plants.
- Selected cell lines often show reduced or no regeneration potential.
- Many selected clones show undesirable features like reduced fertility, growth and even overall performance.

Somaclonal variation represents a useful source of introducing genetic variations that could be of value to plant breeders. Single gene mutation in the nuclear or organelle genome may give the best available variety in vitro that has a specific improved character. In this manner, somaclonal variation could be used to uncover new variants retaining all the favourable characters along with an additional useful trait, such as resistance to diseases or a herbicide. Various cell lines selected in vitro may then prove potentially applicable to agriculture and industry.

Questions

1. The term somaclone was proposed by ……

a). Larkin and Scowcroft b). Skoog
c). Murashige d). None of the above

2. Somaclonal variation includes ……

a). Aneuploids b). Sterile plants
c). Morphological variants **d). All the above**

3. The usefulness of somaclonal variation was first demonstrated through the recovery of disease resistant plants in ……

a). Potato b). Sugarcane
c). Both a and b d). None of the above

4. Epigenetic variation includes ……

a). Non-heritable phenotypic variation b). Temporary
c). Ultimately reversible **d). All the above**

5. Ploidy changes occur due to ……. during mitosis

a). Endomitosis b). Endoreduplication
c). Spindle fusion **d). All the above**

6. Gross structural rearrangements viz., ……. are major cause of somaclonal variation

a). Deletions and duplications b). Inversions
c). Translocations **d). All the above**

7. Ono is a somaclonal variety of …….

a). Sugarcane b). Geranium
c). Alfa alfa d). Citronella

8. Scarlet is a somaclonal variety of …….

a). Sugarcane **b). Sweet potato**
c). Alfa alfa d). Citronella

9. Velvet rose is a somaclonal variety of …….

a). Sugarcane **b). Geranium**
c). Alfa alfa d). Citronella

10. Sigma is a somaclonal variety of …….

a). Sugarcane
b). Geranium
c). Alfa alfa
d). Citronella

11. Bio 13 is a somaclonal variety of …….

a). Sugarcane
b). Geranium
c). *Brassica juncea*
d). Citronella

12. Varuna is a somaclonal variety of …….

a). Sugarcane
b). Geranium
c). *Brassica juncea*
d). Citronella

13. Limitation(s) of somaclonal variation is/are …….

a). Applicable only to those species which regenerate complete plants out of cell cultures
b). Reduced or no regeneration potential
c). Undesirable features like reduced fertility, growth and even overall performance
d). All the above