Somaclonal variation

When the *in vitro* cultured cells have gone through genetic variation it is then called **somaclonal variation** and the plants derived from such cells are called "**somaclones**." Somaclonal variation is always associated with chromosomal variations, which have been generally found in long-term callus, cell suspension culture, and plants regenerated from such cultures. This type of genetic variation generates various potential applications such as crop improvement, in the production of mutants and variants (e.g., disease resistance in potato). Larkin and Scowcroft coined the term "somaclones" for plant variants obtained from tissue cultures of somatic tissues. Similarly, if the somatic tissue-derived variants have a gametophytic origin such as pollen or egg cell, then it is known as "gametoclonal" variation. Several causes of this type of variation are heterogeneity between the cells and explant tissue, spontaneous mutation and activation of culture environment of transposition of genetic materials. In 1980, Shepard and his coworkers screened 100 somaclones produced from leaf protoplasts of large white potato (Russet Burbank). They established that there was a significant amount of stable variation in compactness of growth habit, maturity, date, tuber uniformity, tuber skin color, and photoperiodic requirements. Among the various applications, plant breeding is the major application of somaclonal variations where the new traits with desired or improved characters are introduced into the plants.

Basis of Somaclonal Variations:

Somaclonal variations occur as a result of genetic heterogeneity (change in chromosome number and/or structure) in plant tissue cultures.

This may be due to:

i. Expression of chromosomal mosaicism or genetic disorders.

ii. Spontaneous mutations due to culture conditions.

The genetic changes associated with somaclonal variations include polyploidy, aneuploidy, chromosomal breakage, deletion, translocation, and gene amplifications, besides several mutations. In fact, the presence of several

chromosomal aberrations—reciprocal translocation, deletions, inversions, chromosomal breakage, multi-centric, acentric fragments have been found among the somaclones of barley, garlic and oat. The occurrence of mutations in cultures is relatively low. Mutations may be due varied nutrients, culture conditions and mutagenic effects of metabolic products that accumulate in the medium. Somaclonal variations due to transposable elements, mitotic crossing over and changes in the cytoplasmic genome have also been reported.

Nomenclature of somaclones:

The somaclones that are regenerated from tissue cultures directly are regarded as R_0 or R plants. The self-fertilized progeny of R_0 plants represent R_1 plants. R_2, R_3, R_4 etc. plants are the subsequent generations. Some workers use other nomenclature — somaclones ($SC_1 = R_0$), SC_2, SC_3, SC_4 etc. for subsequent generations.

Isolation of Somaclonal Variants:

There are two procedures commonly used for obtaining the crop plants with somaclonal variations:

1. Without in vitro selection

2. Within vitro selection.

1. Without in Vitro Selection:

The so produced plants are grown in pots, transferred to field, and analyzed for somaclonal variants (Fig. 46.1).

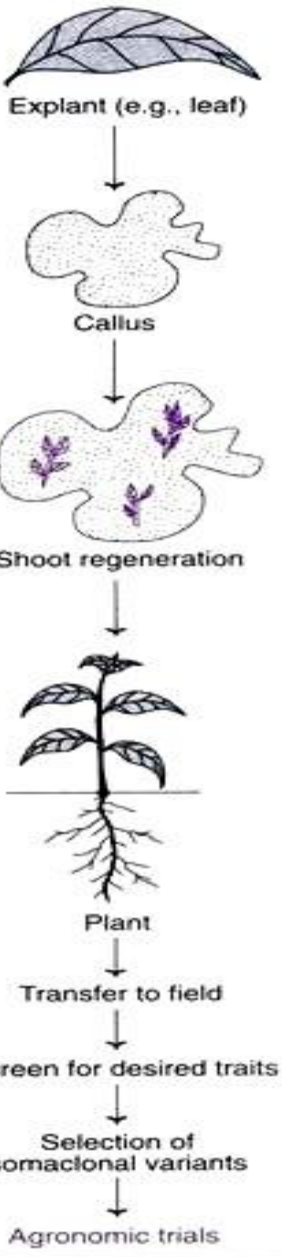


Fig. 46.1 : A diagrammatic representation of isolation of somaclones without in vitro selection.

Somaclonal variants of several crops have been successfully obtained by this approach e.g., sugarcane, potato, tomato, cereals etc.

2. With in Vitro Selection:

Isolation of somaclones with in vitro selection method basically involves handling of plant cells in cultures (protoplast, callus) like microorganisms and selection of biochemical mutants.

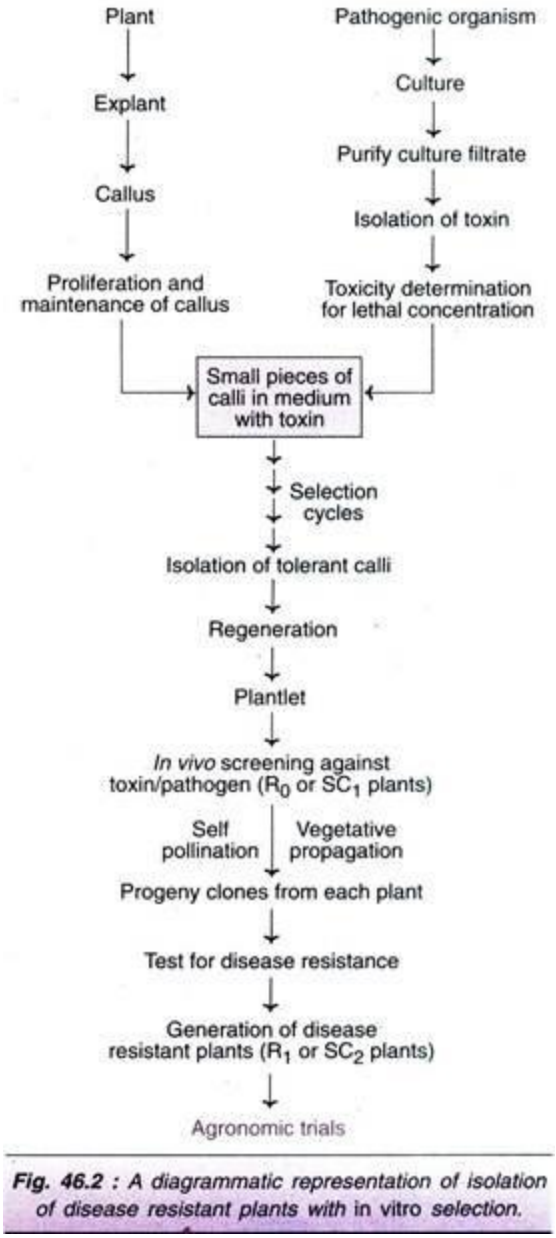


Fig. 46.2 : A diagrammatic representation of isolation of disease resistant plants with in vitro selection.

Mechanism of Somaclonal Variations

1. Genetic (Heritable Variations)

- Pre-existing variations in the somatic cells of explant
- Caused by mutations and other DNA changes

• Occur at high frequency

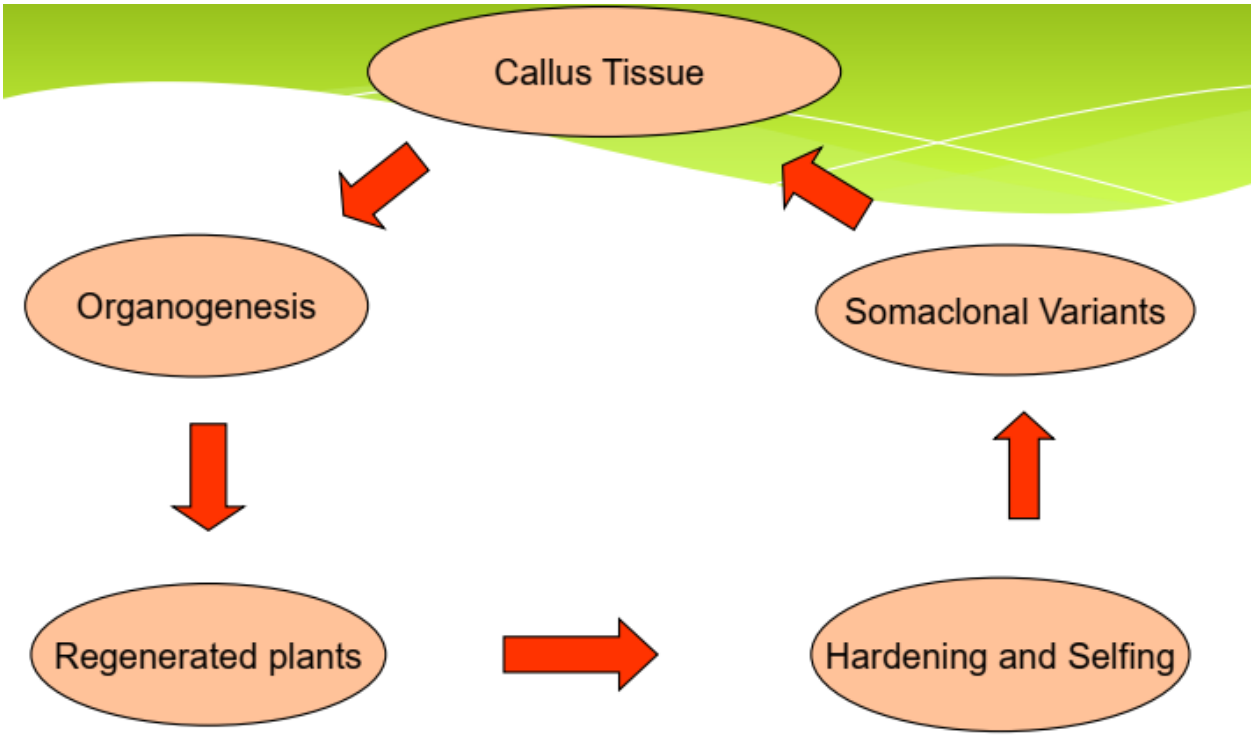
2. Epigenetic (Non-heritable Variations)

• Variations generated during tissue culture

• Caused by temporary phenotypic changes

• Occur at low frequency.

Steps involved in induction and selection of Somaclonal Variations:



Isolation & detection of somaclonal variant

- **Analysis of morphological characters**

Qualitative characters: plant height, maturity date, flowering date and leaf size

Quantitative characters: yield of flowers and seed etc. in different plant parts

- **Cytological studies**

  Staining of meristematic tissues like root tip, leaf tip with feulgen and acetocarmine provide the number and morphology of chromosomes.

- **Variant detection by DNA contents**

  Cytophotometer detection of feulgen stained nuclei can be used to measure the DNA contents

- **Variant detection by gel electrophoresis**

  Change in concentration of enzymes, proteins and hemical products like pigments, alkaloids and amino acids can be detected by their electrophoretic pattern

- **Detection of disease resistance variant**

  Pathogen or toxin responsible for disease resistance can be used as selection agent during culture.

- **Detection of herbicide resistance variant**

  Plantlets generated by the addition of herbicide to the cell culture system can be used as herbicide resistance plant.

- **Detection of environmental stress tolerant variant**

  1. Selection of high salt tolerant cell lines in tobacco
  2. Selection of water-logging and drought resistance cell lines in tomato

3. Selection of temperature stress tolerant in cell lines in pear.
4. Selection of mineral toxicities tolerant in sorghum plant(mainly for aluminium toxicity)

Causes of somaclonal variations:

1. Physiological cause
2. Genetic cause
3. Biochemical cause

Physiological cause

- Exposure of culture to plant growth regulators.
- Culture conditions

Genetic cause

1. Change in chromosome number

- aneuploidy – gain or loss of 1 or more chromosomes
- polyploidy – gain or loss of an entire genome
- translocation – arms of chromosomes switched
- inversion – piece of chromosome inverted

2. Change in chromosome structure

- Deletion
- Inversion
- Duplication
- Translocation

3. Gene Mutation

- Transition
- Transversion
- Insertion
- Deletion

4. Plasmagene Mutation

5. Transposable element activation

6. DNA sequence

- Change in DNA

  Detection of altered fragment size by using Restriction enzyme

- Change in Protein

  Loss or gain in protein band

  Alteration in level of specific protein

- Methylation of DNA

  Methylation inactivates transcription process.

Biochemical cause

- Lack of photosynthetic ability due to alteration in carbon metabolism
- Biosynthesis of starch via carotenoid pathway
- Nitrogen metabolism
- Antibiotic resistance.

Molecular markers

In some instances, discrepancies between molecular markers and phenotypic data are observed. These discrepancies relate to the complexity of the plant genome, and the markers that are normally used often cannot give a complete view. Currently, different molecular analytic techniques have used to point out somaclonal variation in tissue culture and in regenerants of several plants. Randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) markers have been used to study the genetic fidelity or genetic variability in micropropagated fruit crops. RAPD markers are suitable for detecting somaclonal variation and the variation observed is genotype-dependent.

Factors Affecting Production of Somaclonal Variants:

Some of the important factors that influence development of somaclonal variants by without in vitro selection and within vitro selection are briefly described.

Genotype and explant source:

The nature of genotype of the plants influences the frequency of regeneration and frequency of production of somaclones. Explants can be taken from any part of plant — leaves, roots, internodes, ovaries etc. The source of explant is very critical for somaclonal variations. For instance, potato plants regenerated from callus of rachis and petiole are much higher (~50%) compared to those regenerated from callus of leaves (~12%).

Duration of cell culture:

In general, for many plant cultures, somaclonal variations are higher with increased duration of cultures. For example, it was reported that genetic variability increased in tobacco protoplasts from 1.5 to 6% by doubling the duration of cultures.

Growth hormone effects:

The plant growth regulators in the medium will influence the karyotypic alterations in qultured cells, and therefore development of somaclones. Growth hormones such as 2, 4-dichlorophenoxy acetic acid (2, 4-D) and naphthalene acetic acid (NAA) are frequently used to achieve chromosomal variability.

Limitations of Somaclonal Variations:

Despite several applications of somaclonal variations, there are certain

Limitations/ disadvantages also:

i. Most of the somaclonal variations may not be useful.

ii. The variations occur in an unpredictable and uncontrolled manner.

iii. Many a times the genetic traits obtained by somaclonal variations are not stable

and heritable.

iv. Somaclonal variations are cultivar-dependent which a time consuming process

is frequently.

v. Somaclones can be produced in only those species which regenerate to complete

plants.

vi. Many cell lines (calli) may not exhibit regeneration potential.

Advantages & disadvantages of somaclonal variations:

Advantages:

- Help in crop improvement
- Creation of additional genetic variations
- Increased and improved production of secondary metabolites
- Selection of plants resistant to various toxins, herbicides, high salt concentration and mineral toxicity
- Suitable for breeding of tree species

Disadvantages:

- A serious disadvantage occurs in operations which require clonal uniformity, as in the horticulture and forestry industries where tissue culture is employed for rapid propagation of elite genotypes
- Sometime leads to undesirable results
- Selected variants are random and genetically unstable
- Require extensive and extended field trials
- Not suitable for complex agronomic traits like yield, quality etc.
- May develop variants with pleiotropic effects which are not true.

Applications of Somaclonal Variations

The five applications are:

- Production of agronomically useful plants
- Resistance to diseases
- Resistance to abiotic stresses
- Resistance to herbicides and
- Improved seed quality.

Examples of Somaclonal Variation:

- Improvement of existing clonal cultures
- sugarcane – selections for higher yield & disease resistance
- potatoes – yield & disease resistance
- improved geraniums (esp. scented varieties)
- woody ornamentals (e.g., Paulownia – selection for leaf variegation