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Somaclonal variation in micro propagated bananas

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Introduction

Banana is one of the most fascinating and important of all the fruit crops. It is a large monocotyledonous herb that originated in Southeast Asia. Virtually all the cultivars that are grown are thought to have been selected as naturally occurring hybrids in this region by the earliest of farmers. In fact, Norman Simmonds proposed that banana was one of the first crops to be domesticated by man. In writing of the beginnings of agriculture in Southeast Asia, he concluded, "It seems a reasonable assumption that the bananas evolved along with the earliest settled agriculture of that area and may therefore be some tens of thousands of years old." Banana a fruit of tropics is one of the most important fruit crops of the world as well as India. Banana (*Musa* Spp) belongs to family Musaceae. The fruits have attractive yellow color, with weak pedicel attachment, peculiar sugar acid blend, and fetches higher prices among other ruling variants in the local market. Considering the nutritive value of banana, it is so prominent and popular among the people of India that, both poor and rich alike like it. Banana is also called "poor man's apple" as it the cheapest among fruits grown in the country with rich energy and nutritive values. It is also popular on account of its year round availability as compared to seasonal availability of other fruits. The most important banana region in the world is around the Caribbean, Jamaica, Honduras, Costa Rica, Guatemala, Mexico, Panama, Cuba and Nicaragua, in the order of importance. The crop is cultivated on a large scale in Florida in North America, Brazil, Colombia and Ecuador in South America throughout Equatorial Africa, Uganda, Zanzibar, Cameroon, West Africa, Ivory Coast and Eritrea. In Asia India is the chief producing country and the produce is utilized entirely for internal consumption and there are no exports like Malaysia, Ceylon, South China, Formosa, Fiji, and Java. The Philippine Island, Western Samoa and Queens land are the other banana producing countries.

Bananas and plantains together rank as the fourth most important food commodity in the world exceeded only by rice, wheat, milk or milk products. They provide nourishment and a well balanced diet to millions of people around the globe and contribute to livelihood through crop production, processing and marketing. These are largely produced n the tropical and sub tropical regions.[1] The banana growing states in India are Tamilnadu, Maharastra, Karnataka, Assam, Andhra Pradesh, Gujarat, Bihar, Madhya Pradesh, Orissa and west Bengal. Karnataka is leading in production of banana followed by Andhra Pradesh, Madhya Pradesh and Bihar. These five states contribute more than 60% of total banana production in the country. Current world production of banana is estimated at 97.5 million tones per year covering 10 million hectares. India is the largest producer of banana in the world with a total annual production of 16.91 million tones on 0.49 million hectares, contributing 26 per cent of world's production and 37 per cent of the national fruit production.[1,2] Banana ranks as a number one fruit crop in the Indian micro propagation industry. Nearly, over 50 viable micro propagation companies are currently producing micro propagated banana plantlets with a demand, which is ever on the increase. Currently, 5-6 million tissue culture banana plantlets are planted every year. However, Somaclonal variation can pose a problem in any micro propagation program, which is important to produce true to type plant material.[3]

Somaclonal variation

Somaclonal variation is a phenotypic variation, either genetic or epigenetic in origin, displayed among the soma clones (soma = vegetative; clone = identical copy). It results from both pre existing genetic variation within the explants as well as induced during the tissue culture cycle. "*Variation among plants regenerated from any form of cell culture*" is termed as somaclonal variation.[4] Changes in the chromosome number, chromosomal rearrangements, single nucleotide changes, alteration of gene copy number, activation of transposable elements and sequence specific variation in DNA methylation etc., are some of the genetic changes due to which phenotypic variations are known to occur are heritable in nature.[5]

Somaclonal variation in banana

Banana (n=11) appears to be a crop highly sensitive to producing large variations.[6] A majority of the off types recorded in this crop are agronomically inferior to the parental clone. Somaclonal variants of different types have been reported with regard to plant morphology, viz., (1) plant stature, (2) abnormal leaves, (3) pseudostem pigmentation, (4) abnormal bunch orientation, (5) small narrow elongated male bud or bloated male bud, (6) absence of male bud and its reversion, (7) small bunch with short fingers, (8) variation in hand and finger orientation on the bunch, (9) persistence or deciduous nature of floral bracts, (10) hairiness on bunch peduncle and fruit, (11) split and twisted fingers, (12) warty fruits with ugly eruptions.[7]

Dwarfism was the most common variant, whereas, tall mutants were very rare. In banana, where production of somaclonal variants is substantial, only those plants that show side shoots as well as with the same type of variation are considered as 'variants'.[8] Appearance of off types during the *in vitro* multiplication process is an important drawback for mass propagation of bananas. In any micropropagation program, 3-5 per cent somaclonal variation is permissible, but in banana, up to 10 per cent is permitted (as practiced by commercial micropropagation outfits) owing to its flexible genetic makeup of the crop.[9,10] In the genus, *Musa*, somaclonal variation at the phenotypic level has been observed with 6 per cent frequency on average. It is also reported that the occurrence of off types from tissue cultured plantlets ranged from 6 to 38 per cent in Cavendish cultivars. They also reported 29 cases of somaclonal variation in various types of bananas and plantains, with the incidence ranging from 0 to 69 per cent.

Sources of somaclonal variation

There are two important sources of somaclonal variation. They are,

i. **Pre-existing variations** [variants] – sometimes mutated cell arises spontaneously and remains berried among parental somatic tissues. Regeneration of plants from such cells results in creation of somaclones.

ii. **Tissue culture induced variations** [mutants] – tissue culture environment acts as a mutagenic agent and upsets normal control of cell division and chromosome distribution leading to generation of somaclonal variation.

Factors contributing to somaclonal variation

Sahijram et al.,[3] reviewed some of the factors known to contribute to somaclonal variation are listed and explained in brief are,

a. Effect of explant or explant source

b. Effect of culture age or number of subculture cycles

c. Effect of hormonal factors or plant growth regulators *in vitro*

d. Genotype fidelity

e. Flexibility of the genotype

f. Effect of ploidy level

g. Karyotype changes

h. Role of transposable elements

Effect of explant or explant source: Highly differentiated tissues (roots, leaves, and stems) generally produce more variation than explants with pre-existing meristems, viz., axillary buds and shoot or meristem tips. Shchukin et al.,[7] reported that the rate of somaclonal variation was higher in shoot tip derived cultures (5.3%) of cv. 'Grand Naine' as compared to those derived through somatic embryogenesis (0.5% and 3.6%).

Effect of culture age or number of subculture cycles: Culture age enhances variability among the regenerated plants. *In vitro* culture conditions and rapid multiplication of a tissue may affect genetic stability and lead to somaclonal variation. This may be attributed by,

i. Increased number of subcultures and

ii. Longer duration of the culture

Rodrigues et al.,[8] had studied the influence of number of sub cultures on somaclonal variation in micropropagated 'Nanicao' (*Musa* spp., AAA group). They observed that somaclonal variation appeared after 5, 7, 9 and 11 subcultures at a rate of 1.3, 1.3, 2.9 and 3.8%, respectively.

Effect of hormonal factors or plant growth regulators *in vitro:* The primary event that possibly triggers tissue cultured variability is the cell cycle disturbance caused by exogenously applied chemicals. Cytokinins like, BAP (Benzyl Amino Purine) and kinetin were shown to induce genetic variability in banana.

Genotype fidelity: All the modern bananas (*Musa* spp.) are derived from two ancestors: *Musa acuminata* (the A genome) and *Musa balbisiana* (the B genome). Cultivars vary in their response to *in vitro* generation. These differences may in part be attributed to the influence of the microenvironment on cellular behavior. Variability in the presence of the A genome is high while in the presence of the B genome it is lower.

Flexibility of the genotype: In general, *in vitro* conditions can be extremely stressful on plant cells, and may set in motion highly mutagenic processes during explant establishment, callus induction and maintenance, embryo development and plant regeneration. It is reported that there is particularly a labile portion of the genome that is especially susceptible to stress, showing higher rearrangement and mutation rates than other portions of the genome. Therefore, somaclonal variation may not be a random phenomenon as specific loci are prone to higher mutation rates than others during *in vitro* culture.

Effect of ploidy level: variability in plants regenerated *in vitro* is high among the polyploids and higher chromosome number explant donor species than among ones with low ploidy and lower chromosome number.

Karyotype changes: Genetic stability of the somaclonal variants is important. If these variations are stable, selected traits may be found useful. Maintaining bananas in *in vitro* cultures for longer durations, increases the frequency of chromosomal aberrations especially aneuploidy. In vitro proliferation of plant cells from a disorganized callus stage may be accompanied by chromosomal instability. This instability may lead to changes either in phenotype or the genotype, resulting in regenerated plants that are different from the original clone.

Role of transposable elements: Activation of transposable elements may generate either stable or unstable single gene mutations, changes in DNA methylation and activation of transposable, and additional chromosome breakage. Autonomous transposable element activation may be a symptom of genetic instability either in tissue culture regenerated plants or their progeny.

Morphological variation in banana

Israeli et al.,[7] reported the various somaclonal variants in Cavendish sub group of seven different cultivars are agronomically inferior to the normal plants. The variations are observed in plant stature, foliage, pseudostem pigmentation and variations in inflorescence and fruit characteristics.

Variations in plant stature

- Dwarfs,
- Extra dwarfs and
- Giants

Abnormal foliage

- Mosaic,
- Extreme mosaic,
- Variegated leaves and
- Deformed lamina

Variations in pseudostem pigmentation

- Reddish pseudostem
- Black pseudostem and

Green-Red pseudostem

Inflorescence and fruit variations

Persistent flowers and

Split fingers

Detection of somaclonal variation

The various methods of detecting or screening of off types or useful variants in banana are two types, namely,

Morphological screening: Visual screening during acclimatization in the greenhouse or nursery helps to detect putative off types and is satisfactory for commercial purposes. The detection can be done with the help of morphological makers.

Morphological markers

The plant expresses number of morphological character unique to a particular species yet these characters varies with the environment conditions and highly prejudice. Some of the prominent characters are height, branching nature, leaf margin, girth of the stem, flowering pattern, etc. These morphological characters are simple, inexpensive and vary according to the geographical locations. Multivariate analysis has been used to optimize morphological detection of off types during acclimatization.[11] Smith et al.,[10] have detected off type plants in Lady Finger bananas *(Musa* spp., AAB group, 'pome'subgroup) during establishment and growth of plants in the greenhouse with chlorotic streaks in leaves.

Molecular screening: Markers are stable and inherited variation that can be measured or deleted by suitable method. These markers are associated with particular character. There are various types of markers, these are;

Molecular markers include,

1. Biochemical markers/Isozymes
2. DNA based markers

Biochemical Markers

Isoenzymes are based on multiple forms of an enzyme which differ in electrophoretic mobility. They may be visualized following gel separation. These also include proteins and secondary metabolites. These markers are reliable, consistent and not varied due to environmental conditions. At the same time however the content of these biochemical markers change with change in the stage of developing plants.

DNA based markers

The DNA based markers are powerful tools in detecting polymorphism and are used extensively because of the following advantages,

a. Potentially a large number of polymorphisms can be detected.

b. It allows investigation of both coding regions of the whole genome.

c. It is independent of growth and development of the plant.

d. They are seldom influenced by environment.

DNA based markers are RFLP, AFLP, RAPD, SSR etc. are also widely used to detect Somaclonal variation. Randomly amplified polymorphic DNA (RAPD) markers have been used to identify growth habit variants (very dwarf, dwarf and giant) originating from *in vitro* cultures of cvs. Grand Naine and Petite Naine.[11]

Advantages of somaclonal variation

Tissue cultures are providing to be rich and novel sources of variability with great potential in crop improvement without resorting to mutations or hybridizations. Somaclonal variation can also be gainfully exploited for generating disease resistant clones. This is especially useful where no source of natural resistance exists either in the cultivated or the wild relatives.

a. Rapid source of variation is available – somaclonal variation can be created very rapidly as compared to conventional breeding programme. This aspect of somaclonal variation holds great promise particularly in banana which is commercially vegetatively propagated and alternate breeding approaches are limited.

b. Changes can occur in horticulturally important traits – many changes in horticultural traits have been observed in somaclones includes clones having resistance to panama wilt and Sigatoka disease, dwarf growth habit, early flowering, higher yield and improved fruit quality.

c. Changes in characters related to environmental stresses like resistance to aluminum toxicity have also been observed in the somaclones.

d. Novel variants can arise.

e. Changes in characters occur at high frequencies.

f. *In vitro* selection shortens the time in isolation of somaclones having desirable characters.

g. Large population of cells can be used for *in vitro* selection.

Limitations of somaclonal variation

In spite of several advantages, there are a few limitations of somaclonal variation as a tool for banana breeding. These are,

1. Many characters change in opposite or negative direction.

2. May not occur for complex agronomic traits.

3. Variations are unpredictable in nature.

4. Selected somaclones requires extensive field training.

5. Somaclones could be unstable due to DNA methylation and activation of transposable elements.

Measures to reduce somaclonal variation

a) Some of the possible measures to reduce the somaclonal variation in lab and at field conditions are,

b) Source plant must completely match the known description of the cultivar, must have the desired agronomical characteristics and relatively stable in *in vitro* propagation.

c) Number of plants and number of generations produced from a primary explant must be reduced.

d) Many primary explants per batch must be used, to minimize the risk of having a high percentage of variants contributed by a single explant that has mutated in an early stage of propagation.

e) Small undeveloped plantlets in each subculture should be discarded and not used for next generations.

f) Off-type plants should be screened at all stages from regeneration to the final stage in the nursery.[12,13]

Conclusion

In any micro propagation program, Somaclonal variation is not desirable as it defeats the primary objective of clonal propagation. Obvious, chromosome breakages or aberrant number of chromosomes are found even in conventional sucker-grown plants. However, these defects get magnified in plants grown *in vitro* and more incidence is observed with increase in ploidy level. Hence, restricting the number of sub-culture cycles to 5-7 is considered safe.

Although, somaclonal variation produces agronomically inferior off types in banana, it can be used to advantage as a source of new variation in *Musa* spp. by proper methods of detection, evaluation and improvement of useful variants obtained in banana with higher resistance to yellow sigatoka disease wilt, with dwarfism, heavier bunch, shorter growing cycle and tolerance to low temperature or low light.

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None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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