Somaclonal Variation: A new dimension for sugarcane improvement

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Abstract

Plant tissue culture or micropropagation technique is the rapid method to multiply newly released cultivar in limited time. Crop improvement by conventional method in vegetatively propagated crops like sugarcane is very difficult due to its narrow genetic base and other limitations. Somaclonal variations are easily achieved in asexually propagated crops like sugarcane and banana. Tissue culture derived variations are known as somaclonal variation. These variations play an important role in crop improvement program. Genetic variations are heritable in next generation and important for crop improvement, epigenetic changes are temporary ultimately reversible. Mutation breeding is also very advantageous for improving a cultivar. Somaclonal variants of sugarcane are available for several traits like drought, salt tolerance, red rot, eye spot disease, quality and quantity trait. Molecular marker techniques like RFLP, RAPD, AFLP and SSR etc. are regularly used preferentially over traditional phenotypic or cytological methods.

Key words: Somaclonal variation, crop improvement, plant tissue culture, sugarcane, conventional breeding

Introduction

Sugarcane (Saccharum species complex), being the most valuable commercial crop of the world. It is the major source of sugar in the world and recently to produce ethanol, a high energy rich biofuel. The crop improvement in many crops like sugarcane is very difficult and time taking because it has a complex poly-aneuploid, large genome size and long breeding cycle. With the advancement of biotechnological tools for the genetic improvement of many economically important crops like have been highlighting by different researchers in past years. In the past decade considerable progress has been made in understanding and manipulating the sugarcane genome using various biotechnological and cell biological approaches. Notable among them are the crop improvement through somaclonal variation, creation of transgenic plants with improved agronomic or other important traits, advances in genomics and molecular markers, and progress in understanding the molecular aspects of sucrose transport and accumulation. It is anticipated that the rapid advancements in emerging biotechnology innovations would play a significant role in the future sugarcane crop improvement programs and offer many new opportunities to develop it as a new generation industrial crop. Plant tissue culture gave an excellent opportunity for the creation of genetic variability of clones.

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with desired character in sugarcane (1,2,3). Somaclonal variations are important tool to overcome the constrains of conventional breeding (4). The accumulation of genetic variability is an important aspect in plant breeding. Genetic variability has been easily discernible in in vitro regenerated somaclonal variants.

In vitro regenerated variants are known as Somaclonal variation or sub-clone variation (5,6,7) mainly depends on various modes of plant regeneration. Somaclonal variants have been available in many horticultural traits, ornamental plants and recently the selection of new variants of many crops like sugarcane for disease resistance (8,9,10) and various agronomic characters like yield and quality (4). The occurrence of phenotypic instability is a major problem, when our objective to produce the true copies of original plant (11). The occurrence of somaclonal variation in tissue culture derived plants is an alternative method to sort out many barriers of traditional breeding program. Sugarcane is a good candidate for such type of studies due to its complex polyploidy. The use of variation was first established 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).

Somaclonal variation may be of two types 1- genetic variations (may be occurred in somatic cells of the mother plant), 2- epigenetic (may be occurred during in vitro tissue culture practices).

Types of variations

Genetic variation

The variation occurs due to mutations or other changes in the DNA of the tissue are known as genetic variation. These are heritable in next generation and important for crop improvement. Genetic changes behave as Mendelian traits in crosses.

Epigenetic variation

Epigenetic changes are temporary ultimately reversible (plants ‘revert’ to normal phenotype) also known as developmental variation. These are non-heritable phenotypic variation. There are several factors like explants source, age of the donor plant, genotype, number of sub-culture passaging, in vitro culture environment, concentration of plant growth regulators, medium composition etc are the components that might be induce variability in vitro (12). Tissue culture system itself acts as a mutagenic system because cells experience traumatic experiences from isolation and may re-programme during plant regeneration. Reprogramming or restructuring of events can create a wide range of epigenetic variation in newly regenerated plants (13).

Physiological variation

These variations are temporary in response to stimulus and disappear when it is removed.

Need of improved varieties and limitations of conventional approaches

Plant breeders have exploited the germplasm resource to develop new cultivars with desirable traits viz., high yield, diseases resistance and tolerance to many abiotic stresses (14). However enormity of growing world population, crisis of arable land and continual demand for newer improved cultivars by using available natural and induced genetic diversity. Conventional breeding takes approx 8-10 years to develop and commercialize a new selected sugarcane cultivar (15, 9). Another major problem of sugarcane growers its slow multiplication rate cannot fulfill the need of colossal demand of newly released cultivar. In vitro multiplication and mutation breeding is an alternative method to broaden the area of novel cultivar. Due to limited availability of seed cane of a new variety at the time of its release, it further takes several years to cover the desired area for commercial cultivation, by the time the variety starts deteriorating due to biotic and abiotic stresses. Tissue culture derived variations could be very useful in sugarcane crop improvement program for the development of new trait.

Potential role of somaclonal variation

Somaclonal variants of various agriculturally important traits have been studied also in sugarcane. The first in vitro screened somaclone of commercial sugarcane for resistant to Fiji disease reported by Heinz, 1973 (16). Somaclonal variations are highly useful in plant breeding program. Somaclonal variations are easily achieved in vegetatively propagated crops like sugarcane. The potential role of natural or induced genetic variation is a very essential component of crop improvement program. Induced mutations have been used in the improvement of major crops such as sugarcane wheat, rice, barley, cotton, peanuts and beans, which are seed propagated. Mutation breeding is very advantageous for the vegetatively propagated plants like sugarcane, by which a single or few characters of an excellent cultivar can be change. The crop improvement through somaclonal variation was first reported by Heinz and Mee 1971 (17).
Table 1: Crop improvement through somaclonal variation in many crops included sugarcane

<table>
<thead>
<tr>
<th>Crop</th>
<th>Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane</td>
<td>Diseases (Eye spot, Red rot, Fiji virus, Downy mildew, Leaf scald resistant), drought &amp; salt tolerance, improvement of qualitative and quantitative traits viz., sugar content, sugar recovery and cane yield.</td>
</tr>
<tr>
<td>Rice</td>
<td>Plant height, heading date, seed fertility, grain number and weight</td>
</tr>
<tr>
<td>Wheat</td>
<td>Plant and ear morphology, awns, grain weight and yield, gliadin proteins, amylase</td>
</tr>
<tr>
<td>Maize</td>
<td>T toxin resistance, male fertility</td>
</tr>
<tr>
<td>Potato</td>
<td>Tuber shape, maturity date, plant morphology, photoperiod, leaf colour, vigour, height, skin colour, Resistance to early and late blight</td>
</tr>
<tr>
<td>Tomato</td>
<td>Leaf morphology, branching habit, fruit colour, pedicel, male fertility and growth</td>
</tr>
</tbody>
</table>

Studies on Somaclonal variation in sugarcane

Somaclonal variations has been usually reported by many researchers in tissue culture raised plants of sugarcane (18, 19). Variations in morphology, chromosome number and enzymatic pattern in sugarcane plants derived from callus have been reported (17, 20, 21). These make up a basic constraint for a breeding program because the more variable the genetic resources used, the more opportunities to select superior genotype. In sugarcane somaclonal variation have been exloited for the improvement of many economically important traits like salt tolerant, eye spot and red rot resistant. Patade et al (22) studied the effects of salt and drought stresses on irradiated cells of sugarcane and obtained plants tolerant to higher salt stress. Gandonou et al (23) deliberate the effects of salt stress by exposing the callus to a single level of 68 mM NaCl, and observed that physiological and biochemical indicators could play a crucial role in salt tolerance. Salt (NaCl) tolerant sugarcane cultivar CP65-357 developed from callus culture (24). Wagih et al (25) developed eight drought tolerant variants from embryogenic callus of sugarcane (Saccharum hybrids) and grown in a greenhouse for further testing under water stress. They found improved tolerance to drought in amongst the somaclonal variants for different areas of tropics and subtropics. Four salt tolerant somaclonal variants were developed from embryogenic calli of sugarcane variety CP48-103 (26). Clonal variation in combination with in vitro mutagenesis and selection has been applied for the isolation concentration etc. (31). Chromosomal abbreviation and ploidy changes are highlighted by cytogenetic analysis, including chromosome counting under microscope / flow cytometry. Proteins and isozymes also have been used as markers for recognizing somaclonal variants in many fruit species but they are limited in their sensitivity. Cytological evaluation is not often used and can be complicated to detect in numerous crops.

With the advancement of molecular marker techniques like RFLP, RAPD, AFLP and SSR etc. are regularly used preferentially over traditional phenotypic or cytological methods. Restriction Fragment Length Polymorphism (41) analysis is one of the first techniques widely used to detect variation. Later, PCR-based techniques, Random Amplified Polymorphic DNA (RAPD), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), Amplified Fragment Length Polymorphism (AFLP), Simple sequence repeat (SSR) are most extensively used molecular techniques throughout the world. Among these PCR based techniques, the RAPD is faster, inexpensive, simple, less time consuming, most reliable and frequently used to detect genetic variability at DNA level (11, 42) in sugarcane and other crops. As in typical PCR, where a pair of reverse and forward primer is used, in RAPD only single primer amplify the unknown site in the target genome.

Oropeza et al (43) identified somaclonal variants of sugarcane resistant to sugarcane mosaic virus through RAPD marker. The somaclones AT 626 and BT 627 were selected by their resistant to SCMV and analyzed by RAPD. Genetic changes have been detected with the help of RAPD marker in sugarcane during tissue culture. Simple Sequence Repeat (ISSR) technique is a new technique based on the amplification of regions between microsatellites. It is using to check genetic instability at early stages in in vitro.

Detection of Somaclonal variants

Somaclonal variations can be detected easily by morphological characteristics, such as cane height, leaf morphology, bud shape, number of milable cane, sugar concentration etc. (31). Chromosomal abbreviation and ploidy changes are highlighted by cytogenetic analysis, including chromosome counting under microscope / flow cytometry. Proteins and isozymes also have been used as markers for recognizing somaclonal variants in many fruit species but they are limited in their sensitivity. Cytological evaluation is not often used and can be complicated to detect in numerous crops.

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Advantages

- Somaclonal variations occur in high frequencies.
- Some changes can be novel and may not be achieved by conventional breeding.
- In vitro screening reduces the time to isolation a somaclone with desirable trait.
- Sometimes new desirable characters may be occurred which were not available in the germplasm.
- It is a cheaper than other genetic engineering methods

Limitations

- These variations are not stable after selfing or crossing.
- The variations are unpredictable in nature and uncontrollable.
- Selected cell lines often reduced their regeneration potential.
- Many selected clones show undesirable features like reduced fertility, growth and even overall performance.

Strategies to overcome the constraints

1. The breeding objective should be simple and improve one character at a time. If we required more than one trait, stepwise improvement must be possible.
2. An easy and efficient screening technique should be needed to select a desired trait in somaclonal variants.
3. Molecular markers and in vitro selection techniques for various diseases are very helpful in the identification of valuable variants.
4. A comparative study of plants produced through somaclonal variation and conventionally propagated should be tested in field trials before cultivation.

Conclusion

With a continued cultivation of a sugarcane variety for over 15-20 years, tremendous deterioration occurs in variety leading in significant losses in cane yield and sugar recovery. Loss of resistance against major diseases and pests in old elite varieties are the main reasons of deterioration. If the old existing varieties are improved using biotechnological tools such as somaclonal variation, the cane and sugar productivity will be increase significantly. It is more efficient and feasible technology for wider adoption in the field of sugarcane improvement through biotechnological tools.

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