

Micropropagated Plants as Alternative Planting Material to Sugarcane Setts

Nand Lal

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Author's Affiliations: Professor, Department of Life Sciences, Chhatrapati Shahu Ji Maharaj University, Kanpur 208024, Uttar Pradesh, India.

Corresponding Author: Nand Lal, Professor, Department of Life Sciences, Chhatrapati Shahu Ji Maharaj University, Kanpur 208024, Uttar Pradesh, India.

E-mail: nl_pr@yahoo.co.in

Abstract

Sugarcane (*Saccharum officinarum* L., Family - Poaceae) is an economically important sugar and energy crop propagated conventionally by stem cuttings (setts). Low propagation rates, long time demand, huge land requirement and potential transmission of pathogens through seed cane from generation to generation are the major constraints of conventional propagation. In vitro propagation (Micropropagation) is the best alternative to overcome such limitations to produce disease-free genetic stock and sufficient amount of planting material in minimum possible time, however, it is a relatively sophisticated technique requiring aseptic conditions and technical skill at each stage viz. mother plant establishment, initiation and establishment of aseptic cultures, multiplication, rooting, and acclimatization. More knowledge is required regarding sensitivity of the micropropagation protocol for microbial contamination and the economical viability to support the conventional propagation. This review aims towards the scope of micropropagation for increasing yield by enhancing varietal life-span and allow the sugar industry to produce sufficient quality planting material within short period of time and cost effective manner.

Keywords: Cane Setts; Micropropagation; Seed Cane; *Saccharum* Spp; Sugarcane.

Introduction

Sugarcane (*Saccharum officinarum* L., Family-Poaceae), primarily used for sugar production, has a unique source-sink system. Its stem sinks store photosynthate as soluble disaccharide, sucrose, which can reach exceptionally high concentrations, up to 650 mM or 18% of stem fresh weight in commercial sugarcane varieties (Inman-Bamber *et al.*, 2011). Lack of rapid multiplication procedures has long been a problem in sugarcane seed production, particularly in expansion of newly released varieties. Almost a decade is required to conduct various steps of sugarcane variety development i.e. hybridization, selection and different evaluation trials. Subsequently, it takes several years for seed multiplication before reaching to commercial plantations. The time spent for seed multiplication is considered a serious economic loss. The new varieties sometimes enter in degenerative phase due to continued contamination by systemic diseases in open fields. Sugarcane is propagated commercially by vegetative method, involving the planting of the stem cuttings of premature cane (about 8 to 12 months old) grown with special care (Sime, 2013). The seed cane that

is used as planting material may be either whole stalks or stalk cut up in shorter segments called setts (Garside and Braunack, 2001). The growth of sugarcane has different stages: emergency, tillering, stalk growth, and maturation. The germination is a most critical and foundation event in the plant life to assure a good harvest. It is initially dependent on nutrients and water available within sett till developing its own root system for three weeks under proper conditions, though, the initial growth of sugarcane is influenced by several other internal and external factors such as sett age, cultivar, setts nutrients, temperature, soil aeration; setts position on the stalk and humidity (Sime, 2013).

A cane sett is the main conventionally propagation system for majority of the sugarcane growing countries in the world. In some instances, the buds scooped out of the cane using a bud-chipping machine or knives are used for raising the seed nursery. Higher seed rate of 75,000 three-bud setts per hectare is needed for raising breeder's seed to compensate for germination loss due to

heat therapy (Jalaja *et al.*, 2008). In general, non-availability of quality and true to type planting material of newly released varieties is a major bottleneck in their quick adoption for commercial use, and improving sugarcane productivity. Even the cultivation of well adopted commercial varieties requires availability of quality seed to ensure better cane yield, sugar yield, pathogens and pest-free crop (Flynn *et al.*, 2005). Further, traditional method of cultivation using three-budded setts requires large quantity of seed stalks, which is costly, time consuming and land demanding.

Micropropagation has emerged as most successful and commercially viable facet of plant biotechnology and is being routinely used for propagation of a range of species. The critical test for micropropagation is whether it can occur without introduction of genetic alternations. Although tissue culture work was initiated on sugarcane in 1962 in Hawaii, micropropagation studies remained incomplete for next 20 years. Earlier true shoot apex and axillary bud cultures in sugarcane were treated to be extremely difficult. A number of studies conducted between 1978-93 in France, Brazil and India proved that sugarcane can be propagated by induction of axillary shoot proliferation in cultured shoot meristems (Maretzki, 1987, Lal and Singh, 1994). Lee (1987) in Brazil made comparison of various methods of *in vitro* propagation in sugarcane and proposed that shoot apex culture was a better technique for propagation due to faster rate of multiplication and genetic stability of propagules.

Micropropagation in sugarcane results in production of planting material that enjoys a number of positive features such as:

- Faster rate of propagation.
- Availability of planting material throughout the year i.e. in all the seasons.
- Production of uniform plants of a selected genotype.
- Uniform cloning of highly heterozygous *Saccharum* complex.
- Elimination of systemic pathogens before planting.
- Preservation of breeding stocks as juvenile plants.
- International exchange of disease-free genotypes avoiding quarantine regulations.
- Transplanting to replace stalk piece (sett) planting.
- Filling of gaps in plant and ratoon crops.
- Requirement of small laboratory space in comparison to open fields.
- Cleaning of commercial varieties deteriorated due to diseases.

Micropropagation method for sugarcane involves following four stages for generating the planting material (Lal and Krishna, 1994):

- Culture establishment (Fig. 1A)
- Shoot (tiller) multiplication (Fig. 1B)
- Rooting of the tillers (Fig. 1C)
- Hardening of the propagules (Fig. 1D)



Fig. 1: Key steps/stages during Sugarcane micropropagation. **A.** Shoot culture establishment, **B.** Shoot multiplication, **C.** Rooting of tillers (plantlets), **D.** Hardening of plantlets.

Details of micropropagation protocol for sugarcane are well documented and being practiced in India, Brazil, Australia, Hawaii, French territories and African countries.

Use of micropropagated plants for raising seed cane crop is well studied in detail in several sugarcane growing countries. Generally, hardened plants of 30-45 days are used for transplantation in field. Hardening procedure for sugarcane propagules is simple and results in survival and establishment rates exceeding 90%. Transplanting of hardened plants in field in favourable

season shows almost 100% establishment. The hardened plants are transplanted in pits in levelled soil or furrows without disturbing the soil attached to roots. Trimming of leaves prior to transplantation has been found beneficial for establishment and early induction of tillers. A light irrigation is given to the field after transplantation. A single hardened plant is placed in one pit and appropriate amount of insecticide is also applied in pit. Generally the total fertilizer is applied as top dressing in 3 equal split doses however 1/5th of total fertilizers can be also given as basal dressing. Subsequent field care of micropropagated plants is done in same way as practiced for conventional seed nursery.

Studies conducted in various countries have revealed that micropropagated plants do not undergo genetic alterations and show improved tillering, millable stalks, yield and sugar recovery (Lal, 1996). Incidence of disease is also observed to a minimum level in nurseries raised from micropropagated plants (Flynn *et al.*, 2005).

Considering the potential of micropropagated plants in seed multiplication, it has necessitated the development of appropriate management practice. Lal (1997) observed that plants transplanted in deep pits i.e. pits in furrows show better establishment and vigorous growth. The most important consideration for planting is to decide optimum population and configuration to get economically viable seed nursery different inter- and intra-row spacing for transplanting. Comparison of spacing combinations with conventional sett planting system has shown that micropropagated plants transplanted at a low population i.e. 18,518/ha using inter- and intra-row spacings of 90 and 60 cm, respectively showed higher tillers and millable canes. There is only 20% extra cost required for managing seed cane nursery from micropropagated plants and this is credited to higher cost of the planting material (Jalaja *et al.*, 2008). Other inputs like fertilizers, agrochemicals, irrigation etc. are almost same for the seed nursery raised from either of the planting material. This area requires further attention since simplification of inputs for micropropagated plant based nursery can lower down the cost of cultivation. Another way is to further reduce the cost of planting material which is being attempted by integration of hydroponics at hardening stage and inducing one multiplication cycle outside the culture room. This attempt is likely to help in cost reduction of micropropagated plants. A marginal difference in benefit: cost ratio is observed in sett and micropropagated plant-derived crop and this is easily affordable for seed production purpose, particularly for newly released varieties. Development of appropriate agrotechnology can lead to substitution of setts with micropropagated plants for commercial plantations also and this goal seems not very far. Although higher inputs are required with micropropagated plants, net return/ha is almost same if compared with the sett-derived crop. Considering the net return factor, micropropagated plants can be well accepted by rich farmers.

The feasibility of micropropagation for seed multiplication in sugarcane has led to development of biotechnology units at a number of sugar factories and research institutes. A critical appraisal of the work done at various institutes to make micropropagation more economically viable has shown that tissue culture should be only initially used to produce a sizeable quantity of seed and subsequent multiplication should be done by STP technique (multiplication ratio 1:30) and conventional methods (multiplication ratio 1:10).

To bring the feasibility from seed production to commercial plantations for micropropagated plants in sugarcane growing countries following areas require immediate attention:

- Reduction in cost of planting material.
- Development of technology for round the year planting particularly in India and Pakistan.
- Efficient use of inputs to minimize the cost of cultivation.

These challenges can be faced by a co-ordinated effort of tissue culturists, plant physiologists and agronomists. The micropropagated plants are being commonly used for plantations in Australia, Hawaii, Brazil and few Asian countries where the plants are required in low number due to mechanised farming and total cost involved in raising crop from setts and micropropagated plants are almost the same. This is not possible under Indian conditions where more planting material is required due to close inter-row spacings. There seems no scope to reduce the plant population beyond 18,518 per hectare at present. Solution to above mentioned problems can only bring the micropropagated plants within the reach of common farmers. If this happens to be true, micropropagated plants will substitute sett planting and become an ideal planting material for sugarcane cultivation.

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