Rapid *in vitro* Micro-Propagation of Clean Sugarcane Seed

V. Sharma, G.M. Acuna, M.B. Damaj, and N.M. El-Hout
Texas AgriLife Research, Texas A&M System, Weslaco,TX 78596
Corresponding author: vsharma@ag.tamu.edu

Abstract

Sugarcane (*Saccharum* spp.) is a major contributor in the world sugar industry and an emerging bioenergy crop. It would benefit tremendously from the development of high-throughput *in vitro* propagation methods that would allow clean seed production and facilitate varietal commercialization. Conventional propagation of sugarcane from seedcane pieces with bud sets is slow and accompanied by the high risk of disease transmission to the subsequent crop. *In vitro* micro-propagation of sugarcane presents a good alternative, since it produces large quantities of disease-free planting material in a timely manner. A method of direct shoot organogenesis of sugarcane using apical meristems as explants is described herein. Sugarcane tops were collected from the field and surface sterilized (10% sodium hypochlorite solution for 15 min). Apical meristems were recovered and cultured in basal MS (Murashige and Skoog) liquid media containing sucrose (20 g/L), and a combination of the cytokinin benzylaminopurine (0.9 mg/L) and the auxin α-naphthylacetic acid (NAA) (1.86 mg/L) for shoot initiation. After 21 days in culture, the initiated plantlets were transferred to solid shoot proliferation media composed of MS, sucrose (20 g/L), NAA (2.0 mg/L), and the cytokinin kinetin (2.0 mg/L). Multiplying shoots were separated and sub-cultured at 3-week intervals. After reaching a sufficient number, plantlets were transferred to solid rooting media containing MS, sucrose (20 g/L), and the auxin indole-3-butyric acid (2.0 mg/L). The *in vitro* grown plantlets were transferred to the greenhouse where a survival rate of 81% was recorded in 6 weeks. The present *in vitro* tissue culture method provides a simple, high-throughput propagation system for sugarcane. It enables the rapid generation of disease-free planting stocks, thus eliminating the need for field collection of plant material.

Importance of a rapid micro-propagation system for sugarcane breeding program

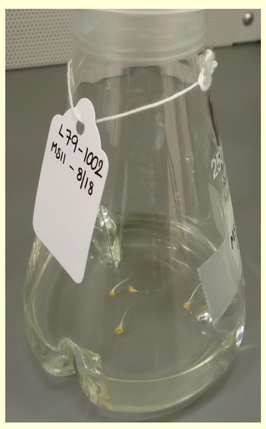
The lack of fast and uniform multiplication procedures has always been a problem in sugarcane breeding programs. An improved variety can be planted commercially only several years later, when enough seedcanes have been produced. This delay causes a serious economic problem, and it is also quite possible that the new variety could enter in its degenerative cycle earlier, because of the continued contamination by systemic diseases during the multiplication stage in the open field (Lee et al. 1987). Sugarcane is conducive to systemic diseases, such as ratoon stunting and several viral diseases. These diseases cause a huge loss in net productivity and is controlled by planting healthy seed-cane, which can be produced by adopting tissue culture techniques. The primary advantage of micro-propagation is the rapid production of high quality, disease-free and uniform planting material. The plants can be multiplied under a controlled environment, anywhere, irrespective of the season and weather, on a year-round basis. Apical meristem culture has been declared to be not only a viable and an efficient method for production of seed-cane propagation but also a rapid and reliable mode of virus elimination (Hoy et al. 2003; Mehrotra et al. 2007). The current study provides a method for the rapid propagation of clean seed cane using apical meristem as an explant.

The *in vitro* propagation system for sugarcane

Explants preparation and culture

10% sodium hypochlorite

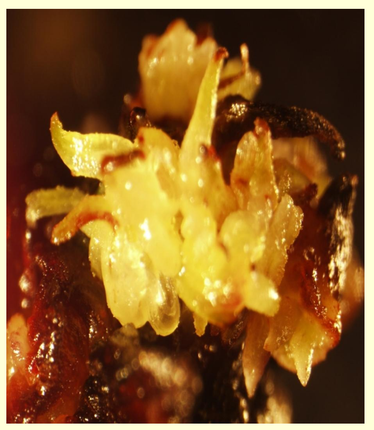
Cutting explant above internode to 7-8 cm and below internode to 1 cm



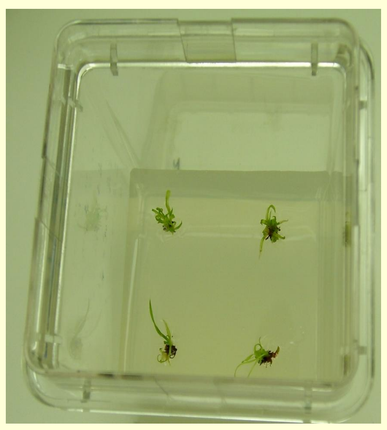
Harvesting of meristem tips

Culturing in liquid media on a shaker
MS + sucrose + BAP (0.9 mg/l) + NAA (1.86 mg/l)

Plant regeneration and proliferation



Shoot organogenesis after 21 days

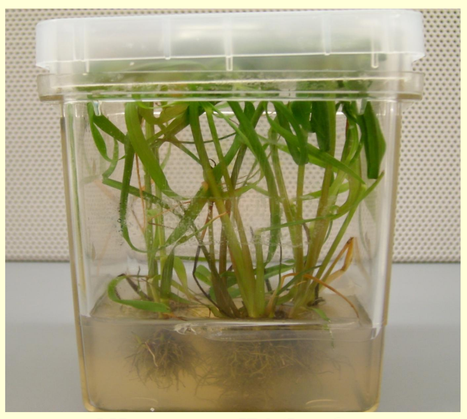


MS + sucrose + cytokinin (2 mg/l) + NAA (2 mg/l)

Shoot multiplication

Root initiation

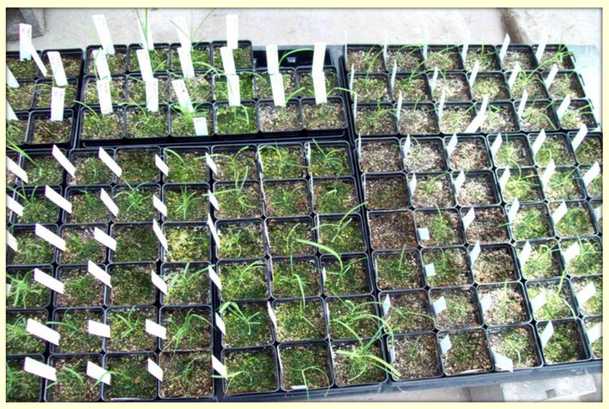
Mass multiplication



MS + sucrose + IBA (2 mg/l)

Greenhouse hardening and field evaluation

Field evaluation of plants



Hardening of seedlings in the greenhouse for 6 weeks

Conclusions

- The present study provides a system for a rapid and uniform *in vitro* propagation of clean sugarcane seedlings round the year.
- The method is applicable to different sugarcane genotypes. Three sugarcane genotypes have been successfully propagated using this method.
- The method produces homogenous plants. Phenotypic variants resulting from somaclonal variation have not been observed.

Future Directions

The yield attributes (stalk count, stalk height and stalk diameter) of sugarcane propagated *in vitro* by the provided method are being evaluated, and they will be compared with those of the cane propagated conventionally by buds.

References

• Hoy JW, Bischoff KP, Milligan SB, Gravois KA (2003) Effect of tissue culture explant source on sugarcane yield components. Euphytica 129: 237-240
• Lee TSG (1987) Micropropagation of sugarcane (Saccharum spp.). Plant cell, Tissue and Organ Culture 10: 47-55
• Mehrotra S, Goel MK, Kukreja AK, Mishra BN (2007) Efficiency of liquid culture systems over conventional micropropagation: A progress towards commercialization. African Journal of Biottechnology Vol.6 (13):1484-1492