academicJournals

Vol. 14(47), pp. 3170-3178, 25 November, 2015
DOI: 10.5897/AJB2015.14440
Article Number: 9664DB556423
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Review

Sugarcane *in vitro* culture technology: Opportunities for Kenya's sugar industry

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Received 20 January, 2015; Accepted 5 November, 2015

Sugarcane (*Saccharum officinarum* L.) is one of the most important crops in Kenya and has wide range of economic importance. The sugar industry contributes up to 15% to the country's agricultural gross domestic product and an estimated 25% of the population depends on the industry for their livelihood. However, the industry has been facing several challenges including declining yields due to use of poor quality planting materials. There is an increasing pressure to enhance the productivity of sugarcane in order to sustain profitable sugar industries in Kenya, while there are several diseases attacking sugarcane and reducing its quality. Seed multiplication of newly released varieties of sugarcane is one of the major constraints in Kenya as it takes 6-7 years to produce sufficient quantity of improved seed material. *In vitro* culture offers a practical and fast method for mass propagation of disease-free clonal materials. Successful protocols for shoot tip culture, callus culture, embryo culture, virus free plant production and somatic embryogenesis have already been established. Thus, *in vitro* technology can be used to enhance productivity of sugarcane in Kenya. Despite several advantages of applying micro-propagation technique in sugarcane such as quick multiplication of newly released varieties, rejuvenation of old deteriorated varieties; production of disease free seed; easy transportation of seed material; elimination of viruses; high cane productivity and sugar yield etc., this technique is not gaining popularity up to the desired extent. There are several constraints like the high cost of production and appearance of some variants in micropropagated population among others. The present article describes the status, challenges and opportunities of *in vitro* technology for the sugar industry in Kenya. Though, some problems have now been resolved to considerable extents which have been described in this review however, some constraints still require intensive research work to be resolved so that a safe and efficient exploitation of this technique can be ensured in sugarcane seed production programmes for enhanced yields and quality.

Key words: Sugarcane, somaclonal variation, *in vitro* culture, meristems, micro-propagation, callus.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a tall-growing monocotyledonous perennial grass that is cultivated in the tropical and subtropical regions of the world, primarily for its ability to store high concentrations of

sucrose or sugar in the stem. The origin of *S. officinarum* is intimately associated with the activities of humans, as *S. officinarum* is a purely cultivated or garden species which is not found in the wild (Sreenivasan et al., 1987). The centre of origin of *S. officinarum* is thought to be in the Indonesia/New Guinea area (Daniels and Roach, 1987) where it has been grown as a garden crop since 8000 B.C. (Fauconnier, 1993). Modern sugarcane varieties are complex interspecific hybrids of *S. officinarum* and *Saccharum robustum*, *Saccharum* barberi, *Saccharum* sinense and related grass genera such as Miscanthus, Erianthus and Nerenga (Altpeter and Oraby, 2010).

Sugarcane is considered the world's most valuable crop estimated to be worth US $ 143 billion (Tecson-Mendoza, 2000). Sugarcane accounts for approximately 70% of the world's sugar and is an economically important cash crop in the tropical and sub-tropical regions of many countries (Chengalrayan and Gallomeagher, 2001). In 2010, world production of sugar from sugarcane was estimated at 1,686 million tons grown on approximately 23.8 million ha (FAOSTAT, 2013). The main product of sugarcane is sucrose, which accumulates in the stalk internodes. Sucrose, extracted and purified in specialized factories, is used as raw material in human food industries or is fermented to produce ethanol, a low pollution fuel. It supplied about 13% of all energy derived from foods (Escolana et al., 1995). It is also used for making paper, livestock feed, chipboard, cane wax, fertilizer, bioethanol, syrup and mulch.

SUGAR CANE PRODUCTION IN KENYA

Industrial sugarcane farming was introduced in Kenya in 1902 (Osoro, 1997). The sugar industry plays a significant role in Kenya's economy, contributing about 15% to the country's agricultural Gross Domestic Product (KSI, 2009). The sector supports more than 250,000 smallholder farmers, who supply over 92% of the sugarcane processed by sugar companies, while the remainder is supplied by factory-owned nucleus estates (KSI, 2009). An estimated 25% of the country's population depends directly or indirectly on the sugar industry for their livelihood. In addition, the industry saves the country in excess of 250 million USD in foreign exchange annually and contributes to tax revenues to the exchequer (KSB, 2010).

Sugarcane performance depends largely on climatic and biophysical (soil and topographic) conditions, which vary significantly throughout Kenya. Sugarcane is mainly cultivated in four major production belts– the Nyando, Western, Nyanza and Coastal Belts primarily located in the southern portion of the country.

Despite government investment in sugar mills, the country still has not reached self-sufficiency in sugar production, as several mills continue to operate below capacity. For this reason, it is unlikely that Kenya will achieve its stated goal of becoming a net exporter of raw sugar in the near future, unless it is able to substantially improve on the sugarcane production. Over the last three decades, sugar consumption in Kenya has grown steadily, outpacing domestic production. Total sugar production grew from 436,238 tons in 1980 to 523,652 tons in 2010, while its consumption increased from 300,000 tons in 1980 to 743,000 tons in 2010 (Figure 1). Kenya produces just about 90% of her domestic sugar requirement. The deficit is met through imports of raw sugar from the Common Market for Eastern and Southern Africa (COMESA) region, which are cheaper than the locally produced sugar (KSB, 2010).

As illustrated in Figure 1, production has increased considerably since 1980, especially over the past decade. Trends suggest that increases in production in recent years have been more correlated with increases in total land planted with cane than with increases in yield, as they were in the past (KSI, 2009). In fact, output of sugarcane per hectare in the 2000s and 1990s has seen a significant decline as compared to yields obtained in the 1980s. Potential reasons for this reduction in productivity include the widespread use of low quality sugarcane varieties, poor agricultural and land management practices and delayed harvesting of mature sugarcane (KSB, 2009).

Challenges facing sugarcane production in Kenya

The shrinking agricultural lands and increasing demand of sugar have compelled the agricultural scientists to devise technologies to increase the sugarcane and sugar productivity per unit area through the development of varieties with high yielding potential. In recent years, Kenya's sugar industry has faced several key challenges, including high costs of production as compared to other sugar producing countries in the region, declining sugarcane yields, and inadequate research and extension services among others (KSI, 2009).

The cost of sugar production in Kenya is high when compared with other countries. The world market price of sugar ranges between US$ 125 and 168 which is well far below the cost of production in Kenya where it averages US$ 500 per ton (Wolfgang and Owegi, 2012). This does not compare well with other regional sugar producers like Sudan, where the average cost of production is US$230

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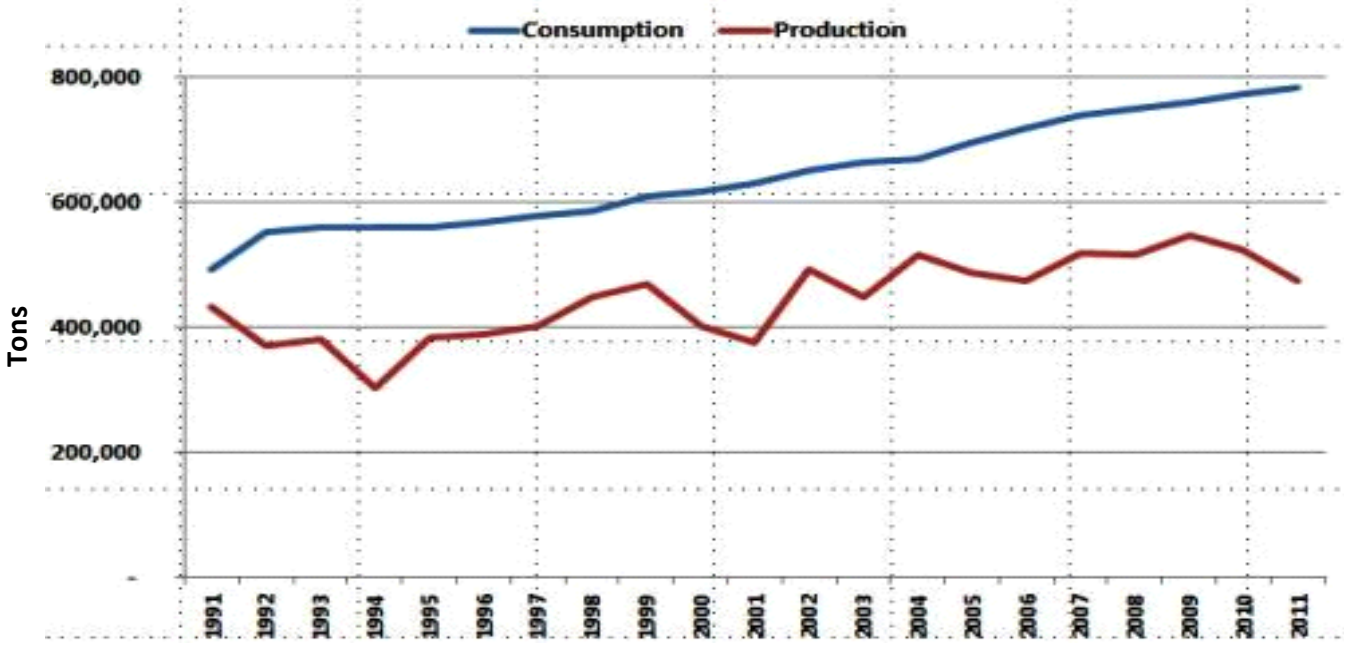

Figure 1. Kenya's sugar production (MT) and consumption 1991-2011 (Source: World Bank estimates based on Kenya Sugar Board and Kenya National Bureau of Statistics data).

per ton. This could be attributed to low sugarcane yields per unit area in Kenya. According to Wolfgang and Owegi (2012), Kenya produces an average of 60 tons of sugarcane per hectare which is just about half of the productivity of Zambia (115 tons per ha) and Malawi (105 tons per ha).

Sugarcane is highly heterogeneous and generally multiplied vegetatively by stem cutting in many countries including Kenya. However, the seed multiplication rate is too low (1:6 to1:8) which makes the spread of newly released varieties slow, taking over 10 years to scale up a newly released variety to the commercial level (Sengar, 2010; Cheema and Hussain, 2004), and also it facilitates the spread of pathogens and may result in epidemics (Schenck and Lehrer, 2000). Moreover, the method requires large nursery space: one hectare nursery for 10 to 15 hectares field planting (Sundara, 2000). This leads to slow release of new sugarcane varieties and spread of diseases. It is worth noting that Kenya still relies on the Coimbatore varieties of sugarcane that were introduced into the country over 50 years ago despite the availability of better and improved varieties from research institutions.

There are a number of diseases of bacterial, fungal, viral and phytoplasmal origin, which affect sugarcane yield and sugar recovery in Kenya (Osoro, 1997). Under field conditions, occurrence of new pathogenic strains of the fungus has been reported from time to time. The red rot pathogen *Colletotrichum falcatum* is a facultative parasite, which keeps on mutating in nature and as a result, new races of the pathogen frequently emerge. Existence of several pathogenic races of smut pathogen has been reported throughout the sugarcane growing zones in Kenya (Osoro, 1997). There are several known sugarcane viruses in Kenya. The Mosaic disease of sugarcane occurs throughout the world except in a few countries.

In Kenya sugarcane planting materials are subjected to hot water treatment by sugar millers as a way of controlling diseases. These materials are then multiplied through several cycles before they are released to farmers. However, hot water treatment alone does not guarantee eradication of all the diseases in the materials. Furthermore, the long multiplication period exposes the materials to re-infection by diseases.

It is therefore imperative that technological interventions that circumvent the problems associated with the conventional propagation methods are found and implemented to address the problem of low sugarcane productivity in Kenya. *In vitro* culture technology is a tool for obtaining rapid, mass multiplication of disease free, true to type planting materials (Singh, 2003).

Opportunities for Kenya's sugar industry

Conventional methods have greatly contributed to sugarcane improvement; however, limitations such as complex genome, narrow genetic base, poor fertility, susceptibility to biotic and abiotic stress, and long duration to breed an elite cultivar still impose challenges (Suprasanna et al., 2011). According to Dookun (1998) and Lakshmanan et al. (2005), sugarcane is a

suitable candidate for the application of biotechnological and genetic engineering tools in increasing its productivity. Researches on sugarcane *in vitro* culture began in the 1960s with culture of mature parenchyma of internodal tissues for some physiological studies (Nickell, 1964). Later, after demonstration of totipotency in callus cultures of sugarcane (Barba and Nickell, 1969; Heinz and Mee, 1969), a rapid progress was made in cell and tissue culture of this crop and it was found that cultures could be raised from any part of the plant. At the moment, studies have been conducted to employ *in vitro* culture combined with radiation/chemical induced mutagenesis for mutant isolation (Synman, 2011).

In Kenya, after the government passed the national biotechnology policy in 2007, many biotechnology projects got a big boost. Conventional biotechnology procedures such as tissue culture have widely been used for production of planting materials for pyrethrum, banana, sugarcane, potato, strawberry, cassava, vanilla, oil palm and flowers (Mtui, 2011). However, commercial application of *in vitro* technology in the sugar industry is still at its infancy and is mainly restricted to research.

The major potential areas identified in *in vitro* culture of sugarcane improvement in Kenya include: i) micropropagation for seed cane multiplication and disease management; ii) somaclonal variation for crop improvement; iii) rejuvenation of older elite varieties; iv) *in vitro* germplasm conservation, etc.

Micropropagation for rapid seed cane multiplication

Vegetatively propagated sugarcane has a low 1:6 to 1:8 seed multiplication rate due to which seed production of newly released varieties is invariably slow (Jalaja et al., 2008). Further, the seed accumulates diseases during several cycles of field production. Non-availability of disease free, true to type planting material is therefore a major constraint in improving sugarcane production.

Micropropagation is an *in vitro* method for clonal multiplication of plants using shoot apical meristems as the explant. During the last 30 years, it has become possible to regenerate plantlets from explants and/or *callus* from all types of plants. As a result, laboratory-scale micropropagation protocols are available for a wide range of species (Debergh and Zimmerman, 1991) and at present micropropagation is the widest used of all plant tissue-culture technologies.

Sugarcane plants can be regenerated *in vitro* via three different pathways: development of axillary shoots from shoot tip or apical meristem explants (direct organogenesis), development of adventitious shoots either directly on the explants or indirectly via *callus* and somatic embryogenesis (Maretzki, 1987). Axillary shoot development has been considered as the most trusted and preferred method of micropropagation for the maintenance of clonal fidelity (Lal et al., 2014).

Several researchers have reported that sugarcane micropropagation is the only realistic means of achieving rapid propagation of new cane varieties, reduction in seed use, and regeneration of large number of true to type plantlets from a small tissue, elimination of pathogens and storage of plant germplasm under aseptic condition (Ali et al., 2004; Lorenzo et al., 2001; Lal and Krishna, 1994). Jalaja et al. (2008) reported that within nine (9) months, callus culture of apical meristem produced planting materials from a single spindle which was sufficient to plant one hectare of land. Ramgareeb et al. (2010) in a study in sugarcane reported propagation of approximately 1300 shoots from a single 2 mm meristem in 11 weeks.

As with other plant species, sugarcane plants propagated *in vitro* from meristems are considered to be more genetically and phenotypically stable than those produced from callus (Hendre et al., 1983). Thus, considerable effort has been expended to investigate the adaptability of meristem culture to commercially grown elite sugarcane cultivars (Hendre et al., 1983; Burner and Grisham, 1995).

Rapid multiplication of disease-free sugarcane planting material through *in vitro* culture technology has been an important step towards quality seed production. Australia, India and Philippines have already embraced the technology for commercial seed production and the benefits are evident in increased sugarcane production (Jalaja et al., 2008).

In India, micropropagation-based seed production technology is accepted by farmers who have obtained higher seed yields (Lakshmanan, 2006). Multiple ratooning in micropropagation-raised crop, due to absence of sett-borne diseases has also been recorded (Jalaja et al., 2008).

In Kenya, sugarcane is propagated vegetatively by nodal cuttings, and for this reason, micropropagation offers a practical and fast method for mass production of disease free true to type quality seed cane.

Despite several advantages of applying micropropagation in sugarcane such as quick spread of newly released varieties (Jalaja et al., 2008), rejuvenation of old deteriorated varieties; easy transportation of seed material; high cane productivity and sugar yield etc., this technique is not gaining popularity up to the desired extent due to the various constraints. Contamination of cultures microbes is a severe problem that not only reduces the frequency of shoot culture initiation from the source explants but also the total number of shoots produced at various cycles due to loss of cultures (Lal et al., 2014).

Further, young meristematic tissues of sugarcane contain high levels of phenolic substances which are enormously released in the medium during culture initiation. The phenolics in oxidized form generally cause mortality of the explants probably by inducing tissue oxidation, retard tissue growth or even promote abnormal

pattern of cell development (Ishaq and Ehirim, 2011). Vitrification also termed as vitricence, glassiness or hyperhydration has been identified as a physiological disorder of shoot cultures (Constantine, 1986). Lower frequency of rooting in vitrified shoots and higher mortality of rooted plants during hardening reduces the production of plants markedly.

Somatic embryogenesis

The recognition of somatic embryogenesis was a turning point in sugarcane biotechnology (Lakshmanan, 2006). Although, developed originally as an alternative system to regeneration, somatic embryogenesis has achieved prominence as an integral part of the genetic transformation system (Bower and Birch, 1992). Somatic cells are theoretically totipotent. However, Namasivayam (2007) stated that chemical stimulus is required in most cases for making them embryogenic. 2,4-Dichlorophenoxyacetic acid (2,4-D) is the most common synthetic auxin used to induce somatic embryogenesis from a range of plant species (Pasternak et al., 2002; Raghavan, 2004) and was found to activate the expression of certain genes essential for embryogenic competence (Boutilier et al., 2002). Inclusion of cytokinin is essential for cell division and hence it contributes towards the initial increase in the number of cells in somatic embryos (Karami et al., 2009).

Ho and Vasil (1983) reported the evidence of embryogenic callus development in monocots. Somatic embryogenesis has been reported from a large number of commercial sugarcane clones and can be obtained directly or indirectly from the leaf tissues (Wekesa et al., 2014; Raza et al., 2012; Manickavasagam and Ganapathi, 1998; Guiderdoni et al., 1995). Embryogenic callus can be maintained for several months without losing its regeneration potential to a significant level (Fitch and Moore, 1993).

Genetic transformation efforts in sugarcane rely heavily on the availability of an efficient system for *in vitro* propagation of sugarcane (Raza et al., 2012). Somatic embryogenesis offers an efficient and high volume regeneration system for the production of a large number of plants within a short period (Shah et al., 2009). The system may be useful for developing transgenic plants through Agrobacterium-mediated method in the future. These transgenic plants with desirable genes may be useful in sugarcane improvement programs. For instance, the Vasantdada Sugar Institute, India has been able to develop sugarcane varieties through *callus* culture that are early maturing, high yielding, with high sucrose content, good ratooning ability and resistant smut disease (VSI, 2013). This technique can therefore be harnessed to develop new sugarcane varieties for the Kenya's sugar industry.

Somaclonal variation for sugarcane improvement

Sugarcane is a genetically complex crop with age of 10-15 years conventional breeding, selection cycle and vegetative propagation of resulting cultivars (James, 2004). Use of somaclonal variation that results from either *in vitro* culture or mutagenic treatments is one of the ways of diversifying the genetic pool and potentially introducing desirable traits (Snyman et al., 2011). The term 'somaclonal variation' is used to describe any kind of genetic or epigenetic variation detected in plants derived from cell cultures, irrespective of the morphogenic route or explant used (Lal et al., 2014; Larkin and Scowcroft, 1981). However, other names such as protoclonal, gametoclonal and mericlonal variation are often used to describe variants from protoplasts, anthers and meristem culture, respectively (Bairu et al., 2010).

There are various reports of somaclonal variation induced by culture media (exposure to growth regulators and length of time in culture) that resulted in desirable traits in sugarcane (Table 1). However, there are no indications in open-access literature sources as to the use of such lines for commercial purposes.

Larkin and Scowcroft (1981) have discussed in detail, various factors responsible for somaclonal variation which include karyotype changes, cryptic changes associated with chromosome rearrangement, transposable elements, somatic gene rearrangements, gene amplification and depletion, somatic crossing over and sister-chromatoid exchanges.

The choice of morphogenic route influences the frequency of somaclonal variation, with indirect somatic embryogenesis resulting in sugarcane plants that are highly variable in chromosome number and agronomic characteristics (Larkin and Scowcroft, 1981; Irvine et al., 1991). Somaclonal variation is a random event, so the identification of desirable somaclones is critical. Selection should be performed either *in vitro*, by the addition of a selective agent (e.g. incorporation of a fungal culture filtrate), through field-based screening of plantlets, or both (Snyman et al., 2011).

To further capitalize on *in vitro* somaclonal variation and to increase the frequency at which it occurs, physical and chemical mutagens maybe applied to callus cultures (Snyman et al., 2011). Such induced mutagenesis has the potential to elicit beneficial modifications in cultivars (Patade and Supra-sanna 2008). Both physical (Saif-Ur-Rasheed, 2001; Zambrano et al 2003a; Khan et al 2007; Patade and Suprasanna, 2008) and chemical (Kenganal et al., 2008; Koch et al., 2010) mutagens have been used successfully in sugarcane to increase somaclonal variation (Snyman et al., 2011).

Many authors have concluded that *in vitro* culture can be applied as a complementary system to conventional breeding to improve its efficiency, create variants and increase variations within sub-clonal populations (Doule et al., 2008; Rajeswari et al., 2009). These somaclonal

Table 1. Examples of beneficial traits induced by *in vitro* culture in sugarcane.

Trait	Beneficial attributes	References
Herbicide tolerance	Glyphosate	Zambrano et al. (2003b)
	Ametryn	Zambrano et al. (1999)
	Imazapyr	Punyadee et al. (2007)
Disease resistance	Fiji disease virus in Sugarcane	Krishnamurthi and Tlaskal (1974) and Heinz et al. 1977)
	Mosaic virus (SCMV)	Nickell and Heinz (1973), Oropeza et al. (1975); Oropeza and de Garcia (1975)
	Red rot (Colletotrichum falcatum)	Heinz (1973), Larkin and Scowcroft (1983)
	Rust (Puccinia melanocephala)	Singh et al. (2008)
	Eyespot (Helminthosporium sacchari)	Sengar et al. (2009) Péros et al. (1994)
	Downy mildew	Krishnamurthi and Tlaskal (1974)
Insect pest resistance	Stalk borer (Diatraea saccharalis)	White and Irvine (1987)
Abiotic stress	Salt tolerance	Saif-Ur-Rasheed et al. (2001), Patade et al. (2005) and Gandonou et al. (2006)
Yield/growth components	Improved erect nature of stalks, stalk diameter, length, colour and density, leaf colour, foliar characters, auricle length, bud groove, bud shape and size, flowering, cane yield, tillering, high silicate deposits on leaf surface and differences in growth habits, higher Brix and sucrose content	Heinz and Mee (1969), Khan et al. (2002), Abo-Elwafa (2004), Doule et al. (2008) and Rajeswari et al. (2009)

variations can be exploited by researchers in Kenya for rapid sugarcane improvement programs.

Production of virus free plants

Sugarcane breeding programs have focused on generation of varieties with increased yields, higher sucrose content, pests and disease resistance, tolerance to biotic and abiotic stress and improved ratooning ability (Brumbley et al., 2008). A significant amount of cane production is lost due to biological pests like viruses. There are no chemical agents to eliminate virus from infected plants. In sugarcane, there are five viral infections viz; mosaic, sereh, streak, ratoon stunting and Fiji (Khani et al., 2012). Viruses that are of notable concern in the global sugarcane growing are sugarcane mosaic virus (SCMV) and sugarcane yellow leaf virus (SyLMV) (Ramgareeb et al., 2010). Unlike fungal and bacterial pathogens, viruses are difficult to eradicate by hot water surface sterilization treatments used in quarantine protocols (Saboohi et al., 2014). Control of these viruses by use of resistant varieties has been limited. The spread of the viruses can be controlled if seed cane nurseries ensure that newly propagated materials are virus-free. Hence, rapid *in vitro* multiplication of virus-free plants sugarcane plants is indispensible. *In vitro* methods to eliminate viruses from infected cane include either apical meristem culture only (Chatenet et al., 2001; Fitch et al., 2001), or combination of thermotherapy and meristem culture (Flynn et al., 2005).

Meristem tip culture is a viable, rapid and reliable method of virus elimination in plants. This technique takes an advantage of the fact that some viruses are unable to invade this region because of inhibition of replication and/or inability of the virus to keep up with the pace of rapidly dividing cells at the meristem tip (Reddy and Sreenivasulu, 2011). Literature review indicates that for virus elimination in sugarcane, the size of the excised meristem should be in the range of 0.2 to 1.5 mm in length (Ramgareeb et al., 2010; Chatenet et al., 2001; Parmessur et al., 2002; Fitch et al., 2001) as only the meristem dome and the immediate covering (1^{st} leaf primordial) are usually virus-free. Hence, the chance of virus elimination is greater and produces plants phenotypically very similar to the original plant.

Meristem culture has been successfully used to eliminate sugarcane mosaic virus (SCMV) (Kristini, 2004); Sugarcane Streak Mosaic Virus (SStMV) (Reddy and Sreenivasulu, 2011), chlorotic streak disease, ratoon stunting disease and white leaf disease (Leu, 1978). In combination with heat treatment, meristem and callus cultures were effective in producing pathogen-free stocks from plants infected with Fiji disease virus (FDV) (Wagih et al., 1995) downy mildew (Leu, 1978) and SCMV (Ramgareeb et al., 2010). Recent researches have shown

that direct plant regeneration using thin cell layer culture could be used for rapid production of disease-free plants from sugarcane infected with FDV, SCMV (Kristini 2004). *In vitro* meristem tip culture, in combination with sensitive molecular detection techniques, can therefore be successfully applied as a rapid and reliable method to generate virus-free planting material, and which can be applied commercially to the Kenyan sugar industry.

Germplasm conservation

The ability to store material for extended periods *in vitro* can alleviate constraints on manpower and capacity in growth rooms and/or glasshouses. Furthermore, it can facilitate maintenance of selected lines without the need for field transfer and, in the case of transgenic material, the associated regulation permits it. Consequently, *in vitro* preservation of sugarcane germplasm using slow growth (Taylor and Dukic, 1993; Chandran, 2010) and cryopreservation techniques has been explored (Engelmann, 2004; Gonzáles- Arnao and Engelmann, 2006). Slow growth is a method of medium-term storage where the growth of explants is slowed down by culture conditions and subculturing is, therefore, minimized. Cryopreservation is the conservation of plant propagules in liquid nitrogen (−196°C), thus ensuring that all metabolic processes are stopped and material can be stored indefinitely (Shibli et al., 2006).

Evidence to date indicates that *in vitro* storage of sugarcane on low maintenance medium for extended periods causes little genetic change, suggesting its potential use for long-term conservation and international exchange of germplasm.

CONCLUSION

Biotechnology is creating technologies that are transforming the world's chemical, pharmaceutical and agricultural establishment. Owing to the advancements made so far in the field of *in vitro* technology, Kenya sugar industry should embrace this technology for mass production of quality disease free clonal planting materials so that the country might reap the benefits of the biological revolution in order to address the numerous problems bedeviling the sugar industry.

Conflict of interests

The author(s) did not declare any conflict of interest.

REFERENCES

Abo-Elwafa A (2004). In field assessment of somaclonal variability in sugarcane through callus culture. Assuit. J. Agric. Sci. 35(4):29-47.

Altpeter F, Oraby H (2010). Sugarcane. In: Kempken F, Jung C (eds.), Genetic modification of plants. Biotechnology in Agriculture and Forestry. Springer-Verlag, Berlin.

Bairu M, Aremu A, van Staden J (2010). Somaclonal variation in plants: causes and detection methods. Plant Growth Regul. 63:147–173.

Barba R, Nickell LG (1969). Nutrition and organ differentiation in tissue culture of sugarcane – a monocotyledon. Planta 89(3):299–302.

Boutilier K, Offringa R, Sharma V, et al (2002). Ectopic expression of baby boom triggers a conversion from vegetative to embryonic growth. Plant Cell 14(8):1737–1749.

Bower R, Birch RG (1992). Transgenic sugarcane plants via microprojectile bombardment. Plant J. 2:409–416.

Brumbley SM, Synman SJ, Gnanasambandam A, et al (2008). Sugarcane. In: Kole C, Hall T (eds) Compendium of Transgenic Crop Plants: Transgenic Sugar, Tuber and Fibre Crops. Blackwell Publishing Ltd. pp. 1–57

Burner DM, Grisham M (1995). Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. Crop Sci. 35:875–880.

Chandran K (2010). In vitro multiplication and conservation of Saccharum germplasm. Indian J. Plant Genet. Resour. 23(1):65-68.

Chatenet M, Delage C, Ripolles M, Irey M, Lockhart BEL, Rott P (2001). Detection of Sugarcane yellow leaf virus in quarantine and production of virus-free sugarcane by apical meristem culture. Plant Dis. 85:1177–1180.

Cheema KL, Hussain M (2004). Micropropataion of sugarcane through apical bud and axillary bud. Int. J. Agric. Biol. 6(2):257–259.

Chengalrayan K, Abouzid AG (2001). Plant Regeneration from Sugarcane seed-derived Callus. Vitro Cell Dev. Biol. Anim 37:3.

Constantine D (1986). Micropropagation in commercial environment. In: Withers LA (ed.), Plant tissue culture and its agricultural applications. Butterworths, London. pp. 175-186

Daniels J, Roach BT (1987). Taxonomy and Evolution. In: Heinz DJ (ed) Sugarcane Improvement through Breeding. Elsevier, Amsterdam, Netherlands. pp. 7–84.

Debergh PC, Zimmerman RH (1991). Micropropagation. Kluwer Academic., Dordrecht.

Dookun A (1998). Biotechnology for sugarcane. Agric. Biotech. News Inf. 10:75–80.

Doule R, Kawar P, Devarumath R, Nerkar Y (2008). Field performance and RAPD analysis for assessment of genetic variation in sugarcane somaclones. Indian J. Genet. 68:301-306.

Engelmann F (2004). Plant cryopreservation: progress and prospects. *In vitro* Cell Dev. Biol. Plant 40:427-433.

Escolana M, Castillo R, Cocepcion O, Bonoto CG, Lorenzo JC, Daqiunt MA, Sanchez M, Puentents C, Chung-shed K (1995). Influence of two calt types on the establishment of Cell suspension in Sugarcane. Cent. Agric. 22:63–70.

FAOSTAT (2013). Crop Production Data, Food and Agriculture Organization of the United Nations. ROME

Fauconnier R (1993). Sugar cane. Macmillan Press Ltd, London, UK.

Fitch MMM, Lehrer AT, Komor E, Moore PH (2001). Elimination of sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. Plant Pathol. 50:676–680.

Fitch MM, Moore PH (1993). Long-term culture of embryogenic sugarcane callus. Plant Cell Tiss. Org. Cult. 32:335-343.

Flynn J, Powell G, Perdomo R, et al (2005). Comparison of sugarcane disease incidence and yield of field-run, heat-treated and tissue-culture based seed cane. J. Am. Soc. Sugar Technol. 25:88–100.

Gandonou CB, Errabii T, Abrini J, Idaomar M, Senhaji NS (2006). Selection of callus cultures of sugarcane (*Saccharum* sp.) tolerant of NaCl and their response to salt stress. Plant Cell Tissue Org. Cult. 87:9-16.

Gonzales-Arnao MT, Engelmann F (2006). Cryopreservation of plant germplasm using encapsulated-dehydration: review and case study on sugarcane. Cryo Letters 27:155-168.

Guiderdoni E, Merot B, Eksomtramage T, Paulet F, Fredman R, Gaszmann JC (1995). Somatic embryogenesis in sugarcane (*Saccharum* species). In: Bajaj YPS (ed.), Biotechnology in Agri-culture and Forestry, 13th edn. Springer Verlag, Berlin, pp. 92–113.

Heinz DJ (1973). Sugarcane improvement through induced mutations using vegetative propagules and cell culture techniques. In: IAEA (ed.), Induced mutations in vegetatively propagated plants. Vienna,

pp. 53–59.
Heinz DJ, Krishnamurthi M, Nickell LG, Maretzki A (1977). Cell, tissue and organ culture in sugarcane improvement. In: Reinert J, Bajaj YPS (eds.), Applied and fundamental aspects of plant cell, tissue and organ culture. Springer, Berlin. pp. 3–17.
Heinz DJ, Mee G (1969). Plant differentiation from callus tissue of *Saccharum* species. Crop Sci. 9(3):346–348.
Hendre RR, Iyer RS, Kotwal M, Khuspe SS, Mascarenhas AF (1983). Rapid multiplication of sug- arcane by tissue culture. Sugarcane May/June: 5–8.
Ho W, Vasil IK (1983). Somatic embryogenesis in sugarcane (*Sacchurum officinarum*). In: The morphology and physiology of callus formation and the ontogeny of somatic embryos. pp. 169–180.
Irvine JE, Benda GTA, Legendre BL, Machado GR (1991). The frequency of marker changes in sugarcane plants regenerated from callus culture. II Evidence for vegetative and genetic transmission, epigenetic effects and chimeral disruption. Plant Cell Tissue Organ Cult. 26:115–125.
Ishaq M, Ehirim B (2011). Reduction of Exudates (Browning) in Sugarcane Micro Propagation. Niger. J. Biotechnol. 23:40–44.
Jalaja NC, D Neelamathi, Sreenivasan TV (2008). Micropropagation for quality seed production in sugarcane in Asia and the Pacific. Rome.
James G (2004). Sugarcane. Blackwell Publishing Ltd, Oxford.
Karami O, Aghavaisi B, Pour A (2009). Molecular aspects of somatic-to-embryogenic transition in plants. J. Chem. Biol. 2:177–190.
Kenganal M, Hanchinal R, Nadaf H (2008). Ethyl methanesulfonate (EMS) induced mutation and selection for salt tolerance in sugarcane in vitro. Indian J. Plant Phys. 13:405–410.
Kenya Sugar Board [KSB] (2009). Kenya Sugar Board Strategic Plan 2009. Retrieved from http://www.kenyasugar.co.ke/
Kenya Sugar Board [KSB] (2010). The Kenya Sugar Industry Strategic Plan 2010-2014. Retrieved from http:/www.kenyasugar.co.ke/new/index.php/help.
Khan AK, Javed IA, Siddiqui MA, Khan MKR, Khanzada MH, Dahar NA, Khan R (2002). Studies on callusing and regeneration potential of indigenous and exotic sugarcane clones. Asian J. Plant Sci. 1(1):41-43.
Khan I, Dahot M, Khatri A (2007). Study of genetic variability in sugarcane induced through mutation breeding. Pak. J. Bot. 39:1489–1501.
Khani U, Muhammad A, Hussain I, et al (2012). Rapid in vitro Multiplication of Sugarcane Elite Genotypes and Detection of Sugarcane Mosaic Virus through Two Steps RT- PCR. Int. J. Agric. Biol. 14:870–878.
Koch A, Ramgareeb S, Rutherford R, Snyman S, Watt M (2010). An in vitro induced mutagenesis protocol for the production of sugarcane tolerant to imidazolinone herbicides. Int. Soc. Sugar Cane Technol. pp. 1–5.
Krishnamurthi M, Tlaskal J (1974). Fiji disease resistant *Saccharum* var. Pindar subclones from tissue culture. Proc. Int. Soc. Sugar Cane Technol. pp. 130–136
Kristini A (2004). The use of tissue culture to eliminate some important diseases in sugarcane. MSc. Thesis, University of Queensland, Australia.
KSI (2009). Kenya Sugar Industry Strategic Plan 2010-2014. Strateg. Plan 2010-2014. Retrieved from http://www.kenyasugar.co.ke/.
Lakshmanan P (2006). Somatic embryogenesis in sugarcane – An addendum to the invited review “Sugarcane biotechnology: The challenges and opportunities.” *In Vitro* Cell. Dev. Biol. Plant 42:201-205.
Lakshmanan P, Geijskes RJ, Aitken KS, Grof CLP, Bonnett GD, Smith GR (2005). Sugarcane biotechnology: The challenges and opportunities. *In Vitro* Cell. Dev. Biol. Plant 41:345-363.
Lal M, Tiwari AK, Gupta GN (2014). Commercial Scale Micropropagation of Sugarcane: Constraints and Remedies. Sugar Tech. 17(4):339-347
Lal N, Krishna R (1994). Tissue culture for pure and disease-free seed production in sugarcane. Indian Sugar 43:847-848.
Larkin P, Scowcroft W (1983). Somaclonal variation and eyespot toxin tolerance in sugarcane. Plant Cell Tissue Organ Cult. 2:111–121.
Larkin PJ, Scowcroft W (1981). Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60(4):197–214.
Leu LS (1978). Apical meristem culture and redifferentiation of callus masses to free some sugarcane systemic diseases. Plant Prot. Bull. 20:77–82.
Lorenzo JC, Ojeda E, Espinosa A, Borroto C (2001). Field performance of temporary immersion bioreactor derived sugarcane plant. Biol. Plant 37:803-806.
Manickavasagam M, Ganapathi A (1998).Direct somatic embryogenesis and plant regeneration from leaf explants of sugarcane. Indian J. Exp. Biol. 832–835.
Maretzki A (1987). Tissue culture: Its prospects and problems. In: D.J. Heinz (ed) In Sugarcane improvement through breeding. Elsevier Science Publishers, Amsterdam, The Netherlands. pp. 343–384.
Mtui GYS (2011). Status of biotechnology in Eastern and Central Africa. Biotechnol. Mol. Biol. Rev. 6:183–198.
Namasivayam P (2007). Acquisition of embryogenic competence during somatic embryogenesis. Plant Cell. Tissue Org. Cult. 90:1-8
Nickell LG (1964). Tissue and cell cultures of sugarcane, another research tool. Hawaii Plant Rec. 57: 223–229.
Nickell LG, Heinz D (1973). Potential of cell and tissue culture techniques as aids in economic plant improvement. In: Srb AM (ed) Genes, enzymes, and populations. Plenum Press, New York. pp. 109–128
Oropeza M, de García E (1996). Somaclonal variants resistant to sugarcane mosaic virus and their agronomic characterisation. *In Vitro* Cell Dev. Biol. Plant 32: 26–30.
Oropeza M, Guevara P, de García E, Ramírez JL (1995). Identification of somaclonal variants of sugarcane (Saccharum spp.) resistant to sugarcane mosaic virus via RAPD markers. Plant Mol. Biol. Rep. 13: 182–191.
Osoro MO (1997). Review of Kenyan Agricultural Research: Sugar. Nairobi. Vol. 18.
Parmessur Y, Aljanabi S, Dookun-Saumtally SS, Dookun-Saumtally A (2002). Sugarcane yellow leaf virus and sugarcane yellow phytoplasma: Elimination by tissue culture. Plant Pathol. 51:561–566.
Pasternak PT, Prinsen E, Ayaydin F, Miskolczi P, Potters G, Asard H, Onckelen HAV, Dudits D FA (2002). The role of auxin, pH, and stress in the activation of embryogenic cell division in leaf protoplast-derived cells of alfalfa. Plant Physiol. 129:1807–1819.
Patade VY, Suprasanna P (2008). Radiation induced in vitro mutagenesis for sugarcane improvement. Sugar Tech 10:14–19.
Patade VY, Suprassana P, Bapat BA (2005). Selection for abiotic (salinity and drought) stress tolerance and molecular characterisation of tolerant lines in sugarcane. BARC Newslett. 273:244-257.
Peros JP, Bonnel E, Roques D, Paulet F (1994). Effect of in vitro culture on rust resistance and yield in sugarcane. Field Crops Res. 37:113-119.
Punyadee P, Throngros M, Pornprom T (2007). Biochemical mechanism of resistance to imazapyr in sugarcane cell selections. Thai J. Agric. Sci. 40:133–141.
Raghavan V (2004). Role of 2,4-dichlorophenoxyacetic acid (2,4- D) in somatic embryogenesis on cultured zygotic embryos of Arabidopsis: cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-D. Am. J. Bot. 91:1743–1756.
Rajeswari S, S Thirugnanakumar K Anandan, Krishnamurthim M (2009). Somaclonal variation in sugarcane through tissue culture and evaluation for quantitative and quality traits. Euphytica 681:71–80.
Ramgareeb S, Snyman S, van Antwerpen T (2010). Elimination of virus and rapid propagation of disease free sugarcane (*Saccharum* spp cultivar NCO 376) using apical meristem culture. Plant Cell Tissue Org Cult. 100:175–181.
Raza S, Qamarunisa S, Hussain M, Jamil I, Anjum S, Azhar A, Qureshi JA (2012). Regeneration in sugarcane via somatic embryogenesis and genomic instability in regenerated plants. J. Crop Sci. Biotechnol. 15:131–136.
Reddy S, Sreenivasulu P (2011). Generation of Sugarcane streak mosaic virus -free sugarcane (*Saccharum* spp. hybrid) from infected plants by in vitro meristem tip culture. Eur. J. Plant Pathol. 130:597–604.
Saboohi R, Syeda Q, Ishrat J, Beena NAA, Qureshi JA (2014). Screening of sugarcane somalones of variety BL4 for agronomic

characteristics. Pak. J. Bot. 46:1531–1535.
Saif-Ur-Rasheed M, Asad S, Zafar Y (2001). Use of radiation and in vitro techniques for development of salt tolerant mutants in sugarcane and potato. IAEA-TECDOC**,** 1227:51–60.
Schenck S, Lehrer AT (2000) Factors affecting the transmission and spread of sugarcane yellow leaf virus. Plant Dis. 84:1085–1088.
Sengar AS, Thind KS, Bipen K, Mittal P, Gosal SS (2009). In vitro selection at cellular level for red rot resistance in sugarcane (*Saccharum* spp.). Plant Growth Regul. 58:201-209.
Sengar K (2010). Developing an efficient protocol through tissue culture technique for sugarcane micropropagation. BioInfoBank Library Acta 01/2010.
Shah HA, Rashid N, Haider MS, Saleem F, Tahir M, Iqbal J (2009). An efficient, short and cost-effective regeneration system for transformation studies of sugarcane (*Saccharum officinarum* L.). Pak. J. Bot. 41:609–614.
Shibli RA, Shatnawi MA, Subaih WA, Mohammed MA (2006). In vitro conservation and cryopreservation of plant genetic resources: a review. World J. Agric. Sci. 2(4):372-382.
Singh R (2003). Tissue Culture Studies of Sugarcane. Thapar Institute of Engineering and Technology, Patiala, India.
Singh RK, Srivastava S, Singh SP, Sharma ML, Mohopatra T, Singh NK, Singh SB (2008). Identification of new microsatellite DNA markers for sugar and related traits in sugarcane. Sugar Technol. 10(4):327–333.
Snyman SJ, Meyer GM, Koch AC, Banasiak M, Watt MP (2011). Applications of in vitro culture systems for commercial sugarcane production and improvement. *In Vitro* Cell. Dev. Biol. Plant 47:234–249.
Sreenivasan T, Ahloowalia B, Heinz D (1987). Cytogenetics. In: Heinz DJ (ed.), Sugar cane Improvement through Breeding. Elsevier, Amsterdam, The Netherlands. pp. 211–253
Sundara B (2000). Sugarcane cultivation. Vikas Publications Pvt., New Delhi, India.
Suprasanna P, Patade VY, Desai NS, Devarumath RM, Kawar PG, Pagariya MC, Ganapathi A, Manickavasagam M, Babu KH (2011). Biotechnological developments in sugarcane improvement: An overview. Sugar Tech 13:322–335.
Taylor PWJ, Dukic S (1993). Development of in vitro culture techniques for conservation of *Saccharum* spp. hybrid germplasm. Plant Cell Tissue Org. Cult. 34:217-222
Tecson-Mendoza ME (2000). Sugarcane tissue culture-trends and prospects. Philipp. J. Crop Sci. 25:73–83.
VSI (Visantdada Sugar Institute) (2013). Technical reports, 37th Annual report 2012-13. Visantdada Sugar Institute. pp. 26-60.
Wagih M, Gordon GH, Ryan CC, Adkins SW (1995). Development of an axillary bud culture technique for Fiji disease virus elimination in sugarcane. Aust. J. Bot. 43(1):135–143.
Wekesa RK, Onguso JM, Wamocho LS (2014). Effect of 2, 4-dichlorophenoxy acetic acid and naphthelene acetic acid concentrations on callogenesis for in vitro regeneration in selected sugarcane varieties. Proceedings of JKUAT Scientific, Technological and Industrialization Conference 13th - 14th November 2014. Jomo Kenyatta University of Agriculture and Technology, Nairobi. pp. 101–113.
White WH, Irvine JE (1987). Evaluation of variation in resistance to sugarcane borer (Lepidoptera: *Pyralidae*) in a population of sugarcane derived from tissue culture. J. Econ. Entomol. 80:182-184.
Wolfgang F, Owegi F (2012). Sweetening Kenya's future – The challenges of the sugar industry. http://blogs.worldbank.org/africacan/sweetening-kenya-s-future-the-challenges-of-the-sugar-industry
Zambrano A, Demey JR, González V (2003b). In vitro selection of a glyphosate-tolerant sugarcane cellular line. Plant Mol. Biol. Rep. 21:365–373.
Zambrano AY, Demey JR, Fuchs M, González V, Rea R, De Sousa O, Gutierrez Z (2003a). Selection of sugarcane plants resistant to SCMV. Plant Sci. 165:221–225.
Zambrano AY, Demey JR, Fuchs M, González V, Rea R, De Sousa O, Gutierrez Z (1999). Selection of an ametryn tolerant sugarcane cellular line. J. Agric. Univ. Puerto Rico 83:47– 54.