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Sugarcane in Vitro Culture Technology: Applications for Kenya's Sugar Industry

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Abstract
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.
Keywords: 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. Commercial sugarcane hybrid cultivars have arisen through intensive selective breeding of species within the *Saccharum* genus, primarily involving crosses between *S. officinarum* and *S. spontaneum*. *Saccharum officinarum* accumulates very high levels of sucrose in the stem but is highly susceptible to diseases (Lakshmanan et al., 2005; Cox et al., 2000) whereas *S. spontaneum* accumulates little sucrose, has thinner stalks and higher fibre content but is a highly polymorphic species with resistance or tolerance to many pests and diseases (Jackson, 2005; Bull & Glasziou, 1979).

The origins of *S. officinarum* are 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 & Roach, 1987) where it has been grown as a garden crop since 8000 B.C. (Fauconnier, 1993). Its cultivation spread along the human migration routes to Southeast Asia, India and the Pacific, hybridizing with wild canes. It reached the Mediterranean around 500 B.C. (Fauconnier, 1993). From there it spread to Morocco, Egypt, Syria, Crete, Greece and Sicily, the main producers until the 15th Century, followed by introduction to West Africa and subsequently Central and South America and the West Indies (Fauconnier, 1993).

S. spontaneum is believed to have evolved in southern Asia (Daniels and Roach, 1987). It accumulates little sucrose content and has thinner stalks and higher fibre content than *S. officinarum* (Jackson, 2005). *Saccharum spontaneum* is an adaptable species and grows in a wide range of habitats and at various altitudes in the tropics through to temperate regions from latitude 8˚S to 40˚N extending across three geographical zones including in Kenya.

Taxonomically, sugarcane belongs to the major grass family, *Gramineae* (now called *Poaceae*), sub-family Panicoideae, super tribe Andropogoneae, sub-tribe Saccharineae and genus *Saccharum* (Watson *et al.*, 1985).

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). At present sugarcane is grown as a commercial crop primarily in South America, North/Central America, Asia, Africa, Australia and the Pacific islands. In 2010, world production of sugar from sugarcane was estimated at 1,686 million tons grown on approximately 23.8 million ha (FAOSTAT, 2013). Brazil was the largest producer at 719 million tons (FAO, 2013). Other countries which produce sugar from sugar cane include Guatemala, Vietnam, South Africa, Cuba, Egypt, El Salvador, Peru and Myanmar (FAO, 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. Ethanol is produced on a large scale by the Brazilian sugar industry. Raw sugarcane can be squeezed or chewed to extract the juice. In some countries in which sugarcane is grown the juice is extracted and bottled for local distribution or sold fresh from juice bars, cafes and restaurants. Outside of commercial processing, artisanal processing of sugarcane occurs where sugarcane juice is boiled and cooled to make cakes of unrefined brown sugar, known as 'jaggery' or 'gur' (Kansal, 1998).

Sugar Cane Production in Kenya

Industrial sugarcane farming was introduced in Kenya in 1902 (Osoro, 1997). The first sugarcane factory was set-up at Miwani 10km north of Kisumu in 1922 and later at Ramisi in the Coast Province in 1927 (Osoro, 1997). After independence, the Government explicitly expanded its vision of the role and importance of the sugar industry (Republic of Kenya, 1965). In pursuit of this, the Government established five additional factories in the 1960s and 1970s, and several more (mainly private) were to come on stream between 1980 and 2010 bringing the total number of milling companies to eleven (11) (EU, 2012).

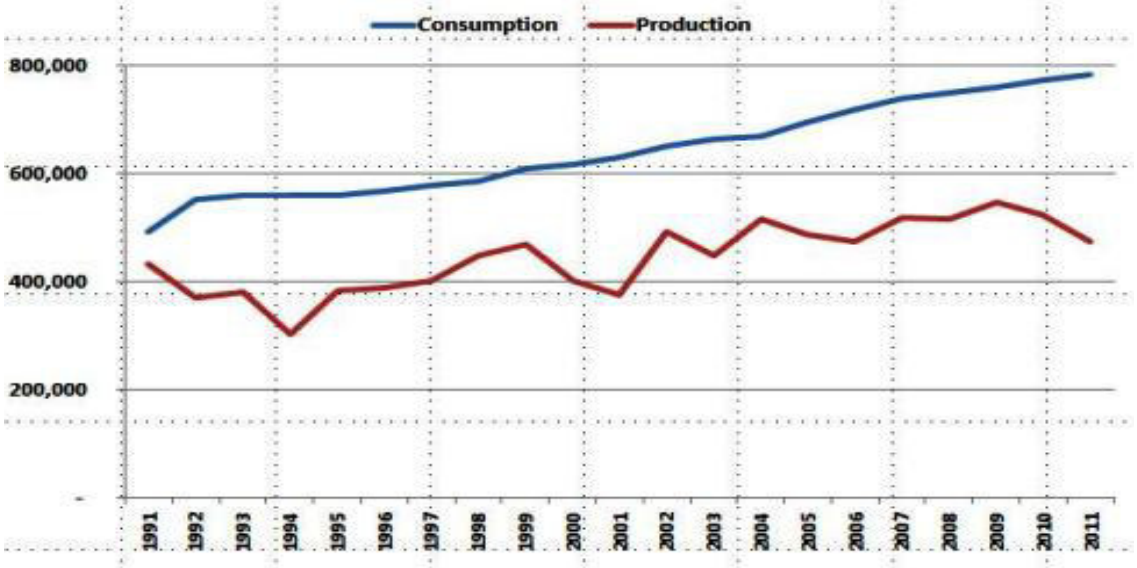
The sugar industry plays a significant role in Kenya's economy, contributing about 15 percent to the country's agricultural GDP (KSI, 2009). The sector supports more than 250,000 smallholder farmers, who supply over 92 percent of the sugarcane processed by sugar companies, while the remainder is supplied by factory-owned nucleus estates (KSI, 2009). An estimated 25 percent 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 USD 250 million in foreign exchange annually and contributes to tax revenues to the exchequer (KSB, 2010).

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 (Fig 2), while sugar consumption increased from 300,000 tons in 1980 to 743,000 tons in 2010 (Fig 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 Fig 2, 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 to 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 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, 2010).

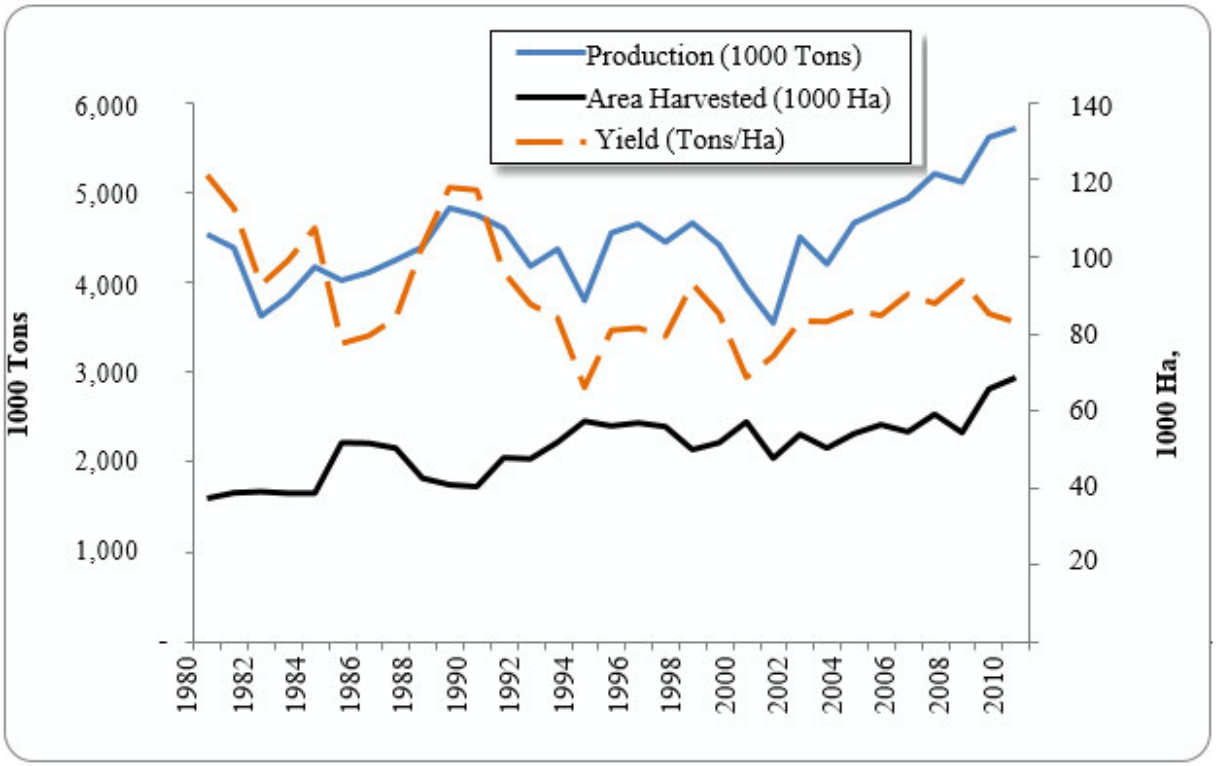
Sugarcane performance depends largely on climatic and biophysical (i.e. soil and topographic) conditions, which vary significantly throughout Kenya. Sugarcane is mainly cultivated in four major production belts – the Nyando, Western, Nyansa and Coastal Belts – primarily located in the southern portion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

Fig 1 Kenya's sugar production and consumption 1991-2011 (in metric tons)



Source: World Bank estimates based on Kenya Sugar Board and Kenya National Bureau of Statistics data

Figure 2: Sugarcane Production, Area Harvested and Yield in Kenya, 1980-2010



Source: FAOSTAT (1975-2004) and CODA (2005-2010)

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.

Challenges facing sugarcane production in Kenya

In recent years, Kenya's sugar industry has faced several key challenges, including high costs of production 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 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 per ton. This could be attributed to low sugarcane yields per unit area in Kenya. 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) (Wolfgang and Owegi, 2012).

As illustrated in fig 2, production has not increased considerably over the last two decades. Output of sugarcane per hectare in the 2000s and 1990s has seen a significant decline 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, 2010).

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 & 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 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. 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. There are several known sugarcane viruses in Kenya. The Mosaic disease of sugarcane occurs throughout the world except few countries (Barber, 1921).

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).

APPLICATIONS AND OPPORTUNITIES FOR KENYA'S SUGAR INDUSTRY

According to Dookun (1998) and Lakshmanan *et al.* (2005) the application of biotechnology to sugarcane has a major role to play in increasing the productivity of this crop. 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.

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) somaclonal variation for crop improvement, ii) micropropagation for seed cane multiplication and disease management, iii) rejuvenation of older elite varieties, iv) *in vitro* germplasm conservation, v) artificial seed etc.

Somaclonal variation for sugarcane improvement

Sugarcane is a vegetatively propagated crop and is a highly polyploidy complex hybrid and sexual crosses can only be interpreted with great difficulty (Lakshmanan *et al.*, 2005). In this regard, tissue culture techniques play important role in creating genetic variability particularly when the opportunities for sexual hybridization are limited. In literature many reports are available on somaclonal variants obtained from tissue culture.

Somaclonal variation in *in vitro*-derived sugarcane has been consistently observed, particularly when plants are produced via a callus stage, which involves long exposure to high levels of certain plant growth regulators (Burner and Grisham, 1995; Lakshmanan & Scowcroft, 1981). The *in vitro* component of the sugarcane transformation process has the potential to generate somaclonal variation to the regenerated plants, and selection by antibiotics or herbicides can add to this increased polymorphism (Carmona *et al.*, 2005).

Sugarcane callus cultures show a considerable variation from cell to cell and among differentiated plantlets. 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.

Research conducted in Hawaii, Fiji, Taiwan, Australia and India clearly demonstrated that somaclonal variation is enormous and provides an opportunity for improvement of highly adapted elite sugarcane varieties with one or two defect(s) by affecting many important agronomic characters. Besides variations observed in morphological characters such as stalk height, girth, stalk colour, leaf colour, foliar characters, auricle length, bud groove, bud missing, bud shape and size, flowering etc, variations were also observed in tillering, high silicate deposits on leaf surface and differences in growth habits (Heinz, and Mee, 1969). Somaclonal variations have led to greater resistance to Fiji disease and Downy mildew in sugarcane while retaining sugar yields (Krishnamuri and Tiaskal, 1974). Resistance to eyespot caused by *Heminthosporium sacchari* generated by tissue culture and sub-culturing through 3-6 vegetation propagation to establish the stability of the trait has also been reported (Tecson-Mendoza, 2000). These variations can be exploited by researchers in Kenya for sugarcane improvement programmes in order to produce quality disease resistant/tolerant planting materials for high yields.

Micropropagation

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 & Zimmerman, 1991) and at present micropropagation is the widest used of all plant tissue-culture technologies.

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; Feldmenn *et al*, 1994; Lal a& Krishna, 1994). Barba, *et al*, (1978) 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. Sauvaire and Glozy, (1978) used auxiliary buds for micropropagation of sugarcane. Lee, (1987) and Heinz *et al*, (1977) also reported shoot tip culture for mass propagation of sugarcane. Conventionally 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 quality clonal planting materials.

Protoplasm and anther culture are the other in vitro culture methods that have been successfully applied in sugarcane. Protoplasm formation and regeneration to complete plants have been successfully undertaken in sugarcane (Srivivasan & Vasil, 1985). In protoplasm culture somaclonal variants can be isolated from individual cells; fusion of protoplasts can generate desirable traits and can be used in physiological and biochemical studies among other advantages. However, Moore (1998) noted the failure to regenerate plantlets from protoplasts. Thus the successful regeneration earlier by Srinivasan and Vasil (1985) could have been genotypic in nature.

Anthers derived haploid of sugarcane has been obtained (Chen *et al.*, 1979) in massive anther isolation programme in China. However, only a few genotypes responded to the technique. This was successfully repeated in Hawaii with modified culture conditions (Fitch and Moore, 1993). Since sugarcane is highly polyploidy, haploidy obtained after only one round of sporophytic development may not carry the basic haploid number of chromosomes for *Saccharum sp*. Nonetheless, doubling resulting haploid number of chromosomes makes the progeny useful for breeding purposes, somatic hybridization and biochemical characterization among other benefits.

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 & Grisham, 1995).

Somatic embryogenesis

Somatic embryogenesis is probably the most intensively investigated method of *in vitro* regeneration in sugarcane. 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 embryogenesis has been reported from a large number of commercial sugarcane clones and can be obtained directly or indirectly from the leaf tissues (Manickavasagam and Ganapathi, 1998; Guiderdoni, *et al.*, 1995; Guiderdoni and Demarly, 1988). Embryogenic callus can be maintained for several months without losing its regeneration potential to a significant level (Fitch and Moore, 1993).

Production of virus and phytoplasma free plants

In Kenya sugarcane crop stands in the field for 18 months or more, and because of intense cultivation practices associated with the crop, new diseases appear to be setting in all the time. There is therefore a need to continuously evolve new varieties to combat several diseases. Since it is not possible to get combined resistance against all or most diseases in a breeding programme, there is a dire need to harness new techniques and methods to evolve new varieties against various diseases and to improve various economically important characters.

Tissue culture techniques have been employed with variable success to recover virus and phytoplasma free sugarcane plants from infected lines. Meristem culture was successfully used to eliminate Sugarcane mosaic virus (SCMV) (Kristini, 2004), 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. Recent researches showed 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 related studies cryotherapy of shoot tips was found to be an efficient method for elimination of SPLL phytoplasma from sweet potato (Wang and Valkonen, 2007). Cryotherapy alone failed to eliminate RBDV that can infect meristematic tissue; however, thermotherapy followed by cryotherapy was able to eliminate RBDV. Furthermore, cryotherapy can be used simultaneously for long-term storage of germplasm and production of virus-and phytoplasma free-plants (Wang and Valkonen, 2007).

Germplasm conservation

Development of in vitro techniques in sugarcane has resulted in more efficient and effective means for international exchange and conservation of germplasm (Taylor and Dukic, 1993). One way of conserving germplasm is in vitro storage under slow-growth conditions (at low temperature and/or with growth-retarding compounds in the medium) or cryopreservation or as desiccated synthetic seed (Villalobos & Engelmann, 1995;

Harry & Thorpe, 1991). These technologies are all directed towards reducing or stopping growth and metabolic activity. Techniques have been developed for a wide range of plants (Bajaj, 1990). The most serious limitations are a lack of a common method suitable for all species and genotypes, the high costs and the possibility of somaclonal variation and non-intentional cell-type selection in the stored material (e.g. aneuploidy) due to cell division at low temperatures or non-optimal conditions giving one cell type a selective growth advantage. 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 to numerous problems bedeviling the industry in the country.

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