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TISSUE CULTURE STRATEGIES IN SUGARCANE (SACCHARUM OFFICINARUM L.)

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) has wide range of economic importance due to its major and by-products. Seed multiplication of newly released varieties of sugarcane is one of the major constraints. Once a desired clone is identified, it usually takes 6-7 years to produce sufficient quality of improved seed material. This long duration causes a major bottleneck in breeding programmes. For this reason, micropropagation offers a practical and fast method for mass propagation of clonal material. Limitations and time consuming conventional methods promoted the need of plant tissue culture and genetic engineering in sugarcane molecular breeding programmes. Plant tissue culture techniques have become a powerful tool for studying and solving basic and applied problems in plant biotechnology. Sugarcane is a suitable candidate for plant biotechnology and genetic engineering tool due to its complex genomic structure, poly-auenploidy, rare flowering, and poor fertility. Successful protocols for shoot tip culture, callus culture, embryo culture, virus free plant production, somatic embryogenesis and genetic transformation have been already established. Thus following technique can be used to enhance mass production of sugarcane crop economically with the present trend of demand of sugarcane in the region.

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KEY WORDS

Direct regeneration, Genetic transformation, Meristems, Micro-propagation, Shoot regeneration.

INTRODUCTION

Sugarcane belongs to the genus *Saccharum oficinarum* L., of the tribe Andropogoneae in the grass family (Poaceae). This tribe includes tropical and subtropical grasses including the cereals genera Sorghum and Corn. Sugarcane is one of the most efficient photosynthesizer, C-4 plant in plant kingdom and commercially propagated through stems cuttings. Saccharum consists of six species Wild: *S. spontaneum* L. and *S.robustum,* Cultivated: S. officinarum L; *S. barberi; S. sinense* and *S. edule*. The four cultivated species are complicated hybrids and all intercross readily. Sugarcane is a highly heterozygous complex polyploidy with meiotic irregularities. *Saccharum officinarum* L (2n=80) or the noble canes accumulates very high levels of sucrose in stems and very susceptible to diseases. *S. officinarum* is less variable then other species (Daniels and Roach, 1987). Sugarcane is the main source of sugar in many countries. India is the world's largest sugar consumer (Murthy, 2010).

The major and by-products of sugarcane (sucrose, bagasses and molasses) have been varied and numerous applied uses. Bagasses is composed of cellulose, hemicelluloses and lignin. Fiber products primarily paper can be obtained from the pentosans and plastics are potentially derived from the lignin. Mollases solids consist of 60% combined sucrose and inert sugars and about 13% inorganic salts. The principle product of sugarcane is sucrose, primarily a food but research has shown that this also can be used as a raw material for production of higher value products. Some natural pharmaceutical compounds are derived from sugarcane (Menéndez et al. 1994); additionally, agricultural and industrial by-products of the sugar production process are extensively employed for animal nutrition, food processing, paper manufacturing and fuel (Patrau 1989). The sugarcane juice is used to be antidote, antiseptic, antivenous, bactericide, cardiotonic, demulcent, diuretic, intoxicant, laxative, pectoral, pesticide, refrigerant and stomachic.

Ikshavo raktapittaghnaa balyaa vrishyaa kaphapradaaha |

Swaadupaakarasaaha snigdhaha guravo mutralaha himaha ||

Sugarcane has the properties of normalizing vitiation of blood and pitta (bile). It rejuvenates liver. Because of this property it is widely used in treating jaundice. It is, however, very essential that the juice, must be clean and devoid of micro organisms. The sugar cane juice instantly energizes our body and provides strength. Sugar cane is rich in iron and carbohydrates. Hence it boosts energy straight away. It acts as an aphrodisiac and increases libido, quality and quantity of semen. It also helps in rectifying erectile dysfunction. It increases kapha and helps in pectoration of it in cough. It acts as a diuretic and helps in detoxifying kidneys. Sugarcane juice has many medicinal properties. It strengthens the stomach, kidneys, heart, eyes, brain and sex organs. The juice is beneficial in fevers. In febrile disorders which causes fever, when there is a great protein loss, liberal intake of sugarcane juice supplies the body with necessary protein and other food elements. Sugarcane is very useful in scanty urination. It keeps the urinary flow clear and helps the kidneys to perform their functions properly. It is also valuable in burning micturation due to high acidity, genorrhoea, enlarged prostate, cyctitis and nepthritis. For better results, it should be mixed with lime juice, ginger juice and coconut water. Studies indicate that it has positive

activities against prostate and breast cancer cells. Studies were carried out to evaluate sugarcane bagasse as an alternative to agar for micropropagation and improve the quality of propagule (Mohan et al., 2004). Brazil is a major grower of sugarcane which is used to produce sugar and provide the ethanol used in making gasoline-ethanol blends (gasohal) for transportation fuel. Commercial sugarcane is propagated vegetatively and germination refers to the initiation of growth from buds present on the stems of the stools that remain in the soil after harvest of the previous crops. Either whole stalks or stalk's cut up in shorter segments called setts are used as planting material (Willcox et al., 2000). There are many causes of low yield, one of which is the lack of rapid multiplication has been a serious problem in Sugarcane breeding (Ali and Afghan, 2001). Once a desired clone is identified, it usually takes 6-7 years to produce sufficient quality of improved seed material. This long duration causes a major bottleneck in breeding programmes (Siddiqui et al., 1994). Time required and continuous contaminations by systemic diseases are the serious problems to multiply an elite genotype of sugarcane in the open field (Nand and Singh, 1994). Important reason for low yield in sugarcane is its susceptibility to attacks by pathogens such as fungi, virus, bacteria and mycoplasma which cause up to 70% in yields reduction (Xue & Chen., 1994; Oropez *et al*., 1995; Bh-avan & Gautam, 2002). For instance, sugarcane mosaic virus (SCMV) is found in almost all the cultivars grown in the sub-continent (Naz, 2003). A significant part of the yield (39-40%) is lost each year due to SCMV (Malik and Munir, 1990). As sugarcane is mostly propagated by vegetative means, once a plant becomes infected by a pathogen it can easily transfer the pathogen from one generation to another. For this reason, sugarcane seed (seed cane) production through micropropagation is a suitable and effective method for rapid propagation in comparison to conventional methods. Sugarcane is a highly poly-aneuploid crop, is impeded by its narrow gene pool, complex genome, poor fertility, and the long breeding/selection cycle makes difficulties for this crop (Manickavasagam, 2004). Conventional propagation of sugarcane suffered from low propagation rates, expensive labour, time consuming and potential transmission of pathogens from the seed cane to the subsequent crop limits the efficiency of this method (Lakshmanan, 2006). Limited availability of seed cane of a newly released variety at the time of its release and reaching the desired area for commercial cultivation it takes long time. However, the variety starts deteriorating due to biotic and abiotic stresses. The growing demand of newly released varieties could not be fulfilled by only conventional propagation methods. Therefore, application of plant tissue culture techniques provides an alternative method for the crop improvement (Sengar et al., 2011). Sugarcane biotechnology researches have been began in 1960s. The pioneer works on induction of callus and production of roots on callus at Hawaiian Sugar Planters (Nickel, 1964; Heinz and Mee, 1969).

Plant tissue culture offers the best methodology through micropropagation of sugarcane for quality and phytosanitary planting material at a faster rate in a shorter period of time. Tissue culture can increase the propagation potential by 20-35 times (Geijskes et al., 2003, Snyman et al., 2006). In addition, plants can be disease-indexed (Snyman et al., 2007) and healthy material multiplied in half the time compared to the conventional vegetative route. Numerous studies on sugarcane plant regeneration have been reported. Essentially, successful culture and regeneration of plants from protoplasts, cells, callus and various tissue and organs have been achieved in this crop. The culture experiments on sugarcane were firstly started in 1961 at Hawaii. Now a days at the many places of world such as Australia, Fizi, Taiwan, Florida, Luciana, Maryland, Phillipines, Brazil, France, Shrilanka and also in India scientists are doing experiments for the improvement as well as for

the development in the production of sugarcane.

A large number of identical clones by in vitro culture were reported by many authors (Hendre et al., 1983; Lee, 1987; Nand and Singh, 1994). Chattha et al. (2001) reported micropropagation by culturing axillary and apical buds on MS medium with 1.5 mg-1 of BA and GA3. Almost 2500 seedling /plants could be generated from one bud in a 12 week period. Kazim and Shahid (2001) worked with micropropagation of 8 sugarcane clones using meristem tip culture method and found that 4-mm size of meristem tips was the most suitable for establishment of culture. Their results indicated that the micropropagated plants were phenotypically similar to the mother plants. Whereas, when variability is required to be induced, cultures are grown from explant sources other than meristem and callogenesis is initiated at first. The use of different gelling agents (agar and agarose) and support materials (filter paper bridge, cotton cloth bridge and adsorbent cotton) as well as shaken and static liquid (control) cultures was studied in order to improve in vitro shoot multiplication and vigour in sugarcane (Lal, et al., 1993). Ramgareeb et al. (2010) established protocol provides for the rapid, proliferation of virus free shoots from infected sugarcane plants and approximately 1,300 shoots were propagated from a single 2 mm meristem in 11 weeks. Viruses that are of concern in the global sugarcane growing areas are sugarcane mosaic viruses (SCMV) and sugarcane yellow leaf virus (ScYLV). Parmessur et al. (2002) reported elimination of phytoplasma from sugarcane crop through tissue culture. Taylor (1994) reported that shoot development was faster from either apical/ axillaty bud than apical apical meristem and shoot growth was more rapid from apical bud than apical meristem (Hendre et al., 1983). Different varieties give shoot on different media with different survivability rate. It may be due to the variable presence of cytokinin in bud. George (1993) reported that young tissue had more cytokinin than the old one with other factors mentioned above. It is possible variable quantity of cytokinin plays the major role in the adjustment of cytokinin in the media for getting the shoot in sugarcane. Plant regeneration from shoot tip culture of sugarcane using Murashige and Skoog (MS) medium supplemented with 0.2 mg/lt BA and 0.02 mg NAA/lt have been reported. Shootlets were multiplied 4-fold every 2 weeks by sub culturing in the same medium. Further multiplication was carried out on solid MS medium containing the same growth regulators, before enhancing shoot growth on medium containing 1 mg 2,4-D/l and 15% (v/v) coconut water, and finally stimulating rooting on MS medium with 1 mg IBA/litre. (Naritoom, et al., 1993). Gosal et al., (1998) reported rapid multiplication in liquid MS medium on BAP (0.5 mg/l) and Kin (0.5 mg/l) and rooting on NAA (0.5 mg/l) and sucrose 70%. Sorory and Hosien (2000) reported that the use of 6% sucrose concentration enhanced shoot regeneration in sugarcane. Lal and Singh (1994) reported that the most efficient auxin for root initiation was NAA. Roots grow from the nodal primordial when the plantlets are well developed (Khan et al., 1998). Rooting was highly influenced by the different types and concentrations of auxin used (Appropriate amounts of auxin in the rooting medium are crucial for root induction. Among three auxins concentrations, IBA at 1 mg/l produced the highest percentage of rooting. Many workers also reported that 5 mg/l NAA was good for rooting (Larkin, 1982; Shukla et al., 1994, Alam et al.; 1995, Islam et al., 1996) and more than 5 mg/l NAA inhibits rooting. The concentration of hormone varies with variety to variety. Hardening of plantlets is an important step in tissue culture studies. Sreenivasan and Sreenivasan (1992) observed 90-95 per cent survival of plantlets in poly house under shade. They used potting mixture of sieved, silt and rotten press mud 1:1:1 proportion. The plantlets with good root system were taken and transplanted into potting mixture by trimming the leaves.

Establishment of callus cultures and regeneration of sugarcane were reported by

(Nickel, 1964; Barba and Nickel, 1969). Callus culture of sugarcane have also been successfully established using young leaves and young inflorescence as explants on MS medium containing 2,4-D and coconut milk (Nadar *et al*., 1978; Liu and Chen, 1984; Bhansali & Sing, 1984). In sugarcane culture, 2,4-D has proved to be indispensable for callus induction, proliferation and even embryogenesis (Brisible et al., 1994, Chengalrayen and Gallo-meaghar, 2001, Kenia et al., 2006). A range of 2,4-D concentrations (2.5-4.0mg/L) were evaluated form callus induction and embryogenic callus production. In all the genotypes highest percentage of explants forming callus was recorded with 3.5mg/L of 2,4-D and callus formation slightly decreased when 2,4-D was increase to 4.0mg/l (83.7%) and progressively decreases with decreasd in the 2,4-D concentration. Although callus induction was higher when MS was supplemented with 3.5 and 4.0 mg/L, embryogenic callus production was significantly higher when MS was supplemented with 3.0mg/L (83.75%) and 2.5mg/L (81.25%).The same concentrations were reported optimum for embryogenic callus prduction in sugarcane by many authors (Khan et al., 1998 and 2004). Selection of resistant cells and callus cultures in vitro followed by regeneration of resistant plants has been utilized as a direct application of the cell culture approach to crop improvement. The most common approach to select disease resistant lines in culture has been to use phytotoxic fungal culture filtrate or purified toxins produced by the pathogen (Behnke, 1979; Strobel, 1982; Wenzel, 1985; Daub, 1986; Chawla et al., 1987; Mohanraj et al., 1995, 2004). In vitro selection in sugarcane has been used for selection of eye spot resistant lines (Heinz et al., 1977; Larkin and Scowcroft 1983; Prasad and Naik, 2000), red rot resistance (Mohanraj et al. 2003), salt stress (Gandonou et al., 2004) and drought stress (Errabii et al., 2006). The embryogenic calli of CoC-671 stressed with different levels of NaCl were also studied. The results showed that a significant decrease in growth of callus and also found variability at higher concentration of salts (Patade et al., 2008). Recently salt tolerant variants from embryogenic calli of sugarcane Varieties CP48-103 were cultured on a selective medium containing different levels of NaCl. A total of four plants which regenerated from the tolerant calli were selected (Shomeili et al., 2011).

In sugarcane, there are only few reports dealing with direct regeneration from different explants (Gill et al., 2006) while there are many available on regeneration and multiplication through organogenesis *via* callus formation. Direct regeneration method enables the exploitation of existing genetic heterogeneity present within cells in the form of different cytotypes. Leaves are good target tissues for genetic transformation of sugarcane as is evident from recent studies (Snyman et al., 2006; Kalunke et al., 2009). For an efficient application of the direct regeneration system, according to Lakshmanan *et al*., 2006 regeneration of plants directly from explants presents an effective strategy to avoid or substantially reduce somaclonal variation but he did not deny the chance of somaclone in population regenerated directly from explants. Direct regeneration is the need of time today. Other reports on direct regeneration in sugarcane has been obtained from thin cell layer (Laxmanan et al., 2006), leaf segments (Gill et al., 2006) and leaf mid rib segments (Franklin et al., 2006). However, genotype constitution influences morphogenic responses in tissue culture systems (Gill et al., 2006; Garcia et al., 2007). High cost involved in micro propagation is another major constraint to its popular use in sugarcane. However a protocol for one step regeneration of complete plantlets and *ex vitro* rooting of micro shoots, raised through auxillary bud culture was developed for sugarcane cultivar CoS96268. Complete plantlets were regenerated in 42 days on regeneration medium using leaf disc explants, pretreated on MS medium supplemented with 3 mg/l 2,4-D for eight days. More than 95% explants exhibited regeneration

with an average of 23 shoots per explants. For *ex vitro* rooting, in vitro shoots of 5 to 6 cm long, treated overnight with 20 mg/l NAA, led to formation of complete plantlets with more than 90% root induction. These plantlets possessed more than 6 roots of 4 cm average length per plantlet and exhibited 95% survival when transferred to polybags containing soil. (Pandey et al., 2011).

Embryos have been induced to develop indirectly via an undifferentiated cell mass or callus stage from leaf discs or floral parts (Bower and Birch, 1992; Gallo-Meagher and Irvine, 1993; Snyman et al., 1996; Ingelbrecht et al., 1999). Establishing, developing and maintaining callus cultures is labour intensive and the recovery of transgenic plants ready for glasshouse planting may take as long as 36 weeks (Bower et al., 1996). To minimise the time spent generating embryogenic callus, one approach would be to employ a route of morphogenesis from leaf discs which is faster than the indirect morphogenic route. Leaf discs cultured for 2 weeks exhibited organised outgrowths on the cut surface of the discs, which by 3-4 weeks were clearly embryonic, with a well defined cellular structure 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 & Gallo-Meagher, 2001). Due to its global importance concerted efforts are being made for sugarcane improvement through plant breeding and more recently through biotechnology. Somatic embryogenesis (SE) is the most common regeneration pathways which have been reported (Nadar et al., 1978; Larkin, 1982; Ho & Guiderdoni & Demarly 1988; Chen et al., 1988; Brisibe et al., 1993; Khan et al., 2004). Ho and Vasil (1983a, b and Ahloowalia and Maretzki 1983) were the first to provide the evidence of plant regeneration through somatic embryos in sugarcane. The first report on SE and subsequent plant regeneration from sugarcane callus cultures (Ho and Vasil, 1983a) at Florida, cell cultures (Ho and Vasil, 1983b), protoplasts (Srinivasan and Vasil, 1986), and cryopreserved embryogenic cell suspension (Gnanapragasams and Vasil, 1990). The development of somatic embryogenesis (SE) was a turning point in sugarcane biotechnology research. The ability to induce SE in protoplast, cell suspension, and callus cultures helps in transgenic technologies for sugarcane improvement (Lakshmanan 2006). Auxin is essential for sugarcane somatic embryogenesis. Various factors like auxin, basal media, carbohydrate source, and cold treatment of inflorescence regulates SE (Brisibe et al., 1994; Liu 1993). Somatic embryogenic callus is the target tissue for genetic transformation studies of sugarcane (Snyman et al., 2001, 2006). In a recent report the first time highlights TDZ induced callus formation, somatic embryogenesis and plant regeneration using leaf explants of different varieties (Co94032, CoC671, Co86032, SNK754, SNK61 and SNK44) of sugarcane. They investigate the high embryogenic potential of leaf explants of tested varieties, and also opened the approach of large-scale clonal propagation of sugarcane for the genetic improvement programmes (Ravindra et al., 2011).

The main morphological characteristic of somatic embryos is the bipolarity and the absence of connection with the explant vascular tissue (Reinert, 1977). These characteristics were described for sugarcane by Guiderdoni (1986) through the histological analysis of somatic embryos which showed a typical bipolar orientation, scutellum, coleoptile and absence of vascular connection with adjacent tissue. More or less same results were observed in our study. Ahloowalia and Maretzki (1983) demonstrated that the absence of coleoptile was due to a premature germination of the embryos in culture medium with plant growth regulators. According to Ho and Vasil (1983a) a typical development of sugarcane embryos may have led to erroneous interpretations of the regeneration pathways. Although shoot apex of zygotic embryos was similar to the shoot apex formed during organogenesis (Falco et al.,

1996). The effects of 2,4-D (2, 3 and 4 mg/l), alone or in combination with benzyladenine (0.5 and 1.0 mg/l), on the somatic embryogenesis of sugarcane cv. Co Si 95071 were investigated. Growing tips collected from 4-month-old plants were placed in Murashige and Skoog medium and incubated at conditions of 24-26 deg C, 5.8 pH, 16 h light and 8 h darkness. After 3 weeks of incubation, non-embryonic calluses, and cream-coloured, compact and nodular somatic embryos, appeared on cut edges of leaf bits. More somatic embryos were formed using 2,4-D combined with benzyladenine.

Somaclonal variation is a phenomenon of all plant regeneration systems that involve a callus phase, whether regeneration occurs through somatic embryogenesis or by adventitious shoot formation (Larkin and Scowcroft, 1981). Somaclonal variation in sugarcane is referred as subclonal variation. This variation in sugarcane plays an important role in varietal improvement programme (Krishnamurthi and Tlaskal 1974). It was found that tissue culture variants are superior than the donor clones in increasing the cane yield, sugar yield and disease resistance (Krishnamurthi 1974; Liu 1983). It is widespread among plant species, including many important crops. One of the earliest reports of somaclonal variation was in *in vitro*-derived plantlets of sugarcane. Among the regenerated plants there were changes in morphology, such as presence or absence of hairs, differences in isozyme profiles, as well as variation in crop parameters such as cane diameter, stalk length and weight, and cane and sugar yield (Bailey and Bechet, 1989; Lyndsey and Jones, 1989). Somaclona1 variation is often heritable (Larkin et al*., 1984;* Brieman et al*.,* 1987) indicating that it results from genetic change (Karp, 1991). Several types of genetic changes associated with somaclonal variation have been reported, notably variation in chromosome number (Karp et al*.,* 1989*;* Karp, 1991),gene copy number (Landsmann and Uhrig, 1985; Zheng et al*.,* 1987), DNA mutations (Brown, 1989; Muller et al*.,* 1990) and transpositional changes (Peschke and Phillips,1991). Several factors are reported to affect the nature and frequency of somaclonal variation in regenerated plantlets. These include the tissue culture procedure employed (callus, cell suspension or protoplast culture), the time spent in culture, the source of the explant cells, and the composition of the culture medium used, in particular the presence of growth regulators such as 2,4-dichlorophenoxyacetic acid (2,4-D) (Karp, 1989). Although much effort has been channelled towards understanding how the above factors interact and whether the influences of some are stronger than others, somaclona1 variation remains unpredictable because it originates from chance events. Although much research has focused on the potential of somaclonal variation for the production of agronomically useful mutants (Masirevec et al*.,* 1988), variation can pose a major problem in genetic manipulation systems such as transformation, where specific genetic changes are desired in otherwise unaltered genomes. The purpose of this study was thus two-fold: first, to establish a rapid and efficient *in vitro* culture procedure for sugarcane and, secondly, to assess the extent of variation produced in the system so that its suitability for use in conjunction with a transformation strategy could be evaluated. Genetic changes include polyploidy, aneuploidy, mutation (point) and new insertions of (retro) transposons (Smulders, 2005). Darke et al., (1998) stated that in plants, nuclear mutations can be directly estimated with the frequency of chlorophyll mutants in the plant population obtained through mutagenic treatments. Plants obtained through *In vitro* cultures can show phenotypic variability which is due to true genetic changes (Orton, 1980). Anbalogan et al., (2000) reported that some phenotypic variability was the result of physiological changes during *In vitro* conditions; hence such plantlets normally revert to their parent type in field conditions. Aneuploids may have lower or higher number of chromosome and plantlets regenerated from these cells could express different genetic behaviour. The first use of induced mutations in

sugarcane has been reported in the twenties of twentieth century by the researchers at Hawaiian Sugar Planter's Association, Hawaii, USA. (Anon, 1928, 1929, 1953). The additional mutagen application can increase somaclonal variation (Maliga et al., 1981). Induced mutations also have useful application in term of providing marker genes for identification of fused protoplasts in somatic hybridization (Maliga et al., 1981).

Protocols for initiation and maintenance of cell suspension cultures have been routinely obtained, however regeneration of plants seems to be a limiting step. Apparently, the regeneration ability of a suspension culture can vary according to its age. Larkin (1982) reported callus cultures which were cultured for 32 months without loss of regeneration ability, while other authors report loss of ability for one-year-old cultures. Ho & Vasil (1983) maintained S.officinarum L. cell suspension cultures for a period of two years without loss of morphogenic ability. Unfortunately, some important traits such as resistance to insect pests and to some herbicides, appear to be absent from the genetic pools of sugarcane cultivars. The use of plant transformation methods to introduce resistance genes into plant genomes may have an important impact on sugarcane yields. The lack of a reproducible methodology for stable transformation of sugarcane was an important obstacle for its genetic manipulation during many years. In 1992, Bower and Birch first time successfully recovered transgenic sugarcane plants from cell suspensions and embryogenic calli transformed by particle bombardment. Simultaneously, Arencibia et al. 1992) developed a procedure for stable transformation of sugarcane by electroporation of meristematic tissue. Later, a method to produce transgenic sugarcane plants by intact cell electroporation was established by the same group. The development of herbicide-resistant plants containing the *bar* gene and derived from the commercial variety NCo 310 by biolistic transformation (Gallo-Meagher and Irvine, 1996) has been recently reported. Further developments in microprojectile technique have been reported by many researchers (Snyman et al., 2000, 2001, 2006; Geijskes et al., 2003; Desai *et al*., 2004; Mulleegadoo and Dookun-Saumtally 2005; Jain et al., 2007; Amala et al., 2009). However, direct plant-transformation systems are known to be traumatic to the cells, expensive due to the need of special equipment, and poorly reproducible because of the variable transgene copy-number per genome. Optimization of the *Agrobacterium*-mediated DNA transfer to sugarcane meristems has recently been reported (Enríquez-Obregón et al.,1997, 1998). It was reported that the enrichment of the media with sugars and low pH were both important factors in achieving efficient transformation of rice and maize by *A. tumefaciens* (Hiei *et al*., 1994; Ishida *et al*., 1996).

Recent works on *Agrobacterium*-mediated genetic transformation of monocotyledonous plant species are focused on the use of the so-called "super-binary" vector systems, i.e. binary vectors carrying a DNA fragment from the *A. tumefaciens* virulence region (Torisky et al., 1997). Our procedures, however, are based on conventional genetic vectors; in addition, acetosyringone - a commonly used inductor of *A. tumefaciens vir* genes (Rashid et al. 1996) is not required during the infection. In this study scientists investigated the regeneration abilities of the obtained calli (Deblaere et al., 1985). The results were similar to those observed under normal conditions and were not affected by the duration of the antinecrotic treatment. They obtained a classical insertion pattern usually observed in *A. tumefaciens*-transformed plants without any discernible genetic rearrangement. The number of independent insertion patterns per individual plant genome was one or two. The results are in good agreement with those reported for other systems of *Agrobacterium*-mediated transformation; they also contrast with the complex multi-copy insertion patterns described for the "first-generation" transgenic sugarcane plants produced by intact-cell electroporation (Arencibia et al., 1995). The

latter phenomena are clearly undesirable due to the lack of control the possibility of gene silencing, cosuppression, etc. In 2004 Univ. of Hawaii started a project entitled "Genetic transformation of sugarcane chloroplasts to improve expression and containment of gene encoding human vaccines". The first successful report on *Agrobacterium* mediated genetic transformation was reported by (Arencibia et al., 1998) in sugarcane. Wang et al., 2003 and Zhang et al., 2005 transformed embryogenic callus with *agrobacterium* strain EHA 105 and transferred the Trehalose synthase gene (TSase) against drought tolerance in sugarcane. Herbicide BASTA resistant sugarcane using axillary bud explants co-cultivated through Agrobacterium strains LBA4404 and EHA105 having neomycin npt II, bar and gus-intron gene (Manickavasagam et al,. 2004). Ai-Qin Wang et al. 2009 transferred the ACC oxidase antisense gene in sugarcane. The somatic embryogenesis is an important tool for mass propagation of plants and genetic transformation (Gopitha et al., 2010) because embryogenic calli is a more reliable and efficient tissue for genetic transformation of sugarcane (Snyman et al., 1996). Most recently Sugarcane cultivars Co 86032 and CoJ 64 were transformed with *cry*1Ab gene driven by maize ubiquitin promoter through particle bombardment and *Agrobacterium*-mediated transformation systems (Arvinth et al., 2010).

This brief review of sugarcane micropropagation system illustrates part of the ground work since the field is vast. The demand of sugarcane's major and by products is increasing but requirement is not completing. To do that we have two options. Either we have to cultivate new land, which is not possible or to increase per unit crop yield, which is in our hands. To increase sugarcane productivity, micropropagation is the best and ecofriendly route. It is evident from ongoing discussion that it is hard to release a new variety of sugarcane by conventional breeding methods due to difficult genetic behaviour of sugarcane. Moreover, it takes a long time to release a new variety with high yield and better sugar recovery. Therefore, the technique is very expensive and time consuming. Hence, tissue culture technique can play an important role in this regard. It is comparatively easy to create somaclonal variation in sugarcane tissue. Moreover, somaclones with superior phenotypes than mother plant can be released as a new variety which is the sole objective of the breeder. But after all these success, acceptance of new varieties by farmers, establishment of culture and cost effective technology development are major challenges in the field of sugarcane micropropagation.

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