Genomics and Applied Biology, 2017, Vol.8, No.1, 1-7
http://gab.biopublisher.ca

Research Report Open Access

Scope of Genetic Transformation in Sugarcane: A Review

Ithape Dinesh M., Maharana M., Tripathy Swapan K.
Nepal Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar, India
Corresponding author email: swapankumartripathy@gmail.com

Genomics and Applied Biology, 2017, Vol.8, No.1 doi: 10.5376/gab.2017.08.0001
Received: 24 Mar., 2017
Accepted: 27 Apr., 2017
Published: 12 May, 2017

Preferred citation for this article:
Ithape D.M., Maharana M., and Tripathy S.K., 2017, Scope of genetic transformation in sugarcane: a review, Genomics and Applied Biology, 8(1): 1-7 (doi: 10.5376/gab.2017.08.0001)

Abstract Sugarcane is a cash crop of national importance. Its complex genome, narrow gene pool, long breeding cycle, rare flowering and complex environmental interactions hinders progress in genetic improvement. But, the crop serves as an excellent material for in vitro culture. Therefore, genetic transformation can be a better alternative to incorporate resistance to diseases and abiotic stresses, and genetic improvement of quality traits. In this pursuit, the authors presented a detailed review of the status of *in vitro* culture and current strategies of genetic transformation in sugarcane using a number of important candidate genes.

Keywords Callusing and regeneration response; Genetic transformation; *Saccharum officinarum L.*

Introduction

Sugarcane (*Saccharum officinarum L.*) is a commercial cash crop. Brazil tops sugarcane production followed by India which contribute nearly 15% of world sugar production. Besides, it has immense potential for production of many diversified products. Its enormous potential for ethanol production has been recognized which is used as a fuel blend with petrol for running automobiles. Besides, green top of sugarcane is in vogue used as fodder and cattle feed. Dried filter cake is used as animal feed supplement, fertilizer and source of sugarcane wax. While, the filter mud resulted from sugar processing is utilized as manure. Bagasse is used as bio-sorbent for waste water purification and also for manufacture of paper, paper board products, hardboard as well as fuel to run boilers for boiling the juice.

In India, sugarcane is constrained with low productivity owing to sensitivity to salt, drought and biotic stresses (Nasir et al., 2000; Khaliq et al., 2005). Drought alone accounts about 17% potential yield loss. Being a typical glycophyte, its growth is severely affected leading to significant reduction in yield potential under salt stress (Suprasanna, 2010). Narrow gene pool, higher ploidy (2n=100-120), rare flowering, low fertility, large genome size, long breeding cycle and complex environmental interactions seem to be major hindrances for breeding of sugarcane. However, production of transgenic sugarcane can be a better alternative to integrate desired gene(s) related to diseases, abiotic stresses, set yield and quality traits. The approach avoids the problem of linkage drag besides shortcutting the period of breeding in sugarcane.

1 Response to *in vitro* Culture

In vitro culture is an integral part of plant genetic transformation (Tiel Kenia et al., 2006). 2,4-D is in vogue reported to produce white creamy embryogenic (nodular) callus from leaf whorl explant which is ideal for genetic transformation. Khamrit et al. (2012) got profused and best callusing response in MS with 3 mg/l 2,4-D + 15% coconut water. Khan et al. (2004) revealed best callus induction and proliferation on MS medium containing 2.0 mg/I 2, 4-D, while combination of 2, 4-D (2 mg/l) with kn (0.5 mg/l) proved to be best for rapid callus growth in all sugarcane genotypes (Srivong et al., 2015). Similarly, combination of 3.0 mg/l of 2,4-D with 0.2 mg/l Kinetin gives best callus from young leaves with callusing response up to 83% in 13-15 days (Satpal et al., 2011).

Direct morphogenesis minimizes somaclonal variation and it can be amenable for genetic transformation. Shoot tip explant (Yadav et al., 2012) and Leaf discs from young leaf whorl is reported be a quick, effective and reproducible direct regeneration system (Ali et al., 2012). Direct regeneration was achieved on MS medium with

MS + BAP+NAA (1.5+0.5 mg/L) + casein hydrolysate (500 mg/L) (Ali et al., 2012), MS + 5 mg/l NAA + 0.5 mg/l Kn (Gill et al., 2006) and MS with 2 mg/l BAP (Biradar et al., 2009). MS with paclobutrazol (40 mg/l) increases the production of plantlets and reduce the number of dead leaves and height of shoots (Panti, 2016). Besides, thiodizuron at lower concentration (0.01 and 0.1 mM) can be used as alternative for BAP and Kinetin (Vinayak et al., 2009).

Somatic embryos (SEs) can serve as excellent material for genetic transformation in sugarcane as these avoid chimerism. Creamy, nodular and friable calli resulted due to somatic embryogenesis may be considered ideal for rapid plantlet regeneration. Gandonou et al. (2005) revealed varied embryogenic callusing response (60 to 100%) in nine elite sugarcane genotypes. The plants derived through direct somatic embryogenesis have been found to be uniform in growth pattern with more vigour compared to plants derived through indirect somatic embryogenesis pathway (Suprasanna, 2010). Somatic embryogenesis has been reported in this crop by several workers (Asad et al., 2009, Ming et al., 2006). MS with 3 mg/L 2,4-D alone revealed highest embryogenic calli (Ijaz et al., 2012; Jahangir and Nasir, 2010). Khamrit et al. (2012) reported maximum percentage of somatic embryogenic callus induction in MS medium supplemented with 3 mg/l 2,4-D and 15% (v/v) coconut water. In contrast, somatic embryos were reported to be induced at lower concentrations of 2,4-D (1 mg/l), whereas higher concentrations induced non-embryogenic calli (Zamir et al., 2014). Sequential removal of 2,4-D followed by sub-culture on MS supplemented with 2mg/l BAP induced 98% shoot induction (after 3 weeks of culture) with maximum shoot elongation (9.4 cm) (Zamir et al., 2014). Similarly, callus induced in MS + 3.0 mg/l 2,4-D and subsequently incubated on 2,4-D-free media was found to be commercially viable for plantlet regeneration on MS + 1.0-1.5 mg/l BAP + 0.2 mg/l NAA in elite sugarcane genotypes (Abdu et al., 2012). Besides, Naz et al. (2008) revealed maximum callusing response from young leaf explants in MS + 3 mg/l 2,4-D; maximum somatic embryogenesis in sub-culture by sequential removal of 2,4-D (up to 2 mg/l) and higher frequency of plantlet formation in hormone-free media. MS with BAP as low as 0.25 mg/l was also reported to be efficient for shoot induction in 80% of embryogenic masses and the hormone free-MS medium proved to be appropriate for elongation and rooting of shoots (Dibax et al., 2011).

Media composition along with hormonal recipes also influenced somatic embryogenesis. Desai et al. (2004) reported high frequency of somatic embryo development in MS with 0.5 mg/l NAA, 2.5 mg/l kinetin, 100 mg/l L-glutamine and 4% sucrose. Besides, MS with 1.5 mg/l 2,4 D + glycine (0.75 mM), arginine (0.5 mM) and cysteine (0.25 mM) showed significant effect on somatic embryogenesis (94%) and shoot production as compared to medium without any amino acid. Among different amino acids, glycine seems to be most effective to promote somatic embryogenesis and maximum shoot regeneration.

A good regeneration system is a pre-requisite for effective exploitation of genetic transformation (Ijaz et al., 2012). The regeneration potential of callus seems to be genotype-specific and dependent on hormonal concentration and combinations. Addition of BAP (2-2.5 mg/l) alone caused an increase in percentage of shoot formation, number of shoot per callus clumps, and average shoot length (Hapsoro et al., 2012). Regeneration response as high as 82.32% was revealed on MS medium supplemented with 0.5 mg/l BAP and activated charcoal (2.0 g/l) (Mittal et al., 2016). Cefotaxime has been found to be a growth promoting substance in sugarcane tissue culture. Therefore, it is used in the medium subsequent to co-cultivation (Kaur et al., 2008). Patel et al. (2015) revealed emergence of microtillering on the media with 1.0mg/l BAP, 0.25 mg/l GA3, 20 g/l sucrose and 7 g/l agar. Whereas, MS with 3.0 mg/l GA3 alone resulted maximum shoot elongation (10.52±1.88) along with the highest number of root emergence (6.51±2.41).

Many workers tried with different types of auxins at different concentrations and combinations to regenerate adventitious roots. In this context; NAA, IAA and IBA seem to have good response and comparatively better response in case of NAA combination with IBA than IAA for profuse rooting (Behera and Sahoo, 2009). Some variety gives normal and healthy rooting within two week in MS or ½ MS medium supplemented with 2.5 to 4 mg/l NAA followed by 0.5 mg/l NAA+2.5 to 3 mg/l IBA with good root length (Mamun et al., 2004; Behera and

Sahoo, 2009; Biradar et al., 2009; Gopitha, 2010; Satpal et al., 2011; Godheja et al., 2014; Tuan et al., 2015; Dinesh et al., 2015). High concentration of NAA (5.0 mg/l) or a combination of NAA and IAA was reported to promote good rooting (Anbalagan et al., 2000). Recently, Tesfa et al. (2016) obtained profuse rooting in half-MS medium fortified with 3 to 5 mg/l NAA and 50 g/l sucrose (Yadav et al., 2012; Tesfa et al., 2016). Many workers also reported that 5 mg/l NAA was good for rooting. More than 5 mg/l NAA could inhibit rooting and the most efficient auxin for root initiation was NAA and IBA at 3mg/l with half MS media (Yadav. et al., 2012; Godheja et al., 2014).

Substrate mixture for acclimatization comprising sand and soil substrate in 1:1 ratio is suitable for plant establishment. However, a mixture of alluvial soil, clean humus, ¼ micro organic fertilizer and sand (1:1:1/ 4:1) was reported to be ideal for best ex vitro acclimatization with higher plantlet survival rate for sugarcane genotypes (Tuan et al., 2015; Tesfa et al., 2016).

2 Transgenic Approaches in Sugarcane

Success of the genetic transformation depends on stable integration of the transgene into the genome of the target tissue, expression of the transgene and selection of transformed cells (Pillay, 2013; Singh, 2013; Rashid and Lateef, 2016). Expression of transgenes requires suitable constitutive promoter sequence. A number of researchers used promoters e.g., Emu, Maize Adh 1, CaMV 35S, Maize ubiquitin promoter, TMV 35S and Rab17 for construction of gene cascade in sugarcane genetic transformation (Pillay, 2013; Kumar et al., 2013; Reis et al., 2014).

In planta genetic transformation using sugarcane seeds (Mayavan et al., 2013), shoot tip explants (Khan et al., 2013), axillary bud explants from 6-month-old plants (Manickavasagam et al., 2004) and young leaf whorl have been reported. Besides, production of transgenic plants via in vitro culture of somatic embryogenic callus (Kumar et al., 2014; de Alcantara et al., 2014) or cell aggregates of suspension culture (Efendi and Matsuoka, 2011) is a method of choice. Embryogenic calli (Taparia et al., 2012), protoplasts (Arencibia et al., 1995), and apical meristems have been used in sugarcane transformation studies. Among these, embryogenic calluses are the preferred explant for transformation owing to their high regeneration response (Taparia et al., 2012). The genetic transformation using polyethylene glycol (Aftab and Iqbal, 2001) microprojectile delivery system (Rani, 2012) and electroporation (Rakoczy-Trojanowska, 2002) are best suited to protoplasts and cell suspension cultures. Bower and Birch (1992) reported production of transgenic plants by bombardment of embryogenic callus with high velocity DNA-coated micro-projectiles.

3 *Agrobacterium*-mediated Genetic Transformation

The Agrobacterium-mediated transformation has the potential advantages over biolistic method owing to its simple methodology and a high efficiency of transgene integration. The selection system and co-cultivation medium were the most important factors determining the success of genetic transformation and transgenic plant regeneration (Joyce et al., 2010). The most important and widely used selectable marker is npt II (neomycin phosphotransferase) gene conferring resistance to phytotoxic amino-glycoside antibiotics, kanamycin and geneticin (Bower and Birch, 1992; Fitch et al., 1995). Inhibitory effect of selective agents is tissue and species specific (Cai et al., 1999; Yu et al., 2003). Therefore, it is necessary to know the minimal inhibitory concentration of selective agent for different sugarcane cultivars before attempting genetic transformation. Genetic transformation in sugarcane also involves use of reporter genes to establish the stability of transgene expression and any other effect of gene transfer process (Hansom et al., 1999).

A reproducible method for transformation of sugarcane using various strains of *Agrobacterium tumefaciens* such as AGL0, AGL1, EHA105 and LBA4404 carring vectors like pAHC27, pEmuKN, pR11F- (Pillay, 2013), pGreen0029, (Kumar et al., 2013), pBract 302 (Reis et al., 2014), pMLH7133 (Efendi and Matsuoka, 2011), Pu912 (McQualter and Dookun-Saumtally, 2007), pGFP35S (Rasul et al., 2014), pWBvec10a (Joyce et al., 2010), pKYLX80 (Gilbert et al., 2005) has been developed. Kumar et al. (2013) employed EHA105 strain of

Agrobacterium harboring pGreen0029 vector containing AVP1 (Arabidopsis Vacuolar Pyrophosphatase-1) gene driven under 35S CaMV promoter for genetic transformation against drought and salinity tolerance in sugarcane. Bax Inhibitor-1 gene from *Arabidopsis thaliana* (AtBI-1) into sugarcane offers suppression of ER (endoplasmic reticulum) stress in C4 grasses which can be an effective means of conferring improved tolerance to long-term water deficit (Ramiro et al., 2016).

In recent years, development of transgenic plants is increasing rapidly in sugarcane. Sugarcane has been also genetically modified for sugar yield and quality traits (Botha and Groenewald 2001; Vickers et al., 2005), pharmaceuticals (Wang et al., 2005), novel sugars with potential benefits to consumer (OGTR, 2004). Besides, many biotic and abiotic stresses related to physiological characters have been studied in transgenic sugarcane. These include resistance to sugarcane mosaic virus (SCMV) (Gilbert et al., 2005), yellow leaf virus (Gilbert et al., 2009), sugarcane borer (Gao et al., 2016) and leaf scald resistance, herbicide tolerance, antibiotic resistance, drought and salinity tolerance (Kumar 2013; Reis et al., 2014). Production of naturally occurring compounds for use in bioplastics, altered plant growth, enhanced nitrogen use efficiency, improved sucrose accumulation, improved cellulosic ethanol production from sugarcane biomass, altered plant architecture, enhanced water use efficiency, incorporation of green fluorescent reporter gene, altered juice colour (Manickavasagam 2004; Mitchell 2011) are the outcome of transgenic technology. Further, genetic engineering of sugarcane varieties that can produce high-value compounds e.g., pharmaceutically important proteins, functional foods, nutraceuticals, biopolymers, precursors, enzymes and biopigments are paving ways to launch sugarcane as a biofactory in coming years (Grice et al., 2003; Suprasanna, 2010).

The expression of *G. frondosa* TSase gene under the control of a promoter CaMV35S improve drought tolerance in sugarcane (Zhang et al., 2006) compared with non-transgenic plants. Similarly, Wang et al. (2005) developed the transgenic sugarcane plants harboring *Grifola frondosa synthase* gene which improved tolerance to osmotic stress. In another study, over-expression of heterologous P5CS gene under stress inducible promoter (AIPC) was also reported to enhance drought tolerance in sugarcane (Molinari et al., 2007). The Arabidopsis CBF4 gene transferred to sugarcane under the control of the maize ubiquitin promoter and the nos terminator was reported to improve drought tolerance (McQualter and Dookun-Saumtally, 2007). Besides, drought tolerance has been attempted in sugarcane by using Arabidopsis Vacuolar Pyrophosphatase (AVP1) gene (Kumar et al., 2014) and induced over-expression of AtDREB2A CA (a transcriptional factor) (Reis et al., 2014), SodERF3 (a novel sugarcane ethylene responsive factor) and Arabidopsis bax inhibitor-1 gene.

4 Conclusions

A ready-in highly regeneration protocol is the pre-requisite for successful genetic transformation in any crop. Several researchers developed cost effective, rapid and efficient regeneration system in elite sugarcane genotypes using varied hormonal recipes, other media supplements and culture conditions. Recently, a number of transgenic techniques have been used for transfer of useful genes from diversified genetic background. However, *Agrobacterium*-mediated genetic transformation proved to have several advantages over direct gene transfer techniques in sugarcane. The present review reveals successful development of genetically modified genotypes with improved quality features and resistance to biotic and abiotic stresses in sugarcane.

Acknowledgements

We sincerely acknowledge and thank all researchers for their valuable contributions included in this pursuit.

References

Abdu S.L., Yahaya M., and Shehu U.I., 2012, *In Vitro* regeneration of commercial sugarcane (*Saccharum* spp.) cultivars in Nigeria, J. Life Sci., 6: 721-725

Aftab F., and Iqbal J., 2001, PEG- mediated somatic hybridization studies in sugarcane (*Saccharum* spp. Hybrid CVS. CoL-54 and CP-43/33), Pak. J. Bot., 33(3):233-238

Ali S.M., Khan S., and Iqbal J., 2012, *In vitro* direct plant regeneration from cultured young leaf segments of sugarcane (*Saccharum officinarum* L.). J. Animal & Plant Sci., 22(4):1107-1112

Anbalagan S., Kalamani A. and Sakila M., 2000, *In vitro* propagation of sugarcane: nature of callus, direct regeneration, regeneration through callus and morphological variations. Res. on Crops, 1(2): 138-140

Arencibia A., Molina P.R., Dela Riva G .and Selman-Housein G., 1995, Production of transgenic sugarcane (*Saccharum officinarum* L.) plant by intact cell electroporation, Plant Cell Rep., 14(5):305 9
https://doi.org/10.1007/BF00232033

Asad S., Arshad M., Mansoor S. and Zafar Y., 2009, Effect of various amino acids on shoot regeneration of sugarcane (*Saccharum officinarum* L.). Afr. J. Biotechnol., 8(7):1214-1218

Behera K.K., and Sahoo S., 2009, Rapid *in vitro* micro propagation of sugarcane (*Saccharum officinarum* L. cv- Nayana) through callus culture, Nature and Science, 7(4): 1-10

Biradar S., Biradar D.P., Patil V.C., Patil S.S. and Kambar N.S., 2009, *In vitro* plant regeneration using shoot tip culture in commercial cultivar of sugarcane. Karnataka J. Agric. Sci., 22(1): 21-24

Botha F.C. and Groenewald J.H., 2001, Method for regulating or manipulating sucrose content and metabolism in sugar storing plants e.g., increasing sucrose content by regulating activity of pyrophosphate- dependent phosphofructokinase enzyme in plants. South Africa Patent Application ZA200101047-A25Jul2001

Bower R., Birch R. G., 1992, Transgenic sugarcane plants via micro-projectile bombardment. Plant J., 2:409-416
https://doi.org/10.1111/j.1365-313X.1992.00409.x

Cai W., Gonalves C., Tennant P., Fermin G., Souza M., Sarinud N., Jan F.J., Zhu H.Y. and Gonsalves D., 1999, A protocol for efficient transformation and regeneration of *Carica papaya*. L. *In Vitro* cell Dev. Biol., 35: 61-69
https://doi.org/10.1007/s11627-999-0011-3

de Alcantara G.B., Dibax R., Ricardo de Oliveira R.A., Filho J.C.B., Daros E., 2014, Plant regeneration and histological study of the somatic embryogenesis of sugarcane (*Saccharum* spp.) cultivars RB855156 and RB72454. Acta Scientiarum Agronomy, 36(1):1-6
https://doi.org/10.4025/actasciagron.v36i1.16342

Desai N.S., Suprasanna P. and Bapat V.A., 2004, Simple and reproducible protocol for direct somatic embryogenesis from cultured immature inflorescence segments of sugarcane (*Saccharum* spp.). Current Sci., 87(6): 764-768

Dibax R., de Alc ântara G.B., Filho J.C.B., Machado M.P., de Oliveira, Y., da Silva, A.L.L., 2011, Plant regeneration of sugarcane cv. RB931003 and RB98710 from somatic embryos and acclimatization. J. Biotech. and Biodiversity, 2(3): 32-37

Dinesh P., Thirunavukkarasu P., Saraniya A.R. and Ramanathan T., 2015, *In vitro* studies of sugarcane variety Co-91017 through micropopagation of shoot tip culture. Adv. plants Agric. Res., 2(6): 00071
https://doi.org/10.15406/apar.2015.02.00071

Efendi and Matsuoka M., 2011, An efficient *Agrobacterium*-mediated transformation method for sugarcane (*Saccharum officinarum* L.) Proc. Annual Int. Conf. Syiah Kuala University, Banda Aceh, Indonesia, November 29-30, 2011

Fitch M., De La-Cruz A. and Moore P. 1995, Effectiveness of different selection markers for sugarcane transformation. Plant Genome II. (Int. Plant Genome Conf), p. 57

Gandonou C., Errabii T., Abrinii J., Idaomar M., Chibi F. and SkaliSenhaji N.S., 2005, Effect of genotype on callus induction and plant regeneration from leaf explants of sugarcane. Afr. J. Biotechnol., 4(11): 1250-1255

Gao S., Yang Y., Wang C., Guo J., Zhou D. and Wu Q., 2016, Transgenic sugarcane with acry1ac gene exhibited better phenotypic traits and enhanced resistance against sugarcane Borer. PLoS ONE 11(4): e0153929
https://doi.org/10.1371/journal.pone.0153929

Gilbert R. A., Gallo-Meagher M., Comstock J.C., Miller J.D., Jain M., and Abouzid A., 2005, Agronomic evaluation of sugarcane lines transformed for resistance to sugarcane mosaic virus strain E, Crop Sci., 45:2060-2067
https://doi.org/10.2135/cropsci2004.0771

Gilbert R.A., Glynn N.C., Comstock J.C., Davis M.J., 2009, Agronomic performance and genetic characterization of sugarcane transformed for resistance to sugarcane yellow leaf virus. Field Crops Res., 111:39-46
https://doi.org/10.1016/j.fcr.2008.10.009

Gill R., Malhotra P. and Ghosal S., Direct plant regeneration from cultured young leaf segments of sugarcane. Plant Cell Tiss. Organ Culture, 84, 227(2006). doi: 10.1007/s11240-005-9015-9
https://doi.org/10.1007/s11240-005-9015-9

Godheja, J., Shekhar, S.K., Modi, D.R.,2014, The standardization of protocol for large scale production of sugarcane (co-86032) through micropropagation. Int. J. Plant, Animal and Environ. Sci., 4(4): 135-143

Gopitha K., BhavaniL A.and Senthilmanickam J., 2010, Effect of the different auxins and cytokinins in callus induction, shoot, root regeneration in sugarcane. Int. J. Pharma and Bio Sci., 1(3): 1-7

Grice J., Wegener M.K., Romanach L.M., and Paton S., 2003, Genetically modified sugarcane: A case for alternate products. AgBio Forum, 6(4): 162-168

Hansom S. and Bower R., 1999, Regulation of transgene expression in sugarcane. In: Proc. Int. Soc. Sugarcane Technologists, XXIII Congress, New Delhi, Feb. 1999, p. 28–290

Hapsoro, D., Astrina, Putri Febrianie A.P., and Yusnita, 2012, In vitro shoot formation on sugarcane (*Saccharum officinarum* L.) callus as affected by Benzyladenine concentrations. J. Agron. Indonesia, 40(1): 56- 61

Ijaz S., Rana I.A.., Khan I.A., Saleem M., 2012, Establishment of an in vitro regeneration system for genetic transformation of selected sugarcane genotypes, Genet. and Mol. Res., 11(1): 512-530
https://doi.org/10.4238/2012.March.6.4

Jahangir G.Z. and Nasir I.A., 2010, Various hormonal supplementations activate sugarcane regeneration *in-vitro*. J. Agril. Sci., 2(4): 231-237
https://doi.org/10.5539/jas.v2n4p231

Joyce P., Kuwahata M., Turner N. and Lakshmanan P., 2010, Selection system and co-cultivation medium are important determinants of *Agrobacterium*-mediated transformation of sugarcane. Plant Cell Rep., 29: 173-183
https://doi.org/10.1007/s00299-009-0810-3

Kaur A., Gill M.S., Ruma D. and Gosal S.S., 2008, Enhanced in vitro shoot multiplication and elongation in sugarcane using cefotaxime. Sugar Tech., 10(1): 60-64. 1 0: 60

Khan I.A., Khatri A., Nizamani G.S., Siddiqui M.A., Khanzada M.H., Dahar N.A., Seema N. and Naqvi M.H., 2004, *In-vitro* culture studies in sugarcane. Pak. J. Biotech., 1(1) 6-10

Khan S.A., Hanif Z., Irshad U., and Rashid H., 2013, Genetic transformation of sugarcane variety HSF-240 with marker gene GUS. Int. J. Agric. and Biol., 15:1258-1264

Khaliq A., Ashfaq M., Akram W., Choi J.K., Lee J., 2005, Effect of plant factors, sugar contents, and control methods on the Top Borer (*Scirpophaga nivella* F.) infestation in selected varieties of sugarcane. Entomol. Res., 35:153-160
https://doi.org/10.1111/j.1748-5967.2005.tb00152.x

Khamrit R, Jaisil P. and Bunnag S., 2012, Callus induction, regeneration and transformation of sugarcane (*Saccharum officinarum* L.) with chitinase gene using particle bombardment. Afr. J. Biotechnol., 11(24): 6612-6618

Kumar T., Uzma, Khan M.R., Abbas Z., Ghulam M., Ali G.M., 2013, Genetic improvement of sugarcane for drought and salinity stress tolerance using *Arabidopsis* Vacuolar Pyrophosphatase(AVP1) gene, Mol. Biotechnol., 56:199-209
https://doi.org/10.1007/s12033-013-9695-z

Kumar T., Khan M.R., Jan S.A., Ahmad N., Ali N., Zia M.A., Roomi S., Iqbal A. and Ali G.M., 2014, Efficient regeneration and genetic transformation of sugarcane with AVP1 gene. American-Eurasian J. Agric. & Environ. Sci., 14 (2): 165-171, doi: 10.5829/idosi.aejaes.2014.14.02.12304

Manickavasagam M., Ganapathi A., Anbazhagan V.R., Sudhakar B., Selvaraj N., Vasudevan A., Kasthurirengan S., 2004, *Agrobacterium*-mediated genetic transformation and development of herbicide-resistant sugarcane (*Saccharum* species hybrids) using axillary buds. Plant Cell Rep., 23(3):134-143
https://doi.org/10.1007/s00299-004-0794-y

Mamun M.A., Sikdar M.B.H., Paul Dipak Kumar, Rahman M., Mizanur, 2004, *In vitro* micropropagation of some important sugarcane varieties of Bangladesh. Asian J. Plant Sci, 3 (6): 666-669
https://doi.org/10.3923/ajps.2004.666.669

Mayavan S., Subramanyam K., Arun M., Rajesh M., Kapil Dev G., Sivanandhan G., Jaganath B., Manickavasagam,M., Selvaraj N. and Ganapathi A., 2013, *Agrobacterium tumefaciens*-mediated in planta seed transformation strategy in sugarcane. Plant Cell Rep., 32(10):1557-74
https://doi.org/10.1007/s00299-013-1467-5

McQualter R.B., and Dookun-Saumtally A., 2007, Expression profiling of abiotic-stress-inducible genes in sugarcane. Proc. Aust. Soc. Sugar Cane Technol., 29: 878-888

Ming R., Moore P. H., Woo K. K., D'Hont A., Glaszmann J. C., Tew T. L., Mirkov T. E., Silva J. D., Jifon J., Rai M., Schnell R. J., Brumbley S. M., Lakshmanan P., Comstock J. C. and Paterson,A. H., 2006, Sugarcane improvement through breeding and biotechnology. Plant Breed. Rev., 27:17-117

Mittal P., Devi R. and Gosal S.S., 2016, Effect of genotypes and activated charcoal on high frequency in vitro plant regeneration in sugarcane. Indian J. Biotech., 15: 261-265

Mitchell H.J., 2011, Regulation of genetically modified (gm) sugarcane in Australia. Proc. Aust. Soc. Sugarcane Technol., 33: 1-8

Molinari H.B.C., Marur C.J., Daros E., Campos M.K.F., Carvalho J.F.R.P., Filho J.C.B., Pereira L.F.P. and Vieira L.G.E., 2007, Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum* spp.): osmotic adjustment, chlorophyll fluorescence and oxidative stress. Physiol. Plantarum, 130: 218-229
https://doi.org/10.1111/j.1399-3054.2007.00909.x

Nasir N.M., Qureshi R.H. and Aslam M., 2000, Effect of salinity on emergence of sugarcane lines. Pak. Sugar J., 15: 12-14

Naz S., Ali A. and Siddique A. 2008, Somatic embryogenesis and plantlet formation in different varieties of sugarcane (*Sacchrum officinarum* L.) HSF-243 and HSF-245. Sarhad J. Agric., 24(4): 593-598

OGTR., 2004, DIR 051/2004 – Field trial of genetically modified (GM) sugarcane expressing sucrose isomerase. www.ogtr.gov.au/ir/dir051.htm

Panti N.C., 2016, Paclobutrazol (PBZ)-Cultar regulator in sugarcane: The effect of different PBZ-Cultar concentrations on sugarcane (*Saccharum*) of variety CPCL99-4455, A Thesis Submitted to the University of Belize in Fulfillment of BIOL 4992 - Independent Research, 2016

Patel V.S., Mehta R., Naik K.H., Singh D., Patel D.U. and Mali S.C., 2015, Callus induction & whole plant regeneration in sugarcane (*Saccharum* spp. complex) variety Co 86032. Green Farming, 6 (5): 935-939

Pillay E., 2013, *In vitro* culture and genetic transformation of selected ancestral and commercial sugarcane germplasm. Dissertation submitted for the degree of Master of Science in the School of Life Science, University of KwaZulu-Natal, Durban, South Africa

Rakoczy-Trojanowska M., 2002, Alternative methods of plant transformation -A short review, Cellular & Mol. Biol. Letters, 7: 849- 858

Ramiro D.A., Melotto-Passarin D.M., Barbosa M.D.A., Santos F.D., Gomez S.G.P., Massola Junior N.S., Lam E. and Carrer H., 2016, Expression of Arabidopsis Bax Inhibitor-1 in transgenic sugarcane confers drought tolerance. Plant Biotech. J., 14:1826–1837
https://doi.org/10.1111/pbi.12540

Rani K.S., Surinder K., Sandhu S. and Gosal, 2012, Genetic augmentations of sugarcane through direct gene transformation with Osgly II gene construct. Sugar Tech., 14(3):229-236
https://doi.org/10.1007/s12355-012-0149-x

Rashid A.H.A. and Lateef D.D., 2016, Novel techniques for gene delivery into plants and its applications for disease resistance in crops. Amer. J. Plant Sci., 7: 181-193
https://doi.org/10.4236/ajps.2016.71019

Rasul F., Sohail M.N., Mansoor S. and Asad S., 2014, Enhanced transformation efficiency of *Saccharum officinarum* by vacuum infiltration assisted *Agrobacterium*-mediated transformation. Int. J. Agric. Biol., 16: 1147-1152

Reis R.R., Cunha B.A.D.B., Martins P.K., Martins M.T.B., Alekcevetch J.C., Júnior A.C., Andrade,A.C., Ribeiro A.P., Qin F., Mizoi J., Yamaguchi-Shinozaki K., Nakashima K., Carvalho J.F.C., Sousa C.A.F., Nepomuceno A.L., Kobayashi A.K., Molinari H.B.C., 2014, Induced over-expression of AtDREB2A CA improves drought tolerance in sugarcane. Plant Sci., 221-222: 59-68
https://doi.org/10.1016/j.plantsci.2014.02.003

Satpal S.B., Routray A.K., Mishra R., 2011, Rapid *in vitro* propagation technique for sugarcane variety 018, Int. J. Pharma and Bio Sci.,2(4): B242-279

Singh R.K., Kumar P., Tiwari N.N., Rastogi J. and Singh S.P., 2013, Current status of sugarcane transgenic: An overview. Adv. Genet. Eng., 2: 112

Srivong T., Zhu Y.J., Pongdontri P., Pliansinchai U., Sakuanrungsirikul S., Borthakur D., Nagai C. and Kosittrakun M., 2015, Optimization of callus induction and plant regeneration in sugarcane (*Saccharum* spp.) for a study of sucrose accumulation in relation to soluble acid invertase expression. Chiang Mai. J. Sci., 42(4): 797-805

Suprasann P., 2010, Biotechnological interventions in sugarcane improvement: Strategies, methods and progress. Nuclear Agric. & Biotech. Div., BARC Newsletter, 316: 47-53

Taparia Y., Gallo M. and Altpeter F., 2012, Comparison of direct and indirect embryogenesis protocols, biolistic gene transfer and selection parameters for efficient genetic transformation of sugarcane. Plant Cell Tiss. Organ Cult., 111:131-141
https://doi.org/10.1007/s11240-012-0177-y

Tesfa M., Admassu B., Bantte K., 2016, *In vitro* rooting and acclimatization of micropropagated elite sugarcane (*Saccharum officinarum* L.) genotypes - N52 and N53., J Tissue Sci. Engg., 7: 164

Tiel Kenia, Enriquez G.A., Ceballo Y., Soto N., Fuentes A.D., Ferreira A., Coll Y. and Pujol M., 2006, Development of a system for rapid plant regeneration from in vitro sugarcane (*Saccharum officinarum* L.) meristematic tissue. Biotecnologia Aplicada, 23(1): 22-24

Tuan V.A., Hanh H.H., Phuong P.T.T., Thuy P.T.T., Thuy P.T., Vinh D.N. and Khanh T.D., 2015, Rapid *in viro* multiplication of some sugarcane cultivars (*Saccharum officnarum* L.) via embryogenic callus culture of young leaf tissues, Int. J. Dev. Res., 5(12): 6139-6146

Vickers J.E., Grof C.P.L., Bonnett G.D., Jackson P.A. and Morgan T.E., 2005, Effects of tissue culture, biolistic transformation and introduction of PPO and SPS gene constructs on performance of sugarcane clones in the field. Aust J. Agr. Res., 56(1):57-68
https://doi.org/10.1071/AR04159

Vinayak V., Dhawan A.K. and Gupta V.K., 2009, Efficiency of non-purine and purine cytokinins on shoot regeneration of sugarcane, Indian Journal of Biotechnology, vol.8, pp 227-31

Wang Z.Z., Zhang S.Z., Yang B.P. and Ruil Y., 2005. Trehalose synthase gene transfer mediated by *Agrobacterium tumefaciens* enhances resistance to osmotic stress in sugarcane. Sugar Technol., 7(1):49-54

Yadav S., Ahmad A. and Lal M., 2012, Effect of different auxins and cytokinins on in vitro Multiplication and rooting of shoot cultures in Sugarcane. Int. J. Biol. & Pharma. Res., 3(6): 814-818
https://doi.org/10.1007/BF02942417

Yu T.A., Yeh S.D. and Yang J.S., 2003, Comparison of the effects of kanamycin and geneticin on regeneration of papaya from root tissue. Plant Cell Tiss. Org., 169:178-74

Zamir R., Khalil S.A., Shah S.T., Khan M.S., Ahma, K., Shahenshah, 2014, Efficient *in vitro r*egeneration of sugarcane (*Saccharum officinarum* L.) from bud explants. Biotech. & Biotechnol. Equipment, 26(4): 3094-3099
https://doi.org/10.5504/BBEQ.2012.0049

Zhang H.X., Zhang B.P., Fang C.L., Chen R.K., Luo J.P., Cai W.W. and Liu F.H., 2006, Expression of the *Grifola* Trehalose synthase gene and improvement of drought tolerance in sugarcane (*Saccharum officinarum* L.). J. Integr. Plant Biol., 48:453-459
https://doi.org/10.1111/j.1744-7909.2006.00246.x