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MULTIPLICATION OF *SACCHARUM OFFICINARRUM* L. CV US 633 TO DEVELOP SEEDING MATERIAL AT MASS SCALE

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ABSTRACT

The aim was to develop a method for mass multiplication of sugarcane (*Saccharum officinarrum* L. cv US 633). Apical bud of *S. officinarum* L. cv US 633 were cultured on Murashige and Skoog (MS) medium augmented with various combinations of 6-Benzylaminopurinn (BAP) (0.5-3.0 mg/L), Kinetin (0.5-1.0 mg/L) and Ascorbic acid (1.0 mg/L) in the initial experiments. BAP (2.5 mg/L) and Kinetin (1.0 mg/L) was determined the most effective growth regulator for mass multiplication and 19.40 ± 0.44 shoots and 8.4 ± 0.05 cm length of tillers per explant were obtained after 25 days of inoculation. The in vitro regenerated shoots were successfully rooted on medium augmented with NAA (5.0 mg/L) in combination with IBA (1.0 mg/L).

Key words: Micro-Propagation; 6-Benzylaminopurine; Kinetin; Mass-Multiplication; Sugarcane.

Introduction

Saccharum officinarrum L (sugarcane) is an important polysomatic, highly heterozygous crop used not only for production of white sugar but also for biofuel and industrially important byproducts (Granja *et al.*, 2018; Tesfa and Ftwi, 2018; Singh *et al*., 2017; Singh *et al*., 2015). In Pakistan *Saccharum officinarrum* L (sugarcane) is the second major cash crop and contribute 3.2% in agriculture and 0.7% in GDP (Economic Survey of Pakistan 2012). Pakistan ranks in top five countries regarding cultivated area but stands at fifteenth and sixteenth position in production and yield (Bashir *et al*., 2012). In Pakistan sugarcane is propagated vegetatively by stem cutting which has always been associated with transmission of pathogens from one generation to another resulting in rapid spread of infection which undermine the quality of good varieties (Khan and Khatri, 2006).

Biotechnological methods had been frequently used worldwide to improve qualitative and quantitative characteristic in sugarcane by *In-vitro* culture techniques including plant tissue culture technique (Mekonnen *et al*., 2014; Kuar, 2014; Dalvi *et al*., 2012 and Koch *et al*., 2012; Arruda, 2012; Srivong *et al*., 2015; Sardar *et al*., 2018). Using this technique one can develop an improved disease-free sugarcane crop with potential of high yield (Mayang *et al*., 2011; Anjum *et al*., 2012; Sengar, 2010).

In nature plant produces hormones to regulate all functions such as cell division cell expansion in different developmental stage in plants which also helps in tissue culture technique to culture plant under *In-vitro* conditions (Tripathy 2019; Pinheiro, 2017; Jimenez 2005; Feher *et al*., 2003). Amino acids and plant growth regulators have been supplemented in media to promote efficient multiplication and growth in plants (Jan *et al.*, 2020); Nieves *et al*., 2003). The sugarcane contains myriad phytochemical such as terpenoids, polyamines, jasmonates, sterols and salicylic acid have specific roles in culture systems (Pinheiro, 2017; Jimenez, 2005). Several reports are available on the in vitro propagation of sugarcane through callus culture, axillary bud, shoot tip and induction of somatic embryogenesis (Tripathy, 2019; Pinheiro *et al*., 2017; Srivong *et al*., 2015; Baksha *et al*., 2002). In Pakistan also there were several efforts made to improve the quality of crop through extensive research on various varieties of sugarcane (Khan *et al*. 2009; Anjum *et al*. 2012; Arshad *et al*. 2017; Sardar *et al*. 2018). This study was conducted on a variety viz *Saccharum officinarrum* L. cv US 633 for the very first time. The objective of the present study the effect of growth regulators on micro propagation of *S. officinarrum* L. cv US 633. To establish the repetitive protocol for *In-vitro* mass multiplication of *S. officinarrum* L. cv US 633) and to produce large number of healthy sugarcane plantlets.

MATERIAL AND METHODS

Culture material

Saccharum officinarrum L. cv US 633 was grown in the experimental field of Plant Tissue Culture Lab at PCSIR Laboratories complex, Karachi. Fresh, young and healthy plants were selected for the collection of apical bud explants from 8 to 10 months old plant.

Explant surface sterilization

Explants were first washed with tap water followed by washing with distilled water and then surface sterilized with sodium hypochlorite solution (35%) containing few drops of Tween-20 for 20 minutes followed by three rinses with sterilized distilled water in a laminar flow cabinet.

Culture media

Murashige and Skoog (1962) basal medium (MS) was used for culture initiation and multiplication. Initiation MS media was supplemented with BAP (1-2 mg/L), kinetin (1 mg/L) and ascorbic acid (1 mg/L) in eleven combinations (Table 1).

Table 1. MS Media for Initiation of *Saccharum officinarrum* L. cv US 633 *In-vitro* culture.

Media	BAP mg/L	Kinetin mg/L	Ascorbic acid mg/L
IM1	0	0	0
IM2	0	1.0	0
IM3	0	0	1.0
IM4	1.0	0	0
IM5	1.0	0	1.0
IM6	1.0	1.0	0
IM7	1.0	1.0	1.0
IM8	2.0	0	0
IM9	2.0	0	1.0
IM10	2.0	1.0	0
IM11	2.0	1.0	1.0

Multiplication MS media for mass multiplication of culture was supplemented with 6-Benzylaminopurine (0.5-3.0 mg/L) and Kinetin (1.0 mg/L) in twelve different combinations (Table 2).

Table 2. Media for Mass Multiplication of *Saccharum officinarrum* L. cv US 633 *In-vitro* culture.

Treatment	BAP mg/L	Kin mg/L
M0	0.0	0.0
M1	0.5	0.0
M2	1.0	0.0
M3	1.5	0.0
M4	2.0	0.0
M5	2.5	0.0
M6	3	0.0
M7	0.5	1.0
M8	1.0	1.0
M9	1.5	1.0
M10	2.0	1.0
M11	2.5	1.0
M12	3	1.0

For the induction of root MS medium was supplemented with different concentration of Naphthalenacetic acid (NAA) in combination with Indolebuteric acid (IBA) (Table 3).

Table 3. MS Media for Rooting in shoots of *Saccharum officinarrum* L. cv US 633.

Media	NAA mg/L	IBA mg/L
RM1	1	1
RM2	2	1
RM3	3	1
RM4	4	1
RM5	5	1

Culture conditions

All culture were maintained at 25 ± 2ºC under 16/8 h day/night photoperiod provided by cool fluorescent lights (35 µmol m-2 s-1). The observation was taken every second day for 25 days after inoculation. The subculture was done after 25 days. Growth parameters recorded were number of shoot proliferation per explants (tillers) and length of shoot (cm.) to identify the efficiency of media for mass multiplication of culture.

Statistics

The experiment followed a completely randomized design with ten replicates. All experimental data used were means of ten replications from all treatments. Statistical analyses were performed using SPSS version 20.0 software (SPSS, Chicago, IL, USA) and Sigma Plot 12.5 (Systat Software Inc) was used to draw graphs.

RESULT AND DISCUSSION

Growth and development of sugarcane by employing conventional methods is hampered by various environmental stresses (FAO, 2009; Munns and Tester, 2008). Therefore, modern techniques like biotechnology had been recognized as potentially valuable tool for addressing the critical problems of crop improvement in agriculture program (Arshad *et al*., 2017). To establish a standardize the repetitive protocol for *In-vitro* initiation and mass multiplication of *Saccharum officinarrum* L. cv US 633 and to obtain pathogen free healthy culture in short time as a healthy seeding material through tissue culture technique Effect of growth regulators on micro propagation of *S. officinarrum* L. cv US 633 was studied. For initiation of culture eleven combinations of BAP (1-2 mg/L), kinetin (1 mg/L) and ascorbic acid (1 mg/L) were tested (Table 1). Many researchers reported that, the pretreatment of explants with plant growth regulators improve the shoot induction (Thomas, 2007; Tie *et al*., 2013). In this study apical buds and axillary buds were used to initiate culture. (Biradar *et al*., 2009) reported that axillary bud is the most suitable explant for the initiation of *In-vitro* culture of sugarcane whereas our results showed that apical buds perform better. Results of the present study showed culture initiation was best on media supplemented with BAP (1mg/L) and Kin (1 mg/L) (Fig. 4). In contrast (Biradar *et al*., 2009) reported that the media containing single growth regulator i.e., BAP (2 mg/l) is suitable for initiation of culture whereas Ali and Afghan, 2001) observed optimum growth regeneration in media containing combination of NAA and BAP in sugarcane Varity SIM-6. The results showed that in media without growth regulators shoot proliferation was lowest (2.07 ± 0.17) after 15 days of inoculation. Several studies reported that the explants cultured on the media which contain different combination of growth regulators exhibited a greater number of shoots compared to media having single PGR (Azade *et al*; 2016; Yi *et al*., 2018). Results showed that combine effect of BAP and kinetin on initiation of culture was positive and combination of BAP and kinetin in ratio of 1:1 and 2:1 supports shoot proliferation (5.34 ± 0.14 and 5.50 ± 0.09). The addition of ascorbic acid enhanced the number of shoot proliferation (8.53 ± 0.18) in media supplemented with 1mg/l 6-Benzylaminopurine, 1mg/l Kinetin and 1mg/l Ascorbic acid after 15 days of inoculation (Fig. 1).

Several scientists reported that the different combinations of plant growth regulators played key role to achieve mass multiplication of shoots under *In vitro* conditions (Kantayos, 2019; Kim *et al*., 2016). Similar results were found in this study that media without growth regulators was unable to produce multiple shoots after 25 days. There was a requirement of growth regulator for multiplication of culture. The response in media without growth regulators was very slow whereas response increases with the increase of growth regulators up to certain level. The growth parameters measured showed that number of tillers and shoot length was lowest in media without growth regulator i.e., control (4.60 ± 0.24 and 5.86 ± 0.05 cm) after 25 days (Fig. 2 and Fig. 3). There were some scientists who reported that only single cytokine (Kinetin) was effective in some cases to get highest multiplication (Patel *et al*., 2001) these results were not in support of this study.

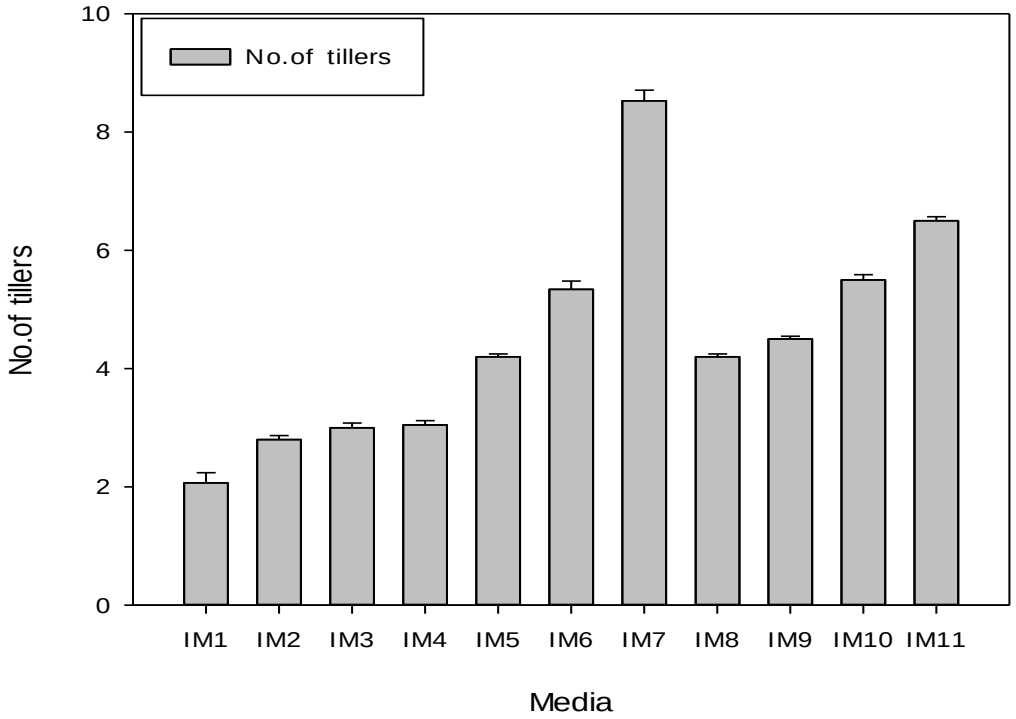

Fig. 1. Effect of various concentrations of BAP and KIN on initiation of *In-vitro* culture of Sugarcane var. US 633 after 15 days.

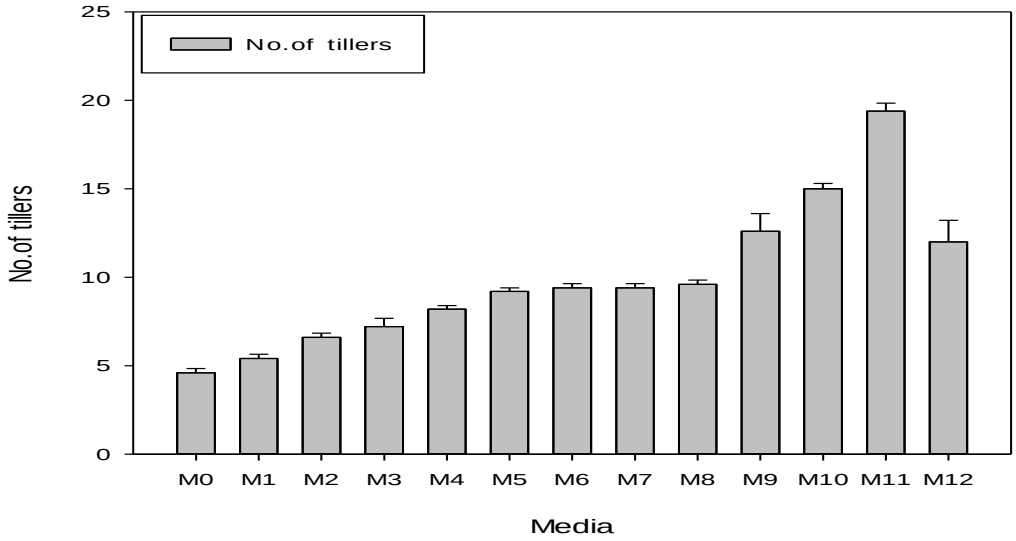

Fig. 2. Effect of various concentrations of BAP and KIN on Mass multiplication of Sugarcane var. US 633 after 25 days of *In-vitro* culture.

For mass multiplication of culture twelve combinations of BAP (0.5-3.0 mg/L), kinetin (1 mg/L) and ascorbic acid (1 mg/L) supplemented in MS media were tested (Table 2). The data analysis of culture showed that with the increase in concentration of growth regulator numbers of tillers increased progressively up to 2.5mg/L BAP and 1mg/L Kin (19.40 ± 0.44) after 25 days (Fig. 2). In contrast Pawar *et al*., (2002) used lower concentration of indole acidic acid (IAA), BAP and KIN for mass multiplication. It has been stated that low concentration of BAP (0.5 mg/L) and Kinetin (0.5 mg/L) are not suitable for vigor multiplication (Chen *et al*., 1987). Several scientists reported that shoot tip explant of sugarcane was best multiplied on MS medium supplemented with BAP, kinetin and coconut water (Bakesh *et al*., 2002; Pawar *et al*., 2002). Results of Geetha and Padmanabhan (2001) that the combination of BAP with kin gave the maximum response in most varieties also supported our results. However other scientist

found that combination of BAP and Indole butyric acid gave best shoot multiplication (Behera and Sahoo, 2009). Similarly shoot length also increases with the increase in concentration of BAP up to 2.5mg/L (8.4 ± 0.05cm) (Fig. 3). On further increase in concentration of BAP from 2.5mg/L to 3 mg/L both number of tillers (12.00 ± 1.22) and shoot length (8.4 ± 0.05cm) start decreasing (Fig. 2). Results showed that higher concentration of BAP was negatively affecting multiplication rate under *In-vitro* conditions. Many researchers reported that NAA and IBA were best for root formation in sugarcane. Baksha *et al*. (2002) observed best root formation in half strength media supplemented with 5.0mg/L NAA, he also tested 0.1-0.5mg/L IBA along with 0.5-2.0 mg/L BAP which was unable to induce root formation. Raman Gill *et al*., (2006) reported that the high concentration of NAA more than 5mg/L is not effective for root induction. Jagadeesh *et al*. (2011) stated that NAA was more suitable than IBA alone or in combination with other hormones rooting of sugarcane. In general, many scientists reported that 5 mg/L NAA was good for induction of root in several varieties of sugarcane (Yadav *et al*., 2012).

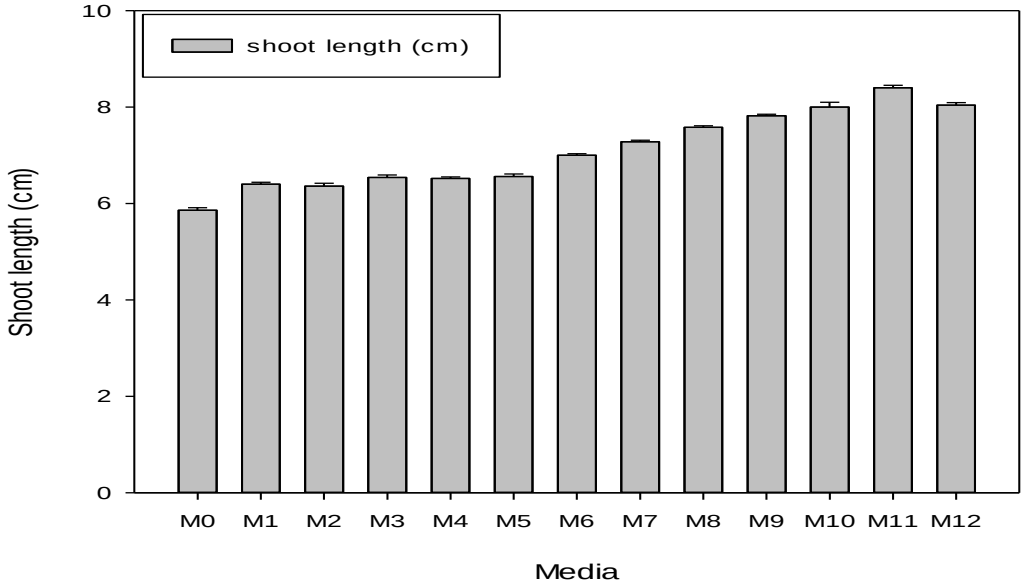

Fig. 3. Effect of various concentrations of BAP and KIN on shoot length (cm) of sugarcane US 633 plantlets after 25 days of *In-vitro* culture.

Fig. 4. The shoots induced on medium containing BAP and Kinetin.

CONCLUSIONS

During the present study twelve different combinations of growth regulators were tested in the MS media and it was revealed that best culture initiation rates (80%) was obtained in media containing BAP (1mg/L), Kinetin (1mg/L) and Ascorbic acid (1mg/L). The highest number of tillers and length of shoot was obtained in media supplemented with 2.5mg/L BAP and 1mg/L kin. The data analysis showed that highest value of shoot length and number of tillers was achieved by the addition of kinetin in nutrient media and the highest multiplication shoot length and number of tillers observe in media containing 2.5 mg/L BAP + 1 mg/L kinetin gave the highest values for shoot length and number of tillers. The combination of NAA and IBA is the best hormones combination for root induction in this species.

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Authors Contribution:
Beena Naqvi: Supervised the experiments and approved the article
Saima Riaz: Performed the experiments and manuscript write up
Bilquees Gul: Design experimental layout
Tour Jan: Critically reviewed the manuscript

REFERENCES

Ali, K. and S. Afghan (2001). Rapid multiplication of sugarcane through micropropagation technique. *Pak. Sugar J.*, 16:11-14.

Anjum, N., S. Ijaz, I.A. Rana, T.M. Khan, I.A. Khan, M.N. Khan, G, Mustafa, F.A. Joiya and A. Iqbal (2012). Establishment of an In Vitro Regeneration System as a Milestone for Genetic Transformation of Sugarcane (*Saccharum officinarum* L.) against *Ustiligo scitaminea*. *Biosci Methods*, 3:7-20.

Arruda. P. (2012). Genetically modified sugarcane for bioenergy generation. *Current Opinion in Biotech*., 23: 315-322.

Arshad, N.A., C.R. Zain, A.K. Ahsan, I. Anizan, M. Azhar and M.W.Y. Wan (2017). Accumulation of antioxidants in rice callus (Oryza sativa L.) induced by β-glucan and salt stress. *Austral. J. Crop Sci*., 11: 118-125.

Azade, L.K., A.S. Indieka, M.O. Adero, S. Kiboi and N.O. Amugune (2016). *In vitro* regeneration of pigeon pea using leaf explants. *African Crop Sci. J.*, 24: 191-201.

Baksha**,** R., **R.** Alam**,** M.Z. Karim**,** S.K. Paul**,** M.A. Hossain**,** M.A.S. Miah and A.B.M.M. Rahman **(**2002). *In vitro* shoot tip culture of sugarcane (*Saccharum officinarum*) variety lsd 28. *Biotech*., 1: 67-72.

Bashir, A., S. Haq, M. Abbas, M.A. Munir and A. Afzal (2012). Impact of sugarcane mills development activities on cane production in Punjab. *Pak. J. Agri. Res.*, 25: 21-27.

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

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

Chen, M.H., P.J. Wang and E. Maeda (1987). Somatic embryogenesis and plant regeneration in *Carica papaya* L. tissue culture derived from root explants. *Plant Cell Reports*, 6:348-351.

Dalvi, S.G., V.C. Vasekar, A. Yadav, P.N. Tawar, G.B. Dixit, P.D. Theertha and R.B. Deshmukh (2012). Screening of Promising Sugarcane Somaclones for Agronomic Traits, and Smut Resistance Using PCR Amplification of Inter Transcribed Region (ITS) of *Sporisorium scitaminae*. *Sugar Tech.*, 14: 68-75.

FAO (2009). *Declaration of the World Summit on Food Security*. 16–18 November, Rome. http://www.fao.org/wsfs/world-summit/wsfs-challenges/en/.

Feher, A., T.P. Pasternak and D. Dudits (2003). Transition of somatic plant cells to an embryogenic state. *Plant Cell, Tissue and Org. Cult.*, 74: 201-228.

Geetha, S. and D. Padmanabhan (2001). Effect of hormones on direct somatic embryogenesis in sugarcane. *Sugar Tech*., 3: 120-121.

Gill, R., P.K. Malhotra and S.S. Gosal (2006). Direct plant regeneration from cultured young leaf segments of sugarcane. *Plant Cell Tissue Organ Cult.*, 84: 227–231. doi: 10.1007/s11240-005-9015-9

Granja, M.M.C., M.J.L. Medeiros, M.M.A. Silva, T.R. Camara, L. Willadino and C. Ulisses (2018). Response to in vitro salt stress in sugarcane is conditioned by concentration and condition of exposure to NaCl. *Acta biologica Colombiana*, 23: 30-38.

Hoy, J.W., K.P. Bischoff, S.B. Milligan and K.A. Gravois (2003). Effect of tissue culture explant source on sugarcane yield components. *Euphytica*, 129: 237-240.

Jagadeesh, B., M. Kumar, M. Shekhar and P. Udhakar (2011). Amenability of the sugarcane variety 2005 t 16 to shoot tip culture. *Journal of Sugarcane Research*, 1(2): 75-77.

Jan, T., B. Naqvi, A. Hazrat, R. Qadri, M. Nisar, N. Khan, M.A. Nawaz and S. Pervez (2020). Impact of Hormones on the Proliferation of Shoots and Initiation of Roots in *Salvia santolinifolia* (Boiss), A High Value Medicinal Herb. *Pak. J. Sci. Ind. Res. Ser. B: Biol. Sci*., 63B (1): 30-36.

Jimenez, V.M. (2005). Involvement of plant hormones and plant growth regulators on in vitro somatic embryogenesis. *Plant Growth Regulation*, 47: 91-110.

Kantayos, V. (2019). *Characterization of plantlet regeneration and genetic transformation of legume plants. School of Plant Production Science*. Ph.D. Thesis, Sunchon National Univ., Korea.

Khan, A. and A. Khatri (2006). Plant regeneration via organogenesis or somatic embryogenesis in sugarcane: histological studies. *Pak. J. Bot*., 38: 631-636.

Khan, I.A., M.U. Dahot, N. Seema, S. Yasmine, S. Bibi and A. Khatri (2009). Genetic variability in sugarcane plantlets developed through In vitro mutagenesis. *Pak. J. Bot*., 41:153-166.

Kim, D.G., V. Kantayos, D.K. Kim, H.G. Park, H.H. Kim, E.S. Rha, S.C. Lee and C.H. Bae (2016). Plant regeneration by in vitro tissue culture in Korean soybean (*Glycine max* L.). *Korean J. Plant Resources.* 29: 143-153.

Koch, A.C., S. Ramgareeb, R.S. Rutherford, S.J. Snyman and M.P. Watt (2012). An in vitro mutagenesis protocol for the production of sugarcane tolerant to the herbicide ppp imazapyr. *In Vitro Cellu. and Devel. Biol.- Plant*, 48: 417-427.

Kuar, K. (2014). *In vitro mass cloning of Dianthus chinensisâ a horticultural important plant*. MSc thesis, Department of Biotechnology, Thapar University Patiala, India Pp. 85.

Mayang, R.B., Hapsoro, D., Yusnita. (2011). Regenerasi *In Vitro Tanaman Tebu* (*Saccharum officinarum* L.), Induksi dan Proliferasi Kalus, serta Induksi Tunas. *Agrotropika*, (16)2: 52-56.

Mekonnen, T., M. Diro, M. Sharma and T. Negi (2014). Protocol optimization for in vitro mass propagation of two sugarcane (*Saccharum officinarum L*.) clones grown in Ethiopia. *Afri. J. Biotech*., 13:1358-1368.

Munns, R. and M. Tester (2008). Mechanisms of salinity tolerance. *Annual Reviews of Plant Biol*.. 59:651-681.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassay with tobacco cultures. *Physiologia Plantar*., 15: 473-497.

Nieves, N., M. Segura-Nieto, M.A. Blanco, M. Sanchez, A. Gonzalez, J.L. Gonzalez and R. Castillo (2003). Biochemical characterization of embryogenic and non-embryogenic calluses of sugarcane. *In Vitro Cellu. and Deve. Biol.-Plant*, 39:343-345.

Patel, A.A., S.R. Patel, C.L. Patel and B.S. Praja-Pati (2001). Effect of media composition on in vitro multiplication of sugarcane. *Indian J. Genet. Plant Breeding*, 61:1-82.

Pawar, S.V., S.C. Patil, V.M. Jambhale, R.M. Naik and S.S. Mehetre (2002). Rapid multiplication of commercial sugarcane varieties through tissue culture. *Indian Sugar*, 52:183-186.

Pinheiro, F.G.C., A.K.L. Soares, S.T. Santaella, L.M. Alexandre e Silva, K.M. Canuto, C.A. Cáceres. M.D.F. Rosa J.P.D.A. Feitosa and R.C. Leitão (2017). Optimization of the acetosolv extraction of lignin from sugarcane bagasse for phenolic resin production. *Industrial Crops and Products*, 96: 80-90.

Sardar, K.S., H.S. Altaf and S. Nusrat (2018). Biotechnological techniques stimulate the production of sugarcane and useful for enhancing of physiological traits. *Pak. J. Biotech*., 15: 517-523.

Sengar, K. (2010). Developing of efficient protocol through tissue culture techniques for sugarcane micropropagation. *BioInfoBank*,18: 56.

Singh, A., U.R. Lal, H.M. Mukhtar, P.S. Singh, G. Shah and R.K. Dhawan (2015). Phytochemical profile of sugarcane and its potential health aspects. *Pharmacognosy Reviews*, 9: 45-54.

Singh, R.B., B. Singh and R.K. Singh (2017). Study of genetic diversity of sugarcane (Saccharum) species and commercial varieties through TRAP molecular markers. *Indian J. Plant Physi*., 22:332-338.

Srivong, T., Y.J. Zhu, P. Pongdontri, U. Pliansinchai, S. Sakuanrungsirikul, D. Borthakur, C. Nagai and M. Kosittrakun (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: 797-805.

Tesfa, M. and M. Ftwi (2018). *In Vitro* Plant Regeneration of Sugarcane (*Saccharum* spp.) Variety Inoculated Under Different Levels of Plant Growth Regulators. *J. Plant Biochem. Physi*., 6: 227.

Thomas, T.D. (2007). Pretreatment in thidiazuron improves the in vitro shoot induction from leaves in Curculigo orchioides Gaertn., an endangered medicinal plant. *Acta Physiologia Plantarum*, 29: 455-461.

Tie, M., Q. Luo, Y. Zhu and H. Li (2013). Effect of 6-BA on the plant regeneration via organogenesis from cotyledonary node of cowpea (*Vigna unguiculata* L. Walp). *J. Agri. Sci*., 5: 5.

Tripathy, S.K. (2019). Scope of in vitro culture targeting genetic transformation in sugarcane *Res. J. biotech*., 14: 95-100.

Yadav, S., A. Ahmad and M. Lal (2012). Effects of different auxins and cytokinins on in vitro multiplication and rooting of shoot cultures in sugarcane. *Int J Biol Pharma Research*, 3(6): 814-818.

Yi, J.Y., K. Balaraju, H.J. Baek, M.S. Yoon, H.H. Kim and Y.Y. Lee (2018). Cryopreservation of *Citrus limon* (L.) Burm. F shoot tips using a droplet-vitrification method. *Korean J. Plant Resources*, 31: 684-694.

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