Pak. J. Biotechnol. Vol 15 (2) 517-523 (2018) ISSN Print: 1812-1837
www.pjbt.org ISSN Online: 2312-7791

BIOTECHNOLOGICAL TECHNIQUES STIMULATE THE PRODUCTION OF SUGARCANE AND USEFUL FOR ENHANCING OF PHYSIOLOGICAL TRAITS

Sardar Khatoon Solangi[1], Altaf hussain Solangi[2], Nusrat Solangi[2]

[1]Institute of Plant Sciences, University of Sindh, Jamshoro and [2]Sindh Agriculture University Tando Jam, Pakistan. E-mails: sardarkhatoon807@yahoo.com, altaf.hussain466@gmail.com, nusratkhatoon934@yahoo.com

Article received 3.2.2018, Revised 12.5.2018, Accepted 20.5.2018

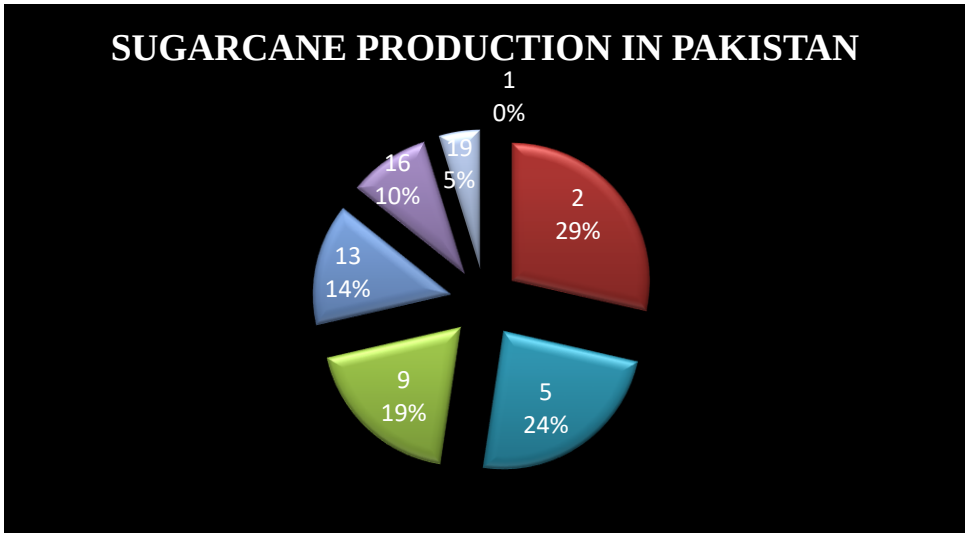
ABSTRACT:
An experiment was designed to develop technological method through which formulate protocol for tissue culture in sugarcane. Shoot tips of Saccharum officinarum L. as explants of three varieties included NIA-2012, NIA-105 and Gulabi-95. The regeneration of regenrable type of callus (whitened and solid type) was using in a basic medium with ABK (Indol acetic acid) (indol butric acid) cytokinin (kintin) added with different content of auxin (2, 4-D), (NAA) and picloram. Main analysis of variance was showing that all the traits are significant in NIA-2012 which fallowed by NIA-105. The best combination for the multiplication and shoot rising of sugarcane the plant growth hormone was used 2, 4-D and NAA. Root proliferated with the NAA at the concentrations of 3.0 mg/L was observed. However, the optimized protocol can be used for rapid in vitro mass multiplication of three sugarcane varieties related to the field parameter enhance production of sugarcane hence minimize the limitations sugarcane planting materials.

Key words: biotechnological techniques, production, sugarcane, agronomic traits, growth hormones.

INTRODUCTION

The large volumes of Sugarcane material used for micro propagation to apical meristems is very useful in sugarcane breeding programs, because of the time it saves in multiplying the promising varieties and clones and in facilitating the acquisition of large volumes of material. Meristems culturing can also be useful in eliminating pathogens (Arencibia *et al*., 1997). Sugarcane (Saccharum *spp*. hybrids) is an important agro industrial crop and information of heritability of phenotypic traits and agronomic characters is vital in breeding programs for globaly level (Anbalgan *et al*., 2000, Alvi *et al.,* 2008). The genetic variability present in the sugarcane cultivars, cultivate by the producers, has hybrid origin, generally thought of researchers (Khan *et al.,* 2009; Ahmed *et al*.,2010; Bairu *et al*., 2011).

In Pakistan requirements of advance in the characters of yield and sugar recovery. Therefore, application of plant tissue culture techniques provides an substitute method for development and improvement of sugarcane which relied on Phytohormone treatments were effective in inducing genetic variability (Mekonnen *et al*., 2014). However, the picloram and 2, 4-D showed stimulating and enhancing effect on plant height and width of internode (Tolera *et al*., 2014). Interactive effect of clones/parents x growth hormones had significant effect on all phenotypic characters. In sugarcane, maximum production means high sugar recovery Experimental work conducted to estimate variability of callus by tissue culture techniques which stimulate physiological trait (Raza *et al*., 2014) of sugarcane and enhancement of the production of sugarcane. Sugarcane is a traditional agricultural field crop with long olden times of safe use (Shimelis *et al*., 2014). It is thought to have become recognized as a domestic garden crop and byproducts of sugarcane correlated with number of tiller and mill able cane, internode width and cane height, cane yield depend on width of internode sugar (Jahangir *et al.,* 2014). Sugar yield using completely randomized block design through two factorial design data analyzed. Varied biotechnological tools have been used to improve qualitative and quantities characteristic of sugarcane crop (Kuar, 2014).



Sugarcane production in Pakistan improved since last five years.

MATERIAL AND METHODS

Experiments were conducted in Laboratory of Nuclear institute of Biotechnology, for micro-propagation. Shoots of sugarcane were used as explants source for the regeneration and micro propagation of Saccharum (fig:1) sterilized with 100% commercial bleach for 25 min and 70% ethanol for 1 minute followed by rinsing three times with sterilized distill water. Whole process was carried out in laminar flow hood.

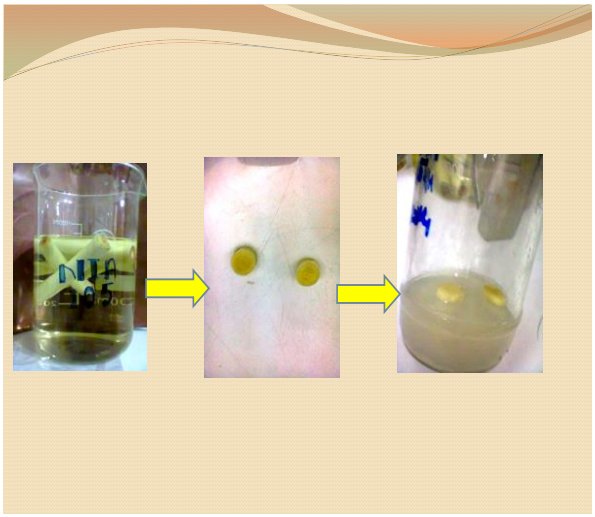


Fig: 1: Steps included in sterilization of explants of sugarcane

Tissue culture of sugarcane for micro propagation and regeneration was conducted according to technique developed by Khan *et al.*, (2008). The shoot tips of three different varieties included NIA-(2012), NIA-(105) and (Gulabi-95) were cutting into small pieces after sterilization process these particles were placed in each of MS basal medium supplemented with different concentration of auxins.

The analysis of variance due to different varieties, concentration and variety x concentration interaction were significant for all agronomic traits Table 2A, B and C. The study showed that shoot tips of sugarcane inoculated aseptically in auxins and cytokinins established the shoot induction and shoot multi-placation at the same concentration, the main parameters of external appearance in the sugarcane plant are number of tillers, height of cane as they directly effect on final phenotypic traits in sugarcane.

Table -2a: Impact of different auxins on NIA-2012 soma clones for phenotypic trait development.

Phenotypic character	**NIA-2012**				
	Growth regulators Concentrations 2,4-D, Picloram, NAA (mg l^{-1})				
	Control (0.00	**0.5**	**1.0**	**2.0**	**3.0**
Number of tillers	4.7778 e-f	6.5556 a-c	7.4444 a	7.3333 a	7.1111 a-b
Height of cane	114.16 e-g	117.49c-d	119.24c	123.05 b	126.47 a
Number of internode	13.778 g-h	16.556 d-e	17.889 c-d	20.222 b	24.889 a
Width of internode	1.5011 e-g	1.6011 d-e	1.7100 c-d	1.8878 b	2.1789 a
Number of tillers	4.3333 d-g	5.0000c-e	6.3333 a-b	5.7778 a-c	6.7778 a
Height of cane	113.44 b	116.76 b	118.25 b	122.08 b	125.20 b
Number of internode	12.667 f-g	14.889 d-e	15.667 d	18.111 b-c	21.667 a
Width of internode	1.4467 d-e	1.5589 c-d	1.6633 b-c	1.8122 b	2.0922 a
Number of tillers	4.7778 a-c	4.3333 a-c	5.8889 a	5.2222 a-c	5.6667 a-b

Height of cane	116.53 c-d	116.69 c-d	117.78 c-d	121.37 a-b	123.85 a
Number of internode	14.444 d-f	16.000c-e	17.111 cb-d	18.222 a-c	21.333 a
Width of internode	1.3733 d-f	1.4911 c-e	1.6111 b-d	1.7378 a-c	1.9578 a

Mean comparison of sugarcane trait Anova at ($p<0.05$) two factorial designs

Table- 2b: Impact of different auxins on NIA-105 soma clones for phenotypic trait development

Phenotypic character	**NIA-105**				
	Growth regulators Concentrations 2,4-D, Picloram,NAA (mg l^{-1})				
	control (0.00)	**0.5**	**1.0**	**2.0**	3.0
Number of tillers	5.1111d-f	4.5556 e-f	5.6667c-e	5.3333d-f	6.1111 b-d
Height of cane	113.63 f-g	115.56 d-f	118.93 c	122.41 b	123.20 b
Number of internode	13.000 e-f	14.00 d-f	14.000d-f	17.111b-d	19.778 a-b
Width of internode	1.1600 g	1.2556f-g	1.3778 e-f	1.4611d-e	1.5589 c-d
Number of tillers	4.7778 c-f	4.2222e-g	4.8889 c-e	5.4444b-c	5.3333 b-e
Height of cane	112.79 b	115.48 b	118.62 b	121.21 b	123.79 b
Number of internode	12.667g-h	15.556 e-f	15.778 e-f	17.556c-d	20.111 b
Width of internode	1.1956 i	1.283 h-i	1.4289 f-h	1.4889e-g	1.5978 d-e
Number of tillers	114.41 d-f	114.4 d-f	115.75 d-e	119.76 b-c	122.17 a-b
Height of cane	12.000 g	13.44 e-g	15.222d-e	16.333b-d	18.111 b-c
Number of internode	3.5556 b-c	4.000a –c	4.3333 a-c	4.3333 a-c	5.2222 a-c
Width of internode	1.3022 e-f	1.4322d-f	1.5567c-d	1.6956 b-c	1.8400 a-b

Mean comparison of sugarcane trait Anova at ($p<0.05$ two factorial designs

Table -2c: Impact of different auxins on Gulabi-95 soma clones for phenotypic trait development.

Phenotypic character	**Gulabi-95**				
	Growth regulators Concentrations 2,4-D, Picloram,NAA (mg l^{-1})				
	Control (0.00)	**0.5**	**1.0**	**2.0**	**3.0**
Number of tillers	2.0000 g	3.0000 g	4.3333 f	4.5556 e-f	6.1111 b-d
Height of cane	110.59 b	114.12 b	117.31 b	115.28 b	223.40 a
Number of internode	12.444 h	14.222 f-g	15.556 e-f	17.222 d-e	19.222 b-c
Width of internode	1.2567f-g	1.4111 d-e	1.5378 c-d	1.6656 b-c	1.7700 b
Number of tillers	5.0000 a-c	3.2222c	3.2222 c	5.4444 a-b	4.4444 a-c
Height of cane	111.05h	112.87g-h	114.85 e-g	116.22d-e	118.38 c
Number of internode	12.111 g	14.667 d-f	14.667 d-f	16.111 c-d	18.333 b
Width of internode	1.2867 h-i	1.3967 g-h	1.5633 d-f	1.6833 c-d	1.8000 b-c
Number of tillers	2.3333i	2.6667h-i	3.6667 f-h	3.4444 g-i	4.2222 e-g
Height of cane	111.52 f	112.46 e-f	112.81 e-f	117.44 c-d	119.68 b-c
Number of internode	12.111 f	12.111 f	12.556 e-f	15.222 c-f	17.000 b-d
Width of internode	1.2122 f	1.2778 e-f	1.4111 d-f	1.5056c-e	1.6233 b-d

Mean comparison of sugarcane trait Anova at ($p<0.05$ two factorial designs.

RESULTS

Variability in plantlets derived from sugarcane callus: The pooled performance of all the treatments and their control for all the characters indicate that parents/soma clones were significantly ($p\leq 0.05$) higher in agronomic trait obtained from high concentration of 2, 4-D in all replicates of sugarcane. In case of picloram high concentration yielded highest thickness of internode. Maximum phenotypic character in case of NIA-2012 was observed at 3.mg/l for all auxins. Highest number of tillers were obtained 7.111a-b in 2,4D and minimum in NAA (5.6667a-b). Maximum height of cane, number of internode and diameter of cane was estimates in soma clones of sugarcane at additive concentration of 2, 4-D and lowest obtained in NAA.

The average performance of all the sugarcane plantlets, under the Concentration of phytohormone and their control for all the characters indicate that were significantly ($p\leq 0.05$) higher in soma clones obtained from high concentration of Picloram and 2,4-D in NIA-105, expressing induced more genetic variability as compared to other auxins under study. In case of NIA-105 Maximum number of cane was obtained when 2, 4-D was applied in 3mg/l. Highest height of cane and number of internode 123.79b, 20.11b was obtainned at 3.0mg/l of picloram, whereas lowest height of cane and number of internode 122.17 a-b, 18.111b-c was observed in NAA. Highest cane thickness was observed (1.8400 a-b) when NAA was applied.

In case of Gulabi-95 all treatments of growth hormones for all morphological characters were

significantly ($p \leq 0.05$) higher in regeneration of clones obtained from high concentration of 2, 4-D, picloram yielded similar results. Maximum number of tillers was obtained 6.1111 b-d in 2, 4-D and minimum was observed 4.2222e-g in NAA were applied. Highest height of cane was estimate 223.40a at 2, 4-d and lowest in NAA was obtained 119.68b-c. In clones of Gulabi -95 maximum number of internode was observed 19.222b-c and minimum were 17.000bd Reduced width of internode found in NAA and increased in picloram at the concentration of 3.0mg/l.

The absence of certain economic trades from the genetic pool of sugarcane like insecticide and herbicide resistance required adoption of genetic transformation as means of improvement. It requires 10 to 15 years to get an improved variety for commercial cultivation. The time spent for this multiplication is considered a serious economic problem, mainly in view of the high yields NIA-2012 showing significant results (Table: 1). that would be obtained by planting the new variety earlier on a large commercial scale, because the propagation of improved or modified clones of sugarcane (Fig:2). Through sets is very slow, usually 1-10 in a year, this is a major hurdle.

Table 1: ANOVA for three different varieties of sugarcane soma clones for phenotypic trait development

Source	DF	Mean square			
		NIA-2012	NIA-105	GULABI-95	
		Number of tillers	Number of internodes	Height of cane	Width of internodes
Replication	2	5.4889	82.689	7445.45	0.58266
Treatment	2	6.556*	79.49**	6947.65*	0.16520**
Concentrations	4	26.204**	193.59**	6652.07ns	1.10429**
VAR	2	78.699 **	34.02**	4570.04*	1.3956**
T x C	8	4.746 **	1.387 *	7564.78*	0.0065ns
T x VAR	4	2.744ns	13.74**	7455.24*	0.02904*
C x VAR	8	4.826*	5.237**	7577.19*	0.0291ns
Error	104	1.595	4.76	7347.88	0.0268
Total	134	CV 23.42	CV 14.00	CV 68.84	CV 10.67

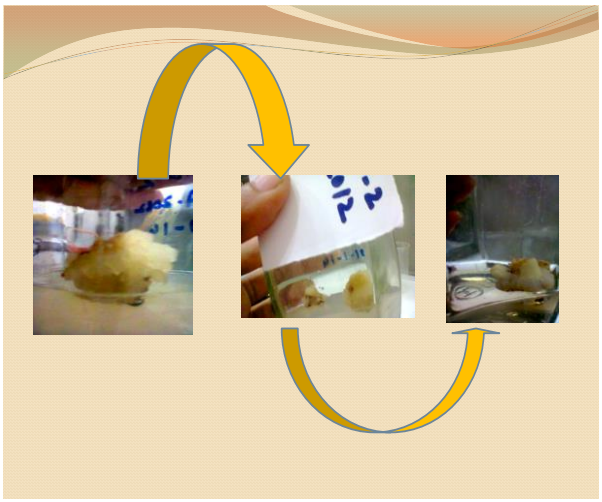


Fig-2: Steps included in callus formation of sugarcane.

Maximum number of tillers cm in case of 2, 4-D was observed in NIA-2012 soma lones 6.8667a followed by NIA-105 (6.667a) and minimum in Gulabi-95 parents 3.60d-e. Highest height of cane was found in NIA-2012 soma clones 180.2a and minimum in Gulabi-95 (115.0 c). Maxi-mum number of internode found in NIA-2012 soma clones 20.13a and Minimum in Gulabi- 95 parent 15.33b-d. Highest width of internode was obtainned in NIA-2012 soma clones 1.867a followed by NIA-105 (1.8253 a) and minimum in Gul-abi-95 (1.51c-d).

The comparison of parents and their clones in case of picloram maximum number of tillers (cm) was found in NIA-2012 soma clones 6.0667a-b followed by NIA-105 (5.6667a-b) and minimum in Gulabi-95 parents 3.2667e. Highest height of cane was obtained in NIA-2012 parents 119.96a and minimum in Gulabi-95 parent 114.26c. Maximum number of internode found in NIA-2012 soma clones 18.467b and minimum in NIA-105 parent 15.200 b-d. Highest width of internode was obtained in NIA-2012 soma clones 1.7713a-b followed by Gulabi – 95 soma c1ones 1.5933c-d and minimum in NIA-105 parent 1.3573e-f.

The data presented in the fig. 3 and 3a,3b and 3c revealed that maximum number of tillers (cm) in case of NAA was observed in NIA-105 parent 5.3333d-e) followed by NIA-2012 parent 5.266-7a-c) and minimum in Gulabi-95 parents (2.9333-e). Highest height of cane was found in NIA-2012 parent 180.22a and minimum in Gulabi-95 parent 114.30c. Maximum number of internode found in NIA-2012 soma clones 20.13a and minimum in Gulabi- 95 parent 15.333b-d. Highest width of internode was obtained in NIA-2012 soma clones

1.6793b-c followed by NIA-105 (1.3700e) and minimum in Gulabi-95 parent (1.3260f).

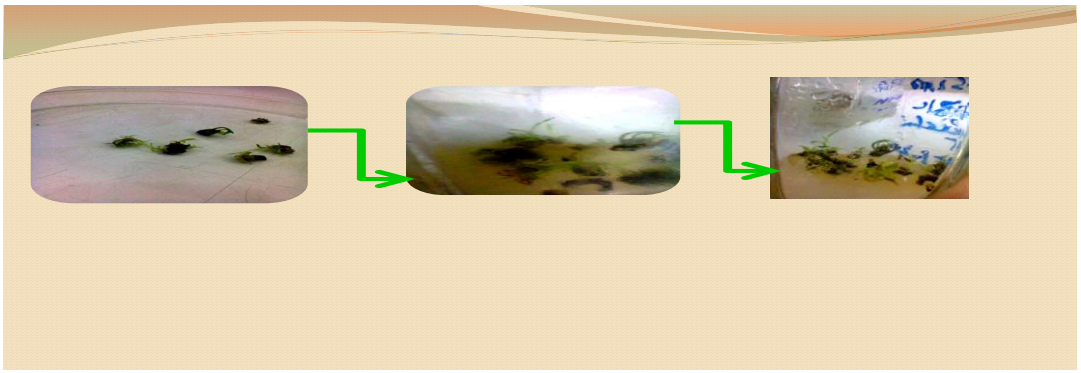


Fig- 3: Different stages included in the regeneration of sugarcane.

(A) (B) (C)

Fig. 3a,b,c: Different stages included in the regeneration of sugarcane.

DISCUSIONS

The evaluation of comparative performance of the selected clones through Randomized complete block design (RCBD) is given in Table (2A, B, C). All the concentration gave best results in same combination for the phenotypic characters of 405 soma clones of three different varieties of sugarcane. 135 clones were observed under the treatment of 2, 4-D for NIA- 2012 responded best as compare to the other growth hormones. Shahid *et al*. (2012) reported 3.mg/l of 2,4D important for inducing variability in the morphological character of sugarcane.

Results reflected that out of the 405 soma clones of genotype evaluated for genetic variation in sugarcane varieties through in-vitro culture techniques. 135 clones of NIA-105 observed in which highest result was found with additive concentration of picloram. Anbalogan *et al*. (2000) reported that some phenotypic variability was the result of physiological changes during in vitro conditions Bairu *et al*. (2011). Present result similar with the findings of Khan *et al*. (2009) and seema *et al.* (2013).

It is evaluated from the current results, Soma clones of Gulabi -95 assessed not more variability in the phenotypic and morphological character as compare to the other clones of varieties.2,4-D and picloram performed better than NAA. Baksha *et al.* (2002), Khatun *et al*. (2003) and Sabaz *et al.* (2008) reported lowest concentration of NAA was better than increasing concentration. Whereas present results reflected with the finding of feyissa *et al*. (2014).

Highly significant ($P \leq 0.005$) differences were observed for four studied characteristics in different parents / soma clones has been given in (Table a,b,c). The maximum number of tillers was observed in NIA-2012 soma clones when 2, 4-d applied. Hoy *et al.*, (2003) also observed smaller cane diameter and increased number of stalks in the plants regenerated from callus culture. However, the finding of Khan *et al.* (2009) and Raza *et al*. (2014) are similar to our findings.

It is optimized from the results number of tillers, height of cane, number of internode and width of internode enhanced and improved these quantitative characters with increase in dose of all

the auxins applied. Present results are similar with the finding of Nawaz *et al*. (2013).

It is understandable from the result that number of internode increases with increase in concentration of picloram applied. Whereas height of cane decreases with increases the concentration of picloram. All the treatment gave best results in 3.0mg/l for the number of internode. Present results are in agreement with the finding of Gaol et *al*. (2010) and Pandey *et al.* (2012). They found best result of at lower concentration of picloram (Shimelis *et al*., 2014).

It is clear from the result fig. 4 that width of internode depends upon the application of all the auxins applied. Present results are concerning with the finding of Khan *et al.* (2004), Roy *et al. (*2010) and Sughra *et al*. (2014).

Fig. 4: Field parameter of sugarcane crop.

Conclusion

Recently research-based work on the biotechnological methods through plant growth hormones, it is possible to deduce that, we have developed a rapid *in vitro* shoot multiplication method protocol for the three sugarcane varieties which can use to complement the conventional propagation method directly significance with sugar production characters of field.

REFERENCES

Ahmed A.O., Obeid A. and B. Dafallah, The influence of characters association on behavior of sugarcane genotypes (Saccharum spp) for cane yield and juice quality. World Journal of Agricultural Sciences 6(2): 207-211 (2010).

Anbalgan S., Kalman A. and M. Sakila, In vitro propagation of sugarcane: nature of callus, direct regeneration, regeneration through callus and morphological variations. Research on Crops 2: 13 8-140 (2000).

Arencibia A., Prieto D. and P. Tellez, Transgenic sugarcane plants resistant to stem borer attack. Molecular Breeding 3(4): 247-255 (1997).

Ali S., Iqbal J. and M.S. Khan, Genotype independent in vitro regeneration system in elite varieties of sugarcane. Pakistan Jornal. Botany. 42(6):3783-3790 (2010).

Alvi A.K., Iqbal J., Shah A.H. and Y.B. Pan, DNA based genetic variation for red rot resistance in sugarcane. Pakistan Journal Botany 40(4): 1419-1425 (2008).

Baksha R., Alam R., Karim M.Z., Mannan S.K., Podder B.P. and A.M.M. Rahman, Effect of Auxin, Sucrose and pH Level on in vitro Rooting of Callus Induced micro Shoots of Sugarcane (Saccharum officinarum). Journal of Biological Sciences 3(10): 915-920 (2003).

Bairu M.W., Aremu A.O. and. J.V. Staden, Somaclonal variation in plants: causes and detection methods. Plant Growth Regul 63:147–173 (2011).

Goel Y., Singh V.P., Lal M. and M.L. Shairma, In vitro morphoginesis in leaf sheath explants of sugarcane hibrid var. cos 99259 sugarcane. Technology 12(2): 172-175 (2010).

Feyissa T., Tariku G., Yoseph B. and W. Girma, Performance of Ethiopian Sugar Estate in Crop season 2008/9 to 2010. Agricultural Operations. Proceedings of Ethiopia Sugar Industry Second Biennial Conference, Adama, Ethiopia. September 23-24 (2010),

Hoy J.W., Bischoff K.P., Milligan S.B. and K.A. Gravois, Effect of tissue culture explant source on sugarcane yield components. Euphytica 129(2): 237-240 (2003).

Jahangir G., Nasir I. and M. Iqbal, Disease free and rapid mass production of sugarcane cultivars. Advance Life Science 1(3): 171-180 (2014).

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

Khan I.A., Effect of NPK fertilizers on the growth of sugarcane clone AEC86-347 developed at NIA, Tando Jam. Pakistan Journal. Botany 37: 355-360 (2005).

Khan I.A., Dahot M.U., Seema N., Yasmin S., Bibi S., Raza S. and A. Khatri, Genetic variability in plantlets derived from callus culture in sugarcane. Pakistan Journal of Botany 40 (2):547-564 (2008).

Khan I.A., Dahot M.U., Seema N., Yasmin S., Bibi S., Raza S. and A. Khatri, Variability in sugarcane plantlets developed through in vtiro

mutagenesis. Pakistan Journal of Botany 153-166 (2009).

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

Khan I.A., Raza G., Muhammad I., Raza S., Seema N. and Y. Shafquat, Assessment of contender sugarcane clones for morphologicl traits and biotic tolerance under Agro-climatic conditions of Tando Jam. Pakistan Journal. Botany 47(1): 43-48 (2015).

Mekonnen T., Diro M., Sharma M. and N. Tadesse, Protocol optimization for in vitro mass propagation of two sugarcane (*Saccharum officinarum L.*) clones grown in Ethiopia. Africa Journal of Biotechnology 13(12): 1358-1368 (201454).

Nawaz M., Ullah I., Iqbal N., Iqbal M.Z. and M.A. Javed, Improving in vitro regenration system of sugarcane with concurrent shoot/ root induction from somatic embryos. Turk Journal Biology (2013).

Pandey, R.N., Singh S.P., Rastogi J., Sharma M.L. and R.K. Singh, Early assessment of genetic fidelity in sugarcane (Saccharum officinarum) plantlets regenerated through direct organogenesis with RAPD and SSR markers. Austrelia Journal. Crop Science 6(4): 618-624 (2012).

Roy M., Hossain M., Biswas A., Islam R., Sarker S.R. and S. Akhter, Induction and evalution of somaclonal variation in sugarcane. Ph.D. thesis, University of Rajshahi, Rajshahi (2010).

Raza S., Qamarunnisa S., Jamil I., Naqvi B., Azhar A. and J.A. Qureshi, Screening of sugarcane soma-clones of variety BL4 for agronomic characteristics. Pak. Journal. Botany 46(4): 1531-1535 (2014).

Raza G.K. Ali, Mukhtar Z., Mansoor S., Arshad M. and S. Asad, The response of sugarcane (Saccharum officinarumL.) genotypes to callus induction, regeneration and different concentrations of the selective agent (Geneticin - 418). Afr. J. Biotechnol. 9: 8739-8747 (2010).

Seema G., Pande H.P., Lal J. and V.K. Madan, Plantlet regeneration of sugarcane varieties and transient GUS expression in calli by electroporation. Sugar Technology 3(1-2): 27-33 (2011).

Shahid M.R., Suhail A., Arif M.J., Gogi M.D., Shahzad M.D. and S. Hussain, Effectiveness of Trichogramma chilonis (ishii) (Hymenoptera: Trichogrammatidae) against sugarcane stem borer (Chilo infuscatellusSnellen) (Lepidoptera: Pyralidae). Pakistan Entomol. 29 (2): 141-146 (2007).

Shahid M.R., Khan F.A., Saeed A., Aslam M. and F. Rasul, Development of somaclones in sugarcane genotype BF-162 and assessment of variability by random amplified polymerphic DNA (RAPD) and simple sequence repeats (SSR) markers in selected red rot resistant somaclones. African J. of Biotecnology 11 (15): 3502-3513 (2012).

Mangrio, G.S., Simiar Altaf, R. Rafique and M.U. Dahot, *In vitro* regenerability of different sugarcane (*saccharum officinarum l.*) Varieties through shoot tip culture. Pak. J. Biotechnol. 11(1): 13-23 (2014).

Shimelis D., Bantte K. and T. Feyissa, Interaction effects of 6-benzylaminopurine and kinetin on in vitro shoot multiplication of two sugarcane (*Saccharum officinarum L.*) genotypes. Advance Crop Science and Technology 2 (143): 1-5 (2014).

Tolera B., Diro M. and D. Belew, Response of sugarcane (*Saccharum officinarum L.*) Varieties to BAP and kinetin on in vitro shoot multiplication. International Journal of Innovative Research and Development 3(5): 694-697 (2014).