ANALYSIS VOL. 5, 2019

ISSN 2347–3819 EISSN 2347–386X

DISCOVERY Agriculture

Different somaclones (r_1) compared to their sources varieties

Kuasha Mahmud[1], **Nasiruddin KM**[2], **Hossain MA**[3], **Hassan L**[4]

[1]PSO & Head, Biotechnology Division, Bangladesh Sugarcane Research Institute, Ishurdi, Pabna, Bangladesh
[2]Prof. Dept. of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh
[3]Director (Research), Bangladesh Sugarcane Research Institute, Ishurdi, Pabna, Bangladesh
[4]Prof. Dept. of Genetics & Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh

Article History
Received: 22 May 2019
Accepted: 04 July 2019
Published: July 2019

Citation
Kuasha Mahmud, Nasiruddin KM, Hossain MA, Hassan L. Different somaclones (r_1) compared to their sources varieties. *Discovery Agriculture*, 2019, 5, 153-160

ABSTRACT

The experiment was conducted in Bangladesh Sugar crop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh to find out performances of somaclones settlings compared to their sources varieties. Four varieties namely Isd 37, Isd 38, Isd 39, Isd 40 and their 20 somaclone's setts were planted in soil bed with three replication including 10 setts (two eyed) per replication on October, 2012 and then transplanted in the field February 2013. Somaclones were produced from different sugarcane varieties through callus culture using 2, 4-D. Data were recorded on eye bud germination %, number of green leaf per plant and height (cm) per plant at one month after planting of sugarcane setts in the soil bed. Besides, brix%, purity% and also pol % cane were observed at twelve months after planting in the field. The highest eye bud germination % (100) were obtained from CC-37-74, CC-37-81, CC-37-83 and also CC-37-86 somaclones of Isd 37 variety followed by CC-37-10, CC-37-51, CC-38-26 and CC-40-07 somaclones with 95% while the lowest from Isd 39 variety among 20 genotypes. Eye bud germination % of each variety was found the lowest than their somaclones. The highest number of green leaf per plant (7) was produced from CC-37-28 somaclone followed by CC-37-10 (6) while the lowest (3) from Isd 38, Isd 39 and as well as Isd 40. The highest height per plant (74 cm) was recorded from CC-38-10 followed

by CC-39-07 (67 cm). On the other hand, the lowest (20 cm) height per plant was produced from Isd 39 variety among 20 genotypes including 4 varieties. Somaclone CC-38-08, variety Isd 37 and somaclone CC-38-2 showed the highest brix% (22.6), purity% (89.29) and Pol% (15.44) respectively while the lowest brix% (16.8), purity% (84.21) and pol% (8.33) were produced from somaclone CC-37-28 among all genotypes.

Keywords: Callus, Sugarcane, Somaclones and Settlings

1. INTRODUCTION

The geographical origin of modern cultivated sugarcane (*Saccharum officinarum*) is New Guinea and distributed throughout the tropics and sub-tropics are generally agreed. Sugarcane is important for sugar, gur and by products such as energy, chemicals, and single cell protein, ethanol, bio-gas, fertilizer, fibre board and paper, polishers, cosmetics and candles. All varieties of sugarcane are species or hybrids of the genus saccharum of the family Gramineae. The genus saccharum contains three cultivated (*Saccharum officinarum, Saccharum sinensis and Saccharum barbari* L) and two wild (*Saccharum robustum* and *Saccharum spontanium*) species (Kochhar, 1998). Sugarcane is globally the main source of raw material for the production of sugar. It is a principal cash crop in North – Western and south western low rainfall belt of the country and the main raw material for sugar and gur industries. Although many countries are producers, only six of them account for 65% of the world's entire sugarcane production. Among these Brazil is the largest one (Viera, 2002). The large barreled high sucrose containing original cane of *Saccharum officinarum* is thought to have originated from the wild species *Saccharum robustum* which is medium thick and low in sugar contend (Brandes *et al*., 1939). The chemical composition of a matured, sound and normal sugarcane stalk of the species *Saccharum officinarum* are water 74.96%, sugar 13.40%, fibre 10.04%, ash 0.64%, N_2 bodies 0.58%, fat and wax 0.38%. Sugarcane is propagated vegetatively for commercial planting by stem cuttings. Production of disease-free large number seedlings during the planting season is laborious and time consuming. Development of more efficient methods for large-scale production of pathogen free planting material would contribute significantly to the overall productivity of the sugar industry. Tissue culture offers an opportunity to mass produce disease free planting material and is now used to supplement commercial sugarcane propagation in many countries in cluding Brazil, the United States, India and Cuba (Lakshmanan *et al.,* 2006). Both average cane yield (45t ha^{-1}) and recovery percentage of sugar (around 8.3 to 8.5%) are fur below as compared to those of the advance sugarcane growing countries. Besides, sugarcane is the greatest source of gur. It is very urgent to increase cane productivity without further area expansion to meet the future need of sugar and gur. Furthermore, it takes several years of painstaking labour to produce a pure line of sugarcane by selfing due to its high polyploidy and extreme heterozygosity (Narayana Swamy, 1994). Plant tissue culture is the most commercially successful aspect of plant biotechnology, which has introduced an exciting new phase into plant propagation and breeding (Roy Kabir, 2007). Plant regenerated from tissue and cell culture show heritable variation for both qualitative and quantitative trait. Variants are obtained in homozygous condition from the cell in-vitro (R_0 generation), but most variants are recovered in the second generation of the tissue culture regenerated plants (R_1 generation). Somaclonals variation reduces the time for releasing new variety by at least two years as compared to mutation breeding by three years in compared to back cross method of gene transfer. Somaclonal variation had produced desirable agronomic changes in the progeny for example eye spot resistant and increase in sugar yield in sugarcane (Evans *et al*., 1984). In case of sugarcane, tissue culture induces considerable phenotypic variability (Burner and Grishan, 1995). Callus masses from different explants sources were reported by many scientists like Laurens *et al*. (1987), Chen *et al*., (1998), Shaheen and Mirza *et al*., (1989) and Sajid-ur-Rahman *et al*., (2002). Use of different growth regulators in culture medium was reported by Bhansali and Kishan (1982). Many scientists have reported organogenesis and differentiation of shoot from callus culture (Shaheen and Mirza, 1989 and Sajid-ur-Rahman *et al*., (2002). Plant tissue culture is considered as a powerful tool for crop improvement within limited time period. For the purpose development of regeneration protocols is prerequisite. In-vitro regeneration of sugarcane has been reported by Barbara and Nickell (1969), Heinz and Mee (1969), Heinz *et al*. (1965) and Barbara *et al*. (1977). Leaf tissue of modern hybrids of sugarcane clones is usually more responsive to tissue culture than those of traditional varieties or wild relatives (Chen *et al*., 1998; Taylor *et al*., 1997). Hence tissue culture research using modern sugarcane varieties of Bangladesh deserves due attention. The sugarcane breeding programme has been under serious problem due to lack of suitable multiplication procedure (Lal and Sing, 1994). Conventional breeding method usually required 10-15 years of work to complete a section cycle for release of a variety and an important variety can be planted commercially several years later when enough seed canes will have been produced. Time required and continued contaminations by systemic diseases are serious problem to multiply a-elite genotype of sugarcane in the open field (Lal and Sing, 1994). The technique of plant tissue culture is being routinely used for producing large number of clonal plants by in-vitro culture of explants from wide range of species throughout the world. It has become now a viable alternative

to the conventional breeding and the clonal propagation methods. Due to increased demand of sugar and gur for local consumption, sugarcane is being cultivated years together without adopting modern technologies. To meet the future requirement of sugar it is essential to develop some improved varieties by tissue culture. Although, sugarcane is one of the most important industrial crops, very limited effort have been made on tissue culture and in-vitro propagation for variety development and multiplication of Bangladesh. Therefore, this experiment was conducted to find out plant regeneration, as well as performance of somaclones (R_1) settlings compared to their sources varieties.

2. MATERIALS AND METHOD

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh to obtain in-vitro plant regeneration as well as performance of somaclone (R_1) settlings compared to parents. The explants materials were used from the young leaf sheath of BSRI released Isd 37, Isd 38, Isd 39 and Isd 40 varieties from 8-10 months old field grown sugarcane of BSRI experimental field. At first MS medium with green coconut water (10%) containing 3 mg/l concentration of 2, 4-D was prepared for callus induction. After five weeks of explantation, the calli were inoculated for shooting on MS medium supplemented with 3 mg/l concentration of BAP and maintained by sub culturing every two weeks and then regenerated shoots were inoculated for rooting by sub culturing every two weeks on MS medium supplemented with 5 mg/l NAA. Rooted plantlets were acclimatized and transplanted to polybag and then field respectively.

Mercuric Chloride ($HgCl_2$) was used as sterilizing agent while savlon was used as antiseptic, detergent and surfactant. The Explants were taken in a beaker and treated with 3% (w/v) savlon for 5-6 minutes with constant shaking and washed thoroughly with distilled water for 3-4 minutes. The explants were transferred in autoclaved conical flask (500 ml) treated with 1% mercuric ($HgCl_2$) for 10 minutes and washed by 3-4 times rinsing with sterile distilled water to remove traces of $HgCl_2$ from outer surface of leaf sheath segments. Explants (approximately 1 cm x 0.5 cm) were prepared in laminar air flow cabinet from sterilized leaf sheath segments and cultured on MS medium with green coconut water containing 3 mg/l concentration of 2, 4-D. Mediums were adjusted to pH (5.7). Agar (0.6%) was added with medium. Media was sterilized by autoclaving at 1.2 Kg cm^{-2} pressure at 121° C for 30 minutes. Cultures were incubated at 25±2°C and kept 16h under fluorescent tube light. Plantlets were produced from explants of different varieties of sugarcane via callus, shoot and also then root regeneration. Polybeg seedlings (R_0) which derived from callus culture were produced after hardening. Polybeg seedlings of somaclones and their parents (two eyed setts) were planted in the field. Somaclones (R_1) and their parents (two eyed setts) were planted in the soil bed with three replications having ten setts per replication and then data were collected at one month after planting. Different types of soil bed settlings (R_1) of genotypes including varieties were planted at four months after planting of setts in the soil bed. Data on brix%, purity% and pol% were taken from 10 matured canes at twelve months after planting of settlings (R_1) in the field.

3. RESULTS

Explants from different varieties were inoculated on MS medium with green coconut water (10%) supplemented with 2, 4-D 3 mg/l and different types of callus were produced (Figure 1).

Different types of callus from different varieties were inoculated on MS medium supplemented with BAP 2 mg/l + Kn 1 mg/l. The different types of shoots were regenerated (Figure 2). Besides, regenerated shoots were inoculated on MS medium containing NAA 5 mg/l and different types of roots were regenerated (Figure 3). This finding supported the results of Ali *et al.*, (2010); Gill *et al.*, (2002); Singh *et al.*, (2001) and Punia *et al.*, (2001). Regenerated plantlets (Figure 4) of callus culture produced R_0 somaclones (Figure 5) in the field.

Figure 1 Callus induced under concentration of 3 mg/l 2, 4–D with MS media

Figure 2 Shoots produced under concentration of 3 mg/l BAP with MS media

Figure 3 Roots produced under concentration of 5 mg/l NAA

Figure 4 Plantlets

Figure 5 Somaclone in the field

Different types of settlings (R_1) were produced from four varieties namely Isd 37, Isd 38, Isd 39, Isd 40 and their 16 somaclone's setts (two eyed) in soil bed (figure 6) and R_1 somaclones were developed in the field (figure 7). Data were recorded on eye bud germination %, number of green leaf per plant and height (cm) per plant at one month after planting of sugarcane setts in the soil bed. The highest eye bud germination % (100) were obtained from CC-37-74, CC-37-81, CC-37-83 and also CC-37-86 somaclones of Isd 37 variety followed by CC-37-10, CC-37-51, CC-38-26 and CC-40-07 somaclones with 95% while the lowest from Isd 39 variety among 20 genotypes (Table 1). Further more, somaclone CC-38-08, variety Isd 37 and somaclone CC-38-2 showed the highest brix% (22.6), purity% (89.29) and Pol% (15.44) respectively while the lowest brix% (16.8), purity% (84.21) and pol% (8.33) were produced from somaclone CC-37-28 among all genotypes (Table 2).

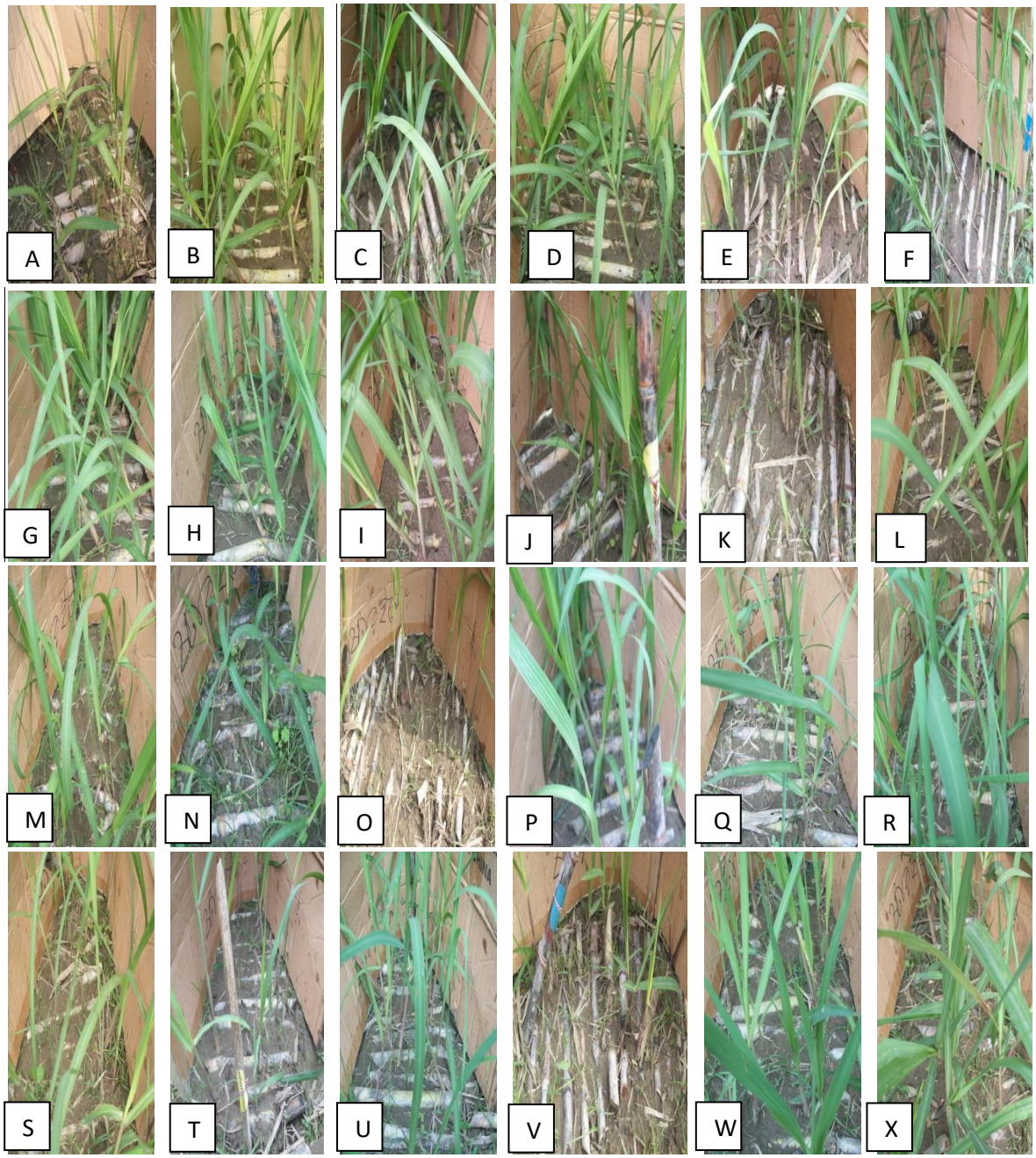

Figure 6 Different somaclones (R_1) settlings (Soil bed) and their parents: A) Isd 37, B to J) somaclones from Isd 37, K) Isd 38, L to N) Somaclones from Isd 38, O) Isd 39, P to U) Somaclones from Isd Isd 39, V) Isd 40 and W to X) Somaclones from Isd 40

Table 1 Performance of somaclones (R_1) settlings compared to parent varieties at one month after planting in soil bed

Variety/ Somaclone	Number of sett (two eyed bud)	Eye bud germination (%)	Number of green leaf per plant	height (cm) per plant
Isd 37	10	75	4	32
CC-37-10	10	95	6	64
CC-37-12	10	90	5	43
CC-37-28	10	75	7	64
CC-37-51	10	95	5	55
CC-37-65	10	90	5	58
CC-37-74	10	100	5	48
CC-37-81	10	100	4	46
CC-37-83	10	100	4	52
CC-37-86	10	100	5	61
Isd 38	10	60	3	20
CC-38-02	10	90	4	61
CC-38-08	10	80	4	34
CC-38-10	10	70	4	74
Isd 39	10	50	3	20
CC-39-01	10	65	4	33
CC-39-05	10	90	5	50
CC-39-07	10	70	5	67
CC-39-12	10	60	5	57
CC-39-25	10	90	5	64
CC-39-26	10	95	5	65
Isd 40	10	65	3	28
CC-40-07	10	95	5	65
CC-40-08	10	85	5	60

Table 2 Performance of Brix%, purity and pol% cane somaclones (R_1) settlings compared to parent varieties at one month after planting in soil bed

Variety/Somaclone	Brix%	Purity	Pol%
Isd 37	21.0	89.29	14.63
CC-37-10	20.4	87.19	13.87
CC-37-12	20.2	87.89	14.03
CC-37-28	16.8	84.21	11.04
CC-37-51	19.8	89.08	13.76
CC-37-65	18.5	84.59	12.36
CC-37-74	20.4	87.19	13.87
CC-37-81	18.6	85.27	12.37
CC-37-83	18.8	87.51	13.00
CC-37-86	18.6	85.92	12.47
Isd 38	21.5	88.37	14.82
CC-38-02	22.5	86.84	15.44
CC-38-08	22.6	86.63	15.27
CC-38-10	22.0	88.78	15.24
Isd 39	21.5	88.59	15.05
CC-39-01	21.5	88.14	14.78

CC-39-05	21.5	88.25	14.80
CC-39-07	20.0	87.88	13.88
CC-39-12	21.7	88.69	15.01
CC-39-25	20.2	87.17	13.74
CC-39-26	21.5	88.62	15.05
Isd 40	21.2	89.28	14.76
CC-40-07	21.5	88.14	14.78
CC-40-08	21.2	89.28	14.95

Figure 7 R_1 somaclones were developed in the field

4. DISCUSSION

Explants from different varieties were inoculated on callusing media (MS + green coconut water (10%) + 2, 4-D 3 mg/l) and different types of callus were produced. The finding of callusing is in agreement with the results of Ali *et al.*, (2008); Alam *et al.*, (2003); Karim *et al.*, (2002); Hossain *et al.*, (1996) and Gopitha *et al.*, (2010). Different types of callus from different varieties were inoculated on shooting media (2 mg/l BAP + 1 mg/l Kn) and then regenerated shoots were inoculated on rooting media (MS + NAA 5 mg/l). This finding supported the results of Ali *et al.*, (2010); Gill *et al.*, (2002); Singh *et al.*, (2001) and Punia *et al.*, (2001). The highest eye bud germination % (100) were obtained from CC-37-74, CC-37-81, CC-37-83 and also CC-37-86 somaclones of Isd 37 variety compared to other somaclones of Isd 38, Isd 39 and Isd 40. Eye bud germination % of each variety was found the lowest than their somaclones. Somaclone CC-38-08, variety Isd 37 and somaclone CC-38-2 showed the better performance brix% (22.6), purity% (89.29) and Pol% (15.44). The highest number of green leaf per plant (7) was produced from CC-11(37)-28 somaclone followed by CC-37-10 (6) while the lowest (3) from Isd 38, Isd 39 and as well as Isd 40. The highest height per plant (74 cm) was recorded from CC-11(38)-10 followed by CC-39-07. On the other hand, the lowest (20 cm) height per plant was produced from Isd 39 variety among 20 genotypes.

5. CONCLUSION

Different somaclones can be showed better performance compared to mother plant regarding germination, eye bud, brix%, Purity%, pol% and height etc. However, some traits of somaclones may be negative or positive compared to mother plant. Besides, traits of somaclones depend on the sources of variety. Some somaclone such as CC-37-74, CC-38-2 and CC-38-08 could be used for crop improvement as well as further research.

Funding: This study has not received any external funding.

Conflict of Interest: The authors declare that there are no conflicts of interests.

Peer-review: External peer-review was done through double-blind method.

Data and materials availability: All data associated with this study are present in the paper.

REFERENCE

1. Alam, R.; Mannan, SK.; Karim, Z. and Amin, M. N. 2003. Regeneration of sugarcane (*Saccharum officinarum*) plantlet for callus. Pak. Sugar J. 18(1): 15-19.
2. Ali, A.; Naz, S.; Siddique, F. A. and Iqbal, J. 2008. Rapid clonal multiplication of sugarcane (*Saccharum officinarum*) through callogenesis and organogenesis. Pak. J. Bot. 40(1): 123-138.
3. Ali, S.; Iqbal, J. and Khan, M. S. 2010. Genotype independent in vitro regeneration system in elite varieties of sugarcane. Pakistan Journal of Botany 42(6): 3783-3790.
4. Barba, R. and Nickell, L. G. 1969. Nutrition and organ differentiation in tissue culture of sugarcane-a monocotyledon. Planta, 89: 299-302.
5. Barba, R. C.; Zamora, A. B.; Mallion and Linga, C. K. 1977. Sugarcane tissue culture research. Plant physiol., 2: pp. 1843-1863.
6. Bhansali, R. R. Kishan, S. 1982. Callus and shoot formation from leaf of sugarcane in tissue culture. Phytomorphology, p. 167-170.
7. Brandes, E. W.; Sartoris, G. B. Grassl, Co. 1939. Assembling and evaluating wild forms of sugarcane and closely related plants. Pro. Int. Soc. Sugarcane Tech., p. 1938.
8. Burner, D. M. and Grishan, M. P. 1995. Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. Crop Science, 35: pp. 875-880.
9. Chen, W. H.; Davey, M. R.; Power, J. B. and Cocking, E. C. 1998. Control and maintenance of plant regeneration in sugarcane callus culture. J. Expt. Bot., 39: pp. 251-261.
10. Evans, D. A.; Sharp, W. R. and Medmi-Filho, H. P. 1984. Somaclonal and gametoclonal variation. American Journal of Botany, 71: 759-774.
11. Gill, N. K.; Rahman, G. and Gosal, S.S. 2002. Somatic embryogenesis and plant regeneration in some commercial cultivers of sugarcane. Crop Improvement, 29(1): 28-34.
12. Heinz, D. J. and Mee, G. W. P. 1969. Plant differentiation from callus tissue of *saccharum* spp. Crop Science, 9: 346-348.
13. Hossain, M. A.; Begum, S.; Miah, M. A. S. Uddin, M. J. and Miah, A. J. 1996. Plant Tissue Culture. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, pp. 76-79.
14. Karim, M. Z.; Amin, M. N.; Hossain, M. A.; Islam, S.; Hossain, F. and Alam, R. 2002. Micropropagation of two sugarcane (*Saccharum officinarum*) varieties from callus culture. On line J.Biological Sci. 2(10): 682-685.
15. Kochhar, S. L. 1998. Economic Botany in the tropics. Second Edition Macmillan. India Ltd., pp. 1-476.
16. Gopitha, K.; Bhavani, A. L. and Santhilmanickam J. 2010. Effects of the different auxins and citokininsin callus induction, shoot, root regeneration in sugarcane. International Journal of Pharma and Bio Science 1(3): 1-7.
17. Lal, N. and Singh H. N. 1994. Rapid clonal multiplication of sugarcane through tissue culture. Plant Tissue Cult., 4(1): 1-7.
18. Lakshmanan P.; Geijskes R. J.; Wang L.; Elliott A.; Grof C. P. L.; Berding N. and Smith G. R. 2006. Development and hormonal regulation of direct shoot organogenesis and somatic embryogenesis in sugarcane (*Saccharum* spp. Interspecific hybrids) leaf culture. Plant Cell Rep. 25: 1007-1015.
19. Laurens, A. G. and Martin, F. A. 1987. Evaluation of *In vitro* propagated sugarcane hybrids for somaclonal variation. Crop Science, 27: 793-796.
20. Narayna S. 1994. Cereal and grass tissue culture. In plants Cell and Tissue Culture, Maheshwari, P. and Rangaawamy, N. S. (eds). Academic Press. Pp. 173-322.
21. Punia, A.; Navinder, S.; Jain, R. K.; Chowdhury, V. K. and Sehrawat, A. R. 2001. Micropropagation in sugarcane. Current Research, University of Agricultural Sciencies, Bangalore, 30(3/4): 40-42.
22. Roy, P. K. and Kabir, M. H. 2007. In vitro mass propagation of sugarcane (*Saccharum officinarum* L.) var. Isd 32 through shoot tips aqnd folded leaves culture. Biotechnology 6(4): 588-592.
23. Sajid-ur-Rahman, M. T. H., Shahid, M. Hossain, Tanveer, M. K. and Javed, M. A. 2002. Genotype effect on callogenesis in sugarcane. Pak. Sugar J., XVII(6): 13-20.
24. Shaheen, M. S. and Mirza, M. S. 1989. *In vitro* production of plants from sugarcane tissue. J. Agric. Ress., 26(6): 302-312.
25. Singh, B.; Yadav, G. C. and Lal, M. 2001. An efficient protocol for micropropagation of sugarcane using shoot tip explants. Sugar Tech. 3(3): 113-116.
26. Taylor, P. W.J. 1997. Micropropagation of sugarcane (*Saccharum spp*. Hybrid). Biotechnology in Agriculture and Forestry, 39: pp. 256-268.
27. Viera, 2002. Genetic stability of sugarcane plants derived from meristem culture. Genetic and Molecular Biology, 235(1): 91-96.