Indian Res. J. Genet. & Biotech 7(3) : 329 – 333 (2015)

Studies On Effect Of Bap On Regeneration Of Sugarcane From Meristem Tip And Eye Bud

Yogendra Singh

Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482004.

Received: May 2015; Revised: June 2015; Accepted: July 2015)

Abstract

Sugarcane belongs to the grass family (Poaceae), an economically important seed plant family that includes maize, wheat, rice, and sorghum and many forage crops. The main product of sugarcane is sucrose, which accumulates in the stalk internodes. Sugarcane is one of the most efficient converters of solar energy into sugars and other renewable forms of energy. Micropropagation is a methodology through which plants are multiplied rapidly by aseptic culture of meristematic regions under controlled nutritional and environmental conditions. When disease-free material is used as the source of explant or the explants are heat-treated to eliminate diseases, the resultant micropropagated plants are disease free and healthy. Micropropagation also helps in the rapid seed multiplication of newly released varieties which is of particular importance in sugarcane where, as mentioned earlier, the normal seed multiplication rate is very low. In present investigation role of BAP growth regulator in direct regeneration of sugarcane was studied. For this different concentration (mg/ml) of BAP were used for regeneration of sugarcane plant from meristem tip and eye-bud. Among different concentrations and combinations for shoot multiplication, best performance was showed on MS medium supplemented with BAP (3mg/l) followed by MS medium supplemented with BAP (4mg/l).For initiation BAP (4mg/l) showed best performance. Rooting was best in MS basal medium.

Keywords: Sugarcane, meristem tip, eye-bud, BAP, basal medium, multiplication

Introduction:

The sugarcane crop stands out in the world mainly because it is a renewable energy source for producing ethanol and contributing to produce 65% of world sugar. Sugarcane is cultured in tropical and subtropical regions of more than 70 countries. The plant was domesticated by the Polynesians **(Brandes, 1958)** for its sweet stem, but presently it has emerged as a multipurpose crop providing not only sugar but also a series of value added products such as paper, ethanol and other alcohol derived chemicals, animal feed, antibiotics, particle board, bio-fertilizer and raw material for generating electricity. Global sugar consumption has been increasing at a steady rate of 2 per cent per annum **(http://www.illovo.co.za/worldofsugar/internationalSugarStats.htm).** Besides sugar, almost all of the consumer sweeteners are exclusively produced from sugarcane.

Although sugar beet can a substitute to sugarcane for white sugar production but carbohydrates of sugarcane are sweeter in taste than those extracted from sugar beet; and bowing to the technical limitations and huge energy inputs this crop is not popular **(Vaccari et al, 2005).** Furthermore, sugarcane is also demanded as raw material for a variety of industries like ethanol from cane molasses, alcohol, bagasse, and chip boards **(Nguyen et al, 2010).** In **2012,** FAO estimates it was cultivated on about 26.0 million hectares, in more than 90 countries, with a worldwide harvest of 1.83 billion tons. Brazil was the largest producer of sugar cane in the world. The next five major producers, in decreasing amounts of production, were India, China, Thailand, Pakistan and Mexico. Brazil led the world in sugarcane production in 2013 with a 739 267 TMT harvest India was the second largest producer with 341 200 TMT tons, and China the third

Corresponding authors- e-mail: yogendrasinghbt@gmail.com.
Published by the Indian Society of Genetics, Biotechnology Research and Development, Biotech Bhawan 5 E Nikhil state, DPS Road, Shastripuram, Agra 282007, Online management by www.isgbrd.co.in

largest producer with 125 536 TMT tons harvest. The average worldwide yield of sugarcane crops in 2013 was 70.77 tons per hectare. The most productive farms in the world were in Peru with a nationwide average sugarcane crop yield of 133.71 tons per hectare.

In vitro multiplication of sugarcane has received considerable research attention because of its economic importance as a cash crop. Micro propagation is currently the only realistic means of achieving rapid, large-scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in Sugarcane. **(Feldmenn et.al. (1994, Lal and Krishna, 1994, Lorenzo *et. al.* 2001).** Micro propagation is a part of plant tissue culture. Basically it is the branch of biotechnology, which is used to clone plants at a very high speed without the restriction of the season **(Bhojwani and Razdan (1983).** Plant tissue culture research is multi-dimensional and it has direct commercial applications as well as value in basic research into cell biology, genetics and biochemistry. Instead of being a tool for rapid propagation, tissue culture became an alternating tool of breeding by selecting and testing for useful variants **(Heinz *et. al.*, 1971, Babra *et. al.* 1978).** Crop production through micropropagation also eliminates the possibility of any interruption in the growing season because it can be carried out inside the carefully regulated environment of a greenhouse. Because the chemical and physical environment inside a greenhouse can be closely monitored, any lull in production that might typically occur as a result of seasonal change can be avoided. **(Lineberge , 2002).** The main objectives of this study were role of BAP in shoot initiation and multiplication for developing disease free plants using micropropagation technique.

Materials and Methods:

The present study was conducted in the Plant Tissue Culture laboratory, Biotechnology Centre, JNKVV, Jabalpur, Madhya Pradesh (India). MS media (**Murashige and Skoog (1962**) fortified with different types of plant hormones in varying concentration was used for this study. For convenience and to reduce time taken for weighing individual ingredients each time, concentrated stock solution of selected components of the medium were prepared and stored in a refrigerator. At the time of media preparation they were brought to room temperature and mixed proportionately to get the desired the medium. At time of working 1 litre of MS medium was prepared by adding the stock solution in manner Macro nutrients 25ml, Micro nutrients 5ml, Iron stock 5ml , Vitamin stock 1ml , Amino acid 1ml, Myoinsitol 100mg, Sucrose 30gm, Growth regulators as a required and Agar 8gm. Above component were added one by one in 800ml of tissue culture grade distilled water and mixed properly. The pH of medium was adjusted to 5.8 with addition of 1N HCL or 1N NaOH. Then the final volume of media made up to 1L and gelled with adding 8gm agar powder. The medium was heated to melt agar. The molten agar medium was poured into the culture bottles and covered with autoclavable plastic caps which were then autoclaved at 120 degree c and 15 psi for 25 to 30 min. sterilized medium was allowed to cool at room temperature.

For this study meristem tip and eye bud of sugarcane were obtained from green house of Biotechnology Centre. Explants were obtained from sugarcanes plants grown in a greenhouse at ambient temperatures under natural lighting, supplemented when necessary to a 14h photoperiod with illumination from PAR lamps. Healthy fresh and vigorously grown plants were selected for experimentation. Meristem tip and eye bud were taken in a glass beaker and washed under running tap water. Then were treated with few drops twenty for 20 min. Again Meristem tip and eye bud were washed with running tap water and with distilled water for 8 to 10 times until traces and foam of tween twenty disappeared. Explants, immediately after excision were inoculated in pre sterilized glass bottles containing about 40ml agar gelled medium fortified with different type of growth regulators for different types of growth requirement. For the purpose of shoot regeneration from apical meristem and multiple shoot formation, various concentrations of BAP were used. For rhizogenesis, the plantlets were transferred to rooting media on achieving the maximum number of multiple shoots, which comprised of MS media and different concentrations of IAA. Full strength MS medium without any hormonal supplementation (MS basal) was also tested for the purpose.

Results and Discussion:

Besides, availability of disease and pest-free, true to type planting material is an important prerequisite for achieving the desired yield improvement. Sugarcane, being a vegetatively propagated crop, has a low 1:6 to 1:8 seed multiplication rate. Hence, non-availability of quality seed material is one of the major problems faced by farmers in developing countries. Further, the bulky cane cuttings used for planting as seed harbor many pests and diseases thereby decreasing cane yield and quality drastically. Accumulation of diseases over vegetative cycles leads to further yield and quality decline over the years. In fact, poor quality seed is a major constraint in sugarcane production.

In present study Shoot formation was highly influenced by concentrations and type of the growth regulators used in the experiment. Among different concentrations and combinations for shoot multiplication, best performance was showed on MS medium supplemented with BAP (3mg/l) followed by MS medium supplemented with BAP (4mg/l) **(Fig 01).** The primary aim to present study was to recover somaclonal variants from short-term (4-6 months) in vitro culture (direct shoot induction from meristem tip and leaf roll) of sugarcane. In present investigation, direct shoot induction from meristem tip and Eye bud were accomplished. Such shoot grown through in vitro culture were compared with parent plant. Shoot tip explants gave higher response to initiation and multiplication than eye bud explants. Highest regeneration and multiplication of shoots from shoot tip was found on MS medium supplemented with BAP 3 mg/l followed by BAP 4 mg. For direct shoot induction the meristem tips were transferred to the medium containing 3mg/l and 4mg/l BAP in different concentration. In this type of medium the secretion of phenolics quantity is directly proportional to shoot proliferation and elongation.

BAP has been extensively used by the investigators, in a similar range of concentrations as reported in the presented study, to study the regeneration response of apical meristem of *Sacchrum officinarum* and have been reported as effective choice. They found 1.5mg/l BAP best responsive in CP 77,400 and the combination of 0.5 mg/l BAP with 0.25 mg/l Kinetin in BL-4 for shoot formation from apical meristems of different sizes as explant. Some other researchers also used four levels of BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and reported its promotive effect on the shoot regenerated from apical meristem of sugarcane cultivars (Co-86032, Co-740 and Co-8014) and found the highest number of leaves on the main shoot on the 1.0 mg/l BAP (**Pawar et al 2002). Hegde and Kuruvinashetty (2010)** also used BAP to study the micropropagation of sugarcane**. Goasal *et. al.*, 1998,** reported the effect of size of meristem on shoot regeneration through apical meristem in sugarcane varieties Co.J.64, Co.J.83 and Co.P.84-211.**Baksha *et. al.* (2002)** established plant regeneration protocol through shoot tip culture in sugarcane using variety Isd-28. They obtained multiple shoot from shoot tip explants by supplementing MS salts with BAP (2.0mg/l), kinetin (1.0mg/l) and IBA (0.5mg/l) and roots in NAA (5mg/l) with half MS medium. The similar results have been reported by various workers **(Khan *et. el.* 2008, Behera and Sahoo, 2009, Ali *et. al.* 2012, Srivastava et al 2014 , Bhuria et al 2014 .**

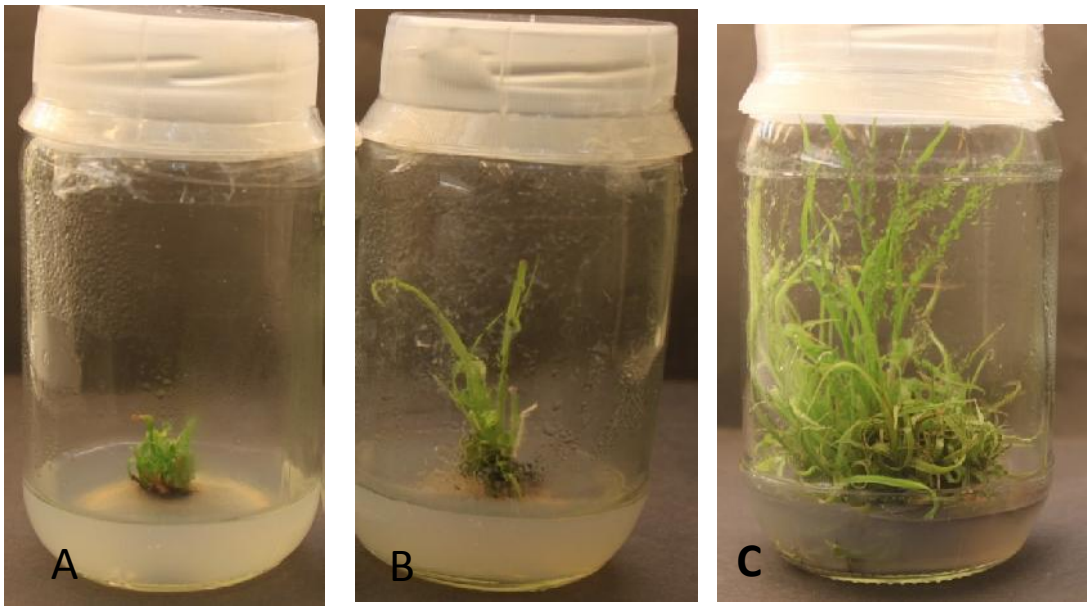

Fig.01: Micropropagation of Sugarcane A,B: Initiation, C: Multiplication D: Developed multiple shoots

Micropropagation is not only feasible but it can be used as the helpful tool for rapid multiplication of disease free, high yielding and premium quality planting material of highly adapted, genetically stable and newly released varieties of sugarcane**.(Ali et al 2008).** Lack of rapid multiplication has been a serious problem in Sugarcane breeding (Ali and Afghan (2001). Micropropagation can also be used for production of disease/pathogen free stock material (widely used in Horticulture industry) - as thorough meristem culture. Apart from these conventional benefits, research on various aspects has widened the scope of Plant tissue culture. The technique of plant tissue culture may play a key role in the "Second Green Revolution'' in which biotechnology and gene modification are being used to improve crop yield and quality.

References:

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

2. **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.

3. **Baksha R.; Alam R.; Karim M.Z.; Paul S.K.; Hossain M.A.; Miah M.A.S and Rahman A.B.M.M.** (2002) *In vitro* shoot tip culture of sugarcane (*Saccharum officinarum*) variety LSD28. *Biotechnology*, **1(2-4):** 67- 72.

4. **Barba R.C.; Zamora A.B.; Malion A.K.; and Linga C.K. (**1978) Sugarcane tissue culture research. *Proc. Int. Soc. Sugarcane Technol*, **16:** 1843-1863.

5. **Bhojwani S. S. and Razdan**. **M. K. (**1983) Plant Tissue Culture: Theory and Practice. Elsevier Science Publishers B. V. 502 p. ISBN 0-444-42526-8

6. **Heinz D.J and Mee G.W.P**. (1971) Plant differentiation from callus tissue of Saccharum sp. *Crop Sci.* **9:** 346-348.

7. **Khan S.A.; Rashid H.; Chaudhary M.F.; Chaudhary Z.; and Afroj A. (**2008) Rapid micropropagation of three elite Sugarcane (*Saccharum officinarum* L.) varieties by shoot tip culture. *Afri. J. of Biotech*, **7 (13):** 2174-2180.

8. **Lineberger R. D.** (2002) Horticulture in the Post-Land Grant Era. *Hort Science* **7:**1147-1149.

9. **Murashige T. and Skoog F.A** .(1962) A revised medium for rapid growth and bioassay with tobacco cultures. *Physio. Plant* , **15:** 473– 497.

10. **Feldmenn P.; Sapotille J.; Gredoire P. and Rott P. (**1994) Micro propagation of sugarcane. In: Teisson C, ed. *In vitro* culture of tropical plants. France: CIRAD: 15-17.

11. **Lal N. and Krishna R.** (1994) Sugarcane and its problems: Tissue culture for pure and disease free seed production in sugarcane. *Ind. sugar*, **44:** 847-848.

12. **Lorenzo J.C.; Ojeda E.; Espinosa A. and Borroto C.** (2001) Field performance of temporary immersion bioreactor derived sugarcane plant ys. *In vitro cell Dev. Biol., Plant*, **37:** 803-806.

13. **Brandes E.W.** (1958) Origin, classification and characteristics. In: Artschwager, E. and E.W. Brandes (eds.) Sugarcane (*Saccharum officinarum* L.). U.S. Dept. Agric. Hand book **122**. Pp. 1-35.

14. **FAO** (Food and Agriculture Organization of the United Nations). (2012).

15. **Vaccari G.; Tamburini E.; Sgualdino G.; Urbaniec K.; and Klemes J.** (2005). Overview of the environmental problems in beet sugar processing: possible solutions. Journal of Cleaner Production. 13(5): 499-507.

16. **Nguyen T.L.T.; Gheewala S.H. and Sagisaka M.** (2010) Greenhouse gas savings potential of sugar cane bio-energy systems. Journal of Cleaner Production. **18(5)**: 412-418.

17. **Pawar S.; Patil S.; Jambhale V.; Naik R. and Mehetre S**. (2002) Rapid multiplication of commercial sugarcane varieties through tissue culture. Indian Sugar. **52(3)**: 183-186.

18. **Hegde G. and Kuruvinashetty M** (2010) Standaridization of Protocols for the Micropropagation of Sugarcane. Karnataka Journal of Agricultural Sciences. **13(4)**:21-26

19. **Gosal S.; Thind K. and Dhaliwal H**. (1998) Micropropagation of sugarcane an efficient protocol for commercial plant production. Crop Improv. **(2)** 167-171.

20. **Bhuria P, Singh Yogendra, Tiwari S. and Chaturvedi M.** (2014). Multiple shoot regeneration from nodal explant of Ashwagandha (Withania somnifera)**.The Indian Research Journal of Genetics and Bio Technology.**06 (03):**521-525.**

21. **Srivastava B, Singh Yogendra, Tiwari S. and Chaturvedi M**. (2014). Studies on Micropropagation of Sugarcane from leaf roll, eye bud and meristem tip". **The Indian Research Journal of Genetics and Bio Technology.06 (01)**: 319-323.

22. Ali A.; Shagufta N.; Siddiqui F.A. and Iqbal J. (2008) An efficient protocol for large scale production of Sugarcane through micropropagation. ***Pak. J. Bot.*, 40(1):** 139-149.