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Differential behavior of sugarcane (*Saccharum* species hybrid) genotypes towards varying concentrations of pH for rapid *in vitro* micropropagation

SURESH YADAV[1], V N PATEL[2], B T RAVINDRABABU[3], VEERASENA REDDY[4] and T E NAGARAJA[5]*

Zonal Agricultural Research Station, GKVK, University of Agricultural Sciences, Bengaluru 560 065, India

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ABSTRACT

Sugarcane (*Saccharum* species hybrid) is not only an important cash crop but also major source of sugar industry worldwide. An effort was made to understand the effect of varying levels of medium pH for testing differential response of sugarcane genotypes (Co 86032, Co 85004 and Co 99004) on *in vitro* shoot initiation, induction, multiplication and root formation. The shoot apical meristem of the three varieties was cultured on MS medium with different pH levels. Aseptic meristem cultures of the three sugarcane genotypes were cultured *in vitro* on the medium with five pH concentrations, viz. 4.8, 5.0, 5.4, 5.6 and 5.8. Co 85004 was found to show its response towards the medium pH at 5.6 in producing highest number of shoots (~22). However, Co 99004 responded well towards medium pH at 5.4 which produced ~21 numbers of shoots and Co 86032 exhibited its potentiality towards pH only at 5.8 levels (~17) on 180 days after inoculation. The response of all the genotypes were on par towards pH 7.0 which produced highest number of roots. At pH 5.8 genotype, Co 86032 produced highest shoot length (~3 cm) followed by Co 85004 (~2 cm) and Co 99004 (~2 cm). The study revealed that medium adjusted with pH 4.8, pH 5.8 and pH 7.0 levels resulted in optimum *in vitro* shoot initiation, induction, multiplication and root formation responses in Co 85004, Co 99004 and Co 86032, respectively.

Key words: Cellular growth, Meristem, Micropropagation, pH, Sterilization

Sugarcane (*Saccharum* species hybrid) is one of the most valuable global crops, widely grown in tropical and subtropical regions of the world. Sugarcane contributes 70% to the total sugar pool at the global level, rest being contributed by sugar beet and other sources. Globally, sugarcane is cultivated over an area of 26.52 million ha with an annual production of 1877 million tonnes and a productivity of 70.77 tonnes/ha. India ranks second among the sugarcane growing countries of the world in both area and production. In India, sugarcane is grown in area of 4.20 million ha with a production of 355.90 million tonnes and productivity of 74.4 tonnes/ha. The shrinking agricultural lands and increasing demand of sugar have compelled the agricultural scientists to increase the sugarcane and sugar productivity per unit area through the development of high yielding varieties. New varieties of sugarcane are developed through breeding methods involving a multistage selection procedure which takes about 8 to 10 years. Due to slower multiplication ratio, it takes a further period of 10 to 12 years to reach in remote areas for general planting if multiplied through conventional means. By that time the varieties start deteriorating in yield and quality parameters. Plant tissue culture techniques are used worldwide by many researchers over the last two decades in the development of useful genetic variability in the economically important crops. Initially attempt was made by Nickell in 1964 for the regeneration of the plants through *in vitro* technique (Nickell 1964). Successful regeneration and somatic embryogenesis were further studied in sugar cane using different explant medium composition. 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. Usually, the plant part (explant) is placed in a suitable tissue culture media, proliferation of the lateral buds or adventitious shoots or the differentiation of the shoots results in tremendous increase in the number of shoots available for rooting. Micropropagation using shoot tip or apical meristem culture has been widely used to produce virus-free plants with rejuvenation and mass production of true to type and uniform planting materials from old diseased sugarcane plants. Moreover, tissue culture raised sugarcane plants were reported to give superior cane and sugar yield as compared to their donors from conventional seed source under similar climate and management practices. In line with this, many researchers have developed *in vitro* propagation protocols for sugarcane. But every genotype needs specific protocol for rapid and

* Corresponding author e-mail: tenagaraja@gmail.com

[1]Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012

efficient propagation. In addition, the universal application of the protocols was limited by various factors like genotype and explants type, position of explant on the stock plant age, endogenous levels of plant growth regulators, time of the year and physical growth factors and explant size. One of the major obstacles is genotype to media or plant growth regulators interactions and media reaction that is hydrogen ion concentration. Hence, the present investigation was formulated for establishment of hydrogen ion concentrations for better propagation of superior sugarcane varieties via tissue culture and hence the research is targeted to meet the present-day requirement.

MATERIALS AND METHODS

The experiment consisted of three elite sugarcane clones (Co 86032, Co 85004 and Co 99004), which were tested on five different levels of pH (pH 4.8, 5.0, 5.4, 5.6 and 5.8) for shoot induction and three other pH levels (5.8, 6.0 and 7.0) for root formation. The laboratory experiments were laid out in completely randomized design with three replications at well-established Sugarcane Tissue Culture Laboratory at Jaggery Park, V C Farm, Mandya.

Explant preparation

Apical portion from the shoot of sugarcane varieties were excised from 4 to 6 months old field growing plants and surface sterilized. The shoot meristem measuring approximately 0.1 mm in diameter and 0.25 mm to 0.30 mm in length was exposed by carefully removing the surrounding leaf sheaths. The cells of the meristem are genetically highly stable hence, the plants produced from them are generally identical to the donor plants except for the occurrence of rare mutations (Hendre *et al.* 1983; Sreenivasan and Jalaja 1995).

Shoot initiation and induction from apical meristem

Explants were collected from top one third portion of sugarcane plant and they were subjected to sterilization with surgical spirit using cotton. Then surrounding leaf sheaths of tops were removed until inner white sheaths were exposed. After excising, the meristem was transferred immediately on the medium. Full strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) was used for initiation or establishment of the shoot tip explants of the selected sugarcane clones. The shoot initiation medium was adjusted with five different levels of pH, viz. 4.8, 5.0, 5.4, 5.6 and 5.8 before adding agar and autoclaved at 121^0C, 15 psi for 20 minutes and maintained separately in sterile culture bottles in order to facilitate inoculation of sugarcane clones. The inoculated tubes were kept in the culture room under light intensity (2500 lux) at 24°C and 16 hr light and 8 hr dark photoperiod regimes were maintained under fluorescent light along with 64-70% relative humidity in the incubation chamber.

Multiplication of shoots

The developing shoots were transferred to fresh containers with liquid differentiating medium (LDM) for shoot multiplication and elongation. The process was repeated in every 30 days depending upon the rate of multiplication. For shoot multiplication the full-strength MS medium was adjusted with three different levels of pH, viz. 5.8, 6.0 and 7.0 before adding agar and autoclaved at 121^0C, 15 psi for 20 minutes in the similar way as done for shoot initiation. The inoculated tubes were kept in the culture room under light intensity (2600-3000 lux) at 24°C with 16 hr light and 8 hr dark photoperiod regimes maintained under fluorescent light and having 64-70% relative humidity to study the effect of pH on formation of number of tillers per explant with time interval of 90, 120, 150 and 180 days after inoculation (DAI) with three replications.

Transfer of shoots to rooting medium

Only well grown shoots with three to four leaves were transferred to rooting medium. Dry leaves were removed and green leaves were trimmed at the tip. While separating, care was taken to prevent damage to the basal portion of the shoots from where the roots emerge. Clumps of five to six shoots were placed in bottles containing rooting medium. The rooting media (MS) containing NAA (5 ml/l), coconut water (100 ml/l), sucrose (30 g/l), macronutrients, micronutrients and vitamins were prepared and adjusted to pH levels of 5.8, 6.0 and 7.0 in different culture bottles in an order to study the effect of different levels of pH on producing number of roots, days to rooting, percentage of cultures rooted, root length and shoot length in the test genotypes.

RESULTS AND DISCUSSION

All the three sugarcane genotypes showed response to *in vitro* growth initiation of meristem, multiplication of shoot cultures, shoot length and number of roots. The method of producing large number of identical clones by *in vitro* culture was used routinely for wide range of plant species (Biondi 1986), but there were marked differences in response of genotypes at different levels of pH. The results of present study demonstrated the regeneration potential of shoot apex of different genotypes (Co 86032, Co 85004 and Co 99004). The best response of genotypes for shoot characters like, days taken by explant to become autotrophic, frequency of shoot induction, shoot induction percentage, number of shoots and shoot length and root characters like days for root initiation, percentage of cultures rooted, number of roots and root length to different pH levels is explained below.

Shoot initiation and induction from apical meristem

To study the effect of pH on shoot initiation and induction, five different levels of pH were used to understand the response of three varieties namely, Co 86032, Co 85004 and Co 99004. The observations were recorded for number of days taken for explants to turn autotrophic, days taken for shoot induction and establishment. The results revealed highly significant differences existed among different levels

Fig 1 Effect of different levels of pH on shoot multiplication in genotype Co 86032. a. 5 b. 5.2 c. 5.6 d. 5.8.

of pH under study at all the stages of observation with respect to shoot initiation and induction (Table 1). Genotype Co 86032 responded better in terms of shoot initiation which turned autotrophic in ~10 days in MS medium at pH 4.8 and 73% of explants showed good response of producing shoot induction in the period of 56 days as compared to rest of the pH levels tested in the medium. In case of sugarcane clones, Co 85004 and Co 99004, MS medium with pH 4.8 showed 66% shoot induction response within the 51 to 57 days and days to turn autotrophic was ~11 and ~13 days, respectively. Among the genotypes and pH levels, the best response was obtained by Co 86032 when cultured in MS medium at pH 4.8. MS medium with pH 5.0 level has taken more number of days (58-64 days) in producing 60% shoot induction in all the three genotypes. In the present investigation it was observed that number days taken for shoot induction was increased at higher pH level of medium. Hence, it proved to be time consuming and not appropriate for *in vitro* propagation (Table 3).

Multiple shoot formation

After 4–5 weeks of shoot growth, actively growing shoots of different genotypes (Co 86032, Co 85004, and Co 99004) were transferred on liquid differentiating medium (LDM) adjusted to different levels of pH, 4.8, 5.0, 5.4, 5.6 and 5.8 in culture bottles for further growth and proliferation. After 30 days, these shoots were further sub cultured in liquid medium. In this way shoot multiplication was maintained for several passages by regular transfer to fresh medium. Number of shoots per bottle was recorded at 90 days, 120 days, 150 days and 180 days for all the three sugarcane clones. Genotype, Co 86032 produced the highest number of shoots per culture (~17) at pH 5.8 (Fig 1) in 180 days of time interval. Genotype, Co 85004 showed highest shoot multiplication rate (~22) in MS medium with pH 5.6 (Fig 2) at 180 days of time interval. At pH 5.4, Co 99004, put forth highest number of shoots per culture with the highest shoot multiplication rate in 180 days of time interval (~21). Very slow growth rate was observed during 90 to 150 days interval compared to 180 days in all the three genotypes. However, the highest number of shoots per culture was recorded between 90 and 150 days. It was observed that in all the three sugarcane clones, the rate of shoot multiplication increased with increasing in levels of

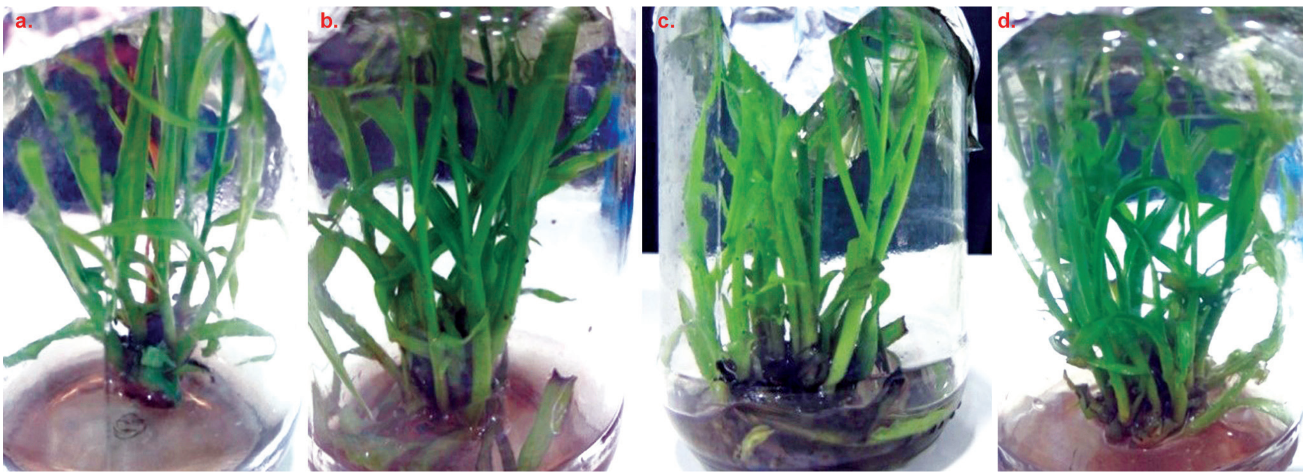

Fig 2 Effect of different levels of pH on shoot multiplication in genotype Co 85004 a. 5 b. 5.2 c. 5.6 d. 5.8 e. 5.8.

Table 1 Response of sugarcane genotypes to different pH concentrations on multiple shoot formation

	T1G1	T1G2	T1G3	T2G1	T2G2	T2G3	T3G1	T3G2	T3G3	T4G1	T4G2	T4G3	T5G1	T5G2	T5G3	S.EM	CD@1%	CV%
90 DAI	2.42	4.97	4.13	2.25	4.13	2.60	2.13	3.92	2.25	1.97	3.60	2.25	1.90	3.30	2.53	0.23	0.90	13.56
120 DAI	7.75	9.05	7.72	6.93	8.73	6.73	6.89	9.43	8.27	6.27	10.07	9.21	6.13	10.38	9.53	0.34	1.32	7.15
150 DAI	13.03	12.27	13.95	12.98	11.35	14.42	12.25	10.37	13.40	12.43	11.25	15.11	12.65	14.30	15.82	0.34	1.34	4.58
180 DAI	15.92	16.22	20.43	16.40	17.90	19.23	15.32	17.83	20.82	16.75	21.77	19.09	17.25	18.25	17.60	0.37	1.43	3.54

G1- Co 86032, G2- Co 85004 and G3- Co 99004; T1- pH 4.8, T2- pH 5.0, T3- pH 5.4, T4- pH 5.6 and T5- pH 5.8.

pH 5.4, 5.6 and 5.8 (Table 1).

Rooting of regenerated shoots

Culture medium for root differentiation was adjusted to three different levels of pH, *viz.* 5.8, 6.0 and 7.0 for all three sugarcane clones. Micro shoots developed on shoot differentiation medium were cultured on root differentiation medium. Observations were recorded on various root and root associated characters, viz. number of roots, days to rooting, the percentage of micro shoots rooted, root length and shoots length per culture. Significant differences were observed among all the three levels of pH on rooting. Genotype, Co 86032 found optimum at pH 7.0 in terms of highest number of roots (~10), lowest number of days to rooting (4 days), maximum number of cultures rooted (95%), root length (4 cm) and shoot length (28.2 cm) (Fig 3). Similarly, the pH 7 was found to be optimum for genotype, Co 85004, it showed highest number of roots (~11), lowest number of days to rooting (5 days), highest percentage of cultures rooted (78%), root length (3.0 cm) and shoot length (~28 cm) as depicted in Fig 4. Highest number of roots (~11), lowest number of days to rooting (7 days) and shoot length (~25 cm) was observed for genotype, Co 99004 at pH 7.0. There was no significant difference found in the explants for root initiation in all three sugarcane clones at pH 5.8 and 6.0, besides also observed the reduction in root induction with lowering in levels of pH. Among, all the three elite sugarcane clones, highest root formation was found in genotype, Co 85004 followed by Co 99004 and least recorded in Co 86032. In contrast, shoot length was recorded highest in Co 86032 followed by Co 85004 and Co 99004 (Table 2).

The present investigation revealed that, the MS medium containing pH 4.8 was the optimum for shoot initiation and establishment for all the three sugarcane clones. Results also showed that MS medium adjusted at pH 5.8 was most appropriate for shoot multiplication for genotype, Co 86032 whereas, the MS medium with pH levels of 5.6 and 5.4 proved to be optimum for the sugarcane clones, Co 85004 and Co 99004, respectively for shoot multiplication. The studies on different levels of pH of rooting medium on root regeneration for all the three sugarcane clones revealed that MS medium with pH level 7.0 was optimum for root formation. The improvement in shoot and root regeneration efficiency require a better understanding of the influence of *in vitro* culture conditions. The pH of the medium is greatly important factor, as it favours the assimilation of ions by the cultured cells from the medium and in turn regulation of metabolic pathway of cultured cells which facilitates better proliferation of shoots since optimal level metabolic process. The pH of medium influences the uptake of nutrients, plant growth regulators and also affects the availability of nutrients and growth hormones in the culture medium. Ramanad and Lal (2004) and Singh (2005) in

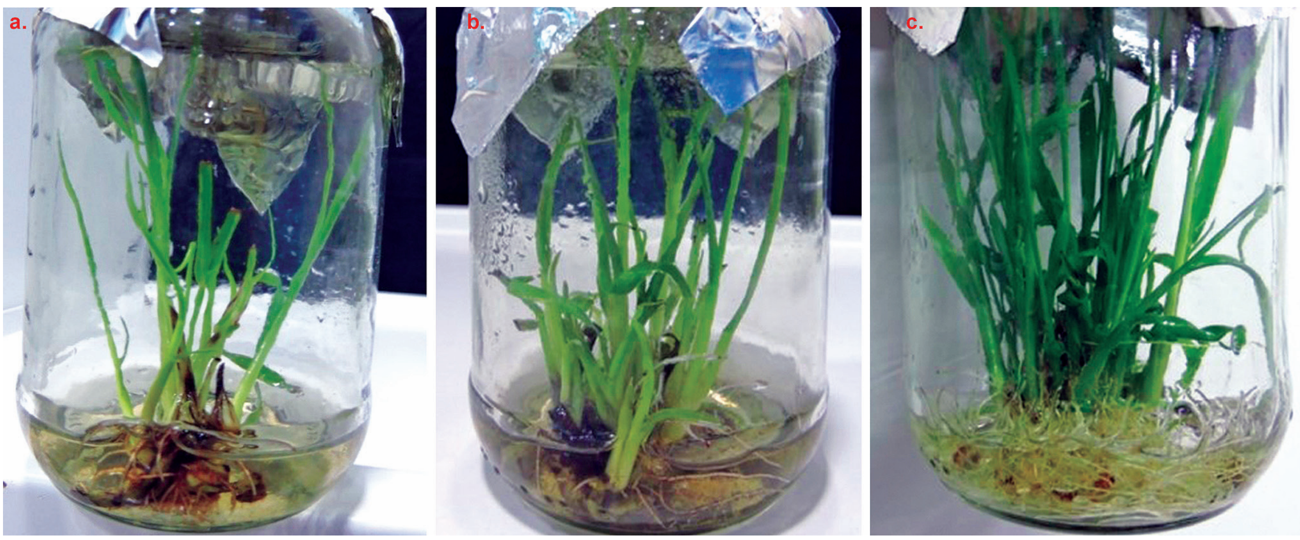

Fig 3 Effect of different pH levels on root regeneration in genotype Co 86032 a. 5.8, b. 6.0, and c. 7.0.

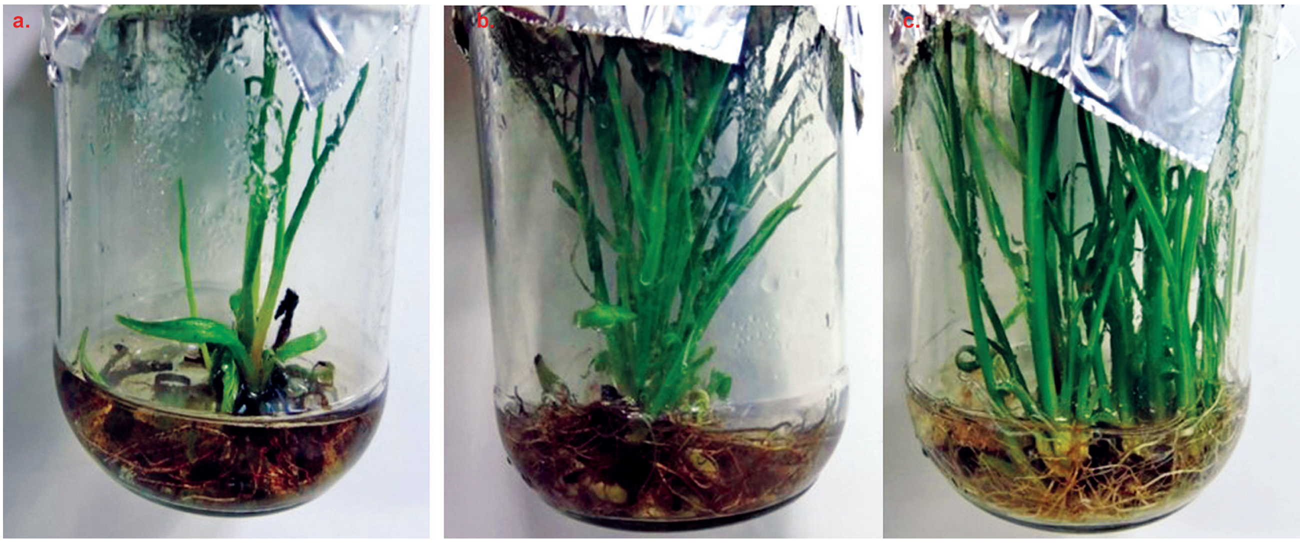

Fig 4 Effect of different pH levels on root regeneration in genotype Co 85004 a. 5.8, b. 6.0 and c. 7.0.

Table 2 Response of sugarcane genotypes on rooting and shoot length at different pH concentrations

	T1G1	T1G2	T1G3	T2G1	T2G2	T2G3	T3G1	T3G2	T3G3	SEm	CD@1%	CV%
Number of roots	5.08	3.95	5.22	5.28	5.17	7.43	9.48	10.98	10.87	0.27	1.10	6.65
Days to rooting	12	12	14	7	8	10	4	5	7	0.17	0.85	2.54
% of cultures rooted	45	30	30	83	75	52	95	78	75	1.35	2.51	4.52
Root length (cm)	1.2	1.0	0.65	1.5	1.3	1.1	4.0	3.0	1.8	0.41	1.20	6.21
Shoot length (cm)												

G1- Co 86032, G2- Co 85004 and G3- Co 99004; T1- pH 5.8, T2- pH 6.0 and T3- pH 7.0

Table 3 Effect of pH on the shoot induction in sugarcane genotypes

pH of medium	Sugarcane variety	Number of meristems inoculated	Number of meristems survived	Days for explants to turn autotropic	Number of Meristems showing shoot induction	Number of days taken for shoot induction	Shoot induction %	Shoot length (cm)
4.8	Co 86032	15	13	9.13	11	56	73(58.69)	1.65
	Co 85004	15	12	11.25	10	51	66(54.33)	1.42
	Co 99004	15	13	12.30	10	57	66(54.33)	1.05
5.0	Co 86032	15	11	9.77	9	58	60(50.77)	1.98
	Co 85004	15	11	14.03	9	62	60(50.77)	1.65
	Co 99004	15	12	13.51	9	64	60(50.77)	1.33
5.4	Co 86032	15	8	9.90	8	70	53(46.72)	2.20
	Co 85004	15	11	14.40	6	62	40(39.23)	1.76
	Co 99004	15	9	14.06	7	75	46(42.71)	1.68
5.6	Co 86032	15	8	9.78	7	65	46(42.71)	2.12
	Co 85004	15	10	10	6	66	40(39.23)	2.02
	Co 99004	15	7	11.23	6	84	40(39.23)	1.95
5.8	Co 86032	15	9	10.67	8	82	53(46.72)	2.92
	Co 85004	15	8	11.23	7	90	46(42.71)	2.24
	Co 99004	15	7	12.21	6	89	40(39.23)	2.13

Figures in the parenthesis indicate angular transformed values; T1- pH 4.8, T2- pH 5.0, T3- pH 5.4, T4- pH 5.6 and T5- pH 5.8; G1- Co 86032, G2- Co 85004 and G3- Co 99004 their investigation also reported that multiplication rate in terms of number of shoots per cultures were higher at pH 6.0 while, pH 5.6 and 6.4 resulted in poor response. The pH of medium possibly plays significant role as reported by Gopitha *et al.* (2010). Mishra (2011) reported that the pH of medium plays role in regulating the cellular metabolic activities through its effect on related enzymes further affects the cellular growth and differentiation. This argument was also supported by the fact that each of the metabolic enzymes has pH optima at which it is most active. Faisal *et al.* (2006) tried a wide range of pH for shoot induction and reported maximum multiplication rate at pH of 5.8. Similar, findings were also reported by Siddique and Anis (2007), Naik *et al.* (2010) and Perveen *et al.* (2011). These studies indicated that the effect of pH value on plant regeneration depends on genotype of the crop species.

Conclusion

The present experiment was carried out to standardize pH of MS medium for not only shoot induction and its associated characteristics but also for rapid root regeneration for *in vitro* propagation of three elite sugarcane clones, Co 86032, Co 85004 and Co 99004. The results revealed that, the MS medium at pH 4.8 was found to be optimum for shoot initiation and establishment in the genotypes tested. Co 86032, on liquid differentiation medium at pH 5.8 was most appropriate for producing shoot multiplication at a much faster rate, while the genotypes Co 85004 and Co 99004 showed optimum response for shoot proliferation in MS medium at pH 5.6 and 5.4, respectively. The effect of different levels of pH, *viz.* 5.8, 6 and 7 of root regeneration medium on number of roots, days to root initiation, percentage of cultures rooted and root length in three genotypes, viz. Co 86032, Co 85004 and Co 99004 revealed that the pH 7 was most suitable for rooting. It also observed that root formation was enhanced in liquid medium at pH 7.0 in all three genotypes. Hence, the medium should be standardized for each genotype, in an order to reduce the duration of *in vitro* protocol for micropropagation.

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REFERENCES

Biondi. 1986. Practical application of *in vitro* propagation: Present situation and future prospects. *Giomale Botanico Italiano* 120:29-42.

Faisal M, Ahmad N and Anis M. 2006. *In vitro* plant regeneration from alginate encapsulated micro-cuttings of *Rauvolfia etraphylla L. World Journal of Agriculture Sciences* **1**:1–6

Gopitha K, Lakshmi B A and Senthil M J. 2010. Effect of the different auxins and cytokinins in callus induction, shoot, root regeneration in sugarcane. *International Journal of Pharmaceuticals and Biological Sciences* **1**(3): 55-11.

Gosal S, Thind K S and Dhaliwal H S. 1998. Micropropagation of sugarcane an efficient protocol for commercial plant production. *Crop Improvement* **25**(2):167-171.

Hendre R R, Iyer R S and Kotwal M. 1983. Rapid multiplication of sugarcane by tissue culture. *Sugarcane* **1**:58.

Khan I A, Dahot M U, Yasmin S, Khatr A and Seema N. 2006. Effect of sucrose and growth regulators on the micropropagation of sugarcane clones. *Pakistan Journal of Botany* **38**: 961-967.

Lal J, Pande H P and Awasthi S. K. 1996. A general micropropagation protocol for sugarcane varieties. *New Botany* **23**:13-19.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bio assays for tobacco tissue cultures. *Physiology Plant* **15**: 473-497.

Nadagauda R S. 2002. Need for tissue culture. Tissue culture pilot plant, National Chemical Laboratory, 1-4.

Naik P M, Manohar S H, Praveen N and Murthy H N. 2010. Effects of sucrose and pH levels on *in vitro* shoot regeneration from leaf explants of *Bacopamonnieri* and accumulation of bacoside A in regenerated shoots. *Plant Cell Tissue and Organ Culture* **100**:235–239

Nand L and Ram K. 1997. Yield comparison in sugarcane crop raised from conventional and mericlone derived seed cane. *Indian Sugarcane* **47**(8): 617-621.

Nickell L G. 1964. Tissue and cell culture of sugarcane: Another research tool. *Hawaii Planters Records* **57**: 223-229.

Perveen S, Varshney A, Anis M and Aref I M. 2011. Influence of cytokinins, basal media and pH on adventitious shoot regeneration from excised root cultures of *Albizia lebbeck. Journal of Forestry Research* **22**:47–52

Ramanand and Lal M. 2004. An efficient protocol for *in vitro* micropropagation of sugarcane. *Sugar Tech* **6** (1&2): 85-87.

Shaw M E A. 1990. Biotechnology in sugarcane agriculture. *Proceedings of the Annual National Conference on Science and Technology,* part 2, pp 31-35.

Siddique I and Anis M. 2007. *In vitro* shoot multiplication and plantlet regeneration from nodal explants of *Cassia angustifolia* (Vahl.): a medicinal plant. *Physiology Plant* **29**:233–238.

Singh A. 2005. Physiological and biochemical basis of *in vitro* morphogenesis in sugarcane hybrids. Thesis submitted to CSM University, Kanpur, UP, India.

Sreenivasan T V and Jalaja N C. 1995. Utility of tissue culture technology in sugarcane improvement. Application of biotechnology for sustainable sugarcane production, 44th annual convention DSTA, Pune, pp 1-9.