OPTIMAL PHYTO-HORMONE LEVELS FOR MERISTEM CULTURE OF SUGARCANE (*SACCHARUM* HYBRID SPP.): VARIETY SL 96 328

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Abstract

Sugarcane plants can be multiplied rapidly by aseptic culture of meristematic *explants* under controlled nutritional and environmental conditions to produce disease free planting materials even at commercial scale. Though there is a standard meristem culture protocol, perfection of this protocol with respect to phyto-hormone levels is important in maximising the efficiency of this technology when it deals with different varieties. This research was conducted to find out the optimum cytokinin [6-Benzylaminopurine (BA) and Kinetin] levels for shoot germination from meristematic *explants* and for shoot multiplication under passage culture of variety SL 96 328. Optimum Auxin [α-Naphthalene acetic acid (NAA) and Indol-3-butyric acid (IBA)] levels for root development in *in-vitro* generated multiple shoots were also investigated for the variety SL 96 328. The results revealed that increased levels of BA lead to increase the mortality of meristematic *explants* in culture. The treatment combinations with BA and Kinetin levels (mg/l) 0.01 BA + 0.05 Kinetin, 0.01 BA + 0.02 Kinetin, 0.05 BA + 0.05 Kinetin, 0.05 BA + 0.001 Kinetin, 0.1 BA + 0.02 Kinetin and 0.1 BA + 0.001 Kinetin were found as the best combinations to be used in shoot germination. The tested combinations of same hormones with levels, BA and Kinetin (mg/l) 0.05 BA + 0.01 Kinetin, 0.03 BA + 0.02 Kinetin, 0.2 BA + 0.05 Kinetin, 0.03 BA + 0.01Kinetin, 0.01 BA + 0.1 Kinetin were found to be the best combinations for shoot multiplication in passage culture. Use of auxins with concentrations (mg/l) of IBA and NAA (mg/l) 0.5 IBA + 0.05 NAA mg/l, 3.0 IBA + 0.2 NAA mg/l, 3.0 IBA + 0.05 NAA mg/l and 3.0 IBA + 0.03 NAA mg/l were found to be the best for root development in multiple shoots of variety SL 96 328.

Key words – auxins, cytokinins, sugarcane, meristem culture, *in-vitro* propagation.

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Introduction

Non-availability of quality planting material is identified as one of the major constraints for sugarcane production and industry expansion. Currently, seed material for commercial sugarcane cultivation is produced through conventional 3-tier nursery system involved with hot- water treatment of seed cane. However, the insufficient amount of seed-cane produced through this system compels the industries and farmers to obtain unsuitable seed-cane from commercial cane fields resulting spread and buildup of systemic sugarcane diseases in commercial plantations and low sugarcane yields (Wijesuriya *et al.*, 2002). At present, use of such unsuitable seed-cane affects more adversely to the cane production under the recent epidemic situation of sugarcane White Leaf Disease (WLD) / Grassy Shoot Disease (GSD) caused by phytoplasma. Hence, there is an absolute necessity to introduce an effective system capable of producing quality seed material and also an efficient seed distribution system for uplifting the local sugar industry. The conventional 3-tier seed-cane nursery system starting with hot-water treatment of seed-cane could eliminate some sugarcane seed-borne diseases and restrict the building up of pathogen population in the commercial plantations. However, this system is ineffective in eliminating viral and phytoplasma diseases, which are presently threatening the local sugar industry.

Adoption of a seed-cane nursery system involving production of healthy cane through meristem culture and subsequent planting material multiplication in secondary nurseries using rapid seed-cane multiplication methods such as spaced planting and spaced transplanting techniques will be able to produce healthy seed-cane and maintain healthy sugarcane plantations. The Crop Improvement Division of the Sugarcane Research Institute of Sri Lanka actively engaged in the development of protocols in planting material production through *in-vitro* micro- propagation and the other rapid sugarcane multiplication techniques such as Lateral Shoot Multiplication (LSM) and Spaced Planting of Seed Setts (SPoSS) (Wijesuriya *et al.*, 2010). The division was able to optimise the methods of *explanting* tissues, composition of media and culture conditions to obtain maximum output from meristem culture in particular to the commercial varieties of sugarcane. However, the past researches revealed that the existence of varietal differences in terms of the rate of multiple shoot formation and root development in the media tested (Anon, 2013). Concentration of phyto-hormones has been identified as the most critical factor that controls and interacts with the varieties in formation of multiple shoots and root formation in culture (Wijesuriya and Teruya, 1988). The Sugarcane Research Institute usually uses the standard MS medium with the concentration of 0.2 mg/l 6-Benzylaminopurine (BA) and 0.001 mg/l Kinetin (KIN) for multiple shoot formation and gelrite medium for first culture and liquid medium for passage culture. For root formation in multiple shoots, MS medium added with α-Naphthalene acetic acid (NAA) 0.2 mg/l, Indol-3 butyric acid (IBA) 2 mg/l and sucrose 60 g/l have been identified as the most effective combination. However, these phyto-hormone combinations may or may not produce optimum output for different varieties under culture. This study was conducted to determine the optimal levels of shooting hormones (BA and KIN) and rooting hormones (NAA and IBA) in particular to the meristem culture of newly-bred sugarcane variety SL 96 328 that is needed rapid multiplication for commercialization.

Materials and Methods

This experiment was conducted at the Sugarcane Research Institute, Uda Walawe, Sri Lanka during the period, May to August 2013. Five levels of BA and five levels of Kinetin (Table 1) were tested for their effects on multiple shoot formation and shoot multiplication in passage culture in variety SL 96 328. For root initiation and development in multiple shoots, five levels of NAA and five levels of IBA (Table 2) were tested. Ten and 30 replicates were used respectively, in culturing meristem *explants* in gelrite medium and in passage culture of single shoots in liquid medium in each hormone combination tested. For rooting, 20 replicates of multiple shoots with more or less similar size and vigour were used for each hormone combination. Liquid medium was used for rooting of multiple shoots.

Mortality of meristem *explants* (0-dead, 1-live), vigour of the shoots generated from the *explants* and length of the shoots were recorded at 10, 20, 30 and 40 days in culture. In passage culture, number of shoots formed, the length and vigour of multiple shoots were recorded after 14 days in culture. In rooting, number of roots per clump, root length and vigour were recorded after 21 days in culture. Vigour of shoots and roots was quantified using a 1-5 scale. Logistic regression analysis, Kruskal Wallis test, Wilcoxon rank sum test and analysis of variance were used in processing of these data.

Table 1: Combinations of BA and Kinetin levels tested in meristem culture and passage culture and their assigned treatment numbers

BA mg/l → ↓ KIN mg/l	0.01	0.03	0.05	0.1	0.2
0.001	1*	2	3	4	5
0.01	6	7	8	9	10
0.02	11	12	13	14	15
0.05	16	17	18	19	20
0.1	21	22	23	24	25

*Treatment number

Table2: Combinations of NAA and IBA levels tested in rooting and the treatment numbers assigned

NAA mg/l → ↓ IBA mg/l	0.03	0.05	0.1	0.2	0.4
0.3	1*	2	3	4	5
0.5	6	7	8	9	10
1.0	11	12	13	14	15
2.0	16	17	18	19	20
3.0	21	22	23	24	25

*Treatment number

Results and discussion

Meristem culture

The mortality of *explants* determined by the probability of success for 10, 20, 30 and 40 days after inoculation is depicted in Figure 1 and, is clearly indicated that the frequency of dead *ex-plants* increased with the increasing level of BA. Significant effects of BA, KIN and BA x KIN were found in shoot vigour and length of shoots. Less *explant* mortality, high vigour and higher length of the shoots developed in 40 days of culture were considered in the selection of optimal hormone combinations for shoot germination. The treatment combinations common for these three criteria *viz.* 3,4,11,14,16 and 18 (Table 1) were selected as the best combinations for meristem culture of variety SL 96 328.

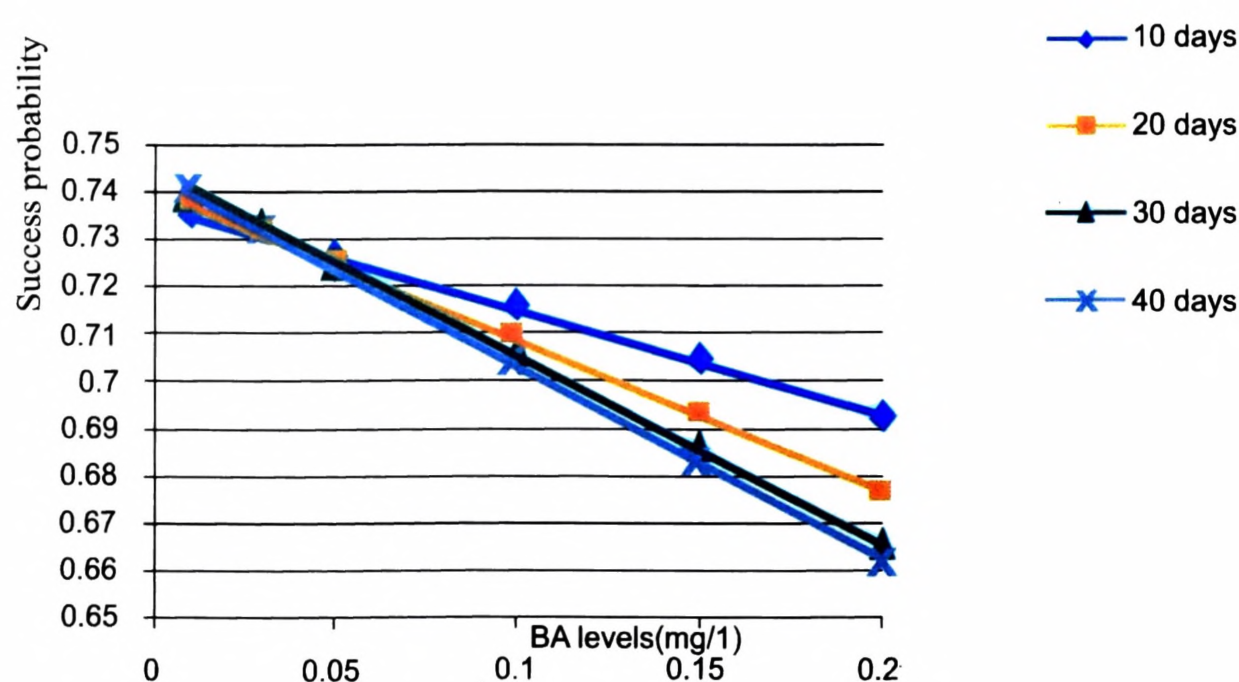

Figure 1: Fitted probability of success in shoot formation with tested BA levels for 10, 20, 30 and 40 days after inoculation

Passage culture

The mean scores for shoot vigour classified by BA levels for Kinetin levels are presented in Figure 2. Mean number of shoots and mean shoot length produced under each treatment combinations are graphically presented in Figures 3 and 4.

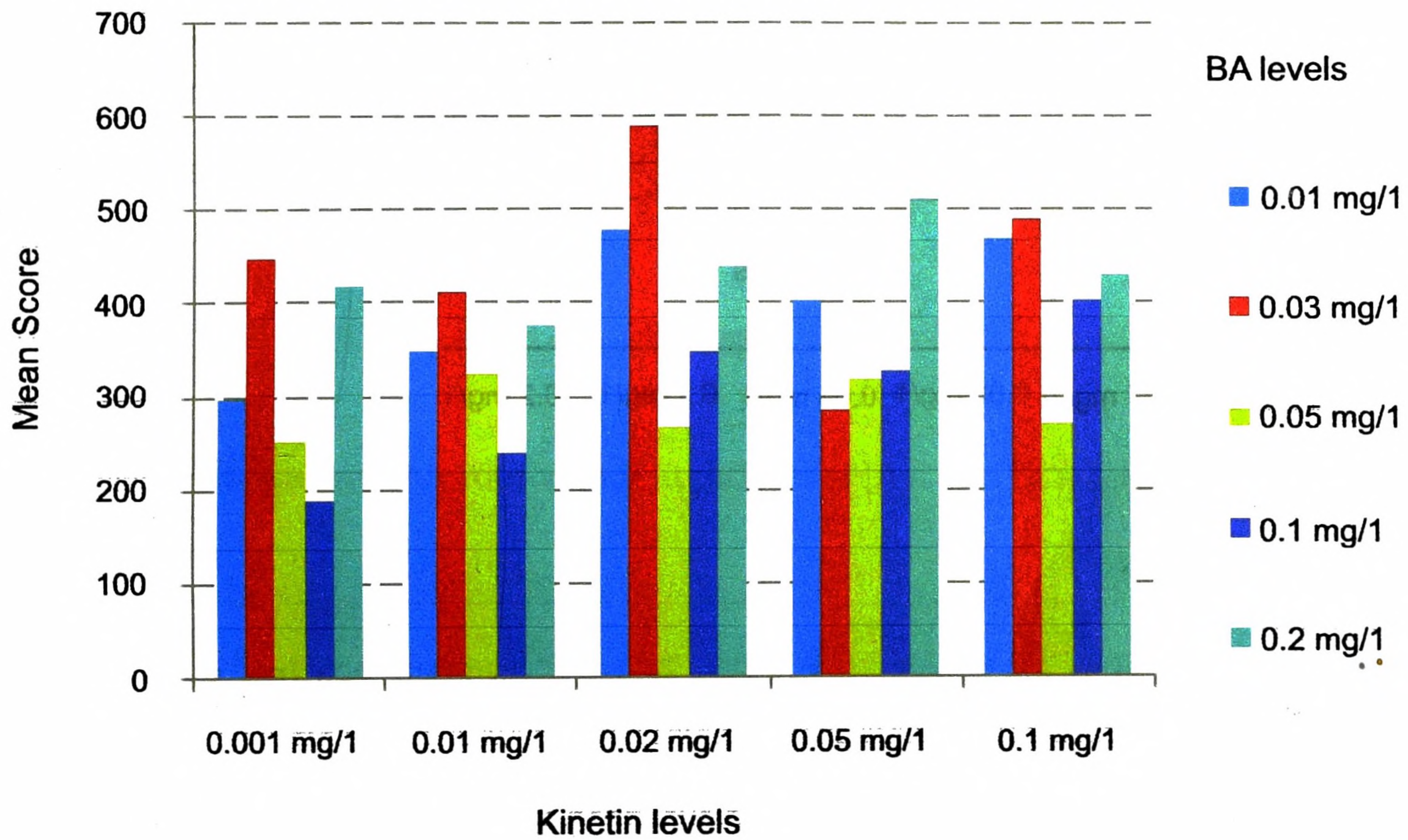

Figure 2: Mean scores for vigor of multiple shoots at different BA and Kinetin levels

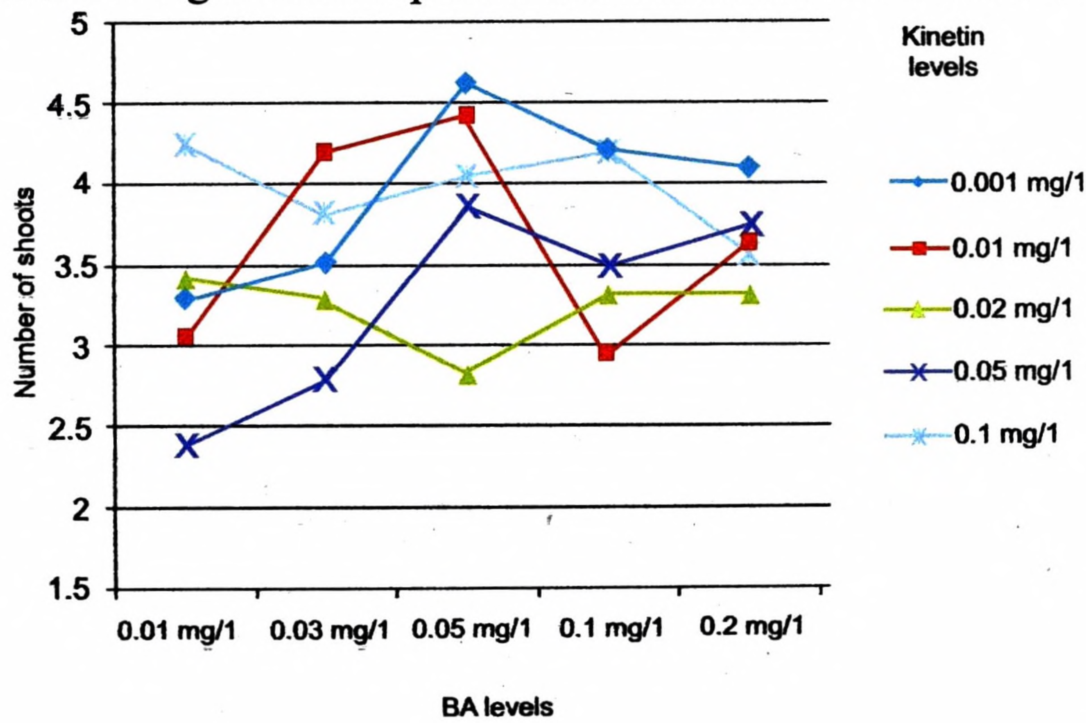

Figure 3: Mean number of shoots at different BA and Kinetin levels

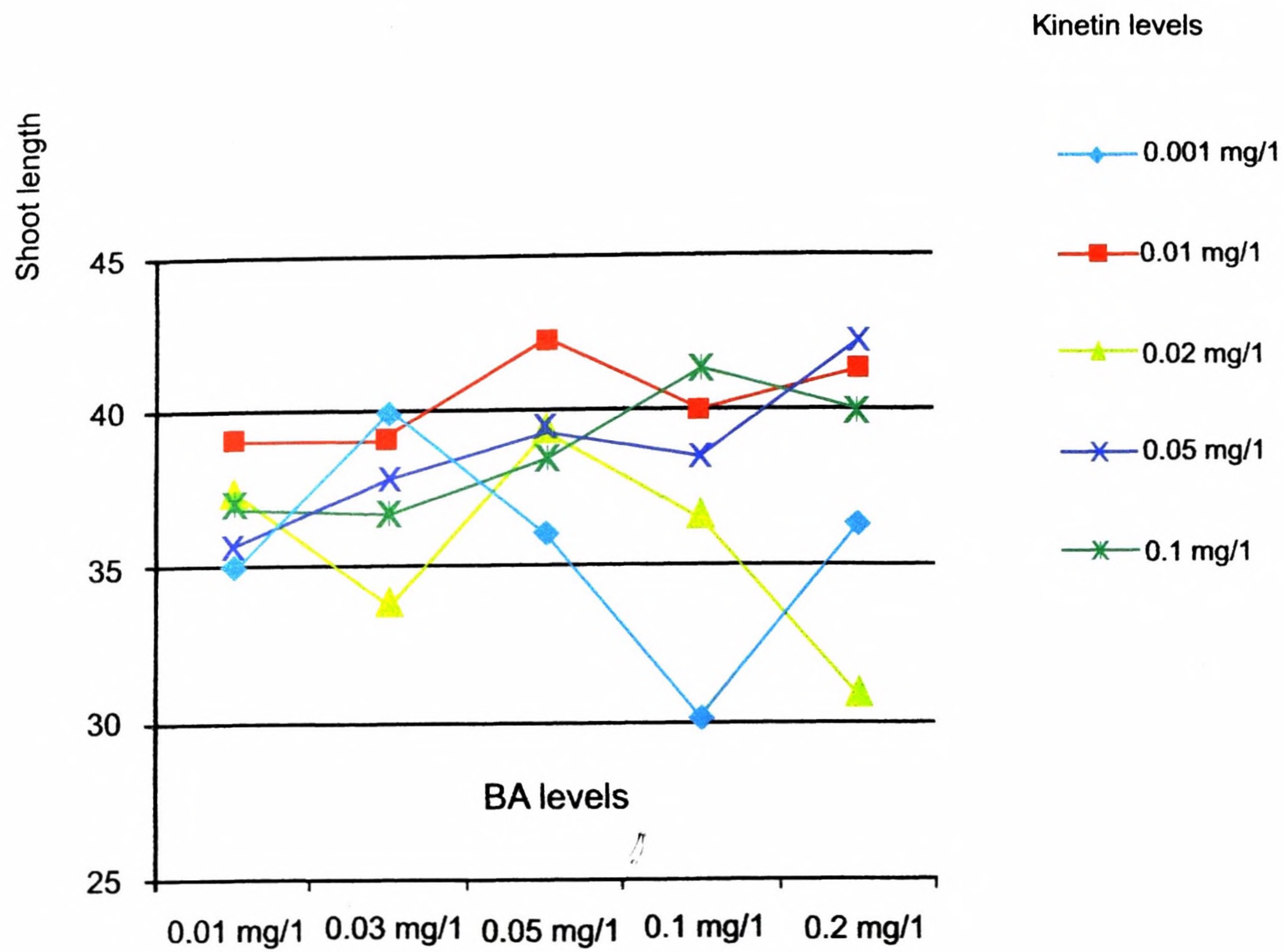

Figure 4: Mean number of shoots at different NAA and IBA levels

The appropriate levels for BA and Kinetin to be used in *in-vitro* shoot multiplication were decided on the basis of higher shoot vigour, higher number of shoots and higher shoot length. Accordingly, five treatment combinations *viz.* 7, 8, 11, 20 and 21 (Table 1) were selected as the best treatment combinations for passage culture of variety SL 96 328.

Rooting of multiple shoots

Mean scores for root vigour and mean root length at different NAA and IBA levels are shown in Figures 5 and 6, respectively. Analysis of variance showed that IBA, NAA and IBA x NAA interaction have significant effects on development of number of roots in multiple shoots and root length under culture in different rooting media. The best levels of NAA and IBA were selected on the basis of higher root vigour, higher number of roots and higher root length and the treatments 4, 6, 7, 21, 22, 24 and 25 (Table 2) were selected for rooting of multiple shoots of variety SL 96 328.

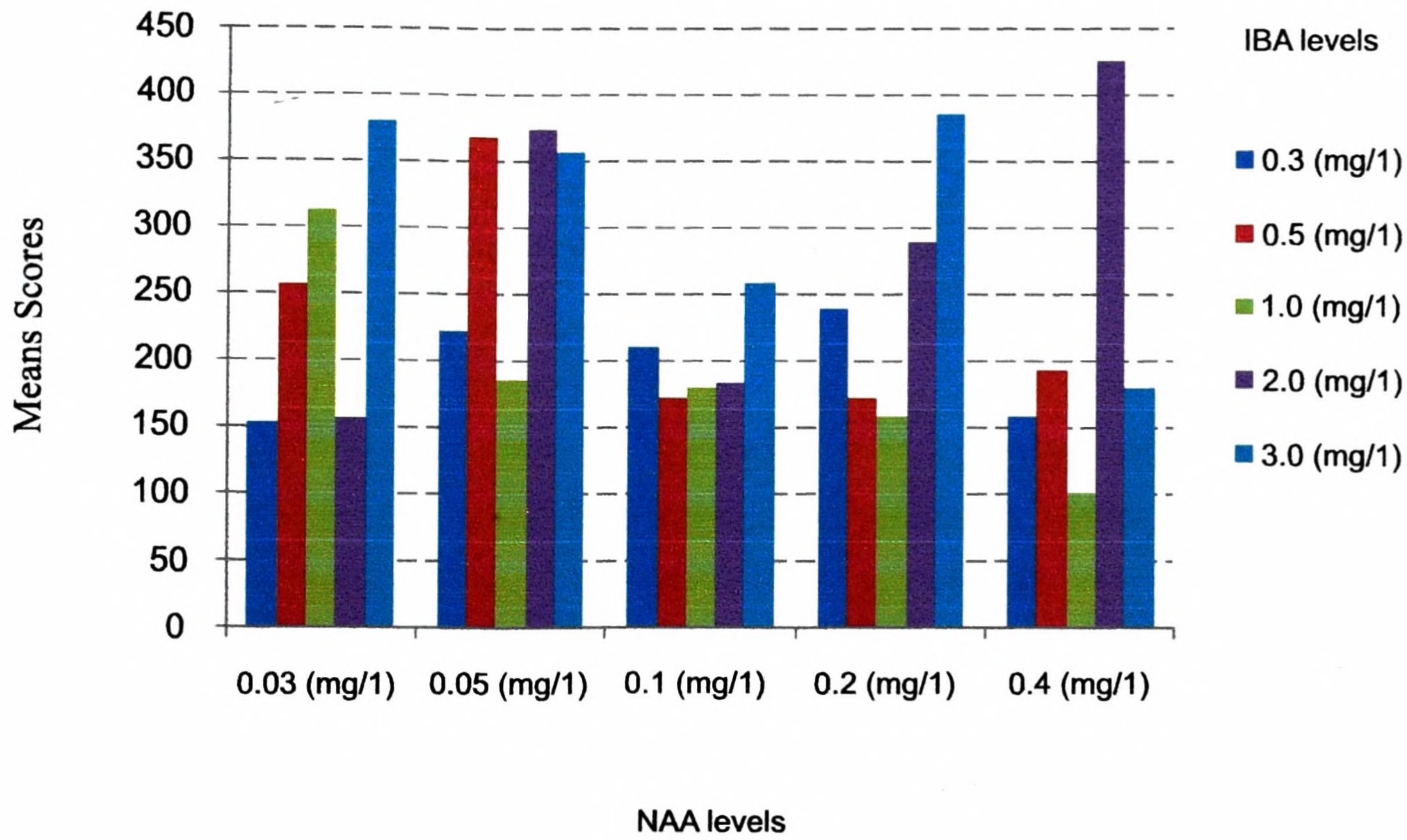

Figure 5: Mean scores for root vigour at different NAA and IBA level

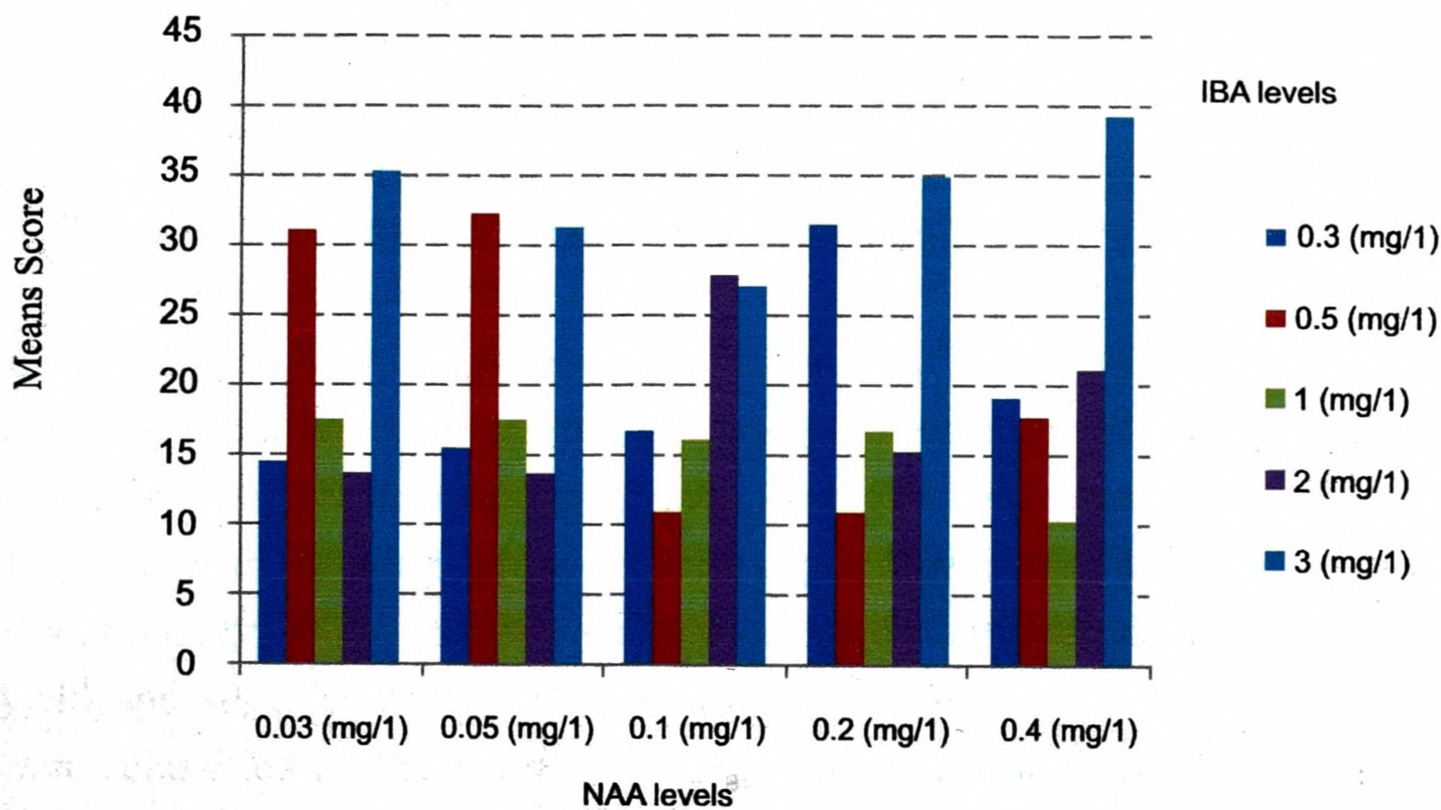

Figure 6: Mean root length at different NAA and IBA level

Conclusion

BA has a negative effect on the viability of meristem *ex-plants* at higher concentrations though it is essential in shoot germination, multiplication and elongation. The best BA and Kinetin levels (mg/l) for culturing meristem *ex-plants* of variety SL 96 328 are 0.01 BA + 0.05 Kinetin, 0.01 BA + 0.02 Kinetin, 0.05 BA + 0.05 Kinetin, 0.05 BA + 0.001 Kinetin, 0.1 BA + 0.02 Kinetin and 0.1 BA + 0.001. The best combinations of BA and Kinetin (mg/l) to be used in shoot multiplication in passage culture are 0.05 mg/l BA + 0.01 mg/l Kinetin, 0.03 mg/l BA + 0.02 mg/l Kinetin, 0.2 mg/l BA + 0.05 mg/l Kinetin, 0.03 mg/l BA + 0.01 mg/l Kinetin, 0.01 mg/l BA + 0.1 mg/l Kinetin for the same variety. For rooting of *in-vitro* generated multiple shoots of variety SL 96 328, the appropriate combinations of IBA and NAA (mg/l) are 0.5 IBA + 0.05 NAA mg/l, 3.0 IBA + 0.2 NAA mg/l, 3.0 IBA + 0.05 NAA mg/l and 3.0 IBA + 0.03 NAA mg/l.

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