Determination of Optimal Auxin and Cytokinin Levels for Meristem Culture of Sugarcane (*Saccharum* Hybrid Spp.): Variety SL 96 328

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Introduction

Non-availability of quality sugarcane planting material is a major constraint for cane production and sugar industry expansion in Sri Lanka. The Crop Improvement Division of the Sugarcane Research Institute of Sri Lanka actively engaged in the development of protocols in quality planting material production through micro propagation and in the other rapid sugarcane multiplication techniques (Wijesuriya *et al*., 2010). 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.

Methodology

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 ex-plants 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.

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

KIN mg/l \ BA 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

Table 2. Combinations of NAA and IBA levels tested in rooting and the treatment numbers assigned.

IBA mg/l \ NAA 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

Mortality of meristem *ex-plants* (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 were quantified using a 1 to 5 scale. Logistic regression analysis, Kruskal Wallis test, Wilcoxon rank sum test and analysis of variance were used in processing of these data.

Results and Discussion

Meristem culture: he mortality of *ex-plants* 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 explants 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 treatments (Table 1) were selected as the best combinations for meristem culture of variety SL 96 328.

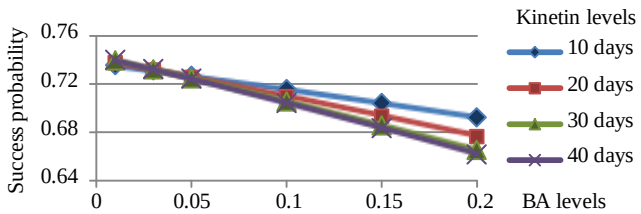

Figure 1. Fitted probability of success in shoot formation with tested BA levels for 10, 20, 30 and 40 days after inoculation

Passage culture: the mean scores for shoot vigour classified by BA and 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.

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, 12, 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 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 7, 24, 22 and 21 (Table 2) were selected for rooting of multiple shoots of variety SL 96 328.

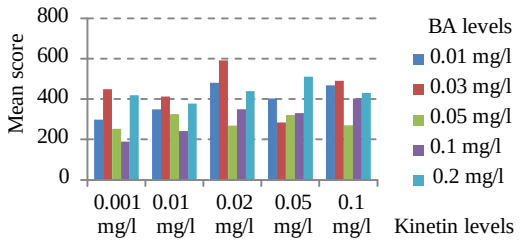

Figure 2. Mean scores for vigor of multiple shoots at different BA and Kinetin levels.

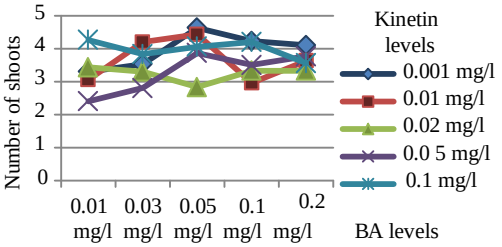

Figure 3. Mean number of shoots at different BA and Kinetin levels.

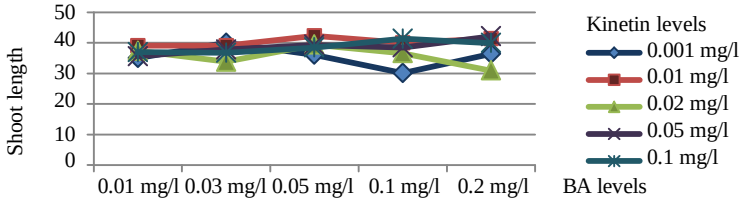

Figure 4. Mean number of shoots at different NAA and IBA levels.

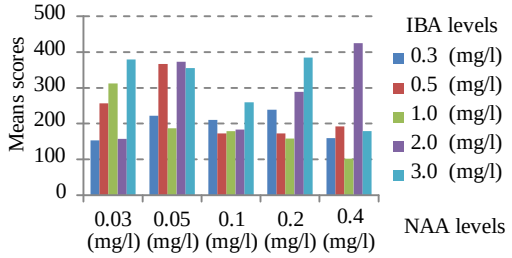

Figure 5. Mean scores for root vigour at different NAA and IBA level.

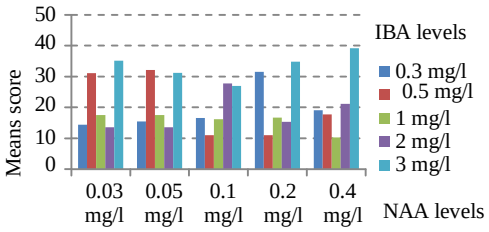

Figure 6. Mean root length at different NAA and IBA level.

Conclusions

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 *explants* 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.

References

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