Plant Tissue Cult. & Biotech. **32**(1): 89-101, 2022 (June) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v32i1.60475

PTC&B

Variable Responses of Two Banana Varieties during *in vitro* Multiplication

R. Reni Krishna, M.K. Sabitha[1], M.K. Gayathri, R. Aswini[2], E.K. Radhakrishnan[2] and A. Remakanthan*

Department of Botany, University College, Thiruvananthapuram, Kerala, India-695034

*Keywords:*Plant Growth regulators*, in vitro*culture, Banana, var. Robusta, var. Nendran, commercial plant tissue culture.

Abstract

Banana is the choicest crop for the micropropagation industries due to the recurrent requirement of large volume of plants in the domestic market. However, all the banana varieties cannot be cultured in media with the same composition to get the optimum shoots in multiplication cycleswith desirable shoot qualities. Banana varieties like Robusta grow optimally in Murashige and Skoog medium containing 3-5 mg/L BA (6-benzyl adenine). Under the same condition, the growth of Nendran is found to be poor with a low number of rootable shoots. The poor multiplication ratio causes a higher cost for the micropropagated plantlets of var. Nendran. Hence the current study was carried out to optimize the shoot multiplication of the two varieties of banana (Nendran and Robusta) in MS medium supplemented with various combinations and concentrations of plant growth regulators. The multiplying shoots were cultured in MS medium supplemented with different concentrations of BA, combinations of BA, NAA (α-naphthalene acetic acid) and combinations of KN (kinetin) and NAA. Combinations of BA and NAA (0.5 mg/L BA+0.1 mg/L NAA or 0.5mg/L BA+0.5 mg/L NAA) was found to result in responses ideal for commercial production of var. Nendran. For the var. Robusta, 2 mg/L BA was ideal for shoot multiplication and 1 mg/L BA+0.5 mg/L NAA could be optimal for use at elongation stage to get maximum desirable shoots for rooting. The study could optimize a suitable plant growth regulator regime for the commercial production of banana var. Nendran and could impact cost reduction of plantlets.

*Author for correspondence: <remakanthana@gmail.com>. [1]Department of Botany, SNGS College, Pattambi, Palakkad, Kerala, India. [2]School of Biosciences, M.G. Univesity, Kottayam, Kerala, India.

Introduction

Banana is a major food crop grown and consumed in more than 100 countries throughout the tropics and subtropics. In developing countries, they are the fourth most important food crop after rice, wheat, and maize (INIBAP 2000).The total annual world production of banana is estimated to be 113.91 million tonnes. India leads the world in banana production by contributing about 26.7% of world production (FAOSTAT 2017). Conventional propagation methods through suckers (Cronauer-Mitra and Krikorian 1988) are not ideal for banana as they carry pathogens, nematodes and viruses and also they have slow multiplication rate (Sagi et al. 1998). Hence clonally propagated banana through tissue culture is widely used.

The micropropagation of banana has facilitated the supply of high quality, disease free and uniform planting material to the farmers at an affordable price. Here the farmers are greatly benefitted by the large scale availability of disease-free planting material, high yield, low crop duration and high uniformity in the field in terms of vegetative growth, flowering, fruiting and timely harvest (Jasrai et al. 1999, CIAT 2006). In India, banana is micropropagated and planted in large numbers by virtue of being the choicest crop of Indian micropropagation industry. In India, the states which plant largest numbers of banana include Maharashtra, Gujarat, Karnataka, Andhra Pradesh, Tamilnadu etc. where the single variety 'Grand Naine' is preferred. Banana var. Nendran (*Musa* X *paradisiaca* var. Nendran AAB) and var. Robusta (*Musa acuminata* var. Robusta AAA) are grown in the large areas in the South Indian states Kerala and Tamilnadu (APAARI 2019). The Nendran variety of Banana has got starchy pulp on ripening and hence is used as a dessert fruit. Also this variety is most suitable for the banana chips preparation (raw and ripe fruits), and also for the baby food production. It is also the second banana variety being exported after Grand naine. Banana var. Robusta belong to Cavendish group and is also used as a dessert fruit.

Most of the farmers prefer to plant the suckers of banana due to the less availability and higher cost of micropropagated plants.During the industrial micropropagation of banana cv.Nendran, inherent problems could be observed during the multiplication cycles in MS medium supplemented with 6-benzyladenine (BA) alone (personal experience of the corresponding author). Here the explants respond slowly with lower multiplication ratio and mostly without leafy shoots. Sometimes leafless corm like structures are also formed with meristematic points in the cultures of Nendran. Due to these undesirable responses, the shoot cultures generally yield fewer rootable shoots in each multiplication cycle, which ultimately leads to the higher pricing of micropropagated plantlets of banana var. Nendran.

Plant growth regulators (PGR) are essential media components for manipulating the growth and development of explants *in vitro.* Their concentration and ratio in the medium often determines the pattern of development. Under the *invitro* conditions banana cultivars of different genomic groups have already been reported to behave

differently (Reshmi and Nair 2011). Cytokinins and auxins are generally preferred growth regulators for *Musa* tissue culture. Here, the shoot proliferation and elongation rates are affected by the cytokinin type and concentration. Murashige and Skoog (MS) (Murashige and Skoog 1962) medium supplemented with BAP has been generally used for themicropropagation of several *Musa* spp. (Madhulatha et al. 2004 Suada et al. 2015 and Choudhary et al. 2014). As the concentration of PGR optimal for one variety may not be suitable for other varieties, standardization of concentration of plant growth regulators for the enhanced proliferationof different varieties is essential for industrial application. Eventhough Devi and Nayar (1992) have described the micropropagation of banana var, Nendran using the MS medium supplemented with 22.22 μM BA, we could get only poor quality shoots in each multiplication cycle. Resmi and Nair (2011) have also reported the banana var. Nendran to have high multiplication rate in media containing 8.9 μM BA and 4.9 μM 2-iP. In our preliminary studies using this media,the response was same as that of media supplemented with BA (3 mg/L) alone and was without desirable shoot qualities. As the media composition from previous studies did not provide high quality shoots, the reported methods are not desirable for industrial level micropropagation. Hence the current study has been designed to optimize the culture media suitable for the increased multiplication rate of banana var.Nendran and to compare its performance with that of var. Robusta.

Materials and Methods

The medium employed throughout the study was MS medium with 30g/l sucrose, 5.8 g/l agar and growth regulators. All the chemicals used were from Himedia, India or SRL, India. The pH of the media was adjusted to 5.7. The media was dispensed in 300 ml bottles (50 ml/bottle) and autoclaved at 121°C for 15 min.

For the study, healthy sword suckers of banana varieties, Nendran and Robusta were collected from the farmers field in Thiruvananthapuram. The sheathing leaf bases were removed from the pseudostem and cut into 5 cm long pieces leaving the young leaf bases around the meristem with about 2 cm of corm tissue. The explant material was washed in running tap water for 15-20 min, after that it was kept in 5% teepol solution for 10 min and again washed to remove the traces of teepol. Subsequently, these were treated with 0.1% mercuric chloride solution for 10 min and washed thrice in sterilized distilled water. The explants were then trimmed from both ends and the outer sheaths were peeled off gradually. Intact shoot apex covered by a few leaf primordia with 1.5-2 cm length were dissected and placed on medium in an upright position and were maintained at a temperature of 25 ± 2°C for 16 h/day under 50μE m^{-2} s^{-1} light intensity provided by fluorescent lamps. The data were recorded and subcultures were carried out at 4 weeks interval. Meristem started to become swollen by the 1st week after inoculation. Later, the main shoot (apical meristem) and side shoots (axillay meristems) emerged and by 8 weeks, each meristem explants became shoot clumps with about 6-8 shoots. Transfer of

the meristem explants to fresh media was carried out in this period to reduce phenolic exudation and browning of explants. Initial culture of explants was carried out in MS medium with 3 mg/l BA.

Once the individual meristem explants became shoot clumps, it was further split into small clumps with 3 shoots each. For this, five clumps each with 3 shoots were transferred into fresh medium periodically at regular four weeks intervals. In order to optimizethe medium for multiplication of banana cv.Nendran, the explants were cultured in different combinations and concentrations of auxin and cytokinins. The shoot clumps were cultured in different concentrations of BAP (0-5mg/l), combinations of BAP (0.5 - 2 mg/l) andNAA (0.1- 1 mg/l), and combinations of kinetin (1-5 mg/l) and NAA (0.1 and 0.5 mg/l).Here, 10 replications were carried out for each medium combination. The multiplication of shoot culture was carried out for six successive cycles in the above selected media and the data collected in the fifth cycle were selected for detailed analysis. Here data collected for number of shoot, length of shoot, girth of shoot, number of leaves and length of leaf were statistically analysed by ANOVA and comparison among individual treatments were performed with Duncan's multiple range test using SPSS statistical software version 13.0.

After the multiplication studies, shoots from various cultures were isolated and rooted in MS medium supplemented with 0.5 mg/L IBA. For the acclimatization, rooted plantlets were washed with tap water and planted in polybags filled with potting mixture containing 1:1:1 (Sand : Soil: leaf mould). The plants were then covered with polythene sheets and kept in plastic trays filled with water upto 2 cm height for 2 weeks in polyhouse. Afterwards the tray with water and polythene sheet were removed and the plants were watered daily and fertilizer solution (mixture of rock phosphate - 4g/l, urea - 1.5 g/l, potash - 1.5g/l, $MgSO_4.7H_2O$ - 0.4 g/l and $CaCl_2.2H_2O$ –0.4 g/l) was also applied (50 ml/bag) at weekly interval.

Results and Discussion

The hormonal requirements of different banana varieties with different genomic combination varies for micropropagation (Reshmi and Ashalatha 2011). During multiplication cycles leafy shoots were rarely occurred in Nendran when cultured in MS medium with 3 mg/l BA which is suitable for multiplying Cavendish varieties (Jasrai et al. 1999, Suada et al. 2015, Chowdary et al. 2014 and Jafari et al. 2011). Therefore experiments were carried out to improve the culture qualities of Nendran by altering the plant growth regulator concentrations and combinations in the medium. Various concentrations of BA ranging from 0-5 mg/l were incorporated in the medium used for the shoot multiplication of banana var. Nendran and var. Robusta. Here, the shoot number was observed to be minimum in basal medium with just elongation of existing shoot buds (Tables1-2 and Fig. 1). Similar observation was also reported in Banana by Lohidas and Sujin (2015). Obviously, in the absence of external hormones, the existing

shoot buds in the explants elongate rather than producing multiple shoots. Sholi etal.(2009) reported that different concentrations of plant growth regulators are required for the multiplication of *Musa* cultivars in which BAP helps in the effective production of multiple shoots. Other parameters like the length of shoots, number of leaves, length of longest leaf etc. were maximum in the basal medium. In most of the previous studies on micropropagation of banana var. Nendran, shoot length was not considered as a parameter and only the shoot numbers were given importance (Reshmi and Nair 2011, Devi and Nayar 1992). In the commercial propagation, shoot number and shoot quality in terms of moderate length and girth and 1-2 leaves per shoots are considered desirable in multiplication cycles.

Table 1. Effect of various concentrations of BA on shoot proliferation in shoot clumps of Banana cv. Nendran.

Sl. No.	BA mg/l)	No.of shoots (Mean±SE)*	Length of shoots in cm (Mean±SE)*	Girth of shoots in cm (Mean±SE)*	No. of leaves (Mean±SE)*	Length of leaf in cm (Mean±SE)*
1	0	3.3±0.42	2.63±0.44	0.25±0.01a	0.90±0.21b	1.57±0.19c
2	0.5	6.9±0.40a	1.82±0.11	0.30±0.01ab	0.46±0.07a	0.88±0.23b
3	1	6.6±0.57a	1.15±0.10a	0.31±0.02b	0.88±0.17b	0.43±0.07ab
4	2	9.6±0.79	1.34±0.05a	0.42±0.01c	0.16±0.05a	0.50±0.00ab
5	3	6.6±0.42a	1.30±0.26a	0.31±0.0b	0.26±0.18a	0.44±0.02c
6	5	6.6±0.76a	1.20±0.08a	0.42±0.02c	0.34±0.17a	0.31±0.02a

*Values followed by same alphabet are not significantly different by DMRTat $P \geq 0.05$ within a column.

Table 2. Effect of various concentrations of BA on shoot proliferation in shoot clumps of Banana cv. Robusta.

Sr. No.	BA (mg/l)	No. of shoots*	Length of shoots*	Girth of shoots*	No. of leaves*	Length of longest leaf*
1	0	3.50 ± 0.52	3.81 ± 0.61	0.30 ± 0.02 b	2.02 ± 0.20	1.72 ± 0.10 b
2	0.5	6.80 ± 0.90ab	2.70 ± 0.25 bc	0.28 ± 0.01 a	1.85 ± 0.15 b	1.07 ± 0.11 a
3	1	5.50 ± 0.52a	3.04 ± 0.21 c	0.23 ± 0.01 a	2.18 ± 0.17 b	1.58 ± 0.17 b
4	2	8.10 ± 0.97b	2.31 ± 0.17 ab	0.37 ± 0.03 b	0.98 ± 0.13 a	1.00 ± 0.08 a
5	3	4.80 ± 0.41 a	2.07 ± 0.21 a	0.27 ± 0.01 a	1.14 ± 0.21 a	1.10 ± 0.10 b
6	5	5.40 ± 0.96a	1.99 ± 0.15 a	0.23 ± 0.01 a	1.22 ± 0.18 a	0.97 ± 0.09 a

*Values followed by same alphabet are not significantly different by DMRT at $P \geq 0.05$ within a column.

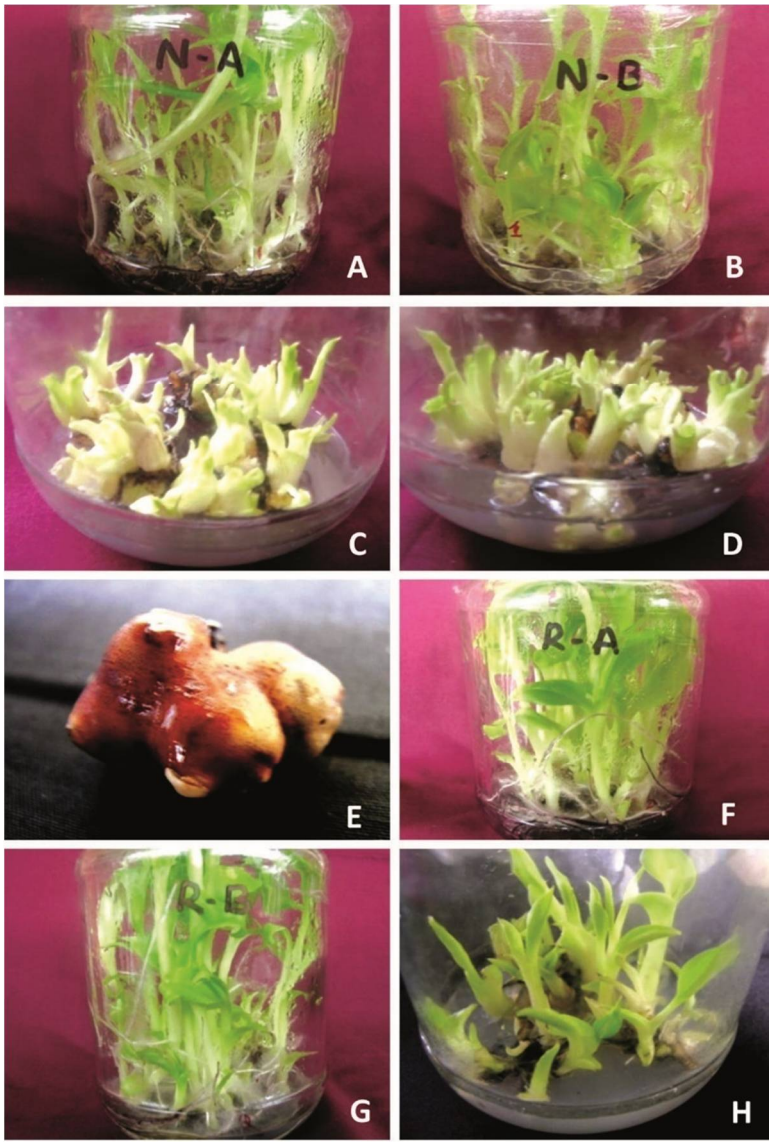

Fig 1. Effect of Various levels of BA on shoot proliferation of Banana var. Robusta and var. Nendran. A-E. Response of Banana cv. Nendran: A. basal medium. B . 0.5 mg/l BA. C. 2 mg/l BA, D. 3 mg/l BAP. E. Corm like structures without leaves and shoot in cultures supplemented with 3 mg/l BAP. F-H. Response of Banana var. Robusta: F. basal medium. G - 0.5 mg/l BAP. H. Response 3 mg/l BAP.

Increasing the concentration of BA from 0.5-5 mg/l was found to have a positive influence on the shoot multiplication in terms of number of shoots but higher levels reduced the length of shoot, number and length of leaves. The number of shoots were found to be in the range of 6-9 shoots per clump in all with maximum were observed in cultures supplemented with 2mg/l BA in Nendran (9.6 $\pm$ 0.79 shoots/clump). In the case

of var. Robusta, 2 mg/l BA induced maximum shoot proliferation (8.19 ± 0.97 shoots/clump). At higher concentrations of BA, shoot number was found to get reduced in Nendran. Here, the shoots formed were very small and mostly leafless. These types of shoot cultures are not ideal for commercial micropropagation as the number of shoots obtained with shoot length desirable for rooting was very low. In Nendran, shoot length, was found to be maximum and shoot girth was minimum in the basal medium (Table 1). A similar trend could also be observed with respect to the shoot number in var. Robusta (Table 2). In the case of Robusta maximum shoot proliferation could be observed in the medium supplemented with 2 mg/l BA. However, the shoot length was maximum in basal medium followed by medium containing 0.5 mg/l BA. Even in high concentration of BA (5 mg/l), the shoot length of 1.99 ±0.1 could be observed which was comparable to the length of shoots of Nendran in basal medium. From these results, the genotype can be considered to have important effect on the shoot length response of tissue cultured banana. Many previous studies have reported that 5 mg/l BAP as the optimum concentration for most of the banana cultivars (Vuylsteke and De Langhe 1985 ,Venkatachalam et al. 2007, Bairu et al. 2008). Jasrai et al. (1999) and Suada et al. (2015) have used 5.0 and 3.0 mg/l BAP for micropropagation of banana var. Robusta and var. Grandnaine respectively. Arinaltwe et al (2000) have reported that the *in vitro* shoot bud induction in banana explants is cultivar dependent. Eventhough Van den et al. (1998) and Victer et al. (1999) have reported higher BA levels in the medium to have effect to increase the number of shoots per explants, the shoot height was decreased or even abnormal shoot buds were developed in these cases.

In the present study, leaf number was observed to be highest in cultures developed in basal medium. Other parameters like the length of shoots, leaf number and leaf length showed a decreasing or comparable trend with increasing BA concentrations. Medium with 0.5mg/l BA was found to be better for shoot multiplication of Nendran since there was more shoots, good shoot length, number of leaves and length of leaves among the BA alone treatments. Higher levels of BA was found to reduce the length of shoots, number and length of leaves and also induced abnormal leafless corm like structures (Fig1E). Number of leaves was not significantly different in explants grown in basal medium, 0.5 and 1 mg/lBA supplemented media for Robusta (Table 2**)**. The length of leaves did not show much variation among the treatments for the var. Robusta.

To improve the shoot qualities of Nendran and Robusta in culture, combinations of auxins (NAA - 0.1, 0.5 and 1 mg/l) and cytokinin (BA - 0.5, 1 and 2 mg/l) were tried (Table 3 and Table 4). For the var. Nendran the number of shoots were highest in combinations of 0.5 mg/l BA + 0.1 mg/l NAA as well as 0.5mg/l BA+0.5mg/l NAA (Table 3). Here, 7-10 shoots per explant were obtained in these combinations. This response was in range, comparable or slightly higher than those observed in treatment of 0.5mg/l BA alone (Table 1)**.** Increasing the concentrations of NAA from 0.5 to 1 mg/l was found to have a negative effect on shoot number. At higher concentrations of NAA, the shoot numbers were reduced to 4-6 per explants. The shoot length in Nendran was increased

very much with incorporation of NAA alongwith BA. Maximum shoot length was obtained in 1mg/l BA and 1 mg/l NAA supplemented medium, but with reduction of shoot numbers. Optimum shoot length without compromising on the shoot number was obtained in combination of 0.5 mg/l BA and 0.1 mg/l NAA as well as 0.5 mg/l BA and 0.5 mg/l NAA. The leaf number/plant was also appreciable in these combinations.The results show that the optimal concentrations of BA and NAA are crucial to get the maximum shoots with desirable shoot length in Nendran.

Table 3. Effect of various concentrations of BA and NAA on shoot proliferation in shoot clumps of banana cv.Nendran.

Sl. No.	BA (mg/l)	NAA (mg/l)	No. of shoots*	Length of shoots*	Girth of shoots*	No. of leaves*	Length of longest leaf*
1	0.5	0.1	7.8 ± 1.11d	1.94 ± 0.21ab	0.25 ± 0.01ab	2.53 ± 0.23bc	0.47 ± 0.04a
2	1	0.1	5.3 ± 0.55bc	2.97 ± 0.34cd	0.27 ± 0.01abc	2.50 ± 0.21bc	0.63 ± 0.08ab
3	2	0.1	4.4 ± 0.61abc	1.60 ± 0.18a	0.27 ± 0.01abc	1.61 ± 0.25a	0.50 ± 0.06a
4	0.5	0.5	8.1 ± 0.80d	1.99 ± 0.12ab	0.34 ± 0.01d	2.74 ± 0.14c	0.36 ± 0.02a
5	1	0.5	5.5 ± 0.40bc	2.86 ± 0.23cd	0.31 ± 0.01cd	2.85 ± 0.16c	0.95 ± 0.08cd
6	2	0.5	5.1 ± 0.34bc	2.52 ± 0.26bcd	0.24 ± 0.01a	2.00 ± 0.27ab	0.79 ± 0.08cd
7	0.5	1	4.2 ± 0.41ab	2.37 ± 0.42abc	0.29 ± 0.02bc	1.92 ± 0.26ab	0.90 ± 0.17bc
8	1	1	3.1 ± 0.27a	3.29 ± 0.49d	0.25 ± 0.01ab	2.51 ± 0.42b	1.18 ± 0.14d
9	2	1	6.0 ± 0.51c	2.17 ± 0.19bc	0.25 ± 0.01ab	1.31 ± 0.18a	0.78 ± 0.10bc

*Values followed by same alphabet are not significantly different by DMRT at P≥0.05 within a column.

Table 4. Effect of various concentrations of BA and NAA on shoot proliferation in shoot clumps of Banana cv. Robusta.

Sl. No.	BA (mg/l)	NAA (mg/l)	No. of shoots*	Length of shoots*	Girth of shoots*	No. of leaves*	Length of longest leaf *
1	0.5	0.1	5.80 ± 0.69 a	4.09 ± 0.31 ab	0.25 ± 0.00 abc	2.77 ± 0.14 bcd	1.32 ± 0.08 bc
2	1	0.1	5.10 ± 0.65 a	3.36 ± 0.24 ab	0.20 ± 0.01 a	2.05 ± 0.19 a	0.81 ± 0.07 a
3	2	0.1	5.10 ± 0.34 a	3.15 ± 0.27 a	0.23 ± 0.01 ab	2.21 ± 0.19 ab	0.92 ± 0.07 ab
4	0.5	0.5	4.50 ± 0.30 a	5.25 ± 0.40 ab	0.34 ± 0.01 d	3.84 ± 0.21	1.81 ± 0.15 d
5	1	0.5	4.70 ± 0.30 a	5.50 ± 1.78 b	0.33 ± 0.01 d	3.31 ± 0.27 de	1.57 ± 0.16 cd
6	2	0.5	3.80 ± 0.35 b	4.87 ± 0.39 ab	0.26 ± 0.01 bc	3.07 ± 0.25 cd	1.68 ± 0.21 cd
7	0.5	1	3.70 ± 0.42 b	5.05 ± 0.43 ab	0.25 ± 0.01 abc	2.37 ± 0.18 ab	1.85 ± 0.15 d
8	1	1	4.80 ± 0.44 a	4.45 ± 0.40 ab	0.27 ± 0.04 bc	2.52 ± 0.20 abc	1.39 ± 0.12 c
9	2	1	4.30 ± 0.70 ab	3.13 ± 0.33 a	0.30 ± 0.02 cd	2.65 ± 0.27 abc	1.27 ± 0.17 bc

* Values followed by same alphabet are not significantly different by Duncan's Multiple range test at p≥0.05 within a column.

In Robusta, the incorporation of NAA has decreased the shoot numbers when compared to BA alone treatment. Shoot length of cultures was increased with the incorporation of NAA in medium and in most of the Robusta cultures the shoots reached a length of 4-5 cm which could not be achieved in any concentration of BA alone treatment. In the present study, incorporation of NAA was found to significantly affect the shoot length as well as number of leaves formed per shoot in Robusta (Table 4). These are desirable qualities as it provide more number of quality shoots at the rooting stage. The number of leaves formed was also found to get improved with the incorporation of NAA in the medium for the Robusta cultures. Some of the media combinations like 0.5 mg/l BA+0.1 mg/l NAA or 0.5 BA mg/l+0.5 NAA could be used at the elongation phase of Robusta cultures to maximize the quality shoot formation for rooting. Some commercial labs used to transfer the cultures to lower concentrations of BA (0.5 - 1.0 mg/l) to get elongated shoots desirable for rooting especially for the Cavendish varieties of Banana (personal experience of the corresponding author). Medium supplemented with BA and NAA combinations standardized in the present study could be used as elongation medium for Robusta. Here, the number of leaves increased significantly when compared to the BA alone treatment. But among the different concentrations of NAA used, the number of leaves did not show much variation **(**Fig. 2).

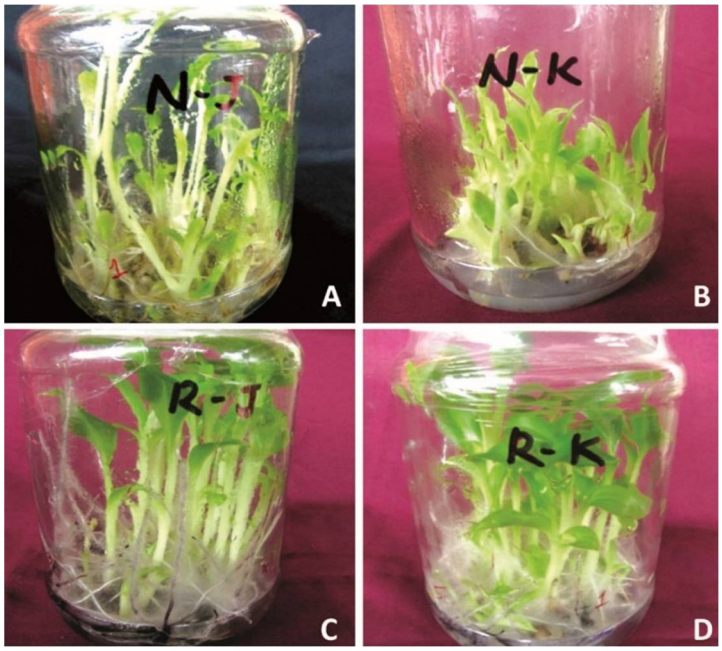

Fig. 2. Effect of BAP and NAA on shoot multiplication of Banana. Nendran: A. 0.5 mg/l BAP +0.5 mg/l NAA, B. 1 mg/l BAP and 0.5 mg/l NAA. Robusta: C. 0.5 mg/l BAP + 0.5 mg/l NAA. D. 1 mg/l BAP + 0.5 mg/l NAA.

The results showed that the incorporation of NAA at 0.5mg/l along with 0.5, 1 and 2mg/l BA to have significant effect to improve the shoot length and leafiness without

affecting the multiplication rate in Nendran. By incorporating the NAA to the medium, all undesirable features of Nendran cultures like poor shoot length, formation of corm like structures etc. did not occur. Banana shoots cultured in medium supplemented with 0.5 mg/lBA+0.5 mg/lNAA or 0.5 mg/lBA+0.1 mg/lNAA displayed highest shoot numbers with desirable shoot length and leafiness suitable for commercial level production. For the Robusta, 2 mg/l BA was ideal for shoot multiplication and 1 mg/l BA +0.5 mg/l NAA could be optimal for use at elongation stage to get maximum desirable shoots for rooting. From these results of the study, growth variation between Nendran and Robusta in response to BA and NAA treatment was evidence for the genotypic difference. This indicates that optimization of media for each variety/ genotype is essential for the cost effective production of banana at commercial level. Banana micropropagation supplemented with media using various plant growth regulator combinations has already been reported for various varieties of banana (Lohidas and Sujin2015, Sholi et al. 2009, Cronauer and Krikorian 1984, Rajoriya et al. 2018 and Rahman etal. 2004). Choudhary et al. (2014) have reported that the maximum number of shoot formation in MS medium supplemented with 2 mg/l BA + 0.5 mg/L NAA for the Robusta. At the same time, 3 mg/l BA and 0.2 mg/l NAA were reported to yield maximum shoot length and shoot number in Red banana (Rajoriya2018). Rahman et al. (2004) have reported the BAP (4 mg/l) + NAA (1.5 mg/l) to be effective in shoot proliferation of banana var. BARI-I.

Table 5.Effect of various concentrations of KN and NAA on shoot proliferation in shoot clumps of banana cv.Nendran.

Sl. No.	Kn (mg/l)	NAA (mg/l)	No. of shoots*	Length of shoots (cm)*	Girth of shoots (cm)*	No. of leaves*	Length of longest leaf (cm)*
1	1	0.1	2.7±0.49a	3.71±0.44b	0.18±0.01a	2.32±0.34bc	1.17±0.21 bc
2	3	0.1	3.5±0.54a	1.88±0.42a	0.26±0.02b	2.12±0.18bc	0.64±0.12a
3	5	0.1	3.7±0.61a	1.15±0.18a	0.35±0.02c	1.64±0.30ab	0.59±0.12a
4	1	0.5	3.3±0.21a	3.79±0.57b	0.16±0.01a	2.42±0.39bc	1.70±0.15d
5	3	0.5	2.9±0.43a	1.85±0.32a	0.27±0.02b	2.34±0.27bc	0.76±0.13ab
6	5	0.5	3.6±0.33a	1.84±0.35a	0.20±0.00a	1.27±0.26a	0.98±0.16abc

*Values followed by same alphabet are not significantly different by DMRT at $p \geq 0.05$ within a column.

Three levels of Kn (1,3 and 5mg/l) were also tried in combination with two levels of NAA (0.1 and 0.5mg/l). From the results, Kn was not found to have beneficial effect on shoot number compared to the basal medium. (Table 5, Table 6 and Fig.3). In addition, the shoot length, number of leaves and length of leaves obtained were very much similar to that formed in basal medium. Hence Kn can have no effect on the shoot multiplication of both banana varieties. This observation is in correlation with the findings of Shirani et

al. (2010). In contrast, Muhammad et al. (2007) have reported that the number of shoot formation increases with increase in Kn concentration up to a certain level after that higher concentration will decrease the formation of multiple shoots.

Table 6. Effect of various concentrations of Kn and NAA on shoot proliferation in shoot clumps of Banana cv. Robusta.

Sl. No.	Kn (mg/l)	NAA (mg/l)	No. of shoots	Length of shoots (cm)	Girth of shoots (cm)	No. of leaves	Length of long-est leaf (cm)
1	1	0.1	2.10 ± 0.17 a	2.66 ± 0.50 a	0.21 ± 0.01 a	1.47 ± 0.28 a	1.34 ± 0.15 a
2	3	0.1	2.80 ± 0.55 ab	4.09 ± 0.48 bc	0.51 ± 0.14	2.45 ± 0.29 bc	1.81 ± 0.24 ab
3	5	0.1	2.90 ± 0.72 ab	3.04 ± 0.41 ab	0.30 ± 0.01 a	1.65 ± 0.23 ab	1.34 ± 0.25 a
4	1	0.5	2.40 ± 0.37 ab	4.62 ± 0.60 c	0.27 ± 0.02 a	2.43 ± 0.32 bc	2.10 ± 0.24 b
5	3	0.5	2.60 ± 0.61 ab	4.33 ± 0.57 bc	0.25 ± 0.02 a	2.57 ± 0.30 c	1.66 ± 0.25 ab
6	5	0.5	3.70 ± 0.44 bc	3.46 ± 0.40 abc	0.22 ± 0.01 a	2.32 ± 0.27 bc	1.52 ± 0.18 ab

*Values followed by same alphabet are not significantly different by DMRT at $p \geq 0.05$ within a column.

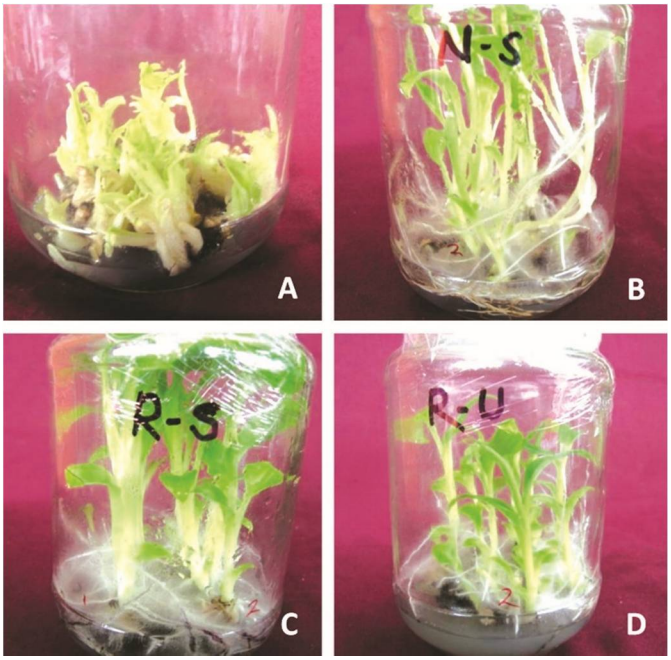

Fig. 3. Effect of Kn and NAA on shoot multiplication of Banana. Nendran :A. 5 mg/l Kn + 0.5 mg/l NAA. B. 1.0 mg/l Kn + 0.5 mg/l NAA. Robusta: C. 1 mg/l Kn + 0.5 mg/l NAA. D. 5 mg/l Kn+ 0.5 mg/l NAA.

The plantlets produced from various media were rooted in MS medium supplemented with 0.5 mg/l IBA. The plantlets after 3 weeks of growth in rooting media were acclimatized, hardened and transplanted in field conditions. There was no visible

difference in growth of plants obtained from various treatments at the rooting and hardening stage except that the number of desirable shoots suitable for rooting obtained in different media varied with media used for shoot multiplication.

From the results of the present study, the role of genotype on the effect of plant growth regulators in micropropagation system is confirmed. This necessitates the need to optimize the growth regulator levels for each variety for commercial production system. A combination of 0.5mg/l BAP + 0.5 mg/l NAA or 0.5 mg/l BA + 0.1 mg/l NAA was found to be the best media for improving the qualities of tissue cultured plants for the multiplication stage in banana cv.Nendran. For the Var. Robusta, 2 mg/l BA was ideal for shoot multiplication and 1 mg/l BA +0.5 mg/l NAA could be optimal for use at elongation stage to get maximum desirable shoots for rooting. On the contrary, Kn incorporation in the medium has reduced the number of shoots, shoot length and number of leaves formed from the explants in both varieties. Increasing the shoots for rooting in the multiplication cycles can enhance the number of plants that could be hardened and so the cost of production of plants could be reduced. So the multiplication medium standardized in the present study will be of immense use for the micropropagation industries engaged in production of banana plantlets of var. Nendran.

References

APAARI (2019) Banana Tissue Culture in India – A Success Story. Asia-Pacific Association of Agricultural Research Institutions, Bangkok, Thailand. xvi+43 p.

Arinaltwe G, Rubaihayo PR and **Magambo MJS (2000)** Proliferation rate effects of cytokinins on banana (*Musa* spp.) culitvars. Sci. Hortic. **8**:13-21.

Bairu MW, Stirk WA, Dolezal K and **Staden JV** (2008) The role of topolins in micropropagation and somaclonal variation of banana cultivars 'Williams' and 'Grand Naine' (*Musa* spp. AAA). Plant Cell Tiss. Organ Cult. **95:** 373-379.

Choudhary D, Kajla S, Poonia AK, Duhan JS, Kumar A and **Kharb P** (2014) An efficient micropropagation protocol for Musa paradisiacal cv. Robusta A commercial cultivar. Annals of Biology **30**(1): 25-31.

CIAT (2006) Annual report, Strategies developed for management of diseases and pests in banana based cropping systems. (Output 3) Genetic resistance to disease. CIAT. Cali Colombia, 1-10pp.

Cronauer SS and **Krikorian AD** (1984) Multiplication of *Musa* from excised stem tips. - Ann.Bot.**53**: 321-328.

Cronauer-Mitra SS and **Krikorian AD** (1988) Plant regeneration via somatic embryogenesis in the seeded diploid banana *Musa ornate* Roxb. Plant Cell Reports **7:** 23-25.

Devi DSR and **Nayar NK** (1992) Micropropagation in banana va.Nendran. Indian J. Genet. **53**(1): 76-78.

FAOSTAT (2017) Banana crop production. The food and agricultural organization of the United Nations (FAO), Rome, Italy.

INIBAP (2000) Networking banana and plantain: INIBAP Annual report 2000.

Jafari N, Othman RY and **Khalid N** (2011) Effect of BAP pulsing on *invitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. Afr. J. Biotechnol. **10**(13):2446-2450.

Jasrai YT, Kannan VR, Remakanthan A and **George MM** (1999) *Ex vitro* survival of *in vitro* derived banana plants without green house facilities. Plant Tissue Cult. **9**: 127-132.

Lohidas J and **Sujin D** (2015) Effect of growth hormones in the micropropagation of Banana cv. Matti. Plant Archives. **15**(1):307-314.

Madhulatha, P, Anbalagan M, Jayachandran S and **Sakthivel N** (2004) Influence of liquid pulse treatment with growth regulators on *invitro* propagation of banana (*Musa* spp. AAA). Plant Cell Tiss. Org. Cult.**76**: 189-191.

Muhammad AL, Hussain H and **Rashid SMS** (2007) Proliferation – rate Effect of BAP and Kinetin on Banana (*Musa* spp. AAA Group) "Basrai". Hort. Sci.**42**:1253-1255.

Murashige T and **Skoog F** (1962) A revised Medium for Rapid Growth and Bio-Assays with Tobacco Tissue Cultures. Physiologia Plantarum, **15**:473-497.

Rahman M Z, Nasiruddin KM, Amin MA and **Islam MN** (2004) *In vitro* response and shoot multiplication of Banana with BAP and NAA. Asian J. Plant Sci. **3**: 406-409.

Rajoriya P, Singh VK, Jaiswal N and **Lall R** (2018) Optimizing the effect of plant growth regulators on *in vitro* micro propagation of Indian red banana (*Musa acuminata*). J. Pharmaco. Phytochem., **2018**: 628-634.

Reshmi L and **Nair AS** (2011) Differential effect of cytokinins in the micropropagation of diploid and triploid Musa cultivars. Int. J. Integr. Biol. **11**(1): 35-38.

Sagi L, Gregory DM, Remy S and **Swennen R**(1998) Recent developments in biotechnological research on bananas (*Musa* spp.). Biotechnol. Genet.Rev.**15**: 313-317.

Sholi NJY, Chaurasia A, Agarwal and **Sarin NB** (2009) ABA enhances plant regeneration of somatic embryos derived from cell suspension cultures of plantain cv. Spambia (*Musa* sp.). Plant cell Tiss. Organ Cult.**99**:133-140.

Shirani S, Sariab M, Zakaria W and **Muziah M** (2010) Scalp induction rate responses to cytokinins on proliferating shoot tips of banana cultivars (*Musa* sp.). Am. J. Agri Biol. Sci. **5**(2):128-134.

Suada EP, Jasim B, Jimtha CJ, Gayatri GP, Radhakrishnan EK and **Remakanthan A** (2015) Phytostimulatory and hardening period reducing effects of plant associated bacteria on micropropagated Musa acuminata cv. Grand Naine. In Vitro Cell. Dev. Biol. -Plant **51**: 682-687.

Van-den HI, Guns J and **Swennen R** (1998) Bacterial contamination in Musa shoot tip cultures. Acta Horticulturae **490**:485-492.

Venkatachalam L, Sreedhar RV and **Bhagyalakshmi N** (2007) Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR makers. Plant Growth Regul. **51**: 192-205.

Victer JMR, Murch SJ, Krishna RS and **Saxena PK** (1999) Somatic embryogenesis and organogenesis in peanut: The role of thiadiazuron and N6-benzylaminopurine in the induction of plant morphogenesis. Plant Growth Regulation **28**:9-15.

Vuylsteke DR and **De Langhe EA** (1985) Feasibility of *in vitro* propagation of bananas and plantains. Trop. Agric. (Trin.). **62**: 323-328.

(Manuscript received on 9 May, 2022; revised on 12 June, 2022)