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Standardization of *in vitro* rooting and *ex vitro* hardening in traditional banana cultivars of Kovvur Bontha (ABB) and TellaChakkeraKeli (AAA)

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Abstract
Banana is most important crop in India and fetching more foreign money in international trade. Banana and plantain are important staple foods that are critical to nutritious and economic well-being of millions of people across the globe. Banana cv. TellaChakkeraKeli (AAA) is choicest table variety in Andhra Pradesh and popular in Godavari districts. Banana cv. Kovvur bontha (ABB) is highly popular in AP and other southern districts mostly used for culinary purpose. Disease free high yielding planting is major constraint for commercial production of both these native genotypes. Poor *in vitro* root induction and *ex vitro* survival are the major hindrance for its large scale commercial production. Therefore, the objective of the present investigation was to develop an efficient culture media for *in vitro* rooting and *ex vitro* performance of both the genotypes. Among the different treatments evaluated, micro-shoots cultured on ½ MS media supplemented with two kinds of auxins (NAA and IBA at lower concentration) were proved to be good in terms of highest root induction in banana cv. TellaChakkerakeli and Kovvur Bontha. Among the different treatments, shoots cultured on ½ MS media supplemented with 0.5 mg l^{-1} NAA gave 100 % rooting, increased girth (2.23, 2.01 mm), pseudostem height (6.09, 5.77 cm), leaf area (7.60, 7.53 cm^2) and no. of leaves per shoot (5.60, 5.23) in both TellaChakkerakeli and Kovvur Bontha respectively. Under *ex vitro* hardening 100 % survival was obtained on the ½ MS media supplemented with 0.5 mg l^{-1} NAA (for cv. TellaChakkerakeli) and on 0.5 mg l^{-1} IBA (for cv. Kovvur Bontha) which are proved to be the best treatments accordingly for most of the characters in both the cultivars.

Keywords: Auxin, native banana, micro-propagation, rooting, hardening

Introduction
Banana is economically most important crop in India and fetching more foreign money in international trade. It is consumed by more than 400 million people in the world and in terms of consumption it is next to rice, wheat and maize. *Musa* spp. are large perennial herbs belongs to the monocotyledonous family *Musaceae*, order Zingiberales. It is originated from South Asia to South-East Asia and Polynesia. It is referred as "Kalpatharu", a plant of all virtues, with each and every part of the plant is being used for various purposes. It is believed to be one of the oldest fruits which have originated from Malaysia through a complex hybridization process (Novak, 1992) [8]. Cultivated banana is a triploid (2n = 3x = 33) derived from diploid species that is *Musa acuminata* (Malaysia) and *Musa balbisiana* (India). Triploid cultivars are the most widely cultivated clones of commerce due to their vigorous growth and higher yield than diploids. Most of the banana cultivars are specific to the regions in different parts of India.

Tella Chakkerakeli (AAA) is considered the best dessert banana in Circar districts of Andhra Pradesh owing to its characteristic taste, aroma and quality. The bunches are small and loose with short stout slightly curved fruits glistening green and fairly rounded with a conspicuous apex. The rind is thick, pulp is yellowish with high TSS and characteristic pleasant aroma. It is tolerant to Panama disease but highly susceptible to *Erwinia*wilt and *Eumusae* leaf spot diseases. Each bunch weighs 6-8 kg with 5-6 hands and 70-80 fruits.

Kovvur Bontha (ABB) is a popular genotypewidely used as cooking banana in most of the states in India. Fruits are larger, stout with less prominent ridges and bottle neck apex. The rind is thick and green with whitish pulp. This variety is tolerant to *Eumusae* leaf spot but susceptible to bacterial rhizome rot disease caused by *Erwinia*. Each bunch weighs 18-20 kg with 5-6 hands and 70-80 fruits.

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In recent times, due to the conventional propagation with suckers, the plantations of Kovvur Bontha and Tella Chakkerakeli are highly suffering with various viral diseases and also losses occurring due to bacterial rhizome rot disease caused by *Erwinia carotovora* sub sp. *carotovora*. Moreover, unavailability of adequate number of quality, disease-free planting material is a major hindrance for area expansion in these native cultivars. Improvement through traditional breeding methods is difficult and time-consuming because of high sterility, polyploidy and long generation timein this crop. By employing tissue culture techniques for these popular traditional genotypes, one can produce large number of genetically uniform quality planting material (QPM) within a short period of time throughout the year. There is lot of demand for tissue culture plants of these native varieties as they are bestowed with highest field establishment, rapid growth, precocious bearing, higher and uniform yields, quality fruits and disease free in nature than conventional propagation method by using sword suckers. Most of the commercial tissue culture labs producing Grand Naine tissue culture banana plants only and not attempted in these traditional varieties owing to the lack of standardized protocols inspite of their huge demand from farmers.

Earlier, several researchers optimized the protocols for local varieties specific to the particular region (Nasiruddin *et al* 2006; Azam *et al*., 2010; Govindaraju *et al*., 2012) [6, 1]. *In vitro* protocol for the commercial production of tissue culture plants of Tella Chakkerakeli and Kovvur Bontha was optimized at Horticultural Research Station, Kovvur for culture establishment and shoot multiplication. However, in both these genotypes some of the abnormalities like poor shoot growth, less rooting percentage, production of unhealthy roots under *in vitro* conditions and weak pseudostems, lanky growth, higher mortality in *ex vitro* hardening are the major limitations which needs optimization to step-up the large-scale commercial QPM production. The benefit of any micro-propagation system can, however, only is fully realized by the successful transfer of plantlets from tissue-culture vessels to the ambient conditions found in *ex vitro.* The present study was therefore conducted with the objective to standardize the suitable growth regulator for *in vitro* rooting and *ex vitro* survival in both the genotypes.

Materials and methods

The present investigation was carried out from July, 2019 to January, 2021 at Tissue culture laboratory, Horticultural Research Station, Kovvur, West Godavari District of Andhra Pradesh. After 8th multiplication cycle, micro-shoots of banana cv. Kovvur Bontha (ABB) and Tella Chakkerakeli (AAA) were collected and used for the experimentation.

Culture media

The mineral formulation employed was MS (Murashige and Skoog, 1962) [5] medium. The culture medium was supplemented with suitable growth regulators in respect of the various stages of *in vitro* culture and MS salt strength was also reduced as per thetreatments. The cytokinin BAP (Benzyl Amino Purine), auxins *viz*., IBA (Indole Butyric Acid) and NAA (Naphthoxy Acetic Acid) were involved in culture establishment and shoot multiplication. The carbon source employed was sucrose and the gelling agent was agar. Individual micro-shoots were obtained by separating the multiple shoot clumps using sterilized scalpel and cultured on rooting medium containing ½ MS medium supplemented with activated charcoal 2.5 g l^{-1} and different auxins in varied concentrations and combinations (Table 1) for four weeks. The cultures were maintained at standard culture conditions of 25 ± 2°C temperature with 70 to 80 % humidity under 2500 to 3000 Lux light at 16 hrs. light / 8 hrs. dark conditions. At the end of experiments, morphological characteristics (percent rooting, avg. no of roots/shoot, days taken for rooting, psuedostem height, girth, number of leaves etc.,) were measured.

Statistical analysis

The data was analyzed using computer software programmed by the method of variance outlined by Panse and Sukhatme (1997) [9]. Observations recorded as percentage were subjected to angular transformation (Snedecor and Cochran, 1980) [12]. The mean values were calculated on various growth attributes were tabulated and analyzed statistically by using Completely Randomized Design. The differences among the treatment means were statistically tested at 5% level of significance.

Results

In vitro rooting

Different growth regulators and their combinations used for in vitro rooting of banana micro-shoots (Table. 1). Under our experimental conditions, among the different treatments tested, the highest rooting percentage was recorded in both ½ MS + 0.5 mg l^{-1} NAA (T_1) and ½ MS + 0.5 mg l^{-1} IBA (T_3) (100%) in both the banana genotypes. However, the least rooting percent was noticed in ½ MS medium devoid of any growth regulators as control (T_0) in Tella Chakkerakeli (62.22%) and Kovvur Bontha (44.44%). Earliest root initiation (15.73 days) was observed in case of the treatment where ½ MS media supplemented with 0.5 mg l^{-1}NAA in Tella Chakkerakeli whereas, in Kovvur Bontha shoots cultured in control recorded the earliest rooting followed by ½ MS media supplemented with 1.0 mg l^{-1}NAA. In both the genotypes, more number of days required for rooting at higher auxin concentration in combination (Table 1). Maximum number of roots per shoot (10.97) was observed in ½ MS media supplemented with 0.5 mg l^{-1}NAA in Tella Chakkerakeli whereas, in Kovvur Bontha shoots cultured in 0.5 mg l^{-1} IBA recorded more number of roots (10.73) per shoot.

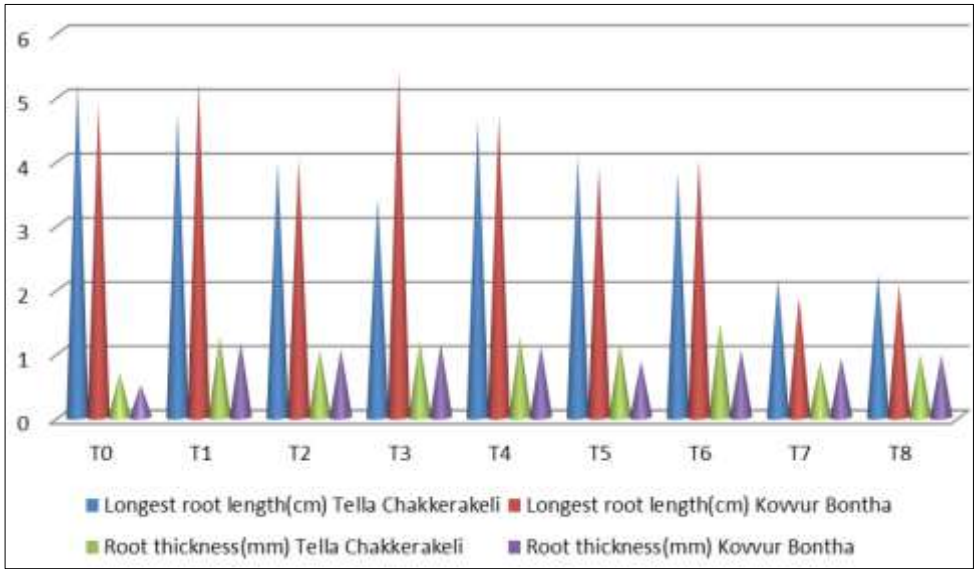

Fig 1: Effect of different growth regulators and their combinations on root length and thickness in banana cv. Tella Chakkerakeli (AAA) and Kovvur Bontha (ABB)

In both the genotypes, lengthiest roots were obtained in control followed by ½ MS media supplemented with 0.5 mg l^{-1} NAA in Tella Chakkerakeli (4.76 cm) and 0.5 mg l^{-1}IBA in Kovvur Bontha (Fig. 1). Though, longest roots recorded in control, the roots were very thin might be non-functional in nature. Among the treatments, in both the genotypes, maximum root thickness was recorded in ½ MS media supplemented with 0.5 mg l^{-1}NAA. The roots induced in IBA were pure white in colour whereas, roots induced in NAA media pale white in colour.

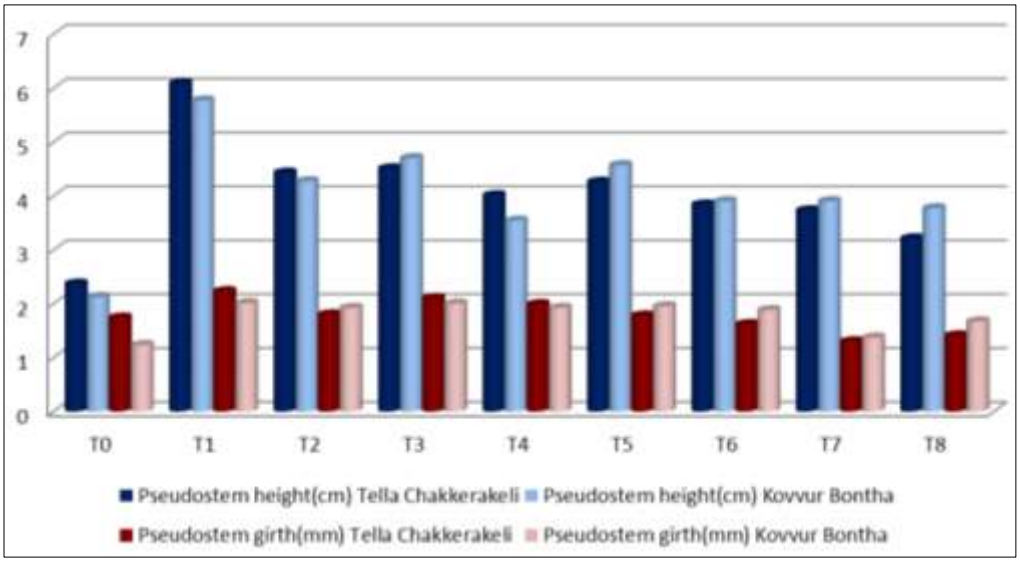

Fig 2: Effect of different growth regulators and their combinations on shoot length and thickness in banana cv. Tella Chakkerakeli (AAA) and Kovvur Bontha (ABB)

Among the different treatments, significant differences were observed for *in vitro* shoot length and girth. In banana cv. Tella Chakkerakeli maximum shoot height was recorded in ½ MS medium supplemented with 0.5 mg l^{-1} NAA (6.09 cm) followed by ½ MS + 0.5 mg l^{-1} IBA (4.51 cm) whereas minimum shoot height was recorded on control (2.37 cm) (Fig. 2). Similar trend was recorded in banana cv. Kovvur bontha in which lengthiest shoots produced in ½ MS medium supplemented with 0.5 mg l^{-1} NAA (5.77 cm). In a similar way, in both the cultivars the shoot girth was maximum in 0.5 mg l^{-1} NAA supplemented medium (Fig. 2).

Evaluation of different in vitro rooted shoots in ex vitro hardening

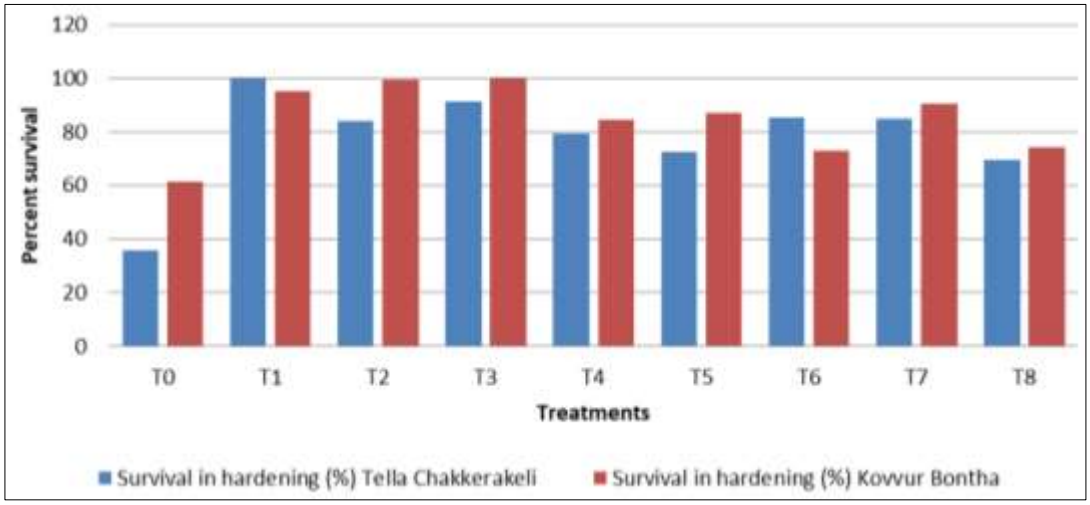

Fig 3: Effect of different growth regulators and their combinations on *ex vitro* survivalin banana cv. Tella Chakkerakeli (AAA) and Kovvur Bontha (ABB)

Among the treatments, in vitro shoots rooted in ½ MS medium supplemented with 0.5 mg l^{-1} NAA recorded 100% survival in cv. Tella Chakkerakeli whereas, only 95.3 % survival recorded in Kovvur bontha in this treatment (Fig 3). In case of Kovvur Bontha, maximum percentage survival recorded with 0.5 mg l^{-1} IBA followed by 1.0 mg l^{-1}NAA. Under our experimental conditions, 6.78 and 6.07 number of roots recorded in shoots rooted with 0.5 mg l^{-1} NAA in cv. Tella Chakkerakeli and Kovvur Bontha respectively. Among the different growth regulator combinations, significantly lowest number of roots per shoot was noticed in control (Table 2).

In banana cv. Tella Chakkerakeli the longest root length (21.45cm) was obtained in ½ MS + 0.5 mg l^{-1} NAA whereas, in cv. Kovvur Bontha the longest root length of 19.86 cm was recorded in same treatment. Significantly, highest root thickness was recorded in 0.5 mg l^{-1}NAA and 0.5 mg l^{-1}IBA in both the genotypes over all other treatments (Table 2).

It was clearly evident from table 2 that, maximum shoot height (17.95 cm) was recorded in shoots rooted in 0.5 mg l^{-1} NAA in banana cv. Tella Chakkerakeli whereas, 0.5 mg l^{-1}IBA recorded high psuedostem height in banana cv. Kovvur Bontha (17.16 cm).

Table 1: Effect of different growth regulators and their combinations on rooting percentage, average number of days taken for rooting and number of roots per shoot in banana cv. TellaChakkerakeli (AAA) and Kovvur Bontha (ABB)

Treatments Details		Percent rooting		No of days taken for root initiation		No. of roots/shoot	
		TellaChakkerakeli	Kovvur Bontha	TellaChakkerakeli	Kovvur Bontha	TellaChakkerakeli	Kovvur Bontha
T_0	Control (½ MS media devoid of any growth regulator)	62.22 (52.16)*	44.44 (41.79)	16.37	14.30	5.87	5.60
T_1	½ MS + 0.5 mg l^{-1} NAA	100.00 (90.00)	100.00 (90.00)	15.73	19.10	10.97	9.77
T_2	½ MS + 1.0 mg l^{-1} NAA	93.33 (77.85)	97.78 (85.00)	17.72	15.23	9.07	9.00
T_3	½ MS + 0.5 mg l^{-1} IBA	100.00 (90.00)	100.00 (90.00)	16.40	19.00	10.10	10.73
T_4	½ MS + 1.0 mg l^{-1} IBA	93.33 (77.85)	86.67 (68.99)	16.40	15.97	6.10	6.17
T_5	½ MS + 0.5 mg l^{-1} NAA + 0.5 mg l^{-1} IBA	95.56 (82.86)	75.56 (60.39)	16.20	15.83	7.23	7.03
T_6	½ MS + 1.0 mg l^{-1} NAA + 0.5 mg l^{-1} IBA	80.00 (63.62)	80.00 (63.62)	18.92	16.50	9.40	7.90
T_7	½ MS + 0.5 mg l^{-1} NAA + 1.0 mg l^{-1} IBA	84.44 (66.86)	88.89 (70.71)	18.50	16.80	5.43	5.83
T_8	½ MS + 1.0 mg l^{-1} NAA + 1.0 mg l^{-1} IBA	77.78 (61.89)	77.78 (61.90)	20.20	17.00	6.97	7.27
SEm±		3.47	2.46	0.71	0.25	0.21	0.11
CD at 0.05		10.40	7.36	2.11	0.74	0.63	0.34

*Figures given in parentheses are angular transformed values

Table 2: Effect of different growth regulators and their combinations on *ex vitro* hardening in banana cv. TellaChakkerakeli (AAA) and Kovvur Bontha (ABB)

Treatments Details		No. of roots/shoot		Longest root length(cm)		Root thickness(mm)		Pseudostem height(cm)	
		Tella Chakkerakeli	Kovvur Bontha	TellaChakkerakeli	Kovvur Bontha	TellaChakkerakeli	Kovvur Bontha	TellaChakkerakeli	Kovvur Bontha
T_0	Control (½ MS media devoid of any growth regulator)	3.45	3.06	6.86	9.38	1.34	1.07	13.43	10.83
T_1	½ MS + 0.5 mg l^{-1} NAA	6.78	6.07	21.45	19.87	2.19	2.61	17.95	16.18
T_2	½ MS + 1.0 mg l^{-1} NAA	3.33	5.14	15.20	17.98	1.74	1.78	14.58	15.34
T_3	½ MS + 0.5 mg l^{-1} IBA	6.44	6.18	15.27	14.89	2.04	2.68	16.6	17.16
T_4	½ MS + 1.0 mg l^{-1} IBA	5.34	4.29	19.97	14.46	1.52	1.84	17.2	15.61
T_5	½ MS + 0.5 mg l^{-1} NAA + 0.5 mg l^{-1} IBA	3.48	4.44	11.41	15.10	1.49	1.86	16.32	14.81
T_6	½ MS + 1.0 mg l^{-1} NAA + 0.5 mg l^{-1} IBA	6.32	5.00	13.42	14.09	1.52	1.76	16.51	14.50
T_7	½ MS + 0.5 mg l^{-1} NAA + 1.0 mg l^{-1} IBA	6.29	4.22	11.20	14.81	1.60	1.68	17.54	14.61

T_8	½ MS + 1.0 mg l^{-1} NAA + 1.0 mg l^{-1} IBA	5.29	5.04	7.23	9.43	1.32	1.82	14.97	15.06
SEm±		0.08	0.19	0.23	0.18	0.144	0.08	0.56	0.35
CD at 0.05		0.24	0.57	0.67	0.52	0.431	0.24	1.68	1.04

Discussion

Auxins are very widely used in banana tissue culture in combination with cytokinins for shoot multiplication and singly for root induction. These are an integral part of nutrient media. At the cellular level, auxins control basic processes such as cell division and elongation. Since, they are capable of ignite cell division they are responsible for formation of meristems giving rise to either unorganized tissue or defined organs like shoot and root. The choice of auxin and the concentration mostly depends on the type of growth required, the natural levels and endogenous synthesis within the explant. Effect of auxins on explants can be represented as bell-shaped activity curve. Depending on the endogenous level of auxins in different plant species, at low concentrations (0.1 – 10 µM) the effect or response usually increases with concentration, but at higher level, these are often inhibitory. This negative effect is usually due to an increase in ethylene production at higher auxin concentrations (George *et al*., 2008) [2]. Most of the investigators have used mixtures of many auxins in different plant species (Blackmon *et al*., 1981b) [14], but as the effect of individual compounds can vary in different genotypes. In our present study similar observations were recorded. For both the genotypes, lower concentration of 0.5 mg l^{-1}NAA or IBA was highly suitable over higher concentrations and combination with other auxins.

In general, half MS media supplemented with 0.18 mg l^{-1} NAA is recommended for rooting in banana cv. Grand naine. Vuylsteke and De Langhe (1985) [13] reported that optimal concentration for IBA was 1 µM (≈0.2 mg l^{-1}) for banana cultivars they were testing. The same concentration is not effective in quality *in vitro* root induction in banana cv. Tella Chakkerakeli and Kovvur Bontha. This might be due to cultivar variability to the response of growth regulators in banana, each variety responds differently to the equal concentration of growth regulators. In the present studies, 100% rooting was recorded in both the cultivars in shortest possible time when the micro-shoots were cultured in ½ MS media supplemented with 0.5 mg l^{-1} NAA or IBA. The longest roots and with more number of roots per shoot were also recorded on this medium. The requirement of higher auxin concentration for these cultivars might be due to presence of low endogenous auxin level. These results are in agreement with the results of Rahman *et al*. 2004 [10] and Govindaraju *et al*. 2012. They also reported the efficiency and suitability of higher concentration of NAA and IBA on banana cv. Bari-1 and banana cv. Rasthali varieties respectively. Munguatosha *et al*. 2013 [4] observed higher response of root initiation with 2 mg l^{-1} IBA in banana cv. Yangambi. In their studies, Raut and Lokhande (1989) [11] also necessitate the use of auxins in *in vitro* root induction. The results from the present study are in conformity with the above reports. In our study, it was evident that best rooting and other rooting related parameters was observed in ½ MS media supplemented with 0.5 mg l^{-1} NAA. This might have a positive influence on the *ex vitro* survival of both these traditional banana cultivars.

For the first time we have studied the effect of different auxin types, concentrations and their combinations on *ex vitro* survival and growth of tissue culture plants. Among the different treatments tested, in banana cv. Tella Chakkerakeli 100% *ex vitro* plant survival was recorded with micro-shoots rooted in ½ MS medium supplemented with 0.5 mg l^{-1} NAA whereas, in banana cv. Kovvur Bontha highest (100%) *ex vitro* survival was recorded with 0.5 mg l^{-1} IBA. These observations were clearly indicating the necessity of the specific rooting hormone for different native banana cultivars for better *in vitro* rooting and higher *ex vitro* survival. Our present study revealed that, the type and concentration of auxins had higher influence on *ex vitro* plant survival and performance. These findings confirmed the results reported by Nazki *et al.* (2015) [7] in gerbera.

Conclusion

Among the different growth regulators testedin both the varieties, ½ MS + 0.5 mg l^{-1} NAA treatment is proved to be the best treatment in terms of improved root and shoot characters with more plant survival percentage followed by ½ MS + 0.5 mg l^{-1} IBA.

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