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Effect of auxin on *in vitro* rooting of the triploid *Musa paradisiaca* L. cv. Nendran (AAB)

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Abstract

Banana is an important fruit crops of the family Musaceae. Tissue culture multiplication of banana is now popular and preferred due to faster multiplication, uniformly and cost-effective production of healthy planting materials. The following work was take on to study the result of various concentrations of auxin (indole-3-butyric acid-IBA). Shoots derived from sucker explants after a series of multiplication (4 to 5), regenerated plants having 4.5 cm length were dissected out and rooted in different concentrations of auxin (IBA) (0.5 to 3.5 mg/L). Root production was started after 10 days of culture and the data taken after 20 days. From all the treatment tested all media concentrations produce roots at varied level with in a period of 15-20 days of culture. Media having concentrations of IBA were used to induce *in vitro* rooting in plantlets of *Musa* cv. Nendran after six sub cultures including elongation. The elongated shoots harvested from culture bottles were transferred to MS solid media supplemented with auxin IBA at varied concentrations. The root induction rate varied from one media concentration to another. The present study revealed that IBA (1.0 mg/L) was found to be most applicable hormone for root induction in sucker shoot tip explants of *Musa* cv. Nendran.

Keywords

banana; *in vitro*; auxin; *Musa*; tissue culture

Introduction

In the world, Banana is considered as the most major and lucrative money crops as well as fruit crops (1). After rice, wheat and maize, Banana (*Musa* spp.) is represented as the fourth most essential food crop (2). Because of their commercial status banana and plantains are referred usually as "poor man's apple". Conventionally, cultivated bananas are propagated through suckers, which is very difficult and time consuming especially when large numbers of planting materials are required round the year. About 67 million tonnes of dessert bananas are produced annually worldwide, but only 20% of them are traded internationally. Micropropagation in the rapid clonal propagation of plants, which is one of the important contributions of tissue culture. Micropropagation techniques have been used in many parts of the world to produce healthy, disease-free banana plants throughout the year that perform better under field condition (3).

In *Musa, in vitro* tissue culture propagation systems are very important. Because it can give superior, invariable plants and it is free from diseases and worms, and a lot of the planting material used in trade plantations, and progressively in smallholder costruction, comes from mass micro propagation. MS (murashige and skoog) is the most commonly used plant tissue culture medium. Shoot tip cultures have been most widely used in *Musa* (7). Bananas have also been

rooted *in vitro* using MS medium supplemented with several auxins, including IBA, IAA, and NAA. By promoting cell proliferation, differentiation, and expansion, auxins promote the initiation of lateral roots and the formation of primordiums (4). As per the findings of (5,6), auxins have a favorable impact on the root system regeneration of plants that have been transplanted. The two auxins that are most frequently employed in nurseries are naphthyl-1-acetic acid (NAA) and indole-3-butyric acid (IBA).

Banana cultivars of different genomic groups also behave differentially under *in vitro* conditions. However, literature available on the characterization of *in vitro* responses of Indian banana cultivars to different plant growth regulators is limited. The majority of researchers have concluded that IBA is the best auxin available for assisting in vitro-developed banana shoots to root (8-11).

Discovery of naturally available plant hormones and making of synthetic growth regulators, auxins have been systematically used to activate rooting of cuttings. Another important role of auxins, especially control of growth and development including formation of adventitious roots (12). Rooting process control was long believed to be attributed to auxin, which is known to be involved in cell expansion. Rooting in micropropagation of *Musa* has been included both in auxin supplemented and auxin-free media. The most commonly utilized auxin is indole-3-butyric acid (IBA) which is a more potent hormone than indolilo-3-acetic acid.

The following study is aimed to study the effect of various concentrations of auxin on *in vitro* response of *Musa* cv. Nendran and to determine the ideal dose of auxin to achieve greater rooting.

Materials and Methods

The explant is taken from *Musa* coming under Musaceae family. The cultivar variety is 'Nendran'. Shoot tip used as the source of explant for *in vitro* culture. Usually healthy and disease free 1 to 3 feet suckers of banana 'Nendran' collected from Nedumangad, brought to JNTBGRI tissue culture laboratory. Shoot tip explants having 1.5'' diameter to 2.5'' in length was isolated.

The explants were washed in distilled water thoroughly and transferred to a beaker containing 5% bleach solution (cleaning agent) and 0.4% soap solution (surfactant) and treated for 50 minutes. After that a few drops of 70% ethanol was added into the above solution and shake gently and kept for 5 minutes. After being cleaned once more in distilled water the explants were cut to a size of roughly 3.5cm in length and 2cm in diameter. Following a 20 minute treatment with 15% bleach solution and a 7 minute surface sterilisation with 0.1% mercuric chloride, the explants were moved to the laminar air flow hood. These explants were again washed using sterile distilled water for at least thrice. The explants were further trimmed (1.5× 2cm) aseptically and inoculated. The explants were inoculated on MS medium.

For culture initiation, a basal medium comprising of MS medium supplemented with 10% tender coconut water and 3% sucrose was prepared and used for inoculation of fresh explants. Culture initiation liquid media were supplemented with 2 mg/L BAP for subsequent sub cultures a basal medium comprising of MS medium supplemented with 10% coconut water, 3% sucrose, 50 mg/L AS and 0.7% agar was used.

After shoot bud formation the shoots formed were subjected for multiplication (3-4) cycles. The elongated shoots formed after a series of multiplication were used for the present study. For which uniform plantlets selected from the multicultures were treated with media having different hormonal concentrations (MS media with IBA 0.5-3.5 mg/L and media devoid of hormone.

In order to study the effect of auxin on root induction the basal media were supplemented with different concentrations of auxin (MS media with IBA 0.5-3.5 mg/L and media devoid of hormone) were used. Required quantity of plant growth regulators and stock solutions kept in refrigerator were added to the media. The pH of culture media were adjusted to 5.7 with the help of either 1 NaOH/HCl and solidified with 0.7% agar prior to autoclaving at 151 b/inch^2 pressure and 121°C for 18 minutes. Distilled water was used for the preparation for stock solutions and media. The aseptic transfer was performed in laminar air flow hood kept in a clean room of tissue culture unit.

Cultures were housed in a growth environment at a temperature of 24±2°C and 1000 lux of cool white fluorescent light. The photoperiod of room was modified to 16-L/8-D hours daily with the help of automatic timers. For checking temperature and relative humidity, thermometer, hygrometer are fixed on the wall of the culture room.

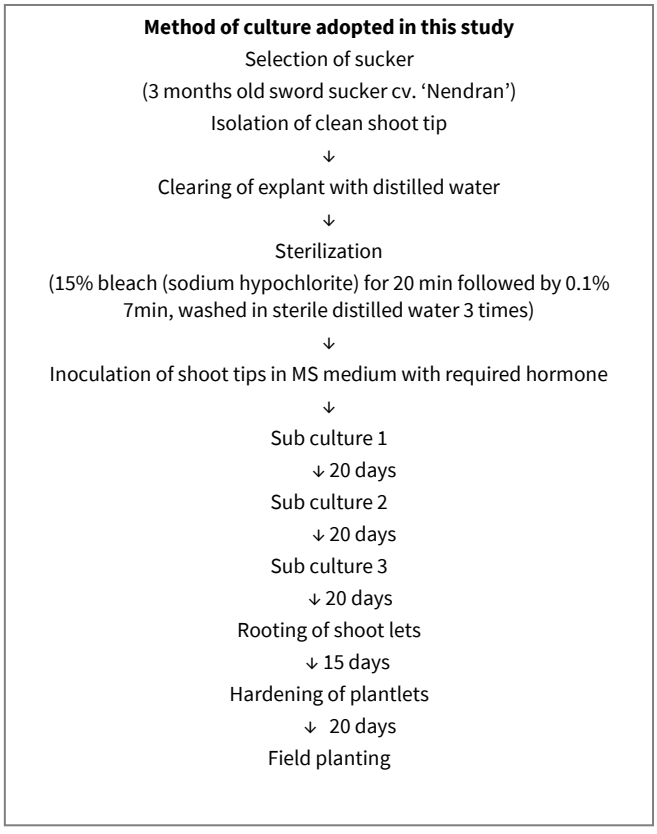

Figure 1. Method of culture adopted in this study

Root production was started after 10 days of culture and the data taken after 20 days. The rooted plants produced through the experiment were deflasked and transferred to green house for further growth.

Results and Discussion

From all the treatment tested different media concentrations produce roots at varied level with in a period of 15-20 days of culture. MS media, supplemented with IBA at 7 concentrations were used for the present study (Fig.1). Media without hormone (basal) also tested for the study as control. The root induction rate varied from one media concentration to another. Application of higher concentration of IBA showed retardation in root production. Results showed that cent percent explants were obtained in contamination free and all the explants were responded positively. The results obtained from the experiments have been presented in Table 1.

It was noticed that the root formation increased from the lower concentration to higher concentration up to 1.0 mg/L. A drastic reduction in the number of root formation can be seen in MS medium supplemented with IBA 3.5 mg/L. when concentration increased from 2.0 mg/L to 3.5 mg/L a gradual decrease in root number as well as root elongation can be seen.

The study revealed that low level of IBA concentration can produce healthy roots and early rooting can be seen in all the treatments except control media. Adventitious rooting can be seen in all the shoots irrespective of the hormone tested. Rooting mediums from control to 3.5 mg/L can be seen in (Fig. 3). Maximum response in 1.0mg/l and minimum response in 3.5 mg/L are represented in (Fig.4). It has been discovered that the root elongation phase is extremely sensitive to auxin concentrations; high auxin concentrations restrict root elongation, whereas medium IBA concentrations (IBA 0.5 and 1.0 mg/L) stimulate both root induction and root elongation (Fig.2). Therefore the optimal medium for *in vitro* rooting of *Musa* cv. 'Nendran' was determined to be MS medium added with IBA (1.0 mg/L), resulting in a maximum root length of (5.60 ± 2.30 cm) and among these concentration IBA 1.0 mg/L showed maximum number of roots (4.10 ± 0.58). The root length of concentration from control to 3.5 mg/L represented as A to H respectively (Fig.5)

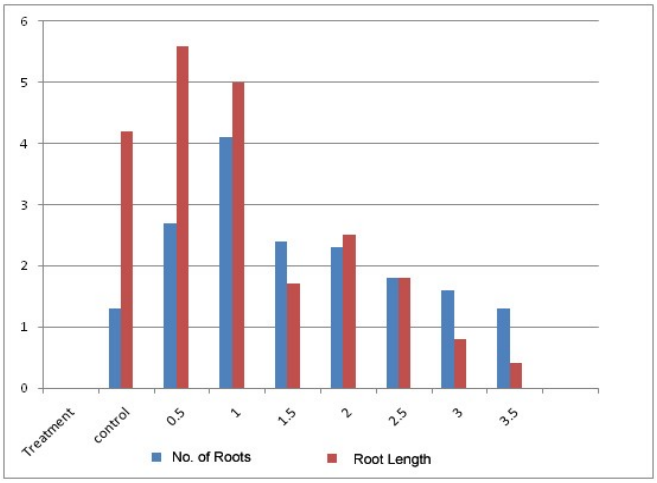

Figure 2. *In vitro* morphogenetic response of *Musa* cv. Nendran at different concentrations of IBA

The present study revealed that IBA (1.0 mg/L) was determined to be the most appropriate hormone for root induction in sucker shoot tip explants of *Musa* cv. Nendran.

Auxin was linked to a variety of plant activities, including stem development, adventitious root formation, lateral bud suppression, fruit and leaf abscission, and cambial cell activation, according to studies on the physiology of auxin action. The usefulness of this substance in promoting root development on cuttings was established by researchers. Approximately at the same time, it was demonstrated that two artificial materials, 2-naphthalene acetic acid and indole-3-butyric acid, were even more successful in rooting than the naturally occurring or artificial IAA.

Table 1. Effect of Auxin (IBA) on *in vitro* rooting of *Musa* cv. Nendran.

Sl. No	Treatment* (mg/ml conc.)	Average no of roots ± SD	Average root length ± SD
T1	Control	1.30 ± 0.20	4.20 ± 1.80
T2	0.5	2.70 ± 0.30	5.00 ± 2.00
T3	1.0	4.10 ± 0.58	5.60 ± 2.30
T4	1.5	2.40 ± 0.27	1.70± 0.70
T5	2.0	2.30 ± 0.22	2.50 ± 0.90
T6	2.5	1.80 ± 0.20	1.80 ± 0.60
T7	3.0	1.60 ± 0.10	0.80 ± 0.20
T8	3.5	1.10 ± 0.10	0.40 ± 0.10

MS Basal medium + 3% sucrose+ 0.7% agar and pH 5

Figure 3. *In vitro* culture of Banana cv. Nendran on rooting medium

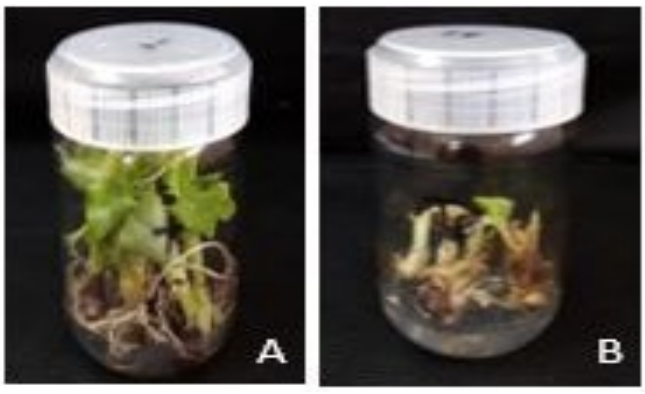

Figure 4. (A). Maximum response in 1.0 mg/L, (B). Maximum response in 3.5 mg/L

Figure 5. (C). Rooting in control media, (D). Rooting in 0.5 mg/L, (E). Rooting in 1.0 mg/L, (F). Rooting in 1.5 mg/L, (G). Rooting in 2.0 mg/L, (H). Rooting in 2.5 mg/L, (I). Rooting in 3.0 mg/L,(J). Rooting in 3.5 mg/L

Now a day, the plant growth regulators are widely used in modern agriculture to promote rooting. (13) reported that good rooting and the best survival were obtained with IBA treatment in *Hibiscus rosasinensis.* (14) reported that IBA had a highly significant effect on the percentage success of rooting, number of root and length of root production *Ixora coccinia*. (15) compared the influence of growth regulators on root induction of *Musa* genus plants cultivated with in *in vitro* conditions. In certain species, the rooting ability of cuttings has been increased by the use of plant growth retardants in addition to auxin. The current study's findings are consistent with (40), They used IBA and IAA at 1.0 and 0.5 mg/l to increase the development of excised potato roots.

In the present study, difference in auxin (IBA) concentrations showed various root initiation and elongation response. Response of *Musa* cv. 'Nendran' explants to auxin was in general experiment with the results reported in various plants. The findings revealed that auxin is also an influential factor in the *in vitro* rooting of banana. In the present root regenerative effect was expressed in varying magnitude according to plant growth regulator treatments. Such morphogenetic response was best expressed in auxin treatments. Auxin is widely used on the stem cuttings for accelerating the formation of adventitious roots (16). Auxin has an effect on speed and increases the percentage of rooting of stem cuttings (17) IBA is the most effective on promoting root initiation and adventitious root production in stem cuttings (18). The first adventitious roots appear from callus and they are main roots for cuttings. Callus contains high amount of auxins (19).

(20) Investigated the effect of auxin concentration on the rooting of *Stewartia pseudocamellia* and announced that cuttings treated with rooting hormones had higher rooting percentages (71.9% to 93.6% as compared with the control 53%). (21) obtained sufficient number of roots in the micropropagated shoots of banana cv. Basari in half MS+2.0 mg/L IBA. (22) Also found similar results in the micropropagation of banana cv. Sagar. (23) Reported that consistently more number of roots are produced when MS media is supplemented with moderate concentration of IBA, NAA and activated charcoal. In the present study IBA alone got good result.

The root elongation phase has been found to be very sensitive to auxin concentration, high concentration of auxin inhibit the root elongation and a medium concentration of IBA supports the induction of root. But in the present study low concentration of IBA supported root elongation and moderate concentration produced more number of roots.

(24) Reported that more roots were produced in the MS with IBA media. This also favours the present study. (25) Recommended 2.5 mg/L of IBA, NAA and 0.5 mg/L activated charcoal for the early rooting of shootlet in the micropropagation of banana. (26) Obtained the average of 8.28 roots per plantlet on 0.5 mg/L IBA, they got 2.60-5.67cms range of root length in 0.5 mg/L of IBA. But the present study 1.0 mg/L IBA got best result. (27) Reported that the number of roots produced per plantlet increased with increasing concentration up to 2 mg/L IBA and declined thereafter up to 5 mg/L.

Here number of roots produced per plantlet increased with increasing concentration up to 1.0 mg/L IBA and declined up to 3.5 mg/L IBA. Among the IBA treatments 2.0 mg/L was found to be the most effective in rooting of banana plantlets (27). The highest number of roots/plantlet was obtained in 2 mg/L IBA. The rate of increase in root length was rapid in the media with 2or 3 mg/L IBA but was slower in the media with 5.0 mg/L and control. In the present study supported the above statement in the case of root elongation.

The greater ability of IBA compared with IAA to promote rooting is due to this relatively higher stability (28). (29) Studied the stability of IAA and IBA under various tissue culture procedures. They showed that the concentrations of IAA and IBA in autoclaved MS medium were reduced by 40% and 20%, respectively, compared with filter sterilized controls. Under growth chamber conditions, IAA and IBA losses from both liquid and agar-solidified MS were significant. In liquid medium, IAA was

more sensitive than IBA to known biological degradation. found that labelled IBA and IAA were metabolised rapidly by cuttings of mung bean (*Vigna radiata* L.), and 24 h after application only a small fraction of radio activity of both auxins corresponded to the free auxin. IAA was metabolised more quickly than IBA by green cuttings of *P. tremula* L. (30) Also observed a rapid disappearance of IBA in pear (*Pyrus communis* L.) plantlets propagated *in vitro*. Only a small fraction of the total extractable radio activity could be identified as free IBA after 12 h of incubation. In apple shoots cultured *in vitro* only 5% of IBA and 1% of IAA were found in the free form. Production of cyclodipeptides with possible roles in auxin signaling was also determined in *P. putida* and *P. fluorescens* culture supernatants by gas chromatography–mass spectrometry. *P. putida* and *P. fluorescens* stimulated lateral root and root hair formation and increased plant biomass, which correlated with an induction of the auxin response (41).

(31) Studied root length in 2.9µm IAA, recorded 2.85cm longest root and 14.5µm IAA produced shortest root (2.23cm). As regard to IBA level, 0.5 mg/L IBA shown long length of roots 4.5 and 5.9 in 15 and 30 DAI respectively, the combined effect among IBA level were not statistically significant, supporting the present study. (32-34) Also reported more or less similar results. In relation to this investigation, different researchers investigated on the influence of media composition on days to rooting. The purpose of multiplication stage is not simply to produce large number of shoots that give minimal difficulties at these subsequent critical stages. (35) Reported that the best quality control during multiplication, grading will be necessary to produce even sized shoots that will root synchronously. The Cascade variant of H. lupulus responded well to several forms of auxin and cytokinin that were introduced to the culture media at varying concentrations (42).

The criteria for the achievement of optimal growth and rooting have included shoot number produced during any one culture. (36) Rooting percentage (37) and the quality of rooting as measured by the number of roots per rooted shots. With this idea the effect of media composition of IAA, IBA hormones were studied. As regard to IBA levels of this study the number of roots concerned was significantly influenced at 20 DAI. (38) Observed maximum rooting on MS medium of half strength supplemented with IBA 1.0 mg/L (39) This result partially supports the present study.

Conclusion

The technology of micropropagation holds great promise for growing high-quality plants and isolating beneficial variants in highly productive genotypes that are better suited to withstand stress and disease. Banana in vitro cultivation has been widely utilized to reproduce plants in large quantities. The findings shown here suggest that, in this widely used variety, a single application of auxin at a relatively low dosage was found to be appropriate for enhanced root formation and growth. In the present study, maximum root induction was achieved from cultures treated with 1.0 mg/L IBA. Plant Tissue Culture directly influences modern agriculture and has been the driving force behind the modernization of agriculture in developed nations. This has been made possible by the vast amount of the intended planting/sowing material that has been obtained by mass micropropagation.

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Author's contributions

The research was planned and designed by SJ and RBJ. SJ did all experiments of tissue culture, collected and analyzed data. RBJ supervised and reviewed the overall research activities of the project.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: "None".

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