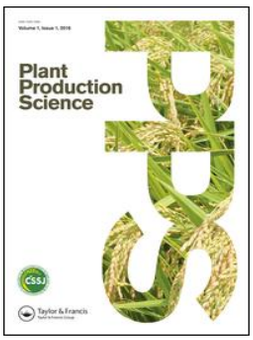

Plant Production Science

ISSN: 1343-943X (Print) 1349-1008 (Online) Journal homepage: https://www.tandfonline.com/loi/tpps20

Nursery Growth of Banana (*Musa* spp.) Plantlets Rooted on Auxin-free and Auxin-supplemented Media

John Nelson Buah, Yoshinobu Kawamitsu & Seiichi Murayama

To cite this article: John Nelson Buah, Yoshinobu Kawamitsu & Seiichi Murayama (1998) Nursery Growth of Banana (*Musa* spp.) Plantlets Rooted on Auxin-free and Auxin-supplemented Media, Plant Production Science, 1:3, 207-210, DOI: 10.1626/pps.1.207

To link to this article: https://doi.org/10.1626/pps.1.207

Plant Prod. Sci. 1(3) : 207—210 (1998)

Tissue Culture

Nursery Growth of Banana (*Musa* spp.) Plantlets Rooted on Auxin-free and Auxin-supplemented Media

John Nelson Buah, Yoshinobu Kawamitsu and Seiichi Murayama

(*College of Agriculture, University of the Ryukyus, Nishihara-Cho, Okinawa 903-0213, Japan*)

Accepted 6 January 1998

Abstract : This paper describes the effects of auxin added to the culture medium on main and branch root formation of banana (*Musa* spp.) shoots and growth characters of the plantlet rooted on the medium with and without auxin. Banana shoots cultured *in vitro* on Murashige and Skoog medium supplemented with 2 μM 1-naphthylacetic acid (NAA), rooted earlier and also had more adventitious roots than those cultured on the medium without NAA. However, the adventitious roots formed on the medium without NAA showed more lateral branching. Plant height and number of leaves per plantlet in *in vitro* culture were not influenced by the addition of NAA but under nursery conditions, plantlets rooted without NAA showed better growth in terms of days to the appearance of new leaf, plant height and number of leaves per plant. This might be due to the presence of abundant lateral roots. Even though auxins are generally known to promote rooting, NAA inhibited the formation of lateral roots in Banana plants.

Key words : Banana (*Musa* spp.), *in vitro*, Lateral roots, Nursery 1-Naphtylacetic acid.

The use of micropropagation of fruit trees including the banana is spreading throughout the world. The success of any *in vitro* rapid multiplication technique is assessed by how well the plantlets grow after transplanting to pots, which largely depends on effective root formation protocols.

The central role of auxin is not only to increase the percentage of shoots with roots but also to increase the number of roots per plantlet (Hartmann and Kester, 1975). However, some studies indicate that root induction is inhibited by auxin in a wide range of concentrations in media (Margareta and Inger, 1981), and that the root promoting effects of auxins are based on some circumstantial evidence (Hart and Carlson, 1967).

Rooting in micropropagation of *Musa* has been induced both in auxin-supplemented and auxin-free media. For example, NAA and IAA have been used as root-inducing hormones *in vitro* (Bulakrishnamurthy and Rangnasamy, 1988 ; Kshanika and Niranjali, 1997). On the contrary, Subramanya and Schwandes (1984), Cronauer-Mitra and Krikorian (1987), Novak (1992) and Bart et al. (1993) observed rooting in all *Musa* shoots cultured *in vitro* on auxin-free medium. It is worth noting that in rooting the shoots of *Musa in vitro*, much concern should be given to the formation of lateral roots (feeder roots) because these are primarily responsible for water and mineral nutrient uptake by the plant ; hence dictating the initial establishment and survival of plantlets after transfer to greenhouse conditions. As there is a good correlation between bunch weight and the quantity of feeder roots (Lassoudiere, 1978), increasing the number of lateral roots is important for the establishment, growth and survival of micropropagated *Musa* plantlets. Lateral root formation in *Musa* is a delicate process which requires a critical balance of specific substances and it is controlled by conditions quite different from those that support general cell proliferation (Torrey, 1956). However, no work has been done on the differences if any, on the roots of *Musa* plantlets formed on auxin-free and auxin-supplemented media in terms of the proliferation of feeder roots and whether these expected differences have any effect on the performance of *Musa* plantlets after transplanting to pots.

In this study we examined differences in the root induction on the two types of media and its effect on initial growth and survival in micropropagated banana plant.

Materials and Methods

1. Plant materials

Shoot tips of shima banana (AAA group), a local type from Okinawa Prefecture, Japan were isolated and cultured *in vitro*. The original materials were collected in December 1994 from sword suckers of banana plants which had been growing in the open and well irrigated field.

2. Preparation of plant material for culture

The top of the shoots (about 8 cm) were cut together with some of the leaf sheath forming the pseudostem. They were then sterilized with 70% alcohol for 3 min. More of the sheathing leaf bases of the pseudostem were removed in a laminar flow chamber until the size of the explant was about 1cm with two or three leaf sheaths

Corresponding author : Y. Kawamitsu (kawamitu@agr.u-ryukyu.ac.jp, fax +81-98-895-8734).

covering the shoot tip. This was followed by 5 min of sterilization with sodium hypochlorite solution (1% active chlorine) containing a drop of tween 20 with occasional shaking (Cronauer and Krikorian, 1984). After rinsing in sterilized distilled water, each shoot tip was divided into two, and sterilized again as above for one min and then rinsed three times in sterilized distilled water before placing each half in a bottle containing 20 mL of the medium. In all, 20 bottles each containing one explant were used in the following experiments.

3. Medium composition and culture conditions

The MS medium (Murashige and Skoog, 1962) containing 20 g L^{-1} sucrose and 5 μM 6-benzyl-aminopurine (BA) solidified with 2 g L^{-1} gellan gum was used to develop the shoots. The medium was adjusted to pH 5.8 prior to autoclaving at 121°C for 15 min. The cultures were kept under a photoperiod of 16 h light at an intensity of 71.6 μmol m^{-2} s^{-1} at 27°C.

4. Subculture and rooting

Explants were transplanted onto fresh medium three weeks after the start of the culture and every two weeks thereafter. Visible shoots were formed four weeks after the start of initial culture. At the seventh week, each shoot was separated and transplanted onto fresh medium at one shoot per bottle. The average plant height at this stage was 5 cm, with an average of one fully opened leaf. The medium used for this subculture was the same as that used for the shoot initiation. At this stage, a total of 45 bottles were obtained.

At the 10th week (in February 1995), two groups of shoots (bottles) randomly selected from the above 45 bottles were transplanted to : (i) MS medium without BA but supplemented with 2 μM 1-naphthylacetic acid (NAA) and (ii) MS medium without plant hormones. The bottles were placed under the same culture conditions as the shoot initiation phase and the days to rooting was examined. The number of roots, plant height, number of leaves and the presence of lateral roots *in vitro* were examined 12 weeks from the start of the experiment.

5. Growth of plantlets after transferrence to greenhouse

Two weeks after the start of rooting treatment, the plantlets, 10 from each group, were transplanted into pots containing vermiculite and transferred to the greenhouse. The days to the emergence of new leaf were recorded. The plant height, number of leaves and leaf and plantlet survival in pots were examined three weeks after transplanting. The data were analysed by perfoming the t-test for the means of the treatments except for the percentages of the plants with particular characterisitcs.

Results and Discussion

Auxins have been reported to shorten the time required for the regeneration of roots as well as increase in the number of roots per culture in *Musa* shoots (Hiratsuka et al., 1989). However, it has also been shown that auxins are not essential for rooting of the shoots of *Musa* (Novak et al., 1986 ; Bart et al., 1993). For example, in the report of Novak et al (1986), 60—80% of shoots formed *in vitro*, rooted on shoot-forming medium even before transplanting onto root-inducing medium. The experiments presented here confirmed both of these observations.

Table 1 shows the days to rooting, root number per plantlet, plant height and number of leaves per plantlet of *Musa* rooted on auxin-supplemented and auxin-free media. Days to the appearance of roots and the number of roots per plantlet were significantly influenced by application of NAA. This is in agreement with the report of Sandra and Krikorian (1984) who induced rooting in banana shoots within five days on NAA-supplemented medium, but Bulakrishnamurthy and Rangnasamy (1988) induced rooting on NAA between 7—10 days. Uptake of auxins as well as their requirement for root initiation has been known to vary with the culture conditions, genotype, physiological and developmental stages of the shoots (Roland and von Arnold, 1987). Therefore the difference between our work and that of Bulakrishnamurthy and Rangnasamy (1988) could be attributed to some of those factors.

The number of roots formed on the NAA-supplemented medium was almost twice that formed on auxin-free medium. However, there were no significant differences in plant height and leaf number per plant between plantlets rooted on NAA-supplemented and NAA-free medium. It has previously been found with the banana as well as other crops that, the number of roots formed *in vitro* usually increases with the addition of auxin (Hansen and Ernstsen, 1982 ; Hiratsuka et al., 1989). There were no comparable data concerning the

Table 1. *In vitro* performance of plantlets cultured on hormone-free and NAA supplemented medium.

NAA treatment (μM)	Days to form the first root	No. of adventitious* roots per plantlet	Plant height* (cm)	No. of leaves* per plantlet
0	6.3±0.8	1.9±0.9	8.8±1.2	2.6±0.5
2	4.0±0.7	3.6±0.5	9.1±1.4	2.7±0.5
t-test	**	**	ns	ns

*, Data were taken at 12 weeks after culture (n=10).

**, significantly different at $P<0.01$; ns, not significant.

Table 2. Root characteristics *in vitro* and leaf and plantlet survival under nursery conditions.

NAA treatment (μM)	Plantlets forming lateral roots (%)	Leaf survival[a] (%)	Plantlet survival[b] (%)
0	80	60	100
2	20	30	70

[a] % of plantlets that showed no leaf withering in nursery (n=10).
[b] % of plantlets that survived in nursery (n=10).

plant height and leaf number per plant cultured on the two types of media but since the plantlets were cultured on the same medium prior to rooting, significant differences were not expected in these two parameters.

In the present study, we examined the growth of plantlets which had been rooted on NAA-supplemented and NAA-free medium after transplantation to pots containing vermiculite. All of the ten plants transplanted from the NAA-free medium into pots survived for three weeks and 60% showed no signs of withering on any of their leaves, and had a 100% plantlet survival at the nursery, while only 30% of plantlets from the NAA supplemented medium showed no signs of withering and had 70% plantlet survival at the nursery.

The plants transplanted from NAA-supplemented medium developed a higher number of adventitious roots per plant than those transplanted from NAA-free medium, but only 20% of them had lateral roots (Table 2). On the contrary, 80% of the plants transplanted from NAA-free medium had lateral roots, although they had a lower number of adventitious roots than those transplanted from NAA-supplemented medium (Fig. 1). This means that the formation of lateral roots is inhibited by the presence of NAA. This is in agreement with the findings reported by Cronauer-Mitra and Krikorian (1987), who observed abundant lateral roots from the adventitious roots of *Musa* plantlets cultured on auxin-free medium. Poor formation of lateral roots in the plants with many adventitious roots might be attributed to the action of some inhibitory substance produced at the root tip (Bowen et al., 1975). To confirm the effects of NAA on the formation of lateral roots, 10 plantlets that had previuosly been rooted on NAA-supplemented medium were transferred to the NAA-free medium. They were then grown under the same culture conditons as previously described. Seven of the 10 plantlets formed lateral roots within 7—10 days confirming the inhibitory effects of NAA on the formation of lateral roots. Banana roots

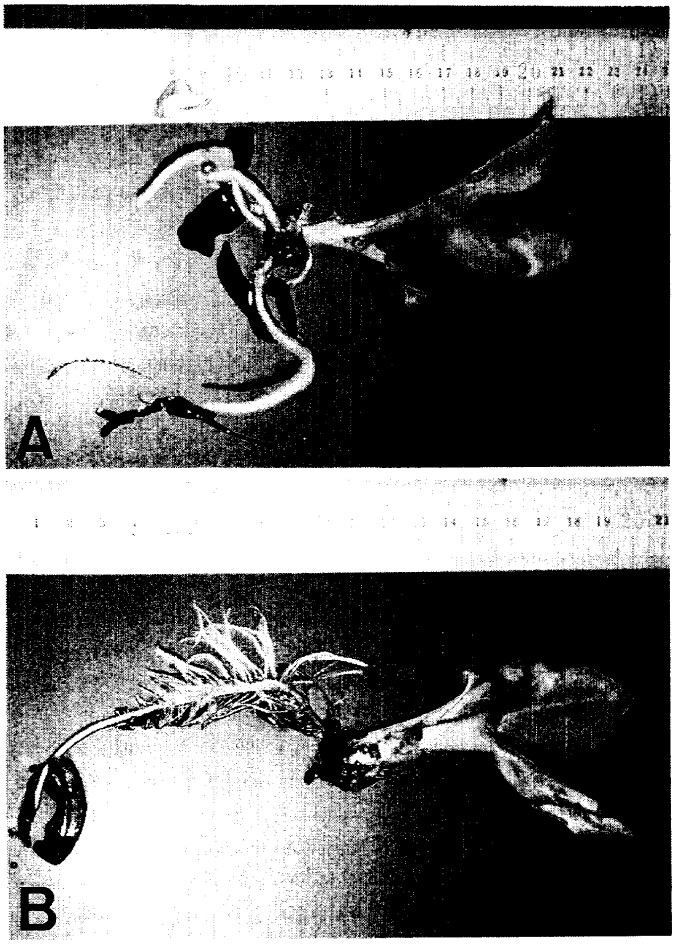

Fig. 1. Banana plants showing root formation in MS medium with 2 μM NAA (A) and without NAA (B).

bear numerous laterals which are smaller in diameter than the adventitious roots, and the lateral roots are considered to be primarily responsible for water and mineral nutrient uptake by the plant. Since there is a good correlation between bunch weight and the quantity of feeder roots (Lassoudiere, 1978), we believe that abundant lateral branching *in vitro* results in a high

Table 3. Nursery performance of plantlets precultured on hormone-free and NAA supplemented medium.

NAA treatment (μM)	Days to the emergence of new leaf	Plant height* (cm)	Number of leaves* per plant
0	5.9±1.1	19.0±2.8	4.8±0.6
2	8.0±1.9	15.0±2.4	3.4±0.5
t-test	*	*	**

*, Data were taken at 3 weeks after transplanting.
*, ** are significantly different at $P<0.05$ and $P<0.01$, respectively (n=10).

initial survival rate and rapid growth and this makes the acclimatization process less delicate.

Other parameters such as the days to the emergence of new leaf, plant height and number of leaves per plant showed highly significant differences between the plants of the two groups from NAA-supplemented and NAA-free media (Table 3). Plants transplanted from auxin-free medium emerged new leaves significantly earlier, had a larger number of leaves per plant and had taller pseudo stems than those transplanted from NAA-supplemented medium. The present findings show that for a better initial establishment of Musa plantlets at the nursery, NAA (auxin) should not be used as a root-inducing agent. However, the effects of other auxins were not studied and further studies with other auxins are necessary.

References

Bart P., Wauwe A.V. and Swennen R. 1993. Plant regeneration through direct somatic embyogenensis from protoplast of banana (*Musa* spp.). Plant Cell Rep. 12 : 403—407.

Bowen M.R., Howarth J. and Longman K.A. 1975. Effects of auxin and other factors on the rooting of *Pinus contorta* Dougl. Ann. Bot. 39 : 647—656.

Bulakrishnamurthy G. and Rangnasamy S.R. 1988. Regeneration of banana plantlets from *in vitro* culture of floral apices. Curr. Sci. India 57 : 270—272.

Cronauer S.S. and Krikorian A.D. 1984. Tropical and subtropical fruits. In W.R. Sharp, D.A. Evans, P.V. Ammirato and Y. Yamada eds., Handbook of Plant Cell Culture. Macmillan Publishing, New York. 338—341.

Cronauer-Mitra S.S. and Krikorian A.D. 1987. Adventitiuos shoot production from Calloid Structures of banana. Plant Cell Rep. 6 : 443—445.

Hansen J. and Ernstsen A. 1982. Seasonal changes in adventitious root formation in hypocotyl cuttings of *Pinus sylvesteris* : Influence of photoperiod during stock plant growth and of indolebutyric acid treatment of cuttings. Physiol. Plant. 54 : 99—106.

Hart W. and Carlson P. 1967. Internal and external factors regulating root initiation. In W. J. Whittington ed., Root Growth. Butterworths, London. 42—64.

Hartmann H.T. and Kester D.E. 1975. Plant Propagation : Principles and Practices. Third edition. Prentice-Hall, Englewood Cliffs. 9—47.

Hiratsuka S., Matsushima J., Kozai T. and Watanabe I. 1989. Effect of IAA on morphogenesis of *Musa* apices and IAA degradation during storage of media. J. Soc. Hort. Sci. 57 : 597—601.

Kshanika H. and Niranjali G. 1997. Cultivar specificity with respect to in vitro micropropagation of *Musa* spp. (banana and plantain). J. Hort. Sci. 72 : 205—211.

Lassoudiere S. 1978. Quelques aspects de la croissance et du development du bananier 'Poyo' en Cote d' Ivoire 2. Le systeme radical. Fruits 33 : 314—338*.

Margareta W. and Inger H. 1981. The rooting ability of shoots raised *in vitro* from apple root stock A2 in juvenile and in adult growth phase. Physiol. Plant. 53 : 301—306.

Murashige T. and Skoog F. 1962. A revised medium for the rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15 : 437—497.

Novak F.J. 1992. *Musa* (bananas and plantains). In F.A. Hammerschlang and R.E. Litz eds., Biotechnology of Perennial Fruit Crops. CAB International, Wallingford. 449—448.

Novak F.J., Afza R., Phadvibulya T., Hermelin T., Brunner H. and Donini B. 1986. Micropropagation and radiation sensitivity in shoot-tip cultures of banana and plantain. In Nuclear Techniques and *in vitro* Culture for Plant Improvement. International Atomic Energy Agency, Vienna. 167—174.

Roland G. and Von Arnold S. 1987. Initiation of roots on hypocotyl cuttings of *pinus contorta in vitro*. Physiol. Plant. 69 : 227—236.

Sandra S. and Krikorian A.D. 1984. Rapid multiplication of bananas and plantains by *in vitro* shoot tip culture. HortScience 19 : 234—235.

Subramanya R. and Schwandes L.P. 1984. *In vitro* propagation of Escarole from leaf explant. HortScience 19 : 235—236.

Torrey J.P. 1956. Chemical factors limiting lateral root formation in isolated pea roots. Physiol. Plant. 9 : 370—388.

*In French with English summary.