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Short communication

Root induction in three species of bamboo with different rooting abilities

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ABSTRACT

Rooting of in vitro axillary shoots from adult field culms of *Bambusa atra*, *Dendrocalamus giganteus* and *D. hookeri* and of juvenile seedling shoots of *D. giganteus* were investigated. *B. atra* rooted spontaneously without exogenous auxin during axillary shoot proliferation, while both *Dendrocalamus* species rooted only on transfer to rooting media with indole-3-butyric acid (IBA). *D. giganteus* required coumarin, an auxin protector, in addition to IBA. Rooting in adult shoots of *D. giganteus* was lower (45.6%) than that in the juvenile shoots (96.7%) but adult shoots of *D. hookeri* rooted well (88.9%). A pretreatment with thidiazuron (TDZ: *N*-phenyl-*N*-[(1,2,3-thidiazol-5-yl)urea]) induced development of axillary buds and subsequently 95% rooting in adult *D. giganteus* shoots but had no significant effect on rooting in juvenile shoots of *D. giganteus* or *D. hookeri*. Pretreatment with tri iodobenzoic acid (TIBA) arrested shoot and bud development leading to very low or no rooting in all three species.

1. Introduction

Large-scale cultivation of bamboo requires development of tissue culture technique to produce propagules. Rooting is a limiting factor in the clonal propagation of bamboo like in many other woody perennials. Auxins play a major role in rhizogenesis. The mechanisms involved in the movement of auxins related to rhizogenesis are rather complex. Auxin interactions with other endogenous and/or exogenous hormones or hormone-like substances as well as environmental cues, plant developmental stages, metabolic activities and many other factors reportedly control rhizogenesis (Hammatt, 1994; Marks, 1996; Couée et al., 2004; Tybursky and Tretyn, 2004). The present investigation deals with in vitro root induction in three species of bamboo with varying rooting abilities.

2. Materials and methods

Continuously proliferating axillary shoots were raised in three species of bamboo, *Dendrocalamus giganteus*, *D. hookeri* and *Bambusa atra* using single node segments of secondary branches bearing unsprouted axillary buds from adult field culms. These were surface sterilized and cultured singly in a basal MS medium supplemented with 5 μM 6-benzyladeneine (BA) as previously described in Ramanayake and Yakandawala (1997). Sprouted shoots were transferred to MS medium with BA at 30 μM for *D. giganteus* or 10 μM for *D. hookeri* and *B. atra* to induce shoot proliferation. Shoots were also raised from seeds of *D. giganteus* germinated in vitro and these proliferated in the medium used for adult shoots but with a lower level of BA at 20 μM.

Axillary shoot clusters bearing three to five shoots were transferred for in vitro rooting to a modified MS liquid rooting medium with macrosalts reduced to half strength, supported by filter paper bridges and enriched with 2% sucrose and IBA or naphthalene acetic acid (NAA). These auxins were added at 10 or 15 μM for *D. hookeri* and *D. giganteus*, respectively. Coumarin at 68.4 μM was also incorporated in the rooting medium of *D. giganteus* as recommended by Ramanayake and Yakandawala (1997). Shoots proliferating in the respective media were directly transferred to the rooting media or they were pretreated before root induction by growing them in the shoot proliferation media to which 0.5 μM TIBA was added. They were also cultured in the presence of 0.5 μM TDZ instead of BA for the duration of 10 days. Cultures were kept at a 12 h photoperiod or under continuous illumination or darkness at 24 ± 2 °C. Osram 36W/10 "Daylight" fluorescent tubes giving an intensity of 48 μmol m^{-2} s^{-1} were used for illumination. Ten to 20 culture tubes, with 15 ml medium, were used for each treatment replicated twice and thrice in *D. giganteus* and *D. hookeri*, respectively.

The rooted shoots were transferred to the same basal medium without growth regulators and grown under 12 h photoperiod. After 2 weeks, plantlets were placed in jars containing tap water

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with roots submerged. Three weeks later these were established in root trainers containing moist coir dust and recommended fertilizer applications were provided with regular irrigation. Observations on number of rooted and non-rooted shoots, days to root emergence, discolouration of shoots and survival after acclimatization were made.

B. atra shoots in the shoot proliferation medium were transferred to liquid MS medium with 10 μM BA and 5, 10 or 15 μM TIBA for 3 weeks. A single shoot cluster was placed in a Magenta jar with 40 ml medium. The number of shoots, roots and root length in each shoot cluster were recorded in 6 replicate jars containing each level of TIBA and in a control set with 10 μM BA at the beginning and end of a subculture cycle of 3 weeks. Shoots bearing roots were acclimatized as described for the two *Dendrocalamus* species.

Completely randomized design was used in all experiments. The effects of treatments (t) on rooting were analyzed by chi-square test with $2 \times t$ contingency tables using data of rooted and non-rooted shoots in the two *Dendrocalamus* species. One way analysis of variance followed by mean separation procedure (Duncan's Multiple Range Test) was used to determine treatment effects on the other parameters (root length, number of roots and shoots, days to rooting and survival). Percentage rooting and survival were also calculated in all treatments in the three species.

3. Results

Axillary shoots of the three species proliferated continuously upon subculture (Fig. 1A). *B. atra* developed adventitious roots spontaneously during shoot proliferation after six subculture cycles from shoot initiation (Fig. 1B). Each cluster of shoots developed both shoots and roots with continuous subculture. When TIBA (0.5, 10.0 and 15.0 μM) was incorporated in the shoot proliferation medium containing BA, each shoot cluster showed a significant reduction in the number of shoots (7.4–12.0) and roots (3.0–5.6) and root length (4.2–6.5 mm) compared to the high number of shoots (31.2 ± 9.2) and roots (24.8 ± 12.5) and longer roots (16.5 ± 3.0) that developed in the presence of BA only. The shoots turned brown and necrotic on repeated transfer to medium with TIBA. Rooted shoots grown without TIBA, on separation into smaller clusters of five or more were readily acclimatized and established in soil with 100% survival.

The axillary shoots of the two *Dendrocalamus* species developed roots after transfer to the respective rooting media (Table 1). NAA was not conducive to rooting in *D. giganteus*. But *D. hookeri* rooted in both NAA (81.9%) and IBA (88.9%) with no significant difference in rooting. Therefore, only IBA was used in pretreatment studies in both these species.

A high percentage of rooting (96.7%) was observed in the juvenile shoots of *D. giganteus*, compared to 45.6% in shoots raised from adult culms when shoots were transferred directly from the shoot proliferation medium to rooting medium (Table 1). Most of the shoots, especially those of adult culms, turned pale green and browned after a few days in the rooting medium (Fig. 1C). Root emergence took over 21 days in adult culms while juvenile shoots rooted earlier in 12.6 days. The TDZ pretreatment before transfer to rooting medium improved rooting of the adult shoots to over 90% (Table 1). New buds developed and the shoots remained green with a relatively low intensity of browning on transfer to rooting medium (Fig. 1D). Exposure to a 12 h photoperiod or continuous light did not significantly improve rooting but survival of rooted plantlets was higher at 85% in continuous light compared to 59.4% at a 12 h photoperiod. The presence of green viable shoots may have enabled acclimatization of these plantlets. Shoots kept in the dark during rooting did not develop roots and turned pale and

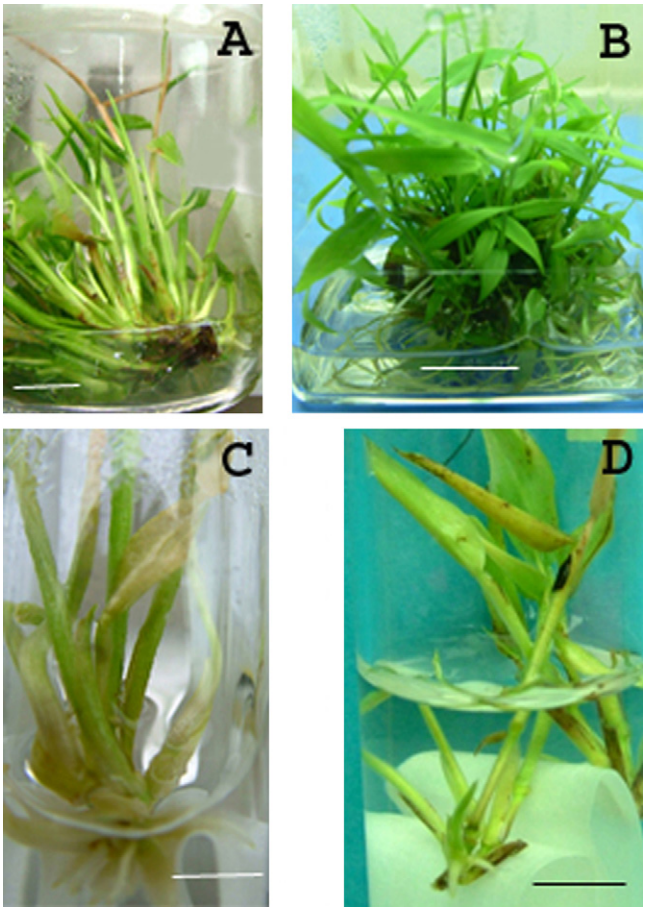

Fig. 1. Axillary shoot proliferation and rooting in *Dendrocalamus giganteus*, *D. hookeri* and *Bambusa atra*. (A) Axillary shoots of *D. giganteus* in shoot proliferation medium (bar = 1.2 cm). (B) *B. atra* axillary shoots bearing roots in shoot proliferation medium (bar = 1.8 cm). (C) Rooted shoots of *D. giganteus* in root induction medium (note pale and browned shoots) (bar = 0.5 cm). (D) Rooted shoots of *D. hookeri* in rooting medium (bar = 0.5 cm).

became brown within 5 days. Shoots given a 0.5 μM TIBA pretreatment also did not develop new shoots or roots on transfer to rooting medium.

Rooting and survival of rooted plantlets were higher in *D. hookeri* than *D. giganteus* adult shoots transferred directly from shoot proliferation medium to rooting media. The shoots were dark green in colour compared to those of *D. giganteus* during shoot proliferation. *D. hookeri* shoots showed no significant difference in rooting even after TDZ pretreatment or when exposed to 12 h or continuous illumination. Rooting decreased to 20% and took a longer time of 18.5 mean days when treated with TIBA before rooting (Table 1). Survival of plantlets was 100% in all rooted shoots including those treated with TIBA. During growth in TIBA supplemented medium, shoot proliferation decreased with the development of a few buds unlike *D. giganteus* which did not develop any new buds or shoots.

4. Discussion

The presence of endogenous auxins has been found to be important during rooting even if these are supplied exogenously (Tartoura et al., 2004). In the present investigation, the competence to root varied in all three bamboo species. The highest competence to root was in *B. atra*, which developed roots spontaneously in the

Table 1
In vitro rooting and survival of axillary shoots of *Dendrocalamus giganteus* and *D. hookeri* pretreated with TDZ or TIBA

Pretreatment and light regime	*D. giganteus* (adult)			*D. giganteus* (juvenile)			*D. hookeri* (adult)		
	% rooting	Days[a]	% survival[b]	% rooting	Days	% survival	% rooting	Days	% survival
No pretreatment, 12 h	45.6 ± 23.7 b	21.7 ± 9.0 a	43.8 b	96.7 ± 5.8 a	12.6 ± 2.8	91.1 ± 8.4	88.9 ± 13.9 a	13.3 ± 1.2 a	100
TDZ, 12 h	91.5 ± 3.7 a	13.9 ± 2.0 b	59.4 b	66.1 ± 33.7 b	14.8 ± 2.1	100	93.3 ± 3.0 a	10.8 ± 0.1 a	100
TDZ, 24 h	95.2 ± 0.0 a	12.7 ± 0.0 b	85.0 a	27.8 ± 27.8 c	13.9 ± 3.5	100	86.7 ± 6.7 a	11.6 ± 0.3 a	100
TIBA, 12 h	0.0	–	–	0.0	–	–	20.0 ± 10.0 b	18.5 ± 1.3 b	100

Means with the same letters along a column are not significantly different ($p = 0.05$).
[a] Mean days to rooting.
[b] Percentage survival of rooted axillary shoots after acclimatization.

absence of exogenous auxin. Jiménez et al. (2006) have described similar spontaneous root development in shoots raised from adult culms of another bamboo, *Guadua angustifolia*. *D. strictus*, *B. stocksii* and *B. bambos* have also developed roots and shoots in the presence of a low level of BA with or without IBA and, thus like *B. atra*, are easy to root bamboos (Shirgurka et al., 1996; Rathore and Rai, 2005; Kapoor and Rao, 2006). The two *Dendrocalamus* species required an exogenous auxin, IBA, for rooting. However, in addition to IBA, *D. giganteus* required an auxin protector coumarin (1,2-benzopyrone), a phenylpropanoid. Coumarin and other compounds like choline chloride and phloroglucinol have enhanced rooting in *B. tulda*, *D. longispathus* and *D. hamiltonii* (Saxena, 1990; Saxena and Bhojwani, 1993; Sood et al., 2002; Mishra et al., 2008). These compounds are reported to enhance rooting by acting as auxin protectors to increase the free endogenous IAA levels during the inductive phase of rooting. (Faivre-Rampant et al., 2004; Tartoura et al., 2004).

The adult shoots of *D. hookeri* and the juvenile shoots of *D. giganteus* showed a higher rooting of 88.9% and 96.7%, respectively compared to 45.6% in adult shoots of the latter (Table 1). Thus the growth phase of *D. giganteus* had a strong influence on rooting. A phase change from adult to juvenile may also occur during repeated subculture to enhance rooting as seen in *B. atra*.

Rooting in woody species is mediated through the basipetal transport of auxin from the shoot apex. These directly induce roots in easy-to-root species but are more dependent on interactions with exogenous auxins in more difficult-to-root species (Marks, 1996). A similar action is evident in the present investigation in relation to axillary bud development and subsequent rooting in the three bamboo species. Induction of axillary buds and shoots enhanced rooting in the easy-to-root *B. atra* growing in the shoot proliferation medium without auxins. The two *Dendrocalamus* species which required exogenous IBA, developed roots only if viable shoots and buds were present. In *D. hookeri* and juvenile shoots of *D. giganteus* that rooted well, shoots remained viable on transfer to rooting medium but the shoots from nodal explants of adult *D. giganteus* turned brown. With a pretreatment of TDZ, that induced viable new shoots and buds to develop, rooting was not only enhanced to 95% but also accelerated by 7 days (Table 1). Similar observations in *B. vulgaris* in vitro shoots treated with TDZ are reported (Ramanayake et al., 2006). TDZ is also reported to enhance auxin absorption and mobility (Murch and Saxena, 2001).

Pretreatment with TIBA, was detrimental to shoot development and rooting in the three species to varying degrees and appeared to correlate with their rooting ability. With a 10-day pretreatment of TIBA, axillary bud development was arrested in *D. giganteus*, shoots turned brown and rooting was inhibited while in *D. hookeri*, axillary bud development decreased and 20% shoots subsequently rooted. *B. atra* however, continued to develop roots and shoots in the presence of TIBA over a culture period of 3 weeks, although the shoot and root number significantly decreased. In vitro shoots of other species of bamboo such as *B. bambos* and *D. hamiltonii*, treated with TIBA, have also turned brown leading to root inhibition (Sood et al., 2002; Kapoor and Rao, 2006). These observations indicate that arrest of axillary bud development by TIBA may have prevented endogenous auxin synthesis required for rooting. Whether TIBA, an auxin transport inhibitor, also inhibited auxin translocation in the three species requires further investigation.

5. Conclusions

This investigation with three species of bamboo showed that growth regulator requirements for in vitro rooting are species dependent. It was possible to achieve a high percentage of rooting in the three species by manipulating the growth regulators, BA and IBA as well as growth regulator-like substances, coumarin and TDZ, in culture media. Understanding auxin interactions leading to rooting responses in bamboo are recommended to make micropropagation of difficult-to-root bamboos a feasible venture.

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References

Couée, I., Hummel, I., Sulmon, C., Gouesbet, G., Amrani, A.E., 2004. Involvement of polyamines in root development. Plant Cell Tiss. Organ Cult. 76, 1–10.
Faivre-Rampant, O., Kevers, C., Gaspar, T., 2004. IAA-oxidase activity and auxin protectors in nonrooting, *rac*, mutant shoots of tobacco in vitro. Plant Sci. 153, 73–80.
Hammatt, N., 1994. Promotion by phloroglucinol of adventitious root formation in micropropagated shoots of adult wild cherry (*Prunus avium* L.). Plant Growth Reg. 14, 192–269.
Jiménez, V.M., Castillo, J., Tavares, E., Guevara, E., Montiel, M., 2006. In vitro propagation of the neotropical bamboo, *Guadua angustifolia* Kunth, through axillary shoot proliferation. Plant Cell Tiss. Organ Cult. 86, 389–395.
Kapoor, P., Rao, I.U., 2006. In vitro rhizome induction and plantlet formation from multiple shoots in *Bambusa bambos var. Gigantea* Bennet and Gaur by using growth regulators and sucrose. Plant Cell Tiss. Organ Cult. 85, 211–217.
Marks, T.R., 1996. The role of shoot apex in controlling rhizogenesis in vitro. Plant Growth Reg. 20, 57–60.
Mishra, Y., Patel, P.K., Yadev, S., Shirin, F., Ansari, S.A., 2008. A micropropagation system for cloning of *Bambusa tulda* Roxb. Sci. Hort. 115, 315–318.
Murch, S.J., Saxena, P.K., 2001. Molecular fate of thidiazuron and its effect on auxin transport in hypocotyls tissues of *Pelargonium* × *hortorium* Baily. Plant Growth Reg. 35, 269–275.
Ramanayake, S.M.S.D., Yakandawala, K., 1997. Micropropagation of the giant bamboo, *Dendrocalamus giganteus* Munro from nodal explants of field grown culms. Plant Sci. 129, 213–223.
Ramanayake, S.M.S.D., Meemaduma, V.N., Weerawardene, T.E., 2006. In vitro shoot proliferation and enhancement of rooting for the large-scale propagation of yellow bamboo (*Bambus vulgaris* 'Striata'). Scientia Hort. 110, 109–113.
Rathore, T.S.S., Rai, V.R., 2005. Micropropagation of *Pseudoxytenanthera stocksii* Munro. In vitro Cell. Dev. Biol. Plant 44, 333–337.
Saxena, S., 1990. In vitro propagation of the bamboo (*Bambusa tulda* Roxb.) through shoot proliferation. Plant Cell Rep. 9, 432–434.
Saxena, S., Bhojwani, S.S., 1993. In vitro clonal multiplication of four year old plants of *Dendrocalamus longispathus* Kurz. In vitro Cell. Dev. Biol. 290, 135–142.

Shirgurka, M.V., Thengane, S.R., Poonawela, I.S., Jan, M.M., Nadgauda, R.S., Mascarenhas, A.F., 1996. A simple in vitro method of propagation and rhizome formation in *Dendrocalamus strictus* Nees. Curr. Sci. 70 (10), 940–943.

Sood, A., Ahuja, P.S., Sharma, M., Sharma, O.P., Godbole, S., 2002. In vitro protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. Plant Cell Tiss. Organ Cult. 71, 55–63.

Tartoura, K., da Rocha, A., Youssef, S., 2004. Synergistic interaction between coumarin 1,2-benzopyrone and indole-3 butyric acid in stimulating adventitious root formation in *Vigna radiata* (L) Wilczek cuttings. I. Endogenous free and conjugated IAA and basic peroxidases. Plant Growth Reg. 42 (3), 253–262.

Tybursky, J., Tretyn, A., 2004. The role of light and polar auxin transport in root generation from hypocotyls of tomato seedling cuttings. Plant Growth Reg. 42, 39–48.