Optimizing Root Initiation by Controlling Exposure to Auxin

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The time of exposure to medium containing 10 μM IBA to achieve maximum rooting (96%) of papaya (*Carica papaya*) shoots *in vitro* was 3 days. One hundred percent root initiation was obtained with neem (*Azadirachta indica*) shoots after exposure to 10 μM IBA for 4 days. For coffee (*Coffea arabica*) shoots rooted *in vivo*, 15 h exposure to 100 mgl^{-1} IBA was optimal in terms of rooting percentage. In lieu of transfer to hormone-free media after 3 days (papaw) or 4 days (neem), good root initiation was achieved by 3 or 4 days dark incubation with medium containing 10 μM IBA and 10 μM riboflavin before transfer to the light, or overlaying of medium containing 10 μM IBA with100 μM riboflavin after 3 or 4 days light incubation.

REVIEW OF LITERATURE

Auxins are used to promote adventitious root initiation *in vitro* (Torrey, 1976). Shoots are usually left on media containing auxins for three or four weeks before transfer. However, it has been known since 1937 that root elongation is inhibited by auxins, such as, IBA (Went and Thimann, 1937). Auxins were also shown to have a dual effect (promotion of root initiation but inhibition of root elongation) in cuttings of *Azukia* (= *Lablab purpureus*)(Mitsuhashi-Kato, et al., 1978) and pea (Mohammed and Eriksen, 1974; Went, 1939) and *in vitro* with apple rootstocks (James, 1983). Thus, there are two distinct phases of adventitious root formation: root initiation, when auxin is essential; and root emergence and growth, when auxin is not required or in inhibitory.

Experiments described in this paper were undertaken to determine the optimum length of exposure to IBA for root initiation *in vitro* of papaya and neem, and for rooting *in vivo* of tissue cultured coffee shoots. In a subsequent experiment, exposure to exogenous IBA is controlled by the addition of riboflavin, which rapidly reduces IBA levels in light (Drew, et al., 1991).

MATERIALS AND METHODS

Effect of Time of Exposure to IBA on Rooting. Papaya shoots were established *in vitro* and multiplied using techniques described previously (Drew, 1988; Drew and Miller, 1989; Drew and Smith, 1986). Axillary shoots, 5 to 10 mm in length, were cultured on rooting medium (RM) containing M (medium) concentrations of minerals and growth factors as described by DeFossard (DeFossard, et al., 1974), without riboflavin, plus 10 μM IBA and 2% sucrose. Fifty replicate explants were removed at intervals (Figure 1) and transferred to hormone-free DS (Drew and Smith, 1986) medium. Control plants were placed on hormone-free DS medium at day 0, or were left on the medium containing IBA for 28 days. Root initiation was assessed daily, and the time required for shoots to reach 50% rooting was calculated. The maximum rooting percentage was the final rooting percent on day 28.

Neem shoots were established in culture using nodal explants from glasshouse-grown plants which were grown from seed obtained from India. Apically dominant shoots were grown on hormone-free MS (Murashige and Skoog, 1962) medium and multiplied by dissection into nodal sections. Actively growing axillary shoots were placed on MS medium plus 10 μM IBA, and 3% sucrose. Twenty replicates were removed on days 2, 3, 4 and 5, and placed on hormone-free MS medium. Control plants were placed on hormone-free medium at day 0 or left on IBA medium for 21 days.

Coffee plants were established *in vitro* using techniques described by Dobson (Dobson, 1991). Axillary shoots, 1 cm in height with 3 pairs of leaves, were dissected from nodal sections and placed in tubes containing 2.5 ml of 100 mgl^{-1} IBA solution. Twenty-four replicates were removed after 5, 10, 15, 20 and 30 hours and planted in peat, perlite and polystyrene foam beads medium (1:1:1, v/v/v). A control treatment was not exposed to IBA, but planted directly into the potting mixture. The plants were maintained in a perspex cabinet at >90% RH with 40% natural light. After 5 weeks, plants were removed from the potting mixture and roots were assessed.

Effect of Riboflavin on Controlling Exposure to Exogenous IBA. The ability of riboflavin to break down auxin in the medium in the light, but not in the dark (Drew, et al., 1991), was utilized to control the length of time shoots were exposed to exogenous auxin. In treatment 1, papaya and neem shoots were cultured for 3 and 4 days respectively, on basal media (RM for papaya, MS for neem)plus 10 μM IBA, then transferred to hormone-free basal media (DS for papaya, MS for neem). In treatment 2, papaya and neem shoots were cultured on basal media (RM, MS) containing 10 μM of both IBA and riboflavin in total darkness for 3 and 4 days respectively, then transferred to a 16 h photoperiod. In treatment 3, papaya and neem shoots were cultured on basal media (RM, MS) plus 10 μM IBA under 16 h photoperiods. After 3 and 4 days respectively, 3 ml of an autoclaved solution containing 100 μM riboflavin was injected from a sterile syringe onto the surface of the agar in each container. Fifty-one replicates were used in each treatment, cultured on 30 ml of medium in 250 ml containers, with 3 shoots in each container. Root initiation was recorded daily.

All tissue culture media contained 8 g l^{-1} Difco bacto-agar and had pH adjusted to 5.6 with 0.1 M KOH before autoclaving at 121°C for 15 min. Cultures were incubated as 25±1° C with cool white fluorescent tubes providing a light irradiance of 55 μmol $m^{-2}s^{-1}$ for a 16 h photoperiod.

RESULTS

Effect of Time of Exposure to IBA on Rooting. Papaya shoots exposed to 10 μM IBA for 3 days before transfer to hormone-free medium had highest final rooting percentage (96%) and shortest time to root (Figure 1A). With neem shoots, 100% root initiation was obtained after exposure to 10 μM IBA for 4 days, and time to 50% root initiation was minimal after 5 days exposure to IBA (Figure 1B). Exposure to IBA for 21 days (neem) or 28 days (papaw) resulted in excess callus production on the base of the shoot and short callused roots with no lateral branches.

Time of exposure to IBA also affected root initiation with tissue-cultured coffee shoots rooted *in vivo*. Fifteen hours exposure to 100 mg l^{-1} was optimal in terms of

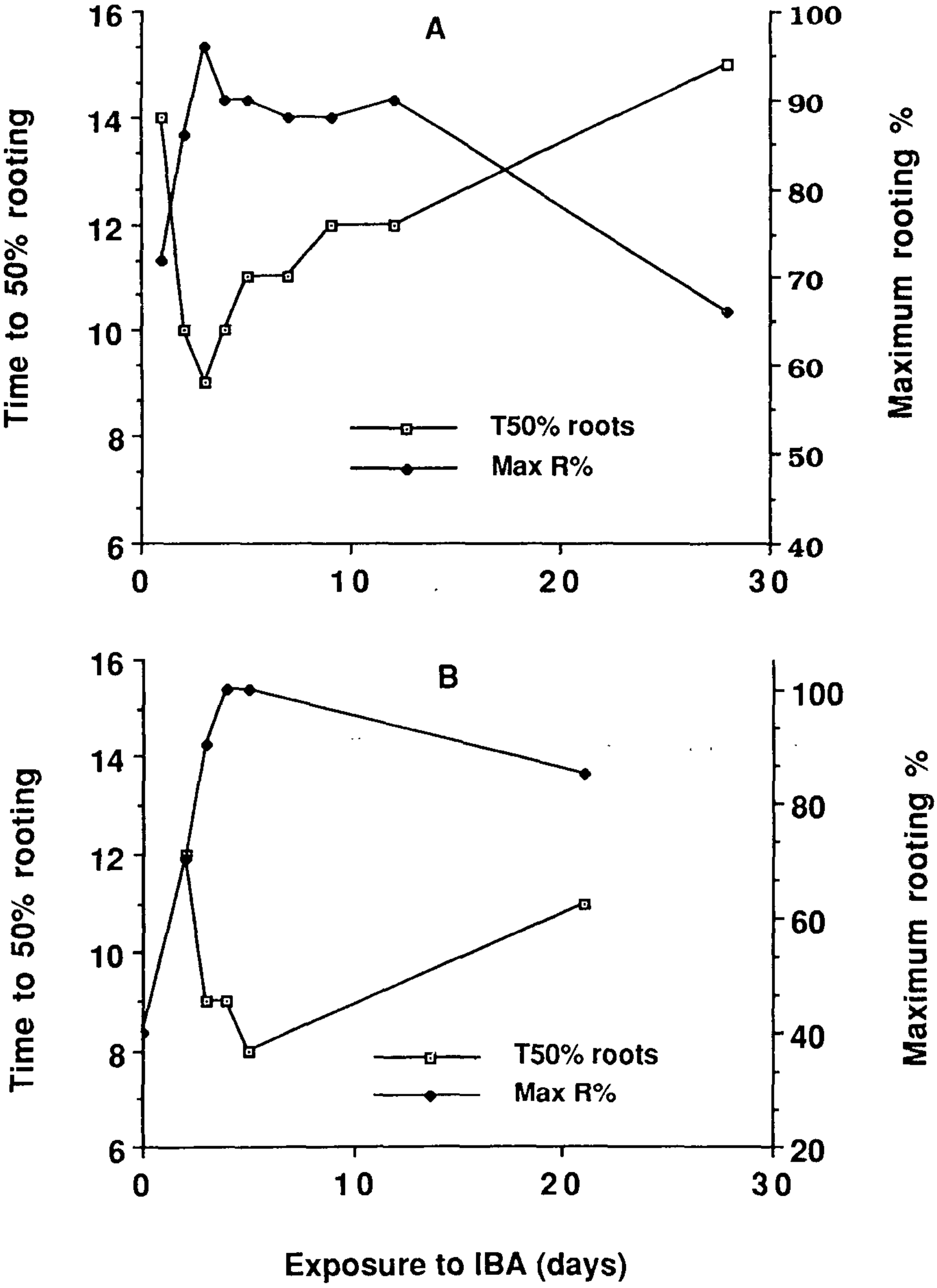

Figure 1. The effect of time of exposure to medium containing 10 μM IBA before transfer to hormone-free medium, on rooting *in vitro* of papaya (A) and neem (B). No papaya shoots rooted on control treatment (0 days). T50% roots = time to 50% rooting, MAX R% = maximum rooting percentage. Results are means of 50 replicates for papaya and 20 replicates for neem.

rooting percentage (92%), and 20 hr as assessed by mean root number per shoot (Figure 2).

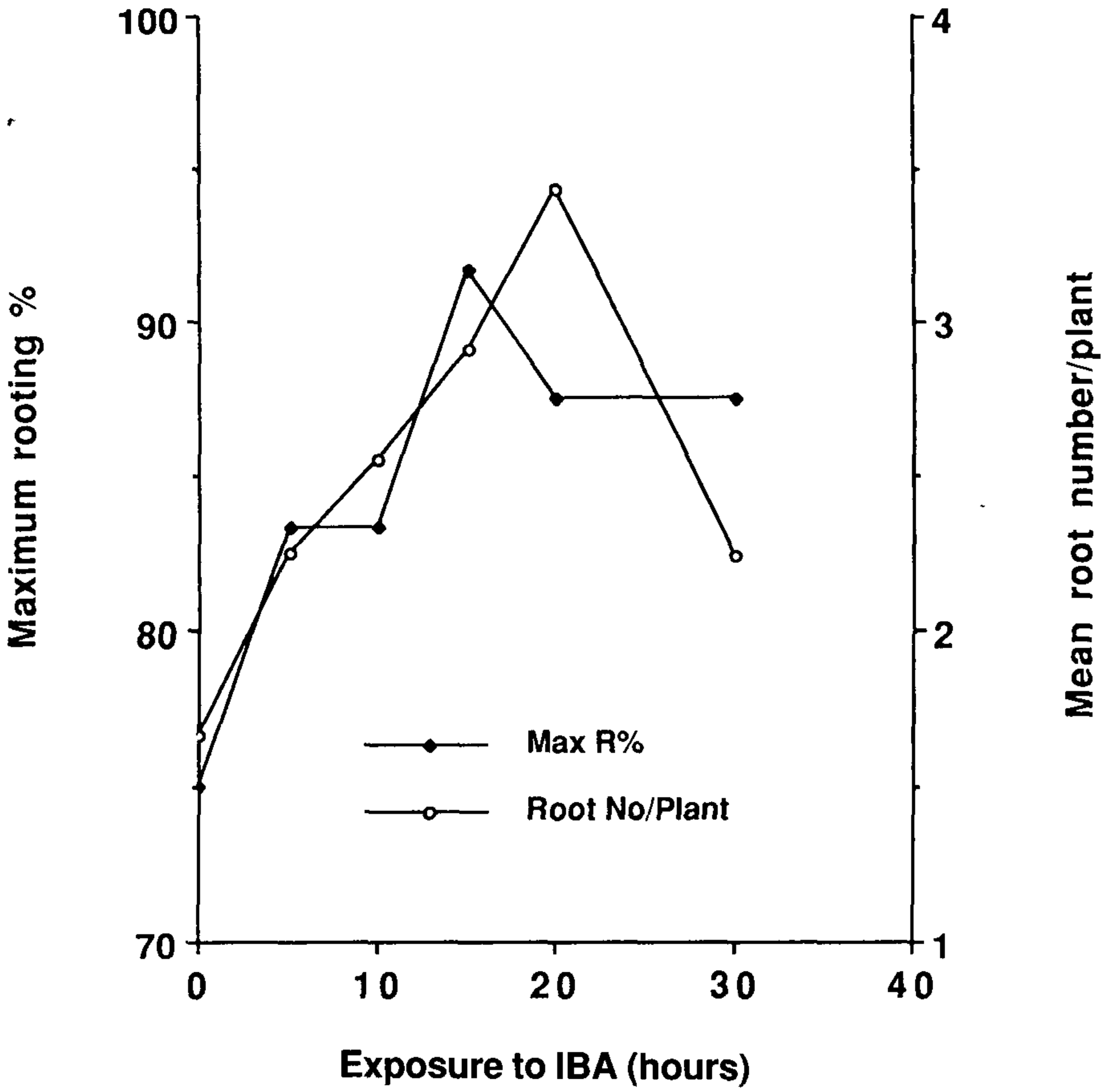

Figure 2. The effect of time of exposure to 100 mg l^{-1} IBA prior to planting, on *in vivo* rooting of coffee shoots which had been multiplied *in vitro*. T50% roots = time to 50% rooting, MAX R% = maximum rooting percentage. Results are means of 24 replicates.

Effect of Riboflavin on Controlling Exposure to Exogenous IBA. Papaya shoots transferred to hormone-free medium after 3 days (treatment 1) gave highest rooting percentages (Table 1), but high rooting percentages were also achieved by incorporation of riboflavin in the medium and exposure to light after 3 days dark incubation (treatment 2), and by overlaying medium with riboflavin on day 3 (treatment 3). Neem shoots showed little difference between treatments in terms of rooting percentages (Table 1). Papaya shoots which were not transferred to hormone-free medium were smaller and had short thick roots, and neem shoots from cultures in which riboflavin was injected onto the surface of the agar had some callus at the base of the explants.

Table 1. Root initiation of papaya and neem *in vitro*.

	Treatment		
	Transfer to hormone-free medium[1]	Dark incubation[2]	Riboflavin overlaid onto medium[3]
Papaya:			
Percent rooted after:			
14 days	92	80	78
28 days	98	88	88
T50 (days)	9	10	10
Neem:			
Percent rooted after:			
14 days	93	90	91
T50 (days)	8	8	9

[1] Shoots transferred from a medium containing 10 µM IBA to a hormone-free medium.
[2] Shoots on a medium containing 10 µM IBA and 10 µM riboflavin, cultured in the dark then transferred to light.
[3] Shoots on a medium containing 10 µM IBA cultured in the light, then overlaid with 100 µM riboflavin. These manipulations were done on day 3 for papaw and day 4 for neem, using 50 replicates per treatment. T50 = time to 50% root initiation.

DISCUSSION

Improved commercial micropropagation systems could be developed for many species if optimum time of exposure to auxin was determined and shoots subsequently grown on auxin-free media. The optimum time of exposure to IBA for papaya *in vitro* has been shown to be 3 days, and 4 days for neem (Figure 1). For micropropagated coffee shoots which can be rooted *in vivo*, a pretreatment of 15 h exposure to 100 mg l^{-1} IBA was optimal for rooting.

Transfer of plants to hormone-free media may be uneconomical for commercial practice. However, exposure to auxin can be controlled by addition of riboflavin to the medium. Drew *et al.* (1991) showed that IBA concentration in media could be decreased rapidly when media containing riboflavin were placed in light. This reduction did not occur with dark incubation (Drew, et al., 1991). Thus cultures can be incubated in darkness on media containing both IBA and riboflavin before being returned to light where the riboflavin will photooxidize the auxin, or cultures can be maintained in light and the media then overlaid with riboflavin solution.

Experiments described in this paper show how optimum time of exposure to auxin can be determined to maximize root initiation. The beneficial effect on rooting of transfer of papaw and neem to media without hormones, can be economically achieved by using exogenous riboflavin and light. These techniques may be applicable to a wide range of species.

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