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Stimulation of Root Formation by Thiol Compounds

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Abstract. **Root formation in shoot cuttings of soybean (*Glycine max* L. 'Williams'), mungbean (*Phaseolus aureas* Mdlbg.), English ivy (*Hedera helix* L.), and apple (*Malus* ×*domestica* Borkh. 'Jork 9') was stimulated by dithiothreitol and reduced glutathione in the presence and absence of auxin (IAA) shock. In soybean, in the absence of auxin, root formation was stimulated to about the same extent by glutathione alone as with auxin alone. The roots induced by thiol compounds were longer than roots induced by auxin shock and were completely normal in appearance. Roots produced with auxin shock alone were short and exhibited characteristic auxin-induced deformations. With a combination treatment of auxin shock and thiol compounds, roots were more numerous than with either alone, somewhat longer than with auxin alone, and exhibited fewer of the usual deformations characteristic of roots grown in the presence of external auxins. The thiol compounds also were beneficial for rooting *Malus* shoots propagated from callus in vitro. The thiol compounds were most beneficial with older cuttings where auxin shock was often insufficient to obtain roots. In shoots where rooting was stimulated by thiol agents, shoots grew more rapidly than in those where rooting was induced by auxin shock alone. These findings suggest a use for thiol compounds alone or in combination with auxin shock to induce differentiation of root primordia as well as for stimulation of root growth. Chemical name used: indole-3-acetic acid (IAA).**

Induction of rooting is an important step in the propagation of woody and herbaceous plants from cuttings or shoots produced from callus (Moncousin, 1992). The classic root induction method is to use an auxin shock of high concentrations of either IAA or other auxins. However, the roots formed frequently are stunted and malformed (Lane, 1978). In our report, we describe using the thiol compounds glutathione and dithiothreitol to induce rooting in shoots of apple, mungbean, and soybean either alone or in combination with an auxin shock.

Materials and Methods

Rooting of shoots of soybean and mungbean. Seeds of soybean ('Williams') or mungbean were soaked in water for several hours, planted in moist vermiculite, and germinated and grown in the greenhouse for ≈2 weeks. Shortly after the epicotyl was fully extended, but before the expansion of the first trifoliate leaf, shoots were harvested by cutting just above the roots. These shoots were transferred to shell vials (10 ml) containing 5 ml of aqueous rooting solution. Rooting was continued in light [fluorescent, 70 μmol·m^{-2}·s^{-1} photosynthetic photon flux (PPF), 8-h light period] for 7 to 10 days. All roots were counted on each of three to five cuttings in three experiments. Results are means ±SD among experiments.

Micropropagation and rooting of apple shoots in culture. Plants were from an apple rootstock ('Jork 9') obtained from B. Kunneman (Lisse, The Netherlands) and were already established in vitro before the start of the experiments.

In the proliferation phase, plants were formed in tufts of shoots varying between 1 and 3 cm long. To multiply plants, 0.5-cm apices were excised and transferred to fresh medium. Apices were cultured in glass jars containing 100 ml of medium and were covered with Saran wrap (PVDC Dow Chemical, Midland, Mich.). The multiplication medium and the culture room conditions were the same as those described by Seingre et al. (1991). The medium contained MS mineral solution (macro- and microelements) (in mg·liter^{-1}) with 100 inositol, 1 thiamine HCL, 0.5 nicotinic acid, 0.5 pyridoxine HCL, the plant growth regulators benzyladenine (1) and IAA (0.1), and sucrose (30,000). The pH was 5.5. The culture conditions were 24 to 26C with a 16-h photoperiod at 70 μmol·m^{-2}·s^{-1} PPF.

For the rooting phase, 2-cm-long shoots were harvested at the end of the proliferation stage and were treated for 1 h with 1 mM auxin (see auxin shock) before transfer to a rooting medium (identical to the multiplication medium but without hormones) to initiate rooting. The thiol compounds were prepared aseptically by filtration and were introduced with the concentrated molten agar rooting medium just before solidification. After transfer, plants were kept in darkness at 26C for 6 days and then returned to the culture room. The auxin treatment and rooting were in glass jars.

Rooting is expressed as the number of shoots per tuft producing >5 roots. Each treatment was 22 tufts, and experiments were conducted three times. Rooting was measured as the ratio of the number of roots produced per total number of shoots.

Rooting of English ivy cuttings. Cuttings of English ivy ≈15 cm long were collected in October. Treatments were exactly as for shoots of soybean and mungbean, except that rooting was in 125-ml Erhlenmeyer flasks containing 75 ml of solution, and the experiments were continued for 30 days.

Auxin shock. The auxin used was IAA. The treatment with auxin involved dipping the base of the cutting (2 to 3 mm) for 1 h in an agarised (apple) or aqueous (soybean, mungbean, and English ivy) solution of 1 mM IAA. The shoots then were rinsed and transferred directly to either agar (apple) or distilled water in shell vials (soybeans and mungbean) or flasks (English ivy) containing the test substances.

Results

The number of roots formed by soybean shoots was increased by adding thiol compounds either in the absence or presence of

Table 2. Rooting of soybean shoots after 14 days promoted by dithiothreitol and effects on root length.

Auxin shock	Dithiothreitol (mM)	Roots No./cutting[z]	Roots Length (cm)[z]
–	0	13 ± 1	2.5 ± 0.5
+	0	19 ± 3	0.45 ± 0.05
+	0.1	40 ± 10	4.1 ± 0.5
+	0.3	53 ± 13	3.0 ± 0.4

[z]Results are means of three experiments of 10 shoots each ± SD among experiments.

Table 1. Rooting of soybean shoots promoted by glutathione and dithiothreitol at 0.1 mM after 14 days in the presence and absence of an auxin shock (1 mM IAA for 1 h) and effects on shoot length.

Thiol	Roots/shoot[z] – Auxin	Roots/shoot[z] + Auxin	Shoot length (cm)[z] – Auxin	Shoot length (cm)[z] + Auxin
None	15 ± 3	28 ± 1	10 ± 2	5 ± 1.5
Glutathione	22 ± 2	45 ± 6	12 ± 3	12 ± 2
Dithiothreitol	21 ± 4	40 ± 5	7 ± 1	9 ± 1

[z]Results are means of 10 shoots in each of three trials ± SD among trials.

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auxin shock alone (Table 1). Comparisons were at near optimum concentrations of reduced glutathione (Table 1) or dithiothreitol (Tables 1 and 2). Mungbean (Fig. 1) and soybean (Fig. 2) yielded an optimum curve in the presence of auxin, with the most roots produced at 0.1 mM for glutathione and at ≈1 mM for dithiothreitol.

In the absence of auxin, overall rooting of mungbean shoots was less, and no clear optimum was present (Fig. 1). However for soybean shoots, rooting in the absence of auxin shock appeared to be stimulated at about the same concentrations of dithiothreitol and glutathione as in the presence of auxin shock (Fig. 2). In the presence of auxin shock, low concentrations of dithiothreitol (0.1 to 0.05 mM) appear to inhibit rooting of mungbean shoots (Fig. 1).

The appearance of the roots formed in the presence of the thiol compounds differed from those formed in their absence. In the presence of reduced glutathione (Fig. 3 b and e) or dithiothreitol (Fig. 3 c and f), there were more roots, and they generally were longer and less deformed than the roots of comparable control shoots treated in the presence of auxin shock alone (Fig. 3d, Table 2). Additionally, roots formed higher on the stem, especially with dithiothreitol (compare Fig. 3 b with a and e with d).

Not only were the number, length, and quality of roots formed in the presence of auxin shock improved by the thiol compounds, but reduced glutathione and dithiothreitol also enhanced shoot elongation in combination with auxin shock (Table 1). With auxin shock alone, shoot elongation was reduced by ≈50%. At optimum concentrations of glutathione and dithiothreitol for rooting, shoot growth in the presence of auxin was restored to about the levels in the absence of auxin.

With *Malus* cuttings growing in agar, thiol compounds also induced rooting with about the same dose relationships as for soybean and mungbean. For dithiothreitol, an optimum curve was obtained, with the greatest enhancement of rooting at ≈0.25 mM (Fig. 4). With reduced glutathione, optimum rooting enhancement was achieved at ≈0.075 mM (Table 3).

With *Malus* cuttings, effects on root length and quality in combination with auxin shock was more evident with thiol compounds than were effects on the number of root primordia (Fig. 5). Roots formed in the presence of auxin shock together with dithiothreitol (Fig. 5b) or reduced glutathione (Fig. 5d) were longer and less deformed than those formed following auxin shock alone (Fig. 5a). As with soybean (Fig. 3), shoots formed with auxin shock plus reduced glutathione or dithiothreitol appeared more robust with longer leaf petioles and more fully expanded leaves (Fig. 5 b and d) compared with auxin shock alone (Fig. 5a). With *Malus*, dithiothreitol generally was superior to reduced glutathione as a rooting enhancer in combination with auxin shock. Neither dithiothreitol alone (Fig. 5c) nor reduced glutathione alone (Fig. 5e) was effective as a root inducer with *Malus* in the absence of auxin shock.

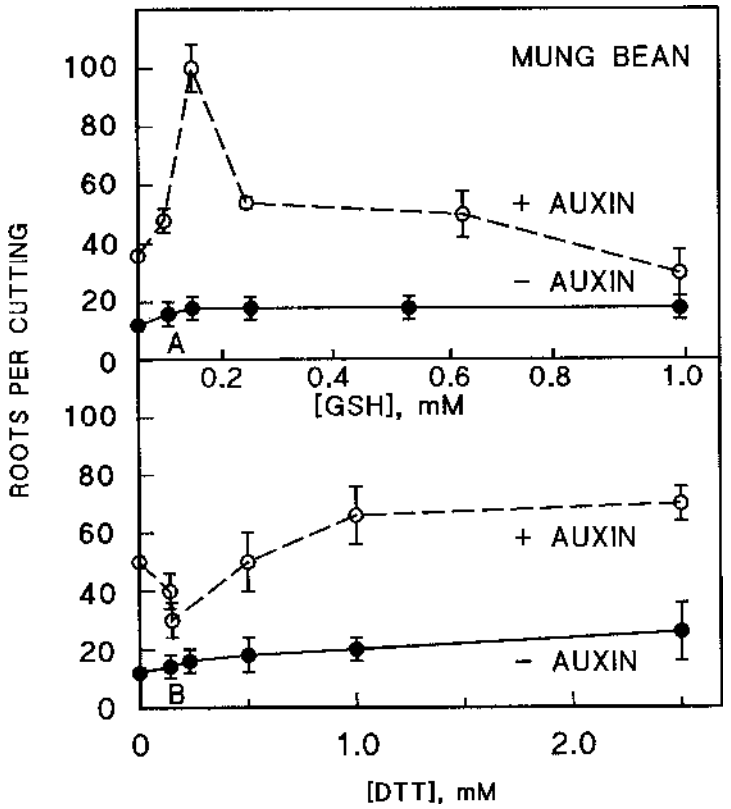

Fig. 1. Rooting of mungbean shoots in the presence (———, ❍) or absence (——, ●) of 2 mM auxin (2 h) as a function of the concentration of thiol compounds. (**A**) Glutathione (GSH) and (**B**) dithiothreitol (DTT).

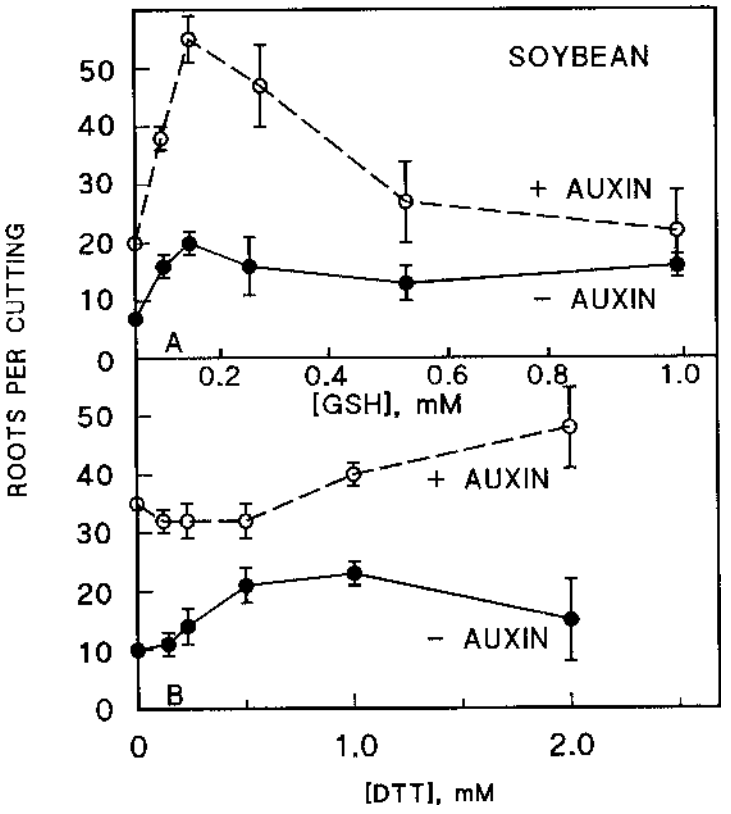

Fig. 2. Rooting of soybean shoots in the presence (———, ❍) or absence (——, ●) of 2 mM auxin (2 h) as a function of thiol compounds. (**A**) Glutathione (GSH) and (**B**) dithiothreitol (DTT).

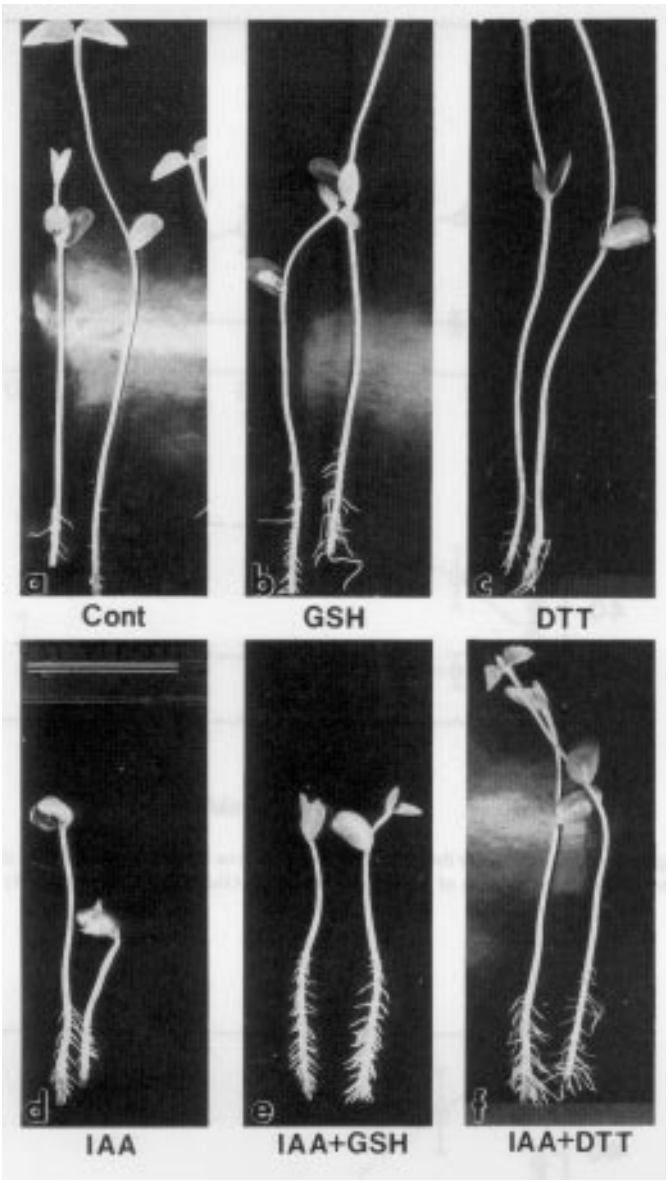

Fig. 3. Appearance of rooted soybean cuttings in the (**a–c**) absence or (**d–f**) presence of 2 mM auxin (2 h). (**a**, **d**) No thiol compound, (**b**, **e**) 0.1 mM glutathione (GSH), and (**c**, **f**) 1 mM dithiothreitol (DTT). Scale bar = 5 cm.

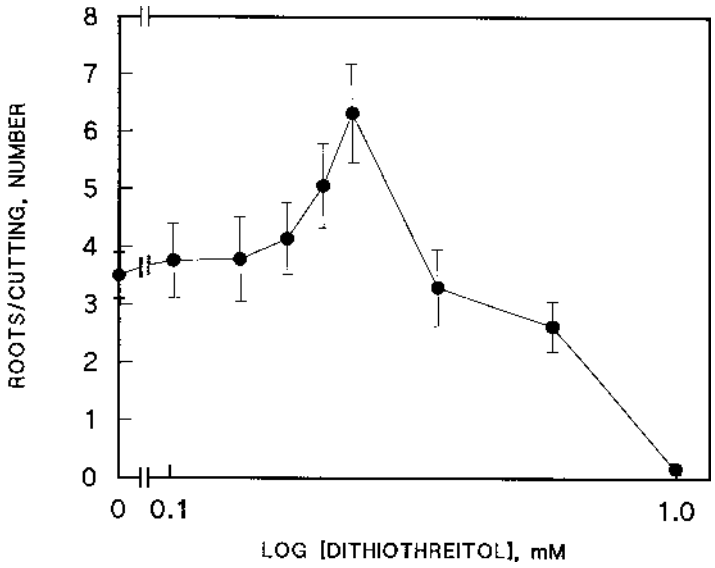

Fig. 4. Rooting of *Malus* ×*domestica* 'Jork 9' cuttings in response to dithiothreitol.

Results for *Hedera* cuttings (Table 4) showed a response to dithiothreitol plus auxin shock similar to that observed with *Malus*. Not only was the number of roots per cutting about doubled, but the number of cuttings producing no roots was reduced from ≈30% to ≤10% by supplementing auxin shock with either 0.1 or 0.3 mM dithiothreitol.

Discussion

There have been few studies related to the ability of thiol compounds, such as glutathione and dithiothreitol, to influence differentiation in plants. Such reagents, which modify thiol groups, have been used extensively to probe the potential role of these substances in maintaining the structure and function of numerous hormone and neurotransmitter receptors (Aronstam et al., 1978; Bottari et al., 1979; Falcone and Aharony, 1990; Larsen et al., 1981; Pavo and Fahrenholz, 1990; Sidhu et al., 1986; Suen et al., 1980), but similar studies have not been performed with plants.

The effectiveness of thiol compounds to induce rooting of soybean shoots was comparable to that of the classical root induction treatment using auxin shock. With soybean, mungbean, and apple, the thiol compounds dithiothreitol and glutathione were less active alone but were strongly interactive with an auxin shock in promoting root growth. With the combination of dithiothreitol or glutathione plus auxin shock, the number of root primordia formed increased compared to auxin shock alone. Additionally, the roots induced by thiol compounds alone or together with auxin shock were generally longer and healthier than those induced by auxin shock alone.

The finding that the formation of root primordia is promoted by thiol compounds, either in the presence or absence of auxin shock, appears to be new. Lis-Balchin (1989) showed promotion and inhibition of adventitious root formation in *Pelargonium* by natural antioxidants (vitamins C and E) or synthetic food antioxidants mixed with gallic acids. Standardi and Romani's (1990) paper dealing with *Malus* and the rooting process reported millimolar concentrations of glutathione where rooting was inhibited.

Using thiol compounds, and especially dithiothreitol, to enhance root formation in combination with auxin shock may have practical application in commercial plant propagation involving potentially difficult-to-root spe-

Table 3. Response of rooting *Malus* ×*domestica* seedlings ('Jork 9') to reduced glutathione 14 days after auxin shock.

Reduced glutathione (mM)	Rooting (%)	Roots/ plant (no.)
0.0 (control)	55 a[z]	2.26 a
0.025	70 a	2.47 a
0.050	77 a	4.59 b
0.075	87 b	4.83 b
0.100	67 a	3.14 a

[z]Dunnett's test at $P \leq 0.05$. Means followed by an "a" do not differ from controls.

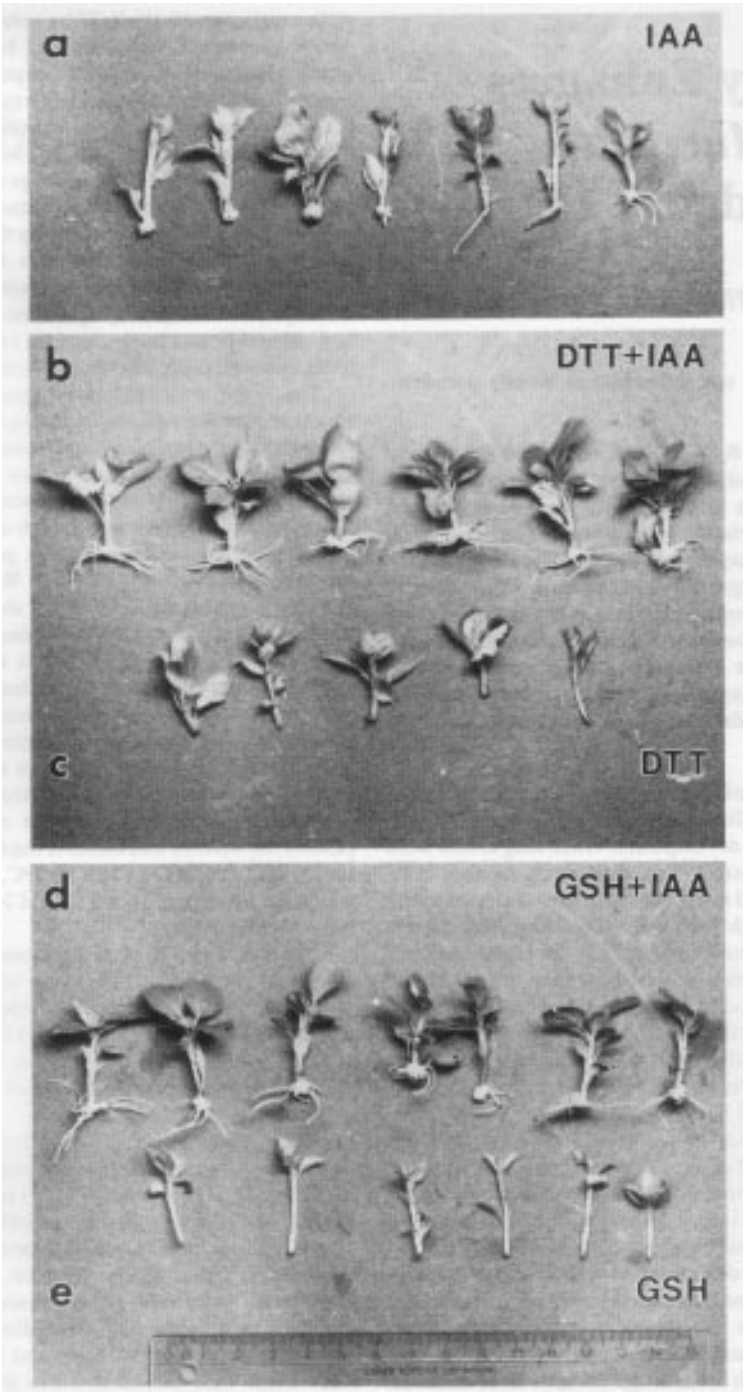

Fig. 5. Appearance of rooted *Malus* 'Jork 9' cuttings in the (**a**, **b**, **d**) presence or (**c**, **e**) absence of 2 mM auxin (2 h). (**a**) Auxin (IAA) shock alone, 1 mM dithiothreitol (DTT) (**b**) with or (**c**) without auxin shock, and 0.1 mM reduced glutathione (GSH) (**d**) with or (**e**) without auxin shock. Scale bar = 15 cm.

cies. *Malus* ×*domestica* 'Jork 9' and *H. helix* are normally considered to be difficult to root. Dithiothreitol dramatically reduced the number of cuttings producing no roots. Even with less difficult-to-root species, the length and quality of roots were improved. Roots were longer and less deformed in response to auxin shock. Shoots were more robust when auxin shock was combined with thiol compounds. Shoot growth increased relative to plants treated with the auxin shock alone.

The mechanism by which thiol compounds might enhance rooting is unknown. For example, glutathione reduces auxin effects by forming conjugates (Farago et al., 1994; Sanderman, 1992). However, there is no precedent for potentiation of an auxin response by thiols. Our findings may be of theoretical importance concerning a potential auxin × thiol interaction in plant growth and differentiation and of practical importance to artificial rooting of woody and herbaceous shoot cuttings.

Table 4. Rooting of *Hedera* cuttings after 1 month as influenced by dithiothreitol (DTT) and auxin shock.

Treatment	DTT (mM)	Roots/cutting (no.)[z]	Cuttings with no roots (%)[z]
Control (water only)	0	2 ± 1	33
Auxin shock	0	5 ± 2	30
	0.1	13 ± 9	10
	0.3	13 ± 0	0

[z]Results are means of replicated determinations of six to nine cuttings per treatment ± mean average deviations between the means.

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