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The effects of benzyladenine and gibberellic acid on adventitious root formation in apple stem discs

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Summary — The effects of BA (benzyladenine) and GA_3 (gibberellic acid) were studied in stem discs of the apple rootstock M9 cv Jork. The disks were first cultured for 24 h in darkness on rooting medium containing 24.6 μM IBA, and then transferred to light onto a hormone-free medium or exposed to a medium supplemented with either BA (1.3 μM) or GA_3 (10 μM) for different periods during the rooting procedure. The BA treatment strongly inhibited root formation especially when applied at the beginning of the rooting period. GA_3 also reduced rooting when applied both at the beginning and at the end of the rooting period.

apple rootstock / adventitious root formation / auxin / benzyladenine / gibberelic acid

Résumé — Effets de la benzyladénine et de l'acide gibbérellique sur l'enracinement de disques de tiges de pommier. *Les effets de la benzyladénine (BA) et de l'acide gibbérellique (GA_3) sur l'enracinement sont étudiés sur des disques de tige du pommier porte-greffe M9 cv Jork. Les disques, d'abord cultivés pendant 24 h à l'obscurité sur un milieu d'enracinement supplémenté de 24,6 μmol.l^{-1} d'AIB, sont ensuite transférés à la lumière sur le milieu de base dépourvu d'hormones, ou exposés au milieu de base supplémenté en BAP (1,3 μmol.l^{-1}) ou en GA_3 (10 μmol.l^{-1}) pendant différentes périodes. Un transfert des disques sur le milieu supplémenté en BA inhibe fortement la formation des racines, spécialement lorsque le traitement a lieu en début de culture. GA3 inhibe également généralement la formation des racines, à la fois lorsqu'il est appliqué en début et en fin de culture.*

pommier porte-greffe / racine adventive / auxine / benzyladénine / acide gibbérellique

INTRODUCTION

The propagation of woody plant species by cuttings or tissue culture is often limited by poor rooting. Moreover, in woody plants rooting ability declines with maturation at the time when interesting traits can be identified. This limits the commercial production of desirable genotypes (Davis *et al,* 1988). Although several physiological and biochemical factors have been identified as essential in the process of rooting, their effect on the initiation of root primordia still needs to be clarified. In cuttings, it is known that applied auxins (indole-3-butyric acid or naphthalene acetic acid) increase, whereas applied cytokinins (van Staden and Harty, 1988) and gibberellins (Hansen, 1988) inhibit adventitious root formation. Van der Krieken *et al* (1991), studying the root formation in woody crops, have developed a model system with 1-mm stem discs of the apple rootstock M9 cv Jork. Using the stem discs system, it was shown that a 24-hour auxin pulse (24.6 μM IBA) in darkness resulted in the formation of adventitious roots in > 90% of the discs (Welander and Pawlicki, 1992). In this sytem, the rooting process has been mapped by microscopic investigations (Welander and Pawlicki, 1992). This study revealed increased cambial activity after 2 d, root primordium initials after 4 d, and more or less differentiated root primordia after 6 d.

Using this stem discs system, the effects of BA and GA_3 on auxin-induced root initiation and subsequent development of the primordia into roots were studied.

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MATERIALS AND METHODS

Shoot multiplication

In vitro shoots of apple rootstock M9 cv Jork (supplied by B Kunneman, Centre for Plant Tissue Culture Research, Lisse, The Netherlands) were subcultured every 4 or 5 wk on medium consisting of MS salts (Murashige and Skoog, 1962) to which 4.4 μM BA, 0.5 μM IBA, 30 g/l sorbitol, and 7 g/l agar (Difco Bacto) had been added. The medium was adjusted to pH 5.5 before autoclaving. The cultures were incubated in growth chambers at 24 ± 1 °C with a 16-h photoperiod at an irradiance of 33 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes.

Rooting experiments

For the rooting experiments, the 10 mm basal part of stems (excluding the callus at the stem basis) from 4 or 5-wk-old axillary shoots were cut into 1-mm thick discs with a razor blade. The stem discs were cultured, basal part upwards, for 24-h darkness on a basal rooting medium (BM) supplemented with 24.6 μM IBA, and then transferred to BM and light conditions. BM consisted of macronutrients in half strength and micronutrients according to Lepoivre (Quoirin *et al*, 1977), COST vitamins (thiamine–HCl 1.0 mg/l, myo-inositol 100 mg/l, nicotinic acid 0.5 mg/l and pyridoxine–HCl 0.5 mg/l), 100 mg/l proline, 30 g/l sucrose, 7.0 g/l agar (Difco Bacto) at pH 5.5. Temperature and light conditions were as described for shoot multiplication.

BA and GA_3 treatments

To examine the effects of BA and GA_3 on rooting, stem discs were incubated after the 24-h dark period on IBA on BM supplemented with 1.3 μM BA or 10 μM GA_3 for different time periods according to the procedure described in figure 1.

Rooting, expressed as the percentage of discs producing at least 1 root and the average number of roots per rooted disc were recorded in every experiment after 20 d of culture. Thirty discs were used per treatment in each of 3 replications.

RESULTS

Effect of BA treatment

Figure 2 shows rooted stem discs after 20 d of culture. The explants were cultured for 24 h in darkness on BM supplemented with 24.6 μM IBA and then transferred to light on a hormone-free medium. As described in a previous paper (Welander and Pawlicki, 1992), 94% of cultured discs produced roots. An exposure time of the discs to BA > 1 d strongly inhibited the formation of adventitious roots, especially when the treatment took place at the beginning of the rooting period (fig 3A). After only 1 d on the rooting medium, 39.5% and 15.1% were obtained for short exposure times to BA (2 and 3 d, respectively). Nevertheless, these percentages were significantly

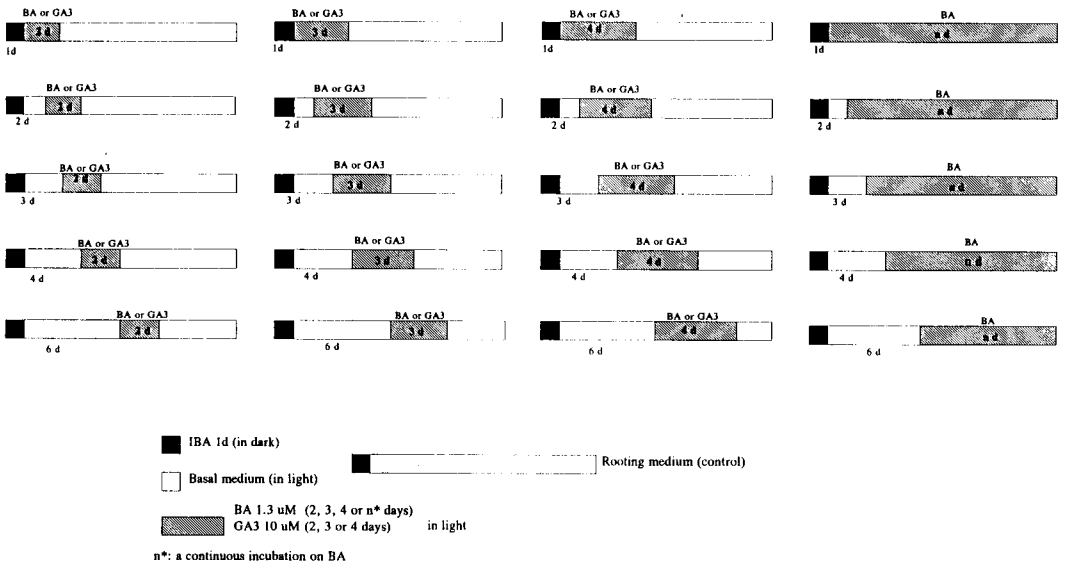


Fig 1. Procedure for BA and GA3 treatments with apple rootstock M9 cv Jork stem discs.

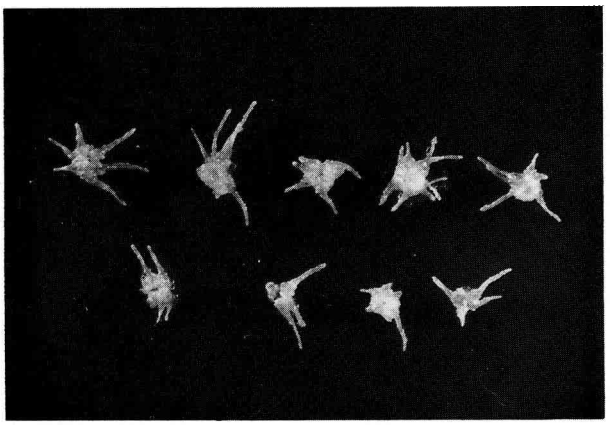


Fig 2. *In vitro* rooting of stem discs from the apple rootstock M9 cv Jork.

lower than the control. After 2 d on the rooting medium, exposure of the discs to BA medium for 2 or 3 d exerted a pronounced inhibition of rooting. Less than 10% of rooting was noted, and callus formation was then observed on the stem discs. When a BA treatment of ≥ 4 d (*n* days) was applied, after 1 or 2 d on the rooting medium no rooting or a very low percentage of rooting (2%) was observed. After extended culture periods (≥ 6 d on the rooting medium) before transfer to BA medium, the rooting percentage was not affected by the length of BA treatment. At this time the root primordia had been formed (Welander and Pawlicki, 1992), indicating that BA does not inhibit root outgrowth. Nevertheless, the observed percentages remained low (≈ 53%) and significantly different from the control (94%). BA treatment also reduced the number of roots per rooted stem disc (fig 3B). For the control (no BA treatment) an average value of 5.9 (± 0.9) roots per rooted disc was obtained, while after the BA treatment the number of roots per rooted discs oscillated around an average value of 1.7 (± 0.6).

Effect of GA_3

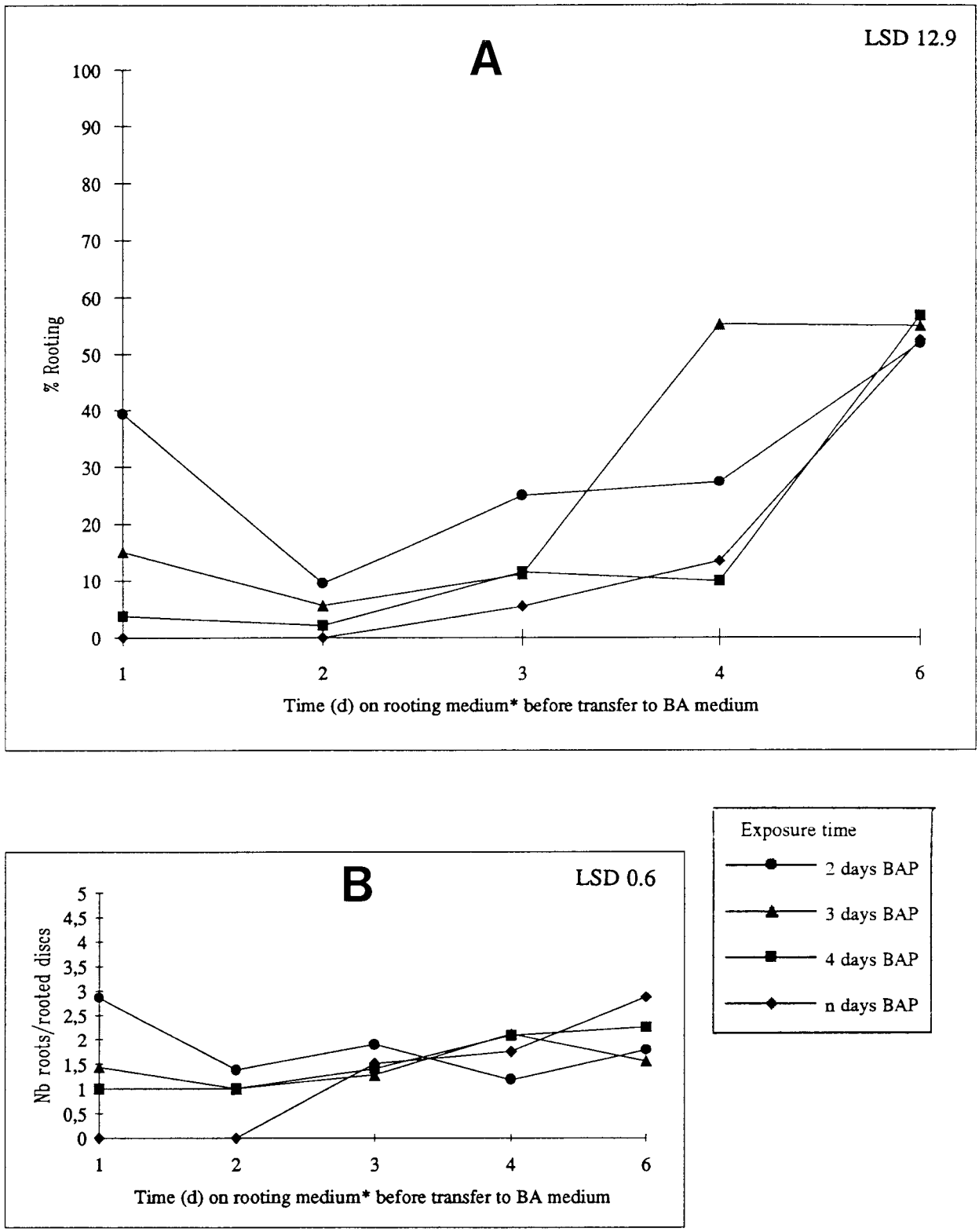
When GA_3 was applied for periods of 2, 3 or 4 d (fig 4A) after 1 d on the rooting medium, the formation of roots on the discs was inhibited (22–44% according to the time of GA_3 exposure) in comparison with the control (94%). Likewise, a GA_3 treatment applied after 6 d on the rooting medium exerted an inhibition of root formation, especially for exposure times of 3 d (36.7%) and 4 d (47.7%). However, when the GA_3 treatment was given after 3 or 4 d on the rooting medium, the rooting percentages were not significantly different from the control, regardless of the time of exposure. One d of GA_3 exposure did not result in the same inhibition, irrespective of the time at which the treatment was given, while continuous exposure always inhibited rooting (data not shown). The number of roots per rooted stem discs is given in figure 4B. For the different treatments, the number of roots which were formed was reduced (an average value of 2.5 ± 0.9 roots per rooted discs) in comparison with the control, but was not completely inhibited (fig 5).

DISCUSSION AND CONCLUSION

Exogenously supplied auxin is essential for root initiation on stem discs, but the rooting response can be modified by other plant hormones such as benzyladenine or gibberellic acid.

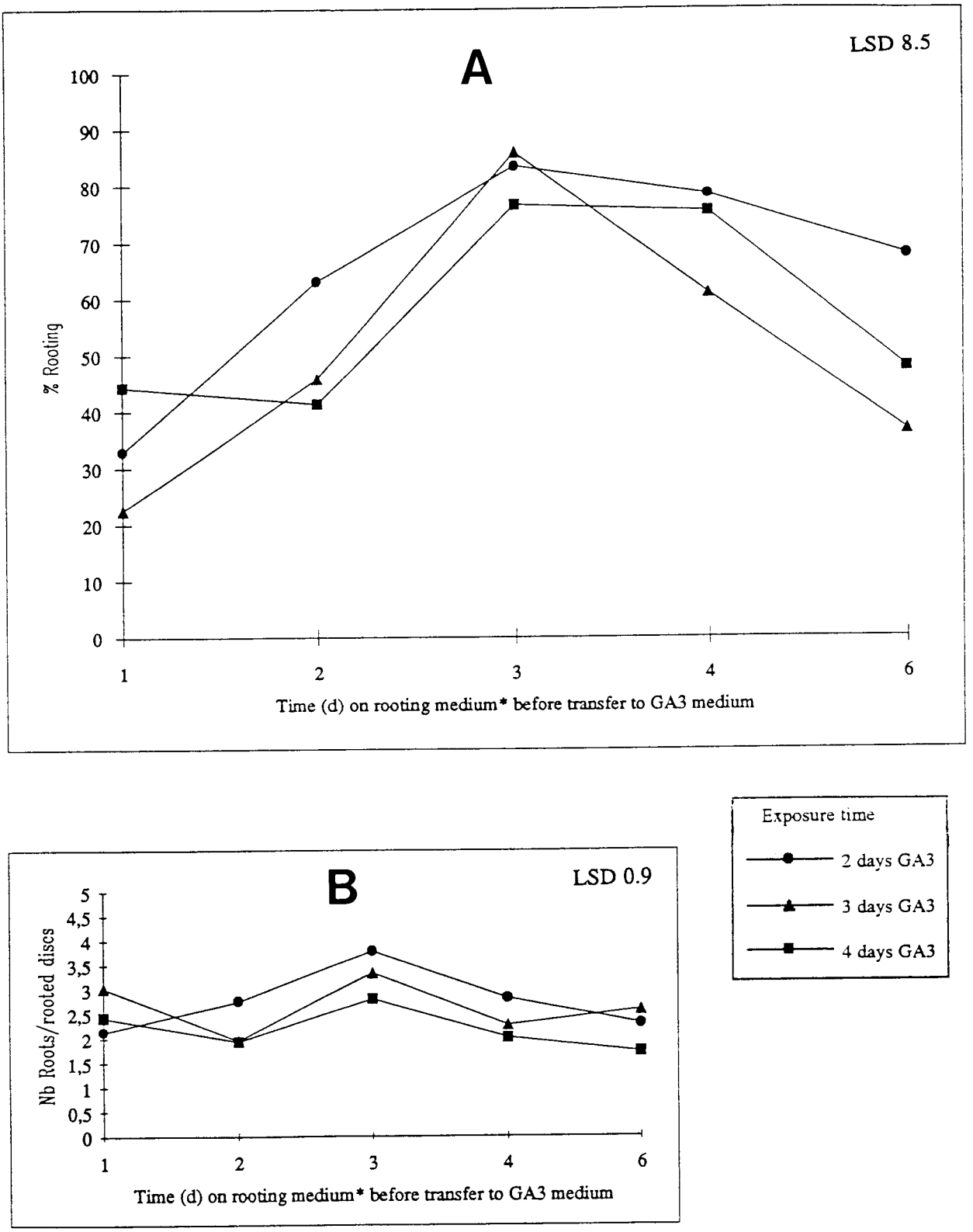
Benzyladenine inhibits adventitious root formation on stem discs, and does this more strongly when applied after 2 d on the rooting medium (fig 4), which corresponds to increased cambial activity (Welander and Pawlicki, 1992). De Klerk *et al* (1992) also found an inhibitory effect of BA (1 μM) using the same system to study rooting in *Malus*. Although rooting was unaffected by the exposure time of BA applied after 6 d on the rooting medium, rooting percentage was still low in comparison to control. This could be due to asynchrony of rooting (Hicks, 1987). Therefore, already formed root primordia could grow out, but new root primordia were inhibited. Indeed, during anatomical studies (Welander and Pawlicki, 1992) root primordia at different stages were observed in the stem discs after 6 d.

An inhibitory effect of GA_3 on adventitious root formation was also observed (fig 4). The inhibition of rooting by GA_3 took place earlier than with BA. This could be related to a partial inhibition of endogenous starch synthesis in the plastids, as described by Coleman and Greyson (1976) in tomato. After 1 d under the normal conditions of rooting, no anatomical changes were noted by light microscopy (Welander and Pawlicki, 1992). However, transmission electron microscopy (TEM) showed a slight augmentation in the number of plastids (Heneen, personal observation). The other inhibitory effect occurred



* Rooting medium: 1 d on BM + 24.6 µM IBA then BM

Fig 3. The effect of benzyladenine (1.3 µM) on the percentage of rooted discs **(A)** and on the number of roots per rooted disc **(B)**. The discs were incubated on the rooting medium for 1, 2, 3, 4 or 6 d before being exposed to 1.3 µM BA for various time periods.



* Rooting medium: 1 d on BM + 24.6 µM then BM

Fig 4. The effect of gibberellic acid (10 µM) on the percentage of rooted discs **(A)** and on the number of roots per rooted disc **(B)**. The discs were incubated on the rooting medium for 1, 2, 3, 4 or 6 d before being exposed to 10 µM GA_3 for various time periods.

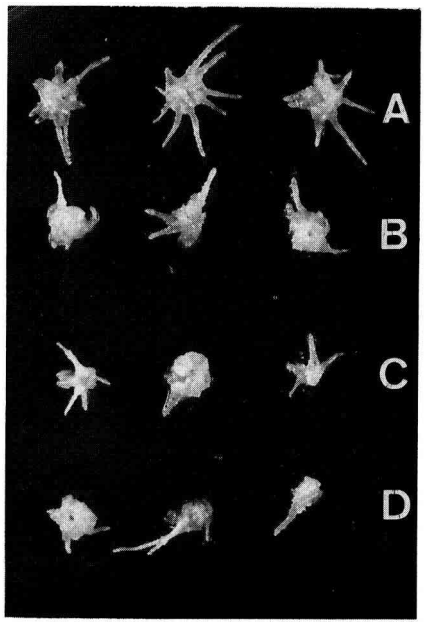


Fig 5. Adventitous root formations on GA_3 treated stem discs. **A**: control, **B**: 2 d rooting treatment followed by 1 d GA_3 treatment; **C**: 4 d rooting treatment followed by 3 d GA_3 treatment; **D**: 4 d rooting treatment followed by *n* d GA_3.

relatively late in the culture period. In this case, gibberellic acid probably inhibited the outgrowth of root primordia. Studying the influence of applied gibberellic acid in brittle willow (*Salix fragilis* L), Haissig (1972) also concluded that GA_3 hindered the development of root primordia *via* an inhibition of mitotic activity within the initiating root primordia.

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