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Factors affecting adventitious root formation in isolated stem segments of *Rhododendron*[1]

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Summary

In vitro culture techniques were applied to investigate factors affecting adventitious root formation in isolated stem explants of the *Rhododendron* cultivars 'Catawbiense Album' and 'Pink Pearl'. Stem segments were isolated in vitro from plants grown in the greenhouse. All experiments were carried out by varying one factor while keeping the other factors constant.
The formation of adventitious roots in isolated stem segments is affected and possibly even limited by different factors. The cultivar 'Catawbiense Album' rooted more easily than the cultivar 'Pink Pearl' agreeing with the experience of nursery practice. Rooting occurred only on segments cut out of young soft shoots. Root formation was strongly promoted when the explants were placed inverted on the medium. Continuous light inhibited while continuous dark promoted rooting. Rooting occurred only in the presence of an auxin together with a sugar in the culture medium. There was no evidence that mineral nutrition and temperatures between 21° and 29°C play an important role.

Introduction

In vitro culture, the technique of growing plants or parts of plants aseptically on artificial nutrient media, has been employed in many fields of research, including horticulture. Several applications of in vitro culture in horticulture were reviewed by Pierik (1968). In vitro culture offers great advantages to the culture of plants or parts of plants in pots or cutting-beds. By means of in vitro culture, plant growth and development can be studied under perfectly controlled aseptical conditions; the quantity of plant material required is relatively small; and the application and uptake of growth regulators and other growth factors is quite easy. Since in vitro culture of small parts of plants saves space and time, and consequently money, the question arises which problems can be studied more easily and more economically using 'in vitro' in stead of 'in vivo' techniques.
The work presented in this paper originated from the observation in horticultural practice that *Rhododendron* shoot cuttings are sometimes difficult to root. These difficulties are sometimes so great that instead of making cuttings, grafting is used for

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propagation. To my knowledge *Rhododendron* has not been cultured in vitro. The objective of the present study is to investigate which plant, environmental and hormonal factors affect adventitious root formation in isolated stem segments of two *Rhododendron* cultivars.

Plant material

All experiments were done with stem segments of plants grown in the greenhouse. In this way suitable plant material was available throughout the year, and greenhouse-grown material gave much less contaminations in vitro than field-grown plants. Vegetatively propagated plants, 3–4 years old, were grown in fertile soil in plastic pots at about 20° C. The temperature was not constant and depended on the weather conditions outside; especially in the summer the temperature was often higher than 20° C. To avoid dormancy and to promote growth, plants were grown under long-day (20 hours) conditions (Doorenbos, 1955), obtained by extending the natural days with light from 160 W Philips ML lamps. During the winter months these lamps were also kept burning during the day. The experiments to be described were carried out in 1967 and 1968 with two *Rhododendron* cultivars: 'Catawbiense Album', which is easy to root, and 'Pink Pearl', which is relatively difficult to root. Terminal buds were removed regularly in order to stimulate the sprouting of lateral buds and to prevent the development of flower buds. Plant material required for the in vitro experiments was always taken from the top of the plants.

Sterile culture techniques

The sterile culture techniques employed were principally the same as those described earlier (Pierik, 1967, p. 31–32). *Rhododendron* stem segments were grown aseptically in standard pyrex glass-tubes plugged with cotton and covered by aluminium foil. Unless otherwise stated, the media contained pyrex-distilled water, 0.6% Difco Bacto agar, Knop's macroelements half strength, Heller's microelements (except $FeCl_3$) half strength, Na-Fe-EDTA 25 mg/l, glucose 20 g/l and IAA 2.10^{-5} g/ml. Before autoclaving pH was adjusted to 5.8. All media were autoclaved at 112° C for 20 minutes. Only young soft shoots were taken of which the bracts had not yet turned brown or dropped off. Before sterilization, leaves, petioles and bracts on the elongated axes were removed. The stems were first immersed in 70% ethanol for a few seconds, followed by sterilization in calcium hypochlorite (20 min, concentration 100 g/l), washed with sterilized tap water and finally prepared in a sterile room.

Cylindrical explants, 1.5 cm in length, were cut out of the elongated part of the stem, situated below the unfolded leaves. The stem segments were always inserted in the medium with terminal ends downwards to a depth of about half of their lengths. For each experiment the material was collected from at least 10 plants in the same developmental stage. The explants were always distributed at random over the different treatments. The number of explants per treatment was 24.

The cultures were usually grown at 25° C in continuous dark, allowing one hour of light per week for observations. Each experiment lasted until 9 weeks after isolation. The experimental results, especially the percentage of rooted cuttings, varied sometimes, depending on the physiological condition of the plant material used and on the

season. All experiments were carried out by varying one factor while keeping the other factors constant.

Experimental results

Cultivar

Several experiments were performed to compare the rooting ability of both cultivars. The formation of macroscopically visible roots started after three weeks of culture. Fig. 1 shows three rooted explants of the cultivar 'Catawbiense Album' after five weeks of culture.

The maximal percentage of rooting for both cultivars is reached 60 days after isolation; for this reason all experiments to be described were stopped after 9 weeks. It was found that the cultivar 'Catawbiense Album' on an average rooted better (62%) than the cultivar 'Pink Pearl' (43%). Experiments performed at the end of 1968, however, indicate that rooting of the cultivar 'Pink Pearl' can possibly be increased by eliminating limiting factors.

Age

The age of the plant material was found to be a major factor in the process of root formation of *Rhododendron*. The capacity to form roots in both cultivars strongly declined with increasing age of the stem segments. Rooting occurred almost exclusively on stem explants cut from young soft shoots on which the bracts had not yet turned brown or fallen off. Older woody stem segments rooted only exceptionally. For this reason all experiments were carried out with young soft shoots. The age factor appeared to be more critical for 'Pink Pearl' than for 'Catawbiense Album'.

Polarity

The influence of polarity on root formation was investigated by inserting stem explants of both cultivars in the basic medium either with their basal ends down or

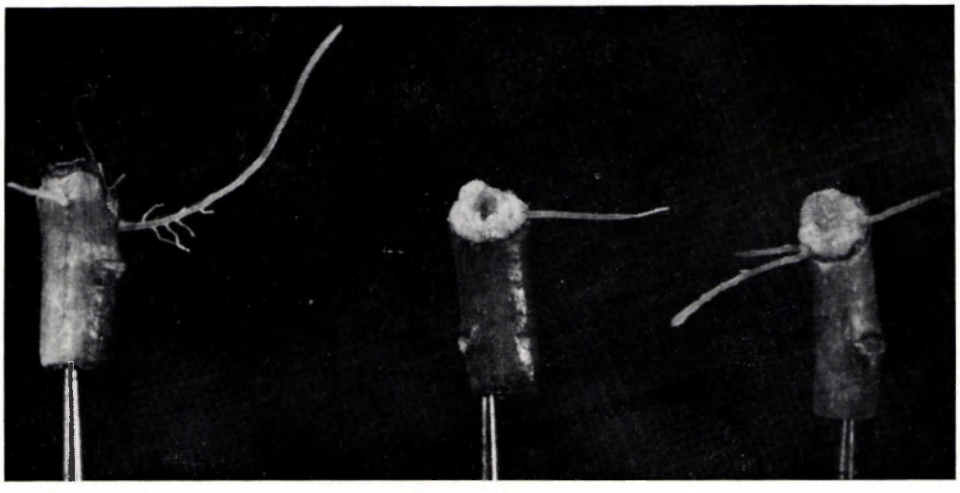

Fig. 1 Formation of adventitious roots in isolated stem segments of Rhododendron *'Catawbiense Album' after 5 weeks of culture*

up. Root formation almost exclusively occurred in inverted stem segments, although occasionally rooting was also observed in normally placed (basal ends down) segments. Hence, all experiments were carried out with inverted stem segments. An additional experiment indicated that root formation, especially in the cultivar 'Pink Pearl', can also occur in normally placed stem segments when grown on liquid media; it is possible that the promoting effect of culture on liquid media is due to a better oxygen supply. It was noted, however, that root formation in inverted stem segments was always superior to normally placed stem segments.

Light and darkness

Several experiments were done to examine the influence of light and darkness on root formation. Stem explants of both cultivars were placed either in continuous light (Philips, TL 40W/55) or in continuous dark. In the cultivar 'Catawbiense Album' light did not prevent root formation, although root formation in dark was both qualitatively and quantitatively much better than in light. In the cultivar 'Pink Pearl' continuous light completely prevented root formation; rooting only occurred in continuous darkness. The one hour of light per week necessary for observation did not inhibit rooting of 'Pink Pearl'. Although the rooting reaction to light and darkness was not fully identical for the two cultivars, all experiments described were carried out in the dark, in order to prevent the introduction of light as a limiting factor for root formation.

Temperature

The effect of temperature was studied by growing stem segments at 21°, 25° and 29° C. Two experiments were performed with each cultivar. The mean percentages of rooted stem segments did not significantly differ at the three temperatures. It was concluded that rooting in both cultivars is not affected by temperatures in the range from 21° to 29° C.

Sugar

Stem segments of both cultivars were isolated on media containing 0, 1, 2 or 3% glucose or sucrose. The presence of a sugar in the media was absolutely essential for rooting; in the abscence of sugar the explants turned black and no rooting occurred. No clear difference could be observed between concentrations of 2 and 3% glucose or sucrose; these, however, appeared to give a slightly better response than 1% glucose or sucrose.

Macroelements

The influence of the macroelements was examined by placing stem segments on media containing different concentrations and compositions of macroelements: control (no macroelements, 0), Knop half strength (K½), Knop full strength (K1), Heller half strength (H½) and Heller full strength (H1). The composition of Knop's or Heller's macroelements is given by Gautheret (1957, p. 12). The highest percentages of rooted stem segments in both cultivars were obtained on the 0 and K½ medium; no difference was noticed between 0 and K½. The media K1, H½ and H1, as compared with 0 and K½, tended to decrease the rooting percentages in the cultivar 'Catawbiense Album', and markedly decreased the rooting percentages in the cultivar 'Pink Pearl'. In conclusion, these results demonstrate that the addition of macroelements to the medium is not essential for rooting.

Auxin

Several experiments were performed in order to examine the influence of IAA and IBA, in the concentrations 0, 2.10^{-6} or 2.10^{-5} g/ml, on adventitious root formation in both cultivars. It was found that rooting occurred only when auxin was present in the culture medium. Percentages of rooted segments in the cultivar 'Catawbiense Album' were not increased by raising the IAA or IBA concentration from 2.10^{-6} to 2.10^{-5}. In the cultivar 'Pink Pearl', however, the rooting percentage significantly increased by raising the auxin (IAA or IBA) concentration. IAA generally appeared to be slightly more effective than IBA in inducing adventitious root formation in both cultivars. For this reason, all experiments described in the previous paragraphs were done with IAA in the culture medium.

Discussion

The results of the in vitro experiments with *Rhododendron* stem segments support the view of Libbert (1956–1957) that a complex of plant and environmental factors determines adventitious root formation. Under the set of environmental growth conditions used in the present study, it seems likely that root formation in *Rhododendron* is limited by certain factors such as sugar, auxin, light, age of the plant material and orientation of the explants. One should, however, be guarded in the conclusion that the principle of limiting factors is applicable in the present case; the question whether a certain factor specifically limits adventitious root formation, can only be answered when a greater diversity of environmental conditions, including physical, hormonal and nutritional factors, is tested.

There are several factors which strongly influence adventitious root formation in *Rhododendron*. Both sugar and auxin are essential for rooting, which was already noticed by many authors (Dore, 1965). The present studies demonstrated that the age of the plant material is a major factor in the rooting of *Rhododendron* stem segments: only segments taken from young soft shoots were able to produce adventitious roots. There is no satisfactory explanation for the fact that in nursery practice older woody *Rhododendron* cuttings from the open can be rooted whereas this is impossible in vitro with plant material cultivated in the greenhouse. The question arises whether plant material grown in the open differs, e.g. anatomically, from plant material grown in the greenhouse.

Almost all the experiments were carried out in continuous darkness because root formation was decreased or prevented in light. Several authors demonstrated that light has an inhibitory effect on adventitious root formation (Galston, 1948; Leroux, 1967) and lateral root initiation (Furuya and Torrey, 1964; Torrey, 1952) in vitro. Only Spanjersberg and Gautheret (1963) showed that adventitious root formation in isolated tuber tissues of *Helianthus tuberosus* was strongly promoted by light. Many authors showed that etiolation of plant material from which cuttings are taken, enhances rooting (Herman and Hess, 1963) and in nursery practice the general observation is that *Rhododendron* shoots grown in the shade root better than shoots grown in full daylight. From all these facts it appears that light is intimately involved in the process of root formation. Galston (1948), who could not give an explanation of the inhibitory effect of light on root formation, concluded that it seems reasonable to postulate the participation of a light-labile rooting factor. It might be possible that the phytochrome pigment participates in root formation, since Furuya and Torrey

(1964) presented evidence for a red/far-red light reversible control of lateral root initiation following auxin stimulation in isolated pea roots.
Rooting was strongly inhibited by placing the explants with their basal ends down; inverted explants, however, easily rooted above the medium. Possibly oxygen supply at the basal part of the stem segments in the medium is limiting root formation. Another explanation may be that basipetal transport of certain unknown factors from the explant to the agar prevents rooting, a phenomenon which cannot occur in inverted explants. Since many authors (Dore, 1965), however, have shown that inadequate aeration inhibits rooting, it seems more reasonable to suppose that oxygen supply at the basal part of the explant is essential for rooting.
The present study emphasizes some physiological aspects of adventitious root formation. Supplementory studies on the anatomy of adventitious root formation in *Rhododendron* will be needed to establish whether reduced rooting capability can be attributed to anatomical barriers. Anatomical studies will also make it possible for the physiologist to discriminate between factors affecting root initiation and/or factors affecting the outgrowth of root initials, which is unfortunately not possible on the basis of the data presented in this study.

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