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Influence of Light on Adventitious Root Formation in Birch Shoot Cultures *in vitro*

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Abstract. The influence of light of different spectral composition and levels of irradiance (2–40 Wm^{-2}) on adventitious root formation (ARF) in birch shoot segments was investigated. Spontaneous rooting of shoot segments occurred in segments with intact apical or axillary meristems. Concerning ARF shoot meristems could be substituted by application of auxin. The very low rooting percentage of shoot segments in darkness was improved considerably by auxin application. Irradiation of cuttings was a requirement for a high percentage of spontaneous rooting. The promoting effect of light was dependent on its spectral composition and was the highest under red followed by white and blue light. The low rooting response under blue light was enhanced almost to the red light level by shielding the root-forming cutting base from light.

In vitro cultured cuttings are a suitable model for characterizing the role of light in adventitious root formation (ARF) (*cf.* FABIJAN *et al.* 1981). Other important factors such as carbohydrate supply (VEIERSKOV *et al.* 1978), nitrogen (WELANDER 1978), plant material and physical conditions can be standardized and easily reproduced. In callus cultures organogenesis was found to be influenced by the spectral composition of light: Regeneration of shoots was induced by light of shorter wavelengths, blue light, regeneration of roots by light of longer wavelengths, red light (KADKADE and SEIBERT 1977, GÖRING and RADKE 1980). To exclude the influence of exogenous hormones on the response of cuttings to light, in our studies shoot segments were used which are able to root spontaneously on a hormone-free medium.

MATERIALS AND METHODS

Birch (*Betula pendula* ROTH) clone propagated for more than 4 years continuously *in vitro* was used for the experiments. The source of explants for establishing the shoot tip culture was a three months old seedling. The propagation medium

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consisted of MURASHIGE and SKOOG's (1962) nutrient elements solution, 1.53 μM thiamine HCl, 0.87 μM biotin, 555.6 μM mesoinositol, 87.7 mM sucrose, 2.5 μM IAA. The medium was solidified with 6 g l^{-1} agar. White light (energy fluence density at plant level 14 W m^{-2}) was provided by fluorescent tubes (*cf.* Fig. 1).

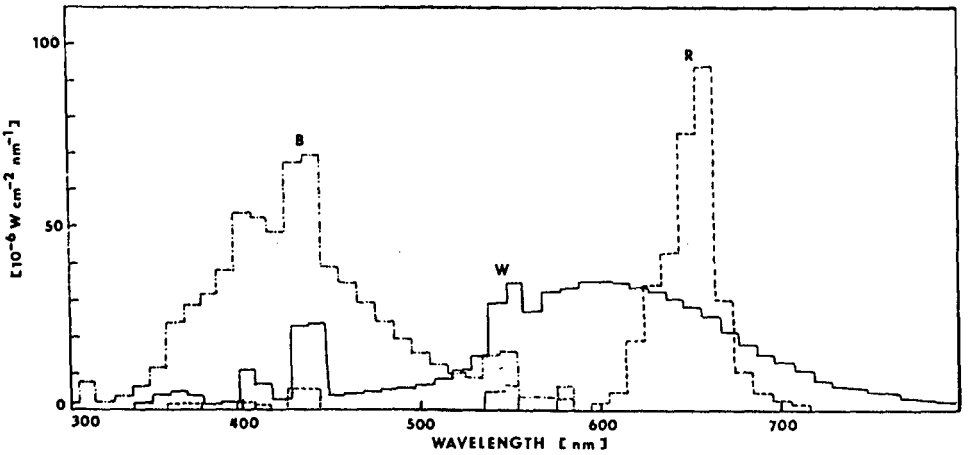

Fig. 1. Emission spectra of fluorescent tubes VEB Narva (GDR): Neutralweiß de luxe (W), red 93 (R) and blue 91 (B).

Shoot segments were taken 4 weeks after the last subculture: 15 to 20 mm long shoot tip segments, 10 to 12 mm long segments from the second or third internode, decapitated segments with one leaf and one axillary bud and isolated leaves were used in the experiments (see scheme on Fig. 2).

All experiments were carried out in test tubes (diameter 18 mm, length 120 mm) containing 8 ml of the basal medium described above but without addition of IAA if not noted in the figures. Usually, the whole segment was irradiated continuously with white (W), red (R) or blue (B) light supplied by fluorescent tubes (Fig. 1). Table 1 represents the data measured at three irradiances using a spherical photometer constructed by Feyerabend in our department. In certain cases segments were kept in darkness (D) or the cutting base was shielded from light by adding washed charcoal powder on the surface of the medium and covering the lower part of test tube with black foil.

TABLE 1

Irradiances measured under white (W), red (R) and blue (B) light [W m^{-2}]

Level	W	R	B
1	–	4	2
2	14	15	10
3	–	40	30

In an additional experiment *Saintpaulia ionantha* shoot cultures of a normal green clone and a chlorophyll deficient clone were used. They were propagated on a hormone-free medium as described by JUNGNICKEL (1977).

The percentage of rooted segments and the number of roots formed per rooted segment were recorded and the mean root length was determined after 28 days of cultivation at 24 ± 1 °C. Experiments were repeated at least twice with 12 segments in each. The U-test of Wilcoxon, Mann and Whitney was used for checking the significance of differences (S. E. is indicated).

RESULTS AND DISCUSSION

The Role of Meristems and Auxins

The investigated shoot segments showed a different rate in ARF, *i. e.* without exogenous auxin supply (Fig. 2). Nearly all shoot tips rooted very rapidly. Shoot segments with an axillary bud rooted less rapidly and to a lower degree depending on the development of the bud. About 30 % of isolated leaves rooted spontaneously.

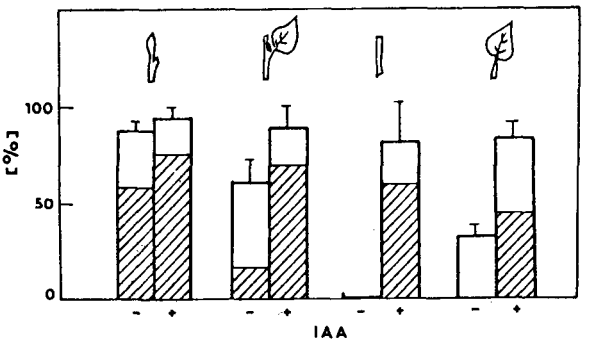

Fig. 2. Adventitious root formation on birch shoot tips, shoot segments with an axillary bud, internodal segments and leaves (from left to right) cultivated on a hormone-free medium (−) or on a medium containing 2.5 μM IAA (+) after 14 d (hatched area) or 28 d (empty area).

Internodal segments, however, did not show any rooting response on a hormone-free medium and died off after a few days, if not treated with auxin. The roots were formed at the morphological base independently of the location of IAA application (Fig. 3c–e).

The importance of apical meristems and outgrowing buds producing the endogenous factor for the induction of adventitious roots (LIBBERT 1956, ERIKSEN and MOHAMMED 1974, FABIJAN *et al.* 1981) was confirmed by our results (Fig. 2). The spontaneous rooting of isolated leaves indicates, however, that they too could be a source of the endogenous factor. In birch shoot segments applied IAA was able to substitute for the effect of original shoot meristems completely (Fig. 2). Thus we assumed that the endogenous auxin supply is the limiting factor for spontaneous ARF and that ARF is positively correlated with the auxin activity in the shoot base.

This activity of auxin, however, is not only dependent on the auxin concentration at the cutting base (MITSUHASHI-KATO *et. al.* 1978) but also on the auxin metabolism (BANSAL and NANDA 1982, MONCOUSIN and GASPAR 1983) and on its relation to cofactors and antagonists such as cytokinins, abscisic acid and ethylene (NORDSTRÖM and ELIASSON 1984, ZOGLAUER *et al.* 1987).

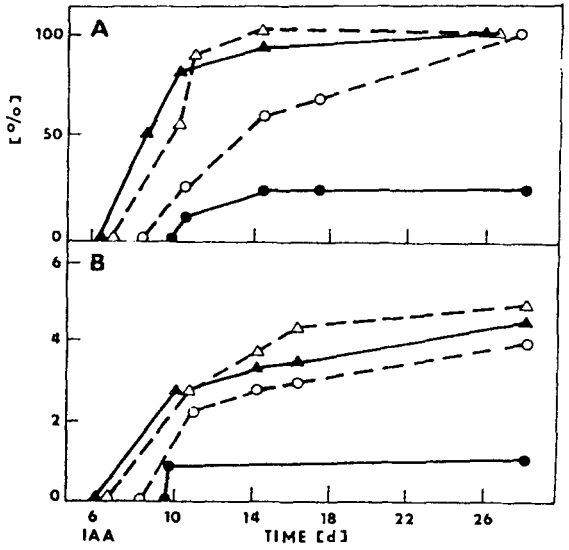

Fig. 4. Time course of adventitious root formation of birch shoot tips cutivated under R light (R, 15 W m^{-2}, △ ○) or in darkness (D, ▲ ●). IAA (2.5 μM) was applied only during the first 5 days of culture (▲ △). – A: Number of shoot tips formed roots; B: number of roots per rooted shoot tip.

The Influence of Light on ARF

A high percentage of spontaneous rooting of shoot tips occurred only in the light (Fig. 4). Application of 2.5 μM IAA at the shoot base for 6 days improved considerably ARF in D. ARF was promoted by R more than by W or B (Fig. 5). Under R about 100 % of cuttings formed roots and there was a tendency to higher root numbers at increased irradiance. On the contrary, the number of roots as well as the number of rooted cuttings was lower under B, especially with increasing irradiance levels.

Saintpaulia leaf cuttings, both green and chlorophyll-free, responded to the light quality in the same manner as birch cuttings (Table 2). Light quality also influenced the emergence of roots. Under R the first roots were visible after 7 days and the number of roots increased substantially during the whole period observed. In the case of high irradiance under R (40 W m^{-2}) and B (30 W m^{-2}), under B even at lower irradiance (10 W m^{-2}) rooting was delayed for 2 to 4 days (data are not shown).

In cuttings of *Sinapis alba* the phytochrome system regulated the availability of the

endogenous factor as it was shown by PFAFF and SCHOPFER (1980). From our results with *Saintpaulia* leaf cuttings we conclude that the influence of light on ARF under *in vitro* conditions, *i. e.* at optimal carbohydrate nutrition, is independent of the presence of chlorophyll and photosynthetic activity. This confirms the results obtained by PFAFF and SCHOPFER (1980) that light acts as a signal system in ARF.

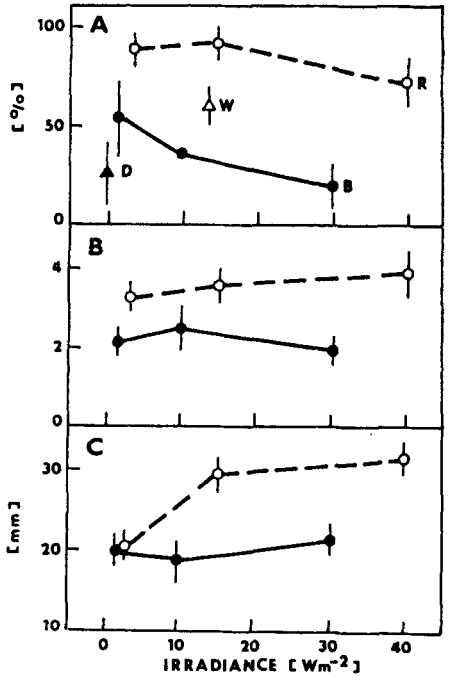

Fig. 5. Influence of red (R) and blue (B) light in dependence of the levels of irradiance on adventitious root formation of birch shoot tips cultivated on a hormone-free medium for 28 days. – A: Number of shoot tips formed roots; B: number of roots formed per rooted shoot tip; C: mean length of roots. D – darkness, W – white light. Vertical bars denote standard mean errors.

TABLE 2

Influence of red (R, 15 $W m^{-2}$) and blue (B, 10 $W m^{-2}$) light on adventitious root formation in normal green (+chl) and chlorophyll-free (−chl) leaf cuttings of *Saintpaulia ionantha* on a hormone-free medium

	+chl		−chl	
Light	Number of rooted cuttings [%]	Roots per rooted cutting	Number of rooted cuttings [%]	Roots per rooted cutting
R	100.0 + 0.0	4.8 + 0.7	73.3 + 12.0	4.8 + 0.7
B	58.3 + 4.8	4.2 + 0.6	48.4 + 15.5	4 3 + 0 6

The Influence of Shading the Cutting Base on Rooting

Rooting was improved by shading the base of the cuttings (Fig. 6) especially under B. Nearly 100 % of shoot tips rooted, *i. e.* about the same number as under R. Nevertheless, the number of roots per cutting was lower under B even with shaded shoot bases.

From our results it can be concluded that light acts directly in the root-forming stem region. Here the main part of inhibitory influence of B is obviously realized (Fig. 6). Two reasons could be responsible for the smaller number of roots of

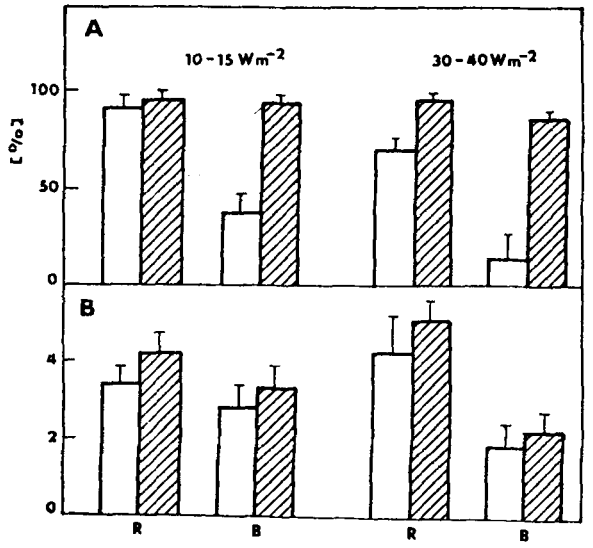

Fig. 6. Adventitious root formation of birch shoot tips on a hormone-free medium under red (R) and blue (B) light if the whole shoot tip was irradiated (empty area) or the lower 5 mm of the shoot tip base were shielded from light (hatched area). A: Number of rooted shoot tips; B: number of roots formed per rooted shoot tip.

B-irradiated shoot tips in comparison to R-irradiated ones. On the one hand fewer root primordia could be initiated, on the other hand the outhgrowth of primordia could be prevented by a stronger correlative inhibition (LIBBERT 1956). It is known that under strong white light the production of inhibitory substances is stimulated in shoots as well as in root tips (FELDMANN 1981). If the production of such substances is also favoured under B a declining auxin effect could be the result. HANSEN *et al.* (1978) and ELIASSON (1978) reported on an inhibitory effect of white light at higher levels of irradiance like those we found under B (Fig. 5). This inhibition was also overcome by darkening the shoot base. On the contrary, under R an increased level of irradiance improved ARF considerably (Figs. 5 and 6) if the shoot bases are irradiated or not. The R effect on rooting of shoot tips is similar to the effect of an auxin application to shoot tips. Perhaps, endogenous auxin is involved in the realization of the R signal.

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Fig. 3 at the end of the issue.