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Hormonal Control of Morphogenesis in Leaf Segments of *Centaurium erythraea*

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Abstract. Transversally cut leaf segments of *Centaurium erythraea* were cultivated on MS medium. Effects of segment polarity, IAA and sucrose concentrations, light and medium volume on morphogenesis were studied. Shoots generally formed at lower (1.3×10^{-6} mol l^{-1}) IAA concentrations than roots and callus (1.1×10^{-5} – 3.4×10^{-5} mol l^{-1}). Leaf polarity strongly modified the effect of IAA concentration, shifting organogenesis at the segment base toward decreased IAA concentrations as compared with segment apex. Light, sucrose concentration above 3 % and high medium volume changed IAA dependence of morphogenesis in various ways and generally suppressed segment polarity.

The regeneration of explants is often used as a model to investigate the role of phytohormones in organogenesis. Some generalizations can be made in this respect. It is assumed that the increase in the auxin/cytokinin ratio (SKOOG and MILLER 1957) shifts morphogenesis from shoots to roots and calli, respectively. Furthermore, the polar transport of IAA is of importance as it interacts with the above effects modifying the auxin/cytokinin ratio. This interaction was studied using leaf cuttings of *Centaurium erythraea*. The investigation was focused on the following question: to what extent might the leaf polarity, position of a leaf cutting within a leaf and conditions of its cultivation such as light, sucrose concentration and volume of cultivation medium exert a modifying effect on morphogenesis determined by auxin/cytokinin ratio.

MATERIAL AND METHODS

Leaves were taken from vegetative *Centaurium* plants grown *in vitro*. Mature leaves were cut transversally into three equal segments. These were laid with adaxial side on medium into deep Petri dishes. MS medium supplemented with sucrose (3 %) and kinetin (10^{-6} mol l^{-1}) was used as a basic one. The IAA concentration varied in the range of 4.2×10^{-7} to 1×10^{-4} mol l^{-1}. The explants were cultivated at 26 °C in darkness. The number of regenerated organs and the size of the callus were evaluated after one month of cultivation. Only basal and central leaf segments were compared as the reactivity of the apical segments lacking the distal cut is entirely different.

RESULTS AND DISCUSSION

The effects of increasing IAA concentration, *i.e.* the rise of auxin/cytokinin ratio as related to segment position and polarity was followed in the first experiment (Fig. 1). The pattern of morphogenesis is in good agreement with

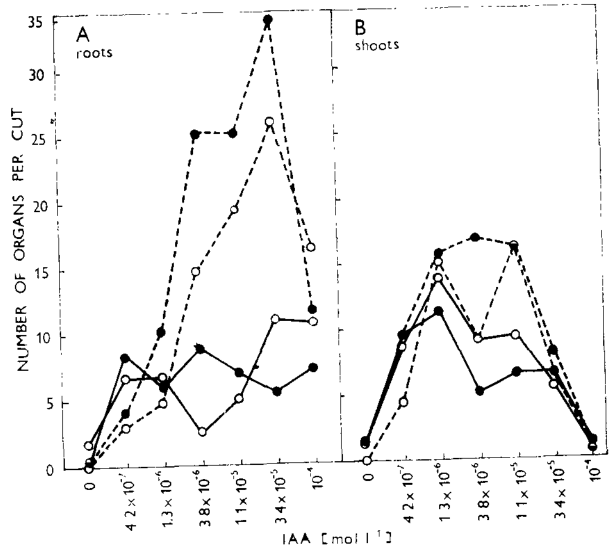


Fig. 1. Influence of IAA concentration on A/rhizogenesis B/shoot formation in darkness; open circles — central segment, closed circles — basal segment, full line — proximal cut, broken line — distal cut.

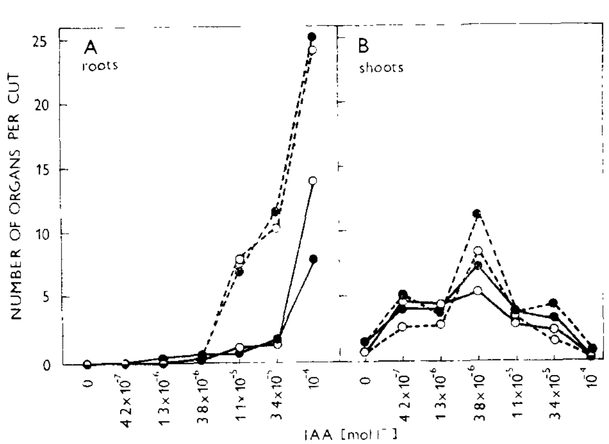
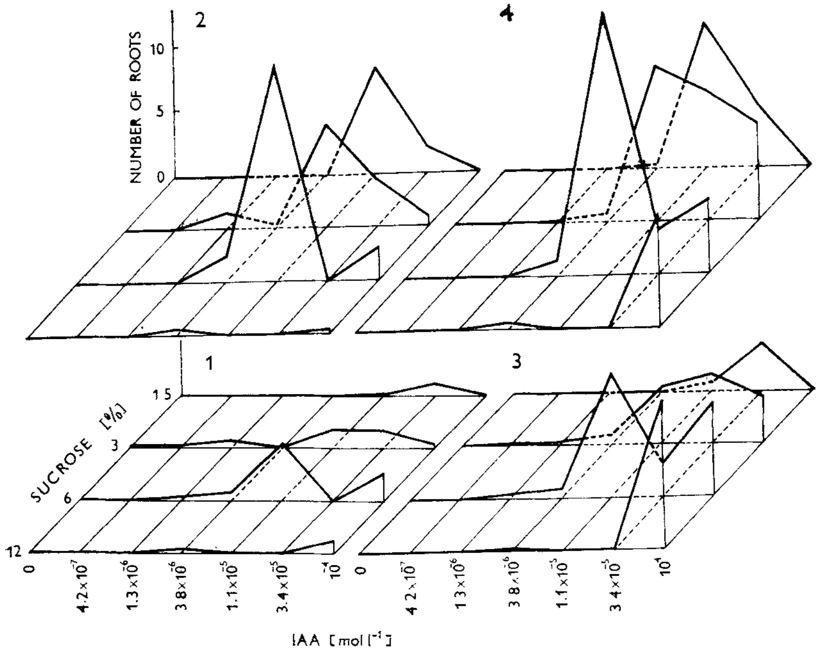
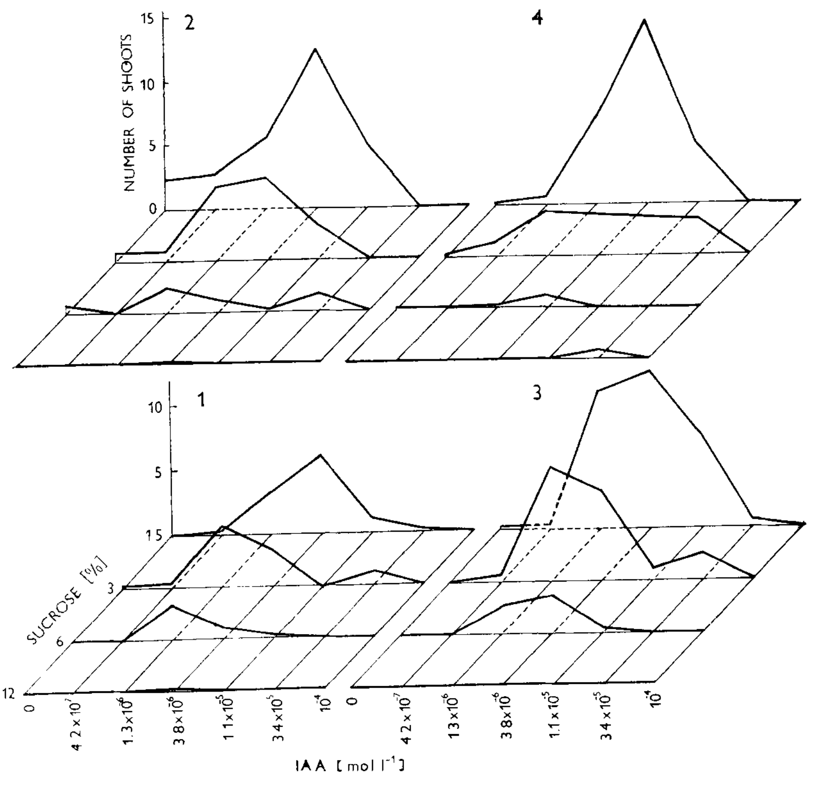


Fig. 2. Influence of IAA concentration on A/rhizogenesis B/shoot formation in light. Explanation of symbols see Fig. 1.


2
4
1
3
NUMBER OF ROOTS
SUCROSE [%]
IAA [mol l⁻¹]


2
4
1
3
NUMBER OF SHOOTS
SUCROSE [%]
IAA [mol l⁻¹]

the results of SKOOG and MILLER (1957) and the assumption of basipetal IAA transport within a leaf. At the lowest IAA concentration the root formation is higher at the proximal cut, but at high concentrations it is much higher at the distal cuts. These effects may be explained by assuming asymmetric IAA distribution due to its polar transport, *i.e.* generally higher IAA concentration at the proximal cuts enables to reach optimal auxin/cytokinin ratio for root formation at lower auxin concentration in the medium. The optimal auxin/cytokinin ratio for shoot formation is shifted to a lower value as compared with the optimum for roots and is lower for proximal cut than for the distal one. Again, the polarity of shoot formation may be explained in the similar way as that for roots. Higher IAA concentrations strongly inhibit shoot production, especially at the proximal cut. Callus is induced by IAA in the range of concentrations employed, but it does not show any significant response to concentration or segment polarity.

In general, a distal cut exhibited a greater reactivity with respect to the level of organogenesis in all experiments. Further, the basal segment appears to be less reactive than the central one, though observed differences proved to be significant only in a few cases.

The effect of auxin/cytokinin ratio on the organogenesis of leaf explants was observed *e.g.* by BEHKI and LESLEY (1976) with tomato and by BAJAJ (1972) with *Torenia*. There are few data on the regeneration pattern as influenced by cut position and explant polarity. Maximum regeneration was observed at the midrib, *e.g.* in *Brassica* and *Crepis* (BROSSARD 1977, DUNWELL 1981). DORE (1965) summarized that when leaves or leaf segments are cultivated on a medium without IAA, morphogenesis is generally higher in the basal part. When auxins are added, they reduce this polarity by inducing regeneration on the distal cut, as demonstrated in *Crassula* by PATERSON (1983). The presented results reveal a similar pattern of regeneration.

In a parallel series of experiments, the effect of light (2500 lx, long days) on the pattern of morphogenesis was investigated. With respect to the instability of IAA under light, it might be assumed that initially higher IAA concentrations would have the same effect as lower concentrations in darkness. Indeed, this expectation is true for both shoot and root formation. Interestingly, morphogenesis displayed reduced polarity under light as compared to that in the dark (Fig. 2.) This might be due to the rapid growth of shoots, whose auxin production may become significant. Callus formation was strongly suppressed by light.

Light was shown to stimulate rhizogenesis in leaf cuttings of *Digitalis purpurea* (RÜCKER *et al.* 1981). Conversely, light has a mild inhibitory effect in present experiments, which was also been reported by COLEMAN and GREYSON (1976) working with leaf cuttings of tomato.

In another series of experiments, the influence of sucrose (1.5, 3, 6, 12 %) on the regeneration pattern induced by the auxin/cytokinin ratio in darkness was studied. As may be expected, *e.g.* from the studies of TRAN THANH VAN (1977) and WELANDER (1976), sucrose concentration exhibited a marked

Fig. 3. Influence of IAA and sucrose concentrations on rhizogenesis (top) and shoot formation in darkness (bottom); 1 — proximal cut of the basal segment, 2 — distal cut of the basal segment, 3 — proximal cut of the central segment, 4 — distal cut of the central segment.

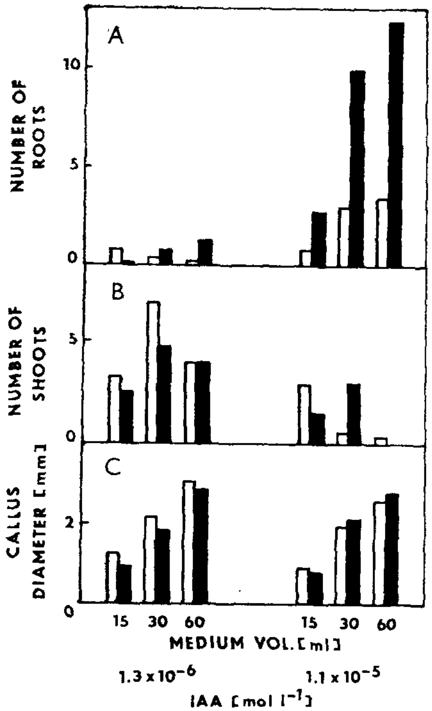


Fig. 4. Influence of the medium volume on A/rhizogenesis, B/shoot formation, C/callogenesis; open blocks — proximal cut, closed blocks — distal cut. Data on both segments were pooled.

effect on morphogenesis. Root and shoot formation were influenced in different ways (Fig. 3). With the exception of the highest concentration, a rising sucrose level did not suppress root formation; conversely, it did strongly suppress shoot formation. The polarity of root formation was also not affected by sucrose. On the contrary, shoot formation lost its clear polarity under higher sucrose concentrations. Moreover, higher sucrose concentrations displaced the optimum for shoot formation toward lower IAA concentrations, *i.e.* rhizogenesis stimulation and inhibition of shoot formation began at lower IAA concentrations. The effect is especially clear-cut at the basal segment.

An increase in rhizogenesis under higher sucrose concentrations was reported by Coleman and Greyson (1976) in tomato leaf cuttings.

Finally, the influence of the volume of medium contained in cultivation dishes on morphogenesis was studied. It was found that increasing medium volume (15, 30 and 60 ml in deep Petri dishes of 9 cm in diameter) inhibited shoot formation and stimulated callus and root formation (Fig. 4). There would appear that several explanations are possible for this effect: 1) Nutrient depletion occurs less rapidly with greater medium volume. 2) Dilution of inhibiting and "conditioning" factors released by the plants into medium is greater in greater medium volume. 3) Finally, the volume effect may be due to the decreasing volume of atmosphere that occurs when the medium volume is increased. The effect of atmosphere volume was demonstrated by Dunwell (1981) working with *Brassica* leaf cuttings.

o In general, the dependence of morphogenesis in *Centaurium* leaf segments n the auxin/cytokinin ratio conforms to the findings of Skoog and Miller

(1957). This effect is modified by the segment polarity, probably owing to the basipetal transport of IAA. However, the interaction of IAA concentration (*i.e.* auxin/cytokinin ratio) and segment polarity is strongly influenced by various external factors, *i.e.* light, varying sucrose concentration and medium volume. These factors shift the optima for root and shoot production in various ways and generally suppress the segment polarity. Therefore, the expected interaction of exogenous IAA concentration and segment polarity in inducing morphogenesis was displayed only in a rather narrow range of cultivation conditions.

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