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BRIEF COMMUNICATION

Biological activity of crenatin, a glucoside from chestnut

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Abstract

Crenatin (3, 4, 5, tri-hydroxybenzyl alcohol, 4-glucoside) isolated from chestnut, was studied for its ability for improvement of rooting in the bean rooting test. Crenatin itself had a positive effect on number of roots. Moreover it enhanced the number of IAA-induced roots. In addition, a retardation of the apical bud growth was observed.

Searching for a suitable method for micropropagation of chestnut, Vieitez *et al.* (1986) reported that buds from 3-month-old plants respond much better to establishment *in vitro* than those from adult plants. Comparative studies of the aqueous extracts of chestnut buds from juvenile and adult plants, purified by *Sephadex G-100* filtration, showed that fractions exhibiting an inhibitory effect on IAA oxidation contained crenatin (3, 4, 5, tri-hydroxybenzyl alcohol, 4-glucoside). This compound was present in buds from juvenile plants in much higher amounts than in adult ones. It was later found that crenatin retarded the IAA oxidation catalysed by horseradish peroxidase and that this effect was concentration dependent (Mato *et al.* 1994).

Consequently, attention was paid to its effect on rooting and to its interaction with IAA, as some polyphenols enhance the auxin-inducing effect on rooting by inhibition of IAA oxidation (Haissig 1986).

Due to the small sample size of buds (2.5 g), an alternative source of crenatin was found in young branches from the same parent tree in which the buds were collected. The branches (116.8 g) were immediately frozen in liquid nitrogen, homogenized and then extracted with methanol. The concentrate was successively chromatographed on 3 MM paper with 15 % acetic acid (R_f 0.73 - 0.87) and butanol-acetic acid-water (4:1:5, upper layer) (R_f 0.39 - 0.47). The bands containing crenatin (purple colour

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with diazotised benzidine) were eluted and the eluates evaporated to dryness and gravimetrically quantified.

The bean (*Phaseolus vulgaris* L. cv. Contender) rooting test was used as previously described (Vázquez and Mato 1991). Each cutting from 9-d-old plants consisting of 3 cm of hypocotyl, the epicotyl, the apical bud and two primary leaves, was placed in a vial containing 10 cm^3 of the test solution (so that the cut end of the hypocotyl was immersed), under the same environmental conditions as for growth of bean plants (photoperiod of 12 h, photon fluence rate of 47 $\mu mol\ m^{-2}\ s^{-1}$, day/night temperature of 25 ± 2/17 ± 2 °C, respectively). When almost all the solution had been absorbed, water was added to maintain the original level. Natural crenatin was tested at the concentration 0.32 mM alone and in combination with 10 μM IAA. Water and IAA controls were included. Ten cuttings were used for each treatment. After 12 d the protruding roots were counted.

The results showed that crenatin itself had a positive effect on number of roots, similar to that elicited by 10 μM IAA. Moreover, it enhanced the auxin-induced rooting initiation. The treatment with IAA in combination with crenatin increased rooting quite significantly althought the response was lower than the additive effect of both compounds at the concentrations tested (Fig. 1).

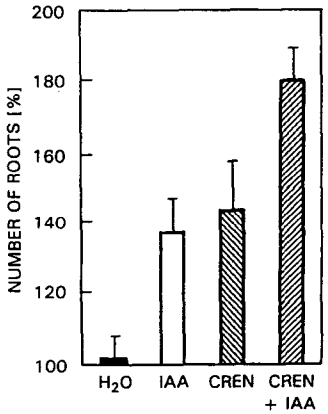

Fig. 1. Rooting response of bean cuttings to IAA (10 μM), crenatin (CREN) (0.32 mM) and CREN plus IAA treatments. Values are the number of roots per cutting as a percentage of those on the water control of three separate experiments, vertical bars indicate S.E.

The positive effect on rooting and the increase in the number of auxin-induced roots by crenatin seems to be a result of an indirect effect through auxin catabolism. As mentioned above (Mato *et al.* 1994), crenatin was shown to inhibit peroxidase-catalysed IAA oxidation; thus it could be involved in the regulation of endogenous auxin content (Kantharaj *et al.* 1979). Another effect observed in the bean cuttings treated with crenatin and crenatin plus IAA was retardation of apical bud growth, quite similar to that obtained with IAA treatment. This also points to the possibility of increasing endogenous auxin levels in cuttings treated with crenatin. Besides that, a swelling of the hypocotyls was observed in cuttings treated with crenatin similarly to that showed by IAA-treated cuttings. Also this effect was greater in treatments

with crenatin and IAA together.

The results are in accordance with the Haissig's thesis (1974) that the type or amount of synergists seems to determine whether a cutting initiate roots easily, with difficulty or not at all. The results presented here also correlate well with the higher rate of the establishment *in vitro* of buds from juvenile plants (Vieitez *et al.* 1986) that are richer in crenatin (Mato *et al.* 1994), and thus probably have a decreased ability to destroy IAA.

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