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BRIEF COMMUNICATION

Rooting, Endogenous Root-Inducing Cofactors and Proanthocyanidins in Chestnut

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Abstract. Rooting cofactors and proanthocyanidins were studied in cuttings of *Castanea sativa* MILL. from a natural mutant Nana and juvenile plants, both of which are easy to root, and from adult plants that do not root. Rooting cofactors and a high amount of proanthocyanidins were found in the easy-to-root cuttings, whereas in the adult plants no rooting cofactors were detected and the amount of proanthocyanidins was very low.

The colourless plant substances which form anthocyanidins when heated with acid were designated collectively by FREUDENBERG and WEINGES (1960) as proanthocyanidins. The anthocyanidins were long regarded as inactive end-products of biosynthesis but the remarkably wide distribution of these substances opened many questions about their possible physiological functions. NITSCH and NITSCH (1962) reported that cyanidin, an anthocyanidin, and goratensidin, a leucoanthocyanidin, were active in promoting the elongation of wheat coleoptiles. OZEKI and KOMAMINE (1981) found in a carrot suspension culture a correlation between anthocyanin synthesis (a metabolic differentiation) and embryogenesis (a morphological differentiation). BACHELARD and STOWE (1963), ARNOLD and ALBERT (1964) and BASTIN (1966) found positive correlation between the number of roots formed at the base of excised hypocotyls and the amount of anthocyanin accumulated in the rooting zone of the cuttings. However, STAFFORD (1968) found that root initiation in sorghum was not always correlated with the accumulation of anthocyanins. The potential for rooting varies greatly among different species and may also differ between juvenile and adult stages of the same species. In general, juvenile shoots, which regenerate roots readily, contain significantly higher anthocyanin levels than do adult shoot apices, leading to the abundance of anthocyanin being reported as a juvenile character. Although

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in the last decade substantial progress has been achieved in elucidating the biosynthesis and enzymology of flavonoid compounds, from a catabolic point of view evidence which links proanthocyanidin and anthocyanidin biogenesis is as yet very scarce. Some biological processes reflect a switch from proanthocyanidin to anthocyanidin metabolism. HASLAM and JACQUES (1974) suggested a relationship between proanthocyanidin and anthocyanidin biogenesis. In the course of a study of phenolic compounds in some woody plants it was observed that the yield of anthocyanidins when the plant material was heated with acid was in general higher in the easy-to-root plants — *Salix* sp., *Cydonia*, *Ribes*, juvenile *Hedera helix* — than in the difficult-to-root — *Quercus* sp., *Juglans*, adult *Hedera helix*, *Robinia* and *Alnus* (unpublished data).

We have investigated this point further in *Castanea*, a difficult rooter when adult, but rooting well at the juvenile stage of growth.

The experiments were performed with: (1) Seven-month-old plants raised from seed in pots in the greenhouse. Cuttings from these plants rooted 60 to 70 % with an auxin treatment. (2) Cuttings taken from the previous year's growth of adult trees of the hybrid 431 (*C. sativa* × *crenata*), which do not root at all. (3) Cuttings from the previous year's growth of adult trees of an easy-to-root natural mutant "Nana". Samples were collected in early November from plants growing in the same orchard.

Analysis of Proanthocyanidins

The cuttings were peeled and the bark cut into little pieces and used immediately for analysis. Samples of 0.5 g were heated at 100 °C with 25 ml of 2 M HCl for 30 min. The extract was then decanted, cooled and extracted with 25 ml of isoamyl alcohol. The mixture was centrifuged at 2 600 × *g* for 5 min. The absorption of the alcohol extract at 530 nm was used as a measure of the amount of proanthocyanidins. All values presented are means of 3—4 runs of three samples each. The anthocyanidins formed were identified in the isoamyl extract by their Rf values in descending paper chromatography with acetic acid : hydrochloric acid : water (30 : 3 : 10, v/v/v) and formic acid : hydrochloric acid : water (5 : 2 : 3, v/v/v/), as solvents.

Extraction and Chromatography

Fresh cuttings were sliced and extracted with water at 4 °C for 24 h (2 ml of water per g of plant material). The filtrate was reduced in vacuo to a tenth of the initial volume, centrifuged, acidified to pH 2.5 and partitioned six times against diethyl ether. Aliquots of the ether extracts equivalent to 100 g of plant (f.m.) were streaked on 20 cm wide Whatman n° 1 paper strips. The chromatograms were developed 40 cm by descending chromatography in isopropanol : ammonia : water (10 : 1 : 1, v/v/v). The paper was cut into 10 equal longitudinal strips and each strip into 5 sections corresponding to 0.2 Rf units, so that ten replicas of each section were tested by the bean rooting test. Blank strips developed in the same solvent served as control. Each section was cut into small pieces and placed in a vial containing 10 ml of 10^{-5} M indol-3-ylacetic acid (IAA). Sections of the blank strips were put in vials containing 10^{-5} M IAA or distilled water.

The bean rooting test was performed as described by VAZQUEZ (1973). Uniform plants of *Phaseolus vulgaris* L. cv. Contender were selected 9 d

after sowing. Cuttings consisted of epicotyl, primary leaves, a small apical bud, and 3 cm of hypocotyl. After 6 h of elution a bean cutting was placed in each vial. The cuttings were allowed to absorb nearly all the test solution and distilled water was then added to the vials daily to maintain the original solution level. The roots formed in the cuttings were counted after 12 d and the results expressed as percentage of the number of roots formed on the control cuttings. Analysis of variance was applied using the F test to obtain the least significant difference between the results from the solutions being tested and the control. The least significant difference is quoted at the 0.05 level.

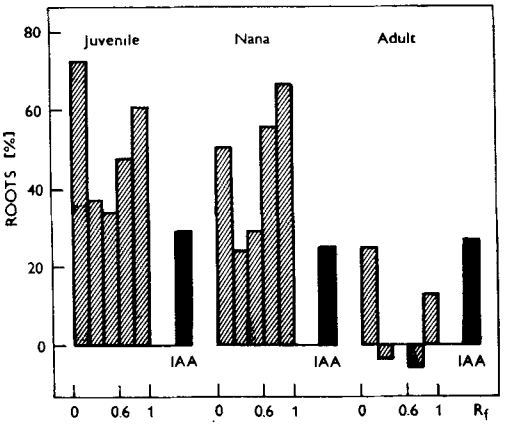

Fig. 1. Rooting activity of the water extracts from chestnut rooting juvenile and Nana plants and non-rooting adult plants as determined by the bean rooting test. LSD at 5 % level 15 (juvenile), 10 (Nana), and 12 (adult).

The cuttings yielded the following total relative amounts of anthocyanidins from the acid hydrolysis (expressed as absorption): juvenile plants 2.000 $\pm$ 0.058, Nana plants 1.200 $\pm$ 0.049, and non-rooting adult plants 0.250 $\pm$ 0.016. Delphinidin and cyanidin were the only anthocyanidins identified in the isoamyl extracts, the former being the most abundant. In easy-to-root cuttings from both juvenile and mutant Nana plants the level of proanthocyanidins was much higher than in cuttings from adult plants which do not root at all. Thus the regulation of biosynthesis of these flavonoids in chestnut was related to the capacity for root differentiation of the plant tissue producing the pigments. This is in agreement with findings of STAFFORD (1968), WIESSENBÖCK and REZNIK (1970), and STAUDE and REZNIK (1973) who reported changes in flavonoid concentration during growth and/or differentiation processes.

The rooting test revealed the presence of two zones of rooting cofactors located at Rf 0.0—0.2 and 0.8—1.0 in the chromatograms of the extracts from juvenile and Nana plants, but not in those of adult plants (Fig. 1). Instead, inhibition of the rooting stimulation caused by 10^{-5} M of IAA was detected from the Rf 0.2—1.0 (Fig. 1). Vanillyl and salicyl alcohols, which are promoters of rooting (VAZQUEZ *et al.* 1983), were located in the second zone promoting rooting (Rf 0.8—1.0). Thus, in chestnut the rooting process

is closely correlated with the presence of rooting cofactors and with anthocyanidin synthesis, a metabolic differentiation.

The loss of rooting capacity at the adult stage of growth seems to be correlated with the presence of an inhibitor and with the fall of proanthocyanidins and rooting cofactors to very low levels. The inhibitor of the rooting stimulating activity of IAA detected at the adult stage might be involved in the minimal capacity for pro-anthocyanidin synthesis observed in adult chestnut plants.

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