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Juvenility and Endogenous Rooting Substances in *Castanea sativa* MILL.

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Abstract. The rooting response to exogenous auxin of cuttings in a juvenile phase of growth from plants of *Castanea sativa* MILL. was determined and simultaneously the rooting potential of the water extracts was evaluated in presence of IAA by a bean rooting test. The level of the extractable rooting promoters was high in the cuttings which exhibited the highest percentage of rooting. An inhibition of the effect of IAA on rooting was detected in the cuttings which showed the lowest rooting response, the histogram differing not much from that of the adult plant. The results indicate that in chestnut the juvenile condition, easy rooting, is associated with high levels of endogenous rooting promoters.

Juvenility appears to be one of the most important factors in the rooting of cuttings. According to DOOREMBOS (1965), the juvenile phase of growth in woody plants is characterized by a greater readiness to form adventitious roots. The internal conditions of the juvenile cuttings seem to be optimally conductive to root formation, but not much is known about it. Research on endogenous cofactors and inhibitors in juvenile plants will hopefully provide a lead to a better understanding both of the limitations that are imposed by ageing in difficult-to-root plants and of the physiological basis of the rooting process in general.

Chestnut (*Castanea sativa* MILL.) is an example of the very close link which exists between rooting ability and juvenility. In our experience (VIEITEZ 1963) relatively easily rooting cuttings can be taken from young plants whereas cuttings from adult plants do not root at all.

The aim of this work was to find out the pattern of the endogenous rooting promoters and inhibitors of chestnut in the juvenile phase of growth. To this end the rooting activity potential of the water extract was tested from cuttings both of young plants growing under different environmental conditions and of the shoots formed at the base of the plant, which according to SCHAFFALITZKY de MUCKADELL (1959) reproduce many characteristics of juvenility.

The rooting response of the cuttings to exogenously applied auxin was simultaneously determined.

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MATERIAL AND METHODS

The cuttings' donors were: 1) Three month old plants growing in pots in a greenhouse. 2) Five month old plants growing outdoors under natural light or under lighting reduced by 75%. 3) Eight month old suckers sprouting from the stubs of four to eight month old plants growing in a greenhouse or from the stubs of two year old plants growing outdoors.

Water Extracts

The 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 (the total of the ether volume being twice that of the aqueous concentrate).

Paper Chromatography

Aliquots of the ether extracts equivalent to 100 g of plant (fresh weight) were line loaded in 20 cm wide Whatman No. 1 paper strips. The chromatograms were developed 40 cm using isopropanol-ammonia-water (10 : 1 : 1, v/v/v) as descending solvent. The chromatograms were cut into ten 2 cm-wide longitudinal strips, each equivalent to 10 g of plant material, and each strip was cut into five sections, so that ten replicates of each section were tested. Blank strips developed in the same solvent served as controls.

Bean Rooting Test

The rooting activity of the different sections of the chromatograms was tested by a bean rooting test which was performed as described elsewhere (VAZQUEZ 1973). Uniform plants were selected 9 days after sowing. Cuttings consisted of epicotyl, a small apical bud and 3 cm of hypocotyl. Each section of the chromatogram was cut into small pieces and placed in a vial containing 10 ml of 10^{-5} M IAA. Sections of the blank strips were put in vials containing 10^{-5} M IAA or distilled water (control). After 6 h a bean cutting was placed in each vial. Cuttings were incubated under the same light and temperature as the seedlings. They were allowed to absorb nearly all the test solution and then distilled water was added daily to maintain the original solution level. Roots were counted after 12 days' incubation, and the results expressed as the percentage of the number of roots formed on the control cuttings. Analysis of variance was employed using the F test to obtain the lowest significant difference (LSD) between the results from the solution being tested and the control (SNEDECOR and COCHRAN 1956). LSD is quoted at the 5% level.

Rooting of the Cuttings

Cuttings, 10 to 15 cm long, were made after removing the apical parts and leaves. The proximal ends of these cuttings were pretreated with a 3 : 3 (m/m) mixture of NAA and IBA in talc powder. The cuttings were then planted in perlite in a mist propagation frame in a greenhouse without photoperiod control. The rooting medium was kept at about 18 °C over the

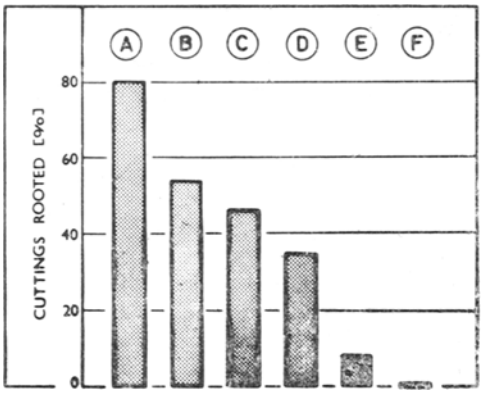

Fig. 1. Rooting response of cuttings taken from: (A) three month old plants; (B) eight month old suckers from young plants; (C) five month old plants growing under reduced lighting; (D) eight month old suckers from two year old plants; (E) five month old plants growing in full light; (F) adult plants. Cuttings were treated with a 3 : 3 (m/m) mixture of NAA and IBA in talc powder. Results recorded after 30 days as percentage rooted cuttings.

rooting period. Two replications of 20 cuttings each were used for each type of cutting. The number of cuttings that had rooted was recorded after 30 days.

RESULTS AND DISCUSSION

The rooting response of cuttings to auxin is given in Fig. 1. The highest rootability was recorded in cuttings from 3 month old plants (Fig. 1A). The cuttings taken from 5 month old plants grown in full light rooted extremely poorly, whereas a good response was found when the plants were cultivated under reduced lighting (Fig. 1C, E). The rooting response of the cuttings from suckers varied, being considerably high in those from young plants (Fig. 1B, D). This difference of the rooting ability is probably associated with the decrease of juvenility retained at the base of the plant (SCHAFFALITZKY de MUCKADELL 1959), which gradually decreases with the height of the plant (PASSECKER 1949). The bean rooting test revealed two root promoting zones located at Rf 0.0—0.4 and Rf 0.6—0.8 (Fig. 2). The level of these extractable rooting promoters was much the highest in the cuttings from 3 month old plants, which were those that exhibited the highest percentage of rooting, whereas it was markedly lower in the cuttings from suckers and from 5 month old plants growing under low light intensity (Fig. 2A). The rooting stimulation detected at the zones mentioned above in the extracts from 5 month old plants growing in full light was similar to that caused by IAA alone, and an actual inhibition of the effect of IAA on rooting was detected at Rf 0.4—0.8 (Fig. 2E). The poor rooting behaviour of these cuttings and the response to the rooting test, which did not differ much from that of the adult plants (Fig. 2F), seems to indicate that these plants have outgrown their juvenile phase. Comparison with the results for the 5 month old plants grown under reduced lighting showed that the juvenile condition of the plant was maintained longer by growing seedlings under these conditions. NJOKU 1956, SCHAFFALITZKY de MUCKADELL 1959, ROGLER and HACKETT 1975 have shown that low light intensity caused rejuvenation or prolonged the juvenile phase in *Hedera helix* and *Ipomea caerulea*.

Results clearly showed that in chestnut the juvenile condition (easy rooting) is associated with high levels of endogenous rooting promoters and the lack of compounds that counteract the effect of IAA on rooting, and that

conversely, the transition to the adult condition seems to occur when the rooting promoter levels fall and inhibitors reach detectable concentrations. PATON *et al.* (1970) suggested that ageing in *Eucalyptus* seedlings involves a direct association between increased levels of inhibitors and decreased rooting ability. Finally, our results for chestnut also agree with those of

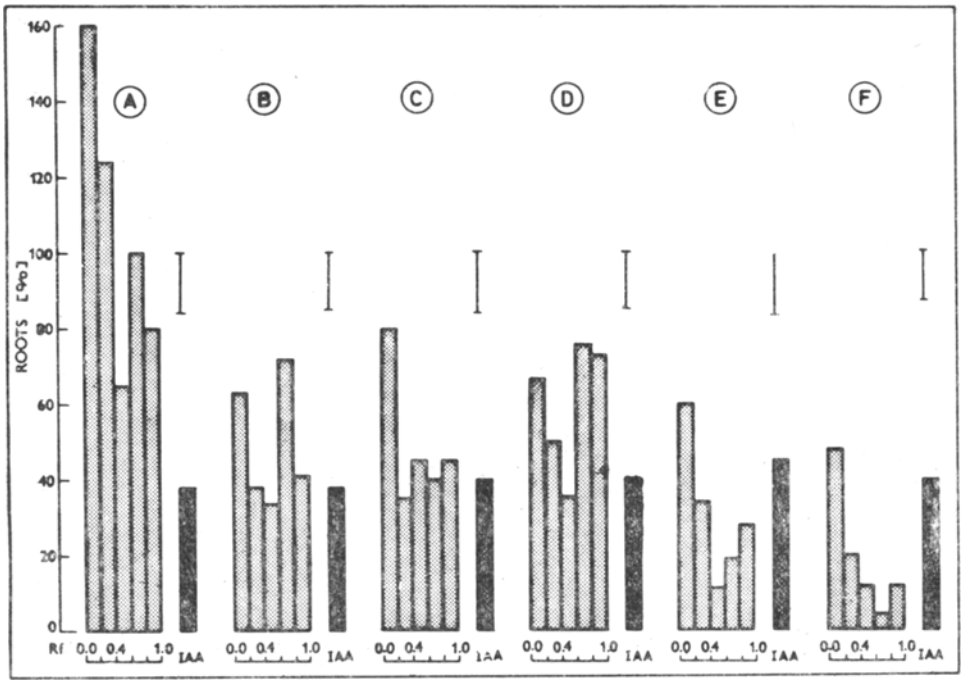

Fig. 2. Rooting activity of the water extracts from cuttings of chestnut as determined by the bean bioassay. Cuttings taken from: (A) three month old plants; (B) eight month old suckers from young plants; (C) five month old plants growing under reduced lighting; (D) eight month old suckers from two year old plants; (E) five month old plants growing in full light; (F) adult plants. Each histogram represents the activity of 10 g fresh weight of plant. Chromatograms were developed in isopropanol-ammonia-water (10 : 1 : 1, v/v/v). Ordinate: Number of roots per cent (number of roots formed in the control cuttings = 100%). The effect of IAA 10^{-5} M is represented. LSD at the 5% level is indicated in each histogram I.

SCHAFFALITZKY de MUCKADELL (1959) in showing that the shoots formed at the base of the plant after the plant stem has been cut off reproduce some characters of juvenility.

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BOOK REVIEW

WOOLHOUSE, H. W. (ed.): ADVANCES IN BOTANICAL RESEARCH. VOLUME 8. — Academic Press, London—New York—Toronto—Sydney—San Francisco 1980. 285 pp., £ 26.60, US $ 59.50.

The eighth volume of this series contains five articles dealing with very different topics. Fast biochemical and chemical events require special techniques for their monitoring. Very promising is picosecond spectroscopy, the biological applications of which are described here by A. H. Reynholds and P. M. Rentzepis. The reader finds here explanation of principles, description of apparatus and methods of data evaluation.Practical application of the picosecond spectroscopy is demonstrated by the investigation of the first triggering step in the oxygen affinity to hemoglobin, of the role of structure of some other heme proteins in energy dissipation, energy relaxation mechanisms of porphyrins, fast events of vision and redox reaction of bacteriochlorophyll. The main value of the presented information is in attracting attention of plant scientists to the technique which can be used for investigation of fast events in plants.

The biochemistry of lignification, which is written by G. G. Gross, is another topic of the volume. The paper contains description of principal steps in lignin biosynthesis and of individual enzymes involved in this process. Special attention is paid to polymerization of cinnamyl alcohols to lignin and to the tissue specificity of enzymes involved in lignification. It is evident that biochemistry of lignification is better understood than are physiological aspects of this process.

Measurement of protein turnover in plants is reviewed by D. D. Davis. There is probably no other field in plant biochemistry where techniques of turnover measurements have met so many difficulties. Discoveries of new factors which affect protein synthesis and degradation, as *e.g.* the existence of separate metabolic pools, compartmentation and recycling of amino acids, have been the main reasons for gradual development and improvement of these techniques. Davis' article can help to understand what are the strong and weak points of the individual methods and which factors can affect results of measurements.

Phosphorus Uptake, Storage and Utilization by Fungi is the title of the next chapter of R. E. Beever and D. J. W. Burns. It collects scattered data in the literature concerning fungal phosphorus physiology and uptake. The review deals with phosphorus economy of the cell, its uptake and utilization, with relation of phosphorus to fungal growth and development, and its translocation. The discussion of this topic reflects the current interest in phosphorus physiology of fungi provoked by recent findings that mycorrhizal fungi may improve nutrition of their host plant and that fungi are a part of chain in the cycling of mineral phosphorus in ecosystems.

The last contribution Plants in Relation to Salinity is written by S. J. Wainwright. Understanding of mechanisms of tolerance of plants to salinity may help to exploit some saline soils in agriculture. This is probably the reason why this problem is so extensively studied and often reviewed. Wainwright focussed his attention on recent advances in the study of salt tolerance, water relations in saline habitats, localization of salt and the role of soluble metabolites and ion toxicity.

As is usual in this series, the book is carefuly edited; it contains high-quality illustrations and extensive author and subject indexes. Individual articles can be recommended to research workers, teaching staff and advanced students in plant biochemistry and physiology.

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