J. Amer. Soc. Hort. Sci. 109(3):284-287. 1984.

Endogenous Content of a Leaf Substance(s) Associated with Rooting Ability of Avocado Cuttings

M. Raviv[1] and O. Reuveni
Department of Subtropical Horticulture, A.R.O., The Volcani Center, Bet Dagan, Israel

ADDITIONAL INDEX WORDS. *Persea americana* Mill., rooting cofactors.

ABSTRACT. Root promotion activity in avocado leaf extracts was determined by the mung bean bioassay. Ten different clones representing a wide range of rooting abilities were compared. Following chromatography of methanol extracts in 8 isopropanol:2 water (v/v), a positive correlation was found between rooting ability of avocado cuttings and a mung bean rooting promoter at R_f 0.9-1.0 of the chromatograms. The same zone inhibited the straight growth of wheat coleoptile.

Attempts to improve rooting of difficult-to-root avocado cuttings by auxin application have met with limited success (12). It seems, therefore, that an endogenous factor(s) other than auxin must play a decisive role in rooting. The rooting ability of cuttings in other plants was found to be correlated with endogenous rooting cofactors which act synergistically with auxin (1. 4. 8, 9. 10. 11, 14. 24).

The objective of the present study was to determine whether the same situation exists for avocado. In a previous paper, the importance of leaf retention was demonstrated for rooting of different avocado clones representing a wide range of rooting abilities (19), and the role of leaves as a source of carbohydrates and nutrients was discussed.

Leaves have been suggested as the source of several factors, other than auxin, essential for rooting (5, 9, 10. 14, 22. 24). Therefore, a correlation was sought between rooting ability of avocado cuttings and endogenous rooting cofactors extracted from their leaves. The possibility of having a common factor associated with rooting of avocado cuttings was studied by comparing 10 different clones. For this purpose, crude methanolic leaf extracts were fractionated by paper chromatography and tested by the mung bean rooting bioassay (10). The disadvantage of such imperfect fractionation is that the response of a specific zone on the chromatogram represents the net effect of both promoters and inhibitors moving to that zone. Further purification is necessary in order to overcome this limitation. It was decided first to check whether rooting activity, as measured in the mung bean bioassay at a specific zone of the chromatogram is associated with rooting of all the avocado clones compared and then, in the 2nd phase,

Received for publication 19 Nov. 1982. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. No. 251-E. 1981 series. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Present address: Dept. of Ornamental Horticulture, A.R.O., Newe Ya'ar Experiment Station, Haifa Post, Israel.

to purify this zone further. If such an association was found, it might be meaningful rather than accidental. A second bioassay, the straight growth of wheat coleoptile (16), was conducted to study the auxin-like activity of the same extracts in order to discriminate between auxinic and nonauxinic effects on the rooting behavior of avocado cuttings.

Materials and Methods

PLANT MATERIAL. Leaves of 10 different avocado clones used in a rooting experiment were sampled, dried, and stored as described previously (19). Their extracts were paper-chromatographed following procedures for dry samples described below and assayed by the mung bean bioassay. Fresh leaves of 2 clones which did not root (Nahlat-7 and Maoz), 1 difficult-to-root (Lula-3), and 1 easy-to-root (Northrop-28/5) were extracted and paper-chromatographed following procedures for fresh samples before mung bean and wheat coleoptile bioassays were run.

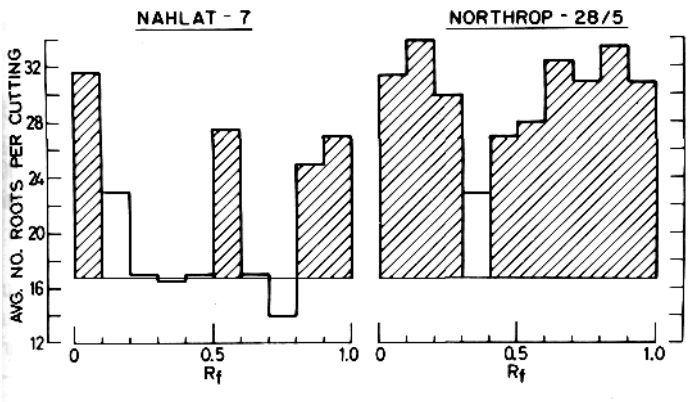

Fig. 1. Histograms showing activity of root promotion in extracts of difficult-to-root (Nahlat-7) and easy-to-root (Northrop-28/5) avocado clones as determined by the mung bean bioassay. Each histogram represents the activity of 50 mg (dry wt) of leaf tissue chromatographed in 8 isopropanol : 2 water (v/v). Significant promotion or inhibition of rooting (greater than twice the standard deviation of the control) is indicated by cross-hatched columns.

EXTRACTION AND CHROMATOGRAPHY. Extraction of dry samples followed Hess's procedure (10, 11) with modifications as described by Reuveni and Adato (18), except that after evaporation of the methanol used for extraction, the aqueous residue was frozen and freeze-dried. The dry residue was dissolved in water and 50-mg equivalents of the original dry samples were spotted on Whatman 3MM filter-paper strips which were chromatographed in a descending system (8 isopropanol:2 water, v/v) to a distance of about 30 cm from the starting line, and cut into 10 sections.

Fresh leaf samples were extracted by absolute methanol. Extracts were treated further in one of the following ways: a) concentrated under reduced pressure at 40°C to dryness, redissolved in methanol, and spotted on Whatman No. 3MM filter-paper strips; or b) evaporated to the aqueous residue, adjusted to pH = 7.0 and then extracted with petroleum ether. Preliminary checks showed that most material with rooting activity was extracted by the petroleum ether. Therefore, the petroleum ether fraction was evaporated to dryness, and the solid residue was redissolved in a minimum amount of petroleum ether and spotted on chromatograms. The paper chromatograms were developed to 20 cm from the starting line by ascending chromatography using 10 isopropanol:1 ammonia:1 water (by volume) as the solvent. Chromatograms were cut into 10 sections, each section assayed for rooting activity as described below.

MUNG BEAN BIOASSAY. A modified version of Hess's method (10, 11) was followed as described by Reuveni and Adato (18), except that indolebutyric acid (BA) (5×10^{-6}M) was used instead of indoleacetic acid (1AA) in the rooting solution. Four cuttings per vial

were used with *5* vials per R_f zone. Light intensity at plant level was 100 μmol s^{-1} m^{-2}, supplied by Growlux fluorescent tubes.

WHEAT COLEOPTILE BIOASSAY. The straight growth bioassay was conducted according to Nitsch and Nitsch (16).

STATISTICAL ANALYSIS. Correlation coefficients were calculated between the number of roots produced in the mung bean bioassay and the rooting percentage of the 10 avocado clones as found in an earlier rooting experiment (19). Coefficients were calculated separately for each R_f value of the chromatograms; thus, for each calculation there were 10 pairs of values (10 clones and 10 R_f values).

Results

A promotive root activity of different rates and at different R_f values of the chromatogram was found in the mung bean bioassay when dry leaf samples of the 10 clones were assayed. The general tendency was for a higher promotive activity in extracts of easy-to-root clones. Two typical histograms of an easy-to-root and a difficult-to-root clone are given in Fig. 1. The question arose as to whether the promotive activity found in the mung bean bioassay at a certain zone of the chromatogram is associated with the rooting ability of avocado cuttings, and whether it is common to all of the 10 clones compared. The correlation coefficients (calculated between number of roots produced in the mung bean bioassay and rooting percentage of the different clones) showed it to be statistically significant only at R_f 0.9-1.0 of the chromatograms, $r = 0.76$, $P < 0.02$.

In order to check this finding, another comparison was made between a difficult and an easy-to-root clone using a different extraction and chromatography procedure (version *a* for fresh material in Materials and Methods). The chromatograms were tested by both the mung bean and the wheat coleoptile bioassays (Fig. 2). Again, the root-promotive activity that was found at different R_f values could not be correlated with the rooting capability of the clones except for the section found at the end of the chromatogram (Fig. 2A). In leaves of Northrop-28/5, a clone which roots easily, very high activity was found at R_f 0.9-1.0 and at lower R_f values adjacent to it, which might be explained by a high concentration which is spread on few zones. No activity was found at R_f 0.9-1.0 in leaves of cuttings of Nahlat 7, a clone which does not root at all.

Greater growth inhibition was found in the difficult-to-root clone Nahlat-7 in the wheat coleoptile bioassay (Fig. 2B). An exception to this general tendency was found at R_f 0.8-0.9 and was even more pronounced at R_f 0.9-1.0, where a greater inhibition was found in the easy-to-root clone.

The finding that at a certain zone of the chromatogram a promotive activity is obtained in one bioassay and inhibitive in another one was checked further. For this purpose, a comparison was made between leaves of the Maoz cuttings which do not root and the moderate- to difficult-to-root Lula-3 cuttings. The crude methanolic extracts of fresh leaves were purified further (version *b* in Materials and Methods for such leaves) before being chromatographed and tested in the mung bean and the wheat coleoptile assays. As in the crude extracts, leaves of the easier-to-root cutting revealed higher rooting activity at R_f 0.9-1.0 of the chromatogram as measured in the mung bean bioassay (Fig. 3A) and greater growth inhibition in the wheat coleoptile at this zone (Fig. 3B).

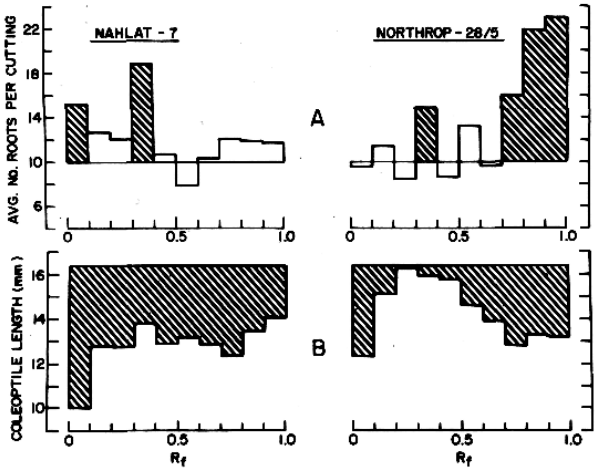

Fig. 2. Histograms showing activity of root promotion (**A**) and coleoptile elongation (**B**) in extracts of difficult-to-root (Nahlat-7) and easy-to-root (Northrop-28/5) avocado clones as determined by the mung bean (**A**) and wheat coleoptile (**B**) bioassays. Each histogram represents the activity of 250 mg (fresh wt) of leaf tissue following ascending chromatography in 10 isopropanol : 1 ammonia : 1 water by volume. Significant promotion or inhibition of rooting or elongation (greater than twice the standard deviation of the control) is indicated by cross-hatched columns.

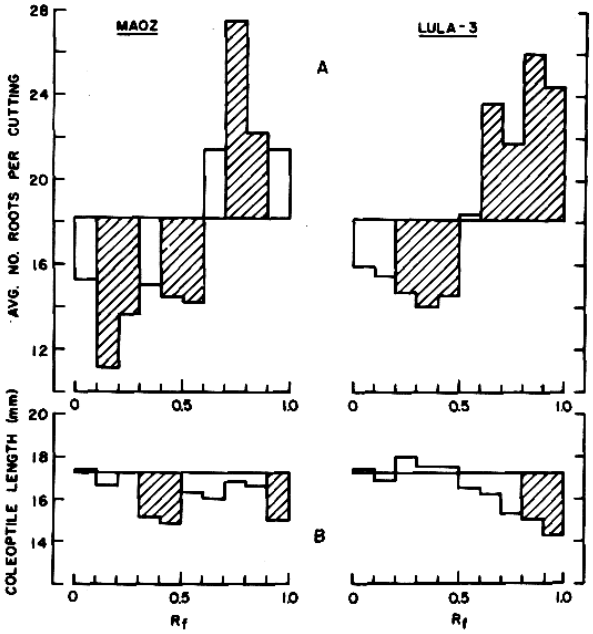

Fig. 3. Histograms showing activity of root promotion (**A**) and coleoptile elongation (**B**) in extracts of difficult-to-root (Maoz) and moderate to difficult-to-root (Lula-3) avocado clones as determined by the mung bean (**A**) and wheat coleophile (**B**) bioassays. Each histogram represents the activity of 250 mg (fresh wt) of leaf tissue following ascending chromatography in 10 isopropanol : 1 water (by volume). Significant promotion or inhibition of rooting or elongation (greater than twice the standard deviation of the control) is indicated by cross-hatched columns.

Discussion

A relationship has been reported for several plants between the endogenous level of auxin in cuttings and their rooting ability (17, 21), whereas in other plants no such relationship could be found (3, 22, 24). Auxin is, therefore, only one of various endogenous factors which are essential for rooting. It is assumed that some substance(s) in addition to auxin and carbohydrates is produced in leaves and transported to the base of cuttings where it stimulates root formation (5, 24, 26). In avocado cuttings, we found a correlation between rooting ability and levels of an activity of endogenous rooting cofactors extracted from leaves. The importance of a minimal level of starch and a low level of manganese for rooting of avocado cuttings was demonstrated in a previous study (19). It is suggested that for good rooting all these requirements must be satisfied. According to Hess and others (8, 10, 11, 14, 24), cofactor 4 is the primary substance involved in the promotion of rooting. In avocado, a root-promoting activity at R_f zone 0.8-0.9 corresponding to cofactor 4 was found as well, but it was not significantly correlated with the rooting ability of all the studied clones.

A significant correlation was noted with activity only at R_f 0.9-1.0. The location of the active zone at the front of the chromatogram led to the question as to whether a high quantity of impurities was concentrated at this zone and, though statistically significant, might not actually be associated with the rooting ability of avocado cuttings. A brief survey of published histograms showing cofactor activities as measured by the mung bean bioassay, and using similar solvent systems, revealed that in other plants as well, high activity was found at R_f 0.9-1.0 (1, 2, 4, 7, 13, 23, 25). Tognoni and Lorenzi (25) found a root-promoting substance (3) at R_f 0.9-1.0 in extracts of *Picea glauca* and

Chamaecyparis lawsoniana. This substance(s) showed a dose-response effect over a wide range of concentration in the mung bean bioassay. The same zone was found to be inhibitory in an auxin activity bioassay. Chromatograms of avocado leaf extracts prepared in a similar procedure and assayed in these 2 bioassays also showed a root-promoting activity at R_f 0.9-1.0 and inhibition of elongation in the same zone (Fig. 2 and 3). That the same active zone is promotive in one test and inhibitive in another was found also by others (6, 15, 20). These findings lend further support to the possibility that the same substance(s) is involved (Fig. 2). In summary, the avocado leaf substance(s) found at R_f 0.9-1.0 calls for further detailed studies of its involvement in the rooting of cuttings and its possible existence in other plants.

Literature Cited

1. Ashiru, G. A. and R. F. Carlson. 1968. Some endogenous rooting factors associated with rooting of East Mailing II and Malling-Merton 106 apple clones. *Proc. Amer. Soc. Hort. Sci.* 92:106-112.
2. Aung, L. H. 1972. The nature of root-promoting substance in *Lycopersicon esculentum* seedlings*. Physiol. Plant.* 26:306-309.
3. Biran, I. and A. M. Halevy. 1973. Endogenous levels of growth regulators and their relationship to rooting of *Dahlia* cuttings. *Physiol. Pl*ant. 28:436-442.
4. Bojarczuk, K. 1978. Studies on endogenous rhizogenic substances during the process of rooting lilac *(Syringa vulgaris L.)* cuttings. *Plant Prop.* 24(4):3-6.
5. Bouillenne, R. and M. Bouillenne-Walrand. 1955. Auxines et bouturage. *Rep. 14th Intl. Hort. Congr.* Vol. 1:231-238.
6. Challenger, S., H. J. Lacey, and B. H. Howard. 1965. The demonstration of root promoting substances in apple and plum root stocks. *Rpt. E. Malling Res. Sta.* 1964. A 48:124-128.
7. Fadl, M. S.,I. Abdel Ghany, O. Bar, and S. Elbosy, 1978. Effect of leaves and natural rooting substances on rooting of sweet potato cuttings. *Egypt. J. Hort*. 5:93-103.
8. Fadl, M. S. and H. T. Hartman. 1967. Relationship between seasonal changes in endogenous promoters and inhibitors in pear buds and cutting bases and the rooting of pear hardwood cuttings. *Proc. Amer. Soc. Hort. Sci*. 91:96-112.
9. Haissig, B. E. 1974. Influence of auxin and auxin synergists on adventitious root primordium initiation and development. *New Zealand J. For. Sci*. 4:311-323.
10. Hess, C. E. 1964. Naturally occurring substances which stimulate root initiation, p. 517-527. *In:* J. P. Nitsch (ed.). Regulateurs naturels de la Croissance Vegetale. C.N.R.S., Paris.
11. Hess, C. E. 1965. Rooting co-factors, identification and functions. *Proc. Intl. Plant. Prop. Sci.* 15:181-186.
12. Kadman, A. and A. Ben-Yaacov. 1965. A review of experiments on some factors influencing the rooting of avocado cuttings. *Yearb. Calif. Avocado Soc*. 49:67-72.

13. Kryzwanski, Z., R. S. Gorecki, and M. Czech. 1976. Some physiological properties of easy-to-root gooseberry *(Ribes grossularia* L.) tumors. *Marcellia* 39:107-112.

14. Lee, C. I., J. J. McGuire, and J. T. Kitchin. 1969. The relationship between rooting cofactors of easy and difficult-to-root cuttings of three clones of Rhododendron. *J. Amer. Soc. Hort. Sci.* 94(1):45-48.

15. Mitsuhashi, M. and H. Shibaoka. 1968. Isolation of an inhibitor of growth and root formation from *Portulaca grandiflora* leaves. *Plant Cell Physiol*. 61:87-99.

16. Nitsch, J. P. and C. Nitsch. 1956. Studies on the growth of coleoptile and first internode sections. A new sensitive straight growth test for auxins. *Plant Physiol*. 31:94-111.

17. Odom, R. E. and W. J. Carpenter. 1965. The relationship between endogenous Índole auxins and the rooting of herbaceous cuttings. *Proc. Amer. Soc. Hort. Sci.* 87:494-501.

18. Reuveni, O. and I. Adato. 1974. Endogenous carbohydrates, root promoters, and root inhibitors in easy- and difficult-to-root date palm *(Phoenix dactylifera* L.) offshoots. *J. Amer. Soc. Hort. Sci.* 99(4):361-363.

19. Reuveni, O. and M. Raviv. 1981. Importance of leaf retention to rooting of avocado cuttings. *J. Amer. Soc. Hort. Sci*. 106(2): 127-130.

20. Shibaoka, H., T. Anazai, M. Mitsuhashi, and M. Shimokoriyama. 1967. Interaction between heliangine and pyrimidines in adventitious root formation of *Phaseolus* cuttings. *Plant Cell Physiol*. 8:647-656.

21. Smith, W. G. and P. E. Wareing. 1972. Rooting of hardwood cuttings in relation to bud dormancy and the auxin content of the excised stems. *New Phytol*. 71:63-80.

22. Steponkus, P. L. and L. Hogan. 1967. Some effects of photoperiod on the rooting of *Abelia grandiflora* Rehd., 'Prostata' cuttings. *Proc. Amer. Soc. Hort. Sci*. 91:706-715.

23. Stolz, L. P. 1968. Factors influencing root initiation in an easy- and a difficult-to-root chrysanthemum. *Proc. Amer. Soc. Hort. Sci.* 92:622-626.

24. Stolz, L. P. and C. E. Hess. 1966. The effect of girdling upon root initiation: auxin and rooting cofactors. *Proc. Amer. Soc. Hort. Sci*. 89:744-751.

25. Tognoni. F. and R. Lorenzi. 1972. Acidic root-promoting growth inhibitor(s) found in *Picea* and *Chamaecyparis. J. Amer. Soc. Hort. Sci*. 97(5):574-578.

26. Van Overbeek. J. and L. E. Gregory. 1945. A physiological separation of two factors necessary for the formation of roots on cuttings. *Amer. J. Bot*. 32:336-341.