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Short Communication

Avocado rooting promoter (ARP) detection in *Laurus nobilis* L. and *Magnifera indica* L. based on gas chromatography

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ABSTRACT

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A qualitative analytical method for avocado rooting promoters (ARP) has been developed based on gas chromatography (GC) of silylated samples.

Leaves of easy and difficult to root clones of *Laurus nobilis* L. and *Magnifera indica* L. were extracted and analyzed for ARP components and for ARP-like activity. Avocado rooting promoter compounds 1I and/or 2I can be detected in leaf extracts of both easy and difficult to root bay laurel. It was found that ARP-like activity was higher in the easy to root than in the difficult to root plants as manifested in the mung bean rooting bioassay. Compounds 1I and/or 2I were found in the easy to root mango clone but not in the difficult to root one. However, ARP-like activity was similar in the two extracts.

Keywords: cutting propagation; mung bean rooting bioassay.

Abbreviations: ARP = avocado rooting promoter; GC = gas chromatography; IBA = indole butyric acid.

INTRODUCTION

Leaf retention on cuttings of evergreen species is indispensable for their rooting in many cases (Hartman et al., 1990). Avocado cuttings behave in a similar manner (Reuveni and Raviv, 1981) and leaves, even of juvenile, easy to root cuttings cannot be replaced by auxin and/or sugar applications to the base of the cutting (Raviv, 1981).

A rooting promoter (ARP) has been detected (Raviv and Reuveni, 1984) in avocado leaves. A positive correlation was found between ARP concentra-

tion in leaves of avocado as expressed in the mung bean rooting bioassay and the rooting ability of those clones. It was found later that the promoter is not an auxin and is inhibitory in auxin bioassays (Raviv et al., 1986b). Its accumulation rate in the rooting zone of avocado cuttings is rapid in juvenile and slow in mature plant material (Raviv et al., 1987) and the accumulation coincides with their first observable rooting events (Raviv, 1981).

The promoter was chemically identified as a mixture of four compounds of which 2II is the most active: 1I, 1 acetoxy-2,4 dihydroxy-*n*-heptadeca-16-en; 1II, 1 acetoxy-2,4 dihydroxy-*n*-heptadeca-16-yn; 2I, 1,2,4 trihydroxy-*n*-heptadeca-16-en; 2II, 1,2,4 trihydroxy-*n*-heptadeca-16-yn (Raviv et al., 1986a). The effect of absolute configuration on rooting activity of compound 2II was determined and it was found that the natural (2R, 4R) form is the most active (Becker et al., 1990).

After completion of the chemical identification, it became possible to develop a specific analytical method for ARP. Such a method would greatly facilitate the study of ARP distribution among plant species.

A qualitative method for ARP components has been developed based on gas chromatography (GC) of silylated samples. Several plant species were analyzed. These analyses were accompanied by mung bean rooting bioassays of the ARP fractions. Of these analyses, results of two difficult to root evergreen species (*Laurus nobilis* L. and *Magnifera indica* L.) are presented here. In these two species we have collections of clones differing greatly in their rooting abilities. One easy to root and one difficult to root clone of each species was chosen for analysis for ARP and ARP-like activity. Rootability of mango cuttings was checked simultaneously with leaf sampling.

MATERIALS AND METHODS

Plant material and rooting experiment. — Bay laurel (*Laurus nobilis* L.) is a tree native to Israel and to other Mediterranean countries. It belongs to the family Lauraceae to which the avocado genus also belongs. In many respects (morphology, growth habit, dioecious mature) it is very different from avocado. However, the rooting capability of its cuttings is very low, as is the case for many avocado cultivars. In previous work (Raviv et al., 1983) we selected and propagated wild clones of bay laurel which varied greatly in their rooting ability. Of these clones, clone number 21 rooted at 94–100% and clone 3 at 24–45%. Since then, several rooting experiments have been conducted which consistently show the same relative rootability between these clones. During the present study, no additional rooting experiment was conducted.

Mango (*Magnifera indica*, Anacardiaceae) clones of different rooting capability were selected and used for repeated rooting experiments (Reuveni et al., 1991). Of these clones, two were chosen for extraction and ARP analysis:

Yotvata 97 and Ein Gedi 3. Their normal rooting percentages are 80–100% for Yotvata 97 and 0–20% for Ein Gedi 3.

ARP extraction. — Young fully expanded leaves were sampled and extracted within 1 h (bay laurel) or 3 h (mango). Leaves were extracted according to the previously described procedure (Raviv et al., 1986b). The crude extract was used for two purposes: GC analysis and rooting bioassay. For the GC analysis, an additional purification was conducted (see below). The detection of the individual ARP components was close to the limit of the GC method. Therefore, we hydrolyzed 1I and 1II to 2I and 2II, respectively. The presence of 1I+2I=A and 1II+2II=B were subsequently determined using the GC analysis described below. For the rooting bioassay, the crude extract was paper chromatographed (Raviv and Reuveni, 1984) and the last section (R_f=0.9–1.0) was used for the bioassay. Additional paper sections were eluted in order to verify ARP existence using GC.

Mung bean rooting bioassay. — The mung bean rooting bioassay, originally described by Hess (1965) was conducted with several modifications, some of which have been described previously (Raviv and Reuveni, 1984). Additional modifications were as follows.

(1) Throughout the present work only one mung bean cutting was inserted in a vial, thus avoiding possible interactions among the cuttings themselves. This also implies that concentration can be expressed on a per cutting rather than on a per vial basis. The solution volume was daily adjusted to 3.5 ml.

(2) A mung bean line, having lower variability and higher responsiveness to rooting promoters was selected in Newe Ya'ar. To select a line of general value, response to indole butyric acid (IBA) rather than to ARP was used as the criterion for selection.

(3) In a previous study (Bassuk and Howard, 1981), it was shown that the rooting response of mung bean cuttings to IBA is age-dependent. When using the standard procedure of cotyledon removal, the cuttings revealed a gradual increase in responsiveness to IBA with age. As it was not certain that the optimally sensitive seedling age for responsiveness is the same for the selected line, it was necessary to determine this empirically. It was found that a 5-day rooting period is optimal in terms of both responsiveness and variability.

(4) Light intensity was 110–120 μM s^{-1}, m^{-2} throughout the growing and rooting periods.

(5) Mung bean seedlings were irrigated with a solution of $Ca(NO_3)_2$ (140 ppm) and H_3BO_3 (1 ppm).

GC analysis of ARP. — For analysis of the ARP compounds by GC the crude methanolic extracts of the leaves or the eluates of paper chromatography were evaporated to dryness, dissolved in a minimal amount of methanol and fil-

tered using 3 mm Whatman filter paper. The extract was then hydrolyzed at room temperature for 4 h in 20 ml of methanol containing 2% KOH (w/v). After hydrolysis was completed, 10 ml of distilled water was added to each sample and the methanol was evaporated. Avocado rooting promoter was extracted three times with CH_2Cl_2 and after evaporation to dryness the residue was redissolved in a minimal amount of CH_2Cl_2 and filtered. The extract was further purified by running the samples through a Florisyl column (Merck 50–100 mesh, 15 cm long, 1 cm internal diameter) using 40 ml of hexane: CH_2Cl_2 (1:1, v/v), 40 ml of CH_2Cl_2, 60 ml of CH_2Cl_2:ethyl acetate (4:1, v/v) and 40 ml of CH_2Cl_2:ethyl acetate (2:1, v/v). Avocado rooting promoter was eluted from the column in the last fraction. After evaporation to dryness, the samples were dissolved in 0.2 ml of dry CH_2Cl_2 and silylated for 20 min at room temperature using 0.2 ml of BSTFA (bis(trimethylsilyl)trifluoracetamide, Aldrich Chemical Co., Milwaukee, WI). Typically, the equivalent of 10 g fresh weight (FW) was partially purified using this procedure, and finally dissolved in 0.4 ml of silylizing agent. Samples of 1 μl were injected into a gas chromatograph equipped with a SE-54 capillary column (25 m long, 0.25 mm internal diameter), splitless injector, Chromosorb precolumn inlet and H_2-flame detector. The column initial temperature was 50°C for 3 min and increased at the rate of 35°C min^{-1} to a final temperature of 200°C. Purge delay was 3 min at 3 ml min^{-1}. Qualitative detection of compounds A and B was performed for all samples. Authentic samples were used for verification of A and B. Retention time under these conditions is about 16.9 min for A and about 17.8 min for B. Currently, this method allows us the qualitative detection of A and B of the ARP composition. It will be possible, however, to calibrate the procedure in the future to enable direct quantitative analysis.

RESULTS AND DISCUSSION

Laurus nobilis *L.* — Results of the test for root promoting activity of the ARP fraction using the mung bean bioassay can be seen in Table 1. There is an apparent association between the clone's rooting capability and the rooting activity of the ARP fraction purified from the leaves. In the GC analysis it was found that both extracts contain compound A.

Magnifera indica *L.* — Unlike the case of bay laurel, there is no apparent association between the rooting capability of the mango clone and the rooting activity of the ARP fraction (Table 2). The GC analysis revealed that neither A nor B were found in the difficult to root extract while A exists in the extract of the easy to root clone. Coupled with the low rooting activity of the ARP fraction of the mango extracts compared with those of bay laurel, it can be

TABLE 1

Average (±SE) number of roots per mung bean cutting as affected by two concentrations of ARP extracted from leaves of an easy and a difficult to root bay laurel clone

Concentration (g equiv. FW)	Clone	
	Easy to root	Difficult to root
0	7.4±1.9	7.4±1.9
0.01	22.0±3.3	17.3±2.8
0.05	23.7±8.3[1]	11.8±3.1[1]

[1]Overdose effects.

TABLE 2

Average (±SE) number of roots per mung bean cutting as affected by four concentrations of ARP extracted from the leaves of an easy to root (Yotvata-97) and a difficult to root (Ein Gedi-3) mango clone

Concentration (g equiv. FW)	Clone	
	Yotvata-97	Ein Gedi-3
0	6.8±0.66	6.8±0.66
0.01	6.4±0.91	7.4±0.67
0.05	8.3±1.01	7.3±0.79
0.1	11.1±2.16	8.6±0.98
0.5	20.6±2.94	21.0±4.55

concluded that ARP is not endogenously involved in the rooting process of mango cuttings.

Compounds 1I and/or 2I of the ARP complex were positively identified using GC analysis of purified extracts of leaves of bay laurel (two clones) and mango (easy to root clone). Components of ARP were not found in leaf extracts of a difficult to root mango clone according to the current sensitivity of the method.

The poor rootability of the cuttings of the difficult to root mango and bay laurel clones cannot be overcome by auxin application (data not shown). It is therefore assumed that auxin is not the limiting factor for their rooting. Avocado rooting promoter was first found in avocado tissues (Raviv et al., 1986b). At present it is still difficult to quantify ARP concentration from the GC results. It is therefore too early to determine whether deficiency in any member of the ARP complex is actually the limiting factor for rooting of these species, although this may be the case for bay laurel. More studies, using ARP

application, are required to further clarify the endogenous role of ARP in the rooting process of these species.

Compounds A and/or B are also found in leaf extracts of *Citrus reticulata* cultivar 'Rangapur', *Citrus grandis* cultivar 'Oroblanco', *Lindera benzoin* and in fruits of *Sassafras albidum* (to be reported in separate communications).

It can therefore be concluded that these compounds are not specific to avocado.

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