Endogenous Carbohydrates, Root Promoters, and Root Inhibitors in Easy- and Difficult-to-Root Date Palm (*Phoenix dactylifera* L.) Offshoots[1,2]

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Abstract. Endogenous root promoting and root inhibiting activity as measured by the mung bean bioassay showed 2 rooting promoters at R_f 0.1-0.2 and at R_f 0.3-0.5, respectively. Extracts were paper chromatographed with isopropanol:water (8:2 v/v). No clear correlation was found between rooting ability and root promoting activity. Root inhibiting activity at R_f 0.5-0.8 was higher in difficult-to-root than in easy-to-root offshoots. The total carbohydrate content was higher in easy-to-root than in difficult-to-root offshoots although the latter contained more reducing sugar.

Date palms are generally vegetatively propagated by offshoots which are in effect side branches. Only rooted offshoots are planted in date gardens, and rooting occurs either while the offshoot is still attached to the palm (16), or in a nursery (4, 22). The rate of rooting varies with the cultivar (22) and with the size and origin of the specimen. Large offshoots root more easily than small ones (17) and offshoots growing low on the trunk root better than those occuring at a higher position (4).

The rooting capacity of many cuttings has been correlated with their carbohydrate content (2, 18). Since the date trunk is known to be rich in carbohydrates (1), we investigated whether a relationship exists between the starch and sugar content of date palm offshoots and their rooting capacity. Other endogenous substances found to be correlated with the rooting ability of cuttings in various species include rooting promoters which act as cofactors of IAA in the mung bean rooting bioassay (5, 8, 9). Endogenous rooting inhibitors have also been found in a number of species and it is suggested that these are the principal reason for rooting failure in certain difficult-to-root cuttings (5, 12, 21).

We sought a correlation between rooting ability of offshoots and endogenous carbohydrates, rooting promoters, and rooting inhibitors in easy- and difficult-to-root offshoots. Easy-to-root specimens were either large offshoots or offshoots growing low on the palm trunk, or cv. Zahidi offshoots. Difficult-to-root offshoots were either small ones or those growing high up on the trunk, or those of 'Halawy'.

Materials and Methods.

Plant material. Offshoots were weighed with their leaves intact after removal from the mother palm, and then cut longitudinally. Samples of tissue were removed from the central zone of the offshoots below the growing point, so that tissue of similar age was sampled from offshoots of different size. The tissue was immediately frozen in liquid air, and then lyophilized. Dry samples were ground and stored in a desiccator over P_2O_5 until required for analysis.

Sugar analysis. Samples of 0.6 g were extracted by shaking with three 20-ml aliquots of 80% methanol. The first extraction lasted for 12 hr and the other 2 for 3 hr, each. The extract was prepared according to Gottreich et al. (7) and reducing sugar was determined by Sumner's method (19, 20).

Starch analysis. In preliminary experiments identical samples were examined by the methods of Nielsen (13, 14) and of Pucher (15). Since identical results were obtained with the 2 methods, Nielsen's more rapid and simpler method was chosen for all subsequent analyses.

Extraction and chromatography of root promoters and inhibitors. One g of dry matter was extracted by shaking 3 times for 30 min with absolute methanol. The extracts were combined and evaporated at 38°C under vacuum, taken up in 1 ml water, and 0.05 ml (50 mg eq.) spotted on 4-cm-wide Whatman 3 MM paper strips. Strips were developed by descending chromatography to a distance of 30 cm from the origin, using isopropanol:water (8:2 v/v) as solvent. Each strip was cut into 15 sections, which were then assayed by the mung bean bioassay. Blank strips, developed in the same solvent, served as controls.

Mung bean bioassay. A modified version of Hess's method (11) was used for assaying rooting. Mung bean (*Phaseolus aureus* Roxb.) seeds were disinfected by ethylene dibromide fumes for 24 hr in air-tight containers. After ventilation, the seeds were stored in dry, closed containers. After rinsing the seeds in tap water for 15 hr they were sown 1-cm deep in vermiculite in plastic flats. The flats were placed at a temperature of 25-27°C and covered with black polyethylene to ensure darkness and the constant relative humidity required for uniform germination. Uniform 3 cm etiolated seedlings were obtained 4 days after sowing. Further growth was continued under a 16-hr photoperiod at light intensity (Sylvania GR-40 lamps) of 1000-1400 ft-c at plant level, and 40-50% relative humidity. Uniform plants were obtained with well-developed, green primary leaves and an unopened first trifoliar leaf 7 days after sowing. Plants of uniform height, hypocotyl thickness and leaf area were chosen for the bioassay. Three cuttings prepared from these seedlings were placed in each 19 x 60-mm vial, containing 8 ml of IAA (1 mg/l) plus boric acid (1 mg/l). Chromatogram sections of the same R_f value from several replicate strips were placed in each vial (5 vials for each R_f value). Distilled water was added to the vials daily. They were incubated under the same light, temperature, and humidity conditions as the seedlings. Roots were counted after 6 days' incubation. Mean root counts for each R_f value are illustrated by histograms.

Statistical analysis. Results of the carbohydrate tests were analyzed by analysis of variance and the Q-test. The histogram differences were considered significant if greater than twice the standard deviation of the control.

Results

Carbohydrate content. Samples for this test were taken during March. "Low" and "high" offshoots of 'Halawy' were compared. "Low" were those appearing below the soil surface

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Table 1. Effects of size and origin on content of reducing sugar, total sugar, starch, and total carbohydrate (dry wt percentage) of 'Halawy' date palm offshoots.

Carbohydrate analyzed	Offshoot size	Growing site "High" −	Growing site "Low" +	Mean for size
Reducing sugar	Small -[z]	7.5	3.3	5.7*
	Large +	5.8	2.1	3.9*
	Mean for height	6.6***	2.7***	
Total sugar	Small -	17.8	17.3	17.6
	Large +	23.0	14.2	18.6
	Mean for height	20.4**	15.8**	
Starch	Small -	20.1	34.4	27.2
	Large +	24.2	43.0	33.6
	Mean for height	22.2***	38.7***	
Total carbohydrates	Small -	37.9	51.7	44.8*
	Large +	47.2	57.2	52.2*
	Mean for height	42.6**	54.5**	

*Difference significant at the 5% level, ** at 1%, *** at 0.1%.

[z](+) Relatively easy-to-root specimen (-) Difficult-to-root specimen.

Table 2. Effect of cultivar and size on content of reducing sugar, total sugar, starch, and total carbohydrate (dry wt percentage) of "high" 'Halawy' and 'Zahidi' date palm offshoots.

Carbohydrate analyzed	Offshoot size	Cultivar Halawy −	Cultivar Zahidi +	Mean for size
Reducing sugar	Small -[z]	7.5	6.2	6.8*
	Large +	5.8	4.1	4.9*
	Mean for cultivar	6.6*	5.1*	
Total sugar	Small -	17.8	25.5	21.7***
	Large +	23.0	33.8	28.4***
	Mean for cultivar	20.4***	29.7***	
Starch	Small -	20.1	24.7	22.4
	Large +	24.2	21.6	22.9
	Mean for cultivar	22.2	23.2	
Total carbohydrate	Small -	37.9	48.9	43.4**
	Large +	47.2	55.4	51.3**
	Mean for cultivar	42.6**	52.2**	

*Difference significant at the 5% level, ** at 1%, *** at 0.1%, interactions not significant.

[z](+), relatively easy-to-root specimen; (-), difficult-to-root specimen.

or up to 20 cm above it, and "high" those at a height greater than 60 cm from ground level. Relatively easy-to-root high 'Zahidi' offshoots were compared with difficult-to-root high 'Halawy' offshoots. In both comparisons, large (12-20 kg) offshoots were also compared with small (4-7 kg) ones. Each test was replicated 3 times for each comparison, and each replicate was composed of combined samples taken from 2 to 5 offshoots. The reducing sugar content of large offshoots was lower than that of small ones (Tables 1 and 2). Total carbohydrates were greater in large offshoots (easier to root) than in small ones. Lower levels of reducing sugars and higher levels of starch and total carbohydrates were found in low (easier to root) offshoots than in high ones. In the case of total sugars, an interaction was found between the size of the offshoot and the position at which it grew on the mother palm. Total sugar content was higher in large offshoots from the high site, but not in the low site.

When offshoots of the easy-to-root 'Zahidi' were compared with those of 'Halawy', a similar trend was found (Table 2). Reducing sugar content of 'Zahidi' was lower than that of 'Halawy', and the total carbohydrate and total sugar content were greater. Size of offshoots and cultivar did not affect starch content.

Rooting promoters and inhibitors. The activity of endogenous rooting promoters and inhibitors was determined in tissue taken from 'Khadrawy' offshoots which were removed from the mother palms in September and were graded according to size. The level of root-promoting activity increased with the size of the offshoot but decreased again in very large offshoots, which are known to root easily (Fig. 1). A negative correlation was found between root-inhibiting activity and offshoot wt.

The effects of cultivar, offshoot size and position on the mother palm are shown in Fig. 2. In all cases, root-inhibiting activity was greater in difficult-to-root offshoots. High concentrations of the inhibitors were found to be toxic to mung bean cuttings; quantities equivalent to 200 mg (dry wt), which was the concentration used in our comparative tests, induced a red coloration in mung bean leaf veins. Higher concentrations caused death within 24 hr.

Discussion

The total carbohydrate content of tissue sampled just below the growing point of easy-to-root offshoots was greater than that of difficult-to-root offshoots, but the reducing sugar levels were always greater in difficult-to-root offshoots. This might indicate a higher rate of hydrolysis of carbohydrates in difficult-to-root offshoots, which may be a result of a higher

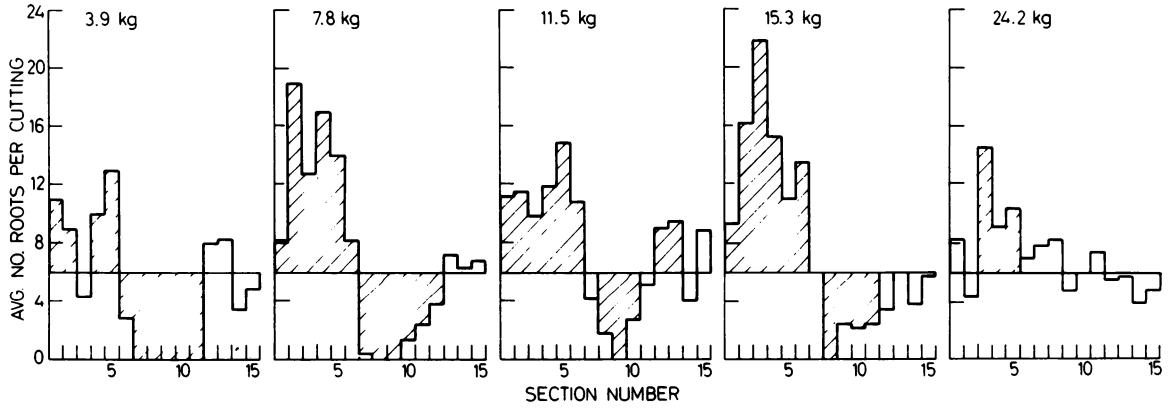

Fig. 1. Effect of offshoot size on activity of endogenous rooting promoters and inhibitors extracted from 'Khadrawy' offshoots, of different size, as determined by the mung bean bioassay. Each histogram represents the activity of 200 mg (dry wt) of tissue from the central zone of the offshoot below the growing point following chromatography of methanol extracts in isopropanol:water (8:2 v/v). Significant promotion or inhibition of rooting (greater than twice the standard deviation of the control) is indicated by cross-hatched columns.

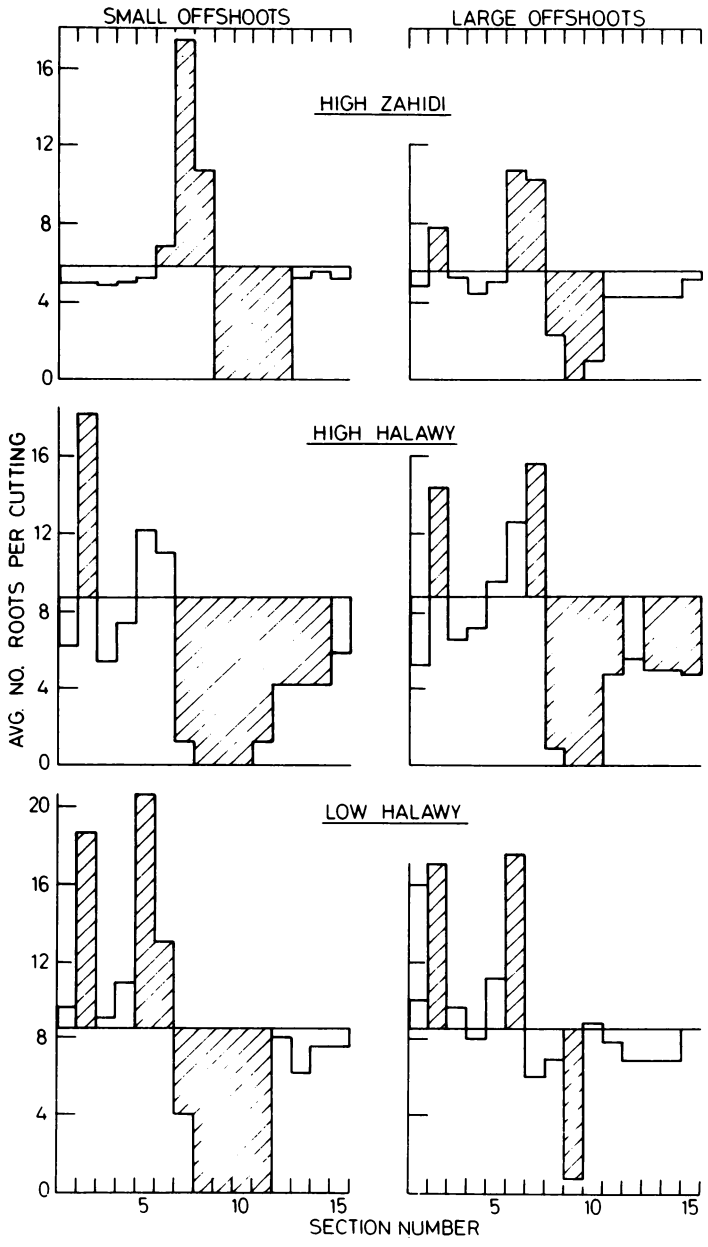

Fig. 2. Effect of cultivar, offshoot size, and source on activity of endogenous rooting promoters and inhibitors extracted from date offshoots, as determined by the mung bean bioassay. Each histogram represents the activity of 200 mg (dry wt) of tissue from the central zone of the offshoot below the growing point, after chromatography of methanol extracts in isopropanol:water (8:2 v/v). Significant promotion or inhibition of rooting (greater than twice the standard deviation of the control) is indicated by cross-hatched columns.

vegetative growth rate. In other plants, high vegetative activity has also been found to reduce rooting rate (2, 23). "Low" offshoots, which are formed while the palm is young, are analogous to juvenile cuttings of other plants. The latter are known to have a high rooting ability. Date palm offshoots resemble other cuttings in that a correlation exists between sugar content and rooting ability (2, 3, 10). Since carbohydrates are such an important source of the energy required for rooting, it seems that large reserves of carbohydrates and little competition from vegetative growth are factors which increase the ability of certain offshoots to root. These 2 prerequisites are generally found in the fall and in winter.

The endogenous levels of root-promoting and root-inhibiting substances have also been correlated with rooting. In the present study a negative correlation was found between the rooting ability of various types of offshoot and the activity of inhibitors as determined by the mung bean test. In all difficult-to-root offshoots, high concentrations of inhibitor were found on chromatogram sections at R_f values of 0.5-0.8.

Four rooting cofactors of IAA have been found in ivy (6, 9). In the present study no promoting activity was found in the region equivalent to Hess's cofactor 4. Two cofactors that may be similar to cofactors 1 and 2 of Hess (9) were found at R_f values of 0.1-0.2 and 0.3-0.5, respectively. Hess's cofactor 3 was not observed, however, inhibitors may have masked its activity.

Since rooting ability of date palm offshoots was not consistently correlated with rooting cofactor content, the major factors involved in rooting appear to be carbohydrates and inhibitors. We conclude that rooting ability is positively correlated with carbohydrate content and negatively correlated with inhibitor content.

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