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Rhizogenesis of GF677, Early Crest, May Crest and Arm King stem cuttings during the year in relation to carbohydrate and natural hormone content

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Abstract

The rhizogenesis of GF677, Early Crest, May Crest and Arm King stem cuttings during the year was investigated. The results showed significant differences in the rooting percentage between different dates of cutting collection and also between the four cultivars. Among these, GF677 generally had the highest rooting percentage. Rooting of cuttings was high in October, November and February and relatively low in the periods March–September and December–January. Interactions among cultivars were also found.

In this study, the possibly effect of endogenous factors (carbohydrate, hormone) was examined. In all cultivars, the starch level in peach shoot cuttings generally increases from January to November. Sucrose content in all cultivars was relatively high during the winter, but dropped during spring and summer and then increased again during the autumn. The level of sugars (soluble reducing sugars) during the year was relatively higher in May and September when the shoot growth rate was high (poor rooting) and rather lower in January–March, August and November–December.

Idole-3-acetic acid (IAA) and abscisic acid (ABA) concentrations in shoot cuttings of GF677 were found to be very high in April and relatively low in the period November–February.

Keywords: Abscisic acid; Cuttings; Idole-3-acetic acid; Starch; Sugar; Sucrose; Rooting

1. Introduction

Increment in volume associated with callus and adventitious root growth must be the result of either cell division or cell enlargement, or both. Auxin, known to be involved in cell enlargement was long thought to be the controlling factor also in the rooting process. Two types of evidence support this reasoning (a) the increased content of endogenous auxin in the base of cuttings during rooting induction (Blachova, 1969) and (b) the rooting response of many plants to exogenous auxin. Blakeslay et al. (1991) found that rooting of two clones of Cotinus was correlated positively with the levels of free idole-3-acetic acid (IAA). Nevertheless, the correlation of rooting with endogenous IAA is still clouded. Stoltz (1968) found that in "*Chrysanthemum* spp." endogenous auxin concentrations did not positively correlate with rooting. Overall the literature dealing with the variations of auxin levels in relation to rooting is still confusing and indicates necessity for further study.

The effect of abscisic acid (ABA) on adventitious rooting is based mainly on the fact that it antagonizes synthesis of gibberellins (normally inhibiting rooting) and reduces shoot growth (Eliasson, 1961). Blazich and Heuser (1977) noted that ABA promotes rooting of mung bean cuttings and Coleman and Greyson (1976) noted that ABA reversed rooting inhibition in cuttings of *Lycopcrsicon esculentum* Mill. only if applied before or during GA_3 treatment. Blakeslay et al. (1991) suggested that ABA is inhibitory to root initiation in *Cotinus* cuttings if the levels of IAA are low and that high levels of ABA alone apparently do not inhibit rooting. Generally it seems that ABA is mainly significant by interacting with other internal or externally applied growth regulators than by itself.

The relationship between tissue carbohydrate contents and rooting has remained controversial for many years. Gunes (1999) found no correlation between carbohydrate levels and callusing of *Juglas regia* L. hardwood cuttings. However, Rowe et al. (1999) reported that the good rooting cuttings of Loblolly

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Pine contained greater amounts of soluble carbohydrate than did the poor-rooting. That endogenous carbohydrate content may be a limiting factor during the rooting process according to Eliasson (1978), who doubled the root number in pea (*Pisum sativum*) cuttings, by supplying a 1% sucrose solution to the medium, while Fuchs (1986) was able to obtain an enhanced rooting response by treating *Rosa mufti flora* cuttings with 5% sucrose solution. A decrease in the total sugar, carbohydrate and phenols were noted at the root initiation stage in the stem cuttings of Heritiera (Das et al., 1997). Possibly when trying to manipulate the carbohydrate content in stock plants, one may easily cause changes in other factors of importance to rooting (Baadsmand and Andersen, 1984).

The aims of this study were to investigate: (a) the effect of season on the rhizogenesis of GF677, Early Crest, May Crest and Arm King stem cuttings and (b) the possibly effect of endogenous factors (carbohydrate, hormone content) on rooting of stem cuttings during the year.

2. Material and methods

All experiments described in this study were performed in two consecutive years (1997, 1998) and all the vegetative materials (shoot cuttings) were taken from the experimental orchards of the Pomology Institute, Naoussa, Greece. The cuttings were collected from the base of annual shoots of the peach rootstock GF677 (almond × peach hybrid), and the peach cultivars Early Crest, May Crest and Arm King (4-year-old).

Shoot cuttings of each of the above cultivars (20 cm in length) were taken at monthly intervals (the first 10 days), from January 1997 to December 1998. The bases of these cuttings (according to season softwood, semi-hardwood and hardwood cuttings), were treated for 15 s with indole-butyric acid (IBA) in a 50% ethanol solution (quick dip) at 2000 mg l^{-1}. After drying, the cut surfaces were treated with Captan 75 (1:9 in talc). The cuttings were then planted in sand on the bottom-heat benches placed in temperature-controlled room with natural light. The temperatures on the bases of cuttings was regulated at 18–20 °C throughout experiments by thermostatically controlled cables; the automatic misting being activated by ‘‘artificial’’ leaf sensors. In leaf cuttings (the period between April–October), two leaves were left in each during rooting period.

The cuttings were sprayed, once per week with (1.5‰ Captan 83VP and 0.4‰ Pyrethroid), successively. The experimental design was completely randomized block with a factorial arrangement. Five replications of 10 cuttings each were used for each treatment. The rooting response of the cuttings (expressed as percentage) was recorded 50 days after planting.

In order to discover possible correlation of rooting with other parameters, contents of carbohydrate, IAA and ABA were measured. The samples for analysis were taken from the base (whole pieces of 5 mm diameter thickness) of cuttings just before dipping in IBA solutions for each collecting date. Samples were freeze-dried and placed in −20 °C until they used. The methods used to measure the sugars, sucrose and starch are described analytically by Tsipouridis (1993). The whole sugar analysis was made by using a spectronic 20D autoanalyser and the reaction peaks were recorded on charts from which calculations were made subsequently. The sucrose concentration was calculated from the difference between the percentage reducing sugar after inversion minus the percentage reducing sugar present before inversion.

Indole-acetic acid and abscisic acid were also measured according to methods described by Hedden and Lewis (1990). This was a method to analyse IAA and ABA simultaneously in the same tissue sample. Two grams of each sample were used. It was involved solvent partition followed by batch purification on anion exchange and reverse phase matrices.

All trees were checked throughout this experiment to confirm that mother trees were healthy (virus by using ELISA test, bacteria by optical observation, fungi by optical observation). Infected trees were rejected. In addition, no rooted cuttings were checked for infections by pathogens (pieces from the bases of cuttings were transferred onto corn meal agar, hemp seed agar, potato dextrose agar and Lutz medium) immediately after recording the results. No pathogen was isolated from the not rooted cuttings.

2.1. Statistical analysis

Data were analyzed by ANOVA. In all experiments for rooting percentages, statistical analyses of the data were carried out after angular transformation to obtain normality. To combine experiments, the Bartlett's test of homogeneity of variance was used and treatment means were separated by Duncan's Multiple Range Test ($P = 0.05$).

Table 1
Rhizogenesis in GF677, Early Crest, May Crest and Armking stem cuttings, sampled monthly throughout year

	Percentage of rooting (%)				Mean
	GF677	Early Crest	May Crest	Arm King	
January	62[a]cd[b]	8gh	8gh	7h	21.3C
February	78b	32f	34f	50de	48.5B
March	18g	7h	7h	9gh	10.3D
April	16g	6h	10g	8gh	10.0D
May	4h	3h	5h	10gh	5.5D
June	2h	44d	30f	11gh	21.8C
July	46de	7h	8gh	9gh	17.5C
August	74b	2h	2h	2h	20.0C
September	10g	3h	3h	2h	4.5D
October	51de	48d	44de	31f	41.0B
November	90a	10gh	56d	68bc	56.0A
December	30f	8gh	40ef	9gh	21.8C
Mean	40.08A	14.83B	20.58B	17.17B	

Each point is the average of 50 cuttings.
[a] Each value represents the mean of two experiments, each with five replicates.
[b] Values followed by different letters are significantly different ($P = 0.05$) according to Duncan's Multiple Range Test.