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Rooting and weaning of apple rootstock YP

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Summary — Rooting and weaning of the Finnish apple rootstock YP were studied. Rooting was affected by carbohydrate type of the *in vitro* medium and age of the shoots. The highest rooting rate was achieved when either 30 g/l sucrose or glucose was used in the rooting medium and shoots were maintained for 5–7 wk on proliferation medium. Shoots > 7 wk rapidly lost their ability to root. In preliminary experiments VA-mycorrhizal inoculation had a positive effect on growth during weaning and subsequent development in the greenhouse.

***in vitro* rooting / *Malus* / carbohydrates / VAM inoculation**

Résumé — L'enracinement et le sevrage du porte-greffe de pommier finnois YP. *L'enracinement et le sevrage du porte-greffe finnois YP ont été étudiés. L'enracinement est influencé par le glucide du milieu et l'âge des tiges. Le meilleur taux d'enracinement a été obtenu avec du sucrose ou du glucose à 30 g/l dans le milieu d'enracinement et quand les tiges ont été conservées pendant 5–7 semaines sur le milieu de prolifération. Les tiges plus vieilles que 7 semaines perdent rapidement leur aptitude à s'enraciner. Dans des essais préliminaires, l'inoculation par des mycorrhizes vasculaires arbusculaires a eu un effet positif sur la croissance pendant le sevrage et sur le développement ultérieur en serre.*

enracinement* in vitro / *pommier* = Malus / *glucide* / *inoculation de mycorrhizes vasculaires arbusculaires

INTRODUCTION

Research on micropropagation of apple trees started > 20 yr ago (Elliot, 1972; Walkey, 1972). Even though micropropagation of apple trees was in most cases successful, rooting of propagules often seemed to be problematic. Wounding of microcuttings, liquid culture (Sriskandarajah and Mullins, 1981) and use of phloroglucinol (James and Thurbon, 1979) have been studied but none of these methods has solved the basic problems related to apple rooting.

The frost-hardy Finnish apple rootstock YP, originating from an open-pollinated seedling of a Siberian crab apple (*Malus baccata* (L) Moench) (Säkö, 1977), has been considered difficult to propagate *in vitro* as a rootstock. At the Elite Plant Unit, rootstock YP has been used as a model plant in studies related to latent bacterial contaminants in tissue cultures and factors affecting rooting and weaning of micropropagated apple shoots. In this study, the effects of different carbohydrates and KIBA concentrations during *in vitro* rooting, the optimal age of the shoots and the effects of VAM inoculation were investigated.

MATERIALS AND METHODS

In all experiments the temperature was maintained at 24 °C during the 16-h light period and 21 °C during the 8-h dark period. All shoots were produced by culturing them for 6–7 wk on G medium (table I) containing 20 g/l fructose, 0.75 mg/l BAP (6-benzylaminopurine, Sigma) and 0.4 mg/l IBA (indole-3-butyric acid, Sigma). The light source consisted of a combination of fluorescent lamps Warm White Super and Floralux, ≈ 3 500–3 700 lux in the tubes (6 500 lux above the tubes). The harvested shoots were 26–32 mm in length. Hormone treatments for rooting were given by immersing the shoots for 15–20 min. After the bath, shoots were placed on hormone-free rooting medium (table I) either in test tubes (160 x 20 mm) or in Sigma's Phytatray II. The rooting experiments were performed using 80 shoots/treatment in 4 repeats.

During *in vitro* rooting, the effects of fructose, glucose and sucrose (30 g/l) were compared using 4 different KIBA (potassium salt of IBA, Sigma) concentrations (25, 50, 100 and 200 mg/l) together with 50 mg/l boric acid (Dirr and Heuser, 1987). Hormone treatment

Table I. G basal medium used in propagation and rooting of apple rootstock YP. This medium was originally developed for strawberry micropropagation.

Chemical compounds	*Amounts of chemicals used during:*			
	Proliferation		*Rooting*	
NH_4NO_3	1 650	mg/l	825	mg/l
KNO_3	250	"	125	"
$Ca(NO_3)2.4H_2O$	1 000	"	500	"
KH_2PO_4	250	"	250	"
$MgSO_4.7H_2O$	200	"	200	"
H_3BO_3	6.2	"	6.2	"
$CoCl_2.6H_2O$	0.025	"	0.025	"
$CuSO_4.5H_2O$	0.025	"	0.025	"
KI	0.83	"	0.83	"
$MnSO_4.4H_2O$	22.3	"	22.3	"
$NaMoO_4.2H_2O$	0.25	"	0.25	"
$ZnSO_4.4H_2O$	8.6	"	8.6	"
Fe(III)Na-EDTA	40.0	"	40.0	"
Nicotinic acid	0.5	"	0.5	"
Thiamine	1.0	"	1.0	"
Pyridoxine	0.5	"	0.5	"
Glycine	2.0	"	2.0	"
Myo-inositol	100.0	"	100.0	"
pH	5.0		5.0	
Roth's agar	4.5	g/l	8.5	g/l
Agar Ph Nord	4.0	"	–	
Bactopeptone	0.27	"	0.27	g/l

with the water-soluble KIBA together with boric acid was chosen instead of alcohol-soluble IBA to avoid possible injurious effects caused by the alcohol solvent. Rooting was evaluated after 3 and 5 wk on rooting medium. Survival during weaning and subsequent growth was observed. The effect of VAM-inoculation was studied by inoculating the *in vitro* rooted shoots with 3 different Finnish VAM isolates when the plants were transplanted to greenhouse conditions. The effect of VAM-inoculation was estimated 3 months after inoculation.

The correct timing for harvesting shoots was studied by rooting shoots which had been on proliferation medium for 4–10 wk. In this experiment, shoots were immersed in a 100 mg/l IBA solution and then placed on the hormone-free rooting medium containing 30 g/l sucrose. Rooting after 3 and 5 wk and survival during weaning were observed.

The effect of light quality on *in vitro* rooting was studied by rooting 6-wk-old shoots; the data have not been presented in this study. The lamp types compared were: 1) Fluora L58W/77 (Osram); 2) Gro-lux F58W/Gro (Sylvania GTE); 3) Cool White Super L58W-2T (Oy Airam Ab); 4) Warm White Super L58W-3T (Oy Airam Ab); 5) Floralux L65/80 W-Fx (Oy Airam Ab); and 6) a combination of Warm White Super and Floralux. The best rooting rate was obtained by using Warm White Super and Floralux together. This combination was therefore used as the light source in all experiments.

RESULTS

On average, sucrose was found to be the best carbohydrate for rooting (fig 1). The optimal hormone concentration varied according to the carbohydrate type used. The mean rooting rate for all KIBA treatments was 60% on sucrose-containing medium, 38% on glucose and 15% on fructose-containing medium. The best rooting rate on sucrose was 72% when 25 mg/l KIBA was used. In another experiment where KIBA was replaced by IBA, the average rooting rate was 75% on sucrose, 72% on glucose and 25% on fructose and in this case the difference between sucrose and glucose was not significant. Weaning survival in this material varied from 0–96%. The best weaning was obtained with shoots originated from sucrose containing medium. The roots regenerated on glucose media seemed to be poorly connected to the vascular bundle of the stems. Shoots from fructose-containing media died during the weaning stage.

The best time for harvesting the shoots was when shoots had been on proliferation medium for 5–7 wk (fig 2). The best rooting (85%) and highest weaning survival were obtained with 6-wk-old shoots.

After VAM inoculation, growth of the young plants was significantly improved in comparison to the controls. The average height of the non-treated controls was 25 mm, and plants inoculated with *Glomus claroideum* V43a, 94 mm, with *G intraradix* V104, 73 mm and with G spp V43a + V104, 63 mm. In this experiment, VAM inoculation did not improve the weaning survival of the shoots.

DISCUSSION AND CONCLUSION

Although micropropagated apple shoots are difficult to root, they are not unable to root. The type of carbohydrate utilised during the rooting stage together with auxin treatment were essential to the rooting success. These factors have also been reported in earlier works (George and Sherrington, 1984; Pua and Chong, 1984; Nemeth, 1986; Yae *et al,* 1986; Chauvin and Salesses,

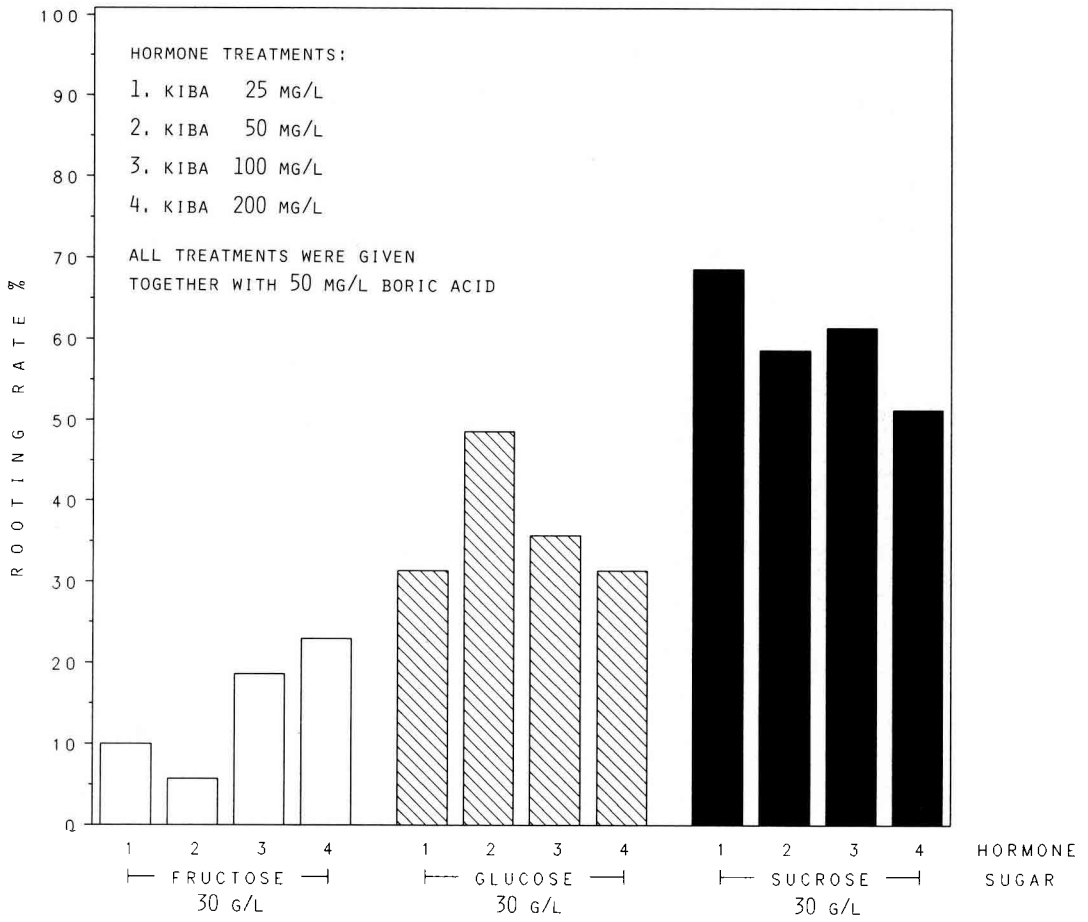

Fig 1. Effect of sugar source of the rooting medium and hormone treatments on the *in vitro* rooting rate of micropropagated shoots of apple rootstock YP.

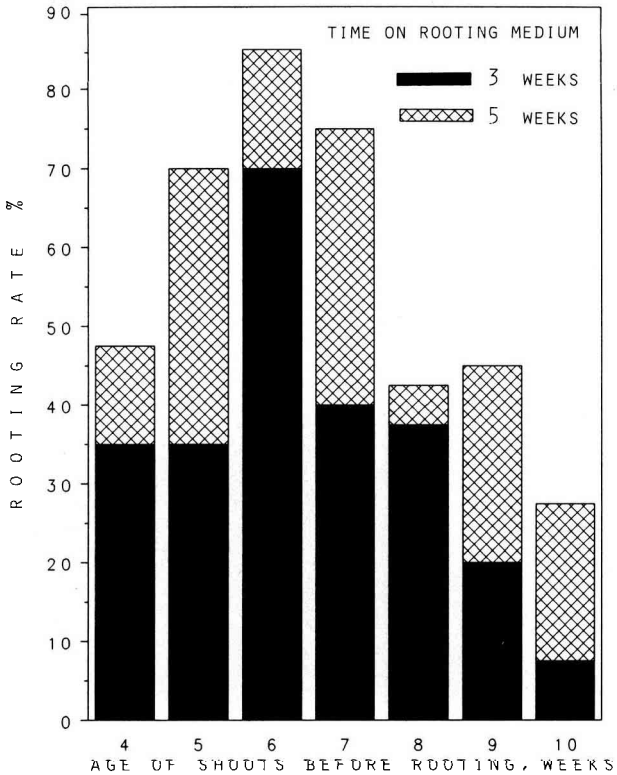

Fig 2. Effect of the age of micropropagated shoots prior to rooting on the *in vitro* rooting rate of apple rootstock YP after 3 and 5 weeks on rooting medium.

1988; Moncousin, 1991). The present study also showed that the optimal hormone concentration was dependent on the carbohydrate source used. This factor as well as the timing of hormone treatment require further investigation.

According to this study, one of the most critical factors in successful rooting was the developmental stage of shoots at the beginning of the rooting stage. The weaning survival and subsequent growth of the young plants were also greatly influenced by this factor. As a result of poor nutrition uptake or because of a possible hormonal inbalance, young apple plants had a tendency to lapse into arrest of growth. It was possible to overcome this problem by VAM inoculation. Light quality could also affect the rooting result. This aspect has until recently been a much-neglected topic in micropropagation research. Because apple is an economically important fruit tree, it is vital that the propagation problems are solved. Therefore the effect of light quality and particularly the utilization of VA mycorrhizas on weaning survival and subsequent growth require further investigation.

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