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Effect of amino acids on rooting of apple dwarf rootstocks *in vitro*

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Abstract

The effect of twelve amino acids and lactalbumin hydrolysate in concentration of 200 mg l^{-1} on rooting of the dwarf apple rootstocks P 2 and P 60 was tested *in vitro*. Arginine, ornithine, glutamic acid and glycine enhanced root number of the P 60 rootstock; proline and lactalbumin hydrolysate were neutral; and asparagine, tyrosine, methionine, cysteine and glutamine lowered the root number. Tyrosine, methionine, cysteine and glutamine reduced almost completely rooting of P 60. In the recalcitrant P 2 rootstock aspartic acid, glutamic acid and ornithine significantly enhanced the number of roots and rooted shoots, arginine and tryptophan increased the root number only slightly, asparagine was neutral, and proline reduced the root number.

Introduction

The most important factors which can improve rooting of woody plants in media containing IBA are: dark treatment (Druart *et al.* 1983, Druart 1986, Orlikowska 1988, 1991), apropriate salt composition (Zimmerman 1983, Druart 1986, Duron *et al.* 1989, Orlikowska 1992), agar brand (Orlikowska 1992), addition of phloroglucinol (Jones 1983, Orlikowska 1991) and riboflavin (Orlikowska 1988, Duron *et al.* 1989). The positive effect of proline on recalcitrant woody species recommended by Boxus (1986) and Druart (1986) suggested experiments with other amino acids. The beneficial effect of proline for rooting of quince S 1 (Orlikowska 1988) and L-arginine for rooting of P 60 apple rootstock (Orlikowska 1991) have already been reported. In this work the 12 amino acids and lactalbumin hydrolysate were tested as rooting media supplements.

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Material and methods

Cultures of dwarf apple *Malus domestica* L. rootstocks were established according to the method given earlier (Orlikowska 1991). Multiplication of shoots was accomplished on Murashige and Skoog (1962) mineral salts with a double dose of $MgSO_4$ with addition of [mg l^{-1}] vitamin B1 - 1.0, B6 - 0.5, nicotinic acid - 0.5, glycine - 2,0, inositol - 100, BAP - 1.2, NAA - 0.05, phloroglucinol - 40, adenine sulfate - 80, 30 g l^{-1} saccharose and 6 g l^{-1} Difco Bacto agar.

Experimental explants about 1.5 cm long were taken from shoots not shorter than 2 cm produced in proliferating cultures at least 1 year old. The control medium for rooting consisted of WPM salts and vitamins (Lloyd and McCown 1981), 30 g l^{-1} saccharose, 6 g l^{-1} Japanese commercial powdered agar, 1.5 mg l^{-1} IBA, and 80 mg l^{-1} phloroglucinol with pH adjusted to 5.0 prior to autoclaving. Amino acids were included separately at a dosage of 200 mg l^{-1}, corresponding to molar concentrarions of 0.98 mM for tryptophan to 1.77 mM for aspartic acid. Amino acids were in L-form with the exception of DL-aspartic acid and DL-tryptophan. For P 2 the most effective amino acids for rooting of P 60 were used, and additionally aspartic acid and tryptophan were tested.

Cultures were kept in the dark during the first 5 d and then transferred to 4 W m^{-2} provided by white fluorescent tubes and 16/8 h ligth regime. Constant temperature of 25 °C was maintained during dark and light periods.

The experiments were accomplished in 100 ml Erlenmayers flasks with 25 ml medium and 4 shoots [P 60] and 3 shoots [P 2] in each flask; with 5 flasks per treatment. All experiments were repeated twice and randomized design was used. An analysis of variance for the root number and root and shoot length was conducted on the means from one flask. The percentage of rooted shoots had to be transformed prior to the analysis according to Bliss. The rooting evaluation combining the rate of rooted shoots, the number and length of roots, the length of shoots and size of callus on the bases of shoots was made after 4 weeks' incubation.

Results

On P 60 rootstock incubated on media containing arginine, ornithine, glutamic acid and glycine the number of roots was significantly higher than in the control. Proline and lactalbumin did not influence the number of roots. Asparagine, tyrosine, methionine, cysteine and glutamine reduced the root number significantly. Arginine and proline enhanced the percentage of rooted shoots, whereas lactalbumin, tyrosine and methionine significantly reduced it. Cysteine and glutamine retarded rooting almost completely (Fig.1). The base of shoots incubated on media containing the last two amino acids did not swell and produced phenolics which coloured the base and the surrounding medium. The shoots rooted on medium with glycine produced abundant callus on their basal parts. The roots on media containing arginine, glutamic acid, asparagine, proline, tryptophan and ornithine had been well visible after 10 d incubation (Fig.3). The number of roots and the percentage of rooting of P 2 rootstocks were significantly higher on the media containing aspartic acid,

glutamic acid and ornithine. Tryptophan and arginine slightly increased, arginine did not affect and proline reduced significantly the number of roots. /Fig. 2/. Rooting of P 2 was accompanied by callusing of shoot basis.

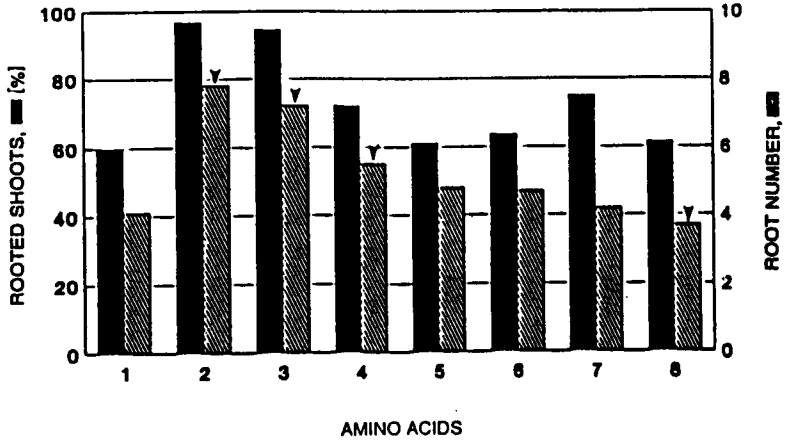

Fig. 1. Rooting of shoots of P 60 apple roostock on media with different amino acids at 200 mg l^{-1}. WPM medium with IBA - 1.5 mg l^{-1}, PG - 80 mg l^{-1}, saccharose - 30 g l^{-1}, 5 d in the dark.
1. control; 2. arginine; 3. ornitine; 4. glutamic acid; 5. glycine; 6. proline; 7. lactalbumin;8. asparagine; 9. tyrosine; 10. methionine; 11. cysteine; 12. glutamine. Values signed by arrow differ significantly from control at $P = 0.05$.

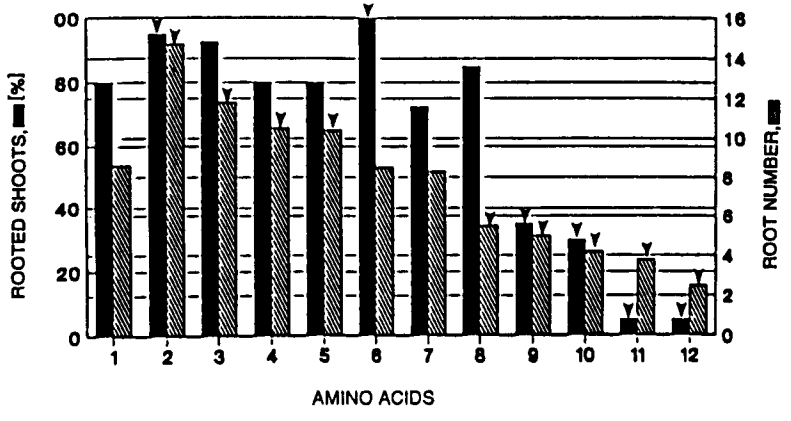

Fig. 2. Rooting of shoots of P 2 apple rootstock on media with different amino acids at 200 mg l^{-1}. WPM medium with IBA - 1.5 mg l^{-1}, PG - 80 mg l^{-1}, saccharose - 30 g l^{-1}, 5 d in the dark.
1. control; 2. aspartic acid; 3. glutamic acid; 4. ornithine; 5. tryptophan; 6. arginine; 7. asparagine; 8. proline. Values signed by arrow differ significantly from control at $P = 0.05$.

Discussion

The addition of amino acids to the media has been observed to stimulate or inhibit growth of plant tissue cultures depending on the kind of amino acid, explant type and genotype (Raghavan 1966, Behrend and Mateles 1975, Marion-Pool and Caboche 1984, Faye *et al.* 1989). Kamada and Harada (1979) reported that 9 out of 16 amino acids tested affected root formation on stem segments of *Torenia fournieri in vitro*. The most effective for the percentage of rooted explants and intensity of root formation were glutamic and aspartic acids, glutamine, and arginine. The authors suggested a possible influence of amino acids on nitrogen metabolism in explants. Proline was recommended for improving the rooting of recalcitrant woody species *in vitro* by Boxus (1986). In the range 10 to 200 mg l^{-1} proline enhanced the percentage of rooting, the number of roots and reduced the length of roots in *Prunus avium* and *P. cerasus* (Druart 1986). A similar effect of proline was reported for quince S 1 (Orlikowska 1988).

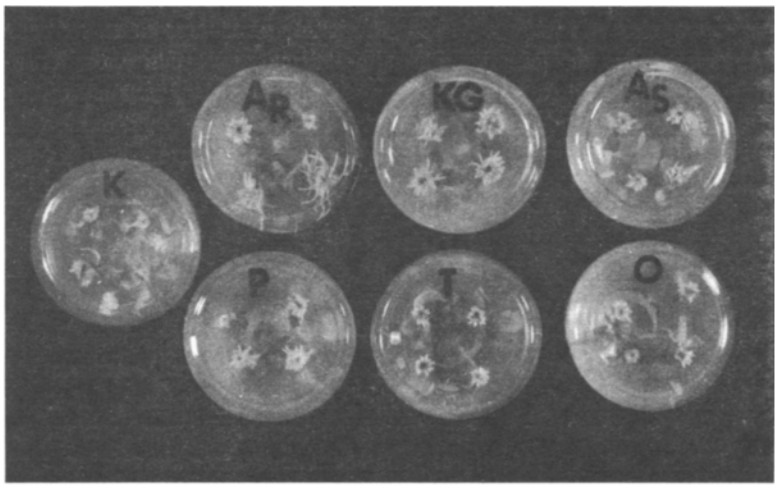

Fig. 3. Rooting of P 60 apple rootstock after 10 d on the media with amino acids at 200 mg l^{-1}. K - control, AR - arginine, KG - glutamic acid, AS - asparagine, P - proline, T - tryptophan, O - ornithine. WPM medium with IBA - 1.5 mg l^{-1}, phloroglucinol - 80 mg l^{-1}, saccharose - 30 g l^{-1}, 5 d in the dark.

The role of amino acids in metabolism connected with rooting is not clear, however. Accumulation of amino acids in the base of cuttings was reported by Haissig (1986). The study of Suzuki and Kohno (1983) indicated that developing callus and roots of mulberry cuttings accumulated 11 amino acids delivered from bark and wood. Welander (1978) attributed the inhibition of rooting of *Pelargonium* petiole explants to increased endogenous concentration of arginine, aspartic acid, alanine, asparagine, glutamic acid and glutamine.

In the present study, rooting of P 60 rootstock was stimulated by arginine, ornithine, glutamic acid and glycine. Glutamic acid, ornithine and arginine were beneficial also for P 2 rootstock as well as aspartic acid, which was not tested for P 60. Asparagine was neutral for rooting of P 2, but reduced the root number in P 60. The opposite influence was seen with proline. The root growth of P 2 was accompanied by callus formation on the bases of shoots. In P 60 cultures only glycine stimulated callusing.

Both rootstocks are used for dwarfing of trees for a size between M 9 and M 26. They have different origin and differ in rooting capacity in the nursery. Also their behaviour *in vitro* is different. In comparison to P 60, P 2 rootstock has a long adaptation period, low and unstable proliferation and rooting of shoots. Elongation of the shoots is difficult and aging of cultures very easy (Orlikowska, unpublished observations).

This work confirms former findings that some amino acids can influence rooting *in vitro* and indicates that they can be used for improving of rooting of woody plants, but their effectiveness may depend on genotype and other rooting factors.

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