Nutrition of the micropropagated fruit trees *in vitro* and *ex vitro*

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Summary: Some experience or details are introduced in connection with the nutrient uptake of micropropagated fruit trees in the different phase of the *in vitro* or *ex vitro* development. It can be stated, that the plants during the micropropagation procedure are overfed. Special careful nutrient supply is necessary during the acclimatization.

Key words: plum, in vitro rooting, carbon dioxide nutrition

Introduction

The micropropagation as a method for mass production of fruit tree rootstocks becomes more popular every day. At the same time the knowledge about the nutrition level of the plants during the *in vitro* stage is often incomplete. Most of the laboratories follow the practical way of research in their work, in order to create some modification of the basic medium used in the laboratory and follow its effect on plants. Some experiments and experiences are shown in connection with the nutrition of the micropropagated plants, which have been done in the Laboratory of the Research Institute for Fruitgrowing and Ornamentals.

The basic media in case of the fruit trees or woody plants are very often the *Murashige–Skoog* (1962) or the woody plant medium, worked out by *Lloyd-McCown* (1981).

The macro- and also the microelement content of this media is very high and it can be stated, that the plants during the *in vitro* propagation procedure are overfed. Most of the plants tolerate this situation or the changes of macro- and microelements in a wide range. They are found to be much more sensitive to the content or changes of the hormonal or sometimes to the vitamin effect, as well as to the quality of the sugar added. In most of the cases even table-sugar is adequate, but there are special cases, for example the micropropagation of most apricot varieties. The apricot varieties grow old very soon during the propagation phase when the medium contains only saccharose. When the saccharose is replaced with a proper mixture of glucose and sorbit the shoots stay in juvenile stage from 4–6 weeks, which means a comfortable period for the *in vitro* manipulation (*Csányi* et. al., 1999).

Much more details are known about the plant nutrition requirements of the rooting phase, perhaps because it is a difficult part of the *in vitro* propagation procedure. For rooting the macro elements are usually used only in half or third concentration of the propagation medium.

The sugar content is a very important component in any nutrient medium. It is essential as carbon source for *in vitro* growth and development, because the CO_2 concentration in the growing vessels is a limited factor for the photosynthesis. High concentration of CO_2 during the acclimatization is favourable for plants (*Vértesy* et al. 1990, *De Riek & Van Huylenbrover,* 1992).

The micropropagated plants usually are rooted under in vitro conditions. Following the root induction period of 5–7 days in a high auxin level, some examinations have been done to root and acclimatize the plantlets at the same time (*Welander,* 1983, *Zimmermann & Fordham,* 1985).

One of the most difficult problems during the procedure is the weaning stage, when plants are transplanted from sterile to semi-sterile glasshouse conditions. The transplantation stress also can be the cause of serious losses, as the root system still does not functioning properly.

Vesicular arbuscular mycorrhizal fungi (AMF) can be beneficial to almost 90 percent of higher plants, improving water and mineral nutrient uptake (*Berta* et al., 1990), protecting against biotic and abiotic stress and certain pathogens (*Gianinazzi* et al., 1990).

Inoculation with selected microsymbionts may improve the survival rate and growth vigour of micropropagated plants (*Calvet* et al., 1989, *Guillemin* et al., 1991, *Gianinazzi & Rancillac,* 1992, *Uosukainen & Vestberg,* 1994).

Materials and methods

Examination of the effect of different sugar content for the *in vitro* rooting

The following fruit tree species and varieties were used: JTEF, a dwarfing apple rootstock originating from the Czech Republic, and K/Sz, a sour cherry hybrid of the Research Station of Újfehértó.

4 weeks old cultures were transferred for 3 days on root induction modified MS medium containing 5 mg/l IBA with 0–, 15– and 60 g/l sugar. The rooting occurred in peat: perlit substrate in a ratio of 3: 1.

The effect of sugar concentration was detected after 4 weeks following the auxin – pre-treatments of *in vitro* propagated shoots.

Trials to replace the sugar content of the medium for CO_2

The test plant of this experiment was a *Sorbus aucuparia* clone selected for the high vitamin "C" content.

CO_2 was added with high partial pressure into the culture vessels during the rooting phase. The MS based rooting medium contained 0–, 15– and 30 g/l saccharose. The culture vessels with the rooting shoots were closed with a semi-permeable plastic foil and placed partly into a glass container, partly into the culture room. The photoperiod, the light intensity and the temperature inside the box were the same as in the other parts of the culture room. The box was hermetically closed and the atmosphere inside permanently controlled. The CO_2 concentration in this atmosphere was increased to 3–4% every day at the beginning of the light period. Due to the phenomenon of diffusion the CO_2 concentration was similar inside the vessels and in the ambient air. The CO_2 concentration inside the box was controlled permanently with "Infralit" (Junkalor, Dessau) equipment.

The rooting rate of the shoots was detected in the medium containing different quantity of sugar with added CO_2 and also without it.

Changing of the cation content of the micropropagated "M 26" during the acclimatization

Experiment was carried out on "M 26" apple rootstocks to follow the change of dry weight and cation content of plants during the *ex vitro* acclimatization.

The "M 26" rootstocks were propagated and rooted on modified MS medium. The rooted plants were acclimatized in a mixture of peat: perlit in a ratio of 3:1. The pH of the substrate was adjusted with 1.75 g/l Futor to 6.2. The nutrient supply was carried out with adding 2 g/l Plantosan or 0.5 ml Humix to the acclimatization substrate. The most important cation concentration (ppm) of the substrate is shown in *Table 1*.

Table 1 Content of the most important cations in the two substrates (ppm)

Cation	Humix	Plantosan
Ca	216.000	257.200
Fe	3.519	6.903
K	38.240	229.700
Mg	15.090	22.560
P	8.892	208.400

Changes of dry weight and cation content during the acclimatization have been examined.

Examination of the effect of some endomycorrhizal (VAM) strains on survival and on growth rate of micropropagated "Fehér Besztercei" rootstocks

In vitro production of "Fehér Besztercei" rootstock is going on routinely in the Laboratory of the Research Institute for Fruitgrowing and Ornamentals. Several AMF isolates have been propagated on white clover to produce inocula for the artificial inoculation of stone fruit rootstocks. The inocula is the homogenized mixture of roots and their surrounding soil of white clover

The inoculation was carried out together with the transplantation of the plantlets from the sterile condition into the greenhouse. Two different substrates were used for the acclimatization. A standardized substrate for micro-propagated plants containing high nutrient level and a nutrient poor, heavy soil from our Research Farm of Érd – Elvira. Both substrates were inoculated with 3 percent root – soil mixture of the AMF – infected clover host.

The effect of the VAM inoculation on the survival and growth of the plantlets during the first vegetation period has been detected.

Results

Examination of the effect of different sugar content of the *in vitro* rooting

It can be seen in *Table 2*, that the lack of sugar from the root induction medium decreases dramatically the rooting rate. The maximum survival and rooting could be achieved when the in vitro propagated shoots were pre-treated for 3 days in a basal medium containing 5 mg/l IBA and 60 g/l saccharose.

Table 2 The percentage of survival and rooting on peat – perlite substrate after 3 days pre-treatment with 5 mg/l IBA in media with different sugar contents (percent)

Plant species	Saccharose					
	0 g/l		15 g/l		60 g/l	
	survival	rooting	survival	rooting	survival	rooting
JTEF Apple rootstock	0	0	79	55	93	71
K/Sz hybrid sour cherry	61	21	80	74	92	84

Trials to replace the sugar content of the medium by CO_2

The artificially added CO_2 to the culture vessel can replace the sugar content of the medium. The rooting of the shoots in the CO_2 atmosphere was the same in case of the 0 and 15 g/l sugar content and the control plants in a continuous 30 g/l sugar containing media, 0–100%. In the culture room no rooting was detected in case of the low sugar containing media, or without sugar.

Changing of cation content of the micropropagated "M 26" during the acclimatization

Slow increase of dry weight was observed during the acclimatization both in case of roots and shoots. *(Fig. 1)*

The cation content of the in vitro cultured plants can be higher than that of the control plants. The changing of iron-, potassium-, phosphorus- and magnesium content is demonstrated in *Fig. 2, 3, 4,* and *5*. During the acclimatization, the cation concentration of plants decreased and reached similar value to control plants in case of iron and potassium, but the uptake began in the third week in case of phosphorus and magnesium. Though plants did not show symptoms of overfeeding, we think that lower nutrient content of medium should be applied. This regards to phosphorus, where high overdosing was observed that affected a faster aging of plants. It can be stated that only minimal nutrient content in the acclimatization substrate is suggested.

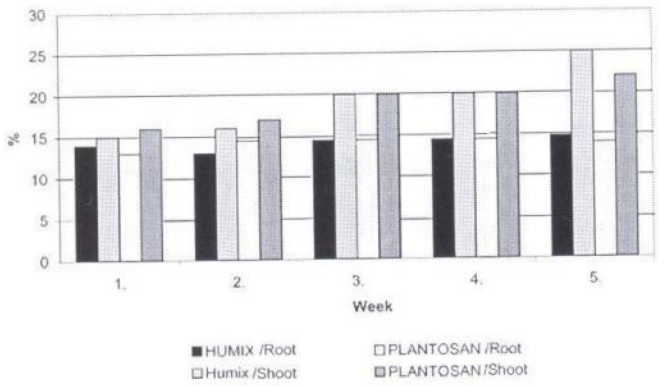

Figure 1 Changing of the dry weight durint the acclimatization

Effect of some endomycorrhizal (VAM) strains on the survival and growth rate of micropropagated "Fehér Besztercei" rootstocks

The survival of the plantlets was strongly determined by the quality of the substrate. But in the nutrient poor substrate, under high pressure of stressed condition – low air humidity, heavy soil – 3 AMF strains from the 7, highly stimulated the survival of the micropropagated plants *(Fig. 6)*. However, 4 AMF strains were found, which have significantly increased the growth of the rootstocks during the first vegetation period *(Fig. 7)*.

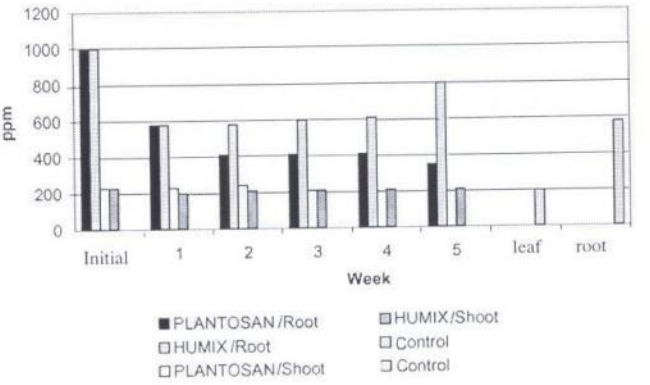

Figure 2 Changing of iron content durint the acclimatization

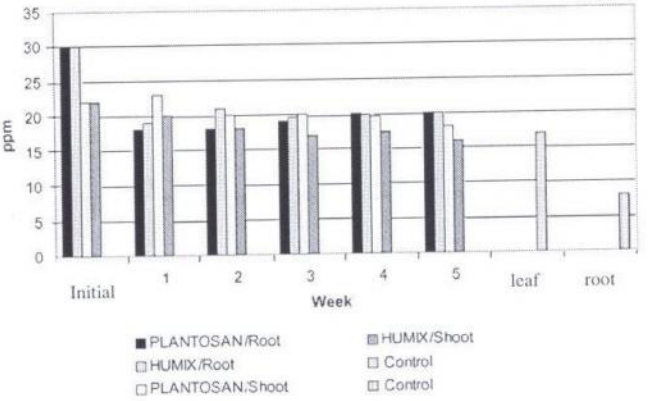

Figure 3 Changing of potassium content during the acclimatization

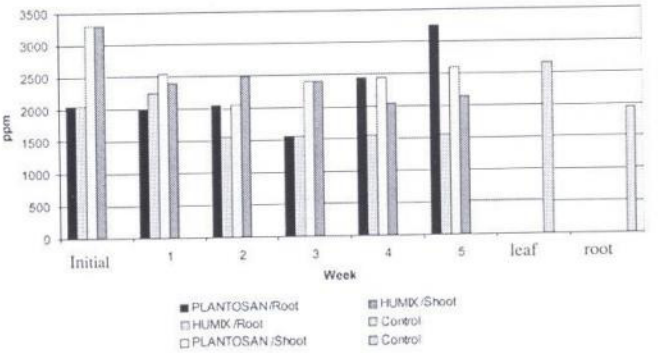

Figure 4 Changing of magnesium content during the acclimatization

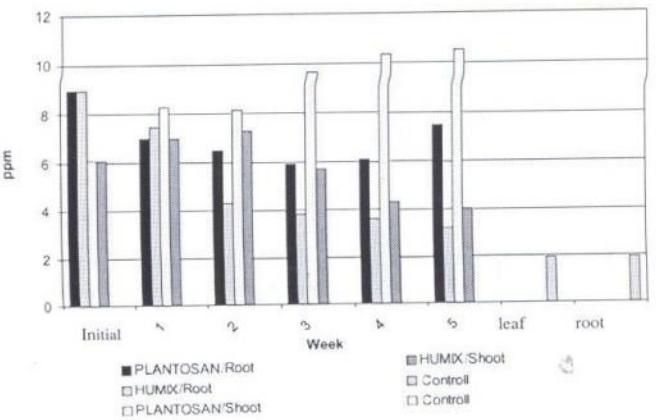

Figure 5 Changing of phosphorus content during the acclimatization

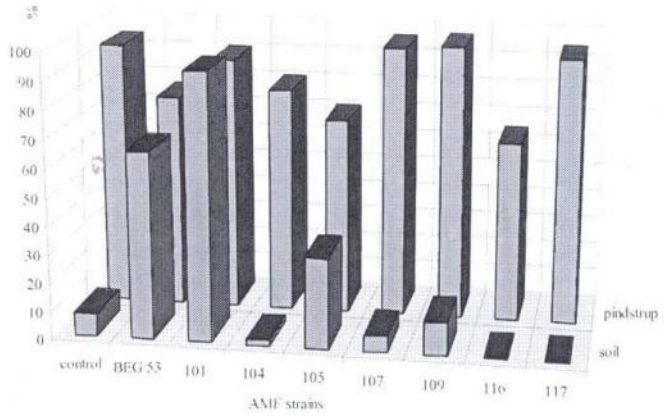

Figure 6 Survival rate of AMF inoculated "Fehér Besztercei" rootstock in two substrates

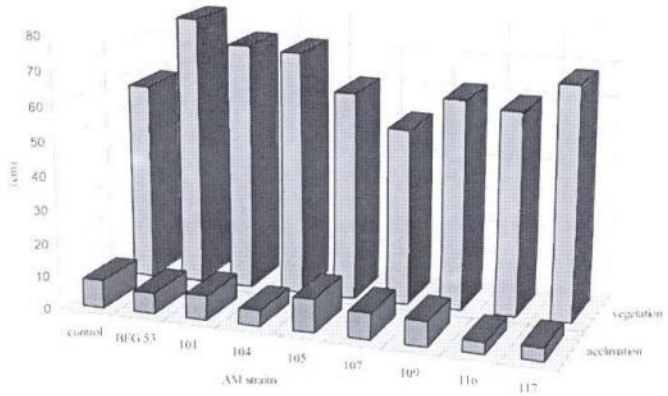

Figure 7 Growth of "Fehér Besztercei" rootstock during acclimation and vegetation inoculated with different AMF stains

Therefore it can be concluded, that the proper compatible strains of AMF improve the water and nutrient uptake of plants, helping the survival and growth under stressed conditions.

Conclusions

1. In most of the cases the micropropagated plants are overfed.
2. Some plant species are sensitive to the quality of the sugar added to the medium.
3. The sugar content of the rooting medium has important effect on the rooting rate.
4. The sugar content of the rooting medium can be replaced with CO_2 artificially increased in the culture vessels.
5. The mineral content of the micropropagated plants is higher than that of the control plants, but during the acclimatization it decreases almost to the value of the control plants.
6. Artificial mycorrhizal inoculation with proper strain can improve the survival and growth of micropropagated plants especially under stressed conditions.

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