Further information to the acclimatization of *"in vitro"* plants

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Summary: The experiment was carried out with *in vitro* propagated `MM 106` apple-rootstock plantlets. The transpiration of the plantlets was examined, and the changes followed by SEM analysis.

Data about the transpiration intensity of the acclimatized plants, of its value under different conditions of relative humidity and influenced by the existence of roots, as well as by the degree of acclimatization are presented.

Leaves were also examined and it was found, that stomata of *in vitro* developed leaves closed slowly, and the number of stomata of newly developed leaves decreased.

It is also shown, that *in vitro* propagated roots, generally, lose their hairs during acclimatization, but these roots are all the same important, as new roots of full value develop out of them

Introduction

During *in vitro* propagation, plants grow under artificial circumstances, hermetically separated from the natural environment. After the propagation period, the plantlets must become acclimatized to field condition, their transpiration has to be controlled, and they must change from contact (medium-plant) nutrition to that through the roots.

Survival depends first of all on the ability to tolerate low humidity. The plants are kept under green-house conditions, where relative humidity is reduced gradually from 95% to field conditions.

Brainerd & *Fuchigami* (1981) examined the process taking place during the acclimatization of `Mac 9` apple rootstocks. They stated, that there is a linear relationship between the opening of stomata and water loss. They describe that at the beginning of acclimatization, under 30 to 40% relative humidity, of closing stomata 9% after 45 minutes, and 30% after 60 minutes was observed. On the fourth day of acclimatization, under similar conditions intense closing of stomata started after 30 minutes, but until that time only 10–20% of stomata were closed. After 60 minutes, closing raised above 80% after 15 minutes, and 95% after 45 minutes.

Warde et al. (1983) studied Chrysanthemum. They showed experimentally, that the efficiency of acclimatization was influenced positively by the existence of roots.

On the other hand, *Vértesy* & *Balla* (1986) found, that a root-induction phase in auxin rich medium was enough to improve acclimatization.

Wetzstein & *Sommer* (1983) examined *Liquidambar styraciflua* and stated, that acclimatized and field plants have an almost equivalent number of stomata, but before acclimatization the number of stomata is higher by 30%.

In the case of strawberry the stomata developed during acclimatization are morphologically an intermediate form of those, found on traditionally grown and *in vitro* plants according to *Fabri* (1986). High concentration of CO_2 during acclimatization is favorable for plants *(Vértesy* et al. 1990; *De Riek* & *Van Huylenbroer* (1992). Transpiration may be modified also by the content of the medium. *Roberts* & *Smith* 1990) stated, that a cellulose based hardening substance in the medium may result in more intensive transpiration. In spite of this, plants wilted less than those grown on agar-agar.

Plantlets are transmitted to the greenhouse usually after *in vitro* rooting. Some authors *(Welander* 1983; *Zimmermann* & *Fordham* 1985) advise to root and acclimatize *in vitro* plants at the same time; they suggest to induce root formation 5 to 7 days before transplantation by a high auxin level. They also state, that plantlets develop better roots than *in vitro* and are more easily acclimatized accordingly.

In vitro roots of `McIntosh` apple were examined by SMITH et al. (1991). They reported, that this kind of roots may contain large and pigmented cells of phloem origine as well as intercellulars in great number. On the contrary, *ex vitro* developed roots show the appearance of the species.

McClelland (1989) wrote, that *in vitro* grown roots do not survive acclimatization but newly developed ones are able to take up the necessary elements from the soil.

Material and methods

In vitro propagated `M 106` apple-rootstocks were used for the experiment. The plants were grown on *Murashige & Skoog* (1962) medium with a reduced mineral concentration, complemented with IBA 0.1 ppm, BA 0.75 ppm, the vitamin mixture of *Barbieri & Morini* (1987), saccharose 30 g/l and agar-agar 5 g/l. The pH was adjusted to 5.6 by KOH.

Rooting was induced in the same medium, but BA omitted and IBA concentration raised to 0.5 ppm. Rooted plantlets received the following treatment:

a., Changes during acclimatization: The plantlets were transferred for acclimatization into a mixture of peat: perlite (3:1) to which 2 g/l BUVILPLANT was added. The low pH of the soil was raised by adding 1.75 g/l FUTOR. Nutritional need of the developing plants was satisfied by weekly sprays of WUXAL solution.

In the greenhouse, high humidity was assured by an artificial mist-system equipment. At the time of transplantation, 95% relative humidity was reduced by 2%, then, from the third day, daily by 3%, and after a week, daily by 4%. Air temperature was 21±3°C.

Changes occurring during acclimatization were studied after a well known usual sample preparation method by a TESLA BS-300 scanning electronmicroscope on 20 KV. Samples were taken at the time of transplantation, then on the 2nd, 5th, and 10th day of acclimatization. Roots as well as leaves of the plants were examined.

b., Transpiration studies. The experiment was carried out in a closed room, where relative humidity was raised to the needed level by an AKA-HYDROMAT cold moisturizer connected with an MC-01 automatic regulator.

Acclimatized plants with roots, as well as without roots and non acclimatized plants without roots were used for the study. Relative humidity was 75%, 85%, and 95%.

The basal part, or the roots of the plants was immerged into water, then a thin oil layer was poured on the surface of the water and a measure was taken of the plants. Thus water loss was determined every minute. Plant weight was also registered at the beginning and after 120 minutes, at the end of the experiment. So net water loss could be measured.

Results and discussion

a., Changes during acclimatization

– **Leaves**: Studying stomata operation during acclimatization, it can be stated, that stomata of newly transplanted, *in vitro* grown plants react late to the changes of humidity, at the beginning. But on the second day of acclimatization 50% of stomata are already closed. *(Fig. 1).* On the fifth day, even 80% of closed stomata can be seen. *(Fig. 2).*, and stomata groups emerging from the leaf surface under *in vitro* conditions, mainly draw back to the leaf level. Developing new leaves have many stomata owing to

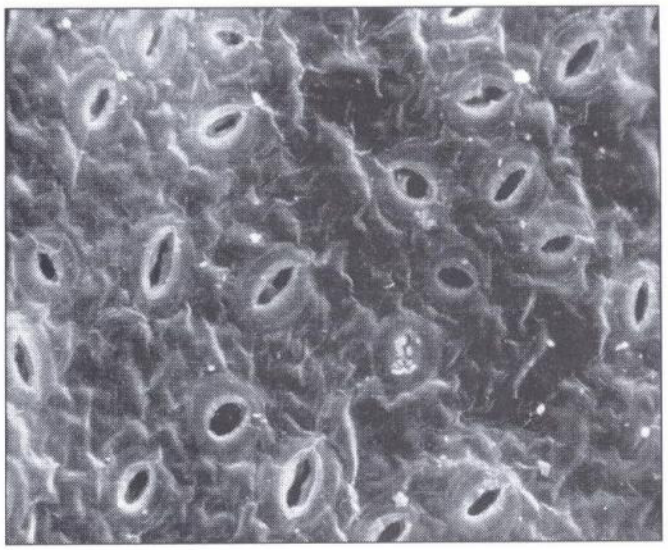

Figure 1 Stomata closing on the 2nd day of acclimatization. (1000X)

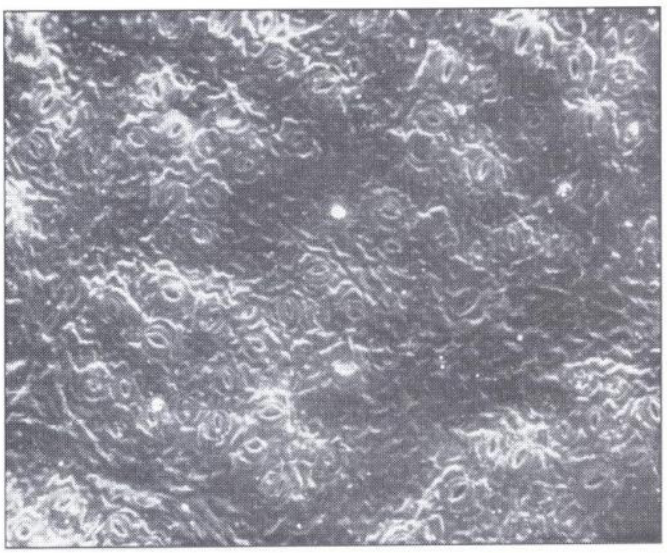

Figure 2 Sinking of stomata into the leafblade on the 5th day of acclimatization. (250X)

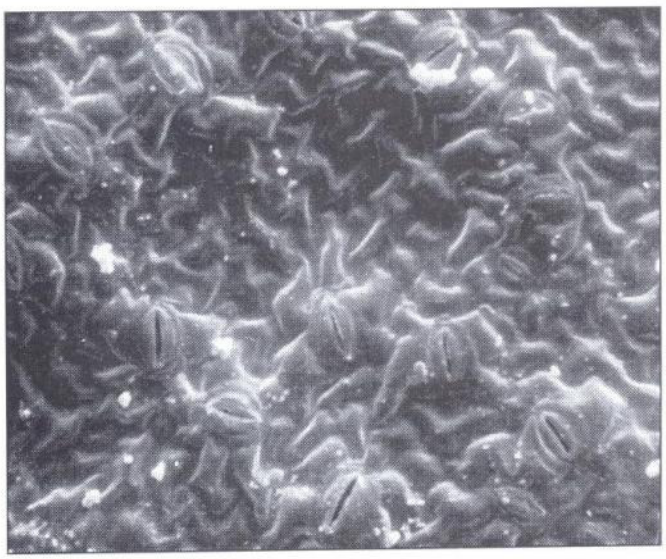

Figure 3 Stomata of young leaves. (500X)

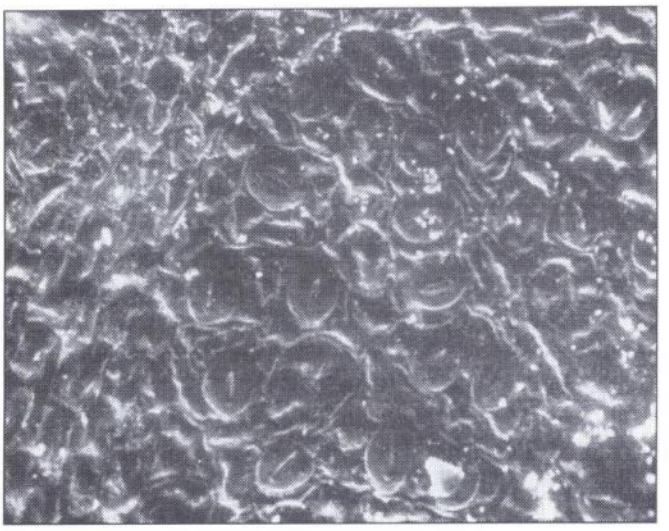

Figure 4 Stomata formed in vitro on the 10th day of acclimatization. (505X)

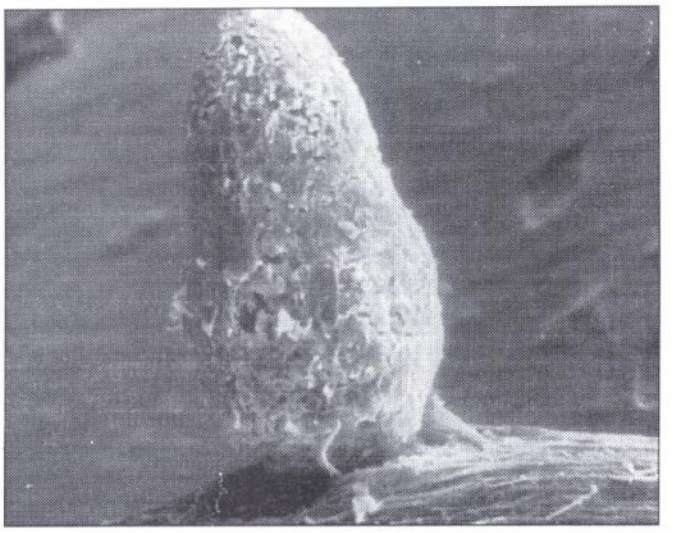

Figure 6 In vitro developed root on the 4th day of acclimatization. (125X)

the still high humidity (about 65%), but their reactions are better compared to the *in vitro* grown leaves. Thus they represent an intermediate stage between *in vitro* and *ex vitro* forms. By this finding FABRI`s opinion (1986) about the intermediate forms may be supported, although upon newly grown leaves stomata occur rather similar to field forms. By that time the regulation of transpiration is assured by the newly developed leaves, which represent already the majority.

– **Roots:** Examining *in vitro* developed roots, it can be observed, that their surface and their apex in addition is densely covered by hairs. *(Fig. 5).* At this stage of the development there are generally numerous callus cells between the hairs.

Observations on the changes of roots during the acclimatization period show that *in vitro* roots lose their hairs, the elongation stops and they sulcate longitudinally *(Fig. 6).* Such roots are possibly notable to draw nutrients from the soil. But they may have importance, as rapidly growing, new roots develop out of them, as shown in *Figure 7.* These new roots also have a broad uptake zone. *(Fig. 8).* It is interesting to observe on the photo, that not only a considerable amount of hairs appear on the surface of these newly formed roots, but callus cells between the hairs are altogether lacking. Unlike *McLelland`s* (1989) results, *in vitro* roots do survive in our case, they induce *ex vitro* root development. It must be also mentioned, that they do not ensure the necessary water supply to the plant, but at that very period of acclimatization apparently the high relative humidity of the greenhouse seems to cover the demands of the plantlets.

Welander (1983) as well as *Zimmermann & Fordham* (1985) expressed the opinion, that *ex vitro* plants are of better quality and it is proved also by the recent study, but the favorable influence of *in vitro*

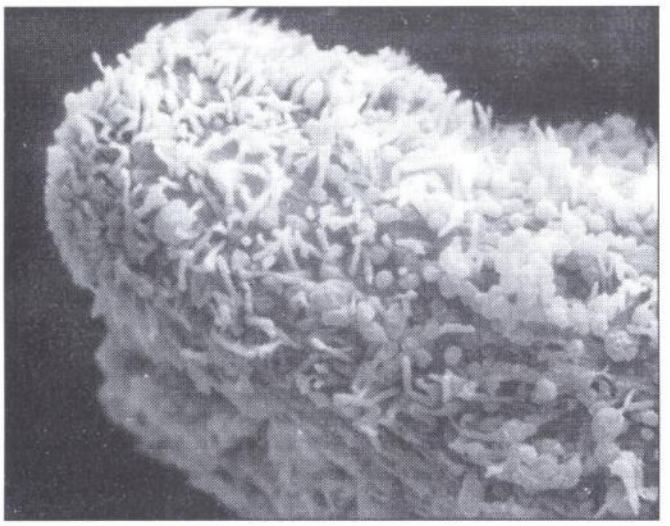

Figure 5 Roots developed in vitro. (75X)

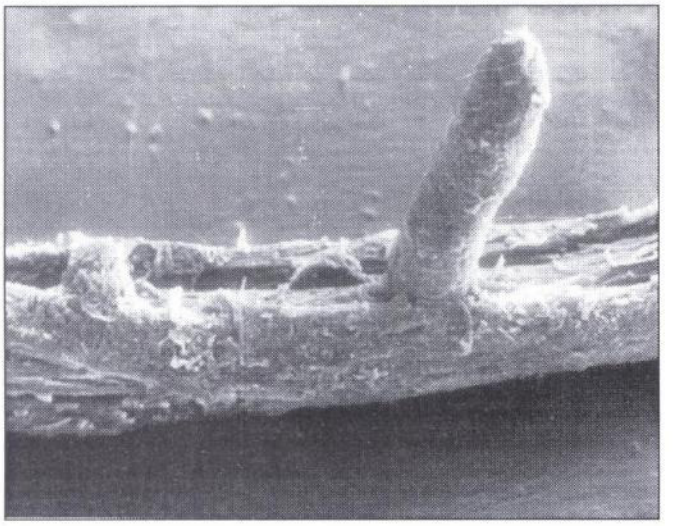

Figure 7 Cracking of roots during acclimatization. (50X)

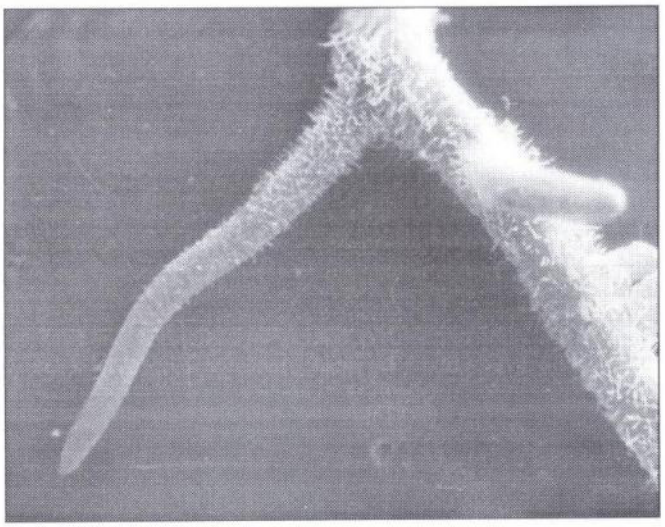

Figure 8 Roots formed during acclimatization. (25X)

roots also must be mentioned here. Finally, we agree with the opinion of *Wardle* et al. (1983) that rooted plants are more efficiently acclimatized, because of the larger uptake zone of newly developed roots.

b., Transpiration studies

Our results show, that the transpiration of plants depends on the relative humidity of the atmosphere. Furthermore, it is influenced by the existence of the roots and the degree of acclimatization.

As a result of our study, it can be stated that transpiration of non-acclimatized plants without roots was the most considerable: it reached 7.2 $mg \cdot g^{-1} \cdot min^{-1}$ at 75% relative humidity. After 120 min, it was 4.8 $mg \cdot g^{-1} \cdot min^{-1}$ at 85 % relative humidity, the starting rate of 5.0 $mg \cdot g^{-1} \cdot min^{-1}$ decreased to 2.8 $mg \cdot g^{-1} \cdot min^{-1}$ after 120 minutes. The quantity of water-loss of one minutes transpiration decreased also at 95% relative humidity *(Fig. 9)*. Net water loss was 10%.

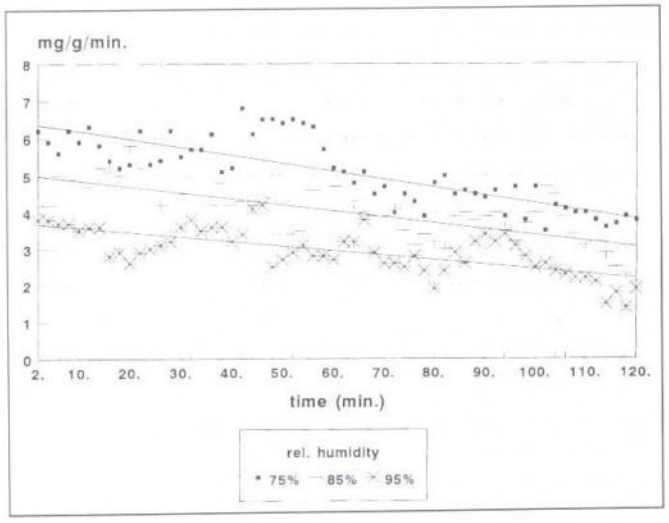

Figure 9 Transpiration of MM 106 apple rootstock variety before acclimatization (without roots).

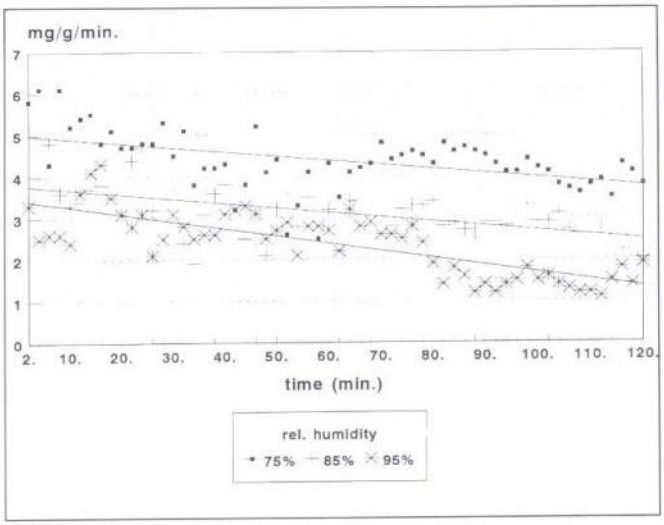

Figure 10 Transpiration of MM 106 apple rootstock variety before acclimatization (rooted)

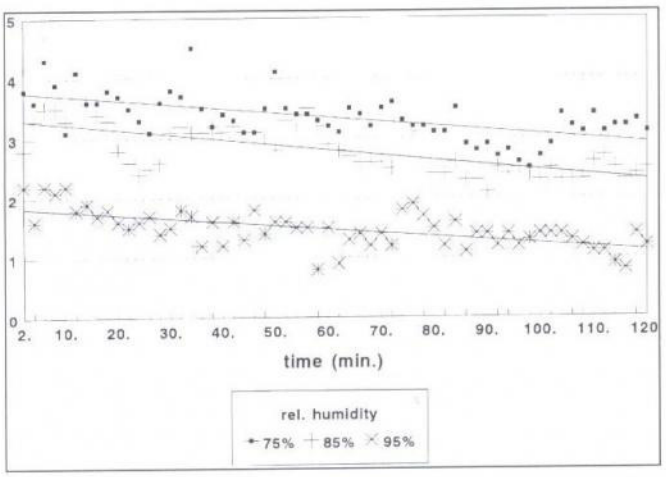

Figure 11 Transpiration of rooted MM 106 apple rootstock variety after acclimatization, under different humidity regimes.

Transpiration of non-acclimatized plants with roots was lower than of those without roots. It seems therefore, that roots of these plants are not of full value, as the plants take up less water by them *(Fig. 10)*. Net water loss reached 15%.

Transpiration rates of acclimatized rooted plants were the lowest, and the value of their transpiration was constant measured by their water loss: it was 0%. It can be stated, consequently, that plants acclimatized 14 day ago are able to regulate their transpiration *(Fig. 11)*.

On the basis of the above transpiration studies too it can be stated, that *ex vitro* developed roots are of better quality, than *in vitro* grown ones.

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