

Dynamics of the uptake of nutrient elements from the medium of *in vitro* cultured apple rootstocks

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Summary: Cation uptake of J-TE-F apple rootstocks propagated *in vitro* was studied by the analysis of culture medium. Test-plants were grown on liquid medium under different light regimes. Samples for tests were taken twice a week. Media without plants served as controls. The analysis of those showed, that only the uptakeable iron-content changed depending upon light treatment. The concentration of all other cations was considered unaltered. As a result of analysis, it could be established, that elements present in the media were taken up in different rates by plantlets: **Cu, P** and **Zn** were utilized totally, but only 50% of **K** and 20 to 40% of **Ca** and **Mg** were taken up under the light treatments applied. The dynamics of the uptake process was also observed. It was registered that they differed in the case of some cations. So **Ba** was utilized at the beginning of subculture, others for example **B** in the later phases. Some elements disappeared unevenly so **K, P** but the whole quantity is taken up during subculture.

Introduction

Plants are able to use only nutrients supplied in artificial media in the course of *in vitro* propagation. The most important elements of plant nutrition are given to the medium as organic or inorganic salts. Consulting literature it becomes obvious that different authors suggest a very wide range of salt-concentrations. In *Table 1* quantities of oligo-elements described by some authors are summarized.

Table 1 The content of some elements in the medium

Author	mM			µM		
	K ⁺	Ca ²⁺	Mg ²⁺	Fe	Mn	Zn
Murashige-Skoog (1962)	20.0	30.0	1.5	100	100	29.9
Quoirin et al. (1977)	37.7	0.85	14.6	100	44.8	3.5
Lloyd - McCown (1981)	12.6	3.0	1.5	100	132.0	30.0
Heller (1953)	1.0	2.1	1.0	3.7	0.5	3.5

In general and mostly *Murashige & Skoog* (1962) medium is used, but often modifications are proposed e.g. half concentration of major elements is quoted (*Mukred*, 1990; *Kiss et al.*, 1994).

As *Murashige & Skoog* (1962) is the basal medium used in most cases of apple micropropagation, its composition

was considered as standard in our experiments, the purpose of which was to establish optimal salt concentrations on the basis of effective demands.

Examinations were directed to solve three problems:

- whether any changes occur in media without plants
- what are the quantities of cations (expressed in percentages) taken up by plantlets *in vitro*
- how rapidly certain cations are taken up.

Materials and methods

Experiments were conducted at the Department of Pomology of the University of Horticulture and Food Industry. *In vitro* cultured plantlets of the dwarfing apple rootstock J-TE-F (*Anonym*, 1983) was used as test plants.

Apple rootstocks were kept on *Murashige & Skoog* (1962) medium supplemented with *Barbieri-Morini* (1987) vitamins, BA 0.75 ppm, IBA 0.01 ppm as well as with sacharose 30 g-l. The pH was measured 4.5 and was not adjusted higher (5.6) as large quantities of potassium would have been introduced into the system.

Comparatively similar number of plantlets - about 0.15 g- were placed into SIGMA culture tubes containing 5 ml of liquid medium.

The plant material was kept under different light regimes: 24/0, 0/24, 4 times 2/4 and 8/16 light/dark periods.

The last treatment was illuminated by 3 types of fluorescent tubes: TUNGSRAFLOOR, WHITE and WARM WHITE ones, as well as with natural light. Temperature was kept at 22 ± 2 °C. The photonfluxus was $37.5 \text{ Mol/m}^2\text{s}$

Samples for analysis were collected two times weekly, the last one on the 34th day of the subculture.

Plantlets were taken by forceps, water drops shaken back into the tubes, subsequently the remaining liquid was measured and refilled to the original level with distilled water, eliminating the increase of the medium salt concentration due to evaporation.

Media was filtered before their cation content was determined by a ICP-AES at the Department of Chemistry and Biochemistry of the University.

The work was performed under contamination free circumstances. In each case 3 parallel analysis were made.

Results and discussion

At a first step, the newly composed medium was analyzed. It could be established, that elements were present in originally intended quantities, except some cases, when an increase was detected, owing – as surmised – to the impurities of sugar.

Iron represented a different case, as of a 5.6 ppm dosage only 4.2 ppm could be demonstrated. During the 34 days of the experimental period cations were present in original quantities independently of treatments. An exception was again represented by the iron quantity, because in media kept in complete darkness it was stabilized around a 3 ppm rate. When media were kept under light conditions concentration decrease could be observed continuously to the 26th day – then 0.7 ppm – followed by a slow increase of concentration (Fig. 1).

The phenomenon can be explained by the lability of Fe-EDTA compound, which begins to dissociate under the influence of light and time, iron possibly accumulating on sites

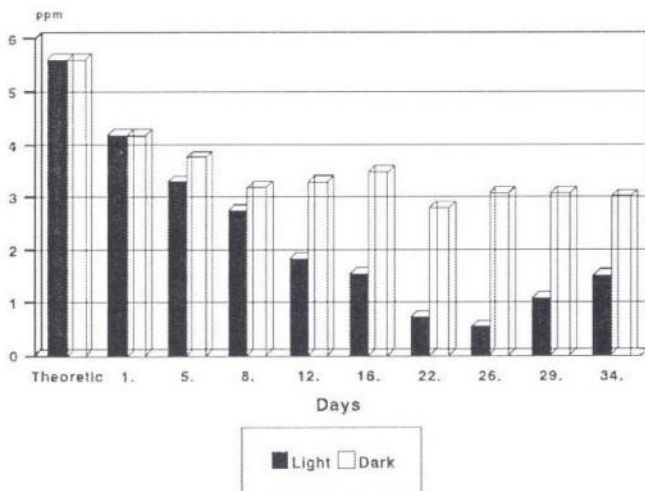


Figure 1 The change of iron content of medium during 34 days (without plants)

inaccessible for plants (e.g. the inner surface of test tubes). It is remarkable, that the concentration decrease of media kept in total darkness is less pronounced, it can be therefore suggested, that dying media to a dark color may be beneficial.

On the basis of our analysis it can be stated, that each cation are uptaken by plants in different degrees during the time of subcultures. The rate of utilization of some cations is demonstrated in Fig. 2, 3, and 4. It can be observed, that plantlets take up 100% of certain elements, such as **Co**, **Cu**, **P** and **Zn**, independently of light treatments.

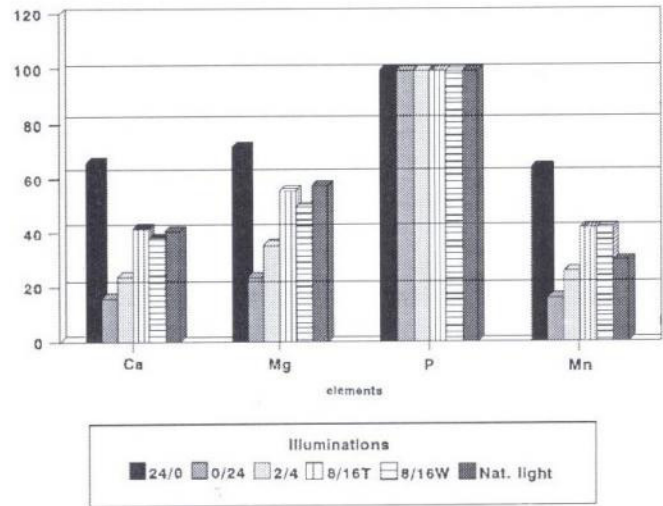


Figure 2 Utilization of some cations depending of illumination I.

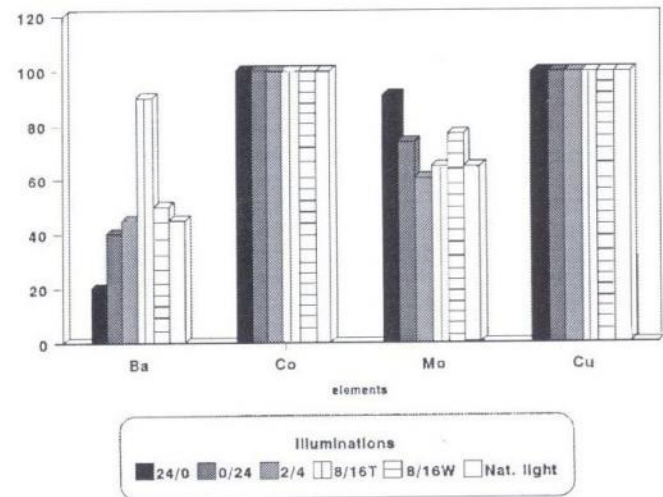


Figure 3 Utilization of some cations depending of illumination II.

In the case of other elements the uptake in general 60–70% of **Mo**, 40–50% of **K**, 20–40% of **Ba**, **Ca**, **Mg**, **Mn** and only 10–30% of **B** depends on light treatment.

On the basis of there findings it can be supposed, that with elements taken up to 100%, there is a deficiency of supply in media (**Co**, **Cu**, **P**, **Zn**). In the case of elements, which remaining high quantity in the medium, where the rate of utilization is lower, are probably oversupplied. It seems lower quantities would be sufficient.

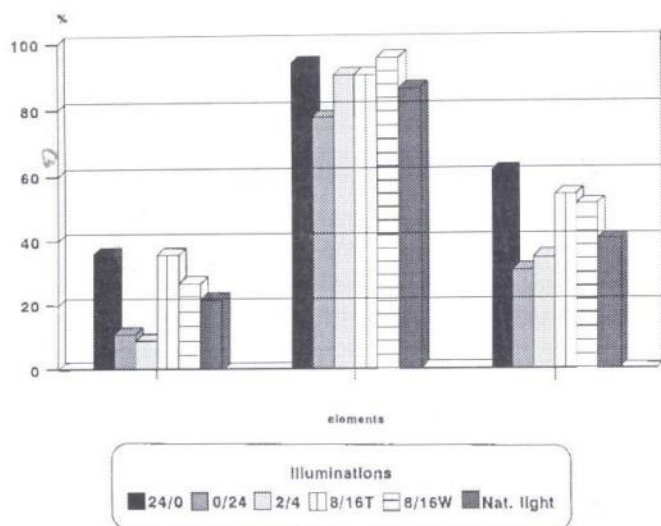


Figure 4 Utilization of some cations depending of illumination III.

Interesting conclusions may be drawn regarding the uptake dynamics of single elements. In Fig. 5 cation uptake dynamics are shown under an 8/16 photoperiod and WARMWHITE illumination.

In the case of the elements, **B**, **K**, **P** special observation were made.

Boron

Practically no uptake can be registered from the beginning to the 23rd day of subculture. From the 23rd to the 26th day 10 % of the total utilization is taken up by plantlets.

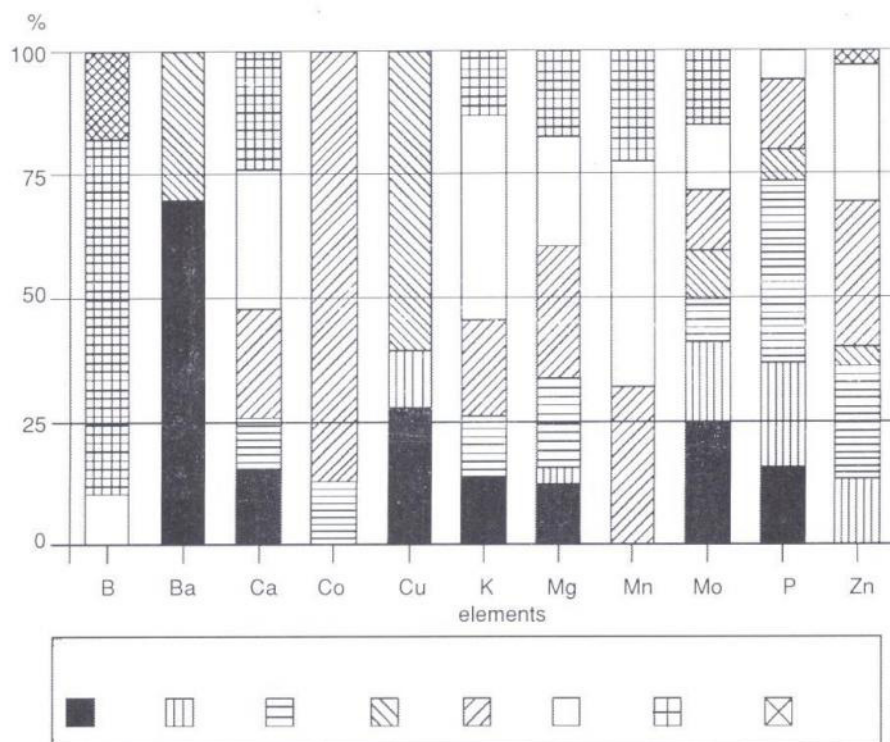


Figure 5 The dynamics of cation uptake in the case of 8/16 photoperiod

Most intense utilization takes place between 27th and 29th day, which means 75 % of the total uptake. At the end of subculture 15 % are (lacking of the medium) taken up.

Potassium

At the beginning of subculture up to the 17th day, take up is fluctuating, then a very intensive absorption can be observed from the 23rd day, about 40 % of the total quantity. At the 29th day potassium disappeared totally from the medium.

Phosphorus

An intensive uptake occurs between the 9th and 12th day of subculture, then dynamics showed down and on the 25th day no more P remains in the medium.

On the basis of our observations it seems necessary to revise the components in order to find the most suitable medium for the respective plants. So we suggest to reduce the concentration those elements, which low utilized by plantlets, such as: Ca, Mg, Mn, K.

The concentration of elements which were taken up the culture completely from the medium should be increased. Those are Zn, P, Cu. In order to determine the optimal concentration of elements for a particular plant genotype further investigations are indispensable.

On the basis of our analysis we can suggest definitely the reduction of K content of the basal medium, whereas the K content tended to increase the concentration during the adjustment of the pH because we used, conventionally, KOH.

It would be expedient to consider the element concentration of the agar and the sugar used in micro-propagation.

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