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Evaluation of the Most Adequate Organ of Reference for Sap Analysis in the Tomato Plant

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Abstract. The most adequate organ of reference for sap analysis is studied during the phenological stage corresponding to the fructification of the second flower bud in the tomato plant grown in hydroponic culture with normal nutrient solution. Thus, the composition of the sap extracted from petioles of leaves in different stages of growth is studied.

The results obtained indicate that the sap extracted from the petioles of fully grown young leaves is the best for achieving a nutritional study of the tomato plant.

The analysis of the watery fluid extracted from the conductive tissues of vascular plants, called "sap analysis", is a very interesting method for studying plant nutrition, since a deficiency can be foreseen well in advance.

The chemical composition of the sap varies according to the type of plant and growth stage (Azuara *et al.* 1985, Garcia López de Sá 1985), and to the chosen organ of reference (Cadahia *et al.* 1965).

Therefore, it is of vital importance to choose an organ of reference for each plant for sap analysis which would provide us with an exact idea of the movement of nutrient from the root up to the apical meristems of the plant, as well as of metabolic reaction processes (Routchenko 1970) which occur during plant development. It is also important for the chosen organ of reference to be sampled at different stages of the crop cycle, in order to check the efficiency of fertilization in the event of a nutritional disorder (Hernando 1973).

Various authors have studied the most adequate organ of reference in different crops, *i.e.* maize (Routchenko 1967), pepper plant (Alcaraz *et al.* 1980), strawberry plant (Casado 1974) and the tomato plant (Hernando *et al.* 1973).

In the case of the tomato (Hernando *et al.* 1973), sap was extracted from the

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different plant tissues, selecting the petioles of the second flower bud as organ of reference, although the authors do indicate that the petioles of the first flower bud were used with satisfactory results.

In subsequent studies (Hernando *et al.* 1982) carried out with tomato plants in the phenological stage corresponding to the first fructification, the sap extracted from the petioles was analysed in leaves with different stages of growth, defining as an organ of reference the petioles of fully grown young leaves corresponding, in this case, to the leaf of the first flower bud, following a downward direction.

The purpose of this study is to define whether in the different phenological stages of the tomato plant the organ of reference for sap analysis corresponds to the petioles of a certain flower bud or to a growth stage of the leaf.

MATERIALS AND METHODS

An experiment was carried out in a glass-house with tomato plants (*Lycopersicon esculentum*, L. cv. Marglobe) in hydroponic culture. Germination was effected in plastic trays containing chemically inert quartz sand. The size of the particles, according to Hewitt (1966), should be between 0.5 mm and 2.0 mm, thus a better relation between sand and water capacities is achieved. The seeds were sown on a sand bedding 10 cm deep. Watering with distilled water was carried out by capillarity in the first few days, and with nutrient solution diluted 10 times after emergence. When the plants were approximately 5 cm, they were transplanted to pots where watering took place by capillarity.

Plant nutrients were maintained in constant amounts throughout the experiment, which concentrations in macro-elements are similar to those of the nutrient solution of Hoagland and Snyder (1933), and using the microelements of Arnon's nutrient solution (Hewitt 1966).

Sampling for sap analysis was made (according to the Hernando-Cadahia method 1973) in the phenological stage corresponding to the second fructification, that is to say, when the fruit corresponding to the second flowering bud has a diameter of approximately 1 cm, during the first two hours of daylight because at this time there are likely to be smaller variations in the concentration of its bioelements (Azuara *et al.* 1982).

The petioles of 4 types of leaves were separated from the plants: a) Petioles of young growing leaves (J), the second in an upward direction from the second flowering bud. b) Petioles of fully grown young leaves (D), corresponding to the leaf of the second flower bud. c) Petioles of mature leaves (M), that is to say, the leaf of the first flower bud and d) Petioles of aged leaves (V), corresponding to the second leaf in a downward direction from the first flower bud.

The following analyses were carried out in the sap extracted from the different petioles: nitric nitrogen N (NO_3^-), sodium, potassium, calcium and magnesium according to Hernando and Cadahía (1973), protein phosphorus Pp, mineral

phosphorus P ($H_2PO_4^-$), protein nitrogen Np, amino acids Nam, and ammonia nitrogen N (NH_4^+), in an autoanalyzer; total soluble sulphur according to Cadahía (1971).

Four replicates were made of each treatment and a statistical study was effected by a simple hierarchic model, once the LSD were calculated at a probability level of 5%.

RESULTS AND DISCUSSION

The results obtained are shown in graphs 1 to 6. They indicate the evolution of bioelement contents in sap for the different growth stages of the leaf.

Nitrogen Fraction

A significant increase of Nm (Fig. 1) is observed in aged leaves, and this is basically due to N as NO_3^-, and to a lesser extent to N as NH_4^+.

N as NO_3^- experiences an increase in fully grown leaves and accumulates significantly in aged leaves. Similarly to the observations made in the first flowering (Hernando *et al.* 1982) an increase in the NO_3^- content occurred at the same time as the metabolic activity of the nitrogen fraction reduced. This occured as the age of the leaf under analysis increased.

On the other hand, there is little variation of N content as ammonium with respect to the age of the leaf, although a more significant concentration is perceived in aged leaves.

The results obtained for the different Norg fractions (Fig. 2) indicate an increase of the Nam content in the sap of the mature leaf with respect to the developed leaf. There was little difference between the Nam content of the young and the developed leaf on the one hand, and the mature and aged leaf on the other.

There was a more significant content of Np in the aged leaf, probably on account of the scarce activity of this leaf.

The Norg experiences an increase from the time the leaf is fully developed. This is possibly due to the scarce necessity of this nitrogen form, once the tissues have been formed.

The increase of N content in the form of NH_4^+ observed in aged leaves could be explained by considering that while the Nam content is similar in mature and aged leaves, NO_3^- concentration in aged leaves is slightly higher. Therefore, this important accumulation of NO_3^- provokes a more significant reduction of NO_3^- to NH_4^+ in aged leaves.

The ratio Nm%NST is relatively stable, thereby increasing in aged leaves.

If in normal conditions there is some problem in the transformation of the elements absorbed, these accumulate in inorganic form (concentration effect). Therefore, for diagnosis purposes the sap of aged leaves should not be analyzed since they accumulate Nm because of their age and not due to a nutritional problem.

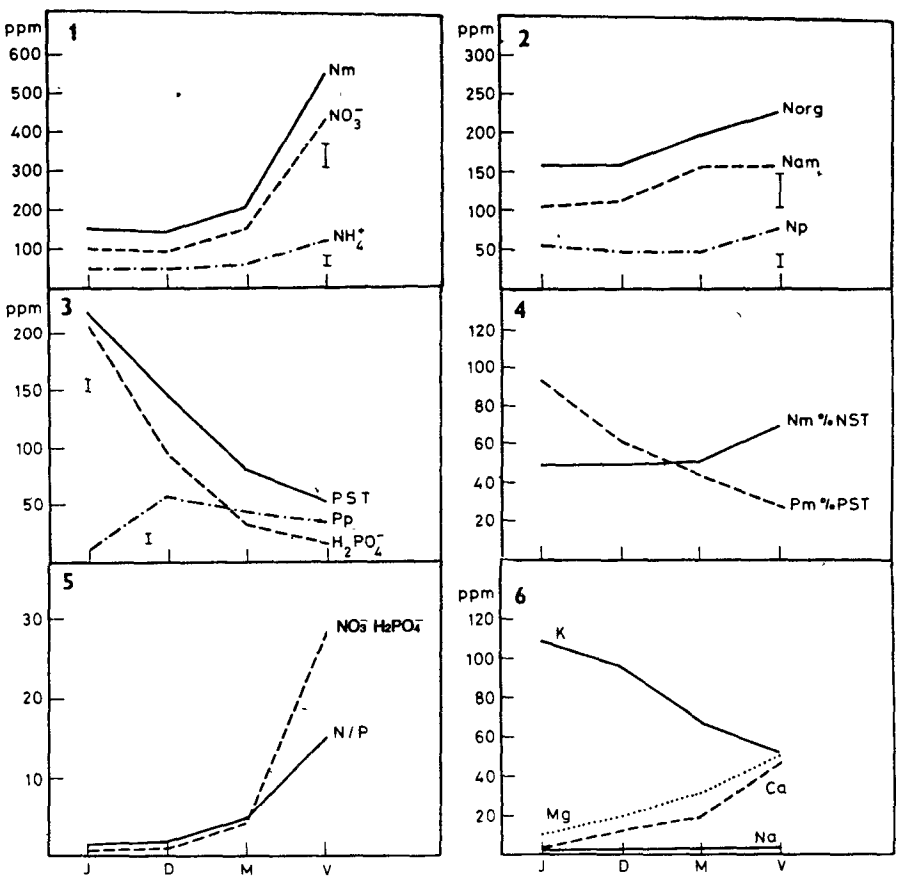
Phosphoric Fraction.

The variations in the content of different P fractions are indicated in Fig. 3.

The Pm fraction ($H_2PO_4^-$) experiences a decrease as the leaf ages, since this is a very mobile element which is found in places of maximum metabolic activity.

A very low content of Pp is observed in young growing leaves, because the tissues are in a phase of having to metabolize Pm to Porg, thus the fully grown young leaf presents the highest content of Porg, since the Pm in mature and aged leaves is able to decrease due to a mobilization of Pm to the places of maximum plant growth.

The PST content decreases as the leaf ages because in the mature leaf the decrease of Pm is more marked than the increase of Pp, and therefore on the whole, a decrease is observed with respect to the age of the leaf. Thus, this value provides us with an index of the metabolic stage of the leaf.



Figs. 1–6. Contents of nitrate nitrogen, ammoniacal nitrogen and mineral nitrogen (Fig. 1), amino acid nitrogen, protein nitrogen, and organic nitrogen (Fig. 2), mineral phosphorus, organic phosporus and total soluble phosphorus (Fig. 3), and of K, Ca, Mg and Na (Fig. 6), and the relations Nm%NST and Pm%PST (Fig. 4) and $NO_3^-/H_2PO_4^-$ and N/P (Fig. 5) in the sap of the petioles.

This decrease of P in relation to the age of the organ under analysis is also noted in the nodes of pepper plants (Alcaraz *et al.* 1980).

The Pm%PST ratio (Fig. 4) indicates a decrease with relation to the age of the organ analysed, due to the reduction of P as $H_2PO_4^-$. This ratio also provides us with an index of the metabolic stage of the leaf.

Therefore, with respect to the different P fractions, a young fully grown leaf ought to be analyzed for diagnosis purposes, since the balance or unbalance of phosphorus nutrition can be detected with more precision.

There is a significant increase in the N/P ratio (Fig. 5) which is basically produced by the variations in the mineral fractions of these two elements, as can be observed in the figure.

Cations and other anions.

The Na content in the sap extracted from the petioles of tomato plant leaves (Fig. 6) remains at a constant level with respect to the age of the leaf.

Of the cations analyzed (Fig. 6) K is the one which presents the highest level in general, and a higher content was noted in the young leaf. This is due to the fact that K is a mobile element, and therefore it is found in higher concentrations in the meristemic parts of the plant (White *et al.* 1988).

On the other hand, Fig. 6 indicates the antagonistic effect with the Ca and Mg, thus the analogous effect between the Ca and Mg.

Regarding total soluble sulphur, the analyses carried out in the sap of the petioles of the 4 types of leaves under examination indicated that it was only found at a trace level.

Thus, it can be concluded that for tomato plants corresponding to the phenological stage of the second fructification the ideal organ of reference for nutritional study is sap extracted from the petioles of young fully grown leaves. Similarly, for the first fructification it was considered convenient to do the sap analysis in the same organ of reference (Hernando *et al.* 1982).

Therefore, in order to recognize the nutritional balance or unbalance of the tomato plant, a sap analysis in the different growth stages should be done in the petioles of young fully grown leaves.

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