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Age-Related Changes in Micronutrients and Related Enzymes in Red Pepper

By

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With 5 figures

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Summary

MORENO D.A., VILLORA G. & ROMERO L. 2003. Age-related changes in micronutrients and related enzymes in red pepper. – Phyton (Horn, Austria) 43 (1): 187 – 195, with 5 figures. – English with German summary.

The change of several micronutrients (Fe, Mn, Cu, Zn and B) and physiological and biochemical indicators (catalase, peroxidase, aconitase and ascorbic acid oxidase activities; phenolic compounds) was studied in mature leaves of red pepper (*Capsicum annuum* L. cv. LAMUYO) grown under controlled greenhouse conditions, during the plant development from flowering to senescence. Biochemical activity changes at different phenological stages may be better indication of the plant nutrient status that the nutrient content itself. Thus, comparisons between biochemical indicators determined at standard conditions against those indicators induced with a given nutrient, as catalase and peroxidase with Fe and Mn, as well as ascorbic acid oxidase with Cu, revealed hiddeninsufficiency situations. The micronutrient concentration itself was not informative enough for the plant nutrient status.

Zusammenfassung

MORENO D.A., VILLORA G. & ROMERO L. 2003. Altersabhängige Veränderungen im Mikronährstoffgehalt und Enzymen im Paprika. – Phyton (Horn, Austria) 43 (1): 187 – 195, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Die Änderung mehrerer Mikronährstoffe (Fe, Mn, Cu, Zn und B) sowie physiologische und biochemischer Indikatoren (Katalase-, Peroxidase-, Aconitase- und Ascorbinsäureoxidase-Aktivitäten, phenolische Verbindungen) wurden in ausgewachsenen Blättern von Paprika (*Capsicum annuum* L. cv. LAMUYO) von der Blüte bis zur Senenszenz untersucht. Die Pflanzen wurden unter kontrollierten Glashausbedingungen gehalten. Änderungen in der biochemischen Aktivität zu verschiedenen phänologischen Zeitpunkten können eine bessere Indikation über den Ernährungszustand der Pflanze ergeben, als der Gehalt an Nährelementen selbst. So weisen Vergleichsuntersuchungen zwischen den biochemischen Indikatoren unter Standardbedingungen und den Indikatoren, welche von einem Nährstoff induziert wurden, wie Katalase und Peroxidase mit Fe und Mn oder Ascorbinsäureoxidase mit Cu, auf verborgene Mangelbedingungen hin. Der Mikronährstoffgehalt selbst war nicht aussagekräftig genug, um den Ernährungszustand der Pflanzen zu charakterisieren.

Introduction

The physiological age of the plant is a factor affecting the mineral composition of each organ (i.e. the leaf tissue) over the course of plant development (ROMERO 1995, ZHANG & al. 1996). Biochemical methods and enzymatic analyses offer approach to asses the mineral nutrition status of plants (MARSCHNER 1995, LAVON & GOLDSCHMIDT 1999). Foliar and biochemical analyses have provided no satisfactory explanation about the plant's nutritional status, possibly because earlier studies did not take into account the changes caused by a transition of a plant from one phenological stage to another. These changes involve different activities and a restructuring of the primary metabolism, which in turn influences the plant as a whole, and thus affect the analytical data (VALENZUELA & al. 1992). Examples given are catalase (CAT) and peroxidase (POX) activities, used for tracing Fe and Mn deficiencies (VALENZUELA & al. 1995, VÍLLORA & al. 2000). The aconitase (ACO) activity is lower under Fe deficiency (DE BELLIS & al. 1993). In several cases, Cu and Zn deficiencies reduce the ascorbic acid oxidase (AAO) activity (MARSCHNER 1995, LAVON & GOLDSCHMIDT 1999). The case of B is more complex. Boron is involved in phenolics/lignin metabolism from control of carbohydrate movement into synthetic pathways to the final polymerisation of lignin (GRAHAM & WEBB 1991, RUIZ & al. 1998).

In the present study, we tested the foliar Fe, Mn, Cu, Zn, and B concentrations and their related biochemical indicators through the plant developmental stages in order to achieve more accurate estimations of the nutritional status of the red pepper plant. The results can be basis for improvements in the greenhouse cultivation of this crop.

Abbreviations: AAO – ascorbic acid oxidase; ACO – aconitase; CAT – catalase; Fe-EDDHA – ferric ethylenediaminedi (*o*-hydroxypenylacetic acid); POX – peroxidase.

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Material and Methods

Red pepper plants (*Capsicum annuum* L. cv. LAMUYO) were grown in cell flats (cell size 3 x 3 x 10 cm) filled with peat-perlite mixture, placed on benches under the greenhouse conditions described below, for a period of 8 weeks; the seedlings were transplanted and grown under controlled conditions in an experimental greenhouse at Almería (southeastern Spain). The relative humidity was 60-80%, a photoperiod of 16 h ($1 \cdot 10^3$ - $3 \cdot 10^3$ Wh/m²/d) and the temperature ranged between 20 and 30 °C. Container-grown red pepper plants were transplanted into 2 rows 100 cm apart and trickle irrigated soil plots of 4 m x 2 m replicated four times and each plot contained 8 plants. The soil used was loamy-sand soil without flodding or ionic accumulation problems with the following characteristics: sand (37.3%), silt (48.6%) and clay (10.1%); $CaCO_3$-equivalent (26.82%), $CaCO_3$-active (14.35%); 3.5 g kg^{-1} total N; 36.1 g kg^{-1} total organic carbon; 890 mg kg^{-1} PO_4^{3-}; 5.34 g kg^{-1} K; pH (H_2O, 8.45; KCl, 8.01) and electrical conductivity (E.C.: 4.63 $dS \cdot m^{-1}$). The soil surface was covered with a 7-cm layer of sand. The irrigation water had the following properties: pH 7.80; E.C. 3.80 $dS \cdot m^{-1}$, Cl^- 484 mg L^{-1}, Na^+ 306 mg L^{-1}, K^+ 10 mg L^{-1}, HCO_3^- 278 mg L^{-1}. Macro and micronutrients were added to the crop: NH_4NO_3 (1 mmol), K_2SO_4 (0.5 mmol), H_3PO_4 (1 mmol); Fe-EDDHA (5 mg L^{-1}); Mn (2 mg L^{-1}), Zn (1 mg L^{-1}), Cu (0.25 mg L^{-1}), and Mo (0.05 mg L^{-1}) as sulphates; H_3BO_3 (0.5 mg L^{-1}); final pH: 5-6. A computerized fertigation system was used. Water and fertilizer are delivered simultaneously to the crop via the nutrient solution (VÍLLORA & al. 2000).

All plants (128 plants) were sampled at 15-days intervals throughout the growth cycle. Leaf samples were taken only from plants with fully expanded leaves of the same size. Mature leaves were picked from about one-third of the plant height below the plant apex, discounting damaged, abnormally large, or pest-infected leaves. In the laboratory, they were rinsed three times in distilled water after desinfecting with 1% non-ionic detergent, then blotted on filter paper and separated into two samples, one for fresh- and the other for dry-matter assays.

The plant material was air-dried at 70°C for 24 h, and 0.2 – 0.3 g of the dried ground matter was subjected to wet digestion with concentrated sulphuric acid in order to determine total Fe, Mn, Zn, Cu levels, using atomic absorption spectrophotometry (HOCKING & PATE 1977). The total B concentrations were determined colorimetrically (WOLF 1971). Concentrations of soluble Fe, Mn, Zn and Cu were measured as indicated above after extraction of 0.2 g dried leaves with 10 ml 1M HCl for 30 min and filtration at the end.

Peroxidase (POX) activity was determined following the method of BAR-AKIVA 1984. Fe- and Mn-induced POX (POX+Fe and POX+Mn) were measured following BAR-AKIVA & LAVON 1968: 1 g of fresh leaf matter was infiltrated under vacuum for 3 min in phosphate buffer (pH 6.8) with 1% $FeSO_4$ for Fe, or 1% $MnSO_4$ for Mn. Catalase (CAT) activity was measured according to the method of MARSH & al. 1963. The same procedure was used for Fe- and Mn-infiltrated catalase activities (CAT+Fe, CAT+Mn), after infiltrating the samples with 1% $FeSO_4$ or $MnSO_4$, respectively. Aconitase activity (ACO) was measured using the BACON & al. 1961 method. Standard and Cu-induced ascorbic-acid oxidase (AAO, and AAO+Cu) activities were also determined (BAR-AKIVA & al. 1969).

The phenols of the plant material were extracted with Methanol:$CHCl_3$:(1%) NaCl (2:2:1). Total phenolic content was assayed quantitatively by absorbance at 765 nm with Folin-Ciocalteau reagent (SINGLETON & ROSSI 1965). The ortho-diphenols were determined by absorbance at 360 nm (JOHNSON & SCHAAL 1957, RUIZ & al. 1998).

Data were analyzed statistically by analysis of variance (ANOVA) using the Statgraphics 7.0 version (MS-DOS). Simple correlation analyses were performed to indicate possible relationships between parameters.

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Results and Discussion

The crop growth conditions of the experiment are intended to imitate as closely as possible techniques used by farmers in southern Spain. The local agricultural production puts a high demand on the water resources, depleting the

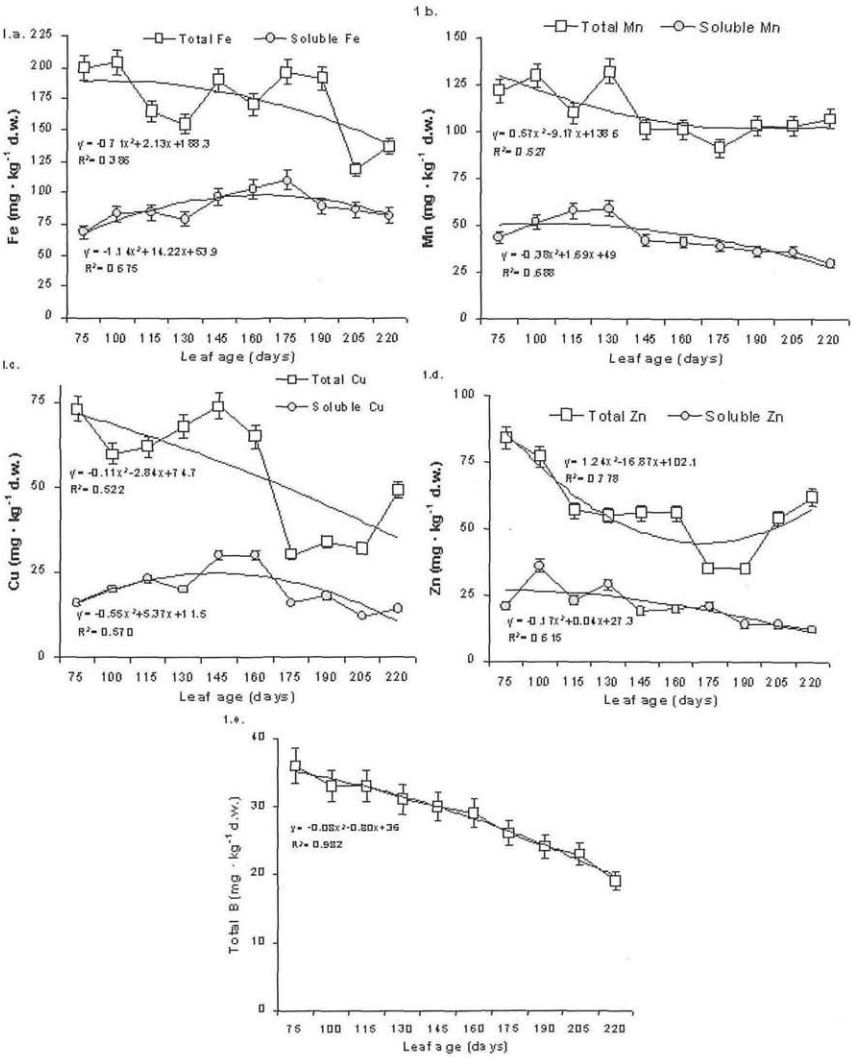

Fig. 1a-e. Changes of micronutrient concentrations in pepper mature leaf during plant age. The polynomial equation and the R2 values represents the correlation between plant age and measurements of all data from the replicates of 10 samplings, not only of the values in the figures.

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local aquifers and causing marine-water intrusion on the groundwater. Therefore, fertigation water has a high saline content as well as pollution from excess fertilizer applied to the crops in the area, with clear predomination of Na and Cl ions (indication its marine origin), alkaline pH, and an elevated electrical conductivity, all of which impose stress to the crop.

The total Fe concentration in red pepper leaf (Fig. 1a.) decreased over time while soluble Fe was significantly highest at 175 d, in fruit production and early senescence period. The foliar concentration of total and soluble Mn (Fig. 1b.), Cu (Fig. 1c.), Zn (Fig. 1d.), as well as total B (Fig. 1e.) decreased significantly over time. Thus, micronutrient trends showed probable dilution or remobilization processes to sink organs in mature leaf as plant growth.

Aconitase activity (ACO) in mature leaves increased as the plants approached senescence (Fig. 2). This trend may be due to the fact that the foliar soluble-Fe activated the enzyme but the possible use as Fe-bioindicator was not relevant (MARSCHNER 1995, ROMERO 1995).

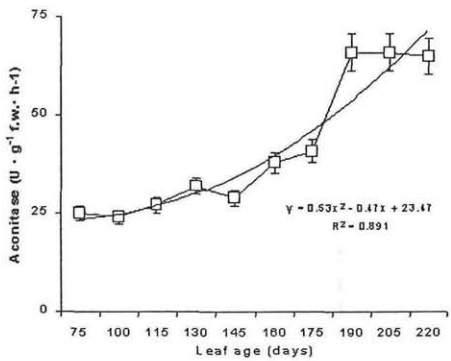

Fig. 2. Changes of aconitase activity in pepper mature leaf during plant age. The polynomial equation and the R2 value represents the correlation between plant age and measurements of all data from the replicates of 10 samplings, not only of the values in the figures.

The CAT (Fig. 3a.), CAT+Fe (Fig. 3b.) and CAT+Mn (Fig. 3c.) increased with plant age, as senescence period is marked by an increase in H_2O_2 formation (BRENNAN & FRENKEL 1977, VALENZUELA & al. 1995), implying an increase in the enzymatic activity to eliminate this product and delay organ senescence (FERGUSON & al. 1983). The plant's Fe requirements are reflected in Fig. 3a-c. The CAT+Fe (Fig. 3b.) was higher than CAT (Fig. 3a.) and the enzyme's requirements for this ion are especially evident during advanced maturity and senescence showing marked 'reactivation' (LAVON & GOLDSCHMIDT 1999, MARSCHNER 1995) in CAT+Fe activity meaning hidden Fe deficiency.

The CAT+Mn increased with plant age (Fig. 3c.) but the activity was lower than the standard CAT. In previous work, infiltration of plant material with the

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element, and determination of the CAT+Mn showed higher activity than the CAT when the element is deficient (LAVON & GOLDSCHMIDT 1999, VALENZUELA & al. 1995). Therefore, as the CAT+Mn was quite lower than CAT, we have a biochemical indication of very high Mn endogenous levels.

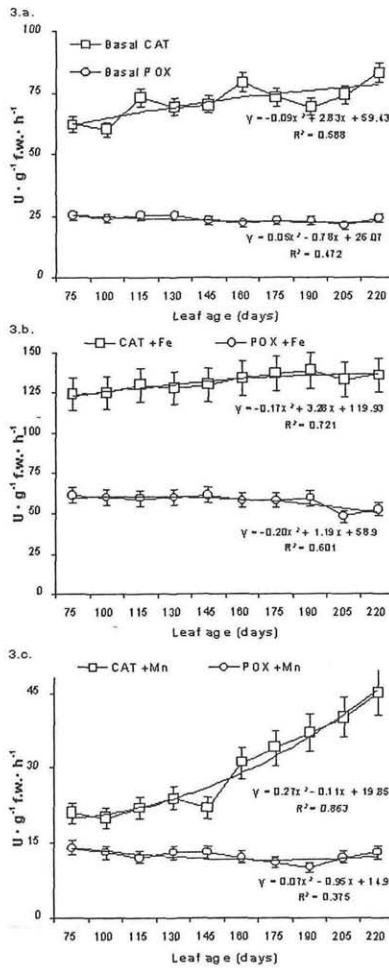

Fig. 3a-c. Changes of Catalase (CAT) and Peroxidase (POX) activities in pepper mature leaf during plant age. The polynomial equation and the R2 values represents the correlation between plant age and measurements of all data from the replicates of 10 samplings, not only of the values in the figures.

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Peroxidase (POX, Fig. 3a.) and POX+Fe (Fig. 3b.) activities declined significantly as plant grew. The transitory drop in POX activity was analogous to the changes in Fe levels with plant age, possibly as a result of the Fe foliar insufficiency or hiddendeficiency also found with the CAT results (MORENO & al. 1996, VÍLLORA & al. 2000). By contrast, POX+Mn (Fig. 3c.) was lower than POX, indicating those high Mn levels in the leaves. These data suggest a phloem retranslocation of Fe from mature leaves causes fluctuations in the amount of ions during the plant's life (ZHANG & al. 1996). These changes could be related and responsible for the alterations in enzymatic activity. Therefore, the comparison between CAT and POX activites under standard and reactivated conditions was useful to trace Fe and Mn nutritional status (MARSCHNER 1995, VALENZUELA & al. 1995).

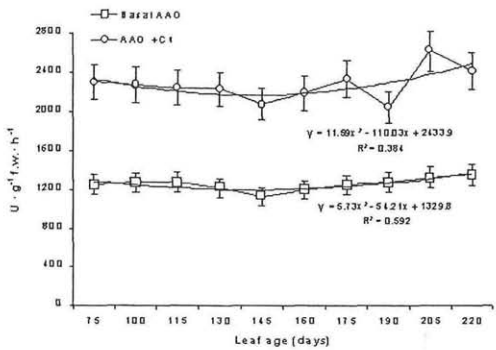

Fig. 4. Changes of Ascorbic acid oxidase (AAO) activity in pepper mature leaf during plant age. bThe polynomial equation and the R2 values represents the correlation between plant age and measurements of all data from the replicates of 10 samplings, not only of the values in the figures.

A close positive correlation between Cu and ascorbic acid oxidase (AAO) activity was found in leaves of different crops (BAR-AKIVA & al. 1969, DELHAIZE & al. 1986), and AAO is markedly reduced under Cu and Zn deficiency conditions (BAR-AKIVA & al. 1969, LAVON & GOLDSCHMIDT 1999). In red pepper plants, AAO and AAO+Cu (Fig. 4.) decreased toward the 145d-160d and then increased to the end of the cycle, and the extent of the AAO+Cu activity practically twice as high as the AAO revealed a hiddendeficiency of Cu (BAR-AKIVA & al. 1969). The relationship with Zn levels was weak and in order to be more specific we would need to assay the enzyme under the induction of Zn in the leaf samples.

We observed B concentrations continuosly and significantly decreasing during the plant growth (Fig. 1e.). The B deficiency is related directly to phenol accumulation in different parts of the plant (ROMERO 1995). The foliar B in red pepper, even decreasing with plant age, was positively related to the phenol compounds (Fig. 5) as supported by their correlations: B—O-diphenols (r=

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0.632***) and B—total phenols (r= 0.724***). Probably plants were not deficient in B, highlighting the usefulness of this physiological parameters under B deficiency conditions (RUIZ & al. 1998) but not being sufficiently informative when the nutrient is within the adequate range.

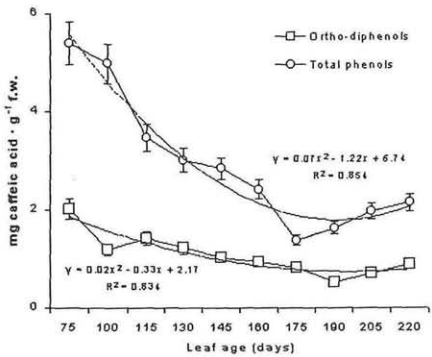

Fig. 5. Changes of ortho-diphenols and total phenols in pepper mature leaf during plant age. The polynomial equation and the R2 values represents the correlation between plant age and measurements of all data from the replicates of 10 samplings, not only of the values in the figures.

Elements and biochemical activities and parameters change depending on many factors intimately related with the plant species and directly influenced by the phenological stage of the plant: as development proceeds, changes occur in biochemical activities and ion demand, as previously established (ROMERO 1995, VALENZUELA & al. 1992, 1995). Thus, the diagnostic methods are useful to solve problems of nutritional disorders and to supplement elemental analyses rather than to replace established techniques (MARSCHNER 1995, VÍLLORA & al. 2000). It should be noted that enzymes chosen as markers of the nutritional status of plants in this experiment gave more information than the chemical analysis not reliable enough by themself. Thus, comparisons of CAT and POX under endogenous or standard conditions with the Fe- and Mn-induced forms, as well as AAO and AAO+Cu, revealed a hiddeninsufficiency of Fe and Cu in red pepper plants, while B-phenolics relationship might not be useful as nutritional marker when the B levels are at adequate levels.

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