Aspects of Leaf Nitrogen Metabolism in Two Cultivars of Tomato Under Water Deficit[1]

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ABSTRACT

The effects of water deficit on free amino acid, proline and protein contents and proteolytic activity were studied in leaves of plants of two cultivars of tomato (Pera Quibor and Río Grande). During the water deficit period, the values of water potential, osmotic potential, osmotic potential at full turgor and relative water content decreased. The leaf free amino acid and proline contents were higher, whereas the protein content decreased and then showed fluctuations in plants under water stress. The leaf proteolytic activity was also higher and fluctuating. Such fluctuations inversely corresponded with observed fluctuations in protein content for both cultivars. The increase and fluctuations in free amino acid content are both a consequence of the changes in proteolytic activity. These results show that during the water deficit period there are alternating intervals of decreased and increased protein content. The former interval is due to increase proteolytic activity and is the main factor responsible for the increased free amino acid content. The latter interval may be the result of the synthesis of new protein at the expense of existing leaf protein. There is a positive correlation between osmotic potential and proline content, but the observed values of osmotic potential at full turgor indicate that osmotic adjustment did not occur.

Key words: Free amino acids, proline, proteolytic activity, protein, drought.

COMPENDIO

Los contenidos de aminoácidos, prolina y proteína y la actividad proteolítica en hojas fueron analizados en plantas de tomate de dos cultivares (Pera Quibor y Río Grande), sometidas a déficit hídrico. Durante el período de déficit hídrico, los valores de los potenciales, hídrico, osmótico y osmótico a pleno turgor y del contenido relativo de agua disminuyeron. En plantas bajo déficit hídrico, los contenidos foliares de aminoácidos y prolina fueron mayores, el contenido foliar de proteína disminuyó y mostró fluctuaciones y la actividad proteolítica foliar fue mayor, mostrando fluctuaciones inversas a las observadas en el contenido de proteínas. El incremento y fluctuaciones en el contenido de aminoácidos son consecuencia de la actividad proteolítica. Se observa que, durante ese período, existen intervalos de menor contenido proteico por una mayor actividad proteolítica, y es el principal factor responsable del aumento en el contenido de aminoácidos, seguido por intervalos de contenido proteico mayor, lo cual pudiera ser el resultado de síntesis de nueva proteína a expensas de la foliar existente. Hay una correlación positiva entre el potencial osmótico y el contenido de prolina, pero los valores obtenidos para el primero a pleno turgor indican que no ocurrió el ajuste osmótico

INTRODUCTION

It has been reported that, in plants under water deficit, protein synthesis decreases (16). Another plant response to water deficit is the accumulation of amino acid, specifically proline, which has been reported for many species (1). Recently, qualitative and quantitative changes have been found (29) in the protein patterns of *Brassica napus* var. Oleifera roots, under physiological conditions of drought, and it has been reported that maize seedlings respond to water stress with the synthesis of stress specific proteins (4). For mesophytic crop plants in drought-prone environments, the identification of adaptative metabolic traits has specific importance because such traits might be exploited in plant breeding for drought resistance (14).

The aim of the present work was to study the effects of water deficit on proteolytic activity as well as protein and free amino acid contents in two tomato (*Lycopersicum esculentum*) cultivars: Pera Quibor and Rio Grande, and to establish the relationship between these parameters. The increase in proline content was also analyzed. Every measurement was done in order to understand how these metabolic parameters change

1 Received for publication 7 December 1990.
Financial support by Venezuelan Council for Scientific and Technological Research (CONICIT) Project S1-1325. Seeds of the two selected cultivars of tomato were supplied by Venezuelan National Fund for Agriculture and Animal Husbandry (FONAIAP)

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and how they interplay during the tested water deficit period.

MATERIALS AND METHODS

Plant material

Seeds from two selected cultivars of tomatoes, Rio Grande and Pera Quibor, were supplied by Venezuelan National Fund for Agricultural and Husbandry (FONAIAP), Maracay. The seeds were germinated on wet paper in plastic trays. After germination, the seedlings were transferred to sand and, at the appearance of the cotyledons, the plants were transferred to 5 l plastic pots (one per pot).

These were kept for daily watering in a greenhouse with 30 ± 2.2°C average maximum temperature; 17.5 ± 2.3°C average minimum temperature; 97.6% average maximum RH; 58% average minimum RH; and 655.7 µmol m^{-2} s^{-1} average sun radiation. Forty-six day-old plants were used for experiments. Watering of 80 plants was withheld (water-stressed plants). Forty plants were watered throughout the experiments and maintained as controls. Measurements on water-stressed plants and control plants (watered) were carried out at two-day intervals over a period of two weeks. The youngest, most expanded leaves were used. A set of four replicates were used for the measurement of all parameters (Y_w, Y_s, Y_{st}, RWC, proteolytic activity, protein, free amino acid and proline contents), resulting in four sets of each.

Water potential (y_w), osmotic potential (y_s) and relative water content

All measurements were made before dawn. For Y_w measurements, four leaflets from each of the four replicates were introduced in a L-44 sealed chamber (Wescor Inc., Logan, Utah, USA) attached to a HR-33T Dew Point microvoltimeter (Wescor Inc. Logan, Utah, USA). After measuring Y_w, the leaves were frozen and Y_s was measured in the expressed sap using the HR-33T dew point microvoltimeter attached to C-52 chambers (Wescor Inc., Logan, Utah, USA). For determinations of Y_{st}, the leaflets were floated in water during 24 h at 4°C; after which the Y_s was measured as before. For RWC measurements, the method reported by Turner (28) was followed.

Free amino acid determination

The amino acid extraction was performed following the method reported by Singh *et al.* (23). The ninhydrin method was used for the determination of free amino acid content. The ninhydrin reagent was prepared according to the method reported by Benson and Petterson (2). The absorbance was measured at 570 nm using a double-beam spectrophotometer (Shimadzu, modUV-150) and the values obtained were extrapolated to mmol of free amino acid using a calibration curve run with sodium -L-glutamic acid (Sigma).

Proline determination

The proline content was assayed using the same extracts employed for amino acid analysis and following the method described by Singh *et al.* (23). The absorbance at 515 nm was measured. The values obtained were extrapolated using a calibration curve generated with DL-proline (Sigma).

Protease extraction and proteolytic activity

The test was performed according to Feller *et al.* (8). The assays used azocasein (Sigma) as substrate. The absorbance at 440 nm was measured. The absorbance values were extrapolated to µg of azocasein in a calibration curve run with azocasein.

Protein content

Aliquots taken from the same extract obtained for proteolytic activity were precipitated with 15% trichloroacetic acid, centrifuged and the pellet resuspended with 0.2 N NaOh; estimation of protein content followed the method of Bradford (5).

Statistical analysis

The regression coefficient, the Pearson and the Spearman rank tests were used for correlation (26).

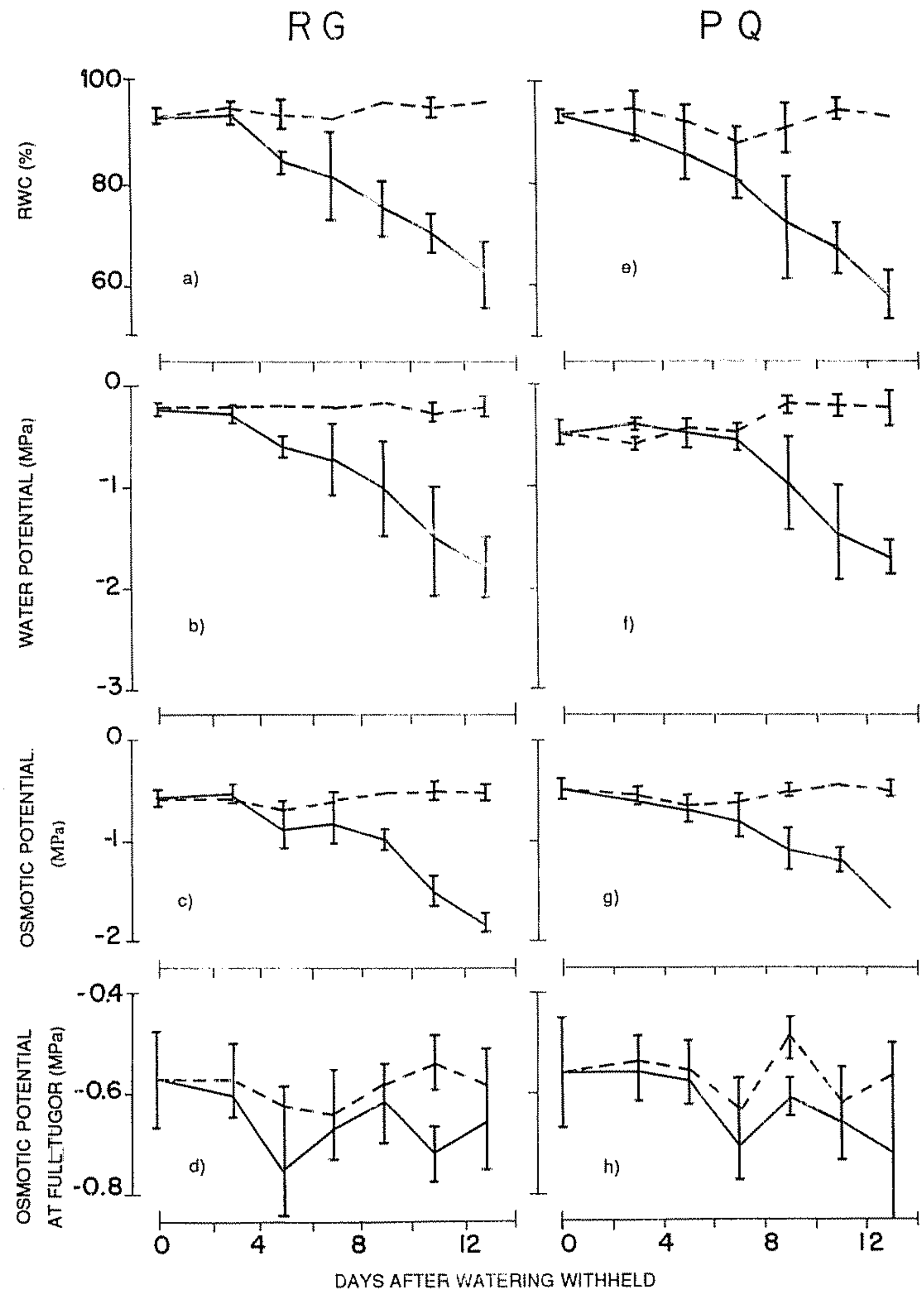

Fig. 1. a- e: Relative water content, RWC (%); b, f:leaf water potential, Y_w (MPa); c, g: osmotic potential, Y_s (MPa); osmotic potential at full turgor, Y_{ft} (MPa) in control and water stressed tomato plants of two cultivars, Pera Quibor (PQ) and Rio Grande (RG), during the treatment period. Each value represents the mean of four replicates, and the bars represent the standard deviation (SD) Dashed lines represent control values.

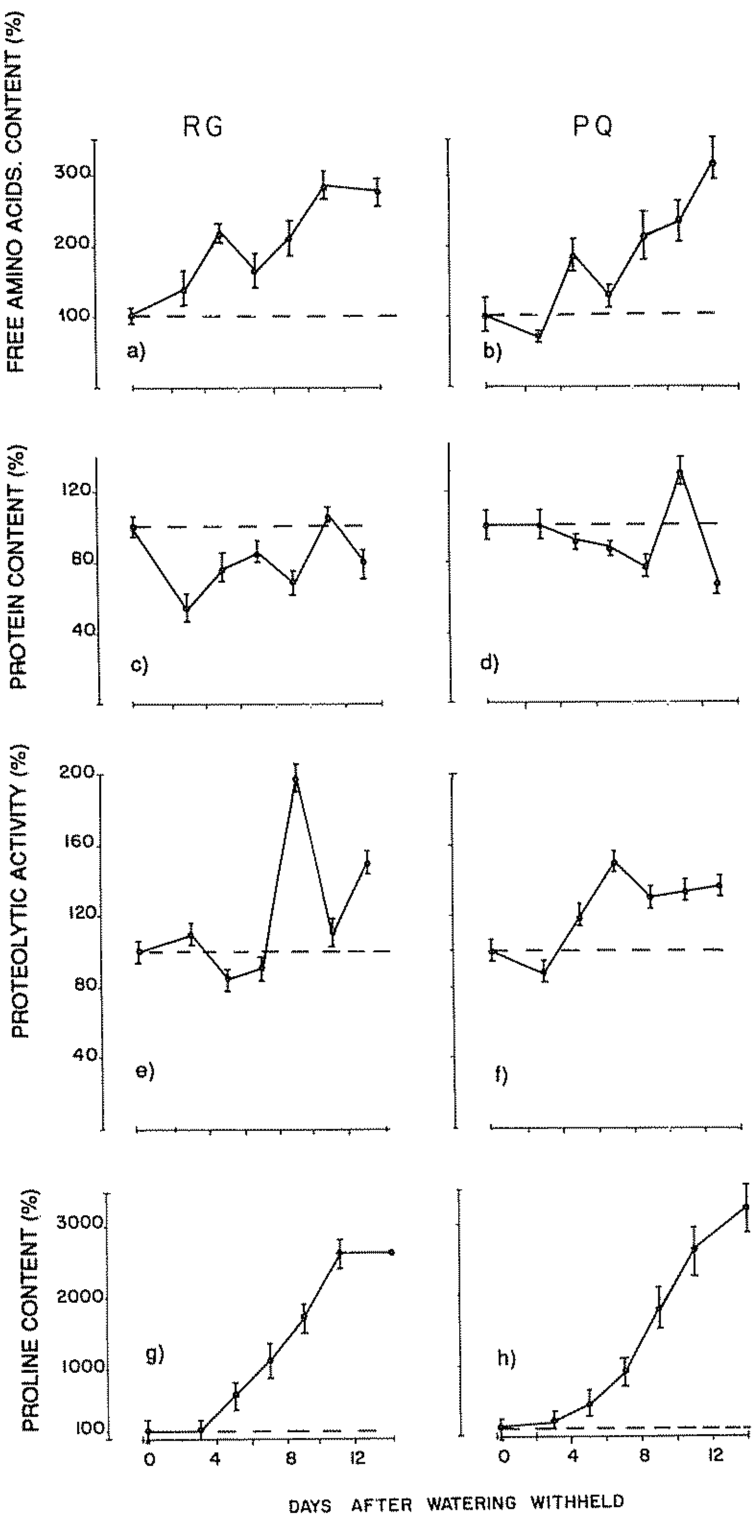

Fig. 2. a-e: % free amino acid content (mol/kgDW); b-f: % protein content (g/kgDW); c-g: % proleolytic activity (g azocasein/kgDWh^{-1}); d-h: % proline content (mol/kgDW) in water- stressed tomato plants with respect to control of two cultivars, Pera Quibor (PQ) and Rio Grande (RG), during the period of treatment. Each value represents the mean of four replicates, and the bars represent the standard deviation (SD). Dashed line represent control values.

RESULTS AND DISCUSSION

The values of Y_w, Y_s and RWC measured throughout the treatment for control plants (watered plants) and water-stressed plants of both cultivars are shown in Figure 1 (a-g). A decrease in these values is observed in water-stressed plants. The values of Y_{st} for control and water-stressed plants of both cultivars showed no differences, as seen in Figure 1 (d-h). Figure 2 shows the % free amino acid content(mol/kgDw) (2a-e); % protein content (g/kgDW) (2b,f); % proteolytic activity (g azocasein/kgDW h) (2c- g) and % proline content (mol/kgDW) (2d, h) in water-stressed plants with respect to control for both cultivars.

In the RG cultivar, the percentage increase in free amino acid content (Fig. 2a) was paralleled by a decrease in protein content (Fig. 2b) below the control levels, and by a slight increase in proteolytic activity (Fig. 2c) on the third day of water withholding. This could suggest that, under mild water deficit, the protein content decreases as a result of an increase in proteolytic activity and possibly a decrease in protein synthesis, leading to an increase in the free amino acid content. On the fifth and seventh days, the protein content showed an increase below control levels, which could be due to a lower proteolytic activity and to new protein synthesis at the expense of available free amino acids.

On the ninth day, the proteolytic activity increased, thus decreasing the protein content and increasing the percentage of free amino acids; but on the 11th day, there was an increase in protein content above control level, possibly due to a decreased in proteolytic activity and an increase in protein synthesis. On the thirteenth day, the protein content decreased due to the increase in proteolytic activity. The free amino acid content remained high from the ninth day onwards, possibly due to a sharp increase in proteolytic activity on the ninth and thirteenth days after watering was withheld.

For PQ cultivar, it was observed that on the third day, the percentage of amino acid content (Fig. 2c) and proteolytic activity (Fig. 2g) were lower than in control plants. The protein content is similar to control (Fig. 2f). Figure 1e shows that the leaf Y_w values of plants of PQ cultivars under water stress did not decrease at the onset of water stress. However, on the fifth day, a decrease of leaf Y_w in water-stressed plants is observed. From fifth day onward, an increase in percentage of free amino acid content (Fig. 2c) and percentage of proteolytic activity (Fig. 2g) above control levels was observed, with a decrease in protein content (Fig. 2f) below control levels; the latter parameter was seen to increase on the eleventh day, suggesting that there was protein synthesis at the expense of available free amino acids and that the protein synthesis could be higher than protein degradation.

There was a positive correlation between Y_s and proline content (Table 1); however, the obtained values of Yst (Fig. 1d and 1h) prove that osmotic adjustment did not occur, indicating that proline does not contribute to important changes in the leaf osmotic potential, as has been previously reported (17).

Table 1. Correlations coefficients (Spearman) among leaf water components and free amino acids, protein content and proteolytic activity, regression coefficient (r^2) for proline content and RWC and Y_s and Pearson coefficient (r) for proline content and Y_w in two tomato cultivars under water deficit.

	Cultivar	RWC	Y_w	Y_s
Free amino acid (mol/kgDW)	RG	0.011	-0 054	0 017
	PQ	-0 551*	-0 428*	-0 457
Protein content (g/kgDW)	RG	-0.500*	-0 485*	-0 531*
	PQ	0 551*	-0.473*	-0 457*
Proteolytic activity (g azocasein/kgDW)	RG	-0 575*	-0 563*	-0 545*
	PQ	-0 245	-0 209	-0 233
		r^2	r	r^2
Proline content (mol/kgDW)	RG	0 89*	-0 892*	0 84*
	PQ	0 88*	-0.906*	0 77*

Significant for α = 0 05

Table 1 presents the correlation coefficients (Spearman) among leaf water components and free amino acids, protein content and proteolytic activity; the regression coefficient among proline content, RWC and Y_s and the Pearson coefficient between proline content and Y_w. There were some significant correlations among the measured parameters and some components of leaf water status for both cultivars, although this significance was more marked in protein and proline contents for both cultivars. For free amino acid content, there was no significant correlation for the RG cultivar; a similar situation was found in the PQ cultivar with respect to proteolytic activity.

A significant increase in the content of amino acids, especially proline, during leaf wilting caused by water deficit has been reported (9, 10, 14, 18, 19, 21, 23). This increase could be attributed to an imbalance between decreased protein synthesis and increased protein degradation as observed during the water deficit period. Lawlor and Fock (18) reported that water deficiency might produce accumulation of certain amino acids due to changes in metabolic paths. The authors explained that the synthesis of amino acids was stimulated by the accumulation of organic acids in plants under water deficit and that the source of NH4 comes from protein degradation.

Working with young bean plants under water deficit, we observed (7) a decrease and subsequent fluctuations in protein content, paralleled by an increase and subsequent fluctuations in the ammonium content. Significant changes in the content of free amino acids in wilted tomato leaves have been reported (2). The results presented in this work show that the increase in free amino acid content is directly related to the increase in proteolytic activity (Fig. 2ae and 2cg).

The leaf proline content increased drastically in both cultivars during the water deficit period (Fig. 2d,h). As is already known, a very marked increase in free proline content occurs in leaf tissue of many mesophytic plants during hours to days of moderate to severe water deficit (12).

It has been indicated (12, 13, 27) that proline accumulation does not present any adaptative value for survival during severe water deficit periods. However, Aspinall and Paleg (1) suggested that the accumulation of proline during periods of water stress has a definite evolutionary advantage in that it endows the cell with a measure of resistance, and that this is not merely a consequence of stress-impaired metabolism. It was reported that in five barley cultivars under water deficit, once the water deficit was removed from all cultivars, the relative growth rate of the cultivars during the recovery period was directly related to the amount of proline accumulated during the water deficit period (24). Blum and Ebercon (3) also suggested that proline may have a positive effect in the immediate recovery of plants after release from stress. Using contrasting sorghum lines under drought stress, it was reported (25) that susceptible lines accumulated less proline than resistant lines. The data, calculated from the immediate recovery of resistant lines after rewatering, compared well with the levels of proline accumulated during the peak stress period. The authors concluded that proline accumulation may, therefore, contribute to the immediate recovery of plants. Working with 39-day-old plants of the same cultivars used in the present work under water deficit and rewatering (6), it was found that all parameters measured showed a gradual recovery after rewatering, reaching or even surpassing the values of control plants, indicating that the high increase in proline content observed in the present work could be associated with a high potential of leaf survival.

In the present work, we found decreased protein content in plants under water deficit, but this decrease showed fluctuations, indicating new protein synthesis and, in some cases, the protein content exceeded that of control plants (105% in RG and 130% in PQ) (Fig. 2b,f). This observed increase in protein content could be due to new protein synthesis geared specifically to produce new water-stress protein. The synthesis of a new water-stress protein has been previously reported (4, 15, 22, 29). It has been concluded that, although the physiological significance of the protein synthesis induced in response to altered environmental conditions is not yet clearly understood, their synthesis appears to be related to the acquisition of a better resistance to the adverse inductive factor. Our results showed that during water deficit in the two studied tomato cultivars, there were intervals of decreased protein content due to an increased proteolytic activity, the main factor responsible for the increased free amino acid content observed, and increased protein content which could be the result of new protein being synthesized at the expense of existing leaf protein.

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