Structural, physiological and metabolic integrated responses of two tomato (*Solanum lycopersicum* L.) cultivars during leaf rehydration

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Abstract

The aim of this work was to characterize the recovery responses of two tomato cultivars, Rio Grande (RG) and Pera Quibor (PQ), to rehydration after water deficit. Recovery responses were measured in terms of timing and magnitude, and analysed as indicative of tolerance. The parameters evaluated were: soil water potential (soil Ψw) and leaf water potential (leaf Ψw); leaf osmotic potential (leaf Ψs); leaf relative water content (LRWC); stomatal conductance (gs); Rubisco activity; protein content; fresh mass (FM) and dry mass (DM); leaf area (LA); specific leaf area (SLA) and leaf density (D). Different types of responses were observed: as common responses of both cultivars, full recovery of soil Ψw, Ψs, and LRWC were found regardless of stress level and length of rehydration time. Recovery of protein was full in PQ and partial in RG. Full recovery of gs and fresh mass and partial recovery of Ψw and leaf area was observed in both cultivars regardless of the level of stress but regarding rehydration time. Full recovery of Rubisco activity was found regarding level of stress and rehydration time. As different responses between cultivars, full recovery of DM was found in PQ and of SLA and leaf D in RG regardless of the level of stress but regarding rehydration time. Partial recovery of SLA and leaf D was found in PQ regarding both level of stress and rehydration time. The delayed recovery of leaf structural changes promoted full recovery of DM and protein in PQ. The correlations between physiological and metabolic parameters and observed sclerophylly indices, reveal integrated tolerance mechanisms intended to protect plant cultivars from intermittent changes in water conditions.

Keywords: leaf density; leaf osmotic potential; leaf relative water content; leaf water potential; protein content ; Rubisco; soil water potential; specific leaf area; stomatal conductance.

Abbreviations: D, leaf density **;** DM**,** dry biomass; FM, fresh biomass; gs**,** stomatal conductance; LRWC**,** leaf relative water content; PQ**,** Pera Quibor cultivar; RG**,** Rio Grande cultivar**;** SLA, specific leaf area; Ψw**,** leaf water potential; sΨw, soil water potential; Ψs**,** leaf osmotic potential.

Introduction

Lack of water is the most unfavorable environmental stress factor affecting cultivars' growth and productivity, its effects being the most harmful among those of other environmental stress agents (Kramer, 1980). Crops grown under rain-fed conditions are usually affected by drought stress at different stages resulting in negative effect on yield (Amjad Ali et al.2011). Water deficit induces changes in leaf anatomical parameters, altering the CO_2 conductance diffusion components, and thus collaborating with the maintenance of photosynthetic rates even at low stomatal conductance (Chartzoulakis et al. 1999). Lowered photosynthetic rate under water deficit has been attributed to both stomatal and non-stomatal limitations (Galle et al. 2010; Souza et al., 2004) involving programmed stomatal closure and reduced photosynthetic enzyme activity (Tabaeizadeh, 1998). Working with chickpea cultivars under water deficit Mafakheri et al (2010) reported that mesophyll resistance is the basic determinate of rate of phototosynthesis under drought stress conditions. A reduction in Rubisco activity during water deficit has also been reported (Castrillo et al. 2001; Vu and Allen, 2009). Water deficit effects in tomato (Torrecillas et al. 1995; Castrillo and Calcagno, 1989) have been reported. Studies show that re-watering after a water deficit period, induces a recovery in assimilation rates. Assimilation rates recovered only partially, despite the

availability of internal CO_2, suggesting some non-stomatal limitation of photosynthesis (Souza et al, 2004). Under normal growing conditions field plants are subjected to environmental fluctuations, such as water deficit periods followed by rainy periods. Reports have shown the recovery of measured parameters following a water deficit period, particularly at the beginning of the rehydration period. However, recovery from water stress on rehydration has not been studied sufficiently, even though intermittent water deficit, on a daily and seasonal basis and for short and long periods of time, is commonly experienced by plants in natural environments. Furthermore, the study of recovery responses on rehydration as an indication of tolerance has not been fully considered. Therefore, the objective of the present work was to analyse the recovery responses to rehydration, in terms of magnitude and timing as a signal of tolerance, of two tomato cultivars, Rio Grande and Pera Quibor, after being subjected to moderate and severe water deficit. Responses are observed at two points: after day one of the water deficit process (both severe and moderate) and after day eight of the water deficit process (both severe and moderate). The parameters evaluated after rehydration at day 1 and day 8 of the water deficit process were soil water potential, leaf water potential, leaf osmotic potential, leaf relative water content, stomatal

Table 1. Correlations among leaf relative water content (LRWC), water potential (Ψw) and osmotic potential (Ψs) and stomatal conductance (gs), soluble protein content (Prot) and Rubisco activity after moderate (WDI) and severe (WDII) water deficit levels, and two rehydration levels (RI and RII) after eight days rehydration; RI 8d and RII 8d, for two tomato cultivars, Rio Grande (RG) and Pera Quibor (PQ),*Spearman coefficient significantly different (P=0.05)

			LRWC	Ψw	Ψs
gs	RG	RI 8d	0.2453	0.1599	0.6439*
		RII 8d	0.2657	0.0262	0.6645*
	PQ	RI 8d	0.1425	0.0517	0.4676*
		RII 8d	0.6755*	0.7834*	0.7463*
Prot	RG	RI 8d	0.6897*	0.0625	0.4134
		RII 8d	0.9733*	0.8757*	0.9563*
	PQ	RI 8d	0.2652	0.0348	0.0047
		RII 8d	0.4993*	0.6765*	0.7989*
Rubisco	RG	RI 8d	0.7289*	0.2721	0.5811*
		RII 8d	0.8923*	0.7929*	0.9633*
	PQ	RI 8d	0.3398	0.0787	0.4795*
		RII 8d	0.6554*	0.6988*	0.7589[*]

conductance, Rubisco activity, protein content, leaf fresh and dry biomass and leaf schlerophylly indices.

Materials and methods

Plant material

Tomato seeds of Río Grande cultivar and Pera Quibor cultivar (nationally produced cultivar in Venezuela) were germinated on wet paper in plastic trays. The seedlings were then transferred to 5L plastic pots (one seedling per pot) containing a mixture of clay and sand (3:2); watered daily; and maintained in a greenhouse at Simón Bolívar University in Caracas, Venezuela. Fifty mL of Hoagland solution plus 0.25mM $CaCl_2$ and commercial fertilizer (POKON, Bendien, Naarden; Netherlands) were added to each pot twice a week.

Treatments

Plants were watered daily for 28 days. After this period, watering was withheld in 80 plants (water deficit plants). When a significant number of plants of both cultivars reached a leaf water potential of between -1.60 ± 0.5 to -1.70 ± 0.5 (which occurred after 8 days without watering), they were re-watered. This moderately stressed group of plants was labeled Rehydration I (RI). Another group was kept under water deficit until its leaf water potential reached a value of between -2.40 ± 0.65 to -2.55 ± 0.70MPa (occurred after 11 days without watering), after which the plants were rehydrated. This severely stressed group of plants was named Rehydration II (RII). The rehydration period lasted 8 days, during which the plants were watered daily. Plant responses to rehydration were evaluated on day 1 (RI 1 and RII 1) and on day 8 (RI 8 and RII 8) after the start of re-watering. An additional group of plants was watered daily and maintained as control. Measurements were made on young, fully expanded leaves of 6 plants (i.e. n = 6) per group.

Water status measurements and stomatal conductance (gs).

To obtain predawn water potential measurements, leaves were sampled in the morning (05:30 - 06:30h) and their water potential was measured on leaf discs in C-52 chambers attached to a HR-33T Dew Point Microvoltmeter (Wescor, Logan, USA). Soil water potential was determined simultaneously using the same technique. After measuring water potential, the leaves were frozen in liquid nitrogen and their leaf osmotic potential was measured using the Hygrometric (dew point) method on sap obtained from samples after thawing. Leaf relative water content was determined according to Turner's method (1981). Stomatal conductance was measured between 07:00 and 08:00h using a porometer (LI-65 with sensor LI-25, Lambda, Lincoln, USA). Abaxial and adaxial conductances were measured and total stomatal conductance was calculated.

Growth and sclerophylly indices

Leaf area was measured by planimetry (Lasiko-Est 1929, Los Angeles Scientific Instrument Co., Inc., Los Angeles, California, USA). Fresh biomass (FM) was determined immediately after sampling, and dry biomass (DM) was obtained through oven drying at 80°C for 48h. Specific leaf area of individual leaves was calculated using the ratio leaf area to dry biomass, and leaf density (D) was obtained using the following formula: D = (DM/FM) x 1000 (Guerfel et al. 2009.
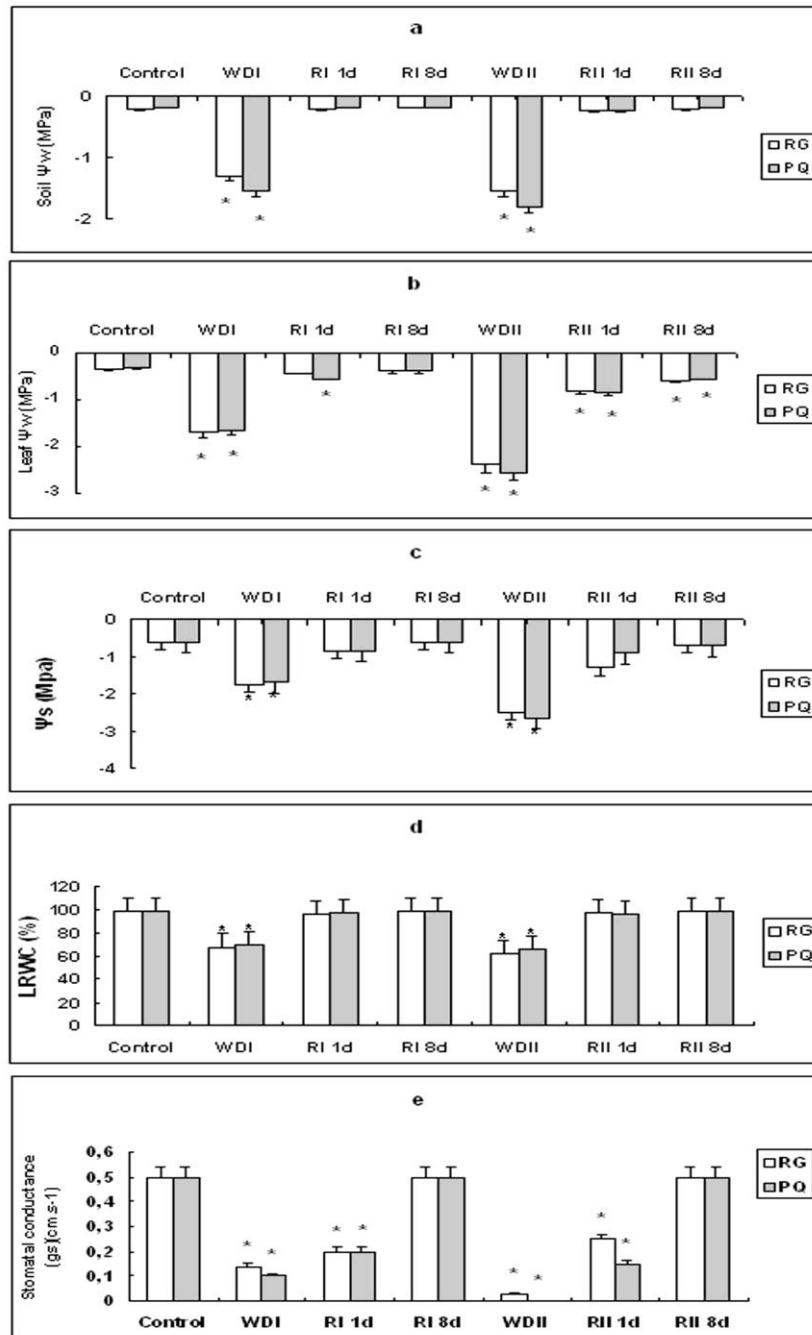
Fig. 1 Soil water potential (sΨw) (MPa) (a), leaf water potential (Ψw) (MPa) (b), and gs (cm s-1) (c); values after moderate (WDI) and severe (WDII) water deficit levels, and two rehydration levels (RI and RI1): RI 1d and RI1 1d, one day after rehydration; RI 8d and RI1 8d, eight days after rehydration and control values (C) for Rio Grande (RG) and Pera Quibor (PQ) cultivars; values are average ± SE (n=6)*Mean is significantly lower from control (P=0.05).

Leaf extraction, Rubisco activity and protein content estimation

Leaf extraction and total Rubisco activity assay was performed following the method reported by Castrillo et al. (2001). Protein content estimation was carried out following the Bradford (1976) method.

Statistical analysis

Pearson and Spearman rank test was used for correlations; Friedman and Mann-Whitney u-test was used for comparison of treatments (P = 0.05) (Sokal and Rohlf, 1995). Analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

Results

The values of soil water potential and leaf water potential, leaf osmotic potential and leaf relative water obtained during recovery from water deficit, in all tested plants, are shown in Figures 1, a, b, c and d respectively. In both moderate and severely stressed plants, soil water potential (sΨw), leaf water potential (Ψw), leaf osmotic potential (Ψs) and leaf relative water content(LRWC) obtained values were significantly lower than those obtained from the control group.During rehydration, soil water potential reached values similar to those of control

697

Table 2. Recovery responses types. PQ: Pera Quibor cultivar; RG Rio Grande cultivar;   WD: Water deficit ; FR: Full recovery; PR: Partial recovery; sΨw: soil water potential; Ψw: leaf water potencial; Ψs: leaf osmotic potential; LRWC: leaf relative water content; Prot: protein content; Rubisco:Ribulose 1,5-Bis-P- carboxylase activity; gs: stomatal conductance; FM: fresh mass; DM: dry mass; D: Leaf density; SLA: Specific leaf area

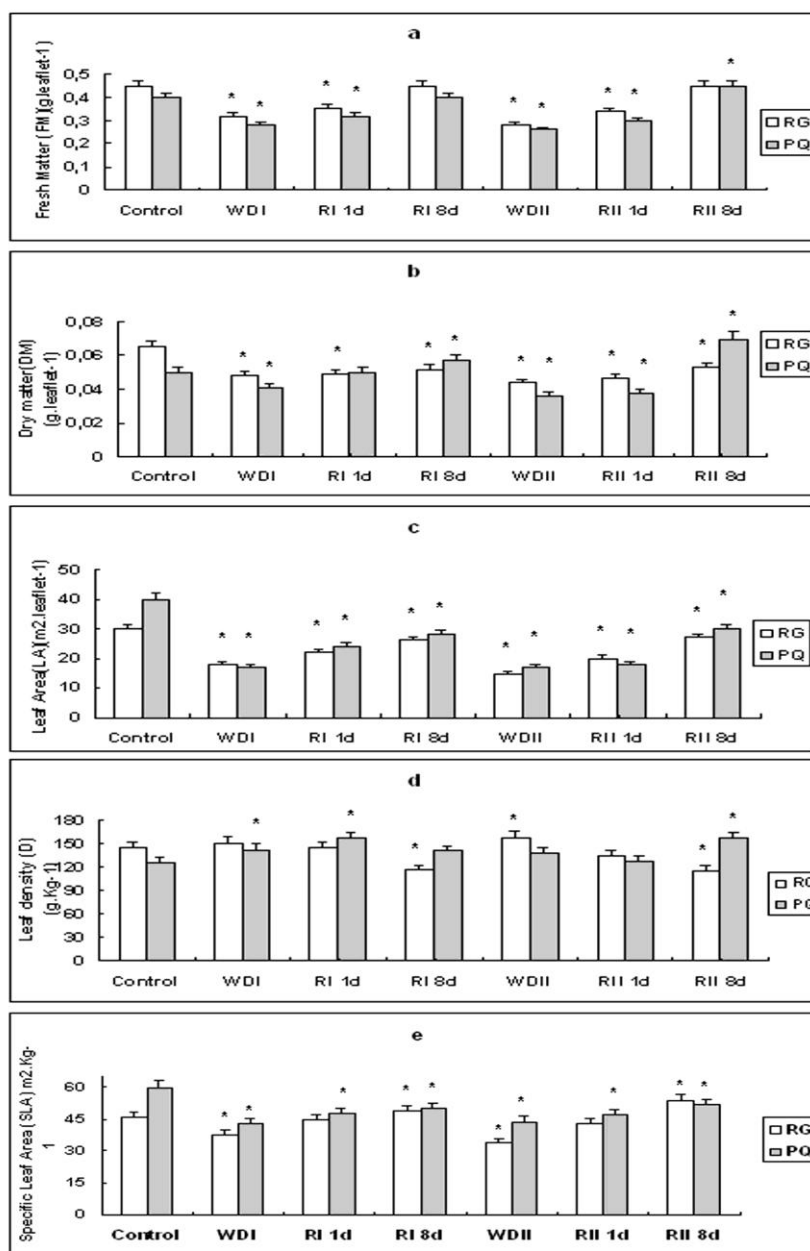| Types	Commons	No commons	
		PQ	RG
WD independent-time independent	sΨw; Ψs;LRWC (FR)	Prot(FR)	Prot(PR)
WD independent-time dependent	gs; FM (FR)	DM(FR) SLA&D(FR)	
	Ψw; LA (PR)		
WD dependent-time dependent	Rubisco act. (FR)	SLA&D(PR) DM(PR)	

Fig. 2 Fresh mass (FM) (a) and dry mass (DM) (b) (g leaflet-1), and leaf area (LA) (c) (m2. leaflet-1) values, leaf density (D) (d) (g kg-1) values calculated as (DM/FM) x 1000, and specific leaf area (SLA) (e) (m 2 kg-1) values determined as the ratio LA to DM of individual leaves   in two tomato cultivars, Rio Grande (RG) and Pera Quibor (PQ), after moderate (WDI) and severe (WDII) water deficit levels, and two rehydration levels (RI and RI1): RI 1d and RI1 1d, one day after rehydration; RI 8d and RI1 8d, eight days after rehydration and control values (C); values are average ± SE (n=6) *Mean is sig- nificantly lower from control (P=0.05).

at both rehydration levels (RI and RII) and measured times (1d and 8d).On re-watering, leaf water potential increased in both cultivars and reached a partial recovery during the 8 days rehydration period. The values of leaf osmotic potential and leaf relative water content showed full recovery at both rehydration levels. Moderate and severely stressed Río Grande and Pera Quibor plants showed a significantly lower stomatal conductance (gs) at the early stage (Fig.1e) of re-irrigation, however, after 8 days of rehydration they showed full recovery at both levels. Fresh biomass values were significantly lower than control in all stressed plants. Full recovery was observed after 8 days of rehydration at moderate and severely stressed plants (RI and RII) of both cultivars (Fig. 2a). Dry biomass (Fig. 2c) values were significantly lower than control values at both levels of water deficit; recovery was observed in Pera Quibor after 8 days of rehydration at both levels of water deficit. Leaf area values were significantly lower than control at both levels of water deficit and increased on rehydration of both cultivars; partial recovery was obtained after 1 day and 8 days of the rehydration period (Fig.2c). Leaf density values increased during moderate and severe water deficit, showing recovery at both levels in Río Grande on days 1 and 8 after rehydration, and in Pera Quibor only on plants subjected to severe stress and after 1 day (Fig. 2d). Specific leaf area values decreased significantly during moderate and severe water deficit in both cultivars; recovery was observed only in Río Grande on 1 day and 8 days of rehydration for both levels of stress (Fig. 2e). Protein content (Fig.3a) decreased significantly in comparison with control values in moderate and severely stressed plants. Full recovery was observed at moderately stressed plants after 1 day in Pera Quibor, and after 8 days at moderately and severely stressed plants in both cultivars. Rubisco activity (Fig. 3b) decreased significantly with respect to control at both levels of water deficit. It showed full recovery in Pera Quibor cultivars under moderate stress after 8 days, and in Río Grande in cultivars under severe stress after 8 days. Table 1 shows correlations obtained at moderate stress (RI) and severe stress (RII) after 8 days of rehydration of both cultivars. Significant correlations in both cultivars were obtained for protein content and Rubisco activity with leaf relative water content, leaf water potential and leaf osmotic potential at severe water deficit, and for stomatal conductance with leaf osmotic potential at moderate and severe water deficit. Similarly in Pera Quibor at severe stress, stomatal conductance correlated with leaf relative water content and leaf water potential, and in Río Grande at moderate stress there was correlation of Rubisco with leaf relative water content and soil water potential. Table 2 shows recovery of the responses common to both cultivars, and the ones that are different between them. The observed responses include: water deficit independent/time independent responses; water deficit independent/time dependent responses; and water deficit dependent/time dependent responses.

Discussion

During the experimentation period, different recovery responses were observed (Table 2); the observed responses common to, and different in both cultivars include: water deficit independent/time independent responses (recovery is not influenced by water deficit intensity, moderate or severe, and was observed at day 1 and day 8 after rehydration); water deficit independent/time dependent responses (recovery is not affected by water deficit intensity, moderate or severe, and was observed only at day 8 after rehydration; water deficit

dependent/time dependent responses (recovery is more affected by severe water deficit and was observed only at day 8 after rehydration). The observed responses that are common to both cultivars are: a) Water deficit independent/time independent responses: full recovery of soil water potential, leaf osmotic potential and leaf relative water content in both cultivars and protein content in Pera Quibor; partial recovery of protein content in Río Grande; b) Water deficit independent/time dependent responses: full recovery of stomatal conductance and fresh biomass, and partial recovery of leaf water potential and leaf area and c) Water deficit dependent/time dependent responses: full recovery of Rubisco activity. The different responses between both cultivars were: a) Water deficit independent/time dependent responses: full recovery of dry biomass in Pera Quibor; specific leaf area and leaf density in Río Grande and b)Water deficit dependent/time dependent responses: partial recovery of specific leaf area and leaf density in Pera Quibor. The full recovery responses in protein content and dry biomass in Pera Quibor, not seen in Río Grande, are the result of structural changes occurred during the water deficit period. These responses are based on delayed sclerophylly recovery through leaf structural changes, which increase leaf thickness, and are maintained after re-watering. The changes in leaf structural parameters are due to reductions in cell expansion and intercellular spacing, producing a compact leaf, with less leaf area and greater leaf thickness. In a C3 crop plant like tomato, an increase in leaf thickness leads to a thicker palisade parenchyma, which could contain larger numbers of CO_2-fixation sites, while a thicker spongy parenchyma could result in easier CO_2 diffusion to these sites. The leaf structure changed to a more compact and thicker one, with a smaller area. This change could be considered as a tolerance structural leaf transition, which occurs during water deficit periods and is maintained at rehydration in order to improve tolerance and increase productivity during recovery events. The mesophyll conductance (g_{mes}), that is, the conductance for CO_2 diffusion from the surface of mesophyll cell walls to the chloroplast stroma via plasma membranes, cytoplasm and chloroplast envelopes under water deficit, could change drastically. The partial recovery observed in leaf water potential and leaf area (water deficit independent/time dependent response) could be due to the fact that leaf water potential depends on its components: leaf osmotic potential and turgor potential, and on tissue rigidity. Leaf area is dependent on leaf cell expansion, which is also affected by turgor potential. With regards to cell expansion it has been reported that in pea plants under water deficit, it could be the cause of changes in the area of leaves experiencing a delayed development after stress (Lecour et al, 1995). It was also reported that leaf expansion and development were nearly halted during water deficit in advanced-stage leaves, being development solely due to cell expansion; this expansion is resumed after re-watering, but not sufficiently for cell size to equal that of controls at maturity (Alves and Setter, 2004). In C3 plants leaf photosynthetic rate depends on photosynthetic components contents, such as Rubisco, cytochrome f, H+-ATPase and reaction centres, among others, but also on structural parameters, such as leaf thickness and area per leaf mass.The sclerophylly indices such as specific leaf area and leaf density have been reported extensively in water deficit studies and in drought tolerant plant varieties or species. There have been many reports on reduced specific leaf area under drought conditions (Guerfel et al. 2009; Bacelar et al. 2006 ; Nautiyal et al, 2002). Moreover, Ennajeh et al (2010) reported that under water deficit conditions, a drought-resistant olive cultivar maintained higher rates of photo-
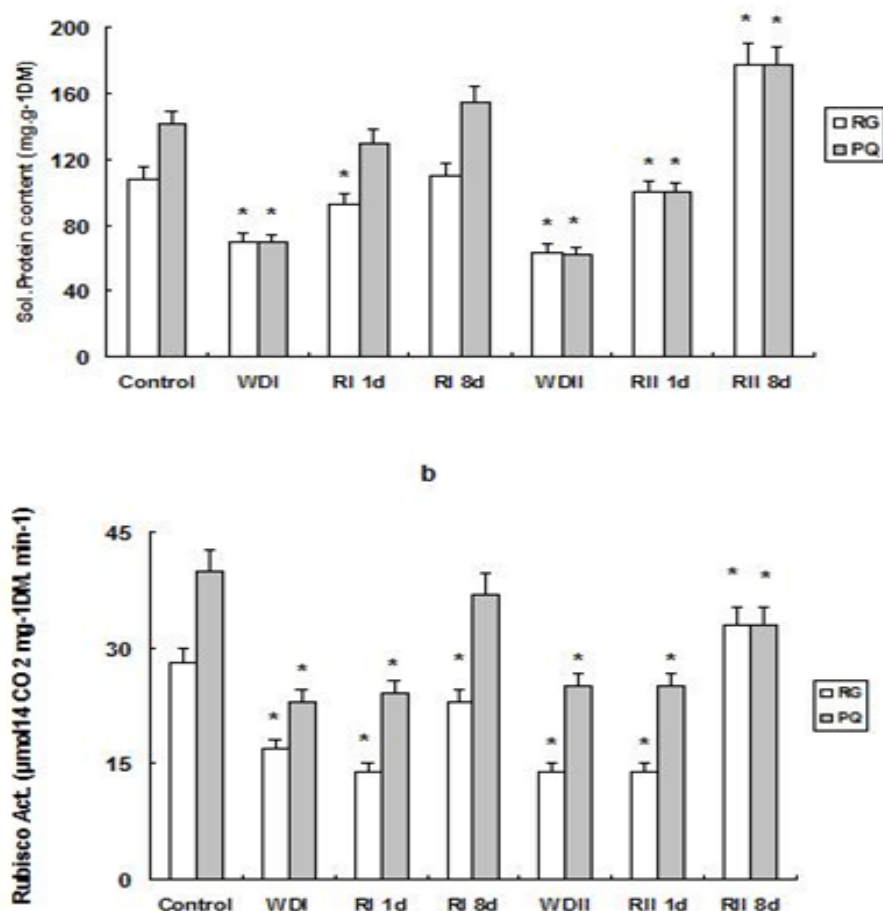
Fig 3 . Soluble protein content (a) (mg g^{-1}DM) and Rubisco (carboxylase activity) (b) (μmol $^{14}CO_2$mg^{-1}DM) values in two tomato cultivars, Rio Grande (RG) and Pera Quibor (PQ), after moderate (WDI) and severe (WDII) water deficit levels, and two rehydration levels (RI and RI1): RI 1d and RI1 1d, one day after rehydration; RI 8d and RI1 8d, eight days after rehydration and control values (C); values are average $\pm$ SE (n=6). *Mean is significantly lower from control (P=0.05)

synthetic assimilation and lower rates of transpiration due to leaf morpho-anatomical adaptations to drought stress. Leaves with high leaf density improve their survival rate during severe drought because of a higher resistance to physical damage by desiccation (Mediavilla et al, 2001). The significant correlations (Table 2) of leaf water potential, leaf relative water content, and leaf osmotic potential with protein content and Rubisco at day 8 of rehydration, after severe stress, revealed a close relationship of these parameters on recovery. Results of response to rehydration after short periods of time have been previously reported. The present study analyses recovery responses to rehydration after moderate and severe water deficit in periods of time of 1 day and 8 days, in attention to the scale and time of recovery of the observed parameters. To conclude, in the present study, the increase in sclerophylly indices resulted in thicker leaves through leaf compaction, and the partial recovery of leaf water potential is indicative of an increased water potential gradient between soil and plant, which could be induced by an increase in cell rigidity in order to improve recovery. These findings show correlations between physiological and metabolic parameters, which in addition to the observed sclerophylly indices, reveal integrated tolerance mechanisms

intended to protect plant cultivars from intermittent changes in water conditions.

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