

Moderate water stress affects tomato leaf water relations in dependence on the nitrogen supply

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Abstract

The responses of water relations, stomatal conductance (g_s) and growth parameters of tomato (*Lycopersicon esculentum* Mill. cv. Royesta) plants to nitrogen fertilisation and drought were studied. The plants were subjected to a long-term, moderate and progressive water stress by adding 80 % of the water evapotranspirated by the plant the preceding day. Well-watered plants received 100 % of the water evapotranspirated. Two weeks before starting the drought period, the plants were fertilised with Hoagland's solution with 14, 60 and 110 mM NO_3^- (N14, N60 and N110, respectively). Plants of the N110 treatment had the highest leaf area. However, g_s was higher for N60 plants and lower for N110 plants. At the end of the drought period, N60 plants showed the lowest values of water potential (Ψ_w) and osmotic potential (Ψ_s), and the highest values of pressure potential (Ψ_p). N60 plants showed the highest Ψ_s at maximum Ψ_p and the highest bulk modulus of elasticity.

Additional key words: *Lycopersicon esculentum*, osmotic adjustment, stomatal conductance, water potential.

Introduction

In Mediterranean environments, yield and quality traits of vegetable crops are adversely affected by drought and/or high salinity of the root zone (Goyal *et al.* 2003). Drought induces a reduction in plant tissue water contents and subsequently water potential, leaf elongation, leaf photosynthesis, and changes in protein synthesis, nitrogen metabolism and cell membrane properties, leading to a reduction in plant productivity (Bogoslavsky and Neumann 1988, Shangguan *et al.* 2000, Saneoka *et al.* 2004, *etc.*), the extent of which depend upon interactions with several environmental factors (White *et al.* 1996).

Plants have developed various mechanisms to withstand drought, such as developing higher root-shoot ratios, fewer and smaller leaves or increasing the concentration of compatible solutes in leaf cells (Snowdown 2000). The accumulation of solutes to decrease water potential may allow plants to maintain a water potential gradient as the soil becomes drier and thus maintain the positive pressure potential required to keep stomata open and sustain gas exchange and growth (White *et al.* 2000). An important environmental factor affecting drought tolerance in plants is nitrogen supply. In

beans (Shimshi 1970), coffee (Tesha and Kumar 1978) and winter wheat (Shangguan 1997, 2000), it has been observed that stomatal conductance increased with nitrogen supply under well-watered conditions and become more sensitive to a decrease in leaf water potential. However, other work on tea (Nagarajah 1981), cotton (Radin and Ackerson 1989) and creeping bentgrass (Saneoka 2004) indicated an opposite response, the stomatal sensitivity to leaf water potential being decreased by high nitrogen supply. Further, it has even been observed in *Lotus corniculatus*, that, depending on the nitrogen source, the accumulation of compatible osmolytes can be different (Diaz *et al.* 2005).

The particular way in which water stress is imposed might be of special importance in understanding the response to drought and also in evaluating the plant capacity to acclimate to stress. For short-term experiments, withholding water is the most common method, but, to simulate more realistic responses to drought, long-term water stress or cyclic water stress is needed (Pennypacker *et al.* 1990). In addition, the development of water stress could be different in plants

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Abbreviations: g_s - stomatal conductance; Ψ_p , Ψ_s and Ψ_w - leaf pressure, osmotic and water potential, respectively.

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of different size, induced, *e.g.*, by different nitrogen nutrition (Yambao *et al.* 1984, Radin *et al.* 1985). Therefore, it is necessary to carry out experiments where the plants grow in a moderate and progressive water stress; in addition, the different treatments assayed should have a comparable water-stress regime, to eliminate the uncertain effects of the treatments on the growth of the plants.

Although the effect of water stress or N supply on tomato plants has been the subject of many studies

(García *et al.* 1996, 2000), it is not known whether nitrogen fertilisation could alter the tolerance of tomato to moderate drought. The objective of this research was to assess the effects of nitrogen fertilisation, with three different doses, on the water relations of tomato plants exposed to drought with the same water deficit regardless the original size of the plants, in order to clearly separate, in accordance with Morgan (1986), the intrinsic effects of N-nutrition from those related to plant growth phenomena, and to assess comparable water-stress regimes.

Materials and methods

Plants and growth conditions: Seeds of tomato (*Lycopersicon esculentum* Mill., cv. Royesta) were germinated in the dark at a constant temperature of 25 °C, in trays containing a Perlite-peat (6:1) mixture wetted with 0.5 mM CaSO₄. When the radicles were 3 - 4 cm in length, the seedlings were transferred to a controlled-environment chamber with a 14-h photoperiod, a 30/20 ± 2°C day/night temperature and a relative humidity of 55 ± 5 %. Fluorescent tubes (Philips, 40 W) and incandescent lamps (200 W) provided a photosynthetically-active radiation of 380 µmol m⁻² s⁻¹ to the upper part of the plants (Licor-LI 1905 quantum sensor, Lincoln, USA). When the plants were two-week-old, the roots were washed with tap water and similar-sized seedlings (12 cm high) were transplanted to 1-dm³ containers containing the *Xerc terriorthent* substrate. Previous to transplanting, deionised water was added to the soil until field capacity was reached.

During one week after transplanting plants were irrigated daily with deionised water, maintaining the soil water status at full field capacity to acclimate the plants to their new root medium. After this, plants were submitted daily to a water regime which consisted of adding an amount of water equal to that evapotranspired the previous day. The difference between the mass of the pot at full field capacity and the mass of the pot 24 h later was used for calculation of amount of water transpired by the plants in every treatment. The drip-irrigation system used employed emitters of 50 cm³ h⁻¹.

Every three days, plants were fertilised, either with Hoagland nutrient solution with 14 mM NO₃⁻ (N14), 60 mM NO₃⁻ (N60) or with 110 mM NO₃⁻ (N110). Extra nitrate fertilisation (N60 and N110) was added as a mixture of K, Ca and Mg nitrates in the same proportion as in the Hoagland solution. The water-stress treatment was started two weeks after transplanting and stressed plants received only 80 % of the water evapotranspired the previous day, while well-watered plants were irrigated daily as above. The water-stress treatment was applied over two weeks. The factorial design (3 × 2) was a completely-randomised design with 6 replicate plants per treatment. The experiment was performed two times.

Measurements: The moisture content of the soil was

measured with a soil moisture meter (Dept. of Research Assistance, University of Murcia, Spain), which consists of two circles of copper wire, 10 cm in diameter and 2 cm apart. This technique consists of the determination of the electric capacity, that changes with the dielectric constant of the medium, which depends on the soil moisture. The probe was placed in the middle third of the containers.

Leaf conductance (g_s), water potential (Ψ_w), osmotic potential (Ψ_s) and pressure potential (Ψ_p) were determined in the youngest fully-expanded leaf every 72 h during the water-stress period. Water relations were measured pre-dawn (07:00). The Ψ_w was measured by a pressure chamber as described by Turner (1988). After measurement of Ψ_w, the same leaves were frozen in liquid nitrogen at -80 °C. The Ψ_s of the cell sap was measured using a Wescor (Logan, USA) 5500 vapor pressure osmometer. Pressure potential (Ψ_p) was calculated by subtracting the Ψ_w from Ψ_s. Leaf conductance was determined at midday in 6 plants per treatment, using a porometer (Li-Cor LI-1600, Lincoln, USA). In addition, on the last day of the experiment g_s, Ψ_w, Ψ_s and Ψ_p were determined every 2 h from the beginning to the end of the light period. In this case the Ψ_w was measured taking single leaves that had been covered with a small, black polyethylene bag, covered by silver foil, for at least 2 h prior to measurements.

Pressure-volume curves were fitted by measuring relative water content at different values of water potential for 12 leaves per treatment. For this, the leaves were water saturated for 24 h in darkness. Dehydration was by transpiration, under ambient conditions, with sequential determination (4-h intervals) of water potential and fresh mass. PV-curves (1/Ψ_w versus relative water content, RWC) were constructed from all the experimental variants. Leaf dry mass (DM) was obtained after drying in an oven for 3 d at 90.8 °C. Osmotic potential at full Ψ_p (Ψ_{sfp}) and the apoplastic water content (RWCapo) were calculated by extrapolating the linear portion of the curve to the y-axis and to the x-axis, where Ψ_{sfp} is the water potential at RWC = 0 and RWCapo is the RWC at 1/Ψ = 0. The bulk modulus of elasticity (ε) at 100 % RWC was estimated by assuming an almost linear relationship between Ψ and RWC, using the methods of Wilson *et al.* (1979). These data were obtained at the end

of the drought period in all the N treatments, in well-watered and water-stressed plants.

At the beginning and end of the experiment, plants were harvested and the total leaf area of each plant (leaf area meter; *Delta-T Devices*, Cambridge, UK) whole-plant dry mass (48 h in an oven at 64 °C). Leaf area ratio (LAR) was calculated as leaf area per unit of whole-plant dry mass. Water saturated mass (SM) and dry leaf mass (DM), to calculate the SM/DM ratio, were measured. For this, leaves were hydrated by immersing in distilled water

Results

The transpiration rate and soil moisture before water stress showed a great homogeneity. After 15-d drought, a 23 % decrease in soil water content was observed compared to the control, there were no significant differences among the three N treatments (data not shown).

Values of Ψ_w in the N110 treatment decreased 3rd day after starting the drought period. However, in N60 and N14 plants, significant differences between the two water treatments were observed at 9 and 12 d, respectively

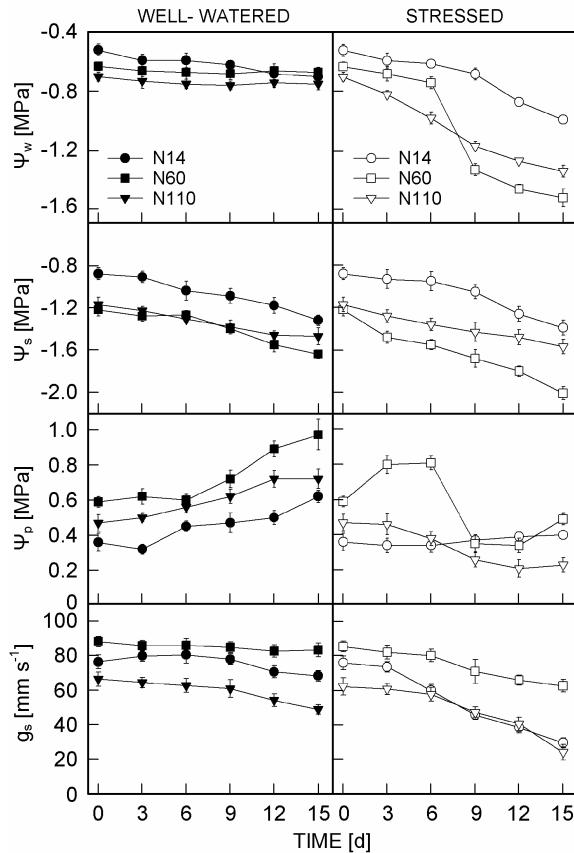


Fig. 1. Effect of N fertilisation (14, 60 and 110 mM NO_3^- ; N14, N60 and N110) on water potential (Ψ_w), osmotic potential (Ψ_s), pressure potential (Ψ_p) and stomatal conductance (g_s) during the stress period, in well-watered and stressed tomato plants. Each value is the mean \pm SE ($n = 6$).

for 24 h, in darkness at high relative humidity (95 %) and low temperature (6 - 8 °C). Hydrated leaves were weighed (SM), dried for 48 h at 64 °C and weighed again (DM).

Statistical analysis: Data were analysed by ANOVA with fertiliser treatment and water-availability treatment as main factors. Mean differences were compared with Duncan's test ($P < 0.05$).

(Fig. 1). The lowest Ψ_w was reached at the end of the experiment in the N60 treatment.

Values of pre-dawn Ψ_s tended to decrease with time in both stressed and well-watered plants, for all three nitrogen doses used. Drought stress reduced Ψ_s in the plants only in the N60 treatment. In the rest of the treatments, significant differences between well-watered and stressed plants were not observed.

Values of pre-dawn pressure potential increased with time in the well-watered plants and they depended on

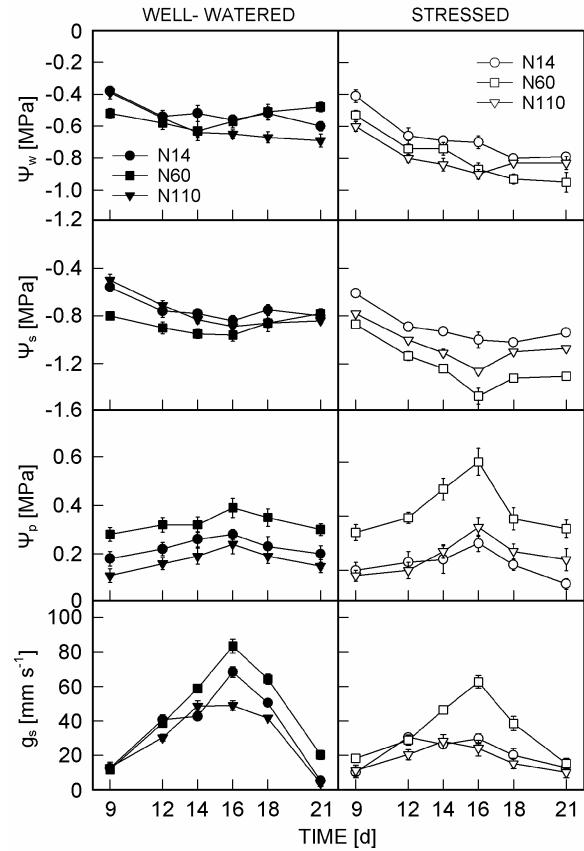


Fig. 2. Effect of N fertilisation (14, 60 and 110 mM NO_3^- ; N14, N60 and N110) on water potential (Ψ_w), osmotic potential (Ψ_s), pressure potential (Ψ_p) and stomatal conductance (g_s) during the last day of the stress period, in well-watered and stressed tomato plants. Each value is the mean \pm SE ($n = 6$).

nitrogen treatment, the highest values being in the N60 treatment. Drought stress increased Ψ_p in N60 plants after 3 and 6 d of the drought period, compared with well-watered plants. However, from day 9, stressed plants had lower Ψ_p than well-watered plants. At the end of the experiment, stressed plants in all three nitrogen treatments had a lower Ψ_p than well-watered plants, N60 and N110 plants reaching the highest and lowest values, respectively.

At the beginning of the water stress (day 0), the plants showed different g_s in different N treatments: the highest values were observed in the N60 treatment and the lowest in the N110 treatment (Fig. 1). For the well-watered plants, g_s values were fairly constant during the experiment, for the three N doses, and the differences among them were maintained throughout the experiment. The g_s of the stressed plants decreased as the experiment progressed, the reductions in N14 and N110 plants being similar and greater than in N60 plants.

The diurnal variations of Ψ_w and Ψ_s at the end of the drought period showed their highest value at the beginning of the light period (09:00), for all the treatments, and after 09:00 these values decreased progressively, reaching at 16:00 their lowest values which were maintained during the rest of the day (Fig. 2). Ψ_p tended to increase in the middle of the day (16:00), especially in stressed plants. In general, as indicated above, the lowest values of Ψ_s and the highest values of Ψ_p were observed for N60 plants, in both well-watered and water-stressed

plants. The diurnal time course of g_s during the last day (day 15) indicates that the maximum values were reached in the middle of the light period, both in stressed and unstressed plants, in all three N treatments, the lowest values occurring at the beginning and end of the light period (Fig. 2).

The imposed drought significantly decreased LAR in N14 and N110, compared with well-watered plants, but did not affect this parameter in N60 plants (Table 1). The SM/DM ratio (indicative of leaf structure) was increased significantly by water stress in N14 and N110, but it was not affected in N60 compared with the well-watered plants, indicating that this mild, long-term stress condition had no effect on leaf structure. Leaf area was affected significantly by N treatment: treatments N110 and N14 showed the highest and lowest values, respectively.

Osmotic potential at full Ψ_p (Ψ_{sfp}), apoplastic water content (RWC_{apo}) and modulus of elasticity (ε) were obtained by fitting pressure-volume curves at the end of the experiment. In general, Ψ_{sfp} was decreased by both the drought and the increasing of nitrogen dose (Table 1). In the well-watered plants, RWC_{apo} was higher for N110 than for both N14 and N60. The drought period increased this parameter in N14 and N60 plants; however, in N110 plants, RWC_{apo} was decreased by the water stress. The values of ε increased with the drought period and with increasing N dose.

Table 1. Effect of nitrogen dose on the leaf area, leaf area ratio (LAR), leaf water saturated mass/dry mass ratio (SM/DM), osmotic potential at full Ψ_p (Ψ_{sfp}), relative apoplastic water content (RWC_{apo}) and bulk modulus of elasticity (ε) for well-watered and stressed plants at the end of the experiment (15 d after the start of the treatment). Means with different letters in the same column are significantly different at $P < 0.05$ according to the Duncan test ($n = 6$; ns - non-significant, *, ** and *** - significant differences at $P < 0.05, 0.01$ or 0.001 , respectively).

Treatment		Leaf area [$\text{cm}^2 \text{ plant}^{-1}$]	LAR [$\text{cm}^2 \text{ g}^{-1}(\text{d.m})$]	Leaf SM/DM	Ψ_{sfp} [MPa]	RWC _{apo} [%]	ε [MPa]
N14	well-watered	856 c	66.7 bc	8.15 b	-0.56 ^z	16.1 ^z	0.21 ^z
	stressed	855 c	64.3 d	8.90 a	-0.59	19.3	0.26
N60	well-watered	1289 b	70.9 a	8.21 b	-0.69	16.5	0.30
	stressed	1325 b	70.7 a	8.24 b	-0.88	21.6	0.68
N110	well-watered	1609 a	68.5 a	8.02 b	-0.71	20.7	0.48
	stressed	1626 a	65.1 b	8.93 a	-0.86	11.6	0.69
Nitrogen	***		***	ns			
Water stress	ns		**	**			
N × water stress	ns		*	*			

Discussion

The essential factor in plant water relations in drought periods is the maintenance of a sufficiently-high water content and Ψ_p to permit growth (Arndt *et al.* 2001). In our experiment, N60 plants showed a better acclimation to moderate water stress than N14 or N110 plants since stressed N60 plants had the highest Ψ_p . These higher values of Ψ_p in stressed N60 plants could be due to a

process of osmotic and elastic adjustment since soil drought caused Ψ_{spt} to decline markedly, with strong increases in ε (Peltier and Marigo 1999, Damatta *et al.* 2003). A high ε in plants under water stress (a more rigid cell wall) means that, within certain limits (a high wall elasticity rapidly plasmolyses leaf cells), an excessive loss of water during prolonged periods of water deficit is

prevented. When water loss increases, a very pronounced gradient develops in the soil-plant-atmosphere continuum, which re-establishes the water balance in the plants (Pita and Pardos 2001). If this mechanism is effective, as it is in the case of stressed N60 plants since they showed, at the end of the experiment, the lowest Ψ_w and the highest Ψ_p , osmotic adjustment promotes water uptake from drying soil to minimise Ψ_p loss as water deficit progresses. Elastic adjustment has been previously observed in some drought tolerant Mediterranean trees species whereas ϵ increased greatly in drought season compared to the rainfall season (Serrano and Peñuelas 2005).

The Ψ_p maintenance not only depends on the extent of osmotic and elastic adjustment but also on the interaction between these adjustments and apoplastic water fraction (Maury *et al.* 2000). In our study, stressed N110 plants also had a higher Ψ_{spt} and ϵ than well-watered plants, so these plants also employed similar physiological mechanisms to maintain Ψ_p although they did not reach the values observed in stressed N60 plants. This could be due to the fact that, in stressed N110 plants, RWC_{apo} was decreased compared with well-watered plants, while in stressed N60 plants this parameter was increased. Thus, changes in the partitioning between the symplasmic and apoplastic water fractions could represent a mechanism developed by N60 plants to maintain Ψ_p during water stress. The apoplastic water content is considered a sort of reservoir that plants turn to in cases of excessive dehydration. In addition, a high content of apoplastic water is a feature shared by all plants that have adapted to dry climates (Cutler *et al.* 1977). In contrast to the stressed N60 and N110 plants, the stressed N14 plants did not show osmotic adjustment, since Ψ_{spt} and ϵ were similar for stressed and well-watered plants and so these plants did not develop the lower Ψ_w necessary to adapt to the drought conditions. It is known that water stress can alter the nitrogen metabolism in plants by decreasing nitrate reductase activity, starch and soluble protein (Garg *et al.* 2001). Therefore, increasing N supply may contribute to drought tolerance as it has been previously observed by Saneoka *et al.* (2004). These authors showed that N supply in plants could ameliorate the negative

effect of drought by preventing cell membrane damage and enhancing osmoregulation. However, Ashraf *et al.* (2001) observed in pearl millet that supra-optimal concentration of N decreased the drought tolerance in plants under water deficits as occurred also in our experiment for N110 plants under drought stress.

The reduction of g_s during the drought period could be mainly attributed to variation in soil moisture. A reduction of g_s by a decrease in soil water availability has been reported previously in tomato plants (Carvajal *et al.* 1998). In addition, in our experiment, we observed that the reduction of g_s by water stress also depended on the N treatment, N60 plants being those which showed the least reduction in this parameter. This may have been because N60 plants were able to adjust osmotically and maintain a high Ψ_p . In water-stressed leaves of barley, a close correlation has been observed between osmotic adjustment and maintenance of g_s (González *et al.* 1999).

Moderate drought, imposed progressively, was not able to decrease leaf area in any of the three N treatments in a short time period (15 d). However, the indices related with the morphological component of the growth, LAR (leaf area per total plant dry mass) and SM/DM (an index of leaf structure according to Rascio *et al.* 1998), responded differently to the water stress, depending on the N treatment. In our experiment, in N14 and N110 plants, the LAR and the SM/DM decreased and increased, respectively. These results are consistent with drought studies which show that leaf expansion, and associated morphological and allocation variables, can be sensitive to water stress; even this parameter can be more sensitive than g_s and net CO₂ assimilation rate (Kalapos *et al.* 1996, Kramer and Boyer 1996, Sadras and Milroy 1996).

In conclusion, water stress tolerance in tomato plants was altered by nitrogen fertilisation. Plants fertilised with 60 mM NO₃⁻ were more drought-tolerant than plants fertilised with 14 or 110 mM NO₃⁻. The mechanism involved in a more-efficient adaptation in N60 plants could have been an osmotic and elastic adjustment, manifested as higher Ψ_{spt} , ϵ and RWC_{apo}. In this way, these plants were able to maintain a higher Ψ_p , preventing the large reduction in g_s observed in the other nitrogen treatments.

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