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ORIGINAL ARTICLE

Eco-physiological response to water stress of drought-tolerant and drought-sensitive tomato genotypes

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Abstract
Water stress is an increasing environmental constraint affecting tomato growth and yield in Mediterranean areas. *Solanum pennellii* is a wild tomato species that exhibits a higher water use efficiency compared with cultivated *S. lycopersicum*. In particular, a cultivated line carrying a small *S. pennellii* region on chromosome 9 (IL 9-2-5) was identified as more tolerant to water deficit. In this work, the tolerant (IL 9-2-5) and the susceptible (M82) genotypes were subjected to three different water regimes: irrigation with 100% (V1), 50% (V2) and 25% (V3) field capacity. To evaluate the physiological response of IL 9-2-5 and M82 to water deficit, leaf functional traits, plant biomass production and maximal PSII photochemical efficiency were measured together with photosynthetic pigments and phenolic compounds. The higher tolerance to water deficiency of IL 9-2-5 was associated with the development of a better antioxidant system, especially in treatment V3. In addition, IL 9-2-5 had higher values of sclerophylly and leaf dry matter content thus confirming that the tolerance of IL 9-2-5 can be attributed to traits related to leaf morphology and physiology. In future, identification of polymorphisms in key-genes controlling these traits can guide breeding efforts aimed at improving susceptible genotypes.

Keywords: *Functional leaf traits, phenolics, photochemical efficiency, tomato introgression lines, water stress*

Introduction

Drought leading to water stress is an environmental constraint in hot and dry climate (e.g. Mediterranean environments) affecting crop growth and yield, and reducing agricultural productivity. In a near future, losses of crop yields due to this abiotic stress may be amplified due to the threats of climate changes emerging from global warming as well as due to the growing scarcity of fresh water available for irrigation caused by urbanization and depletion of aquifers. The ultimate goal is to develop crop plants with improved water use efficiency in order to minimize drought-induced losses of yield and permit the use of cultivable land with limited water supplies (Vitale et al. 2011; Mishra et al. 2012).

Plants develop a range of mechanisms for dealing with low water availability that include: (1) stress escape by completing their life cycle before severe water deficit occurs; (2) stress avoidance by enhancing their capacity to absorb water and conserve it thanks to a large root system, a reduced leaf area and limited transpiration; (3) stress tolerance by improving osmotic adjustment ability and increasing cell wall elasticity; (4) stress resistance by altering metabolic pathways so that the plant can survive under severe stress conditions (e.g. increased antioxidant metabolism) (Xu et al. 2010; Clayes & Inzè 2013; Lawlor 2013). Exposure to water deficit often increases the production of reactive oxygen species and, as a consequence, promotes the concentration of antioxidant compounds as well as the activity of some antioxidant enzymes (ascorbate peroxidase, catalase and superoxide dismutase) (Garg & Manchanda 2009; Sanchez-Rodriguez et al. 2011; Barbagallo et al. 2012). In particular, phenolic compounds can act to detoxify free radicals (Sanchez-Rodriguez et al. 2011). In addition, carotenoids can reduce and eliminate the reactive oxygen damage, serve as precursors of ABA synthesis and

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also participate in photosynthesis as the chlorophylls (Gong et al. 2010).

Water deficit has imposed selective pressure in the evolution of plant morphology and physiology. Selection in water-limited environments can result in populations and/or species with traits that improve their relative fitness in response to drought. Such traits can improve tissue tolerance of desiccation allowing leaves to function longer under drought conditions or improve avoidance of water loss allowing leaves to maintain high water potential (Easlon & Richards 2009).

Tomato (*Solanum lycopersicum*), one of the most important vegetable crops worldwide, is also one of the crops most demanding in water. As with many crop plants, cultivated tomato carries only a very small fraction of the genetic variation that is available in related wild species and landraces (Tanksley & McCouch 1997). It has therefore become a goal of modern breeding to screen wild genetic resources for advantageous traits that could be introduced into modern varieties to enrich the genetic basis of cultivated plants with novel alleles that improve agricultural yield under optimal as well as less optimal field conditions. Many wild relatives of cultivated tomato *S. lycopersicum* exhibit different degrees of tolerances to abiotic and biotic stresses. *Solanum pennellii*, one of the crossable wild relatives of cultivated tomato that originated in the deserts of Peru, displays drought tolerance compared with cultivated *S. lycopersicum*. In particular, it was demonstrated that the desert-adapted *S. pennellii* has higher water use efficiency both in water-stressed and non-stressed conditions and reduced leaf stomatal conductance in response to drought (Easlon & Richards 2009). Eshed and Zamir (1995) generated a collection of introgression lines (ILs) in which defined genomic segments of the wild species *S. pennellii* replaced homologous region in the background of the cultivated variety *S. lycopersicum* M82. Overall, the population of ILs provides complete coverage of the wild-species genome and allows the reservoir of wild genes to be investigated. In particular, a set of such *S. pennellii* ILs has been extensively phenotyped for dissecting traits such as plant yield and fruit quality (Lippman et al. 2007; Alseekh et al. 2013). Previously, a shoot-specific QTL (PW 9-2-5) was identified in the line IL 9-2-5 carrying a 9 cM introgression from the wild species *S. pennellii*, which accounts for an altered growth habit resulting in increases in plant weight, yield and Brix units. Afterward, the tomato IL (IL 9-2-5) was identified as more tolerant to water deficit in terms of yield losses (Vasco et al. 2011). In this work, the tolerant genotype IL 9-2-5 and the drought-sensitive genotype M82 were subjected to three levels of irrigation and their eco-physiological responses were evaluated by measuring leaf functional traits together with photosynthetic pigments and phenolic compounds in order to gain a deeper understanding of the mechanisms that regulate the response to water stress in the selected IL.

Material and methods

Plant material and growth conditions

Plants were grown during the 2012 season at the Department of Agricultural Sciences, Portici, Naples, Italy. Seeds of IL 9-2-5 and M82 were kindly provided by the Tomato Genetics Resource Center (TGRC), University of California, Davis, USA (http://tgrc.ucdavis.edu/). The seeds were germinated in Petri dishes on water-soaked filter paper and subsequently transferred in peat-filled planting tray and incubated in a growth chamber at 22°C with 16 h/8 h light/dark. The plants were transplanted, at the four-leaf stage, in big pots of 100 cm diameter under a plastic cover. At mid-day, the environmental conditions ranged from 29–34°C temperature, 38–64% relative humidity and 1560–1700 μmol photons $m^{-2} s^{-1}$ photosynthetic photon flux densities (PPFDs). Three plants for each pot were used. The pots were filled with soil (characteristics in Table I) and received 300 g of Nitrophoska Blu Gold (N:P:K 12:12:17). The soil water holding capacity (WHC) was estimated according to Rawls and Brakensiek (1989). Soil organic matter (SOM) was evaluated according to Allen (1989) via loss on ignition at 550°C for 2 h of oven-dry samples (75°C). Soil density was calculated as the dry weight of soil divided by its volume. Pots were arranged according to a randomised complete block design with three replicates. Treatments were the genotype (M82 and IL 9-2-5) and the water restitution level. In particular, three water restitution levels were applied consisting of the restitution to plants of 100% (V1), 50% (V2) and 25% (V3) of the lost water, respectively. Lost water was estimated by measuring the reduction in the soil moisture relatively to the field capacity. Water restitution treatments were applied when most of the plants showed fruit set on the first inflorescences. Leaf samples were collected 1 (30 days after water stress, DAWS) and 2 months (60 DAWS) from the application of the first

Table I. Soil texture (sand, loam and clay percentage), SOM, bulk density (BD) and WHC of soil used to fill the experimental pots.

Sand (%)	Loam (%)	Clay (%)	SOM (%)	BD ($kg\,dm^{-3}$)	WHC (% vol/vol)	WHC (% p/p)
78.3	15.8	5.9	0.69	0.90	17.68	0.21

differentiating watering. Leaves were harvested, frozen immediately in liquid N2 and kept at −80°C until analyzed.

Photosynthetic pigments

Total chlorophyll and carotenoids were extracted in ethanol with calcium carbonate (0.3 mg ml^{-1}) and centrifuged at 13,000*g* for 5 min. Thereafter the absorbance of the supernatant was measured at 665, 649 and 470 nm. The chlorophyll *a*, chlorophyll *b* and carotenoids were estimated according to Lichtenthaler (1987).

Total phenolics content

Total phenolics content was assayed using a modified procedure of the Folin–Ciocalteu's test (Singleton & Rossi 1965). In brief, 250 mg of frozen ground tissue were homogenized in a mortar with pestle and extracted using 1 ml of 60% methanol. Samples were left on ice for 3 min in the dark. Crude extracts were transferred in a 15 ml tube and volume was increased to 5 ml adding 60% methanol. The samples were centrifuged at 3000*g* for 5 min; then, 62.5 μl of the supernatant, 62.5 μl of Folin–Ciocalteu's reagent (Sigma, St. Louis, MO, USA) and 250 μl of deionised water were mixed and incubated for 6 min; 625 μl of 7.5% sodium carbonate and 500 μl of deionised water were added to the samples and incubated for 90 min at room temperature in the dark. Absorbance was measured at 760 nm. The concentration of total phenolics was expressed in terms of μg of gallic acid equivalents per 1 mg of fresh weight (FW).

Leaf traits determination

The evaluated leaf functional traits were specific leaf area (SLA), leaf dry matter content (LDMC) and leaf relative water content (RWC). LA for SLA determination was measured using the program Image J 1.45 (Image Analysis Software), whereas SLA, LDMC and RWC according to Cornelissen et al. (2003). More specifically, SLA, which represents the light-intercepting area per dry mass of leaf, was measured as the ratio of leaf area to leaf dry mass and expressed as cm^2 g^{-1} DW. For dry mass determination, leaves were dried at 70°C for 48 h. LDMC was measured as the oven-dry mass of a leaf divided by its water-saturated fresh mass and expressed as g DW g^{-1} FW. The saturated FW was determined by submerging the petiole of leaf blades in distilled water for 48 h in the dark. LDMC is related to the average density of the leaf tissues (Cornelissen et al. 2003). The RWC was expressed as percentage of (leaf fresh mass − leaf dry mass)/(leaf saturated fresh mass − leaf dry mass).

Fluorescence emission measurements

Chlorophyll *a* fluorescence measurements were carried out by means of a pulse amplitude modulate fluorometer (Mini-PAM, Walz, Germany) equipped with a leaf-clip holder (Leaf-Clip Holder 2030-B, Walz), able to record the incident PPFD on the leaf and abaxial leaf temperature. Measurements were performed in early morning (7:00–7:30) on 10 attached leaves *per* replicate of each genotype *per* each water regime, under natural conditions of temperature (20–24°C). More specifically, on 40 min dark-adapted leaves, the background fluorescence signal (F_o) was induced by light of about 0.5 μmol photons m^{-2} s^{-1} at the frequency of 0.6 kHz. Maximal fluorescence (F_m) was obtained by imposing to the leaf 1 s saturating flash of about 10,000 μmol photon m^{-2} s^{-1}. F_o and F_m were used to calculate the maximum photochemical efficiency of PSII as: $[F_v/F_m = (F_m - F_o)/F_m]$ (Maxwell & Johnson 2000).

Total biomass determination

Plants were harvested 95 days post-transplantation. In particular, root system was cut away at collar level and aboveground biomass was weighted, stems were split into main and lateral stems and stems and leaves were counted, as well as the length of main stems was measured. Total plant yield was also assayed for all the genotypes as weight of total collected fruits.

Statistical analysis

Statistical analyses were performed using a two-way ANOVA with treatments (V1, V2 and V3) and genotypes (M82 and IL 9-2-5) as grouping variables both at 30 and 60 DAWS. The Student–Newman–Keuls test was applied for all pairwise multiple comparison procedures. The package Sigma-Stat 3.5 was used (Jandel Scientific, San Rafael, CA, USA). Principal component analysis (PCA) was performed by using the Statistical package for Social Sciences (SPSS) Package 6, version 15.0.

Results and discussion

Antioxidant and photosynthetic pigment analyses

In this study, phenolic compounds and photosynthetic pigments were measured in order to gain a deeper understanding of the mechanisms that regulate the response to water stress in the tolerant genotype IL 9-2-5 and in the drought-sensitive genotype M82 under different watering conditions. At 30 DAWS, a significant increase in the amount of phenolic compounds was observed at V3 for the genotype M82 (Figure 1, Table II). At 60 DAWS, an increase was observed in both genotypes compared

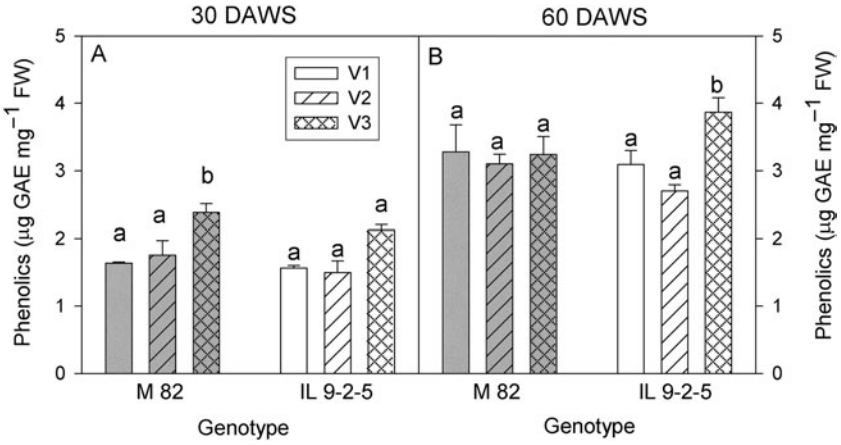

Figure 1. Total phenolics content in the leaves of two genotypes M82 and IL 9-2-5 in response to different water regimes V1, V2 and V3 following 30 (A) and 60 (B) DAWS. Mean (± SE) values are shown. Within each genotype, values marked with different letters indicate significant difference among treatments (Student–Newman–Keuls test, $p < 0.05$).

with the first sampling (Figure 1). In the second sampling, only in IL 9-2-5 a significant increase of total phenolics was measured in treatment V3 compared with treatments V2 and V1 (Figure 1, Table III). The general increase observed moving from 30 to 60 DAWS may be due to the establishment of multiple stresses on the plant. In fact, although in the treatment V1 the water is not a limiting factor, the condition of high temperature and irradiance are exacerbated with the progress of the summer season. Hence, the increase of total phenolics could be a defensive response of the plant to protect the photosystems from the excessive radiation and to mitigate the limited water availability (Ennajeh et al. 2009). The different behavior of M82 and IL 9-2-5 at 60 DAWS is in agreement with the studies from Sanchez-Rodriguez et al. (2011) who demonstrated that polyphenols play a significant role in water-stress tolerance in tomato and that moderate water stress can induce shikimate pathway in tolerant tomato cultivars.

As for the photosynthetic pigments, at 30 DAWS no significant differences in pigment concentrations were found between watering treatments for both genotypes (Figure 2, Table II). The genotype IL 9-2-5 did have a significant higher chlorophyll *a* and *b* concentration regardless of treatments (Table II). At 60 DAWS, for IL 9-2-5 genotype there was a significant increase in the concentration of chlorophyll *a*, chlorophyll *b* and carotenoids in plants subjected to V3 treatment compared with plants of V1 and V2 treatments whereas in M82 an opposite trend was found, thus evidencing a significant interaction between treatments and genotype (Table III). Reduction in leaf pigments induced by drought is considered to be an oxidative stress indicator, which might be attributed to pigment photo-oxidation, chlorophyll degradation and/or chlorophyll synthesis deficiency (Sanchez-Rodriguez et al. 2012). In particular, reduction of chlorophyll concentrations is identified as a drought response mechanism in order to minimize the light absorption by chloroplasts. Accordingly, the decrease in chlorophyll content and carotenoids in M82 and the increase in IL 9-2-5 at 60 DAWS demonstrate that both genotypes perceived the water stress but responded to it in opposite ways, thus confirming the higher tolerance of IL 9-2-5 to water deficiency.

Functional leaf traits

The different water treatments and the extent of the stress affected significantly the leaf functional traits, namely SLA, LDMC and RWC (Figure 3). In response to prolonged water stress conditions, the SLA decrease at 60 DAWS compared with 30 DAWS was evident for both tomato genotypes, and more specifically it was of 33% for M82 and 35% for IL 9-2-5. At 30 DAWS, no statistical difference has been observed in SLA of M82 and IL 9-2-5 among different water regimes as well as no interaction between genotype and treatments was evidenced (Table II). After 60 DAWS, IL 9-2-5 showed a significant reduction of SLA in treatments V2 and V3 compared with V1 (Figure 3B) whereas in M82 an increase of SLA was observed in V3 thus a significant interaction between genotype and treatments was observed (Table III). This result may be ascribed to the development of new generation leaves in both genotypes, which in response to limiting water supply have reduced the leaf expansion to avoid water loss by transpiration and increased the leaf hardness and rigidity (sclerophylly) to control leaf dehydration. Also in this framework, even if in the treatment V1 the water is not a limiting factor, the increasing temperature and irradiance with the progress of the

Table II. Summary of two-way ANOVA statistics for comparison of effects of three different water treatments on the two tomato genotypes: M82 and IL 9-2-5 at 30 DAWS incoming.

	Treatment				Genotype				T × G				Residual	
	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS
Phenolics	2	1.19	9.04	2×10^{-3}	1	0.03	0.20	0.66	2	0.16	1.20	0.33	16	0.13
Chl *a*	2	63.29	1.36	0.29	1	372.91	7.98	0.01	2	22.52	0.48	0.63	16	46.76
Chl *b*	2	20.52	1.65	0.22	1	278.13	22.31	<0.001	2	2.31	0.19	0.83	16	12.47
Car	2	4.80	1.04	0.38	1	7.45	1.62	0.22	2	0.23	0.05	0.95	16	4.61
SLA	2	476.80	0.38	0.69	1	756.15	0.60	0.44	2	1038.08	0.83	0.44	41	1253.03
LDMC	2	1.27×10^{-4}	0.22	0.81	1	8.61×10^{-3}	14.62	<0.001	2	1.33×10^{-4}	0.23	0.80	41	5.89×10^{-4}
RWC	2	156.38	0.86	0.43	1	750.12	4.14	0.05	2	62.93	0.35	0.71	41	181.39
F_v/F_m	2	5.42×10^{-3}	4.32	0.02	1	4.68×10^{-5}	0.037	0.85	2	1.28×10^{-3}	1.02	0.37	41	1.25×10^{-3}

Notes: Treatments: V1, V2, V3; genotypes: M82, IL 9-2-5. T × G: interactions between treatments (T) and genotype (G). SLA, specific leaf area; LDMC, leaf dry matter content; RWC, relative water content; F_v/F_m, maximal PSII photochemical efficiency.

Table III. Summary of two-way ANOVA statistics for comparison of effects of three different water treatments on the two tomato genotypes: M82 and IL 9-2-5 at 60 DAWS incoming.

	Treatment				Genotype				T × G				Residual	
	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS
Phenolics	2	1.24	7.11	0.01	1	0.26	1.48	0.25	2	0.35	1.99	0.18	12	0.18
Chl *a*	2	151.94	2.48	0.13	1	496.92	8.11	0.02	2	1557.28	25.41	<0.001	12	61.28
Chl *b*	2	9.55	0.47	0.64	1	213.72	10.44	0.01	2	250.10	12.21	1×10^{-3}	12	20.48
Car	2	17.33	1.73	0.22	1	7.12	0.71	0.41	2	132.07	13.19	<0.001	12	10.02
SLA	2	189.35	0.30	0.75	1	189.35	0.30	0.75	2	6709.45	10.44	<0.001	42	642.76
LDMC	2	1.89×10^{-3}	1.62	0.21	1	0.02	20.81	<0.001	2	4.55×10^{-4}	0.39	0.68	42	116×10^{-3}
RWC	2	151.20	2.54	0.09	1	222.89	3.74	0.06	2	193.32	3.24	0.05	42	59.61
F_v/F_m	2	1.84×10^{-3}	2.67	0.08	1	3.67×10^{-4}	0.53	0.47	2	8.08×10^{-5}	0.12	0.89	42	6.90×10^{-4}

Notes: Treatments: V1, V2, V3; genotypes: M82, IL 9-2-5. T × G: interactions between treatments (T) and genotype (G). SLA, specific leaf area; LDMC, leaf dry matter content; RWC, relative water content; F_v/F_m, maximal PSII photochemical efficiency.

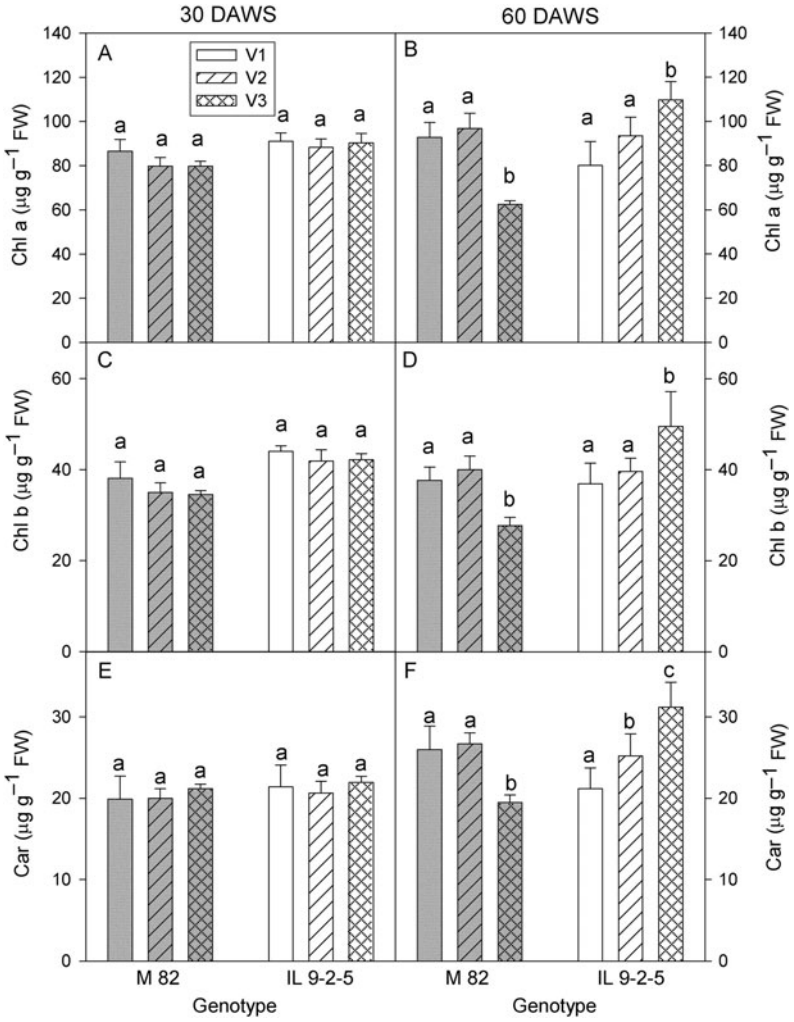

Figure 2. Chlorophyll *a* (A, B), chlorophyll *b* (C, D) and carotenoid content (E, F) in the leaves of two genotypes M82 and IL 9-2-5 in response to different water regimes V1, V2 and V3 following 30 and 60 DAWS. Mean (± SE) values are shown. Within each genotype, values marked with different letters indicate significant difference among treatments (Student–Newman–Keuls test, $p < 0.05$).

summer season may have triggered the formation of new leaves with small SLA. It is well known that the reduction of leaf area as well as the developing of mechanic tissues represents a useful way to cope with environmental harsh conditions, such as high irradiance, temperature and water stress, that may limit plant productivity (Hunt & Cornelissen 1997; Gulías et al. 2003; Poorter & Bongers 2006).

As expected, the LDMC showed an opposite trend compared with SLA (Figure 3(C),(D)). The comparison between the two genotypes demonstrated that IL 9-2-5 showed a significant higher LDMC compared with M82 in all tested water conditions at both 30 and 60 DAWS (Tables II and III). In particular, at 30 DAWS no significant difference was detected in both genotypes in response to different water regimes. At 60 DAWS, LDMC increased significantly (around 30%) in both tomato genotypes, although IL 9-2-5 maintained a higher value compared with M82. Since this parameter can be considered an index of tissue density (Poorter & Bongers 2006), its augment in response to prolonged water stress conditions could indicate the development of a strategy to cope with water stress that implicates the reduction of growth rate and the increase of leaf longevity in order to retain nutrients, as observed by other authors (Poorter & De Jong 1999; Ryser & Urbas 2000). Moreover, due to the higher LDMC observed in IL 9-2-5 under V3 treatment, it could be hypothesized that this genotype under limiting water supply allocates most new photosynthates towards the production of sclerenchymatic tissues in order to avoid leaf dehydration, rather than toward the growth.

The water status of leaves in response to different water treatments and their duration is indicated by RWC (Figure 3(E),(F)). At 30 DAWS, no statistical differences were evident in both tomato genotypes among different water regimes. Conversely, at 60

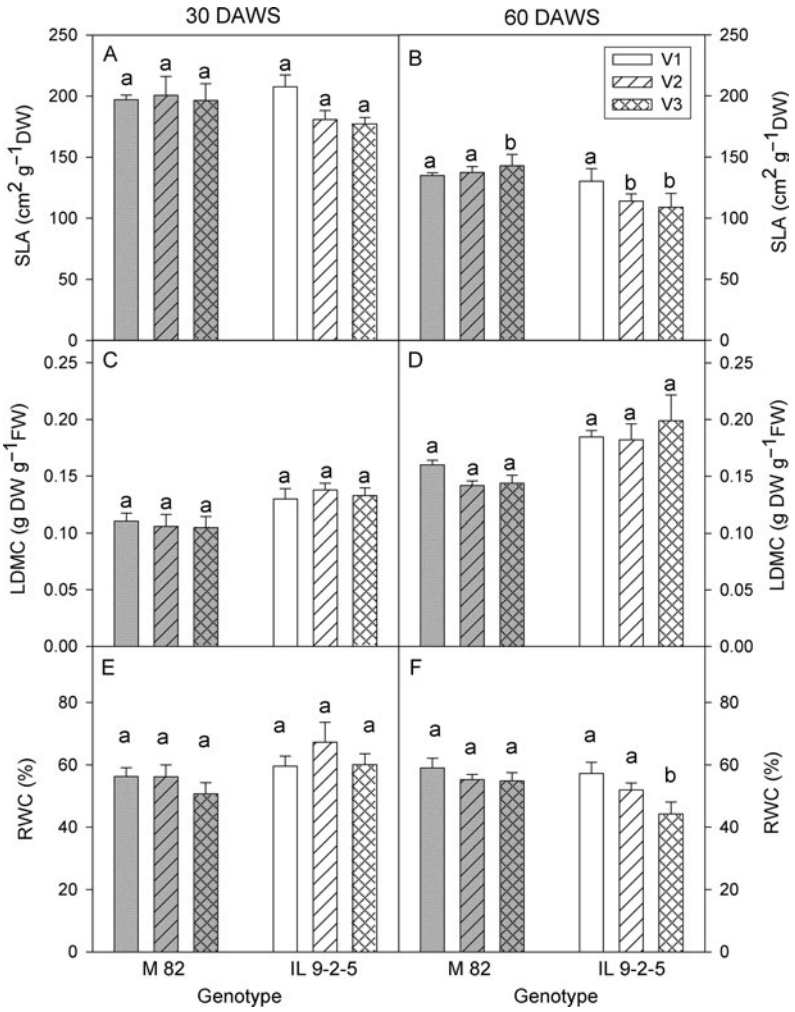

Figure 3. SLA (A, B), LDMC (C, D) and leaf relative water content RWC (E, F) in the leaves of two genotypes M82 and IL 9-2-5 in response to different water regimes V1, V2 and V3 following 30 and 60 DAWS. Mean (± SE) values are shown. Within each genotype, values marked with different letters indicate significant difference among treatments (Student–Newman–Keuls test, $p < 0.05$).

DAWS the genotype IL 9-2-5 showed a reduction of 23% of RWC in the treatment V3 compared with V2 and V1 whereas in M82 RWC values remained comparable to those observed at 30 DAWS. This result evidenced that under prolonged water stress conditions, leaves of IL 9-2-5 are more vulnerable to dehydration compared with the M82 genotype, despite its higher LDMC. The interaction between genotype and treatments was significant only at 60 DAWS (Table III).

Maximal PSII photochemical efficiency

In both tomato genotypes at 60 DAWS, no difference in maximal PSII photochemical efficiency (F_v/F_m) was detected independently of different water treatments (Figure 4). The values of F_v/F_m ratio were comparable for all water regimes and next to 0.8, which is considered the threshold value for plants in healthy status. This indicates that the photochemical apparatus of tomato does not lose its functionality in light conversion to reaction centers even under prolonged periods of limited water supply (Maxwell & Johnson 2000). This result can be considered important since it is well known that water scarcity represents one of the most severe constraints to tomato cultivation (Sanchez-Rodriguez et al. 2011).

Plant biomass production

Regardless of the higher biomass accumulation in IL 9-2-5 previously demonstrated (Fridman et al. 2000), here we confirm that IL 9-2-5 expresses a different response to water deprivation compared with M82 since it retains a higher proportion of its aboveground biomass under water stress (Table IV). In fact, the percentage of aboveground biomass in IL 9-2-5 was higher than M82 at both V2 and V3 and both the water restitution levels and the genotypes significantly affected the reduction in aboveground biomass (Table V). In addition, IL 9-2-5 maintained

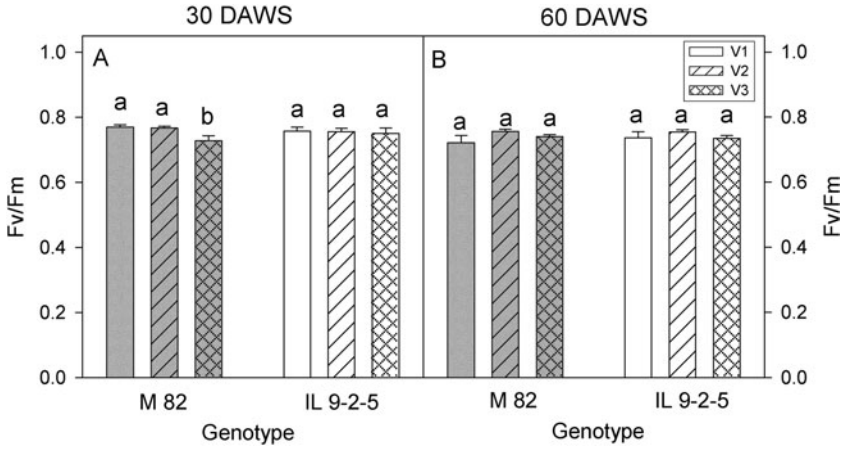

Figure 4. Maximal PSII photochemical efficiency (F_v/F_m) in the leaves of two genotypes M82 and IL 9-2-5 in response to different water regimes V1, V2 and V3 following 30 (A) and 60 (B) DAWS. Mean (± SE) values are shown. Within each genotype, values marked with different letters indicate significant difference among treatments (Student–Newman–Keuls test, $p < 0.05$).

Table IV. Effect of water stress on plant biomass production in M82 and IL 9-2-5.

		Genotypes	
Trait	Treatment	M82	IL 9-2-5
Aboveground biomass	V2	88.65 ± 0.34	93.83 ± 2.50
	V3	49.00 ± 1.47	57.0 ± 11.98
Main stems	V2	95.38 ± 1.78	72.73 ± 18.18
	V3	88.08 ± 6.45	59.09 ± 8.70
Main stem length	V2	107.89 ± 2.41	112.93 ± 14.69
	V3	96.67 ± 4.71	115.13 ± 5.53
Leaves per main stem	V2	115.06 ± 8.99	109.46 ± 12.16
	V3	84.41 ± 4.86	90.65 ± 5.49

Notes: Traits are expressed as a percentage of their values under fully irrigated treatments (V1). Mean (± SE) values are reported.

significantly lower proportions of main stems at reduced levels of water restitution even though this response did not significantly change between V2 and V3 (Tables IV and V). Finally, the response to water deprivation in terms of length of stems and of number of leaves per stem did not account for differences between genotypes. Changes in the length of main stems were statistically the same at V2 and V3 while the level of water reintegration significantly affected the variations in the number of leaves per stem (Table V). Overall, considering the aboveground biomass data reported, IL 9-2-5 expressed lower amplitude than M82 in the plant response to water stress. In addition, preliminary data demonstrates that the genotype IL 9-2-5 maintained a higher percentage of fruit biomass than M82 at reduced levels of water reintegration. In V2 conditions, the percentage of fruit weight compared with that in fully irrigated conditions (V1) was 69.66 ± 4.17 (mean ± SE) in IL 9-2-5 and 40.28 ± 4.18 in M82. The same pattern was observed in V3 conditions where this percentage was 43.86 ± 1.71 in IL 9-2-5 and 22.81 ± 2.15 in M82.

Principal component analysis

Recorded variables on M82 and IL 9-2-5 plants were integrated by a PCA (Figure 5) approach in order to discriminate the effects of genotypes and water restitution treatments (i.e. 100%, 50% and 25% restitution of water loss) on the performances of all traits analyzed. The main three visualized components account for 84% of the overall variability. Component 1 mainly explains variability in the SLA

Table V. Summary of two-way ANOVA statistics reporting effects of two reduced water restitution levels (V2 and V3) on the genotypes M82 and IL 9-2-5.

	Treatment				Genotype				T × G				Residual	
	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P	DF	MS
Aboveground biomass	1	3507.72	512.28	4.87×10^{-7}	1	104.44	15.25	7.93×10^{-3}	1	4.81	0.70	0.43	16	46.76
Main stems	1	138.59	0.53	0.49	1	1772.94	6.81	0.04	1	49.08	0.19	0.68	16	12.47
Main stem length	1	209.75	1.54	0.26	1	496.71	3.64	0.10	1	209.75	1.54	0.26	16	4.61
Leaves per main stem	1	1596.77	8.64	2.60×10^{-2}	1	22.73	0.12	0.74	1	180.69	0.98	0.36	16	0.13

Treatments: V2, V3. Genotypes: M82, IL 9-2-5. T × G: interactions between treatments (T) and genotype (G).

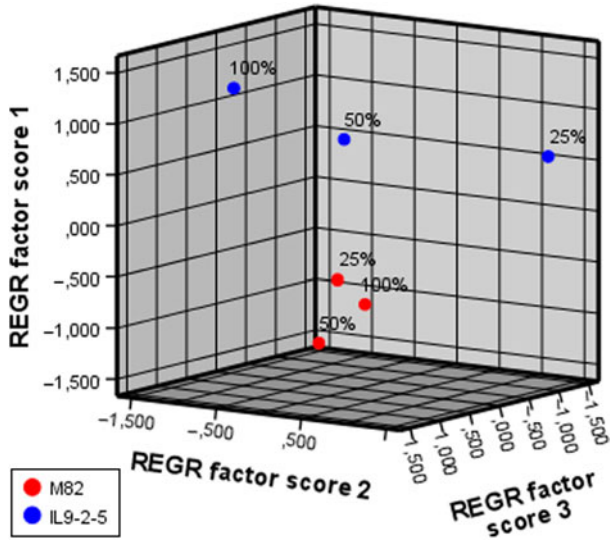

Figure 5. Discrimination of the overall response of M82 and IL 9-2-5 plants to reduced water supply (i.e. 100%, 50% and 25% restitution of water loss) by PCA. Component 1 (REGR factor score 1): SLA (30 and 60 DAWS), LDMC (30 and 60 DAWS), RWC (30 and 60 DAWS), chlorophyll *a* and *b* leaf contents (30 and 60 DAWS), leaf level of carotenoids (60 DAWS) and length of main stems. Component 2 (REGR factor score 2): F_v/F_m (60 DAWS), leaf level of total phenols (30 and 60 DAWS), green FW and number of main stems. Component 3 (REGR factor score 3): F_v/F_m (30 DAWS), level of carotenoids in leaves (30 DAWS) and number of leaves *per* main stem.

(30 and 60 DAWS), LDMC (30 and 60 DAWS), RWC (30 and 60 DAWS), chlorophyll *a* and *b* leaf contents (30 and 60 DAWS), leaf level of carotenoids (60 DAWS) and length of main stems. Component 2 mainly explains variability in the F_v/F_m (60 DAWS), leaf level of total phenolics (30 and 60 DAWS), green FW and number of main stems. Finally, component 3 mainly explains variability in the F_v/F_m (30 DAWS), the level of carotenoids in leaves (30 DAWS) and the number of leaves *per* main stem. The PCA output shows an evident separation between the tolerant genotype IL 9-2-5 and the susceptible genotype M82 that is mainly attributable to the component 1. In addition, in case of the tolerant genotype IL 9-2-5 the response to the three different water regimes can be mainly explained by the component 2. This allows us to point out at the latter traits as effective components of the complex response of IL 9-2-5 to limited water supply.

Conclusion

In this paper, we provided evidences that the IL 9-2-5 performs better than the genotype M82 to reduced water regimes. In particular, we demonstrated that both genotypes perceived the water stress since they react to it by modifying the photosynthetic pigment content in leaves; however, they react in a contrasting way evidencing a higher tolerance of IL 9-2-5 to water deprivation. We showed that the main factors responsible for this better response generally include a more efficient antioxidant system joined with the modification of functional leaf traits associated with an increase of leaf mechanical resistance (namely sclerophylly and LDMC). In addition, we showed that IL 9-2-5 subjected to the water regime V2 (irrigation with 50% field capacity) maintained a higher percentage of fruit weight and aboveground biomass compared with M82 suggesting that IL 9-2-5 could be cultivated in semi-arid environments with reduced losses of yield. Future studies will be required to evaluate if the cultivation of the genotype IL 9-2-5 under the regime V2 can, not only permit the use of marginal lands or cultivation with a more sustainable use of water, but also promote the quality and the nutritional properties of the tomato fruits. Finally, due to the information deriving from the complete sequencing of the tomato genome (Sato et al. 2012) the identification of candidate genes controlling the physiological and morphological traits analyzed is underway by exploring those mapping in the introgression region 9-2-5. Identification of polymorphisms in key-genes controlling these traits can guide in future the breeding efforts aimed at improving susceptible genotypes.

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References

Allen SE. 1989. Chemical analysis of ecological materials. Oxford: Blackwell Scientific Publication.

Alseekh S, Ofner I, Pleban T, Tripodi P, Di Dato F, Cammareri M, et al. 2013. Resolution by recombination: Breaking up Solanum pennellii introgressions. Trends Plant Sci. 18(10): 536–538.

Barbagallo RN, Di Silvestro I, Patanè C. 2012. Yield, physicochemical traits, antioxidant pattern, polyphenol oxidase activity and total visual quality of field-grown processing

tomato cv. Brigade as affected by water stress in Mediterranean climate. J Sci Food Agric. 93: 1449–1457.

Claves H, Inzè D. 2013. The agony of choice: How plants balance growth and survival under water-limiting conditions. Plant Physiol. 162: 1768–1779.

Cornelissen J, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich D, et al. 2003. Handbook of protocols for standardised and easy measurements of plant functional traits worldwide. Austral J Bot. 51: 335–380.

Easlon HM, Richards JH. 2009. Drought response in self-compatible species of tomato (solanaceae). Am J Bot. 96: 605–611.

Ennajeh M, Vadel AM, Khemira H. 2009. Osmoregulation and osmoprotecion in the leaf cells of two olive cultivars subjected to severe water deficit. Acta Physiol Plant 31: 711–721.

Eshed Y, Zamir D. 1995. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141: 1147–1162.

Fridman E, Pleban T, Zamir D. 2000. A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci USA 97(9): 4718–4723.

Garg N, Manchanda G. 2009. ROS generation in plants: Boon or bane? Plant Biosyst. 143(1): 81–96.

Gong P, Zhang J, Li H, Yang C, Zhang C, Zhang X, et al. 2010. Transcriptional profiles of drought-responsive genes in modulating signal transduction and biochemical pathways in tomato. J Exp Bot. 61(13): 3563–3575.

Gulías J, Flexas J, Mus M, Cifre J, Lefi E, Medrano H. 2003. Relationship between maximum leaf photosyntesis, nitrogen content and specific leaf area in balearic and non-endemic Mediterranean species. Ann Bot. 92: 215–222.

Hunt R, Cornelissen JHC. 1997. Components of relative growth rate and their interrelations in 59 temperate plant species. New Phytol. 135: 395–417.

Lawlor DW. 2013. Genetic engineering to improve plant performance under drought: Physiological evaluation of achievements, limitations and possibilities. J Exp Bot. 64: 83–108.

Lichtenthaler HL. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 148: 350–382.

Lippman ZB, Semel Y, Zamir D. 2007. An integrated view of quantitative trait variation using tomato interspecific introgression lines. Curr Opin Genet Dev. 17(6): 545–552.

Maxwell K, Johnson GN. 2000. Chlorophyll fuorescence – A practical guide. J. Exp. Bot. 51: 659–668.

Mishra KB, Iannacone R, Petrozza A, Mishra A, Armentano N, La Vecchia G, et al. 2012. Engineered drought tolerance in tomato plants is reflected in chlorophyll fluorescence emission. Plant Sci. 182: 79–86.

Poorter L, Bongers F. 2006. Leaf traits are good predictors of plant performance across 53 rain forest species. Ecology 87: 1733–1743.

Poorter H, De Jong R. 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. New Phytol. 143: 163–176.

Rawls WJ, Brakensiek DL. 1989. Estimation of soil water retention and hydraulic properties. In: Morel-Seytoux HJ, editor. Unsaturated flow in hydrologic modeling: Theory and practice. Dordrecht: Kluwer Academic. pp. 275–300.

Ryser P, Urbas P. 2000. Ecological significance of leaf life span among Central European grass species. Oikos 91: 41–50.

Sanchez-Rodriguez E, del Mar Rubio-Wilhelmi M, Blasco B, Leyva R, Romero L, Ruiz JM. 2012. Antioxidant response resides in the shoot in reciprocal grafts of drought-tolerant and drought-sensitive cultivars in tomato under water stress. Plant Sci. 188–189: 86–96.

Sanchez-Rodriguez E, Moreno DA, Ferreres F, del Mar Rubio-Wilhelmi M, Ruiz JM. 2011. Differential responses of five cherry tomato varieties to water stress: Changes on phenolic metabolites and related enzymes. Phytochemistry 72: 723–729.

Sato S, Tabata S, Hirakawa H, Asamizu E, Shirasawa K, Isobe S, et al. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485: 635–641.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult. 16: 144–158.

Tanksley SD, McCouch SR. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277: 1063–1066.

Vasco M, De Stefano R, Di Matteo A, Punzo B, Molisso M, Lotti C, et al. 2011. Candidate genes controlling fruit quality in a tomato introgression line tolerant to water deficit. Joint Meeting AGI-SIBV-SIGA, 19–22 September 2011, Cittadella di Assisi (Italy); pp. 6A.48.

Vitale L, Arena C, Carillo P, Di Tommasi P, Mesolella B, Nacca F, et al 2011. Gas exchange and leaf metabolism of irrigated maize at different growth stages. Plant Biosyst. 145(2): 485–494.

Xu Z, Zhou G, Shimizu H. 2010. Plant response to drought and rewatering. Plant Signal Behav. 5: 649–654.