



Isolating Water and Nitrogen Stress Tolerant Genotypes Among Tomato Landraces and *Solanum pennellii* Backcross Inbred Lines

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Abstract

Improving crop adaptation to reduced agricultural inputs is a fundamental goal of current and future cropping systems, which must cope with resource scarcity and feed an increasing world population. Landraces and wild species showing tolerance traits are an important source of underexploited genetic traits that may contribute to pursue this goal. In this work, we exposed tomato lines of different origin to nitrogen and water deficiency to identify stress tolerance traits. Out of 10 accessions, we identified two genotypes, T292 and T150, with remarkable tolerance to nitrogen and water shortage, respectively. Under nitrogen deficiency genotype T292 did not show significant reduction of the shoot biomass vs a 20.2% reduction observed in the control genotype M82. An increased antioxidant activity and the ability to mobilize nitrogen from roots to the shoot were correlated with the sustained growth of T292 under low nitrogen. In response to drought stress, line T150 was the best performer with no significant reduction of biomass compared with control, well-watered conditions, whereas a 27.6% reduction was observed in M82. Under water deficiency, key adaptive traits of line T150 included the ability to improve the root:shoot ratio by allocating more biomass to the roots, which was correlated with lower levels of key markers of osmotic stress (proline and *LEA*) compared to control plants. Overall, we identified key physiological/molecular traits that mediate nitrogen and water use under resource shortage, and we confirmed that landraces represent a valuable genetic resource to be used in modern sustainability-based tomato breeding.

Keywords Drought · Breeding · Sustainability · Low-input agriculture

Introduction

Tomato is one of the most important crops, with more than five billion hectares cultivated worldwide (Mehle et al. 2023) and high varietal diversity in terms of plant growth habit and fruit morphology and quality. Such diversity has been produced over many years of breeding programs but also maintained for centuries by small farmers, who have selected varieties

that well adapted to different cultivation environments, the so-called landraces (Lázaro 2018). Generally, landraces are highly resource-use efficient, since they have been selected primarily for environmental adaptability rather than high yield and, indirectly, tolerance to biotic and abiotic stresses (Jaiswal et al. 2020; Moles et al. 2016; Silva et al. 2019). Landraces are therefore an important source of tolerance traits, which are pivotal to increase the sustainability of tomato cultivation and protect yields in sub-optimal environments, where modern hybrids would generally underperform with respect to their yield potential (Tagiakas et al. 2022). It is generally accepted that most breeding programs have focused on high yield and pests/diseases tolerance rather than adaptability to environmental/cultural constraints such as sub-optimal temperature conditions and/or water and nutrient scarcity. However, future agricultural scenarios will deal with increasingly frequent drought and heatwaves events, especially in arid and semi-arid environments (Shah et al. 2024; Thabet et al. 2024a), low availability of fertilizers due to their natural scarcity and/or high production and delivery costs, and need to reduce nutrients

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leaching and contaminations of aquifers due to unutilized fertilizers by the plants (Carrillo et al. 2024; Manaigo et al. 2024; Thabet et al. 2024b; Alqudah et al. 2024a). In particular, nitrogen starvation reduces tomato plant growth and productivity because it causes an impairment of aminoacids and protein biosynthesis (Wu et al. 2024). The deficit of this macronutrient has negative impacts on the whole plant physiology, making fertilization essential to achieve high yield (Zayed et al. 2023). In this context, low yielding landraces have gathered a renewed interest, as an alternative model to modern cultivars, for low-input agriculture, and as a source of resilience traits for next generation breeding programs (Caramante et al. 2023; Marone et al. 2021; Ochieng et al. 2021). It has been demonstrated that some tomato landraces were more tolerant to salt and water stress compared to commercial varieties (Fita et al. 2015; Giorio et al. 2020; Landi et al. 2023; Massaretto et al. 2018). Moreover, several tomato local varieties are grown without or with minimal water supplementation during the whole growth cycle, a feature that associates low water requirements to the high quality of the final products (Guida et al. 2017). Similarly, old tomato varieties were found to have higher nitrogen use efficiency (NUE) and water use efficiency (WUE) than modern hybrids when facing combined water and nitrogen stress (Machado et al. 2023). Several genes related with NUE belong to the *NRT* family, and mediate nitrate transport from the soil to plant tissues. It has been reported that *NRT* genes are also highly expressed in nitrogen-starved plants to facilitate nitrogen uptake and phloem loading for its remobilization (Lezhneva et al., 2014; Safi., 2023). Introgression of traits from wild relatives of tomato has long been proposed as a key strategy to improve abiotic stress tolerance. *Solanum pennellii* is a wild tomato species recognized for extreme stress tolerance and introgression lines of *S. pennellii* with M82 commercial tomato variety have been used to identify quantitative trait loci (QTLs) for abiotic stress tolerance (Bolger et al. 2014; Eshed and Zamir 1995; Ofner et al. 2016; Pessoa et al. 2023). Therefore, the aim of this study was to identify genetic and physiological traits, from local varieties or advanced (BILs) inbred lines from wild species, able to improve tomato resource use efficiency (Lázaro 2018). Phenotypic evaluation of ten tomato genotypes allowed us to identify candidate tomato genotypes able to grow under limited water and nitrogen supply. Key biochemical and molecular responses have been assessed on the selected lines and identified as boosters of water and nitrogen use efficiency in tomato.

Materials and Methods

Plant material and Growth Conditions

Ten *Solanum lycopersicum* L. genotypes (Table S1) were chosen among a large tomato core collection (Hebrew

University of Jerusalem), long shelf-life Western Mediterranean tomato landraces, and lines previously identified as good performers under single stress conditions (University of Naples Federico II). The commercial variety M82 was used as internal reference to compare the response of the ten lines to the different growing conditions imposed. The experimental conditions were as described in Ruggiero et al. (2022). Briefly, 30-day old seedlings grown in a nursery were transplanted in a semi-controlled conditions glasshouse to 15 L pots containing sand imbibed with water. At transplant, plants were divided in six blocks, each containing three to four replicates per each genotype and organized according to a randomized block setup. Two blocks per treatment were used and plants were fertigated following three treatments: Control (10.2 mM NO₃⁻, 100% water supply), Low N (2.88 mM NO₃⁻, 100% water supply), and Drought (10.2 mM NO₃⁻, 50% water supply). Nutrient and drought stresses were applied after one week of acclimation. Detailed nutrient solution compositions are described in Ruggiero et al. (2022). Plants were cultivated under stress conditions for 30 days prior to biometric measurements and leaf and root sampling for molecular and biochemical analyses.

Physiological Analyses and Biometric Measurements

Physiological analyses and biometric measurements were carried out on six to eight replicates per genotype per treatment. At 30 days after stress application, chlorophyll content was measured using the chlorophyll meter SPAD-502 Plus (Konika Minolta). On the same day, the stomatal conductance was measured by using the AP4-UM3 porometer (Delta-T Devices), as well as the leaf relative water content (RWC). RWC was measured as follows. Excised leaves were immediately weighed for fresh weight (FW) determination and then hydrated with distilled water for 24 h to obtain the turgid weight (TW). Leaf samples were then oven-dried at 70 °C for 72 h and dry weight (DW) was measured. The RWC was calculated using the following equation:

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

For biometric measurements, aerial parts and roots were collected. Roots were washed to remove residual sand. Shoot fresh weight, shoot dry weight, leaf area, and root dry weight were measured. To obtain shoot and root dry weights, the collected samples were oven-dried at 70 °C until a stable weight was reached. Plant leaf area was measured on excised leaves of all plants using a scanning planimeter (LI – 3400 Leaf Area Meter, Licor).

Biochemical Analyses

At the end of experiment, leaf samples were collected from four biological replicates per genotype and treatment. For proline content measurements, two technical replicates were performed for each sample. Proline content was determined according to the method of Claussen (2005). Lipid peroxidation, estimated as MDA content, was determined by spectrophotometric measurements at 532 and 600 nm, and the concentration was calculated using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Zhang and Kirkham 1996). The content of hydrogen peroxide (H_2O_2) was determined as described by Alexieva et al. (2001), on four replicates per genotype per treatment. Absorbance was determined at 390 nm, and the H_2O_2 content for all samples was quantified using a known H_2O_2 concentration curve as a standard. To normalize between leaves at different levels of water content belonging to control vs drought stress treatments, these parameters were expressed on dry weight basis.

Nitrate Content

The nitrate content was measured on finely ground dried shoot and root tissues. The extraction was performed in Milli-Q water (Merck Millipore, Darmstadt, Germany) at 80 °C for 10 min. The analysis was carried out by ion chromatography using a Dionex ICS-3000 system (Sunnyvale, CA, USA). The evaluation of nitrate was performed with NaOH gradient (1 mM–50 mM; flow rate 1.5 mL/min) using an IonPac AS11HC column with an AG11HC guard column as previously described in Di Stasio et al. (2020).

RNA Isolation, cDNA Synthesis, qRT-PCR

Total RNA was extracted from leaves and roots using RNeasy Plant Mini kit (Qiagen, Germany) following the manufacturer's instructions. RNA was DNase-treated and reverse transcribed as previously described (Batelli et al. 2024). Gene expression analysis through qRT-PCR was performed using three biological replicates and three technical replicates with ABI 7900 HT (Applied Biosystems, United States) as previously described (Ruggiero et al. 2019). Primers used are listed in Table S2. PCR product melting curves were analyzed to confirm the presence of a single peak, indicative of one PCR product per primer couple assayed. For relative quantification of gene expression, elongation Factor EF1- α (*Solyc06g005060*) served as endogenous reference.

Ranking Methodology

The ranking was performed according to the methods described in Biermann et al. (2022) using RStudio. The R

code used is stored at GitHub at the following link: <https://github.com/BiermannIGZ/AbioticStressToleranceClassification>. For the biometric, physiological, and biochemical parameters evaluated, the mean values of relevant parameters were subjected to analysis of variance (ANOVA) and Tukey HSD post-hoc to identify the differences among the means. For qRT-PCR, the differences were identified with Student's t-test ($p < 0.05$).

Results

Genotype Ranking Under Low Nitrogen (LN)

The eigenvalue of the PCA applied to the dataset showed that PC1 and PC2 explained 40.1% and 13.7% of the variability, respectively (Figure S1A). The PC1 was mainly correlated with shoot dry weight, leaf area and shoot fresh weight, while the PC2-3 with RWC, root dry weight and SPAD (Table S3B). The scatter plot resulting from the PCA showed that the low nitrogen points are clearly separated from the control ones (Figure S2), but with different distances among the genotypes (Table 1). Indeed, the genotypes T149, T292 and T313 showed higher tolerance toward low nitrogen, while T150, T151 and M82 were sensitive. Finally, T147, T336, T249 and T327, showed an intermediate behaviour between the tolerant and sensitive genotypes (Table 1).

Biometric and Physiological Measurements (LN)

The results of the ranking identified T292 as the most tolerant genotype under low nitrogen, opposed to M82 that showed high sensitivity to this condition (Table 1). Confirming the results of the ranking, M82 shoot and root dry

Table 1 Ranking of the 10 evaluated genotypes based on the distance between the two treatments (control vs low nitrogen)

Low Nitrogen			
Genotype	Distance	z-score	Sig
T149	2.67	- 0.67	a
T292	2.70	- 0.64	a
T313	2.70	- 0.64	a
T147	3.05	- 0.22	ab
T336	3.38	0.17	ab
T249	3.45	0.25	ab
T327	3.46	0.26	ab
T150	3.61	0.44	b
T151	3.62	0.45	b
M82	3.82	0.69	b

Statistical analysis was performed according to ANOVA and Duncan's post-hoc test ($p < 0.05$)

weights were reduced by 20.2% and 31.3%, respectively compared to the unstressed control (Fig. 1A-B). On the contrary, these parameters did not show a significant change under low nitrogen in T292 (Fig. 1A-B).

Biochemical Measurements (LN)

The ranking provided by the PCA allowed us to select, among the tested genotypes, the most representative in terms of tolerance and sensitivity to a low nitrogen condition. Therefore, further analyses were performed on these genotypes to identify the biochemical responses underlying this differential behaviour. When comparing the effect of low nitrogen on the two genotypes showing opposite responses, the tissue levels of H_2O_2 and MDA were consistent with a stress- and tolerance-status of M82 and T292, respectively, showing significant interaction between the genotype x treatment factors (Table S4). Indeed, the H_2O_2 levels increased significantly in M82 tissues compared to control conditions (+82% %), whereas they did not in T292 plants (Fig. 2A). Similar results were found for MDA, that increased by 34.93% in M82 plants under low N (Fig. 2B). Interestingly, this parameter was reduced by 57% in T292 plants (Fig. 2B). Regarding the nitrate concentration of the leaf, no significant interaction between the genotype and the nitrogen treatment

was found, but only a significant decrease caused by low nitrogen compared to the control (-96%) (Table S5). On the contrary, the root concentration of nitrate was affected by the interaction between the two factors (Table S5). This parameter showed an opposed trend between the two genotypes, with 107.5% increase of root nitrate concentration in M82, and 63.2% decrease in T292 (Fig. 3).

Gene Expression (LN)

Under low nitrogen, *SOD6* (*Solyc03g095180*) expression was significantly upregulated (1.4-fold change) in M82 compared to control conditions, while no change was detected for T292. On the contrary, low nitrogen induced an up-regulation of *CAT* (*Solyc04g082460*) in T292 (1.96-fold change) that was absent in M82 (Fig. 4). For the low nitrogen tolerant genotype T292, expression of the dual/low-affinity nitrate transporter (*NRT1.1*, *Solyc08g078950*) in leaves was significantly upregulated under low nitrogen conditions (2.7-fold change), while in M82 this gene did not show significant changes. On the contrary, the high-affinity nitrate transporter (*NRT2.1*, *Solyc06g074990*) in roots was upregulated both in T292 and M82 (32.5- and 42.1-fold change, respectively) (Fig. 5).

Fig. 1 Biometric response of the two selected tomato genotypes (sensitive M82 vs tolerant T292) to low nitrogen (LN) in terms of **A**) shoot dry weight and **B**) root dry weight. Different letters indicate significant variation between the means according to Duncan's post-hoc test ($p < 0.05$)

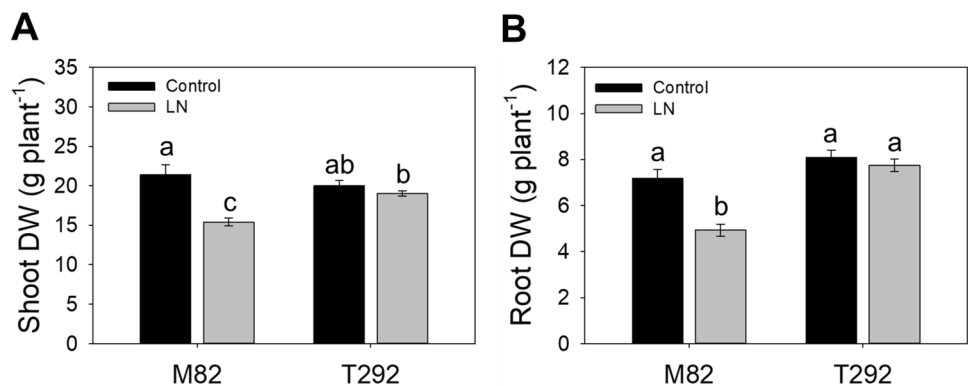
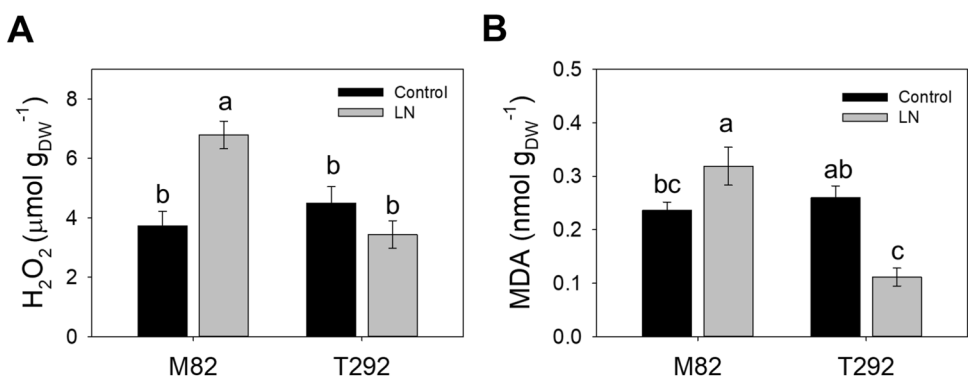


Fig. 2 Biochemical response of the two selected tomato genotypes (sensitive M82 vs tolerant T292) to low nitrogen (LN) in terms of **A**) H_2O_2 and **B**) malondialdehyde (MDA). Different letters indicate significant variation between the means according to Duncan's post-hoc test ($p < 0.05$)



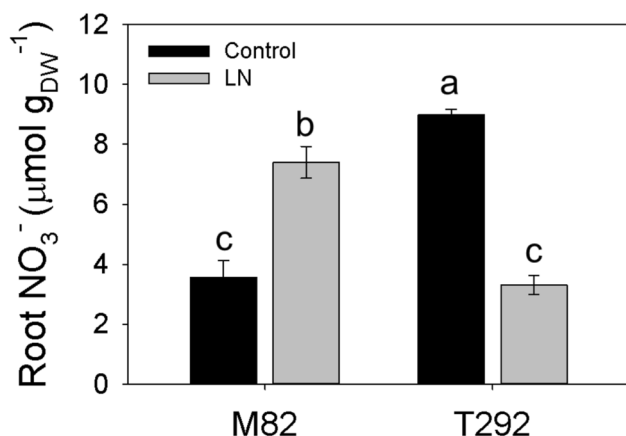


Fig. 3 Root nitrate concentration of the two selected tomato genotypes (sensitive M82 vs tolerant T292) in response to low nitrogen (LN). Different letters indicate significant variation between the means according to Duncan's post-hoc test ($p < 0.05$)

Genotype Ranking Under Drought Stress (DS)

According to the PCA for the dataset on drought, the PC1 and the PC2 explained 50.5% and 15.9% of the variability

Fig. 4 Relative gene expression of **A** *SOD6* and **B** *CAT* of the two selected tomato genotypes (sensitive M82 vs tolerant T292) in response to low nitrogen (LN). Statistical analysis was performed within each genotype. Asterisks indicate significant differences according to Student's t-test (ns = non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$)

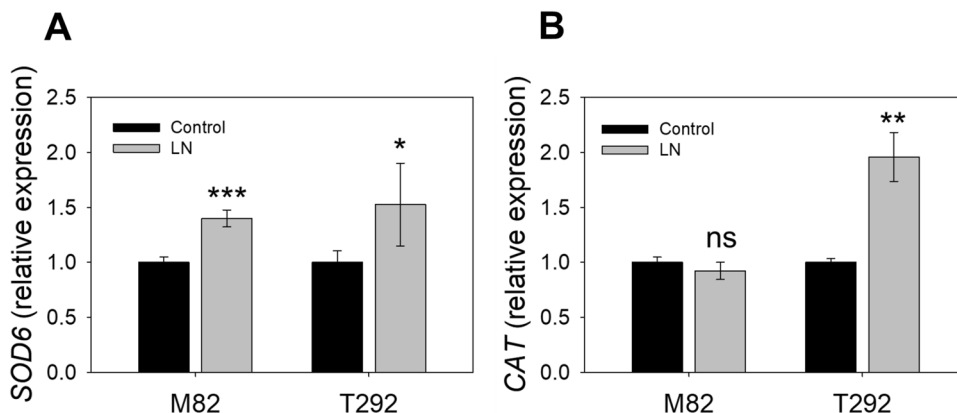
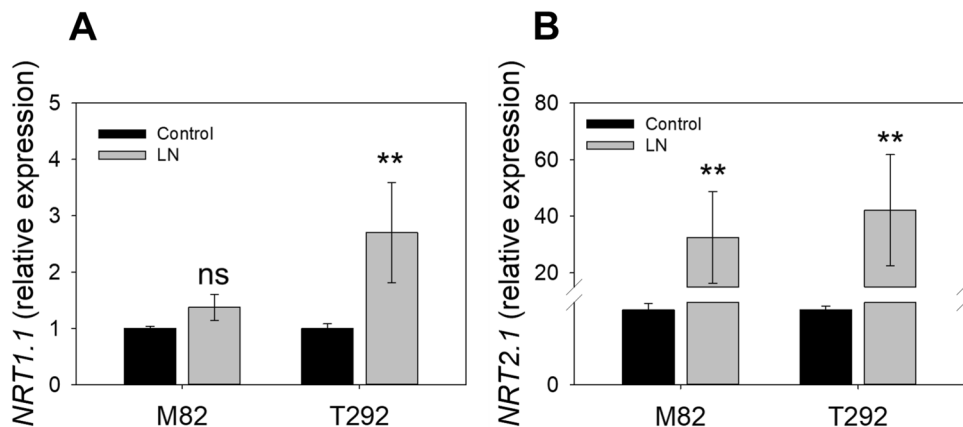


Fig. 5 Relative gene expression of **ANRT1.1 and **BNRT2.1 in the two selected tomato genotypes (sensitive M82 vs tolerant T292) in response to low nitrogen (LN). The expression of the two genes was evaluated in leaves for *NRT1.1* and in roots for *NRT2.1*. Statistical analysis was performed within each genotype. Asterisks indicate significant differences according to Student's t-test (ns = non-significant; ** = $p < 0.01$)****



among the genotypes (Figure S3A). The PC1 was mainly explained by shoot fresh weight, leaf area, shoot dry weight, and transpiration. The PC2 was mainly explained by root dry weight, height, SPAD and RWC (Figure S3B). The PCA points show that the control and the drought plants clearly separate on the chart (Figure S4). The calculated distances on the chart show that the most drought tolerant genotypes, indicated by the lowest distance between control and drought points, were T150, T147, and T149. On the contrary, the most sensitive genotypes were T336, M82, and T151 (Table 2).

Biometric Measurements (DS)

The PCA identified T150 as the most tolerant to drought stress among the tested genotypes, whereas M82 was one of the most sensitive (Table 2). The results show that only the single factor of drought induced 23.3% reduction of shoot dry weight, while the interaction between genotype and drought was not significant (Table S6). In T150, drought caused 12.4% increase in root dry weight compared to the control, while in M82 this parameter did not change (Fig. 6A). With respect to the root:shoot

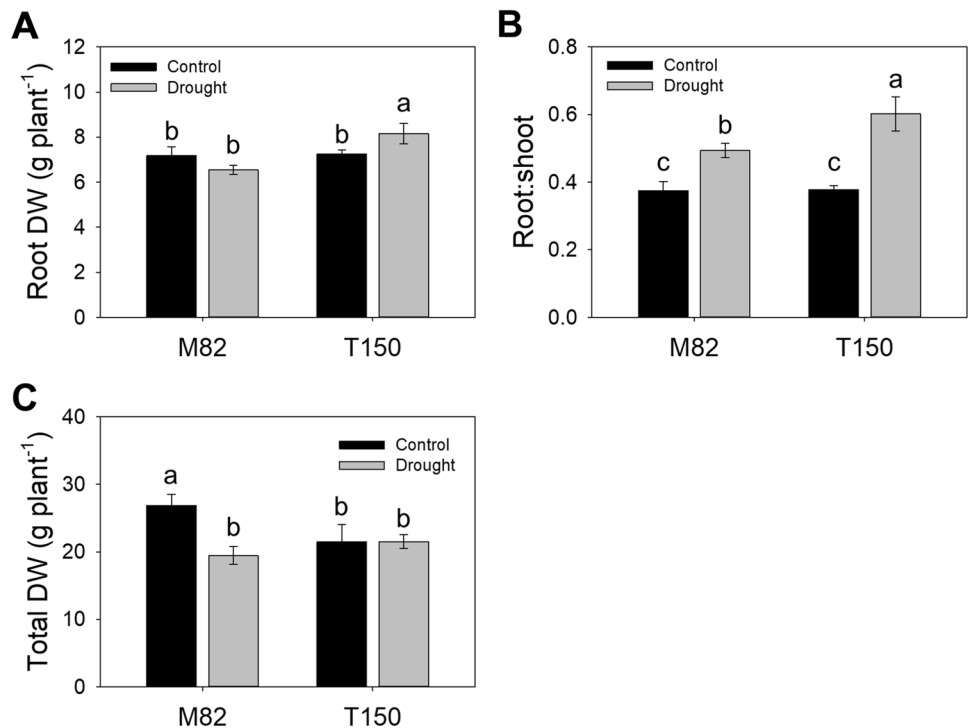
Table 2 Ranking of the 10 evaluated genotypes based on the distance between the two treatments (control vs drought)

Drought			
Genotype	Distance	z-score	Sig
T150	3.08	- 0.89	a
T147	3.18	- 0.77	a
T149	3.21	- 0.72	a
T327	3.70	- 0.12	ab
T313	3.73	- 0.09	ab
T249	4.03	0.27	b
T292	4.03	0.28	b
T336	4.25	0.54	bc
M82	4.27	0.57	bc
T151	4.92	1.36	c

Statistical analysis was performed according to ANOVA and Duncan's post-hoc test ($p < 0.05$)

ratio, both genotypes showed a significant increase of this parameter under drought compared to control conditions, but with a higher intensity in T150 (+75.9% increase) compared to M82 (+31.9%) (Fig. 6B). Finally, the total dry weight was not affected by drought in T150, while it showed significant decrease (-27.6%) in M82 plants (Fig. 6C).

Fig. 6 Biometric response to drought of the two selected tomato genotypes (sensitive M82 vs tolerant T150) in terms of **A** root dry weight, **B** root:shoot ratio, and **C** total dry weight. Different letters indicate significant variation between the means according to Duncan's post-hoc test ($p < 0.05$)



Leaf Transpiration and RWC (DS)

The leaf transpiration rate was only affected by the treatment factor, with -80% reduction in plants subjected to drought compared to control conditions (Table S7). The RWC was significantly affected by the combination of the two factors (Table S7). In particular, M82 showed a significant reduction of RWC compared to the control (-11.9%), while no change was found in T150 (Fig. 7).

Biochemical Measurements (DS)

The analysis of H₂O₂, proline and MDA provided further evidence of the higher tolerance to drought of line T150 compared to M82. The interaction between genotype and drought was significant for all three parameters evaluated (Table S8). Under drought, H₂O₂ and MDA content in tomato leaf were significantly higher in M82 compared to the control (+20.5% and +133%, respectively), while no change was found in T150 (Fig. 8A, B). Drought stress induced a significant increase of leaf proline content in both M82 and T150 plants, with remarkably higher levels in M82 (14-fold change) vs. T150 plants (4.93-fold change) (Fig. 9).

Gene Expression (DS)

Under drought conditions, *CAT* (*Solyc04g082460*) was significantly upregulated only in T150 (2.4-fold change), while its expression did not change in M82 compared to control

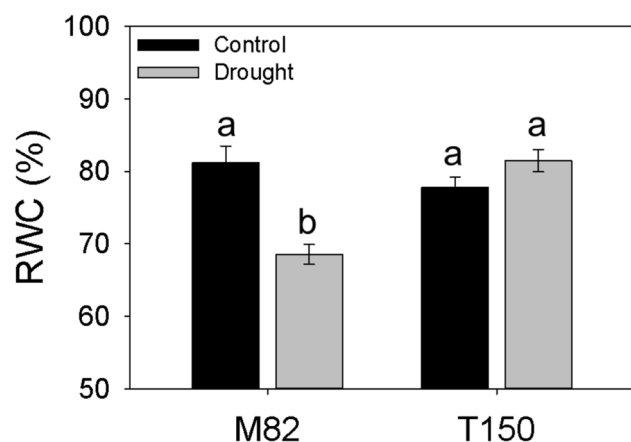


Fig. 7 Relative water content (RWC) of the two selected tomato genotypes (sensitive M82 vs tolerant T150) in response to drought. Different letters indicate significant variation between the means according to Duncan's post-hoc test ($p < 0.05$)

plants (Fig. 10A). With respect to drought stress, *LEA* gene (*Solyc03g116390*) was significantly up-regulated compared to the control in M82 under drought stress (4.1-fold change), while T150 did not show a similar up-regulation (Fig. 10B).

Discussion

Landraces as a Source of Genetic Traits for Low-Input Agriculture

Excess use of fertilizers has a detrimental effect on soil quality and underground water due to mineral leaching in the soil profile (Feng et al. 2024). Moreover, the amount of good quality water for irrigation will decrease in the next years due to climate change (Dasgupta et al. 2017). Increasing the resource use efficiency of tomato cultivation is one of the main goals to improve the sustainability of agricultural productions, as well as to maintain adequate productivity levels for an expanding world population. Different strategies have been proposed to reduce water and fertilizers overuse with

respect to the actual crop needs. Technological advances, including nano-based encapsulated and slow-release fertilizers (Madzokere et al. 2020), and precision irrigation and fertilization (Abioye et al. 2020; Lu et al. 2022) have progressed along with the search for genetic resources that are more efficient in water and fertilizers use (Snowdon et al. 2021). Landraces can have an important role in this context. Although they produce less than commercial hybrids, they have conserved tolerance traits to environmental constraints and yield stability under low input management, both of which are critical in future agricultural scenarios (Fullana-Pericàs et al. 2019; Marone et al. 2021; Ochieng et al. 2021; Thabet et al., 2024c). In addition, wild relatives of tomato such as *S. chilense* and *S. pennellii* represent valuable sources of genetic variation for introgression of traits of interest in cultivated tomato. Landraces and *S. pennellii* introgression genotypes including backcross inbred lines (BILs) can therefore represent an important source of stress tolerance genes to be re-introduced in modern tomato varieties for improving their adaptability to low input agriculture. The ranking obtained from our study shows that

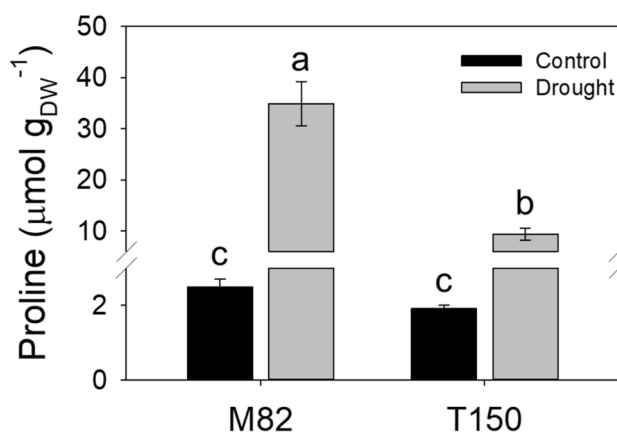


Fig. 9 Proline leaf content of the two selected tomato genotypes (sensitive M82 vs tolerant T150) in response to drought. Different letters indicate significant variation between the means according to Duncan's post-hoc test ($p < 0.05$)

Fig. 8 Biochemical response to drought of the two selected tomato genotypes (sensitive M82 vs tolerant T150) in terms of A H_2O_2 and B) malondialdehyde (MDA). Different letters indicate significant variation between the means according to Duncan's post-hoc test ($p < 0.05$)

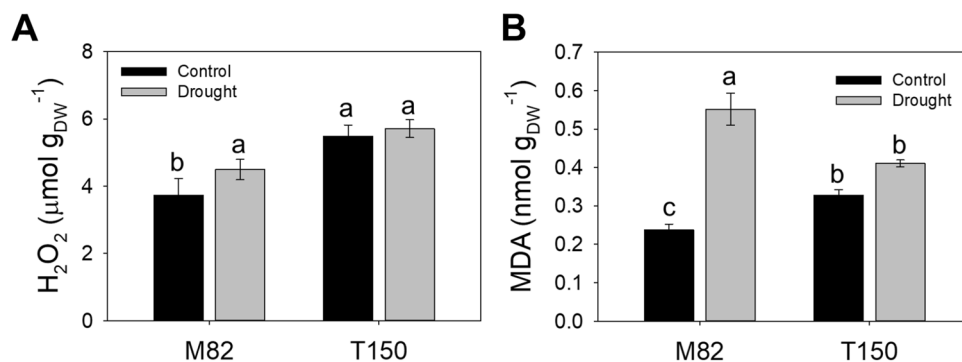
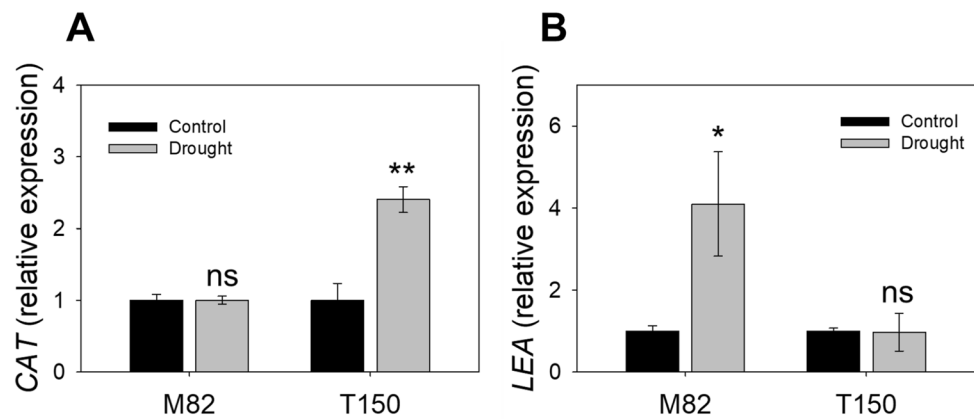


Fig. 10 Relative gene expression of A) *CAT* and B) *LEA* of the two selected tomato genotypes (sensitive M82 vs tolerant T150) in response to drought. Statistical analysis was performed within each genotype. Asterisks indicate significant differences according to Student's t-test (ns = non-significant; * = $p < 0.05$; ** = $p < 0.01$)



M82, a commercial tomato variety broadly used as a control in comparative experiments, was less tolerant both under nitrate limitation as well as under water deficiency (Table 1; Table 2) compared with the landraces we tested. This seems to be consistent with the view that modern varieties have lost tolerance traits and/or adaptation mechanisms in favor of higher yield and quality of the product, along with an agriculture that has increasingly relied on heavy use of chemicals for improving yield rather than resource use efficiency and/or yield stability. Although *yield increase* was and still is necessary to feed a growing world population, *efficiency in resource use* is becoming as well critical and it fits well the overarching goal of increasing the sustainability in agriculture (Ochieng et al. 2021; Tagiakas et al. 2022).

Morpho-Physiological and Biochemical Traits for Enhanced Tolerance to Nitrogen Deficiency

Agriculture-derived nitrate leaching is one of the most challenging sources of water eutrophication and contamination. Therefore, improving nitrogen use efficiency (NUE) in agricultural crops is an important target to reduce nitrate leaching and overall use in agriculture. Our results show that the tested landraces presented different nitrogen needs for an optimal biomass accumulation. When compared to the reference genotype M82, the landraces T292, T149 and T313 were more tolerant according to the results of the Euclidean distance calculated from the PCA (Table 1; Figure S2). It is worth to note that M82 showed one of the lowest degrees of tolerance to nitrate deficiency, confirming the lower adaptability of this modern variety to resource limitations. The aboveground and belowground biomass accumulation was severely impacted by nitrogen deficiency in M82 (Fig. 1A–B). In contrast, the aboveground biomass accumulation was not reduced by nitrogen deficiency in T292 (Fig. 1A), and the root biomass was significantly higher (Fig. 1B). Larger root systems have been proved to be beneficial under nitrogen limitations, since they may explore bigger soil volumes

thus delaying symptoms of nutrient shortage (Islam et al. 2021; Jia et al. 2020). This is particularly true for nitrate, that is a highly mobile ion in the soil solution (Chen et al. 2020). Based on its tolerance to nitrogen deficiency, the landrace T292 was selected for further analysis. Nitrate remobilization has been indicated as an effective strategy to increase NUE in different plant species (Abenavoli et al. 2016; Chen et al. 2020; Islam et al. 2021). *NRT2.1* is a high-affinity nitrate transporter localized in the root cortex. We found that the encoding gene was significantly upregulated both in M82 and in T292 under low nitrogen compared to control conditions (Fig. 5B). On the contrary, the expression of the dual/low affinity nitrate transporter *NRT1.1* in leaf tissues was not upregulated under low nitrogen conditions in M82, while its expression was significantly higher in T292 (Fig. 5A). This appears to be linked with the results on root nitrate content. Indeed, M82 showed significantly higher nitrate content in root tissues under low nitrogen conditions compared to the control, indicating that the missed upregulation of *NRT1.1* in the leaves has probably impaired the ability of this variety to translocate nitrate from the root to the shoot. An opposite response was found in T292, in which the nitrate content in the root was significantly lower under low nitrogen compared to the control, indicating a greater ability for nitrate translocation from root to shoot in T292 compared to M82 under nitrogen starvation. Considering that tomato is an annual crop, the ability to functionalize an internal nitrogen pool, as it occurred in T292, that would otherwise be lost in the environment with plant residues, could be an important trait for crops growing under reduced nitrogen. The ability to store and remobilize nitrate pools in stems and roots has been correlated with higher NUE in rice (Fan et al. 2007). Other than the direct effects of nitrogen starvation, nitrogen deficiency also activated ROS production (Machado et al. 2024; Safi et al. 2021). Therefore, we investigated whether along with expression of genes correlated with nitrate translocation, also the ROS detoxification machinery was more effective in the low nitrogen tolerant

line T292. Notwithstanding the higher *SOD6* expression of M82 plants exposed to low nitrogen conditions (Fig. 4A), this did not protect the membranes from oxidative damage, as suggested by the higher MDA concentration observed in plants grown under low nitrogen, which is a secondary product of oxidative damage (Fig. 2B). This can also be linked with the missing *CAT* upregulation in M82 (Fig. 4A) that did not detoxify H₂O₂ (Fig. 2A) and could have been responsible for further oxidative damage to the membranes (Qureshi et al. 2022). On the contrary, the low nitrogen tolerant genotype T292 showed an upregulation of *CAT* (Fig. 4A) that reduced the H₂O₂ leaf content (Fig. 2A) preventing oxidative damages, as confirmed by the low MDA levels in leaf tissues (Fig. 2B). Upregulation of *SOD6* and downregulation of *CAT* following nitrogen deficiency in nitrogen-starvation sensitive lines has been previously described in Machado et al. (2024) and it confirms the importance of high *CAT* activity to counteract the negative effects of nitrogen deficiency on plant oxidative balance.

Biomass Allocation and ROS Detoxification for Higher Drought Tolerance

Drought causes high yield losses in tomato production. The high-water requirements of this crop cannot always be fulfilled in arid and semi-arid areas, a condition that will likely be exacerbated as consequence of climate change and demographic growth (Alqudah et al. 2024b). Wild relatives of *S. lycopersicum* have been well studied and have been identified as an important source of genes for abiotic stress tolerance (Bergougnoux 2014). Introgression of *S. pennellii* traits into *S. lycopersicum* improved tomato stress tolerance to drought, heat, disease resistance, but also some quality parameters such as firmness and aroma (Henschel et al. 2023; Vitale et al. 2023; Yang et al. 2016). Our results shows that the genotype T150, which is an introgression of *S. pennellii*, had the highest tolerance to drought stress as opposed to the control M82 that had among the lowest degrees of drought tolerance within the tested lines (Table 2). These findings confirmed that the modern varieties have lost drought tolerance traits in favor of yield increase, or other fruit parameters useful for tomato processing (Bai and Lindhout 2007; Silva et al. 2019). The response of T150 seems also to be correlated with the higher biomass allocation to the root compared to M82 when exposed to drought (Fig. 6A). Notwithstanding the similar reduction in shoot biomass under water shortage between T150 and M82, T150 showed an increase of the root:shoot ratio in response to drought stress (Fig. 6B). Therefore, the biomass accumulation in T150 was not impaired as in M82, as confirmed by the results on total dry biomass (*i.e.*, root + shoot dry biomass) (Fig. 6C). A bigger root volume is an important trait for drought tolerance, since it facilitates water uptake

from the soil (Uga et al. 2013). Biomass allocation therefore represents a functional adaptation trait of T150 plants, which had a competitive advantage under water limitation. It can be speculated that the bigger root volume allowed T150 plants to maintain a better water status, as indicated by the higher RWC of these plants compared to M82 (Fig. 7). This was consistent with the unchanged expression of *LEA* gene in T150 drought stressed plants (Fig. 10B), a key marker of osmotic stress, as well as the lower proline content of T150 compared to M82 plants (Fig. 9), which is also a well-known indicator of plant stress (Maggio et al. 2002). Further evidence of the lower sensitivity to drought of T150 is provided by the lower concentrations of H₂O₂ and MDA measured in their leaves compared to M82 under stressful conditions (Fig. 8A-B), both of which are well known markers of oxidative stress in plants. As for other abiotic stresses (Alqudah et al. 2024c; Thabet et al., 2024d), the production of ROS is the main cause of cellular malfunctioning in plants exposed to water shortage (Zou et al. 2020). Therefore, also for drought tolerance, an efficient antioxidant machinery is necessary to overcome membrane damages, which will cause growth reduction. Under drought stress, T150 showed an upregulation of *CAT* compared to control conditions, while in M82 the expression of this gene was not altered (Fig. 10A). This can explain the lower MDA accumulation in leaf tissues found in T150 compared to M82 (Fig. 8B), which clearly indicates that the higher tolerance of T150 to drought stress can be partially mediated by a more functional ROS detoxification system. Therefore, the increased root:shoot ratio under drought stress, with the dual function of reducing plant transpiration in favor of the overall root uptake capacity, combined with higher ROS detoxification capacity mediated by *CAT* upregulation, seemed to be a major adaptation strategy of the T150 genotype to better tolerate drought stress compared to M82.

Conclusions

The results of this study demonstrate that tomato landraces and introgression genotypes from wild species can be an important source of tolerance traits for coping with nitrogen and water shortage, two environmental conditions that will be more frequent in the next future due to climate change. We identified physiological traits of high interest that could be targets to improve resource use efficiency in tomato production. Among them, higher antioxidant activity, functional biomass partitioning, and improved storage and remobilization of the nitrogen pools are the ones that can be of high interest for future sustainability-oriented breeding programs. The definition of the physiological/molecular path mediating nitrogen use efficiency in the genotype T292 is of high importance, since to the best of our knowledge there

is scattered understanding of plant mechanisms that may improve nitrogen use efficiency. Landraces have proven to be a valuable genetic resource to understand critical adaptation mechanisms and eventually improve the sustainability of tomato production. Moreover, the response of *S. pennellii* BIL T150 to water shortage confirmed the higher tolerance of wild tomato relatives and their potential to improve water use efficiency in tomato crop. However, integration of data from metabolomic, genomic, transcriptomic, and proteomic analyses could provide further insights in the complexity of plant-stress interactions. In conclusion, the traits correlated with the higher nitrogen/water use efficiency in T292 and T150 can represent a basis for future breeding strategies oriented to increase sustainability in agriculture. Further experiments will be performed to evaluate the impact of these traits on yield and product quality of plants exposed to nitrogen starvation and/or drought to allow their use in real agricultural contexts.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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References

- Abenavoli MR, Longo C, Lupini A, Miller AJ, Araniti F, Mercati F, Princi MP, Sunseri F (2016) Phenotyping two tomato genotypes with different nitrogen use efficiency. *Plant Physiol Biochem* 107:21–32. <https://doi.org/10.1016/j.plaphy.2016.04.021>
- Abioye EA, Abidin MSZ, Mahmud MSA, Buyamin S, Ishak MHI, Rahman MKIA, Otuoze AO, Onotu P, Ramli MSA (2020) A review on monitoring and advanced control strategies for precision irrigation. *Comput Electron Agric* 173:105441. <https://doi.org/10.1016/j.compag.2020.105441>
- Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ* 24:1337–1344. <https://doi.org/10.1046/j.1365-3040.2001.00778.x>
- Alqudah AM, Elkesh A, Abu-Elsaoud AM, Hassan SE-D, Thabet SG (2024a) Genome-wide association study reveals the genetic basis controlling mineral accumulation in wheat grains under potassium deficiency. *Genet Resour Crop Evol*. <https://doi.org/10.1007/s10722-024-01999-0>
- Alqudah AM, Thabet SG, Safhi FA, Elkesh A (2024b) Unveiling into genetic architecture of transgenerational stress memory via promoting drought tolerance in barley. *J Soil Sci Plant Nutr*. <https://doi.org/10.1007/s42729-024-01864-0>
- Alqudah AM, Elkesh A, Alammari BS, Alsubeie MS, Hamed SM, Thabet SG (2024c) Genetic mapping determining the key Genomic loci/QTNs for stress resilience via controlling antioxidant defenses in barley under salt stress. *Plant Mol Biol Rep*. <https://doi.org/10.1007/s11105-024-01488-9>
- Bai Y, Lindhout P (2007) Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? *Ann Bot* 100:1085–1094. <https://doi.org/10.1093/aob/mcm150>
- Batelli G, Ruggiero A, Esposito S, Venezia A, Lupini A, Nurcato R, Costa A, Palombieri S, Vitiello A, Mauceri A, Cammareri M, Sunseri F, Grandillo S, Granel A, Abenavoli MR, Grillo S (2024) Combined salt and low nitrate stress conditions lead to morphophysiological changes and tissue-specific transcriptome reprogramming in tomato. *Plant Phys Biochem* 215:108976. <https://doi.org/10.1016/j.plaphy.2024.108976>
- Bergougnoux V (2014) The history of tomato: From domestication to biopharming. *Biotechnol Adv* 32:170–189. <https://doi.org/10.1016/j.biotechadv.2013.11.003>
- Biermann RT, Bach LT, Kläring HP, Baldermann S, Börnke F, Schwarz D (2022) Discovering tolerance—a computational approach to assess abiotic stress tolerance in tomato under greenhouse conditions. *Front Sustain Food Syst* 6:1–12. <https://doi.org/10.3389/fsufs.2022.878013>
- Bolger A, Scossa F, Bolger ME, Lanz C, Maumus F, Tohge T, Quesneville H, Alseekh S, Sørensen I, Lichtenstein G, Fich EA, Conte M, Keller H, Schneeberger K, Schwacke R, Ofner I, Vrebalov J, Xu Y, Osorio S, Aflitos SA, Schijlen E, Jiménez-Gómez JM, Rynagillo M, Kimura S, Kumar R, Koenig D, Headland LR, Maloof JN, Sinha N, van Ham RCHJ, Lankhorst RK, Mao L, Vogel A, Arsova B, Panstruga R, Fei Z, Rose JKC, Zamir D, Carrari F, Giovannoni JJ, Weigel D, Usadel B, Fernie AR

- (2014) The genome of the stress-tolerant wild tomato species *Solanum pennellii*. *Nat Genet* 46:1034–1038. <https://doi.org/10.1038/ng.3046>
- Caramante M, Roupheal Y, Corrado G (2023) Genetic diversity among and within tomato (*Solanum lycopersicum* L.) landraces grown in Southern Italy. *Genet Resour Crop Evol* 71:157–166. <https://doi.org/10.1007/s10722-023-01613-9>
- Carrillo V, Castillo R, Magrì A, Holzapfel E, Vidal G (2024) Phosphorus recovery from domestic wastewater: a review of the institutional framework. *J Environ Manage* 351:119812. <https://doi.org/10.1016/j.jenvman.2023.119812>
- Claussen W (2005) Proline as a measure of stress in tomato plants. *Plant Sci* 168:241–248. <https://doi.org/10.1016/j.plantsci.2004.07.039>
- Chen KE, Chen HY, Tseng CS, Tsay YF (2020) Improving nitrogen use efficiency by manipulating nitrate remobilization in plants. *Nat Plants* 6:1126–1135. <https://doi.org/10.1038/s41477-020-00758-0>
- da Silva AC, de Lima M, F., Eloy, N.B., Thiebaut, F., Montessoro, P., Hemerly, A.S., Ferreira, P.C.G., (2019) The Yin and Yang in plant breeding: the trade-off between plant growth yield and tolerance to stresses. *Biotechnol Res Innov* 3:73–79. <https://doi.org/10.1016/j.biori.2020.02.001>
- Dasgupta N, Ranjan S, Ramalingam C (2017) Applications of nanotechnology in agriculture and water quality management. *Environ Chem Lett* 15:591–605. <https://doi.org/10.1007/s10311-017-0648-9>
- Di Stasio E, Cirillo V, Raimondi G, Giordano M, Esposito M, Maggio A (2020) Osmo-priming with seaweed extracts enhances yield of salt-stressed tomato plants. *Agronomy*. <https://doi.org/10.3390/agronomy10101559>.
- 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. <https://doi.org/10.1093/genetics/141.3.1147>
- Fan X, Jia L, Li Y, Smith SJ, Miller AJ, Shen Q (2007) Comparing nitrate storage and remobilization in two rice cultivars that differ in their nitrogen use efficiency. *J Exp Bot* 58:1729–1740. <https://doi.org/10.1093/jxb/erm033>
- Feng Y, Xiong Y, Hall-Spencer JM, Liu K, Beardall J, Gao K, Ge J, Xu J, Gao G (2024) Shift in algal blooms from micro- to macroalgae around China with increasing eutrophication and climate change. *Glob Chang Biol* 30:1–16. <https://doi.org/10.1111/gcb.17018>
- Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O (2015) Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. *Front Plant Sci* 6:1–14. <https://doi.org/10.3389/fpls.2015.00978>
- Fullana-Pericàs M, Conesa M, Douthe C, El Aou-ouad H, Ribas-Carbó M, Galmés J (2019) Tomato landraces as a source to minimize yield losses and improve fruit quality under water deficit conditions. *Agric Water Manag* 223:105722. <https://doi.org/10.1016/j.agwat.2019.105722>
- Giorio P, Cirillo V, Caramante M, Oliva M, Guida G, Venezia A, Grillo S, Maggio A, Albrizio R (2020) Physiological basis of salt stress tolerance in a landrace and a commercial variety of sweet pepper (*Capsicum annuum* L.). *Plants* 9:795
- Guida G, Sellami MH, Mistretta C, Oliva M, Buonomo R, De Mascellis R, Patanè C, Roupheal Y, Albrizio R, Giorio P (2017) Agronomical, physiological and fruit quality responses of two Italian long-storage tomato landraces under rain-fed and full irrigation conditions. *Agric Water Manag* 180:126–135. <https://doi.org/10.1016/j.agwat.2016.11.004>
- Henschel JM, Batista DS, de Souza de Voltare H, Silva Júnior AD, Ribas AF, da Silva DF, Zeist AR, (2023) Selection for drought tolerance in backcross populations derived from interspecific crosses of *Solanum lycopersicum* × *Solanum pennellii*. *Plant Breed*. <https://doi.org/10.1111/pbr.13154>
- Islam S, Zhang J, Zhao Y, She M, Ma W (2021) Genetic regulation of the traits contributing to wheat nitrogen use efficiency. *Plant Sci* 303:110759. <https://doi.org/10.1016/j.plantsci.2020.110759>
- Jaiswal AK, Mengiste TD, Myers JR, Egel DS, Hoagland LA (2020) Tomato domestication attenuated responsiveness to a beneficial soil microbe for plant growth promotion and induction of systemic resistance to foliar pathogens. *Front Microbiol* 11:1–19. <https://doi.org/10.3389/fmicb.2020.604566>
- Jia Z, Giehl RFH, Von Wirén N (2020) The root foraging response under low nitrogen depends on dwarf1-mediated brassinosteroid biosynthesis. *Plant Physiol* 183:998–1010. <https://doi.org/10.1104/pp.20.00440>
- Landi S, Punzo P, Nurcato R, Albrizio R, Sanseverino W, Aiese Cigliano R, Giorio P, Fratianni F, Batelli G, Esposito S, Grillo S (2023) Transcriptomic landscape of tomato traditional long shelf-life landraces under low water regimes. *Plant Physiol Biochem* 201:107877. <https://doi.org/10.1016/j.plaphy.2023.107877>
- Lázaro A (2018) Tomato landraces: an analysis of diversity and preferences. *Plant Genet Resour Characterisation Util* 16:315–324
- Lezhneva L, Kiba T, Ferial-Bourrellier AB, Lafouge F, Boutet-Mercey S, Zoufan P, Sakakibara H, Daniel-Vedele F, Krapp A (2014) The Arabidopsis nitrate transporter NRT2.5 plays a role in nitrate acquisition and remobilization in nitrogen-starved plants. *Plant J* 80(2):230–241. <https://doi.org/10.1111/tbj.12626>
- Lu Y, Liu M, Li C, Liu X, Cao C, Li X, Kan Z (2022) Precision fertilization and irrigation: progress and applications. *AgriEngineering* 4:626–655. <https://doi.org/10.3390/agriengineering4030041>
- Machado J, Heuvelink E, Vasconcelos MW, Cunha LM, Finkers R, Carvalho SMP (2023) Exploring tomato phenotypic variability under combined nitrogen and water deficit. *Plant Soil*. <https://doi.org/10.1007/s11104-023-06023-5>
- Machado J, Fernandes APG, Bokor B, Vaculík M, Heuvelink E, Carvalho SMP, Vasconcelos MW (2024) The effect of silicon on the antioxidant system of tomato seedlings exposed to individual and combined nitrogen and water deficit. *Ann Appl Biol* 184:50–60. <https://doi.org/10.1111/aab.12849>
- Madzokere TC, Murombo LT, Chiririwa H (2020) Nano-based slow releasing fertilizers for enhanced agricultural productivity. *Mater Today Proc* 45:3709–3715. <https://doi.org/10.1016/j.matpr.2020.12.674>
- Maggio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsz B, Narasimhan ML, Hasegawa PM, Joly RJ, Bressan RA (2002) Does proline accumulation play an active role in stress-induced growth reduction? *Plant J* 31:699–712. <https://doi.org/10.1046/j.1365-3113X.2002.01389.x>
- Manaigo F, Rouwenhorst K, Bogaerts A, Snyders R (2024) Feasibility study of a small-scale fertilizer production facility based on plasma nitrogen fixation. *Energy Convers Manag* 302:118124. <https://doi.org/10.1016/j.enconman.2024.118124>
- Marone D, Russo MA, Mores A, Ficco DBM, Laidò G, Mastrangelo AM, Borrelli GM (2021) Importance of landraces in cereal breeding for stress tolerance. *Plants*. <https://doi.org/10.3390/plants10071267>
- Massaretto IL, Albaladejo I, Purgatto E, Flores FB, Plasencia F, Egea-Fernández JM, Bolarin MC, Egea I (2018) Recovering tomato landraces to simultaneously improve fruit yield and nutritional quality against salt stress. *Plant Sci, Front*. <https://doi.org/10.3389/fpls.2018.01778>
- Mehle N, Bačnik K, Bajde I, Brodarić J, Fox A, Gutiérrez-Aguirre I, Kitek M, Kutnjak D, Loh YL, Ferreira MC, O., Ravnikar, M., Vogel, E., Vos, C., Vučurović, A., (2023) Tomato brown rugose fruit virus in aqueous environments – survival and significance of water-mediated transmission. *Front Plant Sci* 14:1–15. <https://doi.org/10.3389/fpls.2023.1187920>
- Moles TM, Pompeiano A, Huaranca Reyes T, Scartazza A, Guglielminetti L (2016) The efficient physiological strategy of a

- tomato landrace in response to short-term salinity stress. *Plant Physiol Biochem* 109:262–272. <https://doi.org/10.1016/j.plaphy.2016.10.008>
- Ochieng G, Ngugi K, Wamalwa LN, Manyasa E, Muchira N, Nyamongo D, Odeny DA (2021) Novel sources of drought tolerance from landraces and wild sorghum relatives. *Crop Sci* 61:104–118. <https://doi.org/10.1002/csc2.20300>
- Ofner I, Lashbrooke J, Pleban T, Aharoni A, Zamir D (2016) Solanum pennellii backcross inbred lines (BILs) link small genomic bins with tomato traits. *Plant J* 87:151–160. <https://doi.org/10.1111/tbj.13194>
- Pessoa HP, Dariva FD, Copati MGF, de Paula RG, de Oliveira Dias F, Gomes CN (2023) Uncovering tomato candidate genes associated with drought tolerance using Solanum pennellii introgression lines. *PLoS ONE* 18:e0287178. <https://doi.org/10.1371/journal.pone.0287178>
- Qureshi MK, Gawronski P, Munir S, Jindal S, Kerchev P (2022) Hydrogen peroxide-induced stress acclimation in plants. *Cell Mol Life Sci* 79:1–16. <https://doi.org/10.1007/s00018-022-04156-x>
- Ruggiero A, Landi S, Punzo P, Possenti M, Van Oosten MJ, Costa A, Morelli G, Maggio A, Grillo S, Batelli G (2019) Salinity and ABA seed responses in pepper: expression and interaction of ABA core signaling components. *Front Plant Sci* 10:304. <https://doi.org/10.3389/fpls.2019.00304>
- Ruggiero A, Punzo P, Van Oosten MJ, Cirillo V, Esposito S, Costa A, Maggio A, Grillo S, Batelli G (2022) Transcriptomic and splicing changes underlying tomato responses to combined water and nutrient stress. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2022.974048>
- Safi A (2023) Starved? Time for SNAC1: a nitrogen starvation-responsive transcription factor that promotes nitrate uptake. *Plant Physiol* 192(4):2580–2581. <https://doi.org/10.1093/plphys/kiad308>
- Safi A, Medici A, Szponarski W, Martin F, Clément-Vidal A, Marshall-Colon A, Ruffel S, Gaymard F, Rouached H, Leclercq J, Coruzzi G, Lacombe B, Krouk G (2021) GARP transcription factors repress *Arabidopsis* nitrogen starvation response via ROS-dependent and -independent pathways. *J Exp Bot* 72:3881–3901. <https://doi.org/10.1093/jxb/erab114>
- Shah WUH, Lu Y, Liu J, Rehman A, Yasmeen R (2024) The impact of climate change and production technology heterogeneity on China's agricultural total factor productivity and production efficiency. *Sci Total Environ* 907:168027. <https://doi.org/10.1016/j.scitotenv.2023.168027>
- Snowdon RJ, Wittkop B, Chen TW, Stahl A (2021) Crop adaptation to climate change as a consequence of long-term breeding. *Theor Appl Genet* 134:1613–1623. <https://doi.org/10.1007/s00122-020-03729-3>
- Tagiakas RI, Avdikos ID, Goula A, Koutis K, Nianiou-Obeidat I, Mavromatis AG (2022) Characterization and evaluation of Greek tomato landraces for productivity and fruit quality traits related to sustainable low-input farming systems. *Front Plant Sci* 13:1–19. <https://doi.org/10.3389/fpls.2022.994530>
- Thabet SG, Alqudah AM (2024) Unraveling the role of nanoparticles in improving plant resilience under environmental stress condition. *Plant Soil*. <https://doi.org/10.1007/s11104-024-06581-2>
- Thabet SG, Elkelish A, Alwutayed KM, Alqudah AM (2024) Genetic insights into stress resilience via enhancing salinity adaptation in barley. *Plant Mol Biol Report*. <https://doi.org/10.1007/s11105-024-01450-9>
- Thabet SG, Safhi FA, Börner A, Alqudah AM (2024a) Genetic associations of transgenerational stress memory in wheat under drought stress. *Environ Exp Bot* 226:105920. <https://doi.org/10.1016/j.envexpbot.2024.105920>
- Thabet SG, Safhi FA, Alqudah AM (2024b) Identification of key genes regulating macronutrient accumulation and final yield in wheat under potassium deficiency. *Plant Biotechnol Rep*. <https://doi.org/10.1007/s11816-024-00916-2>
- Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M (2013) Control of root system architecture by deeper rooting 1 increases rice yield under drought conditions. *Nat Genet* 45:1097–1102. <https://doi.org/10.1038/ng.2725>
- Vitale L, Francesca S, Arena C, D'Agostino N, Principio L, Vitale E, Cirillo V, de Pinto MC, Barone A, Rigano MM (2023) Multitraits evaluation of a Solanum pennellii introgression tomato line challenged by combined abiotic stress. *Plant Biol* 25:518–528. <https://doi.org/10.1111/plb.13518>
- Wu G, Chen X, Zang Y, Ye Y, Qian X, Zhang W, Zhang H, Liu L, Zhang Z, Wang Z, Gu J, Yang J (2024) An optimized strategy of nitrogen-split application based on the leaf positional differences in chlorophyll meter readings. *J Integr Agric* 23:2605–2617. <https://doi.org/10.1016/j.jia.2023.07.014>
- Yang S, Yu Q, Wang B, Yang T, Li N, Tang Y, Aisimutuola P, Wang Q, Xu J, Gao J (2016) Identification of QTLs for red fruit firmness using the wild tomato species Solanum pennellii LA716 introgression lines. *Plant Breed* 135:728–734. <https://doi.org/10.1111/pbr.12423>
- Zayed O, Hewedy OA, Abdelmoteleb A, Ali M, Youssef MS, Roumia AF, Seymour D, Yuan ZC (2023) Nitrogen journey in plants: from uptake to metabolism, stress response, and microbe interaction. *Biomolecules* 13(10):1443. <https://doi.org/10.3390/biom13101443>
- Zhang, J., Kirkham, M., 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. *New Phytol.* 132, 361–373. 1469-8137.1996.tb01856.x
- Zou YN, Wu QS, Kuča K (2020) Unravelling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress. *Plant Biol*. <https://doi.org/10.1111/plb.13161>

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