Received: 26 February 2024 | Revised: 13 September 2024 | Accepted: 1 October 2024

DOI: 10.1111/ppl.14579

ORIGINAL RESEARCH

Physiologia Plantarum

Rye-durum wheat 1BL.1RS translocation: implications for drought tolerance and nutritional status

Giulia Quagliata[1#] | Moez Maghrebi[2#] | Miriam Marín-Sanz[3] | Samuela Palombieri[1] | Francesco Sestili[1] | Domenico Lafiandra[1] | Francisco Barro[3] | Gianpiero Vigani[2] | Stefania Astolfi[1]

[1]Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy

[2]Department of Life Sciences and Systems Biology, University of Torino, Italy

[3]Department of Plant Breeding, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Córdoba, Spain

Correspondence
Stefania Astolfi,
Email: sastolfi@unitus.it

Funding information
Partnership for Research and Innovation in the Mediterranean Area, Grant/Award Number: J89C19000140005

Edited by M.A. Mutwil

Abstract

The translocation of the short chromosome arm 1RS of rye onto the 1B chromosome of common wheat has been shown to improve resistance to stress and yield. Here, translocation was operated in durum wheat and its effects on drought tolerance were evaluated. Both the 1BL.1RS translocation line (Svevo 1BL.1RS) and the corresponding Svevo control were exposed to drought for 7 days. Significant differences were found in root morphology between Svevo and Svevo 1BL.1RS under control and drought conditions. Although Svevo 1BL.1RS experienced more severe growth inhibition due to drought than Svevo, it exhibited greater resilience to oxidative stress. Furthermore, several drought-responsive genes were upregulated in both shoots and roots only in the translocation line. Notably, in roots of Svevo 1BL.1RS, the expression of these genes was also higher in the control condition compared to Svevo, suggesting that these genes could be constitutively expressed at higher levels in the translocation line. Moreover, the 1BL.1RS translocation had a significant impact on the plant's ability to accumulate nutrients under drought. Overall, the impact on sulfate accumulation and the expression of genes associated with its assimilation pathways are particularly noteworthy, highlighting the involvement of sulfur in the plant response to water stress. Additionally, the genetic characterization of Svevo 1BL.1RS revealed variants extending beyond the translocation, located in drought stress-responsive genes.

1 | INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is the most cultivated cereal species in the Mediterranean basin (Xynias et al., 2020). It represents an essential food source, as it provides carbohydrates, proteins, fiber, calcium, zinc, fats, and energy (Boukid et al., 2019). According to Coceral's crop report released in June 2023, Italy is the largest European wheat-producing country, covering over 50% of total European production (COCERAL CROP FORECAST, 2023). Durum wheat is considered rather adapted to high temperatures and semiarid climates and is traditionally grown under rainfed conditions; thus, its growth rate and yield largely depend on the availability of water in its habitat.

Decreased precipitation due to changing climatic conditions affects the whole planet, but the Mediterranean area is far more

[#] Equally contributed to this work

vulnerable, with increasingly hotter and more extended summer periods (Papamichael et al., 2022). Therefore, in this region, drought has become a major challenge for durum wheat cultivation, affecting both yield and also grain protein content and composition (Zhang et al., 2018). For instance, in Spain, the severe and prolonged drought has caused a 30% drop in country's production of durum wheat between 2022 and 2023 (COCERAL CROP FORECAST, 2023). Consequently, the Mediterranean area has become increasingly dependent on food imports, accounting for one-third of the global import of cereals (wheat) and Egypt has become the world's largest importer of wheat to feed its 100 million inhabitants.

The phenomenon of climate change is also combined with external shocks, such as the recent war between Russia and Ukraine, which has dramatically reduced agricultural production and trade, thus increasing the level of vulnerability on the broader region.

Given the increasing demand for food security, it is crucial to address the challenges posed by climate change on cereals. One way to effectively improve performance under water-limited environments is by selecting crop cultivars/genotypes with adaptive mechanisms to drought. To improve yield and resistance to various stresses, many useful genes have been introgressed into wheat through chromosome translocations or substitutions from different relative species (Molnár Láng et al., 2014; Feldman and Levy, 2015; Korobkova et al., 2023).

Rye (*Secale cereale* L.) has great potential for expanding the genetic variability of wheat (Yediay et al., 2010; Ren et al., 2017) and the translocation of the short chromosome arm 1RS of rye (*Secale cereale* L.) to the 1B chromosome of hexaploid wheat (*Triticum aestivum* L.) is a widely used genetic improvement technique for this crop from the late 1930s (Kattermann, 1938; Ren et al., 2017). The resulting translocation line showed a higher grain yield than normal lines due to an increase in shoot biomass and kernel weight (Yediay et al., 2010). In addition, several genes related to biotic and abiotic stress tolerance are located on the 1RS arm (Liu et al., 2020). Specifically, these genes are responsible for the specific resistance to major rust diseases, better adaptation, and tolerance to abiotic stresses such as drought (Kim et al., 2004; Zarco-Hernandez et al., 2005). Some studies also indicate that genes responsible for root development and water-stress resistance are situated in the distal section of chromosome 1RS (Sharma et al., 2009; Howell et al., 2014; Korobkova et al., 2023).

To date, only a few studies have utilized the translocation technique from the rye genome in durum wheat (Korobkova et al., 2023). Therefore, in this work, the 1BL.1RS translocation was obtained in the durum wheat (*Triticum durum* Desf.) variety Svevo and two main hypotheses were tested: (1) the translocation could affect root morphological traits, enabling plants to more efficiently uptake water and nutrients, thus leading to better performance in both control and unfavourable condition; and (2) the translocation directly and/or indirectly modulate plant response to drought and nutrient uptake. For this purpose, both the 1BL.1RS translocation line and the corresponding Svevo control were exposed to water stress for 7 days by applying PEG 6000 at 10% (w/v) to the nutrient solution. Their growth performance and drought tolerance were evaluated by combining morphological, physiological and molecular approaches.

2 | MATERIALS AND METHODS

2.1 | Production of the 1BL.1RS translocation line in the genetic background of the durum wheat variety Svevo

The 1BL.1RS wheat-rye translocation present in the durum wheat cultivar Cando (Friebe et al., 1987) was transferred in the durum wheat cultivar Svevo through crossing followed by five backcrosses.

2.2 | Plant growing conditions

Seeds of both the 1BL.1RS translocation line and the corresponding cv. Svevo were soaked in distilled water for 1 hour and allowed to germinate in aeroponics in the dark at 28°C for 4 days. Homogeneous seedlings were selected and transferred in hydroponic culture in plastic pots (3 seedlings/pot) containing 600 ml of a nutrient solution (Astolfi et al., 2018). Eight days after sowing, half of the plants were exposed to drought by adding PEG 6000 (10%, w/v) to the nutrient solution and the remaining half was kept in the control solution. PEG lowers the nutrient solution's osmotic potential, which then reduces the water availability to plant roots. This mimics the effect of drought in the soil, where water becomes less accessible to plants due to low water content.

Plants were kept in a climate chamber under controlled conditions with a day/night cycle of 16/8 h at 28/20°C air temperature, 80% relative humidity, and 200 µE m^{-2} s^{-1} light intensity. Shoot and root samples were collected 14 days after sowing in hydroponics.

2.3 | Determination of proline concentration

The proline concentration was estimated as described in Quagliata et al. (2023). The plant material (0.1 g) was homogenized in 2 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 16000 x *g* for 10 min. To the supernatant was added 500 µl acid ninhydrin and 500 µl of glacial acetic acid, which was boiled at 100°C for 1 h. The reaction was stopped in an ice bath. Then, the reaction mixture was extracted with 1.5 ml of toluene and absorbance was measured at 520 nm and expressed in µmol g^{-1} FW (fresh weight).

2.4 | Determination of malondialdehyde concentration

The level of lipid peroxidation was expressed by using malondialdehyde (MDA) concentration as a proxy (the higher the MDA content, the higher is the lipid peroxidation level). MDA concentration was determined according to Quagliata et al. (2021), using 2-thiobarbituric acid (TBA) reactive metabolites. Fresh shoot and root tissues (0.2 g) were homogenized in 2 ml of 0.25% TBA made in 10% TCA. The extract was heated at 95°C for 30 min and then quickly cooled on ice. After centrifugation at 16000 x *g* for 20 min, the absorbance of the

supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was expressed as μg g^{-1} FW by using an extinction coefficient of 155 mM cm^{-1}.

2.5 | Extraction and determination of glutamine synthetase and catalase activity

Shoot and root tissues (1 g fresh weight) were powdered in liquid N_2 and then homogenized using a buffer at pH 7.4, added in a ratio of 1:7 for leaves and 1:3 for roots (w/v), following extraction steps according to the method described by Coppa et al. (2018). Glutamine synthetase activity (GS; EC 6.3.1.2) was assayed spectrophotometrically (Agilent Cary 3500 UV-Vis Spectrophotometer) at 540 nm, as described in Astolfi et al. (2004).

Catalase (CAT; E.C. 1.11.1.6) activity was assessed by evaluating the disappearance of H_2O_2 at 240 nm (Agilent Cary 3500 UV-Vis Spectrophotometer) after extraction, according to Zamboni et al. (2017). Enzyme activity was linear with time and proportional to the amount of extract used.

2.6 | Determination of ionomic composition of plant tissues

The concentration of Mg, K, Ca, Cu, Mn, Fe, Zn and Mo was measured using an inductively coupled plasma-mass spectroscopy (Agilent 7850 ICP-MS). Briefly, shoot and root tissues were dried at 80°C for 24 hours. Approximately 0.1 g dry weight (DW) of both tissues were mineralized in 3 mL concentrated ultrapure HNO_3 and 0.6 mL of HCl using a Microwave digestion system (Multiwave Go Plus, Anton Paar GmbH). Subsequently, mineralized samples were filtered and diluted 1:200 with Milli-Q water.

Anion (Cl^-, SO_4^{2-}, NO_3^- and PO_4^{3-}) concentrations in root and shoot tissues were assessed by collecting and washing them with Milli-Q water, followed by a gentle soak with paper, rapid freezing in liquid nitrogen, and subsequent freeze-drying. Samples were ground with mortar and pestle in ultrapure water. Afterwards, the extracts underwent filtration with 0.22 μm filters and were analyzed using capillary electrophoresis (Agilent 7100, Agilent Technologies). Anions, such as chloride (Cl^-), sulfate (SO_4^{2-}), nitrate (NO_3^-), and phosphate (PO_4^{3-}), were analyzed through a bare fused silica capillary column with an extended light path BF3 (i.d. = 50 μm, l = 72 cm, L = 80.5 cm). Sample injection was followed by 50 mbar pressure for 4 s with -30 kV voltage and detection at the 350/380 nm wavelength. All anions were identified using pure standards (Fiorilli et al., 2022). The final anion concentrations in each sample were calculated as mg g^{-1} DW (dry weight).

2.7 | Gene expression analysis

Total RNA was extracted from roots and shoots using TRIzol® Reagent (Life Technologies) and then purified using PureLink® RNA Mini Kit (Life Technologies), according to the manufacturer's instructions. Contaminant DNA was removed on-column using PureLink® DNase (Life Technologies). First-strand cDNA synthesis was carried out using the SuperScriptTM III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen), according to the manufacturer's instructions.

qRT-PCR analysis of drought-responsive genes (*TdP5CS*, *TdDHN15.3*, *TdDRF1*, *TdSHN1* and *TdWRKY2*) and genes involved in sulfur homeostasis (*TdSULTR1.1*, *TdSULTR1.3*, *TdSAT1*, and *TdOASTL1*) was performed on first-strand cDNA in a 20 μL reaction mixture containing GoTaq® qPCR Master Mix (Promega) and specific primers (listed in Table S1), using QuantStudio 3 Real-Time PCR System (Applied Biosystems). The relative transcript level of each gene was calculated by the $2^{-\Delta\Delta Ct}$ method (Livaka and Schmittgen, 2001) using the expression of the *TdACTIN* gene as a reference.

2.8 | DNA extraction and Genotyping-By-Sequencing (GBS)

The total DNA from Svevo and Svevo 1BL.1RS, cultivated in a greenhouse with a 12-hour light/12-hour dark regime and temperatures of 24°C/16°C, was extracted in duplicate from leaves using the CTAB method (Murray and Thompson, 1980). CD Genomics (45-1 Ramsey Road, Shirley, NY, USA) conducted the GBS library preparation, ensuring DNA quality control, enzymatic digestion, and sequencing (Novaseq, 5 M PE150 reads per sample) for each duplicate. The PstI/MspI enzymes were employed for the digestion process.

2.9 | Variant calling analysis

Before the variant calling analysis, the raw reads with low quality and adapter sequences were trimmed using the fastp software with the --detect_adapter_for_pe option (Chen et al., 2018). Subsequently, the trimmed reads were mapped to the Svevo.v1 reference genome (Maccaferri et al., 2019). After mapping, quality control, and marking duplicates using samtools (Danecek et al., 2021), variant calling was executed with GATK (Van der Auwera and O·Connor, 2020). Briefly, the genomic VCF (gVCF) files generated by the HaplotypeCaller command were combined (CombineGCVFs) for joint genotyping of the multi-sample gVCF files (GenotypeGVCFs)). Single nucleotide polymorphisms (SNPs) and Insertions/Deletions (InDels) underwent a hard-filtering process with specific parameter values: (i) for SNPs: QD < 2.0, FS > 60.0, MQ < 40.0, SOR > 3.0, MQRankSum < -12.5, ReadPosRankSum < -8.0; (ii) for InDels: QD < 2.0, FS > 200.0, SOR > 10.0, ReadPosRankSum < -20. The annotation of the variants was carried out using the SnpEff software (Cingolani et al., 2012). For genomic variants-affected functional profiling, the Gene Ontology (GO) enrichment analysis was performed with gprofiler2 (Kolberg et al., 2020), and the heatmap was represented with pheatmap R package (Kolde and Kolde, 2015).

The data analysis was performed in a high-performance computing (HPC) system (24 nodes Bull x440, 192 GB of RAM per node, and

960 total cores) of the Computing Center of the University of Córdoba (Córdoba, Spain).

2.10 | Protein-protein interaction network analysis

The search tool for retrieval of interacting genes (String, 2023; https://string-db.org) database was used to acquire protein–protein interaction (PPI) networks of genes encoding the main transporters and enzymes involved in uptake, translocation and assimilation of sulfur and the main drought stress genes (Table S2). The protein sequences were used as query for STRING analysis and the interaction of known and predicted PPIs was applied to predict functional interactions of proteins in *Triticum aestivum* organism.

2.11 | Root morphology

Analysis of root morphology was performed at harvest (14 days after sowing). Briefly, roots were excised from the stem and subsequently placed in a perspex tray with a shallow film of water to avoid overlapping of roots. Images were analysed using WinRHIZO software (Regent Instruments Inc.) to determine some root-related parameters: total root length, root surface area, root volume, root average diameter and the number of root tips (Quagliata et al., 2023).

2.12 | Measurement of chlorophyll and protein content

Chlorophyll content was measured at harvest in attached leaves of wheat plants using a non-destructive portable apparatus, the Soil Plant Analysis Development (SPAD—502 Plus, Konica Minolta). Three independent measurements within each treatment were detected on the youngest fully expanded leaf, averaged, and expressed as SPAD units.

The protein concentration in crude extracts from shoot and root tissues was determined using the Bradford (1976) method using bovine serum albumin (BSA) as standard.

2.13 | Measurement of shoot and root water content

The amount of water in plant shoots and roots was used as plant water status measurement. Plant water content is the difference between tissue fresh and dry mass, which is that following drying to a constant mass at a specified temperature (usually about 85°C) (Spomer 1985).

2.14 | Statistical Analysis

Each reported value represents the mean ± standard deviation (SD) of measurements carried out in triplicate and obtained from three independent experiments (biological replicates). All data were statistically analysed to compare treatment condition (T) versus control (C) conditions by one-way analysis of variance (ANOVA) with student t-test at $p < 0.05$ by using the statistical software CoStat (version 6.45).

Using PAST 4.0.3 software, two principal component analysis (PCA) were applied: the first for nutrient concentration (Mg, K, Ca, Cu, Mn, Fe, Zn and Mo) and anion concentration (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}) and the second for root trait dataset. The hierarchical clustering was performed on the root traits datasets by complete method and Euclidean distance measurement in R (RStudio Desktop for Windows V. 2022.07.2+576), package heatmap.

3 | RESULTS

3.1 | Effects of translocation on plant growth performance under control and drought condition

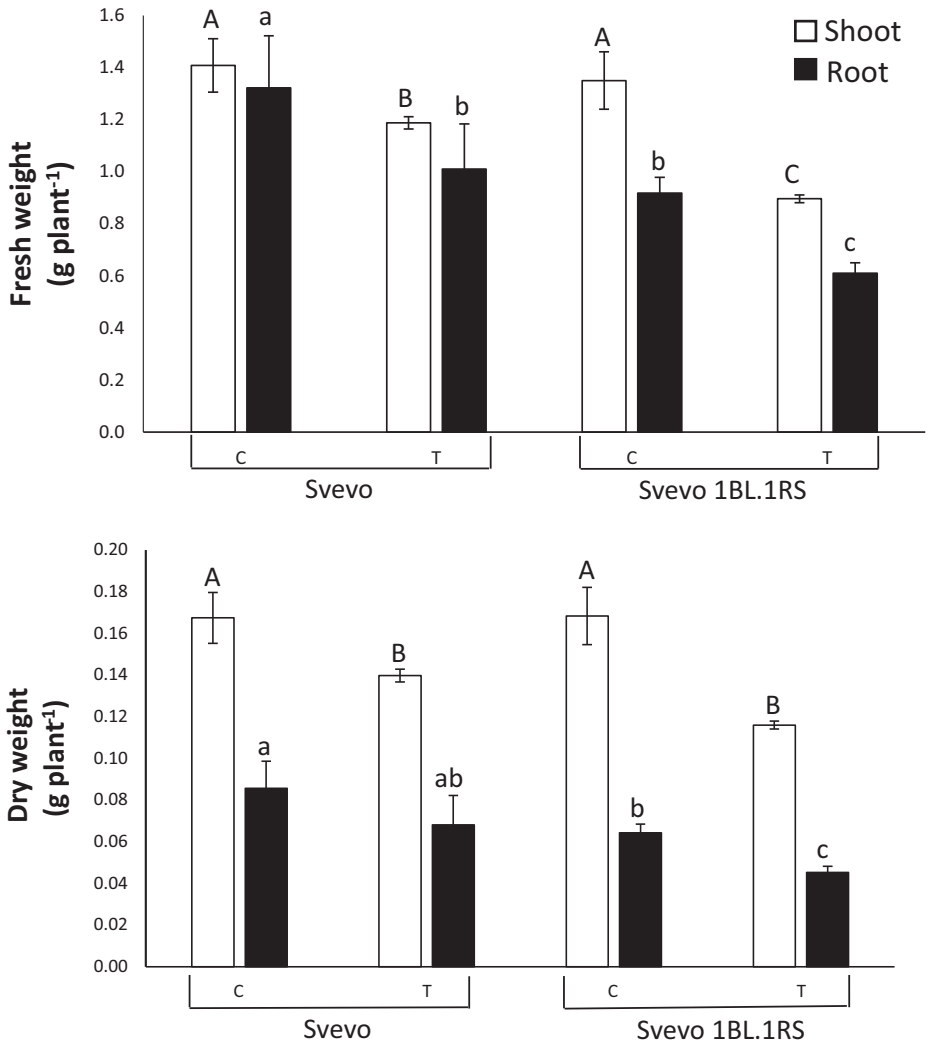
Under control condition, no significant differences were observed in shoot development between the two genotypes when assessed by both fresh and dry mass measurements (Figure. 1). However, Svevo 1BL.1RS exhibited significantly reduced root fresh (-30%) and dry (-25%) biomass compared to Svevo (Figure. 1).

Drought stress significantly inhibited the shoot and root fresh biomass accumulation in Svevo (−16 and 23%, respectively) and Svevo 1BL.1RS (-33% in both tissues), compared to their relative control (Figure. 1). In addition, under drought, Svevo 1BL.1RS showed a lower shoot (-25%) and root (-40%) fresh biomass production when compared to Svevo. As expected, when plants experienced water shortage treatment, both root and shoot water content was reduced by 24 and 16%, respectively, in Svevo and by 33% in both tissues in Svevo 1BL.1RS (Figure. 1).

The shoot-to-root ratio was higher in Svevo 1BL.1RS than in Svevo, irrespective of the growth conditions, and in both genotypes, it was not significantly affected by drought (Figure. 1).

The chlorophyll content was significantly reduced by drought only in Svevo 1BL.1RS (-21%, compared to its control) (Figure. 1). On the other hand, protein concentration was not significantly reduced by drought in both genotypes (Figure. 2).

Root system architecture (RSA) plays a crucial role in water uptake during drought; thus, some parameters related to root morphology, such as the length, surface area, volume, average diameter, and the number of root tips, were considered (Table S3). Principal component analysis (PCA) on the dataset showed that the first two principal components accounted for 96.9% of the variance and discriminated genotypes into four different clusters (Figure 3A). Overall, Svevo and Svevo 1BL.1RS plants clearly separate along the PC1, whereas plants grown in control condition and plants exposed to drought clearly separate along the PC2. This indicates that 72.2% (PC1) of variance is attributable to the



Shoot/root	1.08 a	1.19 ab	1.47 a	1.47 a
SPAD	53.1 a	48.3 ab	53.3 a	42.3 b
WCs	1.24 a	1.05 b	1.18 a	0.78 c
WCr	1.24 a	0.94 b	0.85 c	0.57 d

FIGURE 1 Fresh and dry weight of shoots (white bars) and roots (black bars), shoot-to-root ratio on FW basis, chlorophyll content (SPAD units), water content of shoots (WCs) and roots (WCr) (expressed in g) of both Svevo and Svevo 1BL.1RS durum wheat seedlings grown hydroponically in two different growth conditions: Control (C) and drought-treated (T). Data are reported as mean ± standard deviation (SD) of measurements carried out in triplicate and obtained from three independent experiments (biological replicates). All data were statistically analysed by one-way analysis of variance (ANOVA) with student t-test ($p < 0.05$). Different upper-case and lower-case letters indicate significantly different values among shoots and roots, respectively.

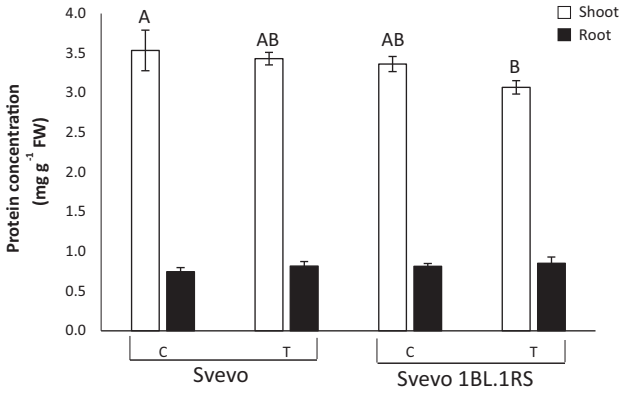
genotype, while 24,7% (PC2) is attributable to the treatment imposed (drought) (Figure. 3A). Notably, surface area, length, volume, average diameter, and number of tips of the root system drove the separation along the positive side of PC1, whereas root average diameter, surface area and volume were the strongest contributors along the positive side of PC2. Moreover, a hierarchical clustering was performed on root traits' values of both genotypes by complete method and Euclidean distance measurement. As shown in Figure 3B, Svevo control plants showed higher values of all the parameters analyzed compared to Svevo 1BL.1RS, indicating a more developed root system (cluster A). On the contrary, Svevo 1BL.1RS showed the lowest values of all parameters (cluster C). Interestingly, Svevo plants subjected to drought showed similar root morphological characteristics of Svevo 1BL.1RS under control condition (cluster B).

3.2 | Physiological and molecular response to drought stress

Proline is known to accumulate in response to drought since it plays a key role both as a compatible solute, helping to maintain cell turgor, and as an antioxidant compound, protecting cellular structures during periods of water stress (Farooq et al., 2012).

Drought induced a significant proline accumulation in shoots of both Svevo and Svevo 1BL.1RS plants (+3148% and +5115%, respectively, with respect to their control) (Figure. 4). On the other hand, we found an increased proline accumulation only in roots of Svevo plants (+365%) under drought (Figure 4). In higher plants, proline can be synthesized through two primary pathways: glutamate and ornithine. Emerging evidence suggests that glutamine synthetase (GS) activity plays a pivotal role in controlling proline accumulation

FIGURE 2 Protein concentration in shoots (white bars) and roots (black bars) of both Svevo and Svevo 1BL.1RS durum wheat seedlings. Growth conditions and statistics as in Figure 1



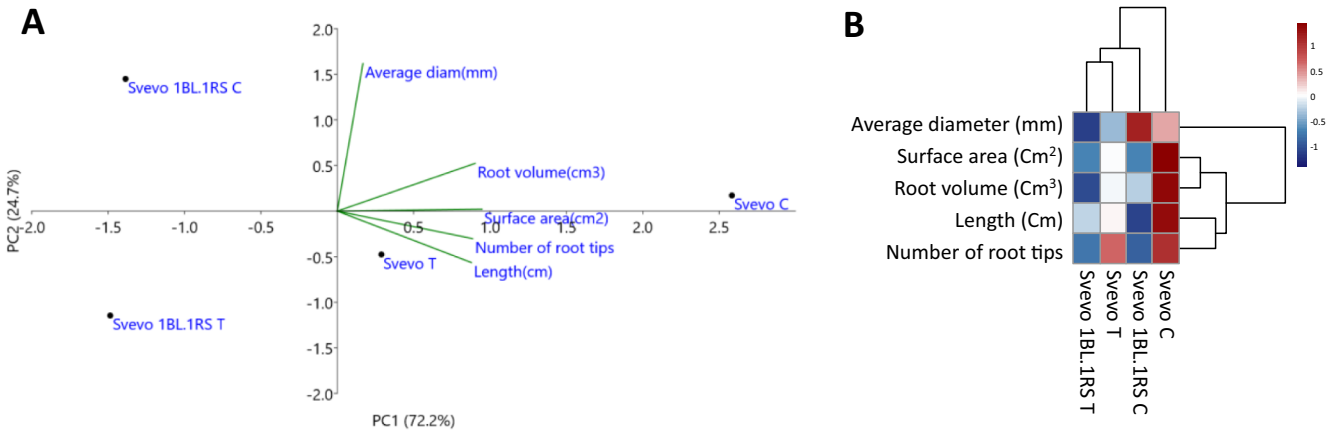


FIGURE 3 (A) Scatter plot of morphological root traits of both Svevo and Svevo 1BL.1RS durum wheat seedlings grown as described in Figure. 1. The first component (PC1) represents 72.2% and the second component (PC2) represents 24.7% of the total variance. PCA was performed using the mean of measurements carried out in triplicate and obtained from three independent experiments (biological replicates). See Supplementary Figure S1 for a scatter plot based on all considered root morphological traits in which each point in the plot represents an individual biological replicate for each condition for the two investigated genotypes. (B) Heatmap representing hierarchical clustering of root traits dataset of durum wheat plants by complete method and Euclidean distance measure. Three major clusters (A-B-C) were found, divided into: high values of root parameters (cluster A)., medium values of root parameters (cluster B) and low values of root parameters (cluster C). The colour bar depicts the gradient of values of root traits.

(Brugière et al. 1999; Meena et al., 2019; Yin et al., 2022). The activity of GS was not affected by drought at both shoot and root levels in Svevo 1BL.1RS plants, whereas Svevo plants showed a reduced GS activity in shoot tissues (-24% compared to the control) (Figure. 4). Interestingly, GS activity in shoot tissues of Svevo 1BL.1RS plants was 69% higher than in Svevo under drought (Figure. 4).

During water deficit condition, increased production of ROS commonly occurs, leading to oxidative stress and activation of antioxidant defence mechanisms by plants (Sallam et al., 2019). Malondialdehyde (MDA), being one of the end products of lipid peroxidation, is often used as a marker to assess the extent of oxidative damage in cells (Tirani and Haghjou, 2019).

Under drought, an increase of MDA production was observed only in Svevo shoots (+107%) compared to the control (Figure. 5), while Svevo 1BL.1RS plants did not show significant changes in MDA accumulation following drought treatment either in shoots or in roots (Figure. 5).

Concerning enzymatic antioxidants, by measuring catalase (CAT) activity, we did not find significant differences between control and treated plants for the Svevo genotype, whereas we found that CAT activity was even reduced in Svevo 1BL.1RS shoots under drought (-23%) compared to the control (Figure. 5).

The response to drought stress in plants also involves the activation of several genes that contribute to drought tolerance such as *TdP5CS* (encoding for pyrroline-5-carboxylate synthetase, involved in the synthesis of proline), *TdDHN15.3* (encoding for dehydrins), and some drought-responsive transcription factors, such as *TdDRF1*, *TdSHN1* and *WRKY2* (Rampino et al., 2006; Szabados & Savouré, 2010; Zhu et al., 2013).

In order to analyze the effect of drought stress on the transcript level of these genes, a qRT-PCR analysis was performed on total RNA extracted from the root and shoot tissues.

In Svevo plants, the steady-state level of the transcripts of *TdP5CS*, *TdDRF1*, *TdSHN1*, and *TdWRK2* genes was not significantly

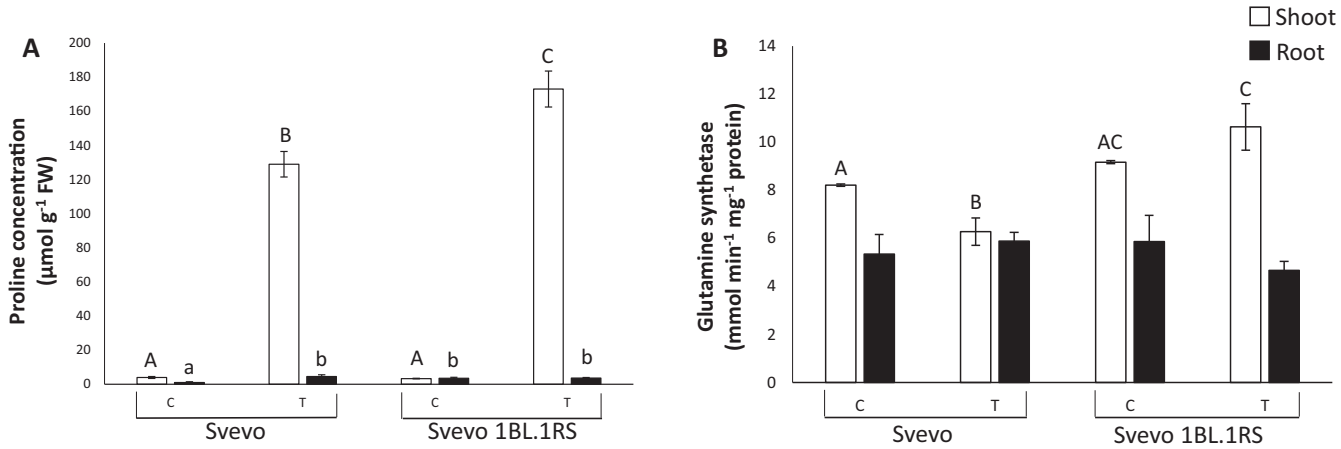


FIGURE 4 Proline concentration (A) and glutamine synthetase activity (B) in shoots (white bars) and roots (black bars) of both Svevo and Svevo 1BL.1RS durum wheat seedlings. Growth conditions and statistics as in Figure 1.

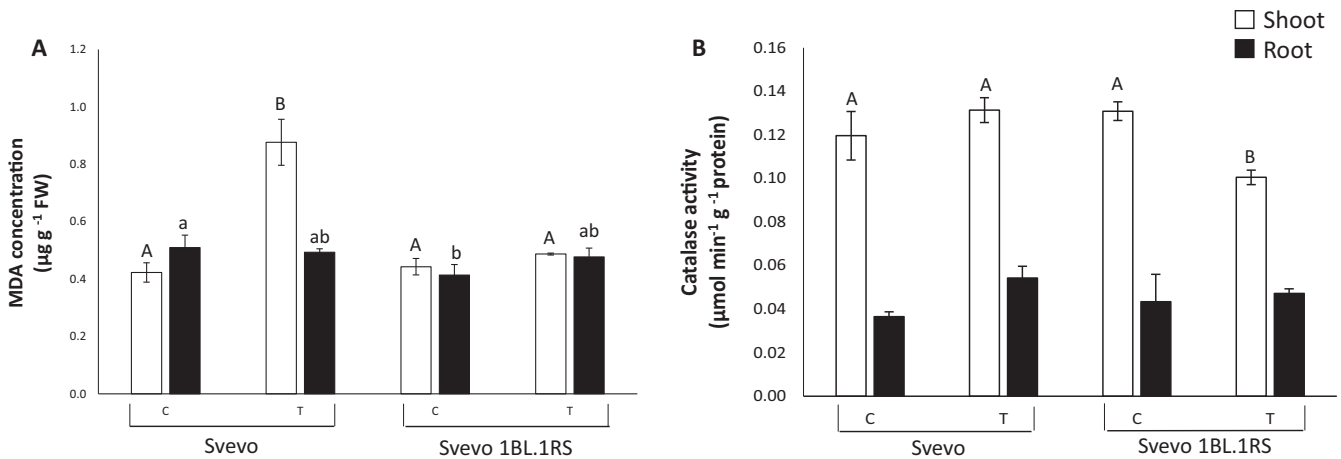


FIGURE 5 Malondialdehyde (MDA) concentration (A) and catalase activity (B) in shoots (white bars) and roots (black bars) of both Svevo and Svevo 1BL.1RS durum wheat seedlings. Growth conditions and statistics as in Figure 1.

affected by drought stress (Figure. 6), whereas the relative transcript levels of *TdDHN15.3* gene increased significantly under drought stress only in root tissue (Figure. 6). A different behavior was observed in Svevo 1BL.1RS plants, where relative transcript levels of the studied genes were strongly increased under drought stress, except for *TdSHN1* and *TdWRK2* genes whose expression was not influenced in shoot tissue (Figure. 6). Interestingly, the relative transcript abundance of the studied genes in the roots of Svevo 1BL.1RS under control condition was significantly higher than those of Svevo control plants. Additionally, a significant difference was found between the *TdDRF1* expression level in shoot tissue of both genotypes under control condition (Figure. 6).

3.3 | Ionomic characterisation of plant tissues

It is well known that drought stress significantly affects nutrient acquisition by plants, often resulting in nutrient imbalances (Chenu et al., 2017). Therefore, the characterization of ionomic profile has been determined on both root and shoot tissues.

Under control condition, Svevo 1BL.1RS accumulated higher amounts than Svevo of some macronutrients, such as K, Mg and Ca (+35, +28 and +21%, respectively), and of all measured micronutrients (+12 % Cu, +42% Fe, +28% Mn, +293% Zn and +40% Mo), at least in shoots. On the other hand, Svevo plants were able to accumulate more nitrate and sulfate in their shoot tissues (+27 and +81%, respectively).

Data reported in Figure 7 show that both Mg and Ca accumulation decreased under drought in shoot tissues of both genotypes (−13% and -15% for Mg and −25% and -32% for Ca, in Svevo and Svevo 1BL.1RS respectively). Drought-stressed Svevo seedlings displayed K and Mn concentration decrease in shoots (−21% and -38%, respectively) (Figure. 7). Moreover, K accumulation also decreased in root tissues of both genotypes (-32% and -20%, in Svevo and Svevo 1BL.1RS respectively), whereas root Mn concentration increased under drought (+30%) in Svevo 1BL.1RS with respect to the control condition (Figure. 7). Under drought, Svevo 1BL.1RS displayed a decreased concentration of both Fe and Zn of about -68% and -70%, respectively, compared to control condition (Figure. 7). Finally, plants of both genotypes exposed to drought showed a significant decrease of Mo concentration in both shoots and roots (-45% and -41% for Svevo; -67% and -98% for Svevo 1BL.1RS) (Figure. 7). The only nutrient whose accumulation was not affected by drought was Cu (Figure. 7).

Besides cations, free anions, such as nitrate, phosphate, sulfate and chloride, are present in plant cells and they play an essential role in plant physiology and nutrition.

Our results reveal that drought imposition resulted in increased accumulation of nitrate, phosphate and chloride only in shoot tissues of Svevo 1BL.1RS plants (+47%, +40% and +42%, respectively) compared to the control condition (Figure. 8). Interestingly, sulfate accumulation was significantly induced by drought in root and shoot of both genotypes. In particular, in drought-stressed plants of Svevo, the concentration of SO_4^{2-} increased by about 72% and 92% at shoot and root level, respectively, whereas it increased at a higher extent in Svevo 1BL.1RS (153% and 190% in shoots and roots, respectively) (Figure. 8).

All dataset was subjected to PCA to distinguish different nutrient accumulation as a function of the growth condition (Figure. 9). For shoots (Figure. 9A), the first two components of PCA described 94.8% of the variance and discriminated genotypes and treatments into four different clusters: Svevo and Svevo 1BL.1RS under control conditions were separated along PC1, while they distributed along PC2 under stress conditions. The distribution along PC1 was driven in the positive direction by Mg, K, Ca, Cu, Mn, Fe, Zn and Fe but in the negative direction by Cl^-, SO_4^{2-}, NO_3^- and PO_4^{3-}. The distribution along PC2 was determined in the positive direction by Mg, K, Ca, Cu, Mn, Cl^-, NO_3^- and PO_4^{3-} but in the negative direction by Fe, Zn, Mo and SO_4^{2-}. For roots (Figure. 9B), PC1 and PC2 represented 89.7% of the variance and discriminated genotypes and treatments into four different clusters, according to genotype (along PC1) and according to treatment (along PC2). Along PC1, the distribution was driven in the positive direction by Mg, Ca, Cu, Mn, Fe, Mo, Cl^-, SO_4^{2-} and NO_3^- and in the negative direction by K, Zn and PO_4^{3-}. Meanwhile, along PC2, the distribution was determined in the positive direction by Mg, K, Ca, Fe, Mo, Cl^- and PO_4^{3-} and in the negative direction by Cu, Mn, Zn, SO_4^{2-} and NO_3^-.

3.4 | Expression of genes coding for high-affinity transporters and enzymes involved in sulfate assimilation

The accumulation pattern of sulfate led us to investigate drought-induced changes in the sulfate metabolic pathway by analysing the expression of genes coding for two high-affinity sulfate transporters (*TdSultr1.1* and *TdSultr1.3*) and enzymes involved in the sulfate assimilation, such as O-acetylserine(thiol)lyase (*TdOASTL1*) and serine acetyltransferase (*TdSAT1*).

All genes analyzed were preferentially expressed in shoots and were upregulated by stress only in Svevo 1BL.1RS shoots, except for *TdOASTL1*, which showed up-regulation also at the root level (Figure. 10A-H).

As already observed for the genes involved in the drought response, the transcript level of all genes analysed in roots of Svevo 1BL.1RS plants was significantly higher than in Svevo under control condition (Figure. 10B-1H).

Finally, to predict the interaction network among proteins encoded by genes of interest, STRING analysis was performed (Figure. 11). Based on these data, we found that the two high-affinity sulfate transporters (*TdSultr1.1* and *TdSultr1.3*) only shared one common partner, *TdP5CS*, which encodes the enzyme P5C synthetase responsible for the conversion of glutamate to P5C (Δ1-Pyrroline-5-carboxylate) which will be then converted into proline (Figure. 11). The latter protein also interacts with *TdDRF1* (Dehydration responsive element-binding factor protein) a transcription factor characterized

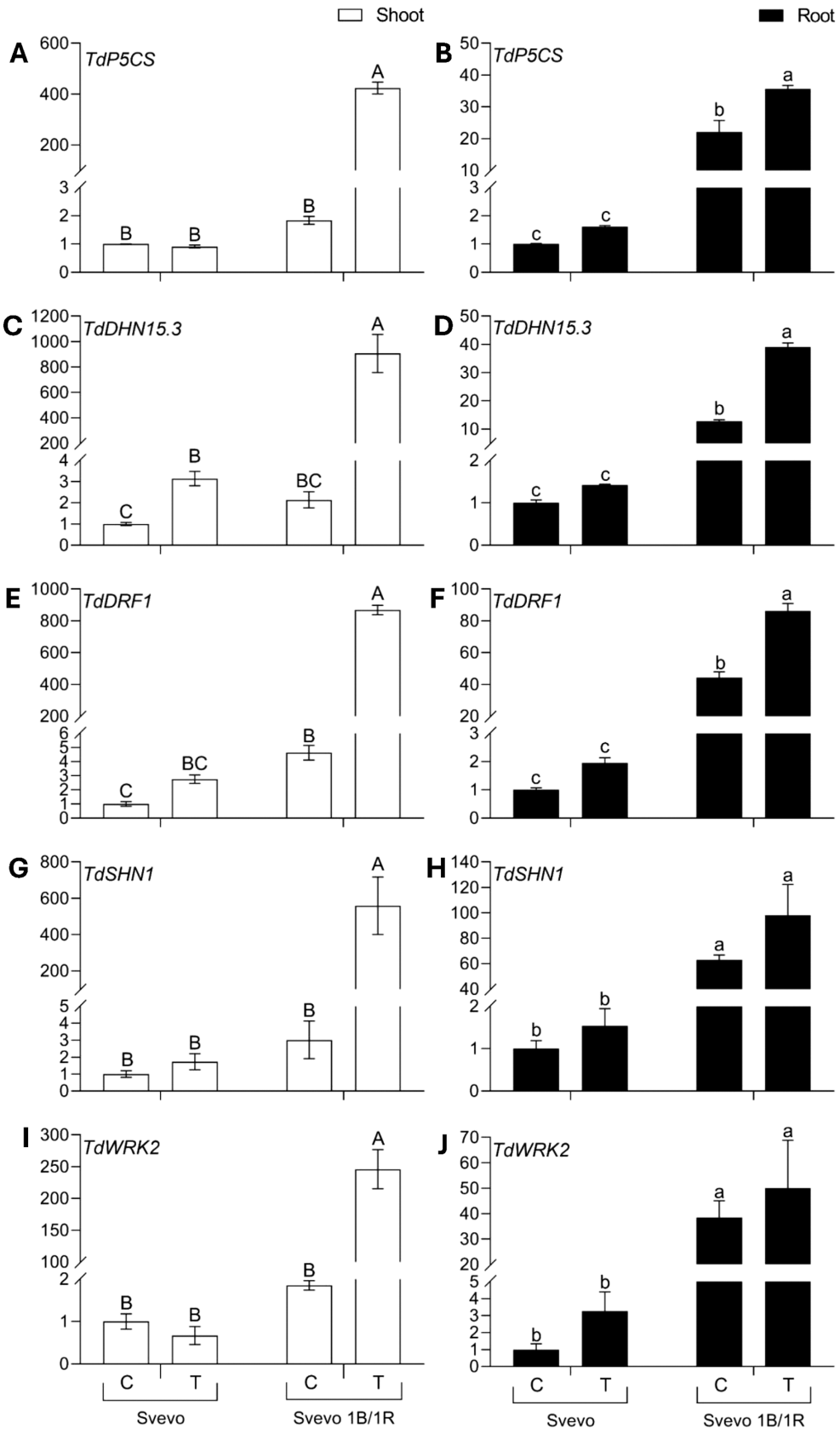


FIGURE 6 Analysis of the expression of several genes involved in water stress response: TdP5CS (A, B), TdDHN15.3 (C, D), TdDRF1 (E, F), TdSHN1 (G, H), TdWRKY2 (I, J), at both shoot (white bars) and root (black bars) level of durum wheat plants. Growth conditions and statistics as in Figure 1.

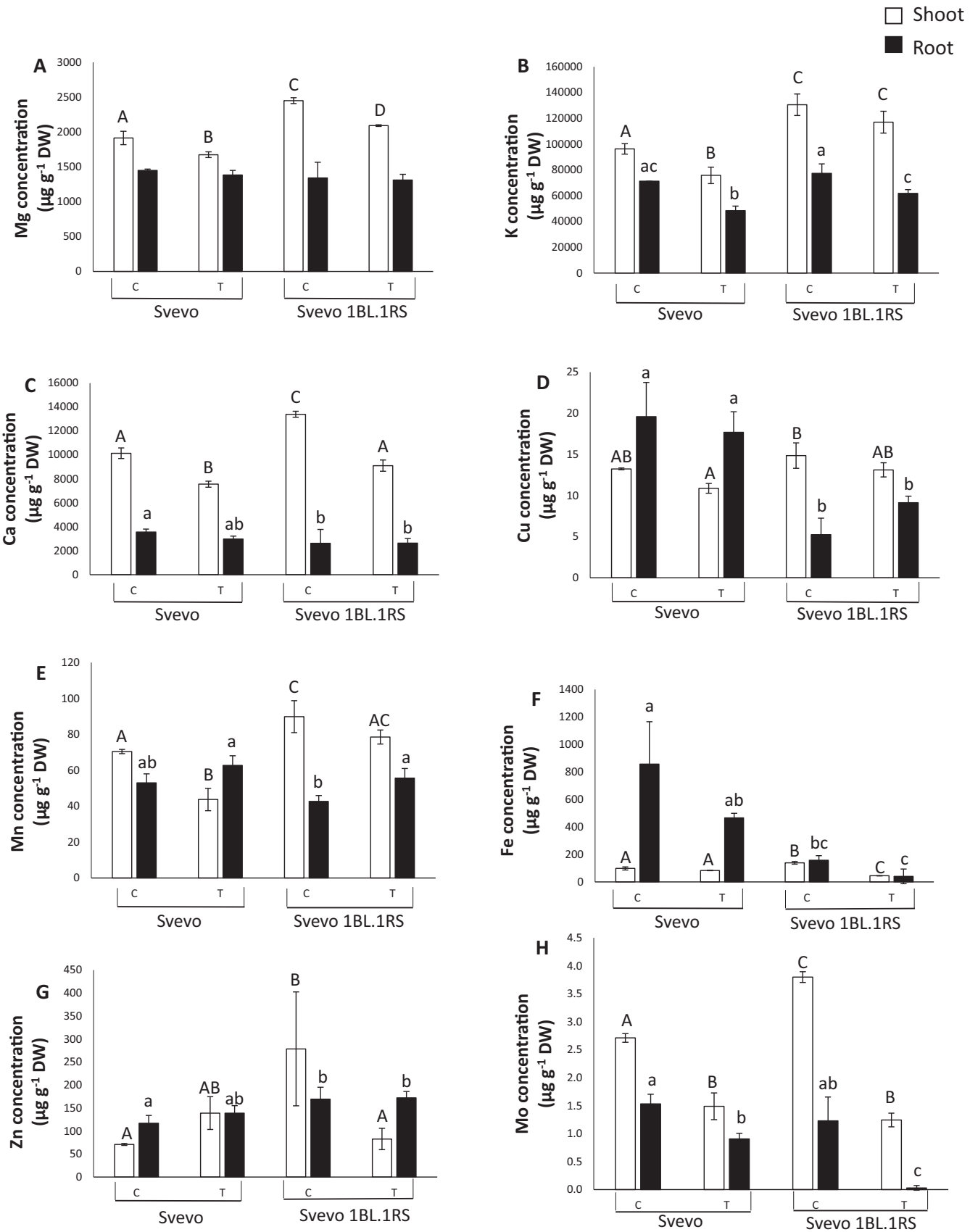


FIGURE 7 Mg (A), K (B), Ca (C), Cu (D), Mn (E), Fe (F), Zn (G) and Mo (H) concentrations in shoots (white bars) and roots (black bars) of both Svevo and Svevo 1BL.1RS durum wheat seedlings. Growth conditions and statistics as in Figure 1.

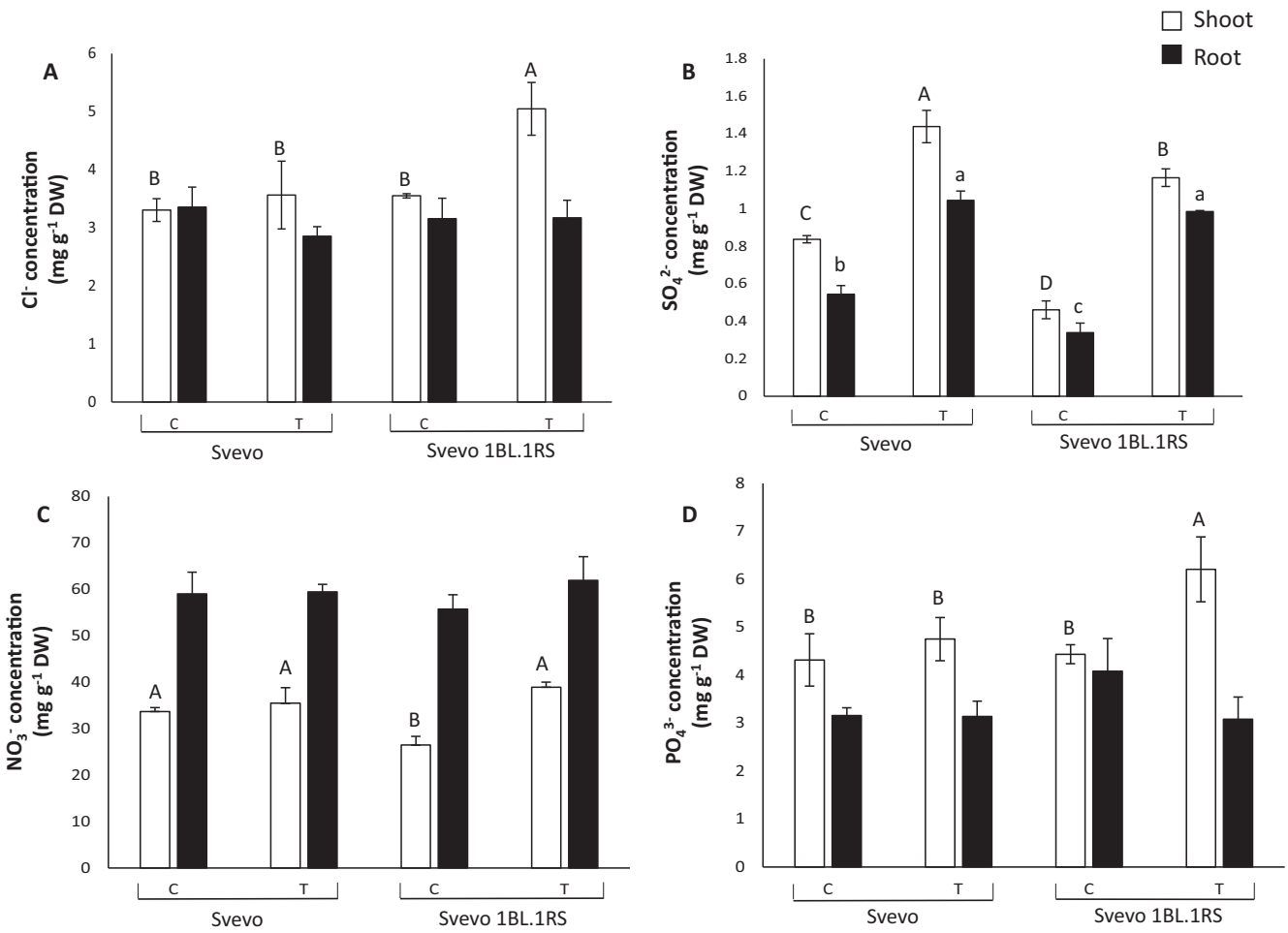


FIGURE 8 Cl^- (A), SO_4^{2-} (B), NO_3^- (C), PO_4^{3-} (D) concentrations in shoots (white bars) and roots (black bars) of both Svevo and Svevo 1BL.1RS durum wheat seedlings. Growth conditions and statistics as in Figure 1.

with an AP2-ERF domain (Figure. 11). The functional enrichment analysis of STRING interaction showed only one enriched category: *the multifunctional anion exchangers*.

3.5 | Genome-wide variants differences between Svevo and Svevo 1BL.1RS

The GBS analysis revealed notable differences in the quantity and impact of genome-wide SNPs and InDels detected between Svevo and Svevo 1BL.1RS, affecting the durum wheat genetic background. Focusing on the variants that differ between both genotypes, Svevo 1BL.1RS presented the highest proportion situated in the intergenic regions (Figure. 12A, Table S4). For the variants located in protein-coding genes and their adjacent regions, the most prevalent ones were identified in regions up- or down-stream of the genes (spanning a length of 5,000 base pairs). Subsequently, gene variants, such as missense, frameshift, or stop-gained mutations, were also relevant in this genotype (Figure. 12A, Table S4). Most of the Svevo 1BL.1RS variants were SNPs (Figure. 12C), with a small proportion of InDels in which the insertions/deletions of 2 and 3 bp were over-represented (Figure. 12B).

These variants affected a substantial number of genes associated with various GOs. Illustrating the functional profile of the affected functions, Figure 12D indicates that biological processes such as response to stress or response to stimulus exhibited a higher proportion of variants in Svevo 1BL.1RS compared to other functions. In processes closely linked to water deprivation or response to water, including dehydrins, Svevo and Svevo 1BL.1RS displayed different proportions of genes related to these GOs with SNPs/InDels (Figure. 12E). A comprehensive list of the variants found in these GOs-related genes is provided in Table S5. For example, Svevo 1BL.1RS exhibited a variant causing a frameshift effect in histidine kinase genes. Similarly, this genotype showed a higher proportion of missense variants than Svevo, affecting genes encoding antiholing-like proteins (Figure. 12E).

Remarkably, almost none of the genes examined for gene expression had variants in any of the genotypes tested (Figure 1D). Although the *TdSULTR1.1* and *TdOASTL1* genes of the B-subgenome showed polymorphisms in their up or downstream regions in Svevo, Svevo 1BL.1RS did not present any variant in either of them. These variations may be associated with the regulation of the expression of these specific genes (Figure. 12F).

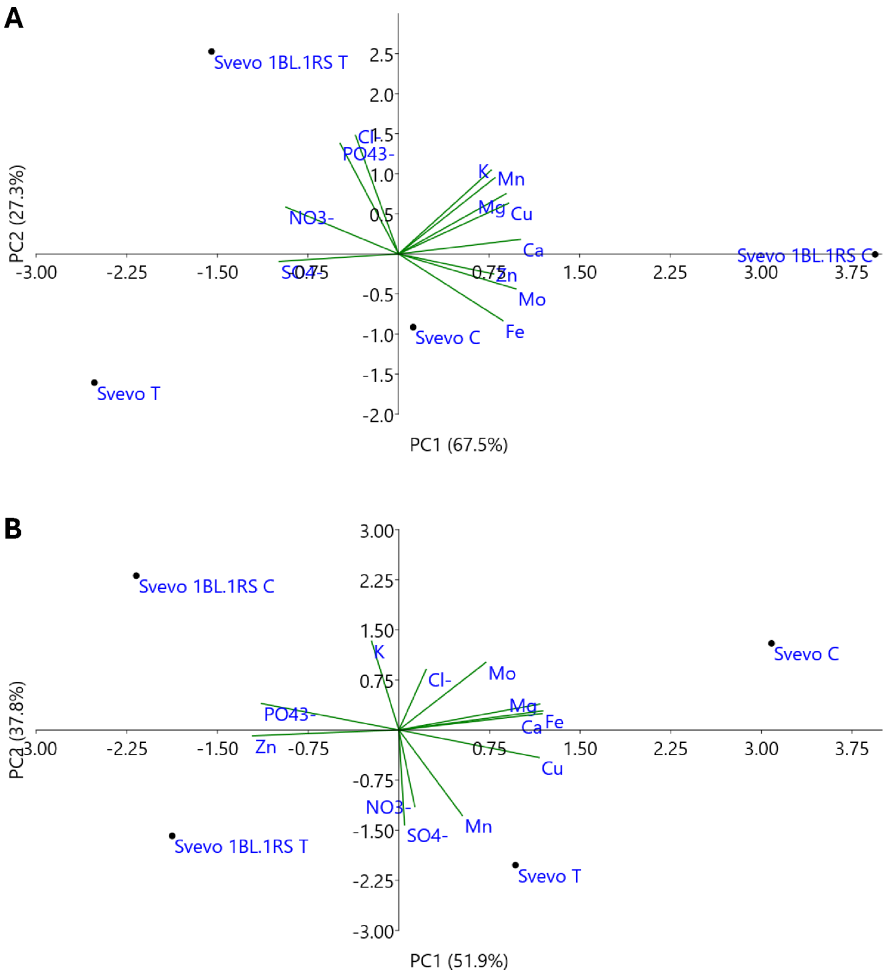


FIGURE 9 Scatter plot of shoots (A) and roots (B) ionomic composition (Mg, K, Ca, Cu, Fe, Mn, Zn, Mo, Cl^-, SO_4^{2-}, NO_3^- and PO_4^{3-}) of both Svevo and Svevo 1BL.1RS durum wheat seedlings grown hydroponically as described in Figure 1. For shoots (A), the first component (PC1) represents 67.5% and the second component (PC2) represents 27.3% of the total variance. For roots (B), the first component (PC1) represents 51.9% and the second component (PC2) represents 37.8% of the total variance. PCA was performed using the mean of measurements carried out in triplicate and obtained from three independent experiments (biological replicates). See Figure S2 for scatter plots in which each point in the plot represents an individual biological replicate for each condition for the two investigated genotypes.

4 | DISCUSSION

Drought tolerance is an important trait for increasing and stabilizing durum wheat productivity, particularly in dry areas such as the Mediterranean basin. This study was conducted to investigate the drought tolerance mechanisms at the seedling stage in two durum wheat genotypes with contrasting drought tolerance, the 1BL.1RS translocation line (Svevo 1BL.1RS) (relatively drought tolerant) and the corresponding Svevo (relatively drought sensitive) (Bacher et al., 2022; Quagliata et al., 2023). Both genotypes were tested by measuring several morphological, physiological, and molecular traits under drought and control conditions.

4.1 | Plant growth performance and root morphology under control and drought condition

In contrast with a previous study in bread wheat, showing that the presence of 1RS arm increased root biomass (Sharma et al., 2010), we found that the 1BL.1RS translocation line showed a lower developed root system when compared with Svevo. This contrast may have resulted from the different stages at which the plants were analysed. Specifically, this study harvested plants at the seedling stage, while the previous study was at the late-tillering stage. However, it cannot be entirely ruled out that the translocation may have a species-specific impact.

In agreement with Sharma et al. (2022) and Bhandari et al. (2021), who reported that drought stress limits plant growth rate, both genotypes displayed a reduction in shoot and root biomass under drought conditions, with the 1BL.1RS line being more severely affected. Svevo 1BL.1RS showed a higher shoot-to-root ratio than Svevo under both control and drought conditions. However, a drought-induced shift in the shoot-to-root ratio was not found in either of the two genotypes. It is widely reported that under drought, plants drive the allocation of biomass to the root system to improve the efficiency of water uptake, resulting in a lower shoot-to-root ratio (Correa et al., 2019). In our experiment, both genotypes maintained the shoot-to-root ratio during drought. This allowed them to find a trade-off between growth and root water absorption. Vegetative shoot growth in wheat has proven to be an important trait for its critical impact on photosynthetic capacity, in turn affecting crop yield (Bacher et al., 2022). Accordingly, the increase of chlorophyll density has also been identified as a drought-tolerant trait (Puangbut et al., 2017). In the present study, it was found that exposure of durum wheat plants to drought significantly reduced the chlorophyll content only in Svevo 1BL.1RS.

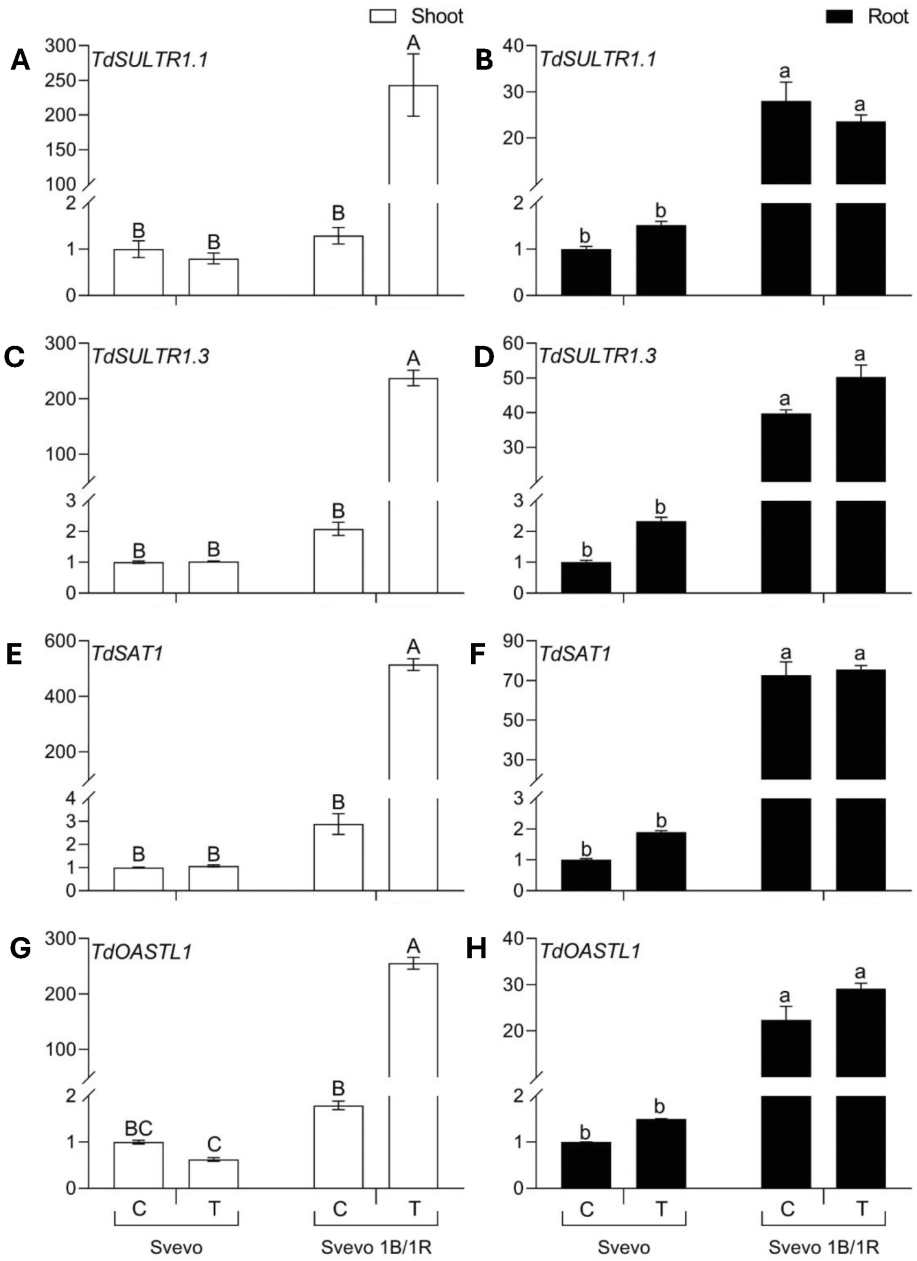


FIGURE 10 Analysis of the expression of two high-affinity sulfate transporters. *TdSULTR1.1* (A, B), *TdSULTR1.3* (C, D) and the *TdSAT1* (E, F) and *TdOASTL1* (G, H) genes coding for two enzymes involved in S assimilation pathway in shoots (white bars) and roots (black bars) of both Svevo and Svevo 1BL.1RS durum wheat seedlings. Growth conditions and statistics as in Figure 1.

It has been previously shown that there is a gene (or genes) on the 1RS chromosome arm that affects root anatomy in bread wheat (Ehdaie et al., 2003; Sharma et al., 2009). Accordingly, the 1BL.1RS translocation affected the shape and structure of the root system. In particular, the roots apparatus of the 1BL.1RS translocation line was characterized by thicker and shorter roots compared to Svevo under control conditions, thus resulting in a lower root surface area. It is well known that under water deficit conditions, plants modify their root systems to cope with the altered situation (Nupur et al., 2020; Robin et al., 2021; Kang et al., 2022). Therefore, under drought, the roots of both genotypes became thinner, but this change was more pronounced in the 1BL.1RS translocation line.

Similarly, previous studies in bread wheat showed that roots of 1BL.1RS translocation wheat were thinner when grown in unfavourable conditions, such as acid soils (Manske and Vlek, 2002). The hierarchical clustering, obtained from the morphological parameters of the root system, highlighted the extent to which the root system of Svevo 1BL.1RS was different from that of Svevo in both control and drought conditions. Interestingly, root morphological characteristics of drought-stressed plants of Svevo were similar to those of Svevo 1BL.1RS under control condition.

4.2 | Mechanisms of drought tolerance

Generally, both organic and inorganic solutes are known to accumulate in plant tissues in response to drought to increase the osmotic potential and allow water uptake. Among organic solutes, proline is

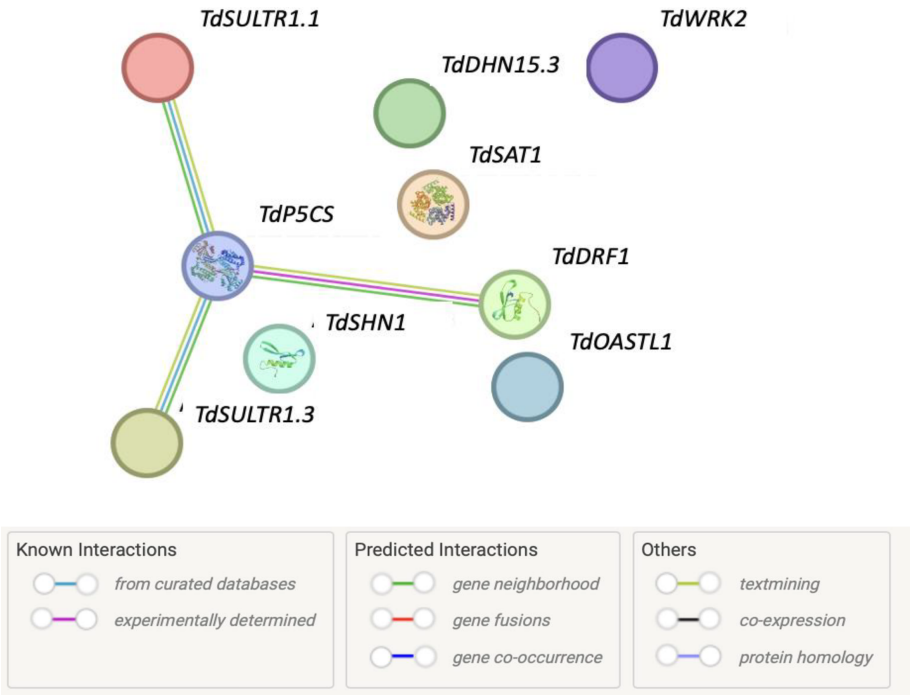


FIGURE 11 Interactions among proteins TdSULTR1.1, TdSULTR1.3, TdDRF1, TdP5CS estimated using STRING database 12.0 (https://string-db.org).

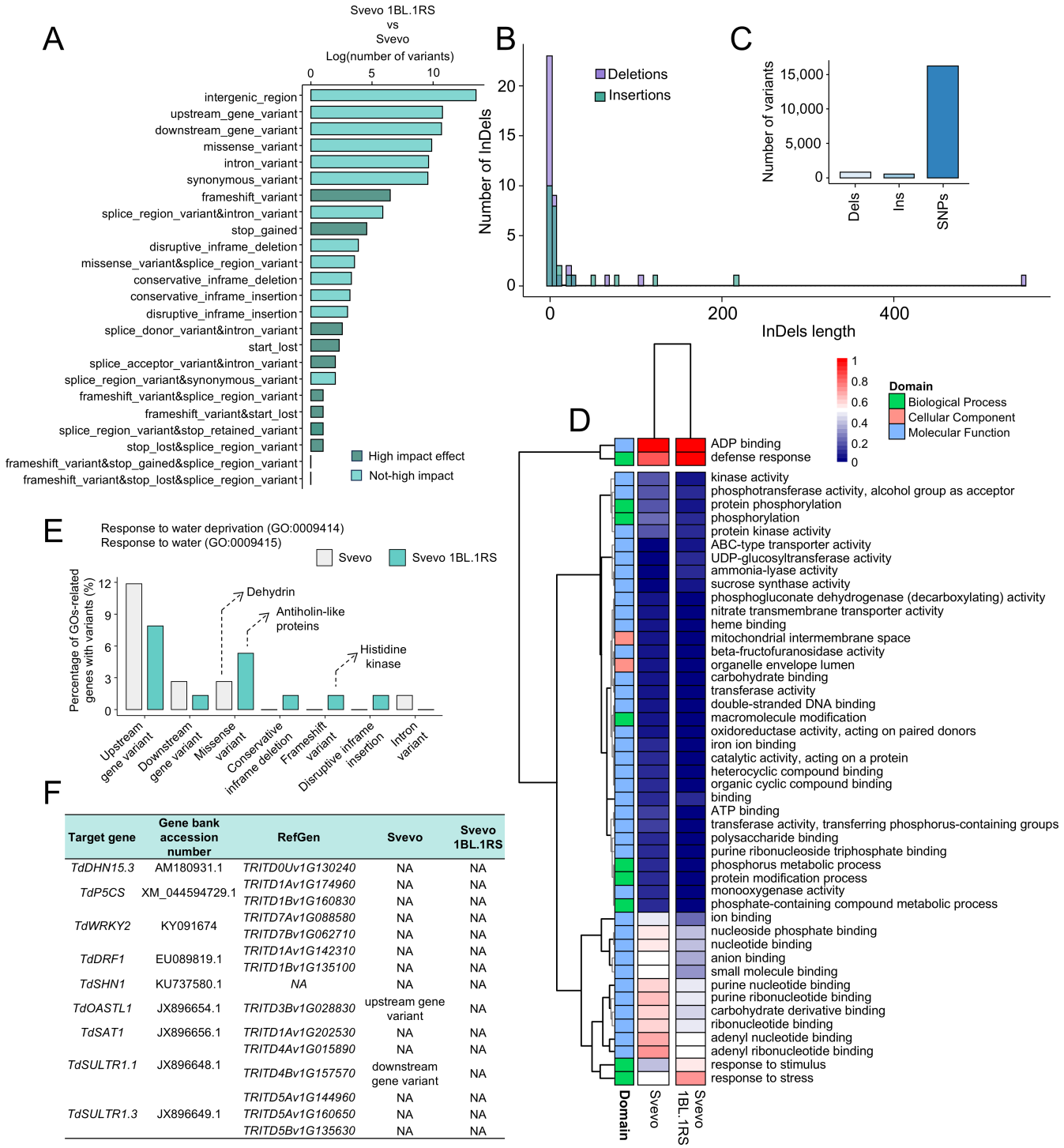
the most widely studied since it plays a key role both as a compatible solute, helping to maintain cell turgor, and as an antioxidant compound, protecting cellular structures during periods of water shortage (Farooq et al., 2012). Proline concentration significantly accumulated in shoots of both genotypes following drought exposure but to a greater extent in Svevo 1BL.1RS. It is widely known that proline is produced in the cytosol in both the root and shoot tissues (Heuer, 2016). However, previous studies have shown that during water stress, proline biosynthesis occurs mainly in the chloroplasts, which are located in the leaves, and this process is light-dependent (Szabados e Savoure, 2010). This could account for the higher concentration of proline in shoots of both genotypes during water stress. In higher plants, proline biosynthesis occurs mainly from glutamate via ∆1 -pyrroline5-carboxylate (P5C) by two successive reductions, which are catalysed by P5C synthetase (P5CS) and P5C reductase (P5CR), or alternatively from ornithine through ornithine δ-aminotransferase – OAT (Meena et al., 2019). In the first case, glutamine synthetase (GS) is involved in proline biosynthesis (Meena et al., 2019). Accordingly, the higher accumulation of proline in shoots of Svevo 1BL.1RS was associated with a significant increase in GS activity in the same tissue. This finding is consistent with a previous study showing that the induction of proline accumulation under stress is usually due to *de novo* synthesis rather than proteolysis (Szabados e Savoure, 2010). Accordingly, no significant changes in protein concentration were found in both Svevo and Svevo 1BL.1RS under drought. Concerning roots, we found an increased proline accumulation only in Svevo plants under drought. Proline translocation to roots could help plants during the recovery from stress as it is also a readily available source of nitrogen, carbon, and reduction equivalents (Hellmann et al., 2000).

During water deficit condition, increased production of ROS commonly occurs, leading to oxidative stress and activation of antioxidant defence mechanisms by plants (Sallam et al., 2019). Malondialdehyde (MDA) production was used as a marker to assess the extent of oxidative damage in cells (Tirani and Haghjou, 2019). The imposition of drought causes greater oxidative damage in Svevo, as evidenced by the increased level of MDA in shoot tissue, despite the high level of proline accumulated in both the shoot and the roots. However, from a recent study, it was clear that the relationship between proline accumulation and stress tolerance still appears controversial (Arteaga et al., 2020).

On the other hand, drought-stressed Svevo 1BL.1RS plants did not show significant MDA accumulation in shoots, together with higher proline accumulation and lower catalase activity, suggesting that Svevo 1BL.1RS was able to cope with imposed stress more efficiently than Svevo. Indeed, catalase is one of the antioxidant enzymes used by plants to protect themselves from the damaging effect of oxidative stress by detoxifying ROS (Demiral and Turkan, 2005; Khan and Panda, 2008)

The drought-induced response of plants also involves the activation of several genes that contribute to drought tolerance, such as *TdP5CS* (encoding for pyrroline-5-carboxylate synthetase, involved in the synthesis of proline) (Zhang et al., 2022); *TdDHN15.3* (encoding for dehydrins) (Rampino et al., 2006) and *TdDRF1*, *TdSHN1* and *WRKY2* as drought-responsive transcription factors (Sakuma et al., 2002; Budak et al., 2013; Baker et al., 2022). Interestingly, most of the genes were upregulated by drought in both shoots and roots only in the genotype Svevo 1BL.1RS. Only *TdWRKY2* showed a different expression pattern, being upregulated in shoots and not affected in roots.

Notably, in roots of Svevo 1BL.1RS, the expression of these genes was higher even in the control condition, compared to Svevo, indicating the 1BL.1RS translocation might impact the expression of drought-responsive genes and, in turn, the ability of this genotype to cope with drought.



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Target gene	Gene bank accession number	RefGen	Svevo	Svevo 1BL.1RS
TdDHN15.3	AM180931.1	*TRITD0Uv1G130240*	NA	NA
TdP5CS	XM_044594729.1	*TRITD1Av1G174960*	NA	NA
		TRITD1Bv1G160830	NA	NA
TdWRKY2	KY091674	*TRITD7Av1G088580*	NA	NA
		TRITD7Bv1G062710	NA	NA
TdDRF1	EU089819.1	*TRITD1Av1G142310*	NA	NA
		TRITD1Bv1G135100	NA	NA
TdSHN1	KU737580.1	*NA*	NA	NA
TdOASTL1	JX896654.1	*TRITD3Bv1G028830*	upstream gene variant	NA
TdSAT1	JX896656.1	*TRITD1Av1G202530*	NA	NA
TdSULTR1.1	JX896648.1	*TRITD4Av1G015890*	NA	NA
		TRITD4Bv1G157570	downstream gene variant	NA
		TRITD5Av1G144960	NA	NA
TdSULTR1.3	JX896649.1	*TRITD5Av1G160650*	NA	NA
		TRITD5Bv1G135630	NA	NA

FIGURE 12 (A) Logarithm of the number of variants (SNPs + InDels) present in Svevo 1BL.1RS and absent in Svevo per effect provoked. (B) Number of the Svevo 1BL.1RS unique InDels per their lengths (bp). (C) Number of Svevo 1BL.1RS unique SNPs, insertions (Ins), and deletions (Dels). (D) Gene Ontology (GO) enrichment analysis of the genes containing variants in Svevo and Svevo 1BL.1RS. (E) Percentage of genes associated with response to water deprivation (GO:0009414) or response to water (GO:0009415) with variants in both genotypes. The arrows highlight the proteins encoded by genes with some type of variants. (F) Presence of variants in a set of genes of interest. NA indicates the absence of SNP/InDels.

The genomic sequence of those genes was studied to identify mutations in Svevo 1BL.1RS. None of these genes presented variants in their protein-coding region and, interestingly, the up- and downstream flanking regions of the drought stress-related genes did not present any variation which could affect their expression level. However, there were other genes related to the response to water

deprivation that presented frameshift, missense mutations, or disruptive inframe insertions in Svevo 1BL.1RS. In particular, the histidine kinase gene family, in which a member (*TRITD3Av1G246370*) presented a variant on its coding region provoking a frameshift, specifically provoked by two insertions of 3 and 8 nt in their coding region. The histidine kinase genes have an important role in the response to a wide range of abiotic stresses, especially drought and salt stresses (Zhao et al., 2023). These findings revealed that the genetic differences extend beyond the 1BL.1RS translocation, increasing the sources of potential tolerance to drought stress.

4.3 | Impact of drought on the ionome of the two durum wheat genotypes

The mineral composition of a plant, including macro- and micronutrients, defined as the ionome, clearly reflects the plant's nutritional status (Salt et al., 2008).

Under control condition, some macronutrients (K, Mg, Ca) and all measured micronutrients (Cu, Fe, Mn, Zn and Mo) were more concentrated in the shoots of Svevo 1BL.1RS than in those of Svevo, suggesting an improved efficiency to uptake/translocate nutrients of the translocation line. On the other hand, Svevo plants were able to accumulate more nitrate and sulfate in their shoot tissue. Higher accumulation of nitrate by Svevo could be attributable to its higher number of root tips, playing an important role in the uptake of mobile resources, as previously reported by Robinson et al. (1991), even if a drought-induced reduced assimilation rate of nitrate cannot be ruled out.

Drought can reduce nutrient uptake and accumulation both indirectly, as the physiochemical properties of the soil can lead to reduced mobility of nutrients, and directly, as mechanisms for nutrient uptake, translocation, and assimilation may not optimally function under drought stress conditions (Amtmann and Blatt, 2009). Accordingly, the imposition of stress had a significant impact on the plant nutritional status of both genotypes.

In particular, K accumulation was significantly inhibited by drought in both shoot and root tissues in Svevo and only in roots of Svevo 1BL.1RS. It is well known that potassium plays a crucial role in modulating stomatal movements and osmotic regulation (Sardans and Penuelas 2015). During drought stress, higher levels of K in the shoots of Svevo 1BL.1RS may assist plants in coping with drought conditions.

Both Mg and Ca showed a lower concentration in shoots of both genotypes grown under drought stress. Being Mg a constituent of the chlorophyll molecule, its lower accumulation led to a reduced level of this pigment, which was particularly significant in Svevo 1BL.1RS. Furthermore, low Mg concentrations are commonly associated with increased oxidative stress and antioxidant response (Tewari et al., 2006; Sánchez-Rodríguez et al., 2010), as previously reported for Svevo plants.

On the other hand, Ca has been shown to play a role in the signalling of drought responses (Shao et al., 2008) and, in particular, in proline accumulation for osmotic adjustment (Sadiqov et al., 2002).

Concerning micronutrients, drought had the greatest impact on Mo, whose accumulation significantly dropped in both tissues of both genotypes, and the lowest one on Cu, which did not show any significant change between control and stressed plants.

Drought and coupled oxidative stress can trigger Mn deficiency, which results in chlorophyll loss (Hajiboland, 2012) and reduced transpiration rate (Hebbern et al., 2009). Our results showed a noticeable decrease in the accumulation of Mn in the shoots of Svevo. However, there was a significant increase in the concentration of Mn in the roots of Svevo 1BL.1RS grown under drought conditions. This suggests that this genotype likely sustains Mn uptake as an adaptive response to drought. Indeed, it has been reported that exogenous supplementation of Mn increases grain yield and stress tolerance in wheat (Gholamin & Khayatnezhad, 2012).

The last two micronutrients, Fe and Zn, exhibited the same accumulation pattern in response to drought:their concentrations were unaffected in Svevo plants and Svevo 1BL.1RS roots, while they reduced in Svevo 1BL.1RS shoots. The reduced Fe accumulation in shoots of Svevo 1BL.1RS grown under drought is likely attributable to the increased Mn uptake, which inhibited Fe uptake through a competition mechanism (in a competitive manner) and reasonably explains the reduced chlorophyll content in the same tissue. On the contrary, the lower Mn concentration in shoots of Svevo-stressed plants most likely prevents competition with Fe uptake.

The same might concern Zn, whose availability is related to both plant growth and drought tolerance (Hajiboland, 2012). Low levels of Zn in plants can increase stomatal restriction and ROS production, leading to reduced plant growth (Hajiboland, 2012). Conversely, the exogenous application of Zn can reduce the damage caused by drought stress by lowering the activity of membrane-bound NADPH oxidase, preventing photooxidative damage, and increasing the activity of enzymes involved in ROS detoxification (Waraich et al., 2011; Hajiboland, 2012).

Concerning anion homeostasis, drought resulted in a higher accumulation of Cl^-, NO_3^- and PO_4^{3-} only in the shoot tissue of Svevo 1BL.1RS. It has been demonstrated that plants able to accumulate Cl^- in their tissues can withstand water deficit more efficiently (Franco-Navarro et al., 2015). Accordingly, the highest concentration of Cl^- was found in Svevo 1BL.1RS, also showing the highest level of proline and the lowest oxidative damage (MDA concentration).

Interestingly, both Svevo and Svevo 1BL.1RS under stress conditions showed an increased sulfate concentration in both shoots and roots. High concentration of S has important implications for its impact on the protein quality of seeds, in turn affecting baking quality (Hagel, 2005; Castellari et al., 2023).

As the modulation of sulfate fluxes is known to play a prominent role in the strategies to adapt to drought conditions (Chan et al., 2013), sulfate uptake and assimilation rate were investigated by analysing the expression of genes encoding two high-affinity sulfate transporters (*TdSULTR1.1* and *TdSULTR1.3*) and two enzymes involved in sulfur assimilation [serine acetyltransferase, *TdSAT1* and O-acetylserine(thiol)lyase, *TdOASTL1*] (Fiorilli et al., 2022). All genes were significantly upregulated after the imposition of drought only in Svevo 1BL.1RS: the two transporters and *TdSAT1* only in the shoot tissue, while *TdOASTL1* also in roots. Furthermore, as already

discussed for drought-responsive genes, these genes were also possibly constitutively expressed in the translocation line.

A recent study by Maghrebi et al. (2024) provides compelling evidence for the connection between sulfate and drought resistance in durum wheat. This study demonstrated that sulfate is a significant macronutrient influencing the genetic diversity of durum wheat under drought conditions. Durum wheat genotypes with a higher capacity for sulfate uptake and assimilation were found to exhibit greater resilience to drought, suggesting that sulfate plays a critical role in the plant's ability to withstand water-limited conditions. In support of this hypothesis, STRING analysis showed a network interaction among both sulfate transporters and the *TdP5CS* protein involved in the proline synthesis. Accordingly, Svevo 1BL.1RS plants under drought showed the highest proline production in shoots associated with high *TdP5CS* expression levels.

5 | CONCLUSION

Although the drought-induced inhibition of growth was more severe on Svevo 1BL.1RS, it was more effective in counteracting oxidative stress, as evidenced by lower MDA levels. Interestingly, the 1BL.1RS translocation in durum wheat also altered the morphology of its root system, thereby significantly affecting its nutrient accumulation ability. The higher expression level of genes involved in S assimilation is particularly noteworthy, highlighting the involvement of S in the plant's response to water stress. Notably, sulfate has been recently indicated as one of the macronutrients potentially linked to the genetic diversity of the durum wheat genotypes under drought (Maghrebi et al., 2024). In particular, durum wheat genotypes showing a greater ability to uptake and assimilate sulfate were identified as having a high drought tolerance degree (Maghrebi et al., 2024). Such findings provide new and exciting information about the changes brought about by the translocation.

AUTHOR CONTRIBUTIONS

SA: conceptualization; GQ and SA: writing—original draft; GQ, MM, MMS, and SP: data curation, formal analysis, visualization; SA and GV: funding acquisition; GQ, MM, MMS, and SP: investigation and methodology; SA: project administration; SA, FS, FB and GV: supervision; SA, GV, FS, FB, DL: writing—review and editing.

ACKNOWLEDGEMENTS

The authors acknowledge the Italian Ministry of University and Research (MUR) and University of Tuscia (Viterbo, Italy) for a grant attributed to Giulia Quagliata in the frame of DM 1061/2021 (Dottorato di Ricerca, PON Ricerca e Innovazione 2014–2020).

We wish to thank Dr. B. Margiotta for providing us with seeds of the translocation line Svevo 1BL.1RS.

DATA AVAILABILITY STATEMENT

The sequencing data analyzed during the current study are available from the corresponding author upon reasonable request.

FUNDING

Research was supported by PRIMA-2019 (EXPLOWHEAT CUP J89C19000140005) and by Ministry for Education, University, and Research (MIUR) initiative "Department of Excellence" (Law 232/2016) DAFNE Project 2023-27 "Digital, Intelligent, Green and Sustainable (acronym: D.I.Ver.So)."

ORCID

Stefania Astolfi https://orcid.org/0000-0002-1450-8783

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Quagliata, G., Maghrebi, M., Marín-Sanz, M., Palombieri, S., Sestili, F., Lafiandra, D. et al. (2024) Rye-durum wheat 1BL.1RS translocation: implications for drought tolerance and nutritional status. *Physiologia Plantarum*, 176(5), e14579. Available from: https://doi.org/10.1111/ppl.14579