

Screening of *Triticum turgidum* genotypes for tolerance to drought stress

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ABSTRACT

Drought is one of the major abiotic stresses leading to reduced yields and economic losses. Effective germplasm screening for drought tolerance particularly under managed water-deficit conditions is an effective way of selecting materials for advanced breeding programs. Here, 37 *Triticum turgidum* genotypes, including landraces, ancient and modern genotypes, along with 2 tritordeum cultivars, were subjected to water-deficit stress through the application of 10% (w/v) PEG 6000 and to re-watering treatment in controlled environment, and at the end of each treatment, several physiological and morphological traits were investigated. Our results revealed large variation in shoot and root fresh weight, proline, chlorophyll, and MDA concentration, and also in root morphological traits across the 37 genotypes. The hierarchical clustering of the physiological and morphological traits led to the identification of tolerant and sensitive genotypes to water-deficit stress and also reveals those genotypes characterized by deep-rooting and shallow-rooting systems. By integrating both datasets, three outstanding genotypes, namely Karim, Svems 20, and Svems 18 were identified as the most tolerant genotypes with deep-rooting system. On the other hand, Iride and Bulel tritordeum, were introduced as the most sensitive genotypes with shallow-rooting system.

1. Introduction

The world population will reach about 10 billion by the year 2050, and a key challenge is to provide food to the entire population in equitable, healthy, and sustainable way (Beddington, 2010). According to the most recent estimates, global production of cereals will have to increase by 70% by 2050 (Godfray et al., 2010). Crop productivity is however threatened by climate change, which has led to increasing water scarcity in various regions of the world, including Europe, and in particular the Mediterranean area (Scherr, 1999; Tilman, 1997). Durum wheat (*Triticum durum* Desf.) is one of the most widely consumed cereals in the world, currently providing 18% of daily calorie intake and 20% of human protein (<http://faostat.fao.org/>) and the most important cultivation area is in the Mediterranean basin, which produces around 60% of world production (Shewry and Hey, 2015; Maccaferri et al., 2014). Here, durum wheat is mostly cultivated in rainy environments, in areas where the amount and frequency of rainfall fluctuate drastically between years and between different areas within the same year, resulting in large variations in yield.

Limited water availability is a major issue for wheat production around the world (Zhang et al., 2018). Approximately 45% of the

wheat-growing lands in developing countries are vulnerable to drought (Macharia and Ngina, 2017). The situation of wheat production may become more problematic since the area affected by the drought stress may increase four times by the middle of the 21st century (Zhao and Dai, 2017). Drought stress significantly reduces cereal crop production globally by 10% due to its negative effects on plant growth and grain productivity (Zhao and Dai, 2017). The best option to mediate the situation is to develop drought tolerant crop varieties. In this regard, developing wheat varieties with efficient root systems that can exploit residual soil moisture under water-deficient conditions in the dry season is crucial. In fact, plants, which are sessile organisms, have developed specific mechanisms that allow them to recognize environmental changes and activate specific responses to stress conditions to survive while minimizing damage (Cruz de Carvalho, 2008).

Plants can cope with environmental stresses at multiple levels such as molecular, cellular, anatomical, morphological, and physiological. These responses may also depend on species and genotypes, the duration and severity of the event, the stage of development of the plant at which the stress occurs and the individual crop conditions. Genotype-dependent adaptation strategies like drought stress avoidance strategy; include the development of a deeper and more expanded root system to

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improve water acquisition from the deep soil layers (Lynch and Wojciechowski, 2015).

In addition, this strategy is correlated with other different root traits such as the number, length, placement, and direction of growth of individual components of the root system, which define the degree of plasticity of the roots (Giehl et al., 2014). Another genotype-dependent adaptation strategy is drought stress tolerance strategies; the characteristics that allow plants to maintain active major metabolic functions and an appropriate cell turgidity under dehydrated conditions are included in drought tolerance mechanisms. Tolerance to drought stress is essentially related to a more efficient use of water and can be obtained by osmotic adjustment or changes in the elasticity of the cell wall and plant tissues (Touchette et al., 2007; Farooq et al., 2009; Scholz et al., 2012). In recent years, the study of root system architecture has received more attention (Bengough et al., 2004; Friedli et al., 2019) and it is generally believed that a deep, extensive, and ramified root system is essential for developing drought tolerant crops (Jackson et al., 2000).

Screening of wheat genotypes for drought tolerance using different indicators have been previously studied (Noorifarjam et al., 2013; Mwadingeni et al., 2016; Haque et al., 2021; Ahmad et al., 2022; Ayed et al., 2021). However, this approach still requires validation for its usefulness in screening germplasm for improved yield under stressed conditions since drought tolerance is a very complex trait. Moreover, information on the correlation between wheat root traits and drought tolerance mechanisms is still limited. Research on drought tolerance still must deal with many underexplored aspects associated with root traits.

The aim of this work was to identify among a collection of 37 *Triticum turgidum* genotypes, including landraces, ancient and modern genotypes, along with 2 tritordeum cultivars (amphiploid between *Hordeum chilense* and durum wheat), those characterised by a better ability to cope with drought stress and to recover after stress. To reach this goal, plants were grown under controlled conditions and subjected to water-deficit stress for six days through the application of 10% (w/v) PEG 6000 to the nutrient solution (Zhang et al., 1991; Robin et al., 2021) that is a commonly used compound in a hydroponic culture (Chazen and Neumann, 1994; Munns et al., 2010). Then, all plants were rewatered (rewatering treatment) and then placed in the same condition as the control plants to evaluate their capability to recover from the stress condition. The physiological characterisation of plant response to drought included the determination of several parameters, such as shoot and root fresh weight, chlorophyll levels, proline and malondialdehyde concentration. Furthermore, the morphological traits of root systems were evaluated and related to the physiological responses.

2. Materials and methods

2.1. Plant growing conditions

The seed collection of *Triticum turgidum* genotypes chosen as the plant material in this study includes landraces, ancient and modern genotypes from Mediterranean area, the two tritordeum cultivars and the SVEMS lines of a durum wheat TILLING platform derived from Svevo (Bovina et al., 2014) which constitutes a unique material (Table 1). Seeds were soaked in distilled water for 1 h and allowed to germinate in aeroponics in the dark at 28 °C for 4 days. Uniform seedlings were selected and transferred in hydroponic culture in plastic pots (3 seedlings/pot), containing 600 ml of a nutrient solution (NS) (Control condition) (Astolfi et al., 2018). After 8 days from sowing, half of the plants were exposed to water-deficit stress by adding PEG 6000 (10%, w/v) to the NS (PEG condition) and the remaining half was kept in the control solution. After 6 days, plants were harvested and analysed. Then, PEG-treated plants were transferred to non-PEG NS (Rewatered condition) and after 7 days, the second plant sampling of both control and rewatered plants were carried out.

Plant cultivation was conducted in a growth chamber under controlled conditions with a day/night cycle of 16/8 h at 28/20 °C air

Table 1

Country and origin of the 37 genotypes chosen as the plant material for this study.

Genotype	Country	Origin	Species
Antalis	France	–	<i>T. turgidum</i> ssp. <i>durum</i>
Aucan tritordeum	Spain	–	Hexaploid wheat-barley hybrid
Aureo	Italy	–	<i>T. turgidum</i> ssp. <i>durum</i>
Aziziah	Italy	–	“
Bulel tritordeum	Spain	–	Hexaploid wheat-barley hybrid
Claudio	Spain	–	<i>T. turgidum</i> ssp. <i>durum</i>
Creso	Italy	–	“
Domino	Italy	–	“
Durum purple	Algeria	–	“
Etrusco	Italy	–	<i>T. turgidum</i> ssp. <i>turanicum</i>
Hyd A1B1	Italy	Garcia-Molina et al. (2021)	<i>T. turgidum</i> ssp. <i>durum</i>
Iride	Italy	–	“
Karim	Tunisia	–	“
Karur	France	–	“
Khair	Tunisia	–	“
Lcye A'B	Italy	Sestili et al. (2019)	“
LG Anubis	France	–	“
Maali	Tunisia	–	“
Nasr	Tunisia	–	“
Om Rabia	Tunisia	–	“
Saragolla	Italy	–	“
Senatore Cappelli	Italy	–	“
Svems 1	Italy	Bovina et al. (2014)	“
Svems 3	Italy	Bovina et al. (2014)	“
Svems 5	Italy	Bovina et al. (2014)	“
Svems 7	Italy	Bovina et al. (2014)	“
Svems 8	Italy	Bovina et al. (2014)	“
Svems 9	Italy	Bovina et al. (2014)	“
Svems 10	Italy	Bovina et al. (2014)	“
Svems 13	Italy	Bovina et al. (2014)	“
Svems 14	Italy	Bovina et al. (2014)	“
Svems 16	Italy	Bovina et al. (2014)	“
Svems 18	Italy	Bovina et al. (2014)	“
Svems 20	Italy	Bovina et al. (2014)	“
Svevo	Italy	–	“
Svevo 1b/1r	Italy	Lafiandra (Unpublished results)	“
SY Leonardo	Italy	–	“

temperature, 80% relative humidity, and 200 $\mu\text{E m}^{-2} \text{s}^{-1}$ light intensity.

2.2. Determination of chlorophyll level

At harvest (14 and 21 days after sowing), chlorophyll concentration was measured in attached leaves of wheat plants using a non-destructive portable apparatus, the Soil Plant Analysis Development (SPAD—502 Plus, Konica Minolta, Osaka, Japan). Three independent replicates within each treatment were detected on the youngest fully expanded leaf, averaged, and expressed as SPAD units.

2.3. Measurement of malondialdehyde concentration

The level of lipid peroxidation was expressed as determination of malondialdehyde (MDA) concentration and was determined as TBA reactive metabolites according to Astolfi et al. (2005). Fresh shoot and root tissues (0.2 g) were homogenized in 2 ml of 0.25% TBA made in 10% TCA. Extract was heated at 95 °C for 30 min and then quickly cooled on ice. After centrifugation at 16,000 \times 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 $\mu\text{g g}^{-1}$ FW by using an extinction coefficient of 155 mM cm^{-1} .

2.4. Measurement of proline concentration

The proline (PRO) concentration was estimated by the method of Bates et al. (1973) (Arteaga et al., 2020; Ghaffari et al., 2019; Lum et al., 2014). The plant tissue (0.1 g) was homogenized in 2 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at $16,000\times g$ for 10 min. Supernatant was used for the estimation of PRO concentration. The reaction mixture consisted of 500 μ l acid ninhydrin and 500 μ l of glacial acetic acid, which was boiled at 100 °C for 1 h. The reaction was stopped in ice bath, then the reaction mixture was extracted with 1.5 ml of toluene and absorbance was read at 520 nm and expressed in μ mol g^{-1} FW.

2.5. Analysis of root morphology

Four days after sowing in control NS, root apparatus of each genotype was analysed using the software WinRhizo software (Regent Instruments Inc., Quebec, Canada) and an Epson scanner. 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 to determine some root-related parameters: total root length, the density of root length, root surface area, root volume, root mean diameter and the number of root tips.

2.6. Statistical analysis

Each reported value represents the mean \pm standard deviation (SD) of measurements carried out in triplicate and obtained from three independent experiments (biological replicates). All the physiological data were subjected to a student t-test analysis to compare treatment (stress and recovery) versus control conditions (p-value cutoff of <0.05). To describe how much a quantity changes between different conditions,

fold changes were calculated (treatment/control) and then were transformed to a logarithmic scale (base 2). Using PAST 4.0.3 software, a principal component analysis (PCA) was applied on the root trait dataset. The hierarchical clustering was performed on the datasets by complete method and Euclidean distance measurement in R (RStudio Desktop for Windows V. 2022.07.2 + 576) package heatmap.

3. Results

To maximize the benefit of this study, as the first stage, we elaborated the obtained results of each indicator across 37 genotypes in details and in the format of bar plots and then as the second stage we describe the clustering results to select the tolerant and sensitive genotypes.

3.1. Changes in plant fresh biomass in response to drought stress and rewatering treatment

At harvest (14 days for PEG treatment and 21 days for rewatered treatment), fresh shoots and roots were separated and weighed and then subjected to a student t-test analysis to compare treatment (stress and recovery) versus control conditions (p-value cutoff of <0.05). Log₂-foldchanges were calculated (treatment/control) to describe how much a quantity changes between different conditions. Our results showed that the imposition of water-deficit stress led to a decrease in shoot fresh weight in 20 genotypes, such as Nasr < Svems 8 < Bulel tritordeum < Domino < Etrusco < Svems 7 < Om Rabia < Maali < Svevo 1b/1r < SY Leonardo < Svems 20 < Hyd A1 B1' < Aziziah < Svems 3 < Aureo < Svems 13 < Svems 5 < Saragolla < Senatore Cappelli < Aucan tritordeum, in a range of log₂FC from -0.3 to -3.3, respectively (Fig. 1A).

Root fresh weight of 24 genotypes (Iride < Svevo < Om Rabia < Domino < Svevo 1b/1r < Svems 18 < Svems 14 < Hyd A1 B1' < Durum

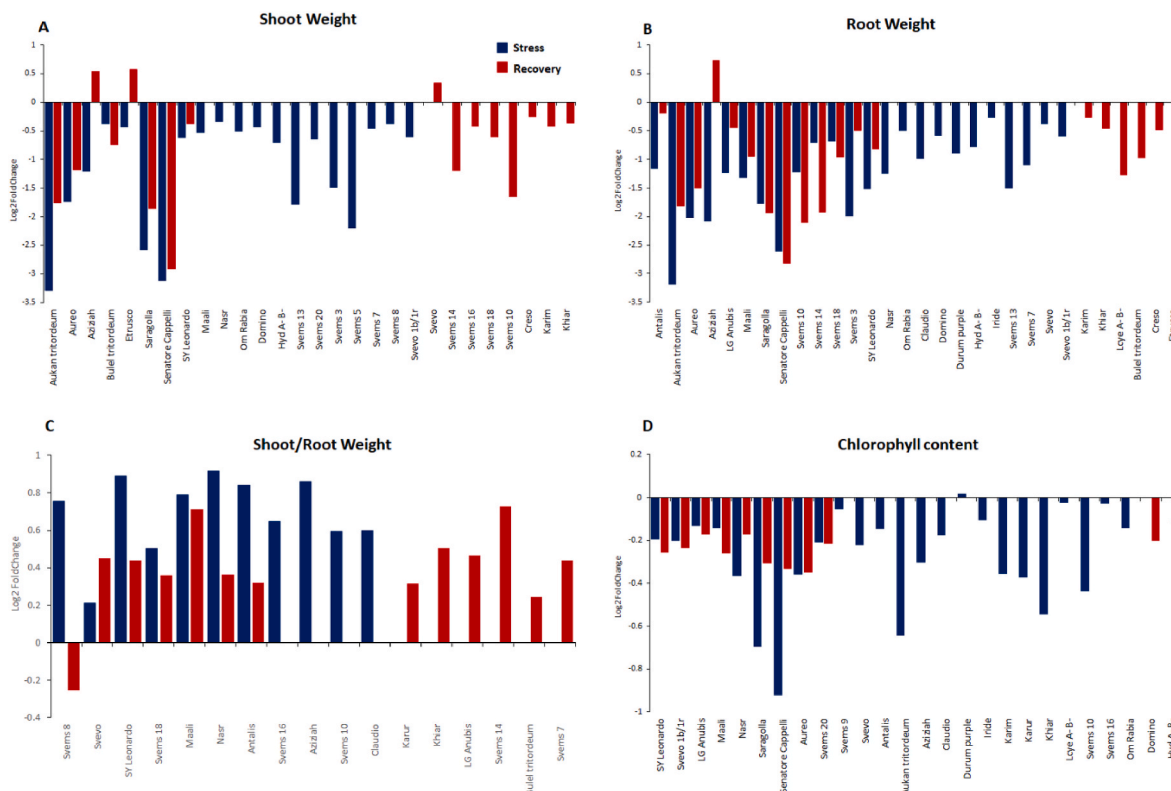


Fig. 1. Fresh weight of shoots (A) and roots (B), shoot/root weight ratio (C) and chlorophyll level (D) of 37 wheat genotypes grown hydroponically in response to two treatments, water-deficit stress (PEG 6000 10% v/v) and recovery. A Student t-test analysis were applied to compare the treatments (stress and recovery) versus control conditions (p-value cutoff of <0.05). Log₂ fold changes were considered for describing the quantity changes between different conditions (treatment/control).

purple < Claudio < Svems 7 < Antalis < Svems 10 < LG Anubis < Nasr < Maali < Svems 13 < SY Leonardo < Saragolla < Svems 3 < Aureo < Aziziah < Senatore Cappelli < Aucan tritordeum) showed decreasing levels under PEG conditions ranging between log₂FC –0.3 to –3.2, respectively (Fig. 1B).

As regards the effect of rewatering treatment on plant shoot fresh weight, only Svevo genotype showed an increase of this parameter (log₂FC 0.3), whereas 13 genotypes (Creso < Khiar < SY Leonardo < Svems 16 < Karim < Svems 18 < Bulel tritordeum < Aureo < Svems 14 < Svems 10 < Aucan tritordeum < Saragolla < Senatore Cappelli) showed decreasing levels of shoot biomass production with a log₂FC ranging from –0.3 to –2.9, respectively (Fig. 1A).

Also, for the root fresh weight only 2 genotypes (Aziziah > Etrusco) showed an increase (log₂FC 0.7, log₂FC 0.2 respectively), while 17 genotypes (Antalis < Karim < LG Anubis < Khiar < Creso < Svems 3 < SY Leonardo < Maali < Svems 18 < Bulel tritordeum < Lcye A' B' < Aureo < Aucan tritordeum < Svems 14 < Saragolla < Svems 10 < Senatore Cappelli) showed decreasing levels with a log₂FC ranging from –0.2 to –2.8, respectively (Fig. 1B).

The shoot/root weight ratio was significantly increased after plant exposure to water-deficit stress in 11 genotypes, including Nasr > SY Leonardo > Aziziah > Antalis > Maali > Svems 8 > Svems 16 > Claudio > Svems 10 > Svems 18 > Svevo, with log₂FC ranging between 0.91 and 0.21 (Fig. 1C). After rewatering treatment, except Svems 8 showing a log₂FC of –0.25, the increasing levels of shoot/root weight ratio were still observed in 6 genotypes (Maali > Svevo > SY Leonardo > Nasr > Svems 18 > Antalis) and the remaining ones revealed no significant changes (Fig. 1C). Moreover, our results led to identification of six genotypes, such as Svems 14 > Khiar > LG Anubis > Svems 7 > Karur > Bulel tritordeum, which cope with rewatering treatment increasing the shoot/root ratio ranging of log₂FC 0.7- log₂FC 0.2 (Fig. 1C).

3.2. Changes in chlorophyll levels in response to drought stress and rewatering treatment

Leaf chlorophyll level of 37 genotypes of wheat were evaluated under control, stress, and recovery conditions. In response to water-deficit stress, significant decrease of leaf chlorophyll level was observed in 23 genotypes, namely Lcye A' B' > Svems 16 > Svems 9 > Iride > LG Anubis > Maali > Om Rabia > Antalis > Claudio > SY Leonardo > Svevo 1b/1r > Svems 20 > Svevo > Aziziah > Karim > Aureo > Nasr > Karur > Svems 10 > Khiar > Aucan tritordeum > Saragolla > and Senatore Cappelli, and ranged between log₂FC –0.02 to log₂FC –0.9 (Fig. 1D). However, rewatering treatment resulted in lower chlorophyll levels in only 9 genotypes out of 23: Aureo (log₂FC –0.3) > Senatore Cappelli (log₂FC –0.33) > Saragolla (log₂FC –0.30) > Maali (log₂FC –0.26) > SY Leonardo (log₂FC –0.26) > Svevo 1b/1r (log₂FC –0.23) > Svems 20 (log₂FC –0.21) > Nasr (log₂FC –0.17) > LG Anubis (log₂FC –0.17) (Fig. 1D). On the contrary, Domino and Hyd A1 B1' showed increased levels of chlorophyll level with log₂FC –0.2 and –0.1, respectively, after rewatering treatment (Fig. 1D).

3.3. Changes in MDA concentration in response to drought stress and rewatering treatment

It has been demonstrated that reactive oxygen species (ROS) are generated in response to the water-deficit stresses. Overproduction of ROS can lead to the lipid peroxidation, which often is monitored by measuring the malondialdehyde (MDA) (Tirani and Haghjoui, 2019). Thus, in this study, we analysed the MDA concentration in root and shoot tissues of each durum wheat genotype grown under three conditions: control, stress, and recovery. The imposition of water-deficit stress increased the MDA concentration in shoots of 22 genotypes, such as Saragolla > Senatore Cappelli > Aziziah > Durum purple > Svems 5 > Aucan tritordeum > Etrusco > Svems 13 > Svevo > Claudio > Svems 10 > Svems 1 > Aureo > Svems 18 > Svems 8 > Om Rabia > Khiar > Bulel

tritordeum > Antalis, with a log₂FC ranging from 1.8 to 0.2, suggesting that they suffered from more severe lipid peroxidation. Only the genotype Svems 20 showed a decrease in shoot MDA concentration with a log₂FC of –0.2 (Fig. 2A). At root level, the imposition of drought stress led to an overaccumulation of MDA in 11 genotypes, such as Svems 3 > Nasr > Senatore Cappelli > Antalis > Maali > Saragolla > Aucan tritordeum > Claudio > Khiar > Lcye A' B' > Svevo 1b/1r, with a log₂FC ranging from 0.8 to 0.2. On the contrary, a decrease in root MDA concentration was observed in 4 genotypes, such as Domino < SY Leonardo < Svems 18 < Karim with a log₂FC ranging from –0.1 to –0.3 (Fig. 2B).

After the rewatering treatment, only 4 genotypes (Om Rabia > Nasr > Svems 7 > Maali, with a log₂FC ranging from 0.8 to 0.5) showed an increased MDA concentration in shoot tissues. On the contrary, 5 genotypes (Karur < Iride < Domino < LG Anubis < Svems 14, with a log₂FC ranging from –0.2 to –0.9) showed a decrease in shoot MDA concentration as (Fig. 2A). Finally, after rewatering treatment, only the Svems 16 genotype showed an increase of root MDA concentration with a log₂FC of 0.3, whereas 10 genotypes, Maali < SY Leonardo < Antalis < Om Rabia < Etrusco < Svems 14 < Claudio < Aziziah < Karur < Nasr, showed lower levels with a log₂FC ranging from –0.2 to –0.7 (Fig. 2B).

3.4. Changes in proline concentration in response to drought stress and rewatering treatment

Free proline accumulation is a typical adaptation strategy that occurs in plants facing drought stress, since proline is a major compatible solute that also acts as reactive oxygen species (ROS) scavenger and even contributes to carbon and nitrogen storage in stressed plants (Szabados and Savouré, 2010). Proline accumulation was measured in root and shoot tissues of each wheat genotype grown hydroponically under control, water-deficit stress, and recovery conditions. Our results showed that shoot proline concentration of 22 genotypes, including Svems 5 > Aureo > Aucan tritordeum > Senatore Cappelli > Saragolla > Svems 13 > Svevo 1b/1r > Svevo > Svems 18 > Etrusco > Svems 9 > Svems 16 > Svems 3 > Domino > Claudio > Antalis > Svems 14 > Khiar > Svems 8 > SY Leonardo > and Iride, significantly increased in response to water-deficit stress condition and in a range of Log₂FC 8 to log₂FC 0.2 (Fig. 2C).

As shown in Fig. 2D, the root proline concentration of Antalis > Domino > Svems 9 > Creso > Svems 14 > SY Leonardo > Karim > Svevo > Aziziah > Svems 7 > Maali > Svems 20 > Lcye A' B' > Durum purple > Aureo > Svems 13 > Senatore Cappelli > and Svems 16 was significantly enhanced under water-deficit stress, while four genotypes, namely Karur < Svems 8 < Iride < and Svems 10 revealed decreased concentration (log₂FC-0.2 to –1.8).

Fig. 2C shows that the shoot proline concentration of 9 genotypes (Aucan tritordeum > Aureo > Saragolla > Senatore Cappelli > Khiar > Svems 13 > Antalis > Claudio > Iride) out of 22 constantly increased in recovery condition ranging between log₂FC 6 to 0.1, while it decreased in 5 genotypes (Domino < SY Leonardo < Svevo 1b/1r < Svevo < Etrusco) out of 22 in a range of log₂FC –3.9 to –0.3. Moreover, our results showed that the shoot proline concentration was significantly increased in Svems 10 > Om Rabia > Maali > Nasr > Lcye A' B' > Karim (Log₂FC 6.2 to Log₂FC 1.6) only after rewatering treatment. Interestingly, LG Anubis, Svems 20, and Hyd A1 B1' showed decreased accumulation of proline only in response to recovery treatment.

We next analysed root proline accumulation and 11 genotypes (Creso > Svems 16 > Durum purple > SY Leonardo > Karim > Iride > Svems 14 > Domino > Karur > Maali > Antalis) out of 18 were found to accumulate higher levels (ranging between log₂FC 2.8 to 0.1) after rewatering treatment, while 4 genotypes, Senatore Cappelli (log₂FC –0.5) < Svems 10 (log₂FC –0.6) < Svevo (log₂FC –1.02) and Svems 20 (log₂FC –12.6) showed lower accumulation (Fig. 2D). As shown in Fig. 2D, the root proline concentration significantly increased in 6 genotypes (Svems 7, Svems 9, Svems 13, Lcye A' B', Aureo, Aziziah) and decreased in one genotype (Svems 8) in response to stress but it did not

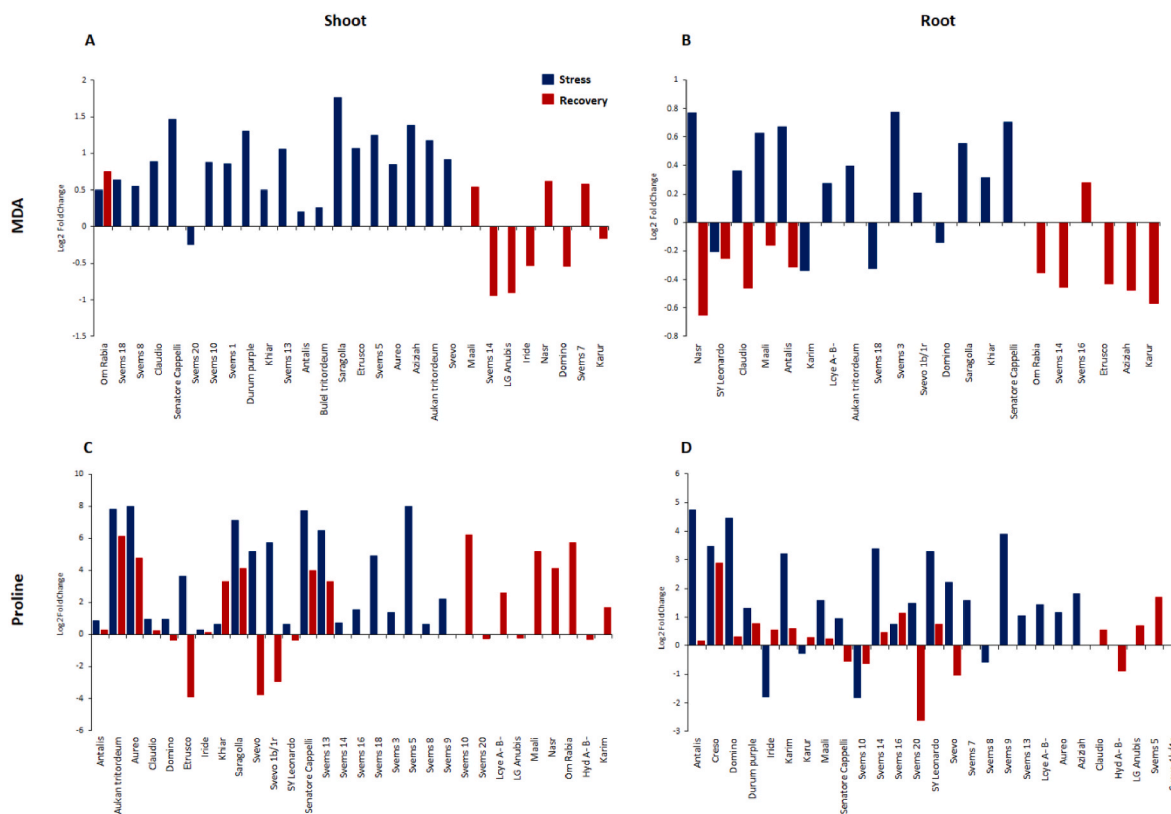


Fig. 2. MDA concentration of shoots (A) and roots (B), and proline concentration of shoots (C) and roots (D) of 37 wheat genotypes grown hydroponically in response to two treatments, water-deficit stress (PEG 6000 10% v/v) and recovery. A Student t-test analysis were applied to compare the treatments (stress and recovery) versus control conditions (p-value cutoff of <0.05). Log₂ fold changes were considered for describing the quantity changes between different conditions (treatment/control).

change after rewetting treatment. On the contrary, 3 genotypes (Claudio, LG Anubis, Svems 5) showed significant increasing and two genotypes showed significant decreasing (Svevo 1b/1r, Hyd A1⁻ B1⁻) showed significant decreasing under recovery condition and no changes under stress condition.

3.5. Clustering the physiological traits across 37 wheat genotypes

To present an overview of the physiological traits and identify major clusters across 37 genotypes under both control and stress conditions, a hierarchical clustering was performed based on the significant fold-change values by complete method and Euclidean distance measurement. The 7 main clusters (A–G) were considered based on MDA and proline changes (Fig. 3). The clusters A, C, and G included 15 genotypes Svems 1, Bulel tritordeum, Om Rabia, Karur, Khiar, Svems 8, Iride, Svems 10, Aziziah, Durum purple, Hyd A1⁻ B1⁻, Svems 3, Nasr, Claudio, and LG Anubis, which could be considered as sensitive genotypes, showing higher accumulation of MDA in both shoot and root tissues, but lower proline accumulation with respect to other clusters. On the contrary, the clusters B, D, and F, including 16 genotypes (Svems 20, Karim, Svevo, Creso, LcyeA⁻ B⁻, Domino, Svems 5, Aureo, Svems 7, Antalis, Svems 18, Svems 14, Svems 16, Svems 9, SY Leonardo, and Svevo 1b/1r), could be identified as the tolerant genotypes, showing lower MDA accumulation in both root and shoot tissues and higher proline production at least in shoot or root tissues (Fig. 3).

3.6. Root morphological traits derived from WinRHIZO scanning

Using WinRhizo scanning equipment, root apparatus of each wheat genotype under control condition was analysed and six root-related indices, including root length, root volume, root diameter, root

surface area, the number of root tips, and the density of root length were measured. Principal component analysis (PCA) on the dataset showed that the first two principal component (PC) dimensions are accounting for 91% of the variation (Fig. 4). It illustrated that the root length, root volume, the number of root tips, and root surface area variables are the most important contributors to the first PC, and they are highly correlated. On the contrary, the root diameter and the density of root length variables are almost uncorrelated with the other variables (Fig. 4).

Moreover, a hierarchical clustering was performed on root traits values across 37 genotypes by complete method and Euclidean distance measurement. As shown in Fig. 5, three main clusters (A–C) were considered as deep-rooting system (cluster A), shallow-rooting system (cluster B) and medium-depth rooting system (cluster C). The cluster A including Svems 20, Khiar, Azizah, Svems 18, Svems 13, Karim, Mali, and Nasr, showing higher values in root length, root volume, root surface area, and the number of root tips compared to other genotypes, while the cluster B including Svevo, Creso, Svevo 1b/1r, Bulel, Iride revealed the lower values (Fig. 5).

Interestingly, 3 genotypes, Karim, Svems 20, and Svems 18 belonging to deep-rooting system clusters, were also considered as the tolerant genotypes based on the physiological traits clustering. Iride and Bulel tritordeum, belonging to shallow-rooting system cluster, also showed susceptibility to water stress according to physiological traits.

4. Discussion

Durum wheat is considered as a strategic crop for many countries bordering the Mediterranean basin, where durum wheat is mostly cultivated in rainfed environments. However, in recent years the Mediterranean climate has changed, resulting in increasing temperatures and precipitation declines (Giorgi, 2006). The current scenario poses a

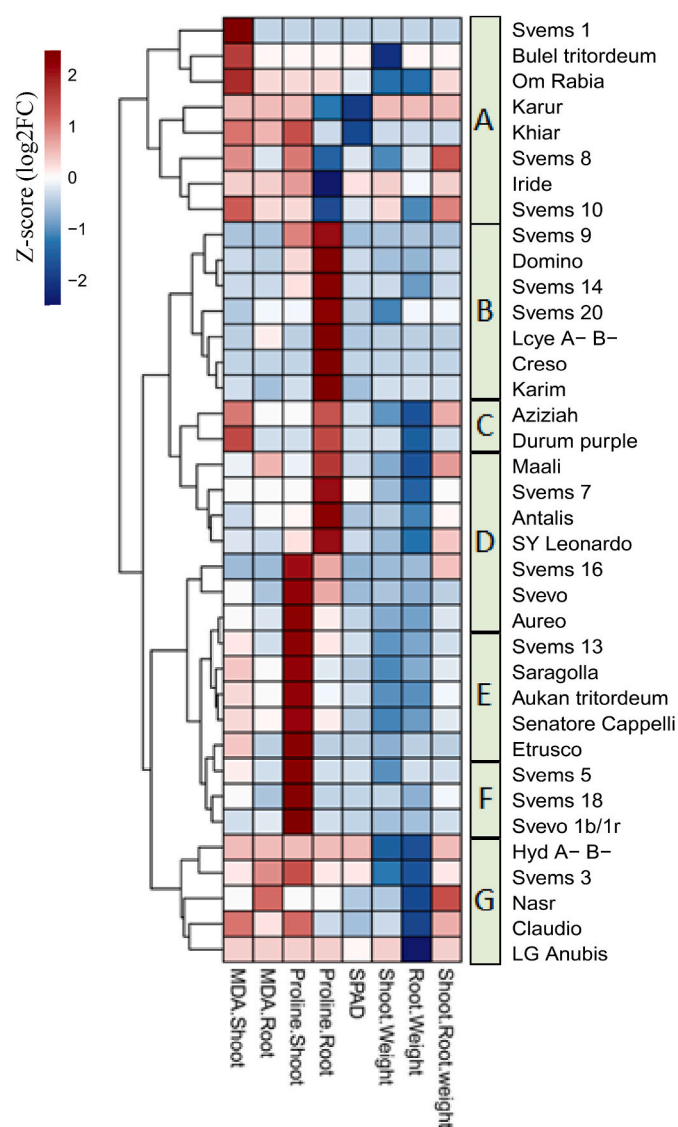


Fig. 3. Heatmap representing hierarchical clustering of physiological traits across 37 wheat genotypes based on the significant log₂fold-change values in response to water-deficit stress (complete method and Euclidean distance measure). The 7 main clusters considered (A–G) were based on MDA and proline changes. The color bar depicts the gradient of log₂fold changes in response to water stress.

significant challenge for survival and productivity of some crops, such as durum wheat. For this reason, the identification of durum wheat genotypes able to efficiently cope with new climatic conditions and to withstand drought is crucial to increase, or at least stabilize, crop production in unfavourable environments (Akitha Devi and Giridhar, 2015). In this study, a large research effort has been made to analyse both root morphological traits and physiological adaptive response to cope with drought of 37 *Triticum turgidum* genotypes, including landraces, ancient and modern genotypes, along with 2 tritordeum cultivars. Aim of this work was the identification of more resilient genotypes able to cope with suboptimal water, which could replace less drought-tolerant one in the Mediterranean area under warming climate. The highly significant genotypic differences recorded among all the traits measured, indicate that the genotypes applied in this study could be a valuable source of genetic diversity for breeding purposes. As expected, we found a large variation in both physiological and morphological indicators across the 37 wheat genotypes under both water-deficit stress and rewatering treatment. With the help of

hierarchical clustering, PCA analysis and the integrating of morphological and physiological data, we succeed to introduce several outstanding genotypes with both deep-rooting systems and tolerance to water-deficit stress.

Gallé et al. (2013) found that drought stress limits relative growth rate of plants. Also, Soni et al. (2014) reported that drought stress decreased most growth parameters, included shoot and root fresh weight. The results of this study clearly indicated that the exposure to drought of durum wheat plants significantly inhibited the shoot and root biomass accumulation in 28 and 30 genotypes, respectively, compared to the control condition. Overall, the group of genotypes characterized by medium-depth rooting system (Aucan tritordeum, Saragolla, Senatore Cappelli) showed a lower growth rate (measured as shoot and root FW) both after drought exposure, and after rewatering treatment. On the other hand, the group of genotypes characterized by a deeper root system included Karim, Svems 18 and Svems 20, was less affected by drought.

The size of the root system, and consequently the shoot/root ratio, determine the plant's ability to acquire water and nutrients, and mainly depend on the concentration of water and nutrients in the rhizosphere. For example, it has been observed in some crops that the shoot/root ratio is considerably lower in dry areas (Gregory et al., 1978; Farrar and Jones, 2000) and in conditions of limited availability of nutrients (Kang and Van Iersel, 2004), because an increased development of the root apparatus would be beneficial for the uptake of water and nutrients, whereas when the supply of water and nutrients is not limiting a small root system may be sufficient (Greenwood et al., 2001).

However, other authors argue that the plant cannot continuously maximize the development of the root system during drought to avoid penalizing the growth of above-ground portion of the plant (Van Noordwijk and De Willigen, 1987). Thus, the combination of both strategies should enable the plant growth and energy management during stress reaching a trade-off between root and shoot development. Our results showing increased levels of shoot/root ratio in 11 genotypes when exposure to water-deficit stress while no significant changes were recorded in the remaining ones. After rewatering, the shoot/root ratio of only 6 genotypes out of 11 still increased. However, shoot/root weight increasing were recorded in 6 genotypes only after rewatering. Karim and Svems 20, as the most tolerant genotypes, showed no significant changes in shoot/root weight ratio in response to both water-deficit stress and rewatering which implies a balance between shoot growth and root growth. However, in Svems 18, as another tolerant genotype, shoot/root ratio increased but less than other genotypes.

Leaf chlorophyll levels of 37 wheat genotypes were evaluated after both drought imposition and rewatering treatment by using SPAD meter. The SPAD chlorophyll meter is a useful method for rapid analysis of chlorophyll status of crops in response to drought (Islam et al., 2014). The chlorophyll meter readings have been positively correlated with destructive chlorophyll measurements in response to adverse conditions such as abiotic stresses (Zhu et al., 2012). Chlorophyll density has been identified as drought tolerant traits and could be used as selection criteria for drought tolerant trait in many plant species (Puangbut et al., 2017). The relationships between chlorophyll level and yield in wheat under water stress are not well documented however, the variation of SPAD under heat (Roy et al., 2021) and salinity (Kiani-Pouya and Rasouli, 2014; Shah et al., 2017) have been previously reported.

In response to water-deficit stress, significant decreasing levels of leaf chlorophyll were observed in 23 genotypes, however, as expected, only 9 genotypes out of 23 showed decreasing chlorophyll after rewatering treatment. Saragolla and Senatore Cappelli with medium-depth rooting systems showed the lowest values compared to other genotypes. The selected most tolerant genotypes with deep-rooting systems such as Karim, Svems 20 and Svems 18 showed no significant changes or less decreasing SPAD units compared to other genotypes.

An important consequence of drought-induced stress is the closure of stomata leading to a restriction in CO₂ fixation and the excessive

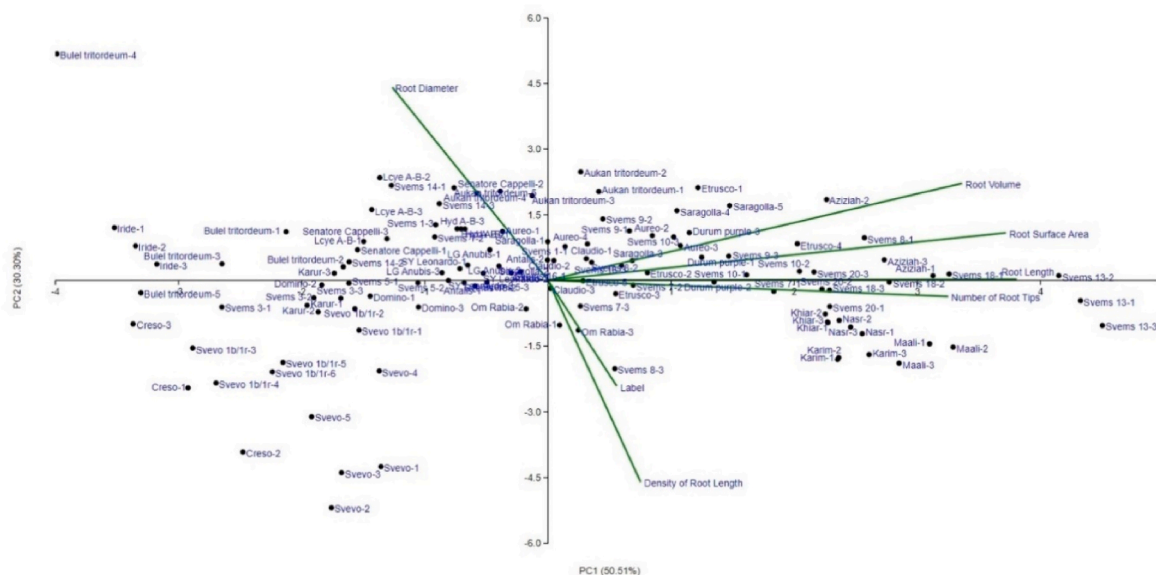


Fig. 4. PCA scatter plot and biplot of morphological root traits across 37 genotypes of wheat. The first component (PC1) represents 50.51% and the second component (PC2) represent 30.30% of the total variance.

production of reactive oxygen species (Abdirad et al., 2020). At times when plants are incapable of performing ROS scavenging, ROS levels may reach a certain threshold and result in progressive damage to proteins, DNA, and lipids, eventually leading to growth retardation and cell death (Foyer and Noctor, 2005). Plant cell membranes are one of the main targets of oxidative damage. Lipid peroxidation referred to as a process through which lipids containing carbon–carbon double bond(s) are oxidized by ROS, leading to cell membrane instability (Ayala et al., 2014). Malondialdehyde (MDA) is one of the key products of lipid peroxidation by thiobarbituric acid reaction. The MDA concentration is an important landmark of membrane damage and the degree of lipid peroxidation which could reflect the degree of damage at water-deficit stress (Yang and Deng, 2015). Therefore, lower MDA concentration means higher antioxidative ability and reflecting higher drought tolerance (Marcinińska et al., 2013). Using MDA concentration towards selecting drought tolerant genotypes of wheat, there are several articles previously reported (Shao et al., 2005; HongBo et al., 2005; Yang and Deng, 2015; Qayyum et al., 2021).

We found a large variation in MDA concentration across the 37 wheat genotypes. The imposition of stress increased the MDA concentration in shoot tissues of 19 genotypes and in root tissues of 11 genotypes. Under recovery conditions, as expected, MDA increasing was observed in shoots of only 4 genotypes and roots of only one genotype. Interestingly, the three genotypes, Karim, Svems 18, and Svems 20, considered as the most tolerant genotypes characterized by deep-rooting system showed no increasing in both shoot and root and in response to both treatments.

Generally, plants accumulate several organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake (Rhodes et al., 1994). Among these solutes, proline is the most widely studied (Delauney and Verma, 1993). In fact, proline is produced by plants under stress to increase the osmotic potential by reducing the hydric potential thus allowing water uptake. Besides its role as compatible solute, proline also acts as reactive oxygen species (ROS) scavenger and even contribute to carbon and nitrogen storage in stressed plants (Szabados and Savaouré, 2010). High proline accumulation under drought is commonly associated with high drought stress tolerance (Bilal et al., 2015). However, the relation between proline accumulation and enhanced or reduced tolerance to stress is still controversial in the literature, having been reported correlation of higher proline concentrations with both higher and lower stress

tolerance (Arteaga et al., 2020).

The imposition of stress increased proline concentration in shoots of 21 genotypes and roots of 18 genotypes. As expected, after rewatering treatment, proline accumulation was observed in shoots of only 15 genotypes and roots of 14 genotype. Svems 18, Karim and Svems 20 considered as most tolerant genotypes with deep-rooting systems showed interesting fluctuation. Svems 18 revealed increasing value of shoot proline concentration only in stress condition, while didn't show significant value of root proline concentration. The other ones, Svems 20 and Karim, showed less increased values of shoot proline concentration in recovery condition. While in root proline concentration, it is interesting to note that they showed increasing values in stress condition. It should be noted that the higher levels of proline as a non-enzymatic antioxidant and reactive oxygen species (ROS) scavenger (Szabados and Savaouré, 2010) were related to lower levels of MDA in tolerant genotypes with deep-rooting system revealing their ability to withstand oxidative stresses.

To understand whether the greater tolerance could be related to a particular root architecture, six root morphological traits including root length, root volume, root diameter, root surface area, the number of root tips, and the density of root length of 37 wheat genotypes were measured. Our PCA analysis revealed that the root diameter and the density of root length variables are almost uncorrelated with the other variables. The hierarchical clustering led to three major clusters representing deep-rooting system, shallow-rooting system, and medium-depth rooting system. Interestingly, three genotypes belonging to deep-rooting system cluster, named Karim, Svems 18 and Svems 20, with higher values in all traits except diameter, revealed tolerance when face to water deficit stress. Under both stress and recovery conditions, no increasing levels of MDA concentration were observed in both shoot and root of Karim and Svems 20 indicating their abilities to withstand oxidative stress. It should be mentioned that a significant increasing was recorded in shoot of Svems 18 but much less than other genotypes. Moreover, under stress condition, root fresh weight and shoot fresh weight of three genotypes revealed no significant decreasing or less decreasing compared to other genotypes representing their normal growth rate in both shoot and root. Morphological root traits play vital roles in crop adaptation and productivity in water-limited environments. A deep and branched root system helps plants to avoid water-deficit stress (Ye et al., 2018). It has been previously reported that large root systems in wheat absorb more water in dry environments to

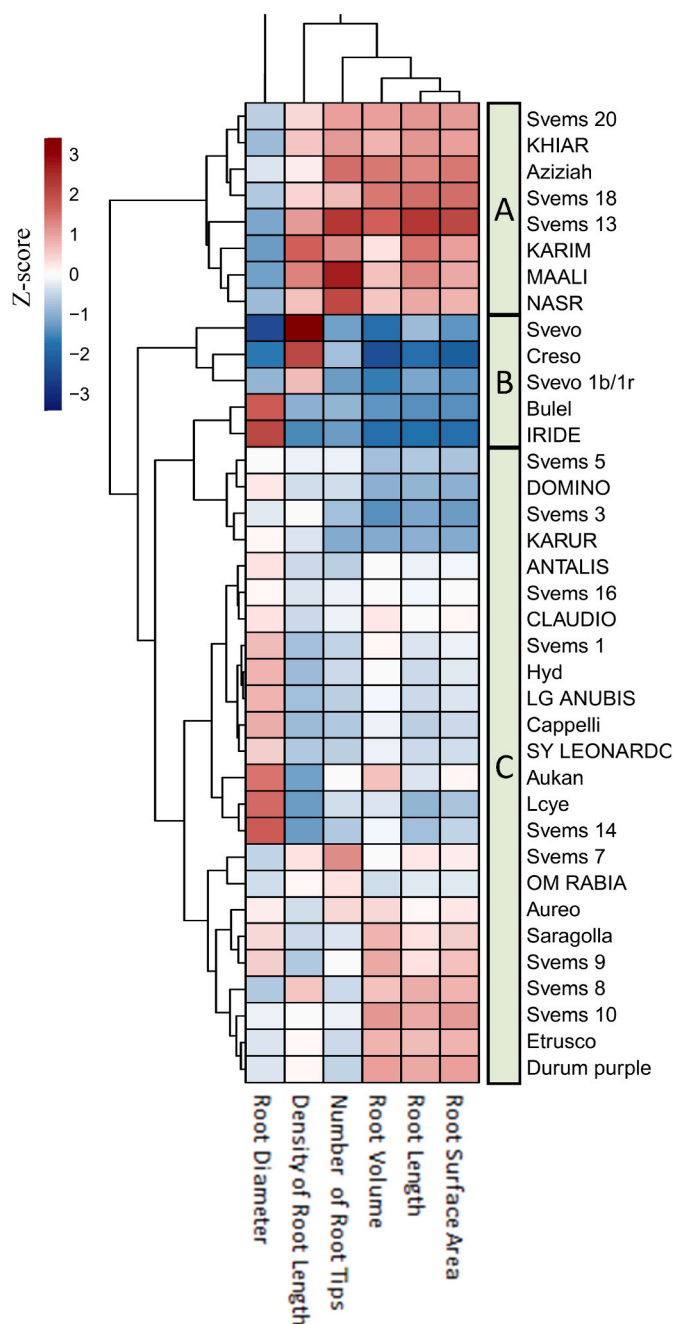


Fig. 5. Heatmap representing hierarchical clustering of root traits dataset across 37 wheat genotypes by complete method and Euclidean distance measure. Three major clusters (A–C) were found divided into: deep-rooting system (cluster A), shallow-rooting system (cluster B) and medium-depth rooting system (cluster C). The color bar depicts the gradient of values of roots traits.

promote access to sufficient water for grain filling (Palta et al., 2011).

5. Concluding remarks

Drought tolerance is crucial for increasing and stabilizing wheat productivity in dry areas worldwide.

A collection of 34 durum wheat genotypes, including landraces, ancient and modern genotypes, along with 2 tritordeum cultivars and Etrusco (*Triticum turgidum* ssp. *turanicum*), were considered for this study to be characterised by a better ability to cope with drought stress and to recover after stress. Several physiological and morphological traits of shoot and root were examined, and the results showed a large variation

in shoot and root fresh weight, proline, chlorophyll, and MDA concentration, and also in root morphological traits across the 37 genotypes. However, the hierarchical clustering of the datasets led to identification of main clusters including drought-tolerant and drought-sensitive genotypes based on physiological traits such as proline and lipid peroxidation. Drought tolerant genotypes showed higher levels of proline concentration along with lower oxidative damage. Moreover, root traits clustering led to identification of main clusters including genotypes with deep-rooting, medium-depth rooting, and shallow-rooting systems. Integrating all the data results led to identification of 3 outstanding genotypes, namely Karim, Svems 20, and Svems 18, as the most tolerant genotypes with deep-rooting system. Significant increased values of proline were recorded in shoot and root of these 3 genotypes while no oxidative damage was observed representing their ability to withstand oxidative stress. The root and shoot weight indicators revealed they were able to maintain their growth rate under water-deficit stress condition. Moreover, two shallow-rooting genotypes, namely Iride and Bulel tritordeum, which showed increasing lipid peroxidation and decreasing chlorophyll level, were introduced as the most sensitive genotypes.

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Author contributions

Giulia Quagliata: Writing - original draft, conducted the experiment and analysis. **Somayeh Abdirad:** Writing - original draft, data statistical analysis, wrote and edited the manuscript. **Silvia Celletti:** conducted the experiment and analysis. **Francesco Sestili:** provided plant material, revised the manuscript. **Stefania Astolfi:** conceived and supervised the study, provided funding, and wrote-revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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