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ORIGINAL PAPER

Morpho-physiological responses of sugar beet (*Beta vulgaris* L.) genotypes to drought stress

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Abstract The identification of morpho-physiological traits related to drought tolerance and high yield potential is a challenge when selecting sugar beet genotypes with greater tolerance to water stress. In this paper, root morphological parameters, antioxidant systems, leaf relative water content (RWC) and H^+-ATPase activity as key morpho-physiological traits involved in drought tolerance/susceptibility of sugar beet were studied. Genotypes showing a different drought tolerance index (DTI) but a similar yield potential, under moderate (−0.6 Mpa) and severe (−1.2 MPa) water stress, were selected and their morpho-physiological traits were investigated. The results showed a wide genetic variation in morpho-physiological parameters which demonstrated the different adaptive strategies under moderate and severe drought conditions in sugar beet. In particular, an efficient antioxidant system and redox signalling made some sugar beet genotypes more tolerant to drought stress. The alternative strategy of other genotypes was the reduction of root tissue density, which produced a less dense root system improving the axial hydraulic conductivity. These results could be considered as interesting challenge for a better understanding of the drought tolerance mechanisms in sugar beet.

Keywords Root morphology · Water stress · H^+-ATPase · Reactive oxygen species scavenging enzymes · Principal component analysis

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Introduction

Water stress is considered as one of the most widespread limitations to crop productivity and yield stability. In sugar beet, drought causes yield reductions between 10 and 30 % in central and western Europe (Ober 2001; Pidgeon et al. 2001; Jones et al. 2003), which increase in arid and semi-arid regions (Sadeghian et al. 2000), especially where precipitation is low. A solution is to improve the drought tolerance of sugar beet varieties and to identify sugar beet germplasm that is drought-tolerant and high yielding so that it can be included in future breeding programmes.

However, developing a reliable method for selecting sugar beet that combines both traits is complex and difficult. The first problem is the lack of a wide range of sugar beet genotypic variation for drought tolerance, in terms of yield and quality, especially among cultivars (Kerr 2000; Bloch and Hoffmann 2005; Bloch et al. 2006). The use of genotypes with a diverse genetic background (Ober et al. 2004; Rajabi et al. 2009), different *Beta* types (Sadeghian et al. 2000; Ober and Luterbacher 2002) and S1 pollinator lines (Ahmadi et al. 2011) could be a valid alternative. A second aspect is related to environmental variability (soil types and/or weather conditions), and to strong genotype × environment interaction, which affects the performance of sugar beet varieties (Pidgeon et al. 2006; Hoffman et al. 2009; Ober and Rajabi 2011). Finally, the

selection of sugar beet for high yield potential did not often ensure the drought tolerance (Sadeghian et al. 2000; Ober et al. 2005). The identification of specific morpho-physiological traits as markers for the selection of drought-tolerant sugar beet genotypes by marker assisted selection could be a promising and interesting approach.

Morpho-physiological responses to drought stress have been generally described by the avoidance/tolerance model (Levitt 1972; Verslues et al. 2006) including stomatal closure and root growth (avoidance mechanisms), solute accumulation and cell wall stiffening (dehydration avoidance) or protective solutes and proteins, metabolic changes and reactive oxygen species (ROS) detoxification (dehydration tolerance). However, some results on physiological traits such as stomatal closure (Clarke et al. 1993; Mohammadian et al. 2001; Ober et al. 2005; Bloch et al. 2006) and osmotic adjustment (Clarke et al. 1993; Gzik 1996; Sharp and LeNoble 2002; Ober et al. 2005; Choluj et al. 2008; Bagatta et al. 2008), appeared to be contradictory to discriminate drought tolerant/sensitive sugar beet genotypes.

Although most drought stress mechanisms are well-known, few studies have focused on the root traits linked to specific components of drought tolerance in sugar beet. Sadeghian and Yavari (2004) observed that root length was maintained by drought-tolerant sugar beet genotypes under water stress in growth chamber experiments.

However, root length ratio (RLR, root length per unit of the plant's dry mass) is a better trait than root length for describing plant's potential for soil resource acquisition under stress conditions (Ryser 1998). This parameter is constituted of the allocation component, root mass ratio (RMR), and two morphological components: root fineness (RF, root length per unit root volume) and tissue density (RTD, root dry mass per unit root volume) (Ryser and Lambers 1995; Ryser 1998, 2006). Plants may produce longer roots either by increasing biomass allocation or root fineness and/or reducing root tissue density, leaving biomass allocation unchanged. For example, changes in RTD seemed to be responsible for plant adaptations to nutritional deficiencies (Ryser and Lambers 1995; Hill et al. 2006) and flooding (Vasellati et al. 2001) or were related to the reduction in water losses at the lowest soil Ψ_w (Cruz et al. 1992; North and Nobel 1996; Noldt et al. 2001). Furthermore, RF was considered to be the functional trait for the drought-tolerant herbaceous tallgrass prairie species (Tucker et al. 2011) and correlated with the root's ability to take up water (Pemàn et al. 2006; Hernàndez et al. 2010).

Although the presence of ROS detoxification mechanisms, such as antioxidants and/or ROS scavenging enzymes, have been considered as a drought-stress mechanism in plants (Apel and Hirt 2004), little attention has been paid to this antioxidant system in the drought responses of sugar beet. Recently, Sayfzadeh and Rashidi (2010) observed a significant irrigation × genotype interaction with catalase (CAT) and guaiacol peroxidase (GPX), both ROS scavenging enzymes. Furthermore, there has been little focus on H^+-ATPase, an important enzyme involved in root growth (Ober and Sharp 2003), in the accumulation of osmolytes in drought-stressed plants (Liu et al. 2005), in the adaptation of the plant to the dry habitat (Chen et al. 2005) and in the "early warning" response to soil drying (Gong et al. 2010), but never reported in drought tolerance sugar beet responses.

Thus, the objectives of this study were to (1) assess the genotypic diversity in experimental lines of sugar beet composed by a diallelic system in terms of shoot and root growth in growth chamber, (2) investigate the morpho-physiological mechanisms with emphasis on new functional traits, such as RLR and related components, antioxidant systems and H^+-ATPase activity, and (3) describe the relationships between genotypes and functional traits using a multivariate approach.

Materials and methods

Yield production in field experiment

The yield data were obtained from field experiments conducted in 2002–2005 at Lion Seeds Ltd (geographic coordinates not available, Maldon, UK) and Research Institute for Industrial Crops (45°04′N, 11°47′E; Rovigo, Italy). Both Maldon and Rovigo have a silt-loam soil, some characteristics of which are presented in Table 1.

A complete randomized block design with four replications was established. All plots received 80 kg ha^{-1} of P_2O_5 splitted into two applications, with 70 % applied after ploughing and 30 % 1 month after sowing. Also nitrogen fertilization was split-applied, with 70 kg ha^{-1} of nitrogen, in the form of urea, at sowing and 30 kg ha^{-1} 1 month later. Pelleted seeds were sown each year in the first week

Table 1 Main properties of both Maldon (UK) and Rovigo (Italy) soils

Parameters	Maldon	Rovigo
Sand (%)	49	46
Silt (%)	38	40
Clay (%)	13	17
pH_{H2O}	7.2	7.8
Total organic C (%)	2.9	2.3
Total N (‰)	2.5	2.2
Assimilable, *P* (ppm)	17	12
Exchangeable, *K* (ppm)	145	116

of March with a drilling machine in six-row plots of 8 m length. Plots were manually thinned to a population density of 96,000 plants ha^{-1}. Sugar beet was irrigated to meet crop water needs and weeds, insects, and diseases were controlled using standard commercial procedures. Beets were harvested each year at the beginning of September. Root and sugar yield were determined according to Märländer et al. (2003) at the laboratory of Research Institute for Industrial Crops. These yield parameters resulted significantly correlated ($r = 0.905$; $P < 0.01$) (E. Biancardi, personal communication).

Growth chamber experiments

Growth conditions and experimental treatments

Sugar beet seeds (*Beta vulgaris* L. subsp. *vulgaris*) were kindly provided by the Research Institute for Industrial Crops, Rovigo Section (CRA-CIN). The seeds belonged to a diallel crossing system consisting of two male-sterile components, three pollinators and their F1 hybrids as shown in Table 2. The commercial cultivar, "Shannon" (Lions Seeds, UK), was included as a control (code "N").

Seeds were scarified with 3 % (v/v) hydrogen peroxide, continuously agitated for about 14 h and then washed thoroughly with deionised water. Then, they were placed in Petri dishes (Ø = 9 cm) containing moistened vermiculite in dark growth chamber at 25 °C for 4 days. Then, seedlings were transferred to modified Hoagland aerated solution (Hoagland and Arnon 1950) that contained 200 μM $Ca(NO_3)_2$, 200 μM KNO_3, 200 μM $MgSO_4$, 40 μM KH_2PO_4 and microelements and placed in growth chamber at 25/18 °C and 70 % relative humidity with a 14/10 h light/dark cycle (PPFD above shoot: 300 μE m^{-2} s^{-1}). The nutrient solution, adjusted to pH 6.0 by 0.1 N KOH, was monitored and adjusted daily and renewed every 2 days.

At 8 days, the seedlings of each genotype were transferred to a nutrient solution with either the same above composition or added with 224 (medium water stress, MWS) or 325 g l^{-1} (high water stress, HWS) of polyethylene glycol (PEG) 8000 (Sigma Aldrich P2139), to reach an osmotic potential of −0.6 and −1.2 MPa, respectively, calculated using the equation by Michel (1983). The final PEG concentration was gradually achieved by the addition of 81.2 and 112 g l^{-1} of PEG every 6 h, on the 8th and 9th day of growth, for the MWS and the HWS treatments, respectively. Plants in the nutrient solution without PEG were used as control.

Table 2 Parental lines and their F1 crosses of sugar beet used in the experiments

Parental line or crosses	Code
Male-sterile	C–D
Pollinator	H–E–M
Hybrid C × E	A
Hybrid D × E	B
Hybrid C × H	F
Hybrid D × H	G
Hybrid C × M	I
Hybrid D × M	L

Morphological root analysis

On day 10 (2 days after drought stress, DADS), ten seedlings of each genotype and from each water stress treatment were collected, divided into roots and shoots, and their fresh weights were immediately determined (RFW and SFW, g, respectively). The roots were immersed in 0.1 % toluidine blue for 5 min and then scanned at a resolution of 300 dpi (WinRhizo STD 1600, Instruments Régent Inc., Canada) to determine the root length (RL, cm) and volume (RV, cm^3) using the WinRhizo Pro v. 4.0 software package (Instruments Régent Inc., Canada). Shoot dry weight (SDW, g) and root dry weight (RDW, g) were determined after oven-drying at 70 °C for 48 h. Based on the above measurements, root length ratio (root length/whole plant dry weight, g cm^{-1}), root mass ratio (root dry weight/whole plant dry weight, g g^{-1}), root fineness (root length/root volume, cm cm^{-3}) and root tissue density (root dry weight/root volume, g cm^{-3}) were calculated.

Leaf relative water content

On day 10 (two DADS), leaves from three seedlings of each genotype and water stress treatment were removed, immediately weighed (LFW, g), put in deionised water and left in the dark for 48 h. After this, they were weighed to determine the leaf turgid weight (LTW, g) and then placed in an oven at 60 °C for 48 h, to determine the dry weight (LDW). The relative water content (%) of the leaves was calculated according to Barr and Weatherley (1962):

$$\mathrm{RWC} = \frac{\mathrm{LFW} - \mathrm{LDW}}{\mathrm{LTW} - \mathrm{LDW}} \times 100$$

Enzyme extractions and assays

At two DADS, three seedlings of each genotype and water stress treatment were collected. Then, the leaf samples (1.5 g) were ground and homogenized with an extraction buffer (1:3, w/v ratio) containing 0.05 M of Na_2HPO_4, 2 mM Na-EDTA at pH 7.8 adjusted by 2-[*N*-morpholino]ethanesulfonic acid (MES), and 2 % polyvinylpyrrolidone (PVP) (w/w). All procedures were carried out at 4 °C. After filtering, the extracts were centrifuged (Eppendorf

model. 5804 R) at 13000 rpm for 40 min at 4 °C. Then the supernatant was collected, immediately frozen in liquid N_2 and stored at a temperature of −80 °C until needed. All the tested enzymes were assayed spectrophotometrically (Lambda 5 double-beam spectrophotometer; Perkin-Elmer, Norwalk, CT, USA). Ascorbate peroxidase activity (EC 1.11.1.11) was monitored by following the oxidation of AsA at 290 nm in the presence of H_2O_2 as the co-substrate (Sanità di Toppi et al. 2005). Catalase activity (EC 1.11.1.6) was determined by following the consumption of H_2O_2 at 240 nm (Sanità di Toppi et al. 2005). Guaiacol peroxidase activity (EC 1.11.1.7) was based on the oxidation of guaiacol (2-methoxyphenol) from H_2O_2 in the presence of the enzyme, with formation of tetraguaiacol (Pandolfini et al. 1992).

PM-H^+-ATPase activity

Isolation of plasma membrane vesicles

Plasma membrane vesicles were isolated from the roots of the sampled sugar beet seedlings using the small scale procedure by Santi et al. (1995). Roots of seedlings from each treatment and genotype were homogenized in extraction buffer (250 mM sucrose, 10 % (v/v) glycerol, 10 mM glycerol-1-phosphate, 2 mM MgSO4, 2 mM EDTA, 2 mM EGTA, 2 mM ATP, 2 mM DTT, 5.7 % (w/v) choline chloride and 25 mM BTP buffered to pH 7.6 with MES and 1 mM PMSF). Before homogenization, 20 mg ml^{-1} chymostatin was added. After extraction, the samples were filtered and centrifuged twice at 12,700 rpm for 3 and 25 min, respectively, at 4 °C. The suspension was layered over a 25/38 % discontinuous sucrose gradient (10 mM DL-α-glycerol-1-phosphate, 2 mM $MgSO_4$, 2 mM EGTA, 2 mM ATP, 1 mM PMSF, 2 mM DTT, 20 mg ml^{-1} chymostatin, 5.7 % choline chloride and 5 mM BTP buffered at pH 7.4 with MES) and centrifuged at 12700 rpm for 60 min at 4 °C. The vesicles, banded at the 25/38 % interface layers, were collected and centrifuged at 14000 rpm for 45 min at 4 °C. The pellets were re-suspended in a medium containing 20 % glycerol (v/v), 2 mM EGTA, 2 mM EDTA, 0.5 mM ATP, 1 mM PMSF, 2 mM DTT, 20 mg ml^{-1} chymostatin, 5.7 % choline chloride and 5 mM BTP buffered at pH 7 with MES). They were then immediately frozen in liquid N_2 and stored at −80 °C until needed.

ATPase activity

ATP-hydrolysis activity was determined by measuring the release of inorganic phosphate, as described by Forbusch (1983), at 38 °C. Assays were performed at 38 °C in a 0.6 ml assay medium containing 50 mM BTP-MES at pH 6.5, 5 mM $MgSO_4$, 5 mM ATP, 0.6 mM Na_2MoO_4, 100 mM KNO_3, 1.5 mM NaN_3 and 0.01 % (w/v) Brij58, with or without 100 μM vanadate (V_2O_5), an inhibitor of P-type H^+-ATPase. Sodium azide and KNO_3 were used as selective inhibitors of mitochondria and tonoplast H^+-ATPase, respectively. The difference between these two activities was attributed to the PM-H^+-ATPase. The reaction was initiated by the addition of 0.5–1.5 μg membrane protein and was stopped after 30 min with a solution containing: 0.6 M HCl, 3 % (w/v) SDS, 3 % (w/v) ascorbic acid and 0.5 % (w/v) ammonium molybdate at 2 °C.

Protein assay

Total soluble protein was estimated according to Bradford (1976) using bovine serum albumin as a standard.

Calculations and statistical analysis

Statistical analysis of the data was undertaken using SPSS Statistics v. 15.0 (IBM Corp. USA) while the graphics were prepared using SigmaPlot v. 8.0 (Jandel Scientific, San Rafael, CA, USA).

Drought tolerance index calculation and correlation between field and hydroponics data

The drought tolerance index (Fernandez 1992), adapted for sugar beet by Ober et al. (2004) was calculated according to the following formula:

$$\mathrm{DTI} = \frac{(\mathrm{PDM_d}/\mathrm{PDM_w})}{(\mathrm{PDMT_d}/\mathrm{PDMT_w})}$$

where PDM_d is the plant dry matter under drought stress ($\Psi_w = -1.2$MPa), PDM_w is the plant dry matter of the control plants ($\Psi_w = 0$ MPa) for each genotype and $PDMT_d$ and $PDMT_w$ are the average values of the plant dry matter for all genotypes under drought stress and control condition, respectively. The DTI was also calculated in terms of root fresh and dry weights.

The correlations between irrigated production (ton/ha of roots) obtained in the field and the growth parameters (shoot fresh and dry weights, root fresh and dry weights) of sugar beet seedlings in the growth chamber at $\Psi_w = 0$ MPa were assessed using the Pearson test ($P < 0.05$).

Morphological and physiological analysis

For plant growth, morphological and physiological analysis, the experiment had a completely randomized design with 10 replicates per treatment (water stress level: 0, −0.6 and −1.2 MPa per genotype). All data were tested for normality (Kolmogorov–Smirnoff test) and homogeneity

of variance (Levene Median test) and, where required, the data were transformed. For each genotype, plant growth (fresh and dry weights of the shoots, roots and whole plant, and the root length), root morphological (root length ratio, root mass ratio, root fineness and root tissue density), and physiological parameters (leaf relative water content, catalase, guaiacol peroxidase, ascorbate peroxidase and H^+-ATPase activities) were statistically analysed using one-way analysis of variance with the water stress level (0, −0.6 and −1.2 MPa) as main factor. Subsequently, the Bonferroni test was used to compare, for each genotype, the mean of the plant growth, morphological and physiological analysis of seedlings grown in PEG (−0.6 and −1.2 MPa) with the control ($P < 0.05$).

Principal components analysis (PCA)

The dataset derived from the morpho-physiological parameters of the selected sugar beet hybrids exposed to MWS and HWS was subjected to principal components analysis based on a correlation matrix of the following variables: RLR, RMR, RF, RTD, RWC, CAT, APX, GPX and H^+-ATPase.

PCA produced uncorrelated multivariate axes that might be interpreted as representing a given sugar beet strategy for adaptation to water stress. Use of the correlation matrix standardizes differences among variables due to the measurement scale. The importance of different traits in a given axis is indicated by the relative loading of the trait in the eigenvector.

Results and discussion

Validation of the diallel system and screening of sugar beet genotypes for drought tolerance in a growth chamber

Before screening genotypes for drought tolerance and studying their morpho-physiological traits, the validation of the diallel system of sugar beet and comparisons between field and growth chamber data for verifying the effectiveness of operating in a controlled growth system were undertaken. During 3 years of field trials, under irrigated conditions, sugar beet genotypes showed a wide variability in root yield. In particular, the "H" and "D" parental lines showed the lowest and highest root yield, respectively, as did "F" and "L" among the hybrids; while "N", the commercial variety, was the most productive genotype (Fig. 1). Similarly, all the genotypes grown in hydroponic culture in the climate chamber at $\Psi_w = 0$ (Fig. 2), $\Psi_w = -0.6$ MPa (Fig. 3a, c) and $\Psi_w = -1.2$ MPa (Fig. 3b, d) showed high genotypic variability in terms of

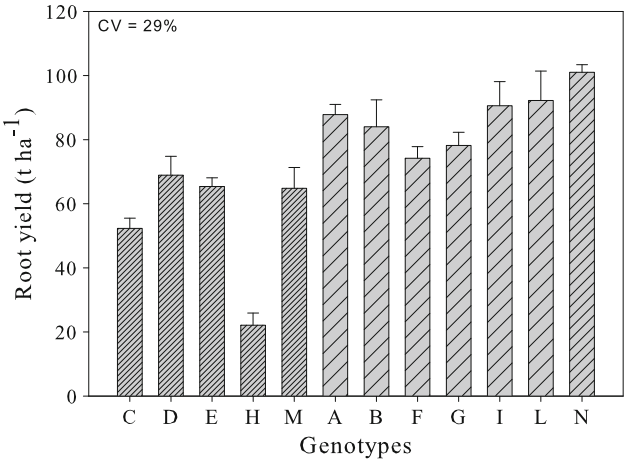

Fig. 1 Root yield (t ha^{-1}) of parental lines (*C*, *D*, *E*, *H*, *M*), hybrids (*A*, *B*, *F*, *G*, *I*, *L*) and commercial variety (*N*) of sugar beet grown in the field with optimal water conditions. The values are the means over 3 years and two locations. *CV* coefficient of variation

shoot and root growth (coefficients of variation between 32 and 47 %). This variability, already reported in terms of total dry matter, relative leaf expansion (Ober and Luterbacher 2002) and sugar yield (Ober et al. 2004) in the irrigated field trials, was an important prerequisite for identifying drought-tolerant sugar beet genotypes in this diallelic system. Furthermore, a higher coefficient of variation for root growth (37–47 %) compared to shoot growth (32–36 %) (Figs. 2, 3), confirmed the validity of using this diallel system. Indeed, the root system can be considered as a source of drought-adaptive traits that could be exploited in sugar beet (Shaw et al. 2002; Sadeghian and Yavari 2004), such as in wheat (Manschadi et al. 2008), white clover (Jahufer et al. 2008), and maize (Hund et al. 2009).

The comparison of field and growth chamber data, in the absence of water stress, showed a high degree of correlation ($R^2 = 0.661$ and $R^2 = 0.615$) and statistical significance ($P < 0.01$) for both root fresh and dry weights and root yield (tonnes of roots ha^{-1}) (Fig. 4b, d). This result not only validated the use of the controlled environmental system to grow the sugar beet genotypes but also suggested that the root system was directly related to yield and could be a potentially useful indirect tool for the selection of drought-tolerant sugar beet genotypes. Conversely, a low level of correlation ($R^2 = 0.191$ and $R^2 = 0.260$) with no statistical significance was recorded for shoot fresh and dry weights and root yield (Fig. 4a, c).

Hybrid selection

The selection of the sugar beet genotypes in response to water stress was carried out plotting DTI against irrigated root yield and plant dry weight. Then, according to Ober et al. (2004), hybrid selection was based on "the genotype

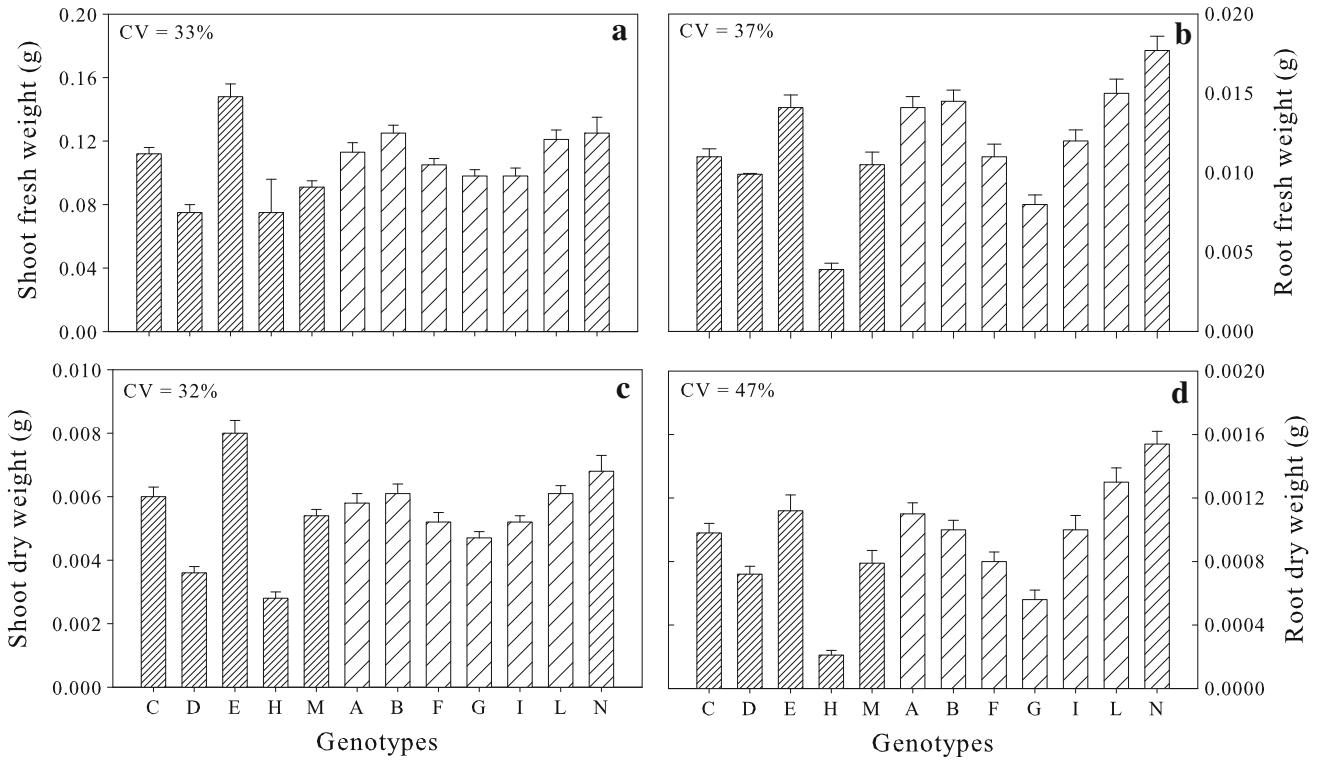

Fig. 2 Shoot fresh and dry weight (**a**, **c**) and root fresh and dry weight (**b**, **d**) of parental lines (*C*, *D*, *E*, *H*, *M*), hybrids (*A*, *B*, *F*, *G*, *I*, *L*) and commercial variety (*N*) of sugar beet seedlings grown on hydroponic system in the climate chamber at $\Psi_w = 0$ MPa (see the "Materials and methods" for the nutrient solution and environmental parameters). *CV* coefficient of variation

pairs that show similar yield potential but contrasted in DTI provide experimental material for dissection of morphological and physiological traits that confer drought tolerance". The plot of DTI (plant dry weight basis) of sugar beet hybrids stressed at −1.2 MPa, (extreme water stress) against irrigated root yields, defined four quadrants: I indicated a high DTI and high yield potential, II corresponded to a high DTI and low yield potential, III represented a low DTI and low yield potential and IV was a low DTI and high yield potential. The hybrids were arranged differently between the four quadrants; "A" and "B" showed a significant difference in DTI but a similar high yield potential, "L" and "I" both had a low DTI and a high yield while "G" and "F" had high DTIs and low yield potentials (Fig. 5a). Therefore, according to Ober et al. (2004), "A" and "B" were the hybrids with the best genetic background for investigating the morpho-physiological traits for drought tolerance. However, hybrid "G", which had the highest DTI, could also be very interesting for selection programmes (Fig. 5a). This result was further supported by the data obtained by plotting the DTI (plant dry weight basis) against plant dry weights at unstressed conditions in a growth chamber (Fig. 5b) and a plot of DTI (root fresh and dry weight basis) against the root yield in the field experiment (Fig. 5c).

Morpho-physiological and biochemical traits

Root system is the main organ responsible for plant water uptake and essential for crop productivity, especially under water stress (Ober and Sharp 2007). Although root length represents the morphological trait which better describes the capacity of the root to explore the deeper layers of soil (Ryser 1998), root length ratio could represent a more functional trait for plant adaptation to drought-prone environments. The results indicated that both moderate ($\Psi_w = -0.6$ MPa) and severe ($\Psi_w = -1.2$ MPa) water stress reduced significantly the RLR of the "A" and "B" hybrids and the "N" commercial cultivar compared to the control ($\Psi_w = 0$ MPa) but did not affect the RLR of the "G" hybrid (Fig. 6a). These results suggested that, under low water availability, the "A" and "B" hybrids and "N" cultivar did not produce an efficient root system while the "G" hybrid, by maintaining a long root system, showed the best drought tolerance response.

The RLR consisted of three morphological components, namely root mass ratio (biomass allocation parameter), root fineness and tissue density (structural parameters) (Ryser and Lambers 1995), which change in response to edaphic and environmental fluctuations. Therefore, the question as to which morphological components drove the variation in

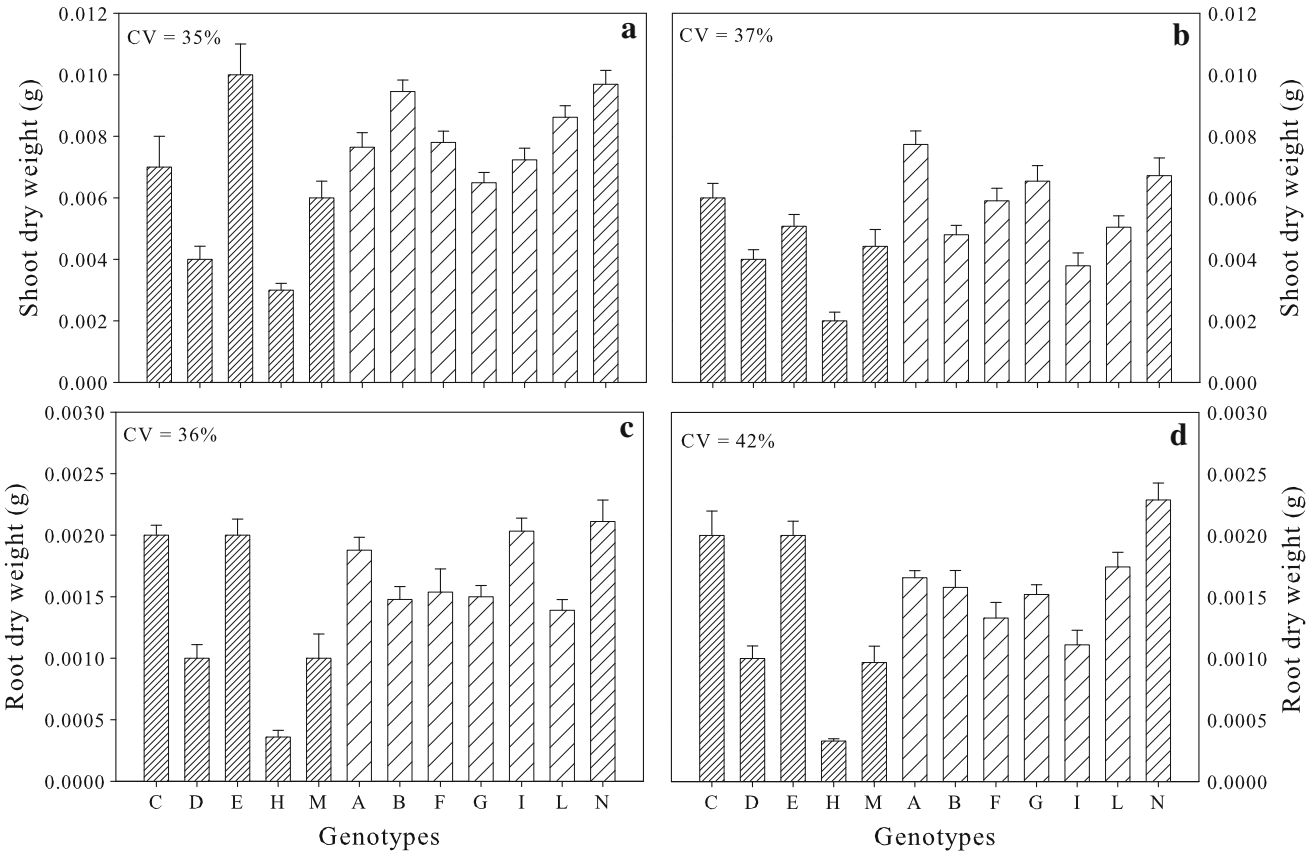

Fig. 3 Shoot dry weight (**a**, **b**) and root dry weight (**c**, **d**) of parental lines (*C*, *D*, *E*, *H*, *M*), hybrids (*A*, *B*, *F*, *G*, *I*, *L*) and commercial variety (*N*) of sugar beet seedlings grown on hydroponic system in the climate chamber at $\Psi_w = -0.6$ MPa (**a**, **c**) and $\Psi_w = -1.2$ MPa (**b**, **d**). *CV* coefficient of variation

RLR in sugar beet genotypes in response to water deficiency remains unclear. At −0.6 MPa, the root mass ratio increased in the "A" and "G" hybrids compared to the control, which was different to the other two genotypes, while at −1.2 MPa, all genotypes, except "A", increased their allocation of biomass to roots (Fig. 6b). In addition, root fineness was strongly reduced by both water stress levels in all genotypes except "G", where it increased by 49 and 41 % under moderate and high Ψ_w, respectively (Fig. 6c). At −0.6 MPa, the root tissue density of the "A" and "G" hybrids increased by 20 and 200 %, respectively, while it did not change significantly in the "B" hybrid and in the "N" cultivar (Fig. 6d). Conversely, at −1.2 MPa, the root tissue density increased significantly by 46, 50, 280 and 46 % in the "A", "B", "G" hybrids and the "N" cultivar, respectively (Fig. 6d). Taken together, the results indicated that the "A" and "B" hybrids and the "N" cultivar had higher RMRs and root tissue densities compared to the control and this was accompanied by a significant reduction in root fineness, producing a thicker and shorter root system at both moderate and severe water stress,. Conversely, the "G" hybrid showed a similar RMR and root tissue density pattern but a sharply increased root fineness, leaving unchanged the RLR parameter. These findings suggested that the "G" hybrid had constructed a thinner and more efficient root system when suffering from water shortage as compared to the other hybrids and cultivars. In particular, thinner roots increasing the root-soil interface pointed out a high root absorption potential (Larcher 1995), radial conductivity by lesser resistance to the radial flow (Huang and Eissenstat 2000), root hydraulic conductance per leaf unit surface area (Pemàn et al. 2006) or per stem cross-section area (Hernàndez et al. 2010). Further, the "G" hybrid showed a large increase in tissue density, an adaptive trait positively correlated with the degree of lignification and cell wall thickness (Ciamporova et al. 1998; Wahl and Ryser 2000; Hummel et al. 2007); anatomical characteristics adopted for water conservation in sorghum (Cruz et al. 1992), *Opuntia ficus-indica* (North and Nobel 1996) and woody species (Noldt et al. 2001).

Under water stressed conditions, the ability of the root system to maintain water levels and make osmotic adjustments was mainly ascribed to the PM-H^+-ATPase activity (Ober and Sharp 2003; Liu et al. 2005; Chen et al. 2005). In all sugar beet genotypes, the PM-H^+-ATPase activity was not significantly affected by either water stress levels,

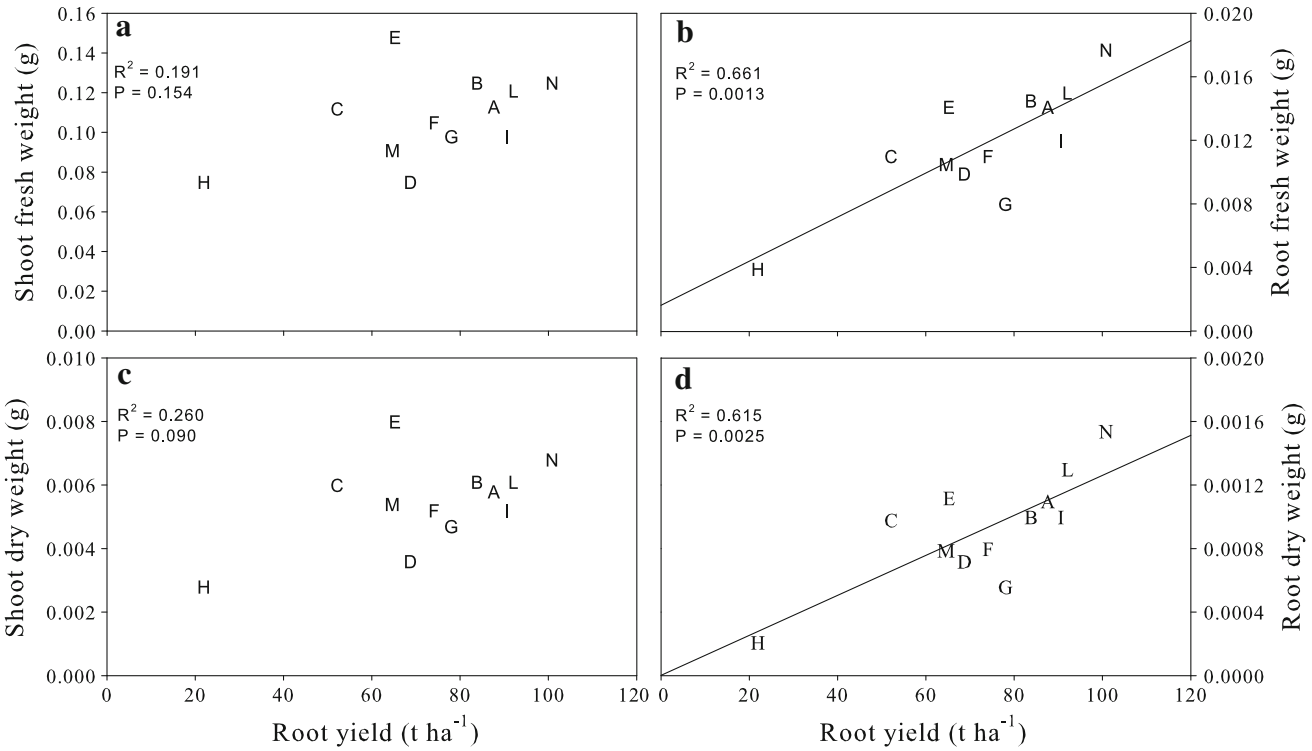

Fig. 4 Correlation between the root yield (t ha^{-1}) obtained under optimal water conditions in field trial and biometric data [shoot fresh (**a**) and dry weight (**c**), root fresh (**b**) and dry weight (**d**)] of seedlings of parental lines (*C*, *D*, *E*, *H*, *M*), hybrids (*A*, *B*, *F*, *G*, *I*, *L*) and commercial variety (*N*) of sugar beet grown on hydroponic system in the climate chamber at $\Psi_w = 0$ MPaR^2 correlation coefficient, *P* significance of correlation (Pearson's test)

although its activity tended to increase and decline at moderate ($\Psi_w = -0.6$ MPa) and severe ($\Psi_w = -1.2$ MPa) water stresses, respectively (Table 3). The increase in PM-H^+-ATPase response under moderate water stress has already been observed in two oat genotypes (Gong et al. 2010) and this suggests its involvement in water stress responses.

In general, drought-induced root mechanisms were accompanied by above ground adjustments including the cuticle and epidermis traits (Luković et al. 2009; Tsialtas and Maslaris 2012) and leaf relative water content and fine tuning responses determined by ROS homeostasis and redox signalling transduction of ABA-mediated physiological events (Miller et al. 2010) that influence the plant water status. According to previous works (Shaw et al. 2002; Ober et al. 2005), the results indicated that the RWC of leaves was significantly reduced by severe drought stress in all sugar beet genotypes, causing a marked turgor loss. Conversely, it was not modified under moderate stress (Table 2).

The activity of the antioxidant system in plants under stress is usually regarded as an indicator of the tolerance/susceptibility of the genotypes against stress conditions. Levels of APX, CAT and GPX did not show any significant changes under drought stress in the leaves of any of the genotypes after 48 h of treatment (Table 3). Although Sayfzadeh and Rashidi (2010) found significant genotype × irrigation differences for CAT in sugar beet, the lack of its involvement in drought responses has also been reported in other plants (Quartacci and Navarri-Izzo 1992; Sofo et al. 2005) suggesting a limited role for this enzyme in cell protection during water stress (Smirnoff 1993). In contrast, the guaiacol peroxidase activity, at both water stress levels, significantly increased in the "B" hybrid and in the "N" commercial variety compared to the control, which was not the case for the "A" and "G" hybrids (Table 3).

Principal components analysis of morpho-physiological and biochemical traits

PCA was conducted to discriminate tolerant and susceptible genotypes via their morpho-physiological and biochemical traits. In particular, it was able to reduce and group the morphological (RLR, RMR, RF and RTD) and physiological traits (H^+-ATPase, CAT, GPX, APX and RWC) into components according to their ability to describe the variability among sugar beet genotypes under water stress. The total variability of the three dimensional space was efficiently summarized by the two principal

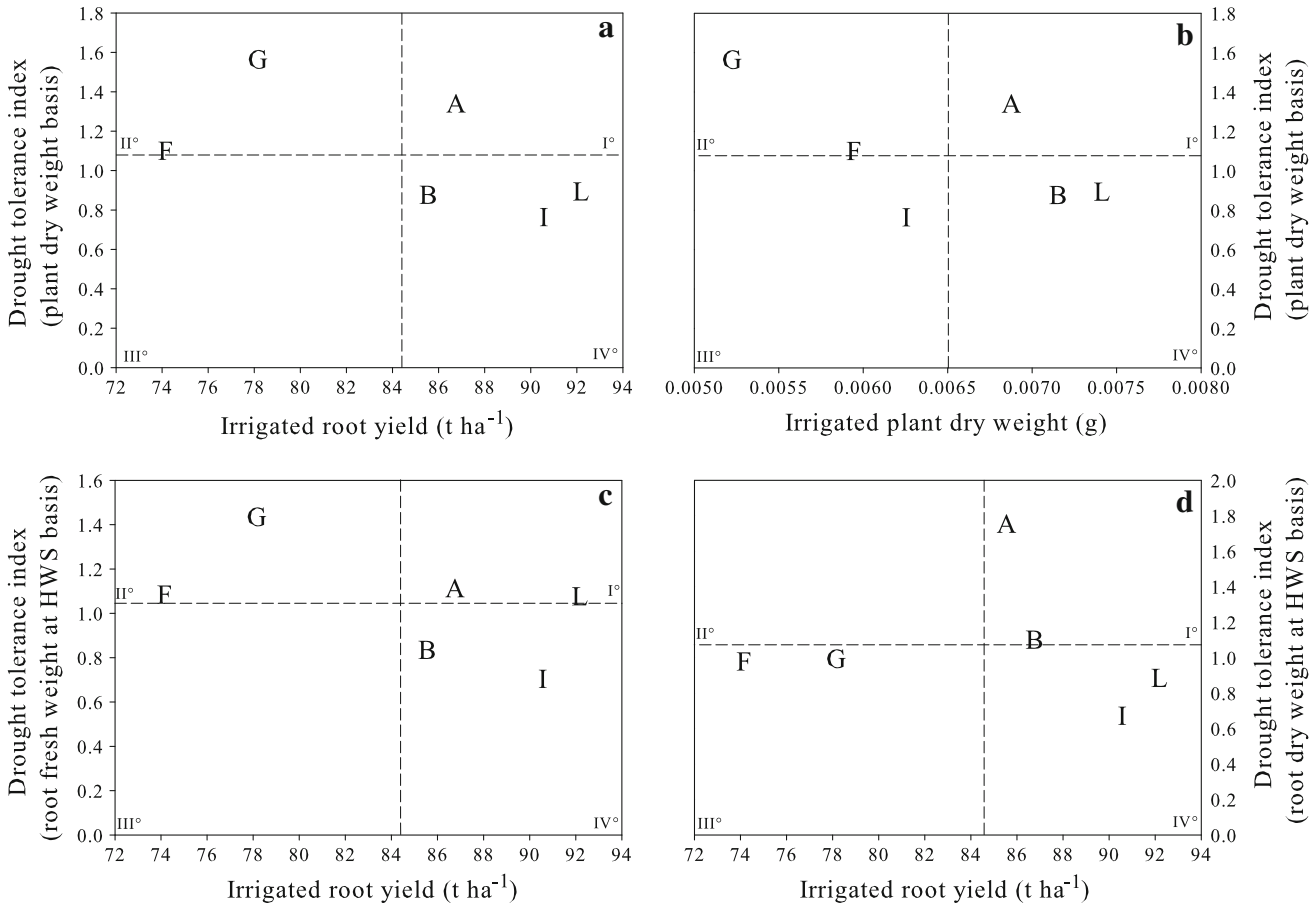

Fig. 5 Drought tolerance index based on plant dry matter (**a**, **b**), root fresh (**c**) and dry weight (**d**) of sugar beet seedlings grown in climatic chamber at Ψ_w of −1.2 MPa plotted against irrigated root yield measured in field (**a**, **c**, **d**) and plant dry weight (**b**). The *lines* indicated the mean value of each parameter of all hybrids

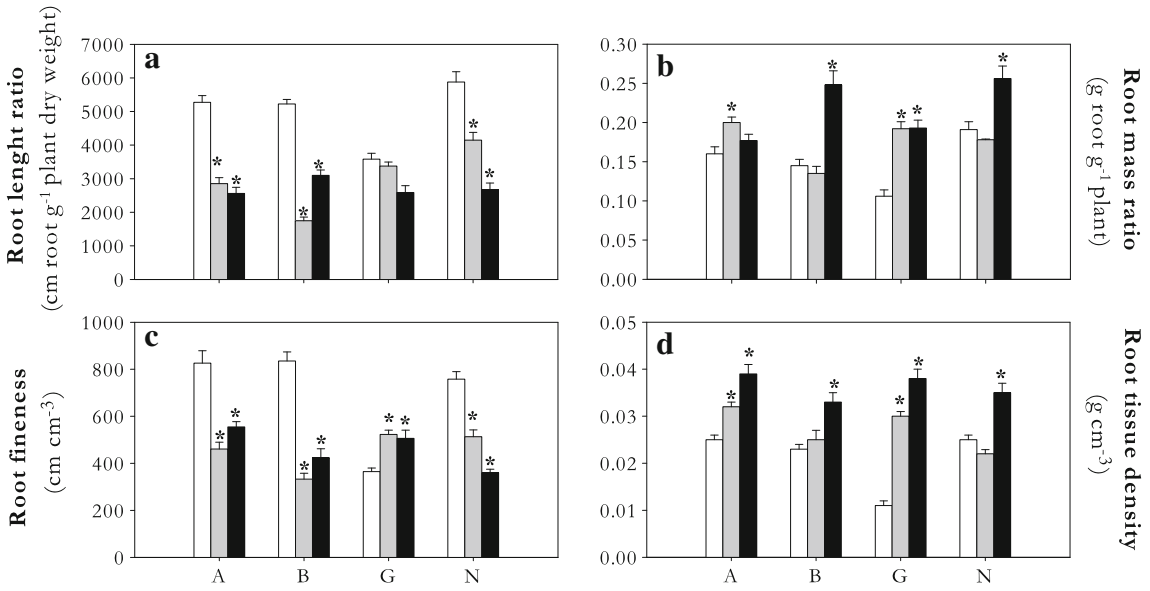

Fig. 6 Root morphology of three hybrids (*A*, *B* and *G*) and the commercial variety (*N*) of sugar beet seedlings exposed to different levels of water stress. [$\Psi_w = 0$ MPa (*open square*), $\Psi_w = -0.6$ MPa (*grey square*) and $\Psi_w = -1.2$ MPa (*black square*)]. *Error bars* indicate the standard error ($n = 10$). Within each hybrid, *asterisk* indicates a significant difference compared to control ($P < 0.05$, Bonferroni test)

Table 3 Physiological parameters of different sugar beet hybrids and commercial variety (*N*) exposed to different water stress conditions

Genotype	Ψ_w (MPa)	H^+-ATPase (nmol Pi mg^{-1} protein h^{-1})	RWC (%)	APX (nmol ascorbic acid mg^{-1} protein)	GPX (unit mg^{-1} protein)	CAT (unit mg^{-1} protein)
A	0	12 (6.0)	71 (2.3)	2.8 (0.5)	4.2 (1.0)	1.40 (0.1)
	−6	17 (3.2)	66 (4.0)	3.3 (1.2)	5.1 (1.2)	1.30 (0.4)
	−12	10 (3.5)	57* (2.0)	2.2 (0.8)	3.2 (1.0)	1.20 (0.3)
B	0	18 (4.5)	68 (1.0)	2.4 (1.4)	2.9 (0.4)	1.80 (0.3)
	−6	22 (7.3)	67 (0.4)	2.5 (1.2)	5.5* (1.7)	1.20 (0.2)
	−12	9 (1.3)	56* (0.8)	1.4 (0.4)	4.7* (1.03)	0.81 (0.1)
G	0	14 (5.2)	70 (1.4)	2.1 (1.2)	2.3 (0.2)	0.93 (0.3)
	−6	16 (1.6)	62 (3.2)	1.8 (0.1)	3.0 (0.3)	0.92 (0.2)
	−12	9 (1.8)	60* (3.0)	1.6 (0.9)	2.0 (0.4)	0.75 (0.4)
N	0	11 (2.8)	66 (0.8)	1.7 (0.5)	4.8 (1.5)	1.02 (0.3)
	−6	14 (4.5)	65 (3.1)	2.0 (0.9)	8.5* (1.4)	1.09 (0.1)
	−12	10 (3.8)	57* (4.5)	1.4 (0.2)	7.9* (0.9)	1.20 (0.3)

* Statistically significance difference between mean of the plant water stressed against the control at $P < 0.05$ (Bonferroni test), within each genotype

Table 4 Principal components of morpho-physiological traits of sugar beet genotypes exposed to moderate ($\Psi_w = -0.6$ MPa) and severe water stress ($\Psi_w = -1.2$ MPa)

	Attribute loading	
	Prin 1	Prin 2
Statistics		
Eigenvalues and variability		
Eigenvalue	1.603	1.448
Proportion of variability (%)	40	36
Variable		
Eigenvectors		
Root tissue density	−0.805	−0.126
APOX	0.258	0.804
CAT	−0.390	0.884
RWC	0.887	0.065

components, which accounted for 40 and 36 % of the variability, respectively (Table 4). The first component (Prin 1) consisted of high negative and positive loadings for root tissue density and RWC, respectively (Table 4). Therefore, Prin 1 included an integrated response of the below-ground (root morphology) with the above-ground parts (leaf water status) of the plant. Positive values for this component indicated a root system with reduced lignification, which, in turn, had led to a higher RWC. The second principal component (Prin 2) had high positive loadings for APX and CAT activities and hence, it could represent the antioxidant system and/or redox signalling under water stress (Pignocchi and Foyer 2003; Dat et al. 2000; Zhang et al. 2001; Miller et al. 2010).

Plotting the single sugar beet genotype by means of their component scores, Prin 1 (positive *vs* negative values) sharply separated the sugar beet genotypes exposed to moderate ($\Psi_w = -0.6$ MPa) from those subjected to severe ($\Psi_w = -1.2$ MPa) water stress (Fig. 7). This finding indicated that higher drought stress led to a denser root system and a lower leaf water content. The increase in root tissue density could have constrained hydraulic transportation towards the above-ground parts of the plant. Indeed, previous studies have shown a negative correlation between root tissue density and axial hydraulic conductivity (Joseph et al. 1998).

In contrast, Prin 2 (positive *vs* negative values) separated the "A" hybrid from "B" and "G" hybrids when they were under severe water stress (Fig. 7), which suggested that the "A" hybrid had adopted a successful strategy by having an efficient antioxidant system and redox signalling mechanisms. This was corroborated by having a higher DTI compared to the "B" hybrid (Fig. 4). Although the "G" hybrid resulted in an higher drought-tolerant genotype, it showed the lowest Prin 2 (Fig. 7) under severe water stress (highest DTI, Fig. 4); probably, the antioxidant and redox signalling systems, differently to the root morphological features characteristics, were not the mechanism basis of the drought tolerance for this genotype.

Conclusion

The diallelic population of sugar beet investigated in this study showed wide genotypic variation in their morpho-physiological responses to moderate and severe drought conditions. The results confirmed that susceptibility/tolerance to drought in plants was a coordinated action involving multiple stress response mechanisms. Using PCA, it was possible to identify the individual morpho-physiological traits behind the different water use strategies adopted by sugar beet genotypes as a response to drought stress. In particular, an efficient antioxidant system and redox signalling made the "A" hybrid more tolerant to drought than

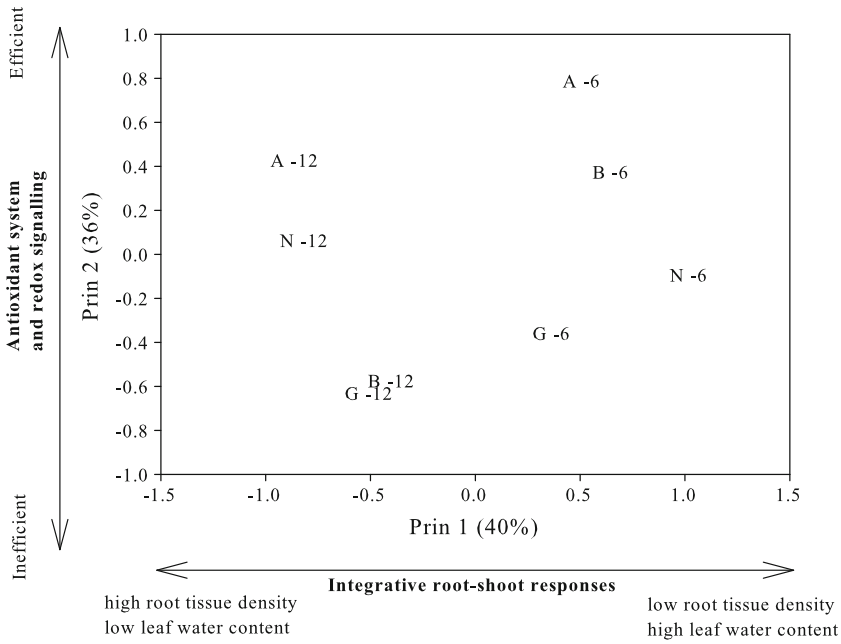

Fig. 7 Scores of the principal components 1 and 2 of the morpho-physiological traits of sugar beet genotypes exposed to moderate and severe water stress. The symbols combined the sugar beet genotypes (*A*, *B*, *G* and *N*; see "Materials and methods") with the moderate ($\Psi_w = -0.6$ MPa) and severe water stress ($\Psi_w = -1.2$ MPa). The *arrows* indicate the interpretation of the principal component and the proportion of explained variability is given within the *bracket*

the "B" hybrid. In contrast, a different water use strategy was employed by the "G" hybrid that led to a reduction in RTD, producing a root system that was less dense, which led to improvement in axial hydraulic conductivity.

The study of morpho-physiological traits should improve understanding of the drought tolerance mechanisms in sugar beet and they need to be further investigated, especially at the molecular and genetic (hereditability) level.

Author contribution Romano Alessandro: Experimental setup and work on root morphology on climatic chamber; Sorgonà Agostino: Experimental setup, work, data elaboration and writing text; Araniti Fabrizio and Lupini Antonio: Experimental setup and work on physiological proprieties on climatic chamber; Stevanato Piergiorgio: Experimental setup and work of field experiments; Cacco Giovanni: Critical revision of the text; Abenavoli Maria Rosa: Writing and critical revision of the text.

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