

REGULAR ARTICLE

Physiological responses to drought in four developed *Triticum aestivum* groups

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Abstract

Restricted water availability and yield reductions derived from climate changes have become a strong concern as regards fundamental crops, such as wheat. There is an increasing need to characterize germplasm diversity in order to highlight drought tolerant genotypes and to assist Portuguese wheat breeding programs. Bread wheat (*Triticum aestivum*) varieties were selected from four different evolutive and/or breeding groups: ancient landraces, traditional varieties, modern currently used and advanced lines. The aim of this work was to identify physiological traits that contribute to drought tolerance during grain filling period. Plants were cultivated in pots, under semi-controlled greenhouse conditions. Drought was imposed by withholding irrigation after anthesis. Well irrigated and water stressed plants were compared as regards leaf gas exchanges (net photosynthetic rate, Pn; leaf stomatal conductance, gs; transpiration, E), instantaneous water use efficiency (iWUE), membrane electrolyte leakage, osmotic potential and leaf pigments. Subsequently, plants were maintained under a controlled irrigation (droughted plants: 50% of the water given to fully irrigated controls) until harvest, to quantify yield. Pn and gs were significantly reduced by drought in all varieties. As regards membrane integrity ancient and traditional varieties presented lower membrane injury, what may reflect a higher protoplasmic tolerance to drought. More evolved varieties (modern and advanced) showed higher spike weight per plant and number of grains per spike, disregard the water regime. Under water deficit 1000 kernel weight was reduced in all varieties except in traditional Pirana, which also showed an increase in the number of spikes per plant. Higher membrane stability, increased pigments and lower osmotic potential under drought may underly such improved response to drought, pointing this variety as an interesting genetic resource for breeding purposes.

Key words: Membrane integrity, Photosynthetic activity, *Triticum aestivum*, Water deficit, Yield

Introduction

In South Mediterranean regions drought periods associated to high temperatures, have become more frequent and strongly affect plants productivity. The lack of information and unpredictability of target environments, especially in the context of climate changes, is still the greatest obstacle to the improvement of wheat yield productivity (Reynolds et al., 2012). Bread wheat (*Triticum aestivum*) is a major crop in South Portugal, where it is exposed to low rainfall and

heat stress at the end of the growing season (Maças, 1996; Almeida, 2007). Local rustic genotypes, generally more adapted to extreme environmental conditions, have been replaced by more productive varieties. There is a continuous need to characterize germplasm tolerance to drought to assist Portuguese wheat breeding programs in Alentejo (Coutinho et al., 2013). This important portuguese wheat producing region is also highly prone to desertification and water shortage (Santos et al., 2001).

Drought induces stomatal closure, resulting in photosynthesis reductions (Campos et al., 1999; Chaves and Oliveira, 2004). Water shortage causes oxidative stress to the cells through reactive oxygen species formation, which result in damaged cellular structures (Blum and Ebercon, 1981), leading to cell compartmentation and death. Membrane injury can be evaluated through increased electrolyte leakage (Campos and Pham Thi, 1997; Matos et al., 2002; Campos et al., 2003;

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Dias et al., 2010; Farooq et al., 2011; Scotti Campos et al., 2014). Plant reproductive development is extremely sensitive to abiotic stresses, so that any environmental perturbations may strongly affect final yield and quality (Dupont and Altenbach, 2003; Prasad et al., 2008). Water stress affects plants metabolism and reduces pollen fertility (Praba et al., 2009).

Plants can develop adaptive strategies to maintain assimilatory activities and membrane stability under water shortage conditions (Matos et al., 2010; Scotti-Campos et al., 2013). Under stress conditions, cell compartmentation must be preserved to maintain plant metabolism. Osmotic adjustment is a heritable character and plays an important role in maintaining yield stability under stress (Blum, 1992). Higher photosynthetic constraints may arrest growth and result in biomass decrease. However reduced water availability influences photoassimilates distribution and biomass partition, and may favour yield. Increasing physiological knowledge concerning mechanisms underlying such responses may reveal useful traits to screen for tolerant genotypes (Blum, 1992).

The use of adapted varieties may contribute to preserve productivity (Dodd et al., 2011) being considered an agricultural measure to cope with changing climate. The aim of this work was to compare 14 bread wheat varieties from different evolutive and/or breeding groups (ancient landraces, traditional varieties, modern currently used and advanced lines) as concerns drought induced physiological responses during grain filling (net photosynthesis, P_n; leaf stomatal conductance, g_s; instantaneous water use efficiency, iWUE; membrane integrity; osmotic potential ψ_s , pigments content) and relate them with yield.

Material and Methods

Plant material

Fourteen bread wheat (*Triticum aestivum* L.) varieties were selected from 4 different evolutive and/or breeding groups: ancient landraces Mocho de Espiga Quadrada (MEQ), Mocho de Espiga Branca (MEB), Gentil Rosso; traditional varieties Restauração, Ardito, Pirana (Vasconcelos, 1933); modern currently used varieties Roxo, Nabão, Ardila, Jordão, Eufrates; advanced lines TE 0205, Flycatcher, TE 0206, resulting from recent breeding work (national and international breeding programs + genetic stocks). Seeds were sown in December 2011, in 5 L pots filled with clay loam soil collected from the field (sowing density of 7

seeds per pot and 2.5 cm depth, eight pots per variety). Germination occurred 7-10 days after sowing (DAS). Two weeks after sowing germinated seeds were thinned to five plants. Pots were maintained in greenhouse under controlled conditions (mean daily temperatures 23-26°C and relative humidity 50-60%), under natural irradiance (PPFD up to 400-1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$). To obtain maximum field capacity, pots were supplied with water until runoff and weighed 24 h later. Seven days after visual assessment of anthesis (89 DAS), drought was gradually induced in four pots of each variety by withholding irrigation for 10 days, until field capacity values < 70% were obtained. These values were maintained through controlled irrigation (drought plants: 50% of water given to fully irrigated controls) until harvest, to obtain grain yield. The remaining pots were kept under full irrigation (field capacity ca. 85%). Gas exchange measurements were performed in flag leaves. Leaf discs and sections were collected from the uppermost fully expanded leaves immediately below. Three replicates were performed per treatment.

Gas exchange measurements

Leaf gas exchanges (net photosynthetic rate, P_n; stomatal conductance, g_s; transpiration, E) were measured using a portable CO₂/H₂O infrared gas analyzer exchange system LI-6400 (LI-Cor, Inc., Lincoln, U.S.A.) with an external CO₂ concentration of ca. 370 ppm, chamber block temperature controlled at 25°C, and artificial light adjusted at 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ supplied by a “cold” lamp LED type. The parameters were calculated according to the equations of Caemmerer and Farquhar (1981). Instantaneous water use efficiency (iWUE) was estimated as P_n/E. The sensor head encloses a leaf surface of up to 6 cm², well stirred to minimize boundary layer resistance as referred by Matos et al. (1998). Measurements were carried in the morning (10:00-12:00). For each parameter, the mean value of three measurements (minimum) is presented.

Membrane electrolyte leakage

For each variety, four leaves (one per plant) were cut into 1 cm sections. Pooled samples of 7 leaf sections were floated on 10 mL of deionized water for 22 h. Conductivity values resulting from electrolytes released by cells were read using a conductimeter Crison GLP 31 (Crison Instruments, Spain), at ca. 20°C. Total conductivity was measured after sample exposure to 90°C in an oven for 2 h, followed by cooling. Membrane leakage was expressed as a percentage

of the total conductivity (Scotti-Campos et al., 2011).

Relative water content

The fresh weight (FW) of pooled samples of 4 leaf discs (0.385 cm² each) was determined, after which the samples were floated on deionised water at room temperature for 18-24 h. The leaf discs surface was carefully blotted dry with tissue paper before turgid weight (TW) was determined. The samples were oven-dried at 70°C for at least 18 h after which dry weight (DW) was measured. Relative water content was calculated using the standard equation $RWC = ((FW-DW)/(TW-DW)) \times 100$ (Barrs, 1968).

Osmotic potential

Osmotic potential (π) was determined with a thermocouple psychrometer (SC-10A, Decagon Devices Inc, Pullman, WA, USA) in pooled samples of 15 leaf discs (0.385 cm² each) frozen in liquid N₂ and kept at -80°C. They were placed in psychrometer steel cups and left to equilibrate for 90 min before measurements and take the reading after 2 min. The μ v output was converted to corresponding pressure values (MPa) using a standard regression curve of 8 known KCl concentrations. The obtained π values were corrected to full turgor with the RWC of the corresponding sample [$\pi_{100} = \pi \times (RWC/100)$] according to Ramalho et al. (2014).

Leaf pigments

Total leaf chlorophylls and carotenoids were extracted from pooled samples of 3 leaf disks (0.385 cm² each) placed in vials containing 5 ml of pure methanol and stored at 4°C in the dark for 72 h. Thereafter, the concentration of the extract was determined spectrophotometrically (Shimadzu UV160A, Japan) at 665.2, 652.4 and 470 nm and estimated by using the equations of Lichtenthaler (1987).

Yield

Plants were individually harvested and threshed manually at full maturation stage (ca. 150 DAS, GS 90). The number of spikes per plant, the number of kernels per spike and the 1000 kernel weight were determined after oven drying of the whole shoots for 35°C for 72 h. Culms dry weight was also evaluated.

Statistical analysis

ANOVA ($P < 0.05$) was applied using Statistix 9 program (Windows 2009, Analytical Software) followed by Tukey for mean comparison. Different letters express significant

differences between genotypes within each germplasm group (a,b,c) or between control and stress in the same genotype (r,s).

Results and Discussion

Under irrigation conditions cultivars Pirana, Eufrates and TE 0206 presented the highest Pn rates, as well as the highest gs values (Figure 1). In well watered plants the lowest gs values were observed among ancient varieties (MEQ and MEB). Drought caused a significant reduction of Pn and gs in all cultivars. However the more evolved Eufrates (modern) and TE 0206 (advanced), as well as Pirana (traditional), showed the strongest gs decreases under drought (Figure 1). Stomatal conductance has been recently considered as an indicator of genotypic differences in the growth response to water stress (Munns et al., 2010). According to these authors, a sensitive growth response to decreases in soil water potential would be beneficial for crops growing without irrigation on stored water and likely to endure a terminal drought.

Lower RWC decreases (ca. 8%) occurred in ancient and traditional cultivars (MEQ and Restauração), suggesting water preservation within the tissues, while the largest reductions (22-24%) occurred in modern Roxo and Jordão, and advanced TE 0206 (results not shown). This, as well as lower gs values under full irrigation, highlight a reduced water loss particularly in ancient MEQ, that may reflect a higher adaptation to dryness, as reported for other species (Campos et al., 1999; Matos et al., 2002; Matos et al., 2010; Scotti-Campos et al., 2013).

Under water deficit, along with Pn reductions, concomitant transpiration decreases were observed in all varieties (Figure 2). As regards iWUE, it increased under drought in all varieties except in MEQ where it decreased. This result is consistent with the lowest evolutive stage and higher rusticity of this ancient variety. The raise in iWUE was particularly high in ancient MEB, modern Roxo, Ardila, Jordão and Eufrates, and in advanced Flycatcher (Figure 2). An increase in water use efficiency under stress is a desirable trait for the preservation of plants production (Coutinho et al., 2013).

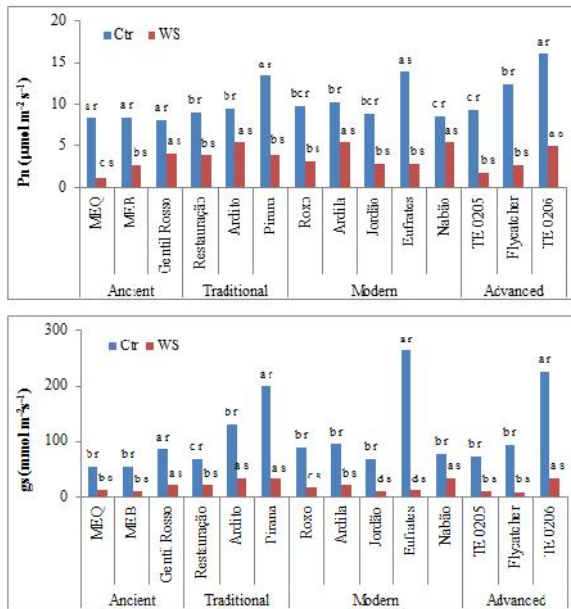


Figure 1. Changes in net photosynthesis (Pn) and stomatal conductance (gs) in leaves of fourteen *T. aestivum* varieties, under control (Ctr) or water stress (WS) conditions imposed after anthesis. Different letters express significant differences between genotypes within each germplasm group (a,b,c,d) or between control and stress in the same genotype (r,s).

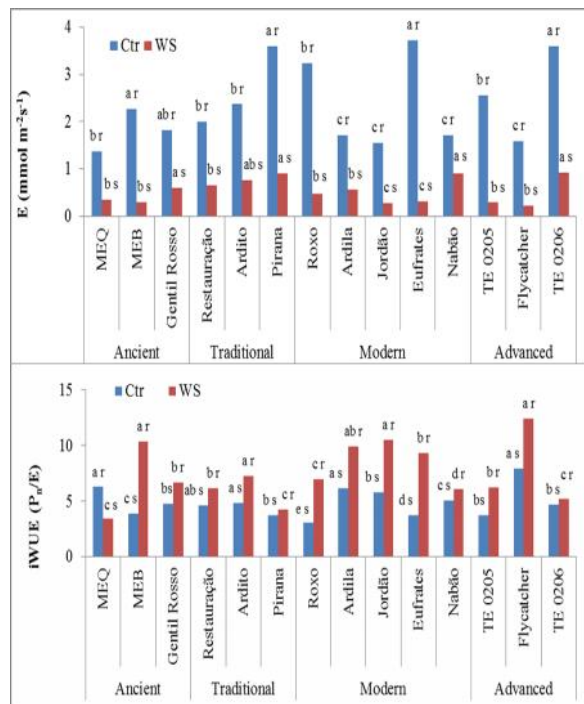


Figure 2. Changes in transpiration (E) and intrinsic leaf water use efficiency (iWUE) in leaves of fourteen *T. aestivum* varieties, under control (Ctr) or water stress (WS) conditions imposed after anthesis. Different letters express significant differences between genotypes within each germplasm group (a,b,c,d,e) or between control and stress in the same genotype (r,s).

In ancient and traditional groups, varieties showed high membrane stability when subjected to water deficit, as inferred from small or unexistent increases of membrane leakage when compared to irrigated controls (Figure 3), what may denote a higher protoplasmic tolerance to dehydration (Campos et al., 1999; Matos et al., 2010). On the contrary, in modern and advanced groups many varieties reached twice the values observed in well watered plants. Membrane injury has been widely used as an indicator of protoplasmic tolerance to abiotic stresses (Matos et al., 2002; Scotti-Campos et al., 2011). Raises in electrolyte leakage are usually related to irreversible membrane damage and the occurrence of membrane lipoperoxidative processes that lead to cell decompartmentation and tissues senescence (Lauriano et al., 2000; Scotti-Campos et al., 2014).

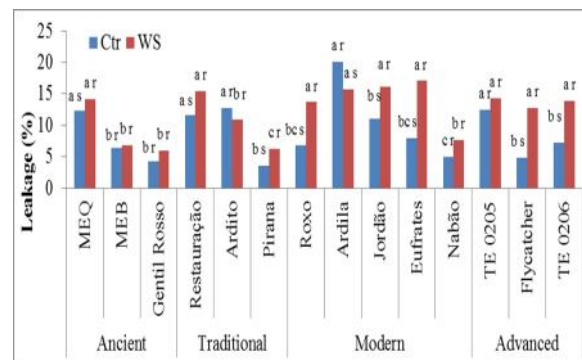


Figure 3. Changes in electrolyte leakage values in leaf sections of fourteen *T. aestivum* varieties, under control (Ctr) or water stress (WS) conditions imposed after anthesis. Different letters express significant differences between genotypes within each germplasm group (a,b,c) or between control and stress in the same genotype (r,s).

Such losses of membrane integrity may also reflect damages occurring at chloroplast level, resulting in photosynthetic activity impairment (Matos et al., 2010; Scotti Campos et al., 2014). As regards leaf osmotic potential ($\Psi_{\pi 100}$), except MEB, Gentil Rosso, Ardito and Nabão, all the varieties presented lower values upon drought (Figure 4), suggesting the occurrence of an osmotic adjustment. In most species, the acquisition of tolerance to water deficit may rely on the ability to maintain osmotic homeostasis, preserving cell turgor through accumulation of compatible solutes (Chaves and Oliveira, 2004). Among these, sugars have important osmoprotective functions since due to their hydrophilic structure they can replace water on protein surfaces, protein complexes or membranes, helping to preserve biological functions (Ramalho et al., 2014).

Chlorophyll reduction is also an indicator of plant senescence, while increasing amounts of carotenoids may reveal a better ability to cope with oxidative stress resulting from dehydration. Restauração, Ardila and Jordão stood out for their highest chlorophyll reductions (26%) under drought, relatively to the control (Table 1). TE 0206 presented a 35% rise in chlorophyll content, followed by Pirana and MEQ (29 and 26% increases, respectively).

Along with chlorophyll decrease, Restauração also showed a decrease in carotenoids content. Such decreases denote enhanced tissue senescence, and correspond to an early maturation stage of this variety which, according to our previous observations (Scotti-Campos et al., 2011) presents a shorter development cycle than the remaining. MEQ, Pirana, Flycatcher and TE 0206 increased 34, 21, 22 and 31%, respectively (Table 1).

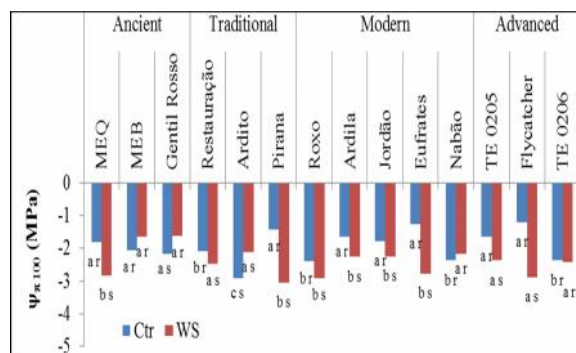


Figure 4. Changes in leaf osmotic potential ($\Psi_{\pi 100}$) in fourteen *T. aestivum* varieties, under control (Ctr) or water stress (WS) conditions imposed after anthesis. Different letters express significant differences between genotypes within each germplasm group (a,b,c) or between control and stress in the same genotype (r,s).

In well watered plants, differences among groups were found as regards the culms weight per plant and the number of spikes per plant, which was higher in ancient varieties contrasting with low values in advanced lines (Figure 5). An inverse tendency was observed in the number of kernels per plant, where the highest values were obtained for advanced lines, denoting the breeding progress undergone to maximize grain yield.

Drought reduced plant biomass in ancient varieties MEQ and Gentil Rosso (Figure 5). As regards the number of spikes per plant, under water deficit it decreased in ancient MEQ and modern Nabão, and increased in modern Pirana. Plants subjected to drought depicted a higher number of kernels per spike in Roxo, while a decrease occurred in MEQ and traditional Ardito, being unaltered in all the others.

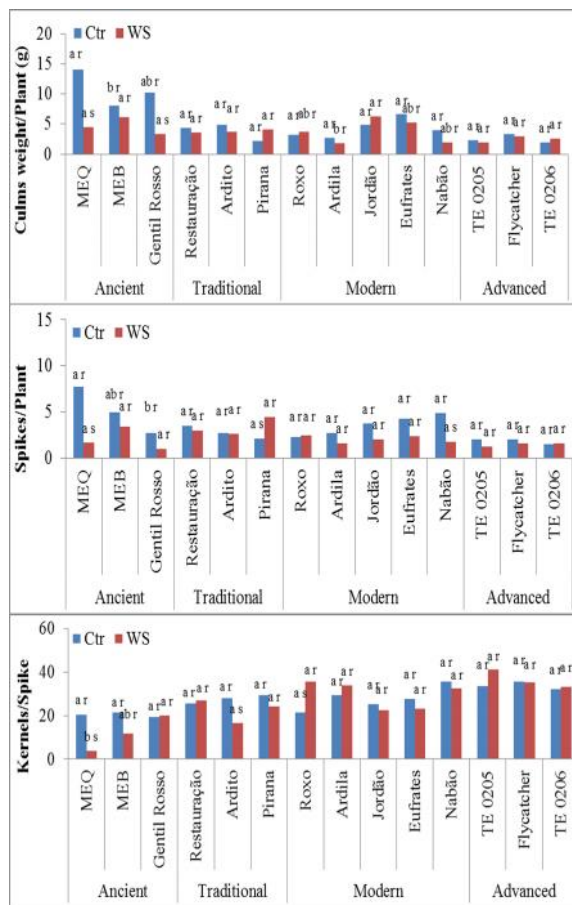


Figure 5. Changes in plant biomass (culms weight per plant) and grain yield (number of spikes per plant and number of kernels per spike) in fourteen *T. aestivum* varieties under control (Ctr) or water stress (WS) conditions imposed after anthesis. Different letters express significant differences between genotypes within each germplasm group (a,b) or between control and stress in the same genotype (r,s).

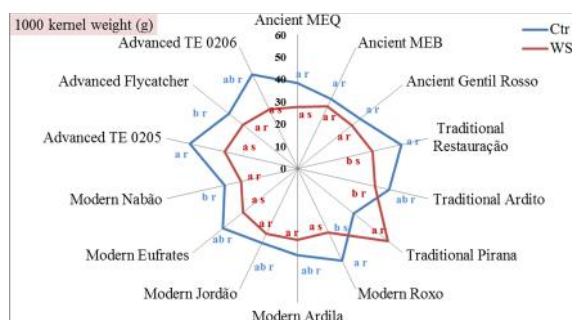


Figure 6. Changes in 1000 kernel weight of fourteen *T. aestivum* varieties under control (Ctr) or water stress (WS) conditions imposed after anthesis. Different letters express significant differences between genotypes within each germplasm group (a,b) or between control and stress in the same genotype (r,s).

Table 1. Changes in chlorophyll and carotenoid contents in leaves of fourteen *T. aestivum* varieties under control (Ctr) or water stress (WS) conditions imposed after anthesis. Different letters express significant differences between genotypes within each germplasm group (a,b,c) or between control and stress in the same genotype (r,s).

Developed group	Genotype	Treatment	Chlorophylls (a+b) mg m ⁻²	Carotenoids mg m ⁻²	
Ancient	MEQ	Ctr	502.94 ^{as}	101.81 ^{as}	
		WS	635.47 ^{ar}	136.36 ^{ar}	
	MEB	Ctr	634.19 ^{ar}	119.38 ^{ar}	
		WS	658.67 ^{ar}	125.55 ^{ar}	
	Gentil Rosso	Ctr	555.40 ^{ar}	126.67 ^{ar}	
		WS	615.59 ^{ar}	130.37 ^{ar}	
Traditional	Restauração	Ctr	573.63 ^{ar}	116.61 ^{ar}	
		WS	420.82 ^{bs}	99.40 ^{bs}	
	Ardito	Ctr	574.45 ^{ar}	128.92 ^{ar}	
		WS	641.36 ^{ar}	135.82 ^{ar}	
	Pirana	Ctr	484.22 ^{as}	106.80 ^{bs}	
		WS	623.61 ^{ar}	129.64 ^{ar}	
Modern	Roxo	Ctr	739.47 ^{ar}	136.19 ^{ar}	
		WS	697.50 ^{ar}	137.48 ^{ar}	
	Ardila	Ctr	527.63 ^{ar}	110.21 ^{ar}	
		WS	391.50 ^{bs}	95.93 ^{br}	
	Jordão	Ctr	627.19 ^{ar}	121.55 ^{ar}	
		WS	465.92 ^{bs}	108.11 ^{ar}	
	Eufrates	Ctr	552.53 ^{ar}	108.07 ^{ar}	
		WS	519.93 ^{ar}	116.22 ^{ar}	
	Nabão	Ctr	633.83 ^{ar}	127.75 ^{ar}	
		WS	547.92 ^{ar}	116.69 ^{ar}	
	Advanced	TE 0205	Ctr	715.08 ^{ar}	137.35 ^{ar}
			WS	644.78 ^{ar}	134.80 ^{ar}
Flycatcher		Ctr	528.08 ^{br}	108.84 ^{bs}	
		WS	594.80 ^{ar}	132.50 ^{ar}	
TE 0206		Ctr	517.96 ^{bs}	110.55 ^{as}	
		WS	698.32 ^{ar}	144.70 ^{ar}	

Under water deficit, except for traditional Pirana, 1000 grain weight was reduced in all varieties (Figure 6). This is probably due to the strong g_s reductions observed in droughted plants, that resulted in P_n decreases. Carbon metabolism is one of the key physiological processes determining crop growth, yield and quality, and is very sensitive to abiotic stresses (Wang et al., 2012). Stomatal conductance responds rapidly and sustainably to changes in soil water potential, and provides the main limitation to photosynthesis and growth (Munns et al., 2010). On the contrary, a greater photosynthetic rate in the flag leaf will sustain current photo-assimilate supply to the grains ensuring grain filling. Remobilization of the carbohydrate reserves from the stem to the grains may also contribute to grain filling in wheat particularly when the plants are grown under stressful conditions (Wang et al., 2012). This could eventually be the case of Pirana, where an increase in 1000 kernel weight was observed (Figure 6), denoting a higher performance during

grain filling, that probably resulted in heavier grains. Such enhanced performance under stress is in accordance with the rise in the number of spikes per plant observed for this variety subjected to drought (Figure 5). The present results suggest that higher membrane stability, increased pigments and lower osmotic potential under drought may underlay the improved response to drought in Pirana.

These results are also in agreement with data obtained from an experiment using the same germoplasm under rainfed conditions in the INIAV/Elvas (Pinheiro, N., Jan. 2013, personal communication), pointing Pirana as an interesting variety to be used as gene donor for breeding purposes.

Conclusion

Under irrigation conditions, results highlight a good photosynthetic performance (high P_n and g_s) of Pirana, Eufrates and TE 0206. When subjected to dehydration, TE 0206 also depicted higher chlorophylls and carotenoids. In controls, the

highest 1000 kernel weight occurred in advanced varieties. Under water deficit, a similar 1000 kernel weight reduction occurred in all varieties, except in Pirana, where this yield component increased, as well as the number of spikes per plant. When subjected to drought, Pirana also presented an increased amount of carotenoids.

Ancient and traditional varieties showed preserved membrane stability (small or no leakage increases) when subjected to water deficit, denoting a higher protoplasmic tolerance to drought than modern and advanced ones. Some of them (MEQ, MEB) also showed lower g_s values, constituting useful genetics resources in wheat breeding programs under mediterranean environment.

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Author contributions

P. S. C., J. N. S. and I. P. P. conducted the experiment and contributed to the writing of the paper. M. O., J. P., A. S. A., A. R. C., J. C. and B. M. were involved in critical reviews and suggestions. P. S. C., J. N. S., J. C., B. M., N. P., C. B., J. C. and A. C. contributed to overall planning of the work and data discussion. Data were obtained by P. S. C., J. N. S., I. P. P., M. O. and M. S.

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