Phenotypical and physiological study of near-isogenic durum wheat lines under contrasting water regimes

Judit Bányai[a*], Magda Pál[a], Maria Angela Cané[b], István Monostori[a], Tamás Spitkó[a], Zoltán Bognár[a], Csaba Kuti[a], Klára Mészáros[a], Ildikó Karsai[a], László Láng[a]

[a] Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, POB 19, 2462 Martonvásár, Hungary.

[b] Department of Agricultural Sciences (DipSA), University of Bologna, 40127 Bologna, Italy.

***Corresponding author:** Judit Bányai

banyai.judit@agrar.mta.hu

Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 2462 POB 19, Martonvásár, Hungary.

Tel./Fax: +36 22569500; +36 22569576.

Abstract

Irrigation treatments involving three different water regimes were carried out in a controlled environment on eight near-isogenic durum wheat lines that differed in a major yield-related QTL region (QYld.idw-3B) in order to assess the relationship between morpho-physiological traits, antioxidant enzyme activities, polyamine contents and drought tolerance. Drought stress, simulated under a rain-out shelter, negatively affected the performance of the isogenic lines, leading to significant reductions in seed yield, tiller number, chlorophyll content, plant height, leaf area and ascorbate peroxidase activity, while the polyamine content and guaiacol peroxidase activity increased. Correlation analysis revealed that the antioxidant enzyme activities in the flag leaf were in significant, negative relationship with several yield-related parameters, while a significant, positive correlation was found between polyamine contents and the seed number or weight in the main spike. The ascorbate peroxidase activity was negatively correlated with seed weight per main (r= -0.446) or side spike (r= -0.465) and the 1000-grain weight of the main or side spike (r= -0.396 or r= -0.49) and the guaiacol peroxidase activity with the number of seeds per main (r= -0.457) or side spike (r= -0.378) and the seed weight per side spike (r= -0.38). GGE biplot analysis showed that lines with the $KK_{2BL}KK_{3BS}$ allele combination had better yield performance under non-watered conditions**,** but their response to drought stress was not uniform in other yield-related traits.

Keywords: antioxidant enzymes**;** drought; polyamines; rain-out shelter; yield components

1. Introduction

The occurrence of drought and dry seasons is a recurrent phenomenon. Since the late 20th century, there have been increasingly higher temperatures, accompanied by less and unpredictable rainfall, and this is expected to continue due to climate change. If the amount of precipitation is insufficient, in the critical phases of plant growth and development, which means flowering and grain-filling in the case of cereals, the genetically encoded yielding ability cannot be fully achieved (Nouri et al. 2011). The yield reduction depends on the abiotic stress tolerance of the plants. Thus, one of the important tasks now facing wheat breeding programmes is to develop genotypes that are heat- and drought-tolerant, high-yielding, with stable properties.

Oxidative stress is induced during drought. The ability of plants to overcome the effect of stress conditions and to sustain productivity may be related to the scavenging of stress-induced reactive oxygen species. Peroxidases are one of the major systems for the enzymatic removal of H_2O_2 in plants (Kocsy et al., 2011). Polyamines (PAs) are aliphatic amines found in all living cells and well known for their direct antioxidant properties and their ability to regulate the expression of genes encoding antioxidant enzymes (Kuznetsov and Shevyakova 2007). The early activation of polyamine biosynthesis in response to abiotic stress has been reported in several cases, and the existence of a relationship between the stress tolerance of plants and their capacity to enhance the synthesis of polyamines on exposure to stress has also been suggested (Fariduddin et al., 2013; Minocha et al., 2014). A recent review discussed the fact that PAs are involved in the grain filling of wheat and rice plants (Liu et al., 2013).

Grain filling and its end result, the grain yield are closely linked to several morphological, anatomical, physiological and molecular characteristics of flag-leaves (Biswal and Kohli, 2013). For example, the net CO_2 assimilation during water deficit displayed a close correlation with the drought sensitivity of cereals (Saeedipour and Moradi 2011). The increased accumulation of osmolites such as proline and sucrose was exhibited by the flag-leaves of tolerant wheat genotypes under induced drought stress (Sawhney and Singh 2002). Despite increasing knowledge on the importance of the physiological condition of cereal flag-leaves under normal or stress conditions, little is known about the relationship between the content of endogenous plant growth regulators, such as polyamines, in flag-leaves and the yield under drought stress conditions.

The approach most widely used for the selection of drought-tolerant cereal genotypes is screening for grain yield under stress conditions (Tardieu and Hammer 2012). Direct selection for grain yield under water-stressed conditions has been hampered by low heritability, polygenic control, epistasis, and significant genotype-by-environment (GxE) and quantitative trait loci (QTLs)-by-environment (QTLxE) interactions (Cattivelli et al., 2008). Many QTLs for yield in drought environments have been identified in durum wheat (Habash et al., 2009). Creating a suitable population for examining QTL effects is a complex task because differential gene expression is caused not only by the trait of interest but also by the variation present in the genetic background. One solution for establishing the functional association between the level of gene expression and a given trait is the use of a set of near-isogenic lines (NILs), which are genetically similar except for a single gene, marker or trait

(Varshney et al., 2005). Although several studies have been made on the physiological aspects of drought stress, mainly under controlled conditions, only the complex analysis of the combined effect of environmental factors and genotypes under field conditions will reveal the real responses.

In the present study near-isogenic durum wheat lines differing for a major grain yield QTL (*QYld.idw-3B)* were evaluated. The main aims were 1) through detailed morphological and physiological analysis to reveal the stability of the lines under drought conditions, 2) to explore the correlation between morphological and physiological parameters and yield components under drought conditions, and 3) to discover how the polyamine content and antioxidant enzyme activity of the flag-leaves were related to yield-related parameters and drought tolerance. In order to achieve these goals the NILs were tested under drought stress conditions controlled by soil sensors, which collected data on the moisture content, temperature and electrical conductivity of the soil hourly throughout the growing season.

2. Materials and methods

106 *2.1. Plant material*

107

108 Near-isogenic durum wheat lines (NILs) derived from 4 different Recombinant imbred
109 lines (RILs) of the original Kofa x Svevo spring durum wheat cross were included in the
110 experiments. These two cultivars were found to be similarly early flowering and to have good
111 adaptation ability in a multi-location experiment around the Mediterranean Basin. Two major
112 QTLs for grain yield, one on chromosome 2B (QYld.idw-2B) and one on chromosome 3B
113 (QYld.idw-3B), were identified across several environments, with significant epistatic
114 interactions between them (Maccaferri et al. 2008). The F4 plants were checked for
115 heterozygosity and marker-assisted selection was used to derive the NIL couples (NIL1++,
116 NIL1--, NIL2++, NIL2--, NIL3++, NIL3--, NIL4++, NIL4--). The NILs were all fixed for the
117 Kofa allele on chromosome 2B. When the allele on chromosome 3B was KK (Kofa) the NILs
118 were coded as ++ ($KK_{2BL}KK_{3BS}$) and when the allele was SS (Svevo) they were coded as --
119 ($KK_{2BL}SS_{3BS}$). Both Kofa and Svevo were included in the experiment.

120

121 *2.2. Field trial and experimental data*

122

123 The experiments were carried out in the rain-out shelter and the surrounding
124 experimental area of the Agricultural Institute, Centre for Agricultural Research, Martonvásár
125 in 2014. The lines were planted on 17 March, 2014 and were grown in three different
126 treatments: (i) non-irrigated (NW), (ii) fully irrigated (W), (iii) rainfed (RF). Individual plots

consisted of 3 rows per line, 10 cm apart, in 1.5 m x 4.8 m plots. There were four plots in each treatment, so measurements were made on 12 rows per line/treatment. The soil texture of the experimental site was chernozem with forest residues, having good water permeability. In NW treatment the plants were grown under a rain-out shelter and drought stress was generated by total water withholding from emergence until harvesting, in 30 cm depth of the soil the value of field capacity was 29 vol% (pF 2.5), the wilting point at 10.3 vol% (pF 4.2), and the water-stress state occurs at 19 vol% (pF 3.4). The field capacity of the rain-fed (RF) plots is 30 vol%, the wilting point at 10.8 vol%, the water-stress state begins when the soil moisture drops to 20.2 vol%. The amount of water per area was regulated using an automatic drip irrigation system (Irritrol Junior Max, The Torro Company, Lyndal, USA). Soil moisture sensors were placed at depths of 10, 20 and 30 cm. Data on the moisture content (vol%), temperature (°C) and electrical conductivity (dS/m) of the soil were collected hourly throughout the growing season. For each plot, phenological development was recorded using the Zadoks score (Zadoks et al., 1974).

The chlorophyll content of the flag-leaf was estimated using a chlorophyll meter (SPAD-502; Minolta, Tokyo, Japan) and expressed as a relative value (SPAD value) at the boot stage (SPAD45), at flowering (SPAD65), in the late stages of milky ripeness (SPAD77), at early waxy ripeness (SPAD83) and at the end of waxy ripeness (SPAD85) in sixteen replications per line for each water regime.

The flag-leaf (FLA) and total plant leaf (PLA) area were defined in eight and twelve replications, respectively, at flowering (ZGS65) using an LI-3100C leaf area meter. The plant

height up, to the flag-leaf collar (FLC), the base of the ear (BE) and the tip of the ear (TE, without awn), the peduncle length (PL, from the flag-leaf to the base of the ear) and the neck size (NL, from the last node to the base of the ear) were measured in twelve replications.

Measurements were made on the spikelet number per spike for 16 main spikes (SKNM) per line, on the grain number and grain weight per spike (SNM) and per metre, and on the number of sterile apical (ASM) and basal (BSM) spikelets per spike. Chemical weed control was applied and no disease symptoms were observed during the growth period.

2.3. Antioxidant enzyme assays and polyamine analysis

The ascorbate peroxidase (APX) and guaiacol peroxidase (G-POD) activities and the polyamine contents were measured in the flag-leaves of the main tiller in five replications on samples collected from irrigated (W) and non-irrigated (NW) plots at flowering (ZDS65).

Enzyme extraction and the analysis of antioxidant enzyme activity, expressed as nkatal g^{-1} DW, were carried out as described by Pál et al. (2013) using a UV-visible recording spectrophotometer (UV-VIS 160A, Shimadzu Corp. Kyoto, Japan), by monitoring changes in the absorbance at 290 nm in the case of APX (EC 1.11.1.11.) and at 470 nm in the case of G-POD (EC 1.11.1.7.).

Polyamine extraction and analysis were carried out as described by Pál et al., (2013). The polyamines were analysed as dansylated derivatives via HPLC using a W2690 separation

module and a W474 scanning fluorescence detector with excitation at 340 nm and emission at 515 nm (Waters, Milford, MA, USA). The values were expressed as μg g^{-1} DW.

2.4. Statistical analysis

Analysis of variance, phenotypic correlation analysis between phenotypic traits and GGE-biplot analysis were performed for each variant using the GENSTAT17 software. Means were compared by using Fisher's least significant difference ($P<0.001$, 0.01 and 0.05).

3. Results

3.1. Soil water conditions in the experiments

In the NW treatment the soil moisture content dropped to below 13 vol% at a depth of 30 cm even before sowing, thus causing water stress (Supplementary Figure 1-3). Because of the wet weather in May the water supplies of the rain-fed (RF) and irrigated (W) areas did not differ from each other, so there were no significant differences between any of the measured properties.

3.2. Effect of drought stress on plant morphology and physiology

The results of variance analysis for chlorophyll content indicated that genotypic differences were highly significant at all the developmental phases except in the early waxy ripeness stage (ZDS83), when the SPAD index was 38% lower in the NW treatment than in the W treatment (Table 1). The effect of the treatment for the chlorophyll content was not significant at the end of waxy ripeness (ZDS85), while there were positive, significant differences between the lines under stress conditions because of the genotypic effect. The chlorophyll contents of NIL3++, NIL1++ and NIL1-- were significantly higher than the experimental mean at ZDS85 (Figure 1).

The different water regimes had a significant effect on both the flag-leaf area and the plant leaf area among the lines. In the case of the W treatment, the flag-leaf area of the NIL1--, NIL1++ lines was significantly larger than the average, while in the NW treatment only line NIL1-- had a larger flag-leaf area (Supplementary Table 1).

The water stress developing in the soil after sowing significantly reduced the number of fertile tillers and thus the size of the entire plant leaf area in the NW treatment. In the case of the W and NW treatments, the NIL1-- and NIL1++ lines had the largest total plant leaf area (Supplementary Table 1).

Analysis of variance showed that the genotypic variance was not significant for plant height up to the flag-leaf collar, while the plant height to the bottom and top of the spike showed greater diversity over treatments and lines. The average height of the plants decreased by 12% due to water shortage. In all the treatments the NIL3++ plants were the tallest. Compared to the irrigated treatment the peduncle length of the NIL1--, NIL3-- and NIL4++

lines was not reduced significantly during drought stress. In the irrigated treatment there was no significant difference in the neck length between the lines, but insufficient water supplies resulted in the shortening of the internode, which was most characteristic of the NIL1++ and NIL2++ lines. The genotype had no significant effect on the main spike size, but lines NIL1-- and NIL1++ had the longest spike size under drought stress (Supplementary Table 1).

3.3. Tthe effect of drought stress on yield components

Analysis of variance on the yield components indicated that genotypic differences were highly significant for all traits except for the apical sterile spikelet number, where neither the genotype nor the treatment effect was significant (Table 1). Due to drought stress the number of basal sterile spikelets significantly increased in the case of lines NIL2-- and NIL3--. In the NW treatment, the average grain number in the main spike decreased by 20%, the grain weight by 30%, and the thousand-kernel weight per main spike by 16%, while in the side spikes these values were 28%, 40% and 17%, respectively. In addition, 13% fewer tillers emerged on average compared to the W treatment. Under NW conditions there were significantly more seeds and significantly higher seed weight in the main spike of line NIL1++, while line NIL3++ line had the highest seed number and seed weight in the side spikes compared to the mean value for this treatment (Supplementary Table 1). GGE biplot analysis showed that PC1 and PC2 accounted a total of 95.12% of the variation (Figure 2). In the NW treatment, when the lines were ranked based on seed number per metre NIL1--, NIL2--,

NIL3-- and NIL4-- were found to have lower than average yield, NIL2++ and NIL4++ near average yield, and Svevo, Kofa, NIL1++ and NIL3++ higher than average yield. The vector of NIL3++ was shorter than that of the other lines, suggesting that it was more stable than all the other genotypes.

3.4. Drought-induced changes in antioxidant enzyme activities and polyamine contents

Under favourable water conditions the lowest ascorbate peroxidase (APX) activity was measured in Kofa and NIL3++, while the highest value was observed for Svevo. Drought stress (NW) significantly decreased the APX activity except in the case of Kofa and NIL3++ (Table 2). The lowest guaiacol peroxidase (G-POD) activity was found in NIL1-- and NIL1++, and the highest in NIL2++ under irrigated conditions (Table 2). Drought stress significantly increased the activity of G-POD in all the lines, with the highest increments in NIL1-- and NIL1++. The lowest increase in G-POD activity was found in NIL2++, where the enzyme activity was already high under favourable water conditions.

The agmatine and cadaverine contents were below the detection limit. Although the patterns of the detectable free polyamine contents, namely putrescine (PUT), spermidine (SPD) and spermine (SPN), were similar in the various lines, the most pronounced differences were observed in the case of PUT. Lower PUT, SPD and SPN contents were detected in line NIL3++, and higher amounts in NIL2-- under irrigated conditions. Water deficit induced greater PUT, SPD and SPN accumulation in NIL3++, than in lines such as NIL2--, where the

polyamine content was already high under irrigated conditions. Drought caused hardly any significant changes in the SPN content (Table 2).

3.5. Correlations between the examined parameters under non- irrigated conditions

Significant relationships were found between several traits or parameters in the non-irrigated treatment. For instance, there was a positive significant correlation between the chlorophyll content of the flag-leaves and the seed number (0.450**), seed weight (0.682***) and 1000-grain weight (TGW) of the main spike at the booting stage (0.580***) and the seed number (0.648***) and seed weight (0.621***) of the side spikes at the ZDS85 stage under drought conditions (Table 3). Similarly, positive significant correlations were detected between the seed weight (0.425**) and TGW (0.520***) per side spike and the flag-leaf area in replications exposed to total water withholding.

There was a positive significant correlation (0.720***) between the APX and G-POD activities under drought stress conditions. Significant negative correlations were found between the APX activity and the seed weight per main (-0.446**) or side spike (-0.465**) and the TGW of the main or side spike (-0.396** or -0.490**), and between the G-POD activity and the number of seeds per main (-0.457**) or side spike (-0.378*) and the seed weight per side spike (-0.380*).

PUT exhibited a high correlation with the SPD content (0.541***) and the SPN content (0.569***), while the PUT, SPD and SPN contents showed a significant positive

relationship with both SNM (0.533***, 0.500*** and 0.481**, respectively) and SWM (0.383*, 0.352* and 0.399**, respectively).

4. Discussion

Several breeding experiments for drought tolerance demonstrated that genotypes with good tolerance of stress conditions are incapable of producing high yields under optimum conditions (Rosielle and Hamblin 1981; Dixit et al. 2014; Spitkó et al. 2014).It would be the idea that high yielding genotypes should be drought-tolerant and have low yield depression when exposed to water shortage. In order to achieve a better understanding of the drought stress responses of plants, complex morphological, physiological and yield component examinations were carried out in an experimental nursery with a rain-out shelter. Near-isogenic durum wheat lines differing only in the QYld.idw-3B region were used to investigate the combined effect of environment, QTL, genotype and treatment. This was the first study to highlight whether the polyamine content or the activities of certain antioxidant enzymes in the flag leaves of NILs are correlated with yield-related QTLs and yield parameters under drought conditions in field experiment.

In the non-irrigated treatment, the plants were subjected to drought stress throughout the growing season, which thus had an impact on inflorescence formation, fertilization and crop formation. The yellowing of the leaves, indicating the aging process, started soon after flowering, the individual isogenic lines showed a decrease with varying degrees of

chlorophyll content. The original expectation was that lines with the Kofa allele on chromosome 2B and the Svevo allele on chromosome 3B would exhibit early senescence so the leaves would begin to wither earlier. The higher chlorophyll values measured at the end of the waxy ripeness stage in isogenic lines NIL1++ and NIL3++, both of which had the Kofa allele on 3B, showed that this allele combination could also sustain photosynthetic activity for a longer period of time under non-irrigated conditions, leading to higher seed number and weight at the end of the growing season. This was supported by the positive, significant correlation between the flag-leaf chlorophyll content and the seed number and weight in the main spike. Marker-trait associacion was detected on chromosome 3B for chlorophyll content at grain filling in genetically diverse elit lines of spring wheat (Sukumaran et al. 2014).

Grain yield was strongly influenced both by genotype and treatment effects, while the genotype by treatment interaction was not significant. In the NW treatment there were significantly more seeds and significantly higher seed weight in the main spike of the NIL1++ line, while line NIL3++ had the highest seed number and seed weight in the side spikes compared to the mean value of the treatment. The positive effect of Kofa QTL on chromosome 3B was observed in two inbreed family under drought stress. It was recently demonstrated that QTL qGYWD.3B.1 on the short arm of chromosome 3B was associated with both increased grain yield and TGW (Shukla et al., 2015). This QTL was co-located with QTLs for yield components, canopy temperature and days to flowering, and was apparently independent of plant height. It was also observed that four QTLs related to yield, which were robust (i.e. across stressed and irrigated environments), appeared in linkage groups 1B-a, 3B-

b, 4A-a, and 4A-b (Pinto et al., 2010). Although drought tolerance were to be found associated with alterations in the antioxidant metabolism in various plant species, changes in antioxidant enzyme activities during drought stress are greatly dependent not only on which enzyme was examined, but also on the plant species and cultivar, and on the severity and duration of the stress (DaCosta and Huang, 2007). Drought caused a reduction in the APX activity in Kentucky bluegrass plants, but the decrease was less severe in the tolerant genotype. Under the same conditions no difference in G-POD activity was observed between the sensitive and tolerant genotypes (Xu, 2011). A similar decrease in APX and increase in G-POD activity were found in wheat plants exposed to drought stress (Chakraborty and Pradhan, 2012). In other experiments on the wheat APX activity increased in both tolerant and sensitive genotypes, but the maximal activity occurred at the end of flowering in the tolerant one, and at the end of ear formation in the sensitive one (Huseynova, 2012). In the present experiment, the APX activity decreased under non-irrigated conditions except for Kofa and NIL3++, which have relatively low APX activity even under irrigated conditions. In contrast, higher G-POD activities were detected in all the lines under non-irrigated conditions than under favourable water conditions. The APX activity showed a significant negative correlation with the seed weight of the main and side spikes, the flag leaf area and the SPD content under drought conditions. The G-POD activity also showed a close, negative correlation with several yield components.

Polyamines (Pas) are thought to play a protective role under stress conditions. However, the data in the literature are contradictory. In some cases a close, positive

correlation was found between the endogenous polyamine content and tolerance of various stress factors (Minocha et al., 2014), while in several plant species the correlations were negative or non-existent (Pál et al., 2015). Increased polyamine contents were reported in the flag-leaves of wheat under drought conditions (Biswal and Kohli, 2013). In the present work, too, the accumulation of polyamines was observed in the flag-leaves of durum wheat lines under water deficit conditions, with the highest accumulation of PUT, SPD and SPN in the case of line NIL3++. Correlation analysis revealed a close, positive correlation between these polyamines. In addition, several close, positive correlations were found between individual polyamine contents and the seed number or seed weight of the main spikes under drought conditions. The protective effect of all studied poliamine compounds were found in this studie.

PAs are involved in the balance of hormones that regulate the grain filling of wheat (Liu et al., 2013), as there is negative feedback between PAs and ethylene and positive feedback between PAs and abscisic acid, which also plays a key role in drought signalling and protection (Alcazár et al., 2011). In agree with our results the endogenous SPD and SPN contents were positively correlated with the grain-filling rate and grain weight of wheat, and the abscisic acid/ethylene ratio was positively and significantly correlated with the maximum grain weight and with the maximum and mean grain-filling rates (Liu et al., 2013). The increased contents of free SPD, free SPN, and insoluble-conjugated PUT in rice cultivars under drought stress were also significantly correlated with the ratio of the grain yields recorded under dry and well-watered conditions (Yand et al., 2007).

Considerable variation was detected between the eight near-isogenic lines in their response to drought stress measured via phenological, physiological and yield component traits. Among these lines, NIL1++ and NIL3++ proved to be the highest drought tolerant because of the depression of yield components were the lowest. Although the selection for QYld.idw-2B and QYld.idw-3B regions appear promising for the development of high-yielding durum wheat lines under water limited conditions even though the clarification of the role of other chromosome regions are required. Yield components showed a close, negative relationship with the antioxidant enzyme activities, which in turn may indicate that changes in these parameters more related the cause of the drought stress. In contrast, yield-related parameters were in close positive relationship with the polyamine contents, suggesting the need for a better understanding of flag-leaf physiology under drought, and of the role of antioxidants, other protective compounds and hormonal balance in the flag-leaf, together with the identification of flag-leaf-specific gene expression.

Acknowledgements

The research leading to these results was conducted as part of the DROPS project, which received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under grant agreement No 244374. Funding from the EU BONUS 12-1-2012-0017 and OTKA 108811 projects is also gratefully acknowledged. We thank Klara Illés for helpful technician work.

References

Alcázar, R., Bitrián, M., Bartels, D., Koncz, C., Altabella, T., Tiburcio, A.F., 2011. Polyamine metabolic analization in response to drought stress in Arabidopsis and the resurrection plant *Craterostigma plantagineum*. Plant Signaling and Behavior 6, 243-250.

Biswal, A.K., Kohli A., 2013. Cereal flag leaf adaptations for grain yield under drought: knowledge status and gaps. Molecular Breeding 31, 749-766.

Cattivelli, L., Rizza, F., Badeck, F.W., Mazzucotelli, E., Mastrangelo, A.M., Francia, E., Mare, C., Tondelli, A., Stanca, A.M., 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Research 105, 1-14.

Chakraborty, U., Pradhan, B., 2012. Oxidative stress in five wheat varieties (*Triticum aestivum* L.) exposed to water stress and study of their antioxidant enzyme defense system, water stress responsive metabolites and H_2O_2 accumulation. Brazilian Journal of Plant Physiology 24, 117-130.

DaCosta, M., Huang, B., 2007. Changes in antioxidant enzyme activities and lipid peroxidation for bentgrass species in response to drought stress. Journal of the American Society for Horticultural Science 132, 319–326.

Dixit, S., Singh, A., Kumar, A., 2014. Rice breeding for high grain yield under drought: a strategic solution to a complex problem. International Journal of Agronomy 2014, 1-15.

Fariduddin, Q., Varshney, P., Yusuf, M., Ahmad, A. 2013. Polyamines: potent modulators of plant responses to stress. Journal of Plant Interactions 8, 1-16.

Habash, D.Z., Kehel, Z., Nachi, M., 2009. Genomic approaches for designing durum wheat ready for climate change with a focus on drought. Journal of Experimental Botany 60, 2805–2815.

Huseynova, I.M., 2012. Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought. Biochimica et Biophysica Acta 1817, 1516-1523.

Kocsy, G., Pál, M., Soltész, A., Szalai, G., Boldizsár, Á., Kovács, V., Janda, T., 2011. Low temperature and oxidative stress in cereals. Acta Agronomica Hungarica 59, 169–189.

Kuznetsov, V.V., Shevyakova, N.I., 2007. Polyamines and stress tolerance of plants. Plant Stress 1, 50-71.

Liu, Y., Gu, D., Wu, W., Wen, X., Liao Y., 2013. The relationship between polyamines and hormones in the regulation of wheat grain filling. Plos One 8 (10), e78196. doi:10.1371/journal.pone.

Maccaferri, M., Sanguineti, M.C., Corneti, S., Ortega, J.L.,Salem, M.B., Bort, J., DeAmbrogio, E., del Moral, L.F., Demontis, A., El-Ahmed, A., Maalouf, F., Machlab, H., Martos, V., Moragues, M., Motawaj, J., Nachit, M., Nserallah, N., Ouabbou, H., Royo, C., Slama, A., Tuberosa, R., 2008. Quantitative Trait Loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. Genetics 178, 489-511.

Minocha, R., Majumdar, R., Minocha, S.C., 2014. Polyamines and abiotic stress in plants: a complex relationship. Frontiers in Plant Science 5, 175.

Nouri, A., Etminan, A., Silva, J.A., Mohammadi, R., 2011. Assessment of yield, yield-related traits and drought tolerance of durum wheat genotypes (Triticum turgidum var. durum Desf.). Australian Journal of Crop Science 5, 8-16.

Pál, M., Kovács, V., Vida, G., Szalai, G., Janda, T., 2013. Changes induced by powdery mildew in the salicylic acid and polyamine contents and the antioxidant enzyme activities of wheat lines. European Journal of Plant Pathology 135, 35-47.

Pál, M., Szalai, G., Janda, T., 2015. Speculation: Polyamines are important in abiotic stress signalling. Plant Science 237, 16–23.

Pinto, R.S., Matthew, P., Reynolds, M.P., Mathews, K.L., McIntyre, C.L., Olivares-Villegas, J.J., Chapman, S.C., 2010. Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and Applied Genetics 121, 1001-1021.

Rosielle, A.A., Hamblin, J., 1981. Theoretical aspects of selection for yield in stress and non-stress environments. Crop Science 21, 943-946.

Saeedipour, S., Moradi, F. 2011. Effect of drought at the post-anthesis stage on remobilization of carbon reserves and some physiological changes in the flag leaf of two wheat cultivars differing in drought resistance. Journal of Agricultural Science 3, 81-92.

Sawhney, V., Singh, D.P., 2002. Effect of chemical desiccation at the post-anthesis stage on some physiological and biochemical changes in the flag leaf of contrasting wheat genotypes. Field Crops Research 77, 1-6.

Shukla, S., Singh, K., Patil, R.V., Kadam, S., Bharti, S., Prasad, P., Singh, N.K., Khanna-Chopra, R., 2015. Genomic regions associated with grain yield under drought stress in wheat (Triticum aestivum L.). Euphytica 203, 449-467.

Spitkó, T., Nagy, Z., Zsubori Tóthné, Z., Halmos, G., Bányai, J., Marton, L.C., 2014. Effect of drought on yield components of maize hybrids (Zea mays L.). Maydica 59, 1-9.

Sukumaran, S., Dreisigacker, S., Lopes, M., Chavez, P., Reynolds, M.P., 2014. Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. Theoretical and Applied Genetics, 128, 2435.

Tardieu, F., Hammer, G., 2012. Designing crops for new challenges. European Journal of Agronomy 42, 1-2.

Varshney, R.K., Graner, A., Sorrells, M.E., 2005. Genomics-assisted breeding for crop improvement. Trends in Plant Science 10, 621-630.

Xu, L., Han, L., Huang, B., 2011. Antioxidant enzyme activities and gene expression patterns in leaves of kentucky bluegrass in response to drought and post-drought recovery. Journal of the American Society for Horticultural Science 136, 247–255.

Yang, J., Zhang, J., Liu, K., Wang, Z., Liu L. 2007. Involvement of polyamines in the drought resistance of rice. Journal of Experimental Botany 58, 1545-1555.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stage of cereals. Weed Research 14, 415-421.

Table 1. Analysis of variance for traits of eight near-isogenic lines and two genotypes of durum wheat under NW, W and RF conditions during the 2014 cropping season in the rain-out shelter.

Source of variation	df	DH	DF	DM	SPAD45	SPAD65	SPAD77	SPAD83	SPAD85	FLC	BE	TE	PL	NL	SS	FTN	FLA	PLA
Genotype (G)	9	65.55	59.86	44.55	20.33	33.91	85.00	56.41	28.64	131.40	174.97	185.19	65.34	80.45	3.18	0.63	190.27	3021.00
Treatment (T)	3	498.71	441.09	1419.05	188.99	451.64	182.77	1985.08	0.44	596.20	775.73	933.85	205.85	302.64	9.82	1.59	550.80	41963.90
G x T	27	3.66	4.11	6.26	8.67	10.70	24.95	25.77	0.22	108.80	10.58	12.15	7.65	9.65	0.86	0.09	16.57	484.10
F pr.		**DH**	**DF**	**DM**	**SPAD45**	**SPAD65**	**SPAD77**	**SPAD83**	**SPAD85**	**FLC**	**BE**	**TE**	**PL**	**NL**	**SS**	**FTN**	**FLA**	**PLA**
Genotype		<.001	<.001	<.001	0.047	0.001	<.001	0.078	<.001	0.425	<.001	<.001	<.001	<.001	0.065	0.003	<.001	<.001
Treatment		<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.156	0.004	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
G x T		0.516	0.372	<.001	0.685	0.426	0.1	0.725	0.662	0.682	0.603	0.534	0.019	<.001	0.98	0.995	0.263	0.24
Source of variation	**df**	**SKNM**	**SNM**	**SNS**	**SNP**	**SWM**	**SWS**	**SWP**	**TGWM**	**TGWS**	**ASM**	**BSM**	**df**	**APX**	**GPX**	**PUT**	**SPD**	**SPN**
Genotype (G)	9	14.67	91.83	750.60	1160.20	0.22	1.60	2.67	101.26	95.94	1.01	24.43	9	112228	1457696	156595	8956.3	4610
Treatment (T)	3	9.48	825.85	2852.60	4781.90	4.81	11.65	26.46	615.84	530.36	1.76	185.81	1	1658496	6850802	265065	265211.5	47529
G x T	27	0.74	9.82	135.20	161.50	0.06	0.35	0.48	27.72	43.91	0.82	9.21	9	94623	1028636	38936	8441.3	2214
F pr.		**SKNM**	**SNM**	**SNS**	**SNP**	**SWM**	**SWS**	**SWP**	**TGWM**	**TGWS**	**ASM**	**BSM**		**APX**	**GPX**	**PUT**	**SPD**	**SPN**
Genotype		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.001	0.522	<.001		<.001	<.001	<.001	<.001	<.001
Treatment		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.197	<.001		<.001	<.001	<.001	<.001	<.001
G x T		0.603	0.99	0.345	0.446	0.89	0.334	0.449	0.272	0.07	0.821	0.13		<.001	<.001	0.002	<.001	0.052

NW: non-irrigated; W: irrigated; RF: rain-fed; DH: days to heading; DF: days to flowering; DM: days to maturity; SPAD45: SPAD value at ZDS45; SPAD65: SPAD value at ZDS65; SPAD77: SPAD value at ZDS77; SPAD83: SPAD value at ZDS83; SPAD85: SPAD value at ZDS85; FLC: plant height up to the flag-leaf collar (cm); BE: plant height up to the base of the ear (cm); TE: plant height up to the tip of the ear (cm); PL: peduncle length (cm); NL: length of the neck (cm); SS: spike size (cm); FTN: fertile tiller number; FLA: flag-leaf area (cm^2); PLA: plant leaf area (cm^2); SKNM: spikelet number per main spike; SNM: seed number per main spike; SNS: seed number per side spike; SNP: seed number per plant; SWM: seed weight per main spike (g); SWS: seed weight per side spike (g); SWP: seed weight per plant (g); TGWM: 1000-grain weight per main spike (g); TGWS: 1000-grain weight per side spike (g); ASM: apical sterile spikelet number per main spike (%); BSM: basal sterile spikelet number per main spike (%); APX: ascorbate peroxidase **(**nkatal g^{-1} DW**)**; G-POD: guaiacol peroxidase **(**nkatal g^{-1} DW**)**; PUT: putrescine **(**mg g^{-1} DW**)**; SPD: spermidine **(**mg g^{-1} DW**)**; SPN: spermine **(**mg g^{-1} DW**)**.

Table 2. Polyamine contents and antioxidant activities in the flag-leaves of near-isogenic durum wheat lines under irrigated (W) or non-irrigated (NW) conditions. Data are presented as means ± SD (n=5). *, ** and *** denote significant differences from the experimental mean at the P< 0.05, 0.01 and 0.001 probability levels, respectively.

	Treatment	KOFA	NIL1--	NIL1++	NIL2--	NIL2++	NIL3--	NIL3++	NIL4--	NIL4++	SVEVO
Polyamine (mg g^{-1} DW)											
Putrescine	***W***	128.3±8.3	252±57.6	125.8±7.6	455.2±73***	323.2±37.5*	284.8±12.3	128.846±47.4	108±31.1	274.9±25.8	331.7±13.6*
	NW	381.6±61	710.4±9.6	454.4±14	893.3±30.5*	627.6±126.6	617.4±18.8	618±64.8	473.6±146.3	680.6±94.3	1159.9±21.2***
Spermidine	***W***	173±20	191.7±31	173.1±8.1	292±13.2***	243.1±25.5***	187.4±12.5	105.5±24.2	121.4±12.7	154.6±11.4	162.5±3.5
	NW	235.1±28.7	366.6±47.8	346.4±12.8	299.4±32.4	289.4±44.8	352.1±6.7	313.8±16.2	261.7±56.6	265.7±11.7	403.8±58**
Spermine	***W***	201.7±11.1	203±20	205.5±15.6	237.9±16	265.6±44*	205.5±15.7	148.6±27.5	194.7±28.3	177.2±19.2	238.1±42.6
	NW	206±11.5	239.9±23.9	253.3±45.1	248.5±25.3	308±49.9	249.9±3.3	274±36.1	258.3±20.1	276.5±22.9	326.5±77.1
Enzyme activity (nkatal g^{-1} DW)											
APX	***W***	676.6±101.6	984.5±153.7	896.4±87.5	1102.4±169.5	1164.1±170.9	1026.5±249.3	730.3±145.4	841.5±6.3	1029.1±184	1294.8±187.6**
	NW	766±71.9	568.5±56.4	664.7±35	969±26***	736±66.8	655.±45.7	658.7±60.1	649.4±119.6	524.1±40.9	674.8±86.6
G-POD	***W***	471±100.8	244.5±82.7	232.1±37.6	798.1±196.4	1169.6±511.5	774.9±493.2	653.7±58.5	876.2±225.3	743.3±150	527±66.3
	NW	2965.2±213	1922.4±344.2	1870.5±198.8	4140.1±257.8	1988.2±298	2437±333.8	2687.7±381	3037.5±224.4	1797.7±257.8	2152±184.5

Table 3. Simple correlation coefficients between values of the eight near-isogenic durum wheat lines and two durum wheat genotypes under non-irrigated condition.

	APX	FLA	GPX	PUT	SPAD45	SPAD65	SPAD77	SPAD83	SPAD85	SPD	SPN	SNM	SNS	SWM	SWS	TGWM	TGWS
APX	-																
FLA	-0.421	-															
GPX	0.721***	-0.234	-														
PUT	-0.015	-0.003	0.146	-													
SPAD45	-0.220	0.141	0.039	0.293	-												
SPAD65	-0.197	0.124	-0.269	-0.329	0.182	-											
SPAD77	-0.006	0.196	-0.093	-0.478	-0.025	-0.012	-										
SPAD83	-0.297	0.220	-0.282	-0.143	-0.428	0.078	0.053	-									
SPAD85	-0.433	0.457	-0.331	-0.195	0.078	0.310	0.060	0.303	-								
SPD	-0.455	0.375	-0.275	0.542***	0.205	-0.191	-0.155	0.138	0.265	-							
SPN	-0.294	-0.071	-0.209	0.569***	0.254	-0.179	-0.357	-0.207	-0.074	0.498	-						
SNM	-0.291	0.077	-0.457**	0.534***	0.450**	-0.102	-0.069	-0.132	0.112	0.496***	0.481**	-					
SNS	-0.292	0.201	-0.378*	-0.133	-0.004	0.246	0.101	0.036	0.649***	0.219	0.154	0.256	-				
SWM	-0.446**	0.141	-0.223	0.383*	0.682***	-0.030	-0.162	-0.118	0.042	0.352*	0.399**	0.668	0.142	-			
SWS	-0.465**	0.426**	-0.380*	-0.162	0.110	0.259	0.154	0.078	0.622***	0.120	0.006	0.152	0.853	0.305	-		
TGWM	-0.396**	0.141	0.021	0.106	0.580***	0.067	-0.177	-0.033	-0.007	0.112	0.153	0.152	0.025	0.835	0.317	-	
TGWS	-0.490**	0.520**	-0.246	-0.062	0.174	0.149	0.120	0.097	0.264	-0.040	-0.156	-0.030	0.226	0.355	0.695	0.505	-

APX: ascorbate peroxidase (nkatal g^{-1} DW); FLA: flag-leaf area (cm^2); G-POD: guaiacol peroxidase (nkatal g^{-1} DW); PUT: putrescine (mg g^{-1} DW); SPAD45: SPAD value at ZDS45; SPAD65: SPAD value at ZDS65; SPAD77: SPAD value at ZDS77; SPAD83: SPAD value at ZDS83; SPAD85: SPAD value at ZDS85; SPD: spermidine (mg g^{-1} DW); SPN: spermine (mg g^{-1} DW); SNM: seed number per main spike; SNS: seed number per side spike; SWM: seed weight per main spike (g); SWS: seed weight per side spike (g); TGWM: 1000-grain weight per main spike (g); TGWS: 1000-grain weight per side spike (g). *, **, *** significant at the P< 0.05, 0.01 and 0.001 probability levels, respectively.

Supplement

Table 1. Means of phenological, physiological and yield component parameters of eight near-isogenic durum wheat lines and two durum wheat genotypes under irrigated (W) and non-irrigated (NW) conditions.

	SPAD77		SPAD83		SPAD85		FLA		PLA		FLC		BE		TE		PL		NL	
	W	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***
KOFA	49.23	47.05	43.13	24.60	6.18**	5.93	25.27	22.11	124.24	50.64	45.08	39.92	61.25	52.25	68.17	58.67	16.17	12.33	33.00	28.50
NIL1--	46.58	41.85	37.95	24.83	5.68	6.10	32,95*	28.29*	151.00**	65.81	41.08	38.17	49.83	47.25	55.42	53.42	8.75	9.08	24.50	25.50
NIL1++	49.90**	47.68	39.15	25.63	7.13***	7.23***	35,76**	26.43	124.35	63.40	45.75	40.67	61.50	52.41	67.33	58.50	15.75	11.75	32.58	27.75
NIL2--	42.20	42.68	35.90	24.48	3.13	3.43	20.37	22.77	106.66	53.46	43.42	40.92	59.25	52.66	65.58	57.67	15.83	11.75	30.50	26.75
NIL2++	42.90	40.70	37.08	23.80	3.78	3.18	21.13	19.56	94.46	43.54	40.67	39.92	58.17	52.00	64.50	57.58	17.50	12.08	32.58	26.67
NIL3--	46.60	43.60	40.73	24.25	3.55	3.73	24.62	21.90	122,64	54.80	43.58	39.50	60.25	54.50	66.33	59.08	16.67	15.00	32.58	30.00
NIL3++	46.40	42.00	39.73	24.08	5.55	6.20***	25.50	24,34	123,96	55.63	48,91*	38.92	65,25**	51.25	71.50*	61.83	16.33	12.33	32.17	28.58
NIL4--	46.75	44.40	39.80	22.60	4.18	4.70	25.29	24.06	107.26	56.02	47.58	41.50	64,41*	53.91	69.92	59.50	16.83	12.42	31.75	29.08
NIL4++	45.60	45.05	40.10	27.45	5.45	5.23	29,31	25.45	122,49	53.50	42.08	38.25	54.08	50.42	59.67	55.58	12.00	12.17	27.25	26.75
SVEVO	44.08	42.58	40.40	24.38	5.50	5.53	24.59	22.11	115.57	59.32	43.50	39.25	59.42	52.41	65.08	58.08	15.92	13.17	31.17	28.91
LSD5%	3.76	5.09	7.06	6.26	0.65	0.55	6.22	3.50	23.33	16.16	3.87	4.65	4.23	5.10	4.58	5.56	3.26	3.42	2.73	2.67
LSD1%	5.07	6.87	9.53	8.46	0.88	0.74	8.40	4.73	31.50	21.83	5.23	6.27	5.72	6.88	6.18	7.51	4.40	4.62	3.68	3.60
LSD0.1%	6.76	9.15	12.70	11.26	1.17	0.98	11.19	6.30	41.95	29.07	6.97	8.36	7.61	9.16	8.23	10.00	5.86	6.15	4.91	4.79

	SS		SKNM		SNM		SWM		BSM		ASM		TGWM		SNS		SWS		TGWS	
	W	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***	***W***	***NW***
KOFA	6.91	6.41	15.75	16.06	42.56	33.19	2.14	1.36	1.86	5.17	0.38	0.74	64.06	39.72	82.37*	49.25	3.88*	1.67	48.00	33.72
NIL1--	5.58	6.16	17.00**	16.68	37.75	29.38	1.77	1.30	1.86	5.02	1.38	0.37	56.73	43.77	68.00	46.75	2.62	1.90	38.28	40,58
NIL1++	5.83	6.08	16.31	17.12	39.75	33.81*	2.07	1.53*	0.00	2.99	0.76	1.08	56.70	44.79	74.37	60.00	3.22	2,42	44.11	40.64
NIL2--	6.33	5.00	14.88	14.38	37.50	28.06	2.00	1.32	1.26	7.32	0.00	0.42	56.35	46.64	59.38	42.75	2.69	1.61	45.52	37.71
NIL2++	6.33	5.58	14.31	15.44	35.31	31.69	2.00	1.52	2.33	6.48	0.00	0.00	55.55	47.71	75.62	48.13	3.76*	1.66	49.76	34.20
NIL3--	6.08	4.58	14.38	14.81	36.25	30.25	2.06	1.42	1.72	8.10	0.79	0.00	53.29	46.42	60.38	43.00	3.07	1.75	50.90	40.53
NIL3++	6.25	5.58	14.56	15.06	38.56	30.56	2.45*	1.59*	2.58	2.88	0.79	0.00	52.19***	52.43	81.62*	63.12*	3.87*	2,85**	47.81	45,38
NIL4--	5.50	5.58	14.31	15.18	34.13	27.69	1.92	1.22	3.02	5.76	0.40	0.43	52.08	43.64	58.88	41.75	3.06	1.71	53.03	40,61
NIL4++	5.58	5.17	14.31	14.69	35.69	30.00	1.99	1.41	3.15	5.60	1.85	0.00	50.22	46.57	76.00*	52.00	3.66*	2.29	48.04	43.90
SVEVO	5.67	5.66	14.88	14.06	39.88	35.00*	2.08	1.61*	1.97	3.18	0.00	0.00	46.77	44.71	62.63	54.37	3.06	1.88	49.06	34.62
LSD5%	0.97	1.07	1.34	1.08	6.18	2.07	0.37	0.11	3.19	3.76	2.14	1.12	4.94	7.69	6.04	12.12	0.37	0.57	7.34	6.81
LSD1%	1.31	1.45	1.81	1.46	8.34	6.85	0.50	0.47	4.31	5.08	2.89	1.51	6.67	10.38	19.90	16.36	0.99	0.77	9.91	9.20
LSD0.1%	1.74	1.93	2.41	1.94	11.10	9.11	0.67	0.63	5.73	6.76	3.84	2.02	8.88	13.82	26.50	21.79	1.32	1.02	13.19	12.25

SPAD77: SPAD value at ZDS77; SPAD83: SPAD value at ZDS83; SPAD85: SPAD value at ZDS85; FLA: flag-leaf area (cm^2); PLA: plant leaf area (cm^2); FLC: plant height up to the flag-leaf collar (cm); BE: plant height up to the base of the ear (cm); TE: plant height up to the tip of the ear (cm); PL: peduncle length (cm); NL: length of the neck (cm); SS: spike size (cm); SKNM:

spikelet number per main spike; SNM: seed number per main spike; SWM: seed weight per main spike (g); BSM: basal sterile spikelet number per main spike (%); ASM: apical sterile spikelet number per main spike (%); TGWM: 1000-grain weight per main spike (g); SNS: seed number per side spike; SWS: seed weight per side spike (g); SWP: seed weight per plant (g); TGWS: 1000-grain weight per side spike (g);