



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



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### ABSTRACT

Irrigation treatments involving two 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, and polyamine contents in the flag leaves, 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. The ascorbate peroxidase activity was negatively correlated with seed weight per main ( $r = -0.446$ ) and side spike ( $r = -0.465$ ) and the 1000-grain weight of the main and side spike ( $r = -0.396$  and  $r = -0.49$ , respectively) and the guaiacol peroxidase activity with the number of seeds per main ( $r = -0.457$ ) and side spike ( $r = -0.378$ ) and the seed weight per side spike ( $r = -0.38$ ). PUT, SPD, and SPN contents showed a significant positive relationship with the number of seeds per main spike ( $0.534^{***}$ ,  $0.496^{***}$ , and  $0.481^{**}$ , respectively) and the seed weight per main spike ( $0.383^*$ ,  $0.352^*$ , and  $0.399^{**}$ , respectively). ANOVA showed that the *QYld.idw-3B*  $++$  lines had significantly better yield performance under non-watered conditions than the *QYld.idw-3B*  $--$  lines, but their response to drought stress was not uniform regarding other yield-related traits.

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## 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 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, such as 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 programs 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). Drought stress inhibits grain filling in crop plants, which strongly influences the grain weight. Moderate soil drying during the grain-filling period of rice can accelerate endosperm cell division and grain-filling rates and increase the grain weight of inferior spikelets, which was associated with an increased spermidine (SPD) and free spermine (SPN) biosynthesis. Severe soil drying, however, leads to the reduction in the grain-filling rate and grain weight which was paralleled by decrease in SPD and SPN contents (Chen et al., 2013). Correlation between the grain filling rate and free SPN contents in grains suggested that PAs are involved in regulating the grain filling of rice plants (Wang et al., 2012). Similarly in wheat, it was found that the grain PA content

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correlated with the grain filling rate and weight of wheat, and exogenous applications of SPD or SPN significantly increased the grain filling rate and weight of wheat (Liu et al., 2013, 2016).

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; Sanchez-Bragado et al., 2014). For example, the net CO<sub>2</sub> 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 polyamines, proline, and sucrose was exhibited by the flag leaves of tolerant wheat genotypes under induced drought stress (Biswal and Kohli, 2013). Increasing knowledge on the importance of the physiological condition of cereal flag leaves under normal or stress conditions suggests that a relationship exists between the content of endogenous plant growth regulators, such as abscisic acid, ethylene, and polyamines in flag leaves during grain filling and yield (Biswal and Kohli, 2013). Most studies focusing on the relationship between plant growth regulators and drought stress were carried out in rice (Yang et al., 2004; Wang et al., 2012; Chen et al., 2013) or in a limited number of genotypes in the case of wheat or investigated the contents of these compounds only in the grains (Yang et al., 2007; Liu et al., 2013, 2016). Knowledge is limited on how effectively flag leaves can function under stress conditions, and very few reports exist on adaptation of the flag leaf to drought at the molecular level. Polyamines regulate various fundamental cellular processes as signaling molecules. It has been shown that abiotic stress tolerance is influenced by polyamines, which are involved in signaling processes via providing direct interactions with other metabolic routes and hormonal cross-talk, and via activating the expression of stress-responsive genes (Pál et al., 2015).

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 (G×E) and quantitative trait loci (QTLs)-by-environment (QTL×E) 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 help to better understand the plant responses and tolerance mechanisms.

In the present study, near-isogenic durum wheat lines differing for a major grain yield QTL (*QYld.idw-3B*) were evaluated. The aims were 1) to reveal the stress responses of the lines in a spring sowing under drought conditions through detailed morphological and physiological analyses, 2) to explore the correlation between morphological and physiological parameters and yield components under drought conditions, and 3) to elucidate how the polyamine content and antioxidant enzyme activity of the flag leaves were related to yield parameters and drought tolerance. To achieve these goals, the NILs were tested under drought stress conditions, the level of which was constantly monitored by soil sensors.

## 2. Materials and methods

### 2.1. Plant material

Near-isogenic durum wheat lines (NILs) derived from four recombinant inbred lines (RILs) of the original Kofa × Svevo spring durum

wheat cross were used in the experiments. These two cultivars were found to be similarly early flowering and to have good adaptation ability in a multi-location experiment around the Mediterranean Basin. Two major QTLs for grain yield, one on chromosome 2B (*QYld.idw-2B*) and one on chromosome 3B (*QYld.idw-3B*), were identified across several environments, with significant epistatic interactions between them (Maccaferri et al., 2008). The F4 plants were checked for heterozygosity and marker-assisted selection was used to derive the NIL couples (2BL: Xgwm846, Xgwm1027, Xwmc361; 3BS: Xgwm1034, Xbarc133, Xgwm493). The NIL couples were obtained by MAS from heterozygous lines from F4 to F6 and have the following structure: the numerical code indicates the original K×S lines and identifies couples of materials contrasting at the 3BS QTL only (the NILs were all fixed for “Kofa KK allele” on chromosome 2B). At the *QYld.idw-3B* QTL region, the isogenicity for the QTL was verified based on long range SSR- and ISBP-haplotypes consisting of markers at a density of 0.2 cM/marker spanning the complete QTL confidence interval region, from Xgwm1034 to Xgwm493 (Maccaferri et al. unpublished data). Each NIL pair consisted of two inbred F4:6 sister lines, one carrying a complete, uninterrupted Kofa haplotype at *QYld.idw-3B* and the other carrying a complete Svevo haplotype. When the *QYld.idw-3B* region on chromosome 3B was “Kofa KK” (KK2BLKK3BS), the NILs were coded as ++ (NIL1 ++, NIL2 ++, NIL3 ++, NIL4 ++), and when the allele was “Svevo SS” (KK2BLS3BS), they were coded as -- (NIL1 --, NIL2 --, NIL3 --, NIL4 --). Both Kofa and Svevo were included in the experiment.

### 2.2. Field trial and experimental data

The experiments were carried out in the rain-out shelter of the Agricultural Institute, Centre for Agricultural Research, Martonvásár, in 2014. The lines were planted on 17 March 2014 and were grown under the rain-out shelter with two treatments: (i) non-irrigated (NW) and (ii) fully irrigated (W). Individual plots consisted of three rows per line, 10 cm apart, in 1.5 × 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 the NW treatment, drought stress was generated by total water withholding from emergence until harvesting using a rain-out shelter. At 30 cm soil depth, field capacity was 29 vol% (pF 2.5), the wilting point at 10.3 vol% (pF 4.2), and the water stress state occurred at 19 vol% (pF 3.4). In the W treatment, 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 16 replications per line for each water regime. Readings were taken at three positions on the flag leaf: the base, the middle, and the tip of the leaf lamina. The average per plot was computed and used in the analysis.

The flag leaf (FLA) and total plant leaf (PLA) area were measured in 8 and 12 replications, respectively, at flowering (ZGS65) using an LI-3100C leaf area meter. Plant height 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 collar to the base of the ear), and the neck length (NL, from the last node to the base of the ear) were measured in 12 replications.

Measurements were made on spikelet number per spike for 16 main spikes (SKNM) per line, grain number and grain weight per main and side spikes, and on the number of sterile apical (ASM) and basal

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