



Research article

Aeluropus littoralis stress-associated protein promotes water deficit resilience in engineered durum wheat

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ABSTRACT

Global climate change-related water deficit negatively affect the growth, development and yield performance of multiple cereal crops, including durum wheat. Therefore, the improvement of water-deficit stress tolerance in durum wheat varieties in arid and semiarid areas has become imperative for food security. Herein, we evaluated the water deficiency resilience potential of two marker-free transgenic durum wheat lines (*ALSAP*-lines: K9.3 and K21.3) under well-watered and water-deficit stress conditions at both physiological and agronomic levels. These two lines overexpressed the *ALSAP* gene, isolated from the halophyte grass *Aeluropus littoralis*, encoding a stress-associated zinc finger protein containing the A20/AN1 domains. Under well-watered conditions, the wild-type (WT) and both *ALSAP*-lines displayed comparable performance concerning all the evaluated parameters. Ectopic transgene expression exerted no adverse effects on growth and yield performance of the durum wheat plants. Under water-deficit conditions, no significant differences in the plant height, leaf number, spike length, and spikelet number were observed between *ALSAP*-lines and WT plants. However, compared to WT, the *ALSAP*-lines exhibited greater dry matter production, greater flag leaf area, improved net photosynthetic rate, stomatal conductance, and water use efficiency. Notably, the *ALSAP*-lines displayed 25 % higher grain yield (GY) than the WT plants under water-deficit conditions. The RT-qPCR-based selected stress-related gene (*TdDREB1*, *TdLEA*, *TdAPX1*, and *TdBlt101-2*) expression analyses indicated stress-related genes enhancement in *ALSAP*-durum wheat plants under both well-watered and water-deficit conditions, potentially related to the water-deficit resilience. Collectively, our findings support that the ectopic *ALSAP* expression in durum wheat lines enhances water-deficit resilience ability, thereby potentially compensate for the GY loss in arid and semi-arid regions.

1. Introduction

Water deficit is one of the most common environmental stresses affecting crop production worldwide [1]. In recent years, approximately 40 million tons of durum wheat (*Triticum turgidum* subsp. *durum* Desf.) were produced, accounting for 5 % of the total global wheat crop. Water deficit and extreme temperature are the primary stresses limiting the cultivation of durum wheat in the

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Mediterranean basin, and their occurrence during flowering, pollination, and grain-filling can lead to a drop in yields. Therefore, it is crucial to develop durum wheat lines that are able to withstand future climatic changes.

Water deficit stress tolerance is a complex polygenic trait impeding dissection of its overall molecular and physiological mechanisms. Compared to conventional long-term breeding programs, the transgenic approach offers an attractive and effective solution for engineering abiotic stress tolerance. Numerous abiotic stress-related genes from a variety of organisms have been inserted into wheat via genetic transformation. Wheat water-deficit has previously been enhanced using late embryogenesis-abundant (LEA) protein-encoding genes [2]; dehydration-responsive element-binding (DREB), nitrogen assimilation control (NAC), and homeodomain-leucine zipper (HD-Zip) protein transcription factors [3–6]; ethylene-response factor families [7]; and the aldo-keto reductase family [8]. The majority of these studies have focused on bread wheat, while considerably less progress has been made in the development of genetically modified durum wheat. Indeed, no transgenic drought-tolerant wheat has been approved for the market yet [9,10]. Because of the complex genetic characteristics of wheat, the literature data considering genetic modification is very poor in comparison to that of other economic crops.

The adaptation of plants to water deficit conditions requires transcriptional changes to the expression of key genes associated with molecular and physiological changes that allow for better tolerance to harmful conditions [10,11]. In general, plants' abiotic stress response is initiated by signal molecules involved in signal transduction, resulting in the activation of key stress-related genes as well as changes in the cellular expression machinery, biochemical response, and physiological response [10,12]. Therefore, this disturbance at the physiological, biochemical, and metabolic levels leads to losses in crops. The plants that have a greater ability to absorb water and minerals, to regulate stomatal function and osmotic adaptation, to maintain and modify cell wall integrity, are more effective to deal with harsh environmental conditions [11,13,14].

Over the past decade, the stress-associated protein (SAP) gene family has taken a wide amplex, multiple members have been identified, and their roles in abiotic stress tolerance have been confirmed [15]. Several members of the SAP family have been isolated from apple [16], cucumber [17], maize [18], rice [19–22], soybean [23], and wheat [24,25] as well as from *Aeluropus littoralis* [26], *Sorghum bicolor* [27], *Lobularia maritima* [28], *Populus trichocarpa* [29], *Tamarix hispida* [30], and *Vitis amurensis* [31]. The potential of these genes to improve tolerance to abiotic stress has been validated in model plants under *in vitro* and greenhouse conditions.

In previous research, our group successfully isolated the *ALSAP* gene from *Aeluropus littoralis* [26]. This gene has been characterized in tobacco [26] and rice [32] under *in vitro* and greenhouse conditions. Recently, the potential for water deficiency tolerance in *ALSAP*-overexpressing rice lines has been tested in the field, with *ALSAP*-transgenic lines showing an increased ability to maintain productivity under stressful conditions compared to non-transgenic plants [33]. Previously, two marker-free transgenic lines of durum wheat var. Karim overexpressing the *ALSAP* gene, K9.3 and K21.3, were found to have appreciable levels of tolerance to abiotic stress under *in vitro* and greenhouse conditions [34]. In the present study, the water deficiency stress tolerance potential of K9.3 and K21.3 lines under on-farm conditions was evaluated.

2. Materials and methods

2.1. Plant materials

The Tunisian wild-type durum wheat cultivar Karim and the two T3 homozygous genetically engineered durum wheat lines (K9.3 and K21.3) expressing low and high levels of *ALSAP*, respectively, used in this study were provided by Dr. Afif Hassairi. These two *ALSAP*-genetically engineered lines were previously described by Ben Saad et al. [34].

2.2. Growth conditions and water-deficit treatment

The on-farm experiment was carried out at King Saud University Dirab Agricultural Research protected Station (24°25'35.7"N, 46°34'11.0"E) in a split-plot design in a randomized complete block (the main plots were subjected to two irrigation regimes, and the

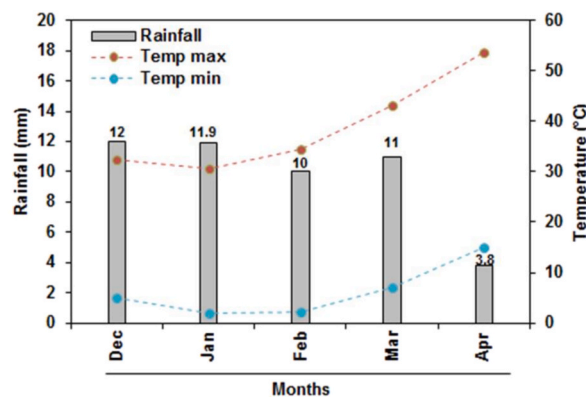


Fig. 1. Climatological data at the experimental site for the durum wheat growing season.

subplots were linked to the three genotypes). The experiment was conducted on sandy loam soil (pH 7.9 and 7.8 g kg⁻¹ organic matter content) with 0.220 and 0.101 m³ m⁻³ as field water capacity and wilting point, respectively. The climatological data collected during the growing period was illustrated in Fig. 1.

The genetically engineered and wild-type seeds were sown at a rate of 150 kg ha⁻¹ on the 15th of December in 10 rows/plots (plot dimensions: 1.5 m × 4 m in width and length, respectively). The experiment was conducted with three replications and two treatments. The ETc was calculated using the crop coefficient (Kc) of durum wheat and the daily reference evapotranspiration (ETo) [35]. A limited water regime was imposed 4 weeks after sowing and the water was administered at the eight Zadoks growth stages (ZS) [36].

2.3. Physiological plant traits measurements

2.3.1. Leaf water status parameters

Green leaves from ten plants randomly selected per plot were harvested and used to determine the leaf water content (LWC) and the relative water content (RWC) following the formulas previously described by Al-Ashkar et al. [37].

The canopy temperature (CT) was assessed using a thermometer (Therma CAM SC 3000 infrared camera, FLIR System Inc., North Billerica, MA, USA)

2.3.2. Leaf parameters

At the ripening stage (ZS 90), ten plants were randomly selected from each plot to determine the flag leaf area (FLA), total leaf area per plant (TLA), and leaf area index (LAI). ImageJ software was used to estimate the FLA and TLA.

2.3.3. Gas exchange measurements

Gas exchange measurements were conducted using a Li-6400 portable photosynthesis system (LI-COR, Inc., Lincoln, NE, USA). The following parameters: photosynthesis rate (Pn), transpiration rate (E), stomatal conductance (Gs), leaf equivalent water thickness (LEWT), water use efficiency (WUE), intrinsic water use efficiency (WUEi), stomatal limitation value (Ls), and intracellular CO2 concentration (Ci) were recorded on the flag leaves during the stage of floret development, specifically between 10:00 a.m. and 11:00 a.m.

2.3.4. Agronomic and physiological features

Several agronomic parameters were estimated at the ripening stage (ZS 90) using ten plants randomly selected from each plot. These agronomic parameters included plant height (PH), leaf number (LN), stem dry weight (SDW), leaf dry weight (LDW), total dry weight (TDW), spike length (SL), number of spikelets (NS), number of seeds per spike (NSS), thousand kernel weight (TKW), and grain

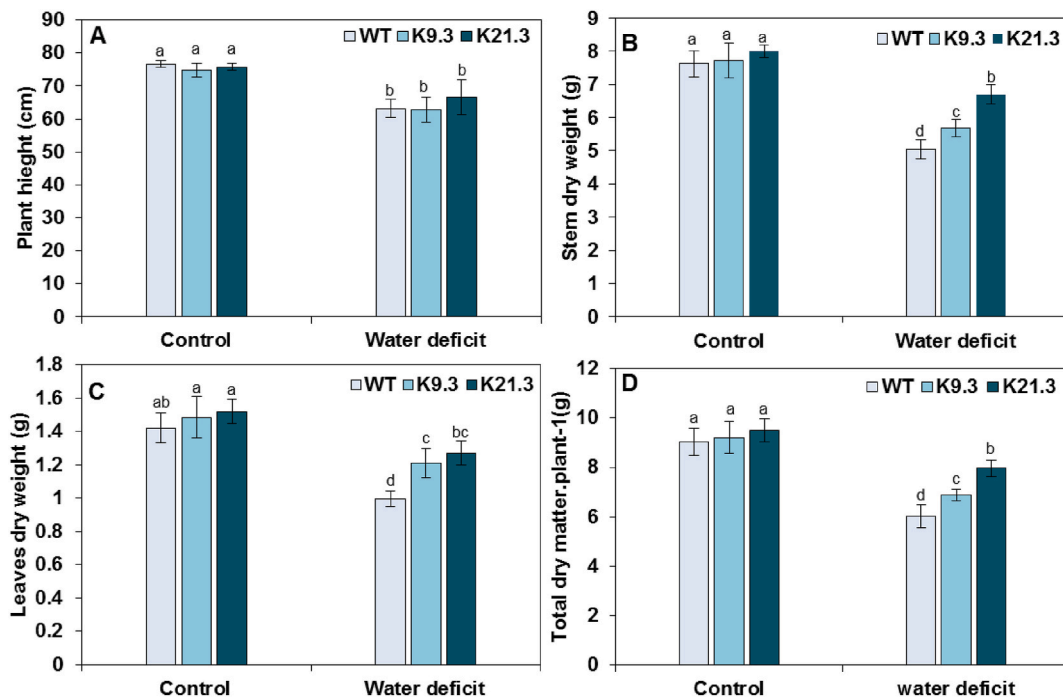


Fig. 2. Influence of water-deficit stress on plant high (A), stem dry weight (B), leaf dry weight (C), and total plant dry matter in ALSAP-lines (K9.3 and K21.3) and WT durum wheat plants. Bars represent the means ± SE of three replicates. Bars designated by different letters for each treatment × genotype combination are significantly different (p ≤ 0.05) according to Duncan’s multiple range test.

yield (GY).

2.4. RNA extraction and quantitative real-time PCR assay

Total RNA was collected from frozen leaves using the E.Z.N.A Kit (Omega Bio-Tek). For RT-qPCR, one μg of total RNA per sample was employed to synthesize first-strand cDNA using a QuantiTect RT-Kit (Qiagen). The transcript accumulation of the selected stress-related genes, including dehydration-responsive element binding transcription factor (*TaDREB1*), plasma membrane protein (*TdBlt101-2*), late embryogenesis abundant (*LEA*) protein (*TdLEA*), and ascorbate peroxidase 1 gene (*TdAPX1*), was monitored via RT-qPCR reactions as previously outlined by Ben Romdhane et al. [38]. Relative expression was determined by the comparative threshold cycle ($2^{-\Delta\Delta\text{CT}}$) method, using the actin gene (*TdActin*) as the internal control (Supplementary Table S1).

2.5. Statistical analysis

All the data were subjected to two-way ANOVA analysis using XLSTAT statistical package (Addinsoft, New York, USA). Differences for each treatment \times genotype combination were estimated using Duncan's multiple range test ($p < 0.05$). Stepwise multiple linear regressions (SMLRA) were used to identify the best traits and their contributions to grain yield.

3. Results

3.1. Evaluation of growth and physiological performance

To assess the water-deficit stress effects on the growth of durum wheat plants, several parameters were monitored under normal and water-deficit conditions for the *ALSAP*-lines and WT plants. Under control conditions (Fig. 2A), no significant differences in plant height (PH) were recorded between the *ALSAP*-durum wheat lines and the WT plants. However, a reduction of PH was noticed for all the evaluated durum wheat genotypes under water-deficit stress conditions, which was estimated to 18 %, 16 %, and 12 % for the WT, K9.3, and K21.3 lines, respectively, with no significant difference (Fig. 2A).

Under well-watered control conditions, no significant differences in SDW, LDW, and TDW were recorded for the *ALSAP*-durum wheat lines compared to the WT plants. However, both *ALSAP*-lines have displayed greater SDW, LDW, and TDW than did the WT plants under water-deficit conditions (Fig. 2B–D). The WT plants appeared more affected by water-deficit stress, and their dry weight

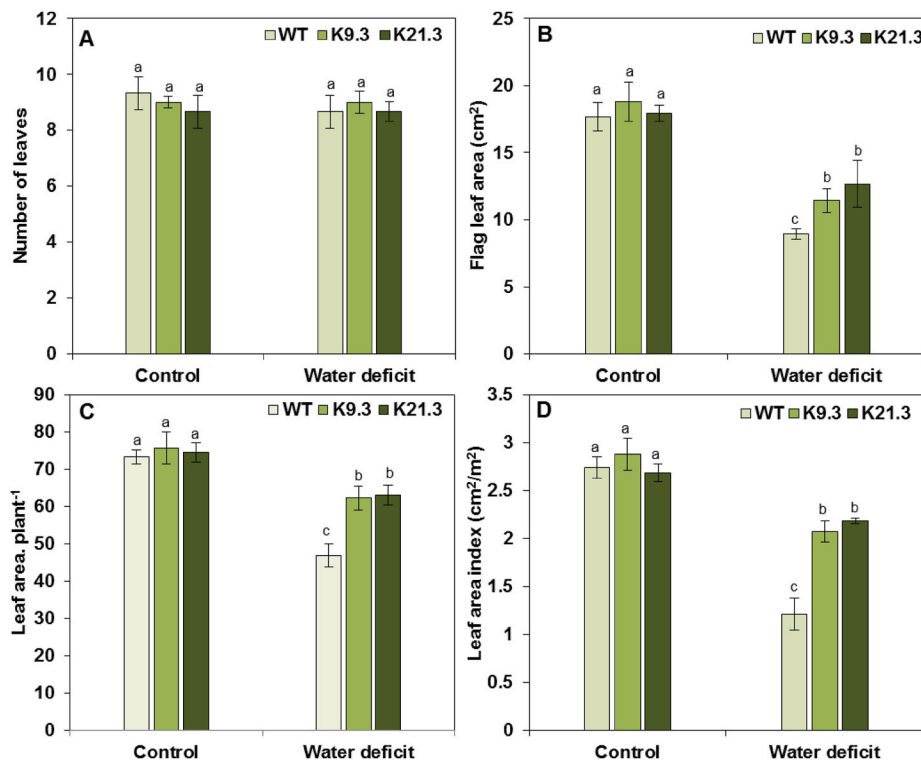


Fig. 3. Effect of water-deficit stress on leaf number (A), flag leaf area (B), total leaf area (C), and leaf area index (D) of WT plants and *ALSAP*-genetically engineered durum wheat lines (K9.3 and K21.3). Bars represent the means \pm SE of three replicates. Bars designated by different letters for each treatment \times genotype combination are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

decreased by 30–33 % compared to those recorded under well-watered control conditions. Compared to the WT plants, K9.3 lines showed a significantly lower decrease (25 %) in the LDW. On the other hand, the K21.3 line showed the most significant performance under water-deficit conditions, as illustrated by the lowest decrease in SDW, LDW and TDW (Fig. 2B–D).

3.2. Leaf area parameters

Under well-watered and water-deficit conditions, four leaf-related parameters, leaf number (LN), flag leaf area (FLA), total leaf area (TLA), and leaf area index (LAI), were monitored in the *AlSAP*-lines and WT plants (Fig. 3A–D). There were no significant differences in any of the leaf-related parameters assessed under normal conditions between the *AlSAP*-durum wheat lines and WT plants (Fig. 3A–D). Under water-deficit conditions, low and non-significant ($P < 0.05$) decrease in LN was noticed for all the evaluated genotypes compared with their counterparts under well-watered control conditions (Fig. 3A). The FLA was reduced in the all evaluated genotypes due to water-deficit stress, by 50 %, 39 %, and 29 % for the WT plants, and the K9.3, and K21.3 *AlSAP*-lines, respectively, compared to their counterparts under well-watered control conditions (Fig. 3B). Similarly, the plant leaf area (PLA) and leaf area index (LAI) significantly decreased under water-deficit stress conditions for the all evaluated genotypes (Fig. 3C–D). Interestingly, the PLA and LAI measurements of the WT plants decreased by 62 % and 56 %, respectively. However, compared with their counterparts under well-watered control conditions, the K21.3 and K9.3 *AlSAP*-plants maintained high ability to maintain their PLA and LAI parameters under water-deficit stress conditions despite reductions of 15–17 % and 18–28 %, respectively (Fig. 3C–D).

3.3. Leaf water status parameters

As shown in Fig. 4, the LWC of all tested genotypes slightly decreased under water-deficit stress conditions compared to that registered under well-watered control conditions. The decrease in LWC was estimated to 3.53 %, 2.71 %, and 1.71 % for the WT, K9.3, and K21.3 durum wheat plants, respectively, compared to the records registered under well-watered control conditions; however, these decreases in LWC were still not statistically significant ($P < 0.05$) (Fig. 4A). In the same trends, the estimated RWC values were not significantly ($P < 0.05$) affected in any of the tested genotypes under water-deficit stress conditions compared to those registered under well-watered control conditions (Fig. 4B). Interestingly, compared with those of the WT and K9.3 plants, the WUE and WUEi of the K21.3 plants under water-deficit conditions were significantly greater (Fig. 4B–D). In addition, the CTs of the WT plants and the

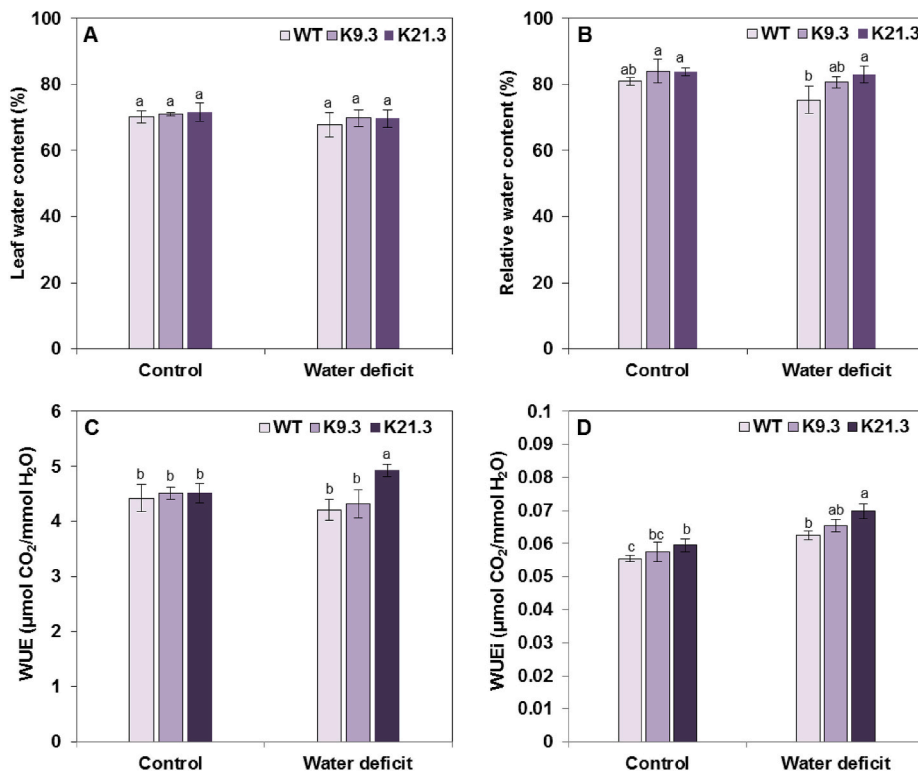


Fig. 4. Effect of water-deficit stress on leaf water status parameters of WT plants and *AlSAP*-durum wheat lines (K9.3 and K21.3). Influence of water-deficit stress on LWC (A), RWC (B), WUE (C), and WUEi (D) in *AlSAP*-engineered lines and WT durum wheat plants. Bars represent the means \pm SE of three replicates. Bars designated by different letters for each treatment \times genotype combination are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

ALSAP-durum wheat lines were similar under well-watered control conditions (Fig. S1). However, under water deficit conditions, the CT increased by 7.3 °C, 2.1 °C, and 1.9 °C in the WT, K9.3, and K21.3 plants, respectively, compared to their counterparts under well-watered control conditions (Fig. S1).

3.4. Leaf gas exchange

The leaf gas exchange rate was monitored through photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) measurements (Fig. 5A–D). There were no significant differences in any of the above-mentioned leaf gas exchange measurements under normal conditions between the *ALSAP*-lines and the WT plants (Fig. 5A–D). Under water deficit stress conditions, the P_n , G_s , C_i , and E of the *ALSAP*-durum wheat lines were greater than those of the WT plants. Compared with those in the WT plants, P_n , G_s , C_i , and E in the K21.3 plants under water deficit stress conditions were 21.8 %, 16.6 %, 13.3 %, and 16.8 %, respectively (Fig. 5A–D). Together, these data suggest that the expression of *ALSAP* gene in the K9.3 and K21.3 durum wheat lines could enhance their gas exchange capacities under water-deficit conditions.

3.5. Yield and yield component parameters

The yield parameters were monitored through spike length (SL), number of spikelet (NS), number of seeds per spike (NSS), thousand-kernel weight (TKW), and grain yield (GY) measurements. As shown in Fig. 6A–D, no significant differences in spike length (SL), and number of spikelet (NS) were revealed for K9.3 and K21.3 durum wheat lines compared to WT plants under both well-watered control and water-deficit conditions. In the same trends, there were no significant differences in the number of seeds per spike (NSS) and thousand-kernel weight (TKW) between the *ALSAP*-durum wheat lines and the WT plants under well-watered control conditions (Fig. 6E and F). However, the NSS and TKW of the K9.3 and K21.3 durum wheat lines were 22.7–31.57 % and 21.96–27.18 % greater, respectively, than those of the WT plants under water-deficit conditions (Fig. 6E and F). Compared to the well-watered control conditions, the K9.3 and K21.3 durum wheat lines showed 16.16 % and 7.4 % decreases, respectively, in their NSS and 8.09 % and 8.83 % decrease respectively in their TKW under water-deficit stress conditions. However, the NSS and TKW of the WT plants decreased by 28.7 % and 26.22 %, respectively, under these conditions (Fig. 6E and F). The revealed high performance of the

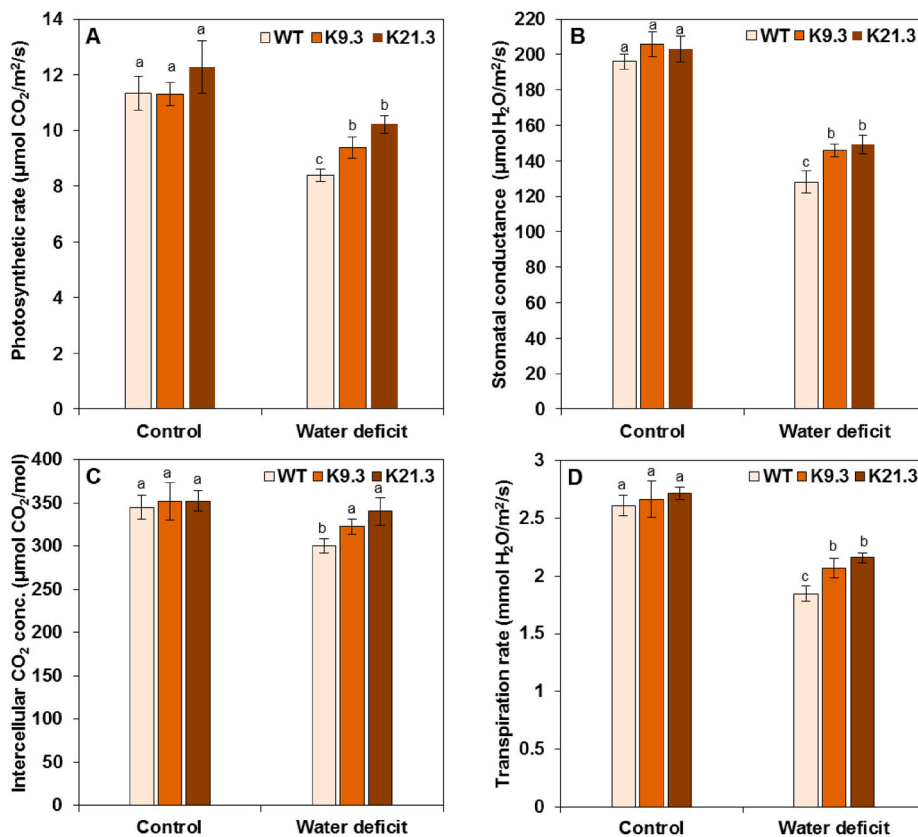


Fig. 5. Effect of water-deficit stress on leaf gas exchange parameters of WT plants and *ALSAP*-durum wheat lines (K9.3 and K21.3). Bars represent the means \pm SE of three replicates. Bars designated by different letters for each treatment \times genotype combination are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

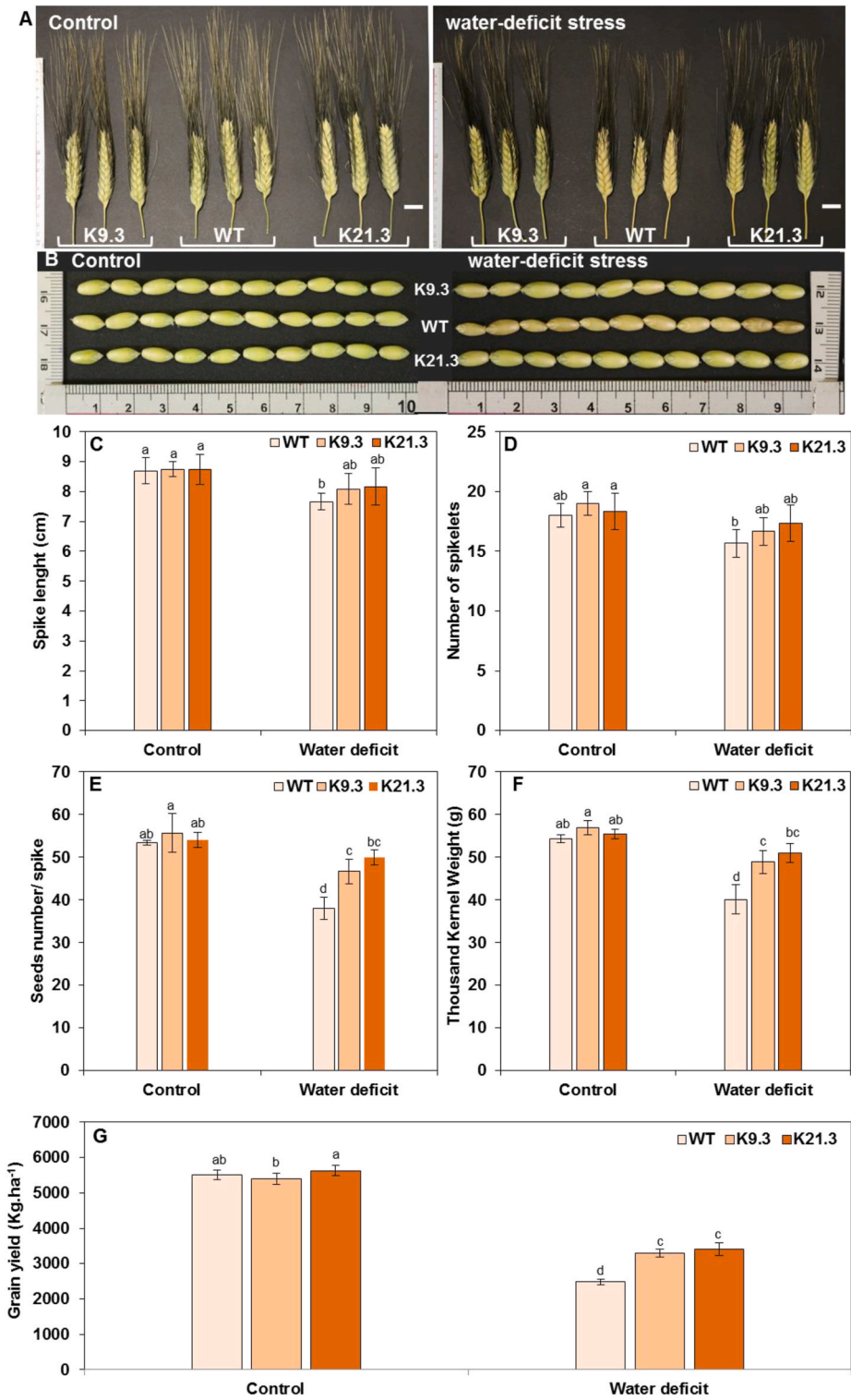


Fig. 6. Effect of water-deficit stress on yield and yield component parameters of WT plants and ALSAP-genetically engineered durum wheat lines (K9.3 and K21.3). Data in B, C, D, E, and F represent the means \pm SE of three replicates. Bars designated by different letters for each treatment \times genotype combination are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

ALSAP-durum wheat lines under water deficit stress conditions in terms of greater number of seeds per spike and greater thousand kernel weight was associated with greater grain yield (GY) than that of the WT plants under such conditions (Fig. 6G). Thus, compared to that under well-watered control conditions, the GY under water-deficit stress conditions decreased by 55 %, 39.4 %, and 38.8 % for the WT, K9.3 and K21.3 plants, respectively (Fig. 6G). Interestingly, the GY of durum wheat plants overexpressing the ALSAP gene (K9.3 and K21.3 lines) was 25 % greater than that of WT plants under water-deficit stress conditions (Fig. 6G).

3.6. Identification of traits associated with water-deficit responses in WT and ALSAP durum wheat

The analysis of the relationships between the variables and the GY revealed high positive correlation values for LAI and GLA (0.971) and high negative correlation values for CT and GY (-0.827). The GY showed highly significant positive correlation with nine variables, namely, NS, PH, FLA, GLA, LAI, NKS, Pn, Gs, and E, and highly significant negative correlation with both the CT and LEWT variables (Fig. 7).

To determine the most closely-related traits and the level of their impact on the performance of each evaluated genotype, a regression analysis was performed using 29 evaluated traits as interpreted variables and grain yield as the dependent variable. The stepwise regression analysis revealed significant associations between the evaluated parameters and the grain yield ($P > 0.0001$). Interestingly, the CT and LEWT traits significantly contributed ($R^2 = 0.953$) with 0.822 and 0.131, respectively, to the grain yield of the WT durum wheat plants. However, the Gs and TKW traits were significant contributors ($R^2 = 0.870$), with values of 0.750 and 0.120, respectively, to the grain yield of the K21.3 ALSAP-durum wheat lines. The mean of the predicted regression GY value ranged from 3.865 to 4.552, the error value ranged from -0.010 to 0.536, the relative error value ranged from -0.005 to 0.162, and the evaluation accuracy (%) ranged from 81.70 to 96.60, with an average value of 91.163 (Table S2).

3.7. Molecular responses of WT and ALSAP-durum wheat plants to water deficit

The analysis of the expression levels of four selected stress-related genes (*TdDREB1*, *TdLEA*, *TdAPX1*, and *TdBlt101-2*) in the leaves of WT and ALSAP-durum wheat revealed a positive deregulation of the selected stress-related genes in ALSAP-durum wheat lines even under well-watered and water-deficit conditions compared to their WT counterparts. The positively deregulated stress-related genes are involved in the regulation of the expression of water scarcity-responsive genes (*TdDREB1*), maintenance of membrane integrity, and regulation of water balance (*TdLEA* and *TdBlt101-2*), detoxification of harmful hydrogen peroxide species and protection of plant cells from oxidative damage, which enhance the ability of plants to cope with stress conditions. Consequently, the integration of the ALSAP gene in the durum wheat genome was able to modulate stress-related genes expression and potentially prime ALSAP-engineered plants to cope with water-deficit conditions (Fig. 8).

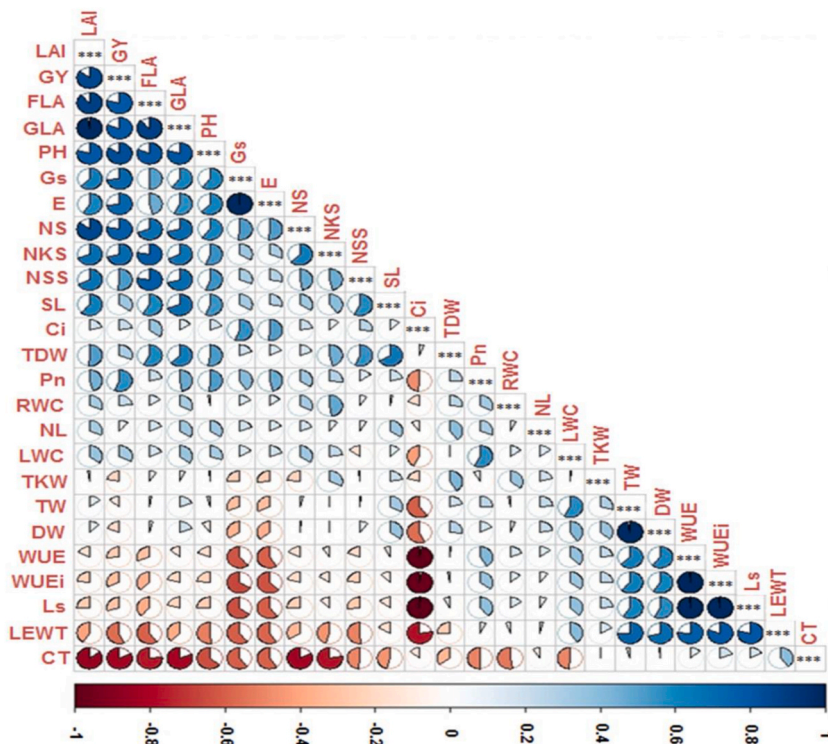


Fig. 7. Correlation among all evaluated traits under water-deficit stress conditions.

4. Discussion

Aggravated climate changes pose considerable risks to food security and negatively affect the global agricultural production of many crucial cereal crops, such as wheat, barley, rice, maize and etc. [39]. Reduced plant productivity due to water deficiency is a major concern for durum wheat farmers, and the development of high-yielding durum wheat cultivars is a primary goal of breeding programs. Therefore, the development of plant varieties with effective agronomic and physiological traits that enable them to withstand abiotic stress (drought, salinity, heat, and cold) has become imperative in arid and semiarid areas.

Water-deficit tolerance is a complicated trait resulting from the contributions of several putative interdependences, including water status parameters, leaf surface area, photosynthetic performance, root characteristics, plant biomass, number of spikes, and kernel features, which are interesting traits for water-deficit tolerance evaluation [40–42]. Maintaining an optimal balance between the water received by roots and the water lost by leaves is a crucial aspect of plant water-deficit tolerance [11,13]. During the vegetative stage in wheat, water deficit restricts leaf elongation, leaf number, leaf area, and increases leaf senescence [40,43,44]. Additionally, water deficit stress during the generative stage in wheat drastically affects the fertilization and grain yield [43]. Therefore, the generation and selection of cultivars that possess efficient water use mechanisms can be considered as approach to improve the yield of durum wheat under water-deficit stress conditions. Previously, Ben Saad et al. [26] isolated the *ALSAP* gene from the halophyte grass *Aeluropus litoralis* and functionally characterized it in tobacco, rice and durum wheat under *in vitro* and greenhouse conditions [26,32,34]. Recently, Ghneim-Herrera et al. [33] reported that, compared with non-genetically engineered plants, *ALSAP*-overexpressing rice lines exhibited marked water deficiency tolerance under field conditions, and an increased ability to maintain productivity under such stressful conditions. Two marker-free genetically modified lines of durum wheat var Karim overexpressing the *ALSAP* gene, K9.3 and K21.3, were previously found to have appreciable tolerance to salinity and dehydration stress under *in vitro* and greenhouse conditions [34]. In this study, we evaluated the water deficiency stress tolerance potential of K9.3 and K21.3 lines under on-farm conditions through morpho-physiological approach.

Under well-watered conditions, our analysis revealed no significant differences ($p < 0.05$) between the K9.3 and K21.3 *ALSAP*-lines and the WT plants for any of the evaluated parameters, including PH, NL, FLA, LWC, RWC, WUE, Pn, Gs, E, NS, LS, and GY. These results suggest that the overexpression of *ALSAP* gene has no detrimental effects on the growth and productivity of genetically engineered durum wheat plants, which is in line with previous findings recorded under *in vitro* and greenhouse conditions reported by Ben Saad et al. [34]. However, the stem- and leaf dry weight and total dry weight of WT, K9.3 and K21.3 plants subjected to water-deficit stress were significantly lower than those of the well-watered control plants. Furthermore, the plant heights of both the WT and *ALSAP*-engineered durum wheat plants were similar under the water-deficit treatment. The ability to maintain high dry weight (stem and leaves) was more pronounced in K21.3 lines, which express high levels of the *ALSAP* gene compared to K9.3 lines. These findings suggest that the expression of the *ALSAP* gene in durum wheat improved the potential of *ALSAP*-engineered lines to maintain their growth and dry matter under water-deficit conditions. Our results are in line with those reported by Ben Saad et al. [34], who demonstrated that *ALSAP* wheat lines exhibited improved biomass production under osmotic stress conditions. In rice, *ALSAP* expression has been found to increase the accumulation of green biomass under drought conditions [33]. Several A20/AN1-zinc-finger domain containing proteins characterized in wheat, rice, maize, and sugarcane were found to confer drought and osmotic stress tolerance as well as an improved biomass and grain yield in transgenic *Arabidopsis*, rice, and tobacco plants [15,45–47].

Leaves, especially flag leaves, are vital sources of carbohydrates. These carbohydrates produced via gas exchange and photosynthesis processes can directly influence wheat grain filling and yield [48]. Despite the similar number of plant leaves under well-watered and water-deficit conditions, the plant leaf area, and particularly the flag leaf area, were markedly affected by water deficiency. However, both the K9.3 and K21.3 *ALSAP*-lines maintained their leaf area indices and, in particular, their flag leaf area, which was

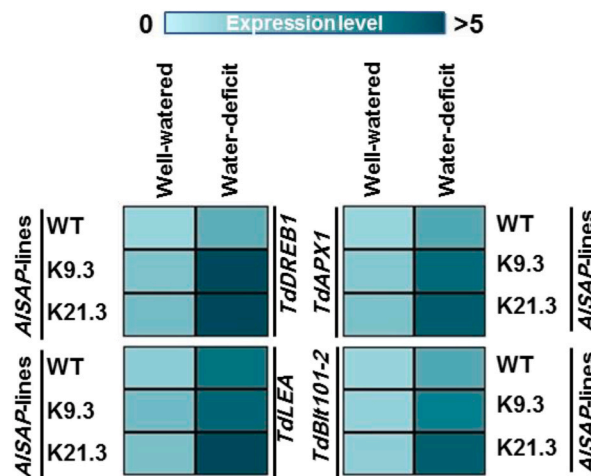


Fig. 8. Expression pattern of stress-related genes (TdDREB1, TdAPX1, TdLEA, and TdBlt101-2) in WT and *ALSAP*-durum wheat lines under well-watered and water-deficit regimes.

27–40 % greater than that of the WT plants under water-deficit conditions. Subsequently, these leaf characteristics of the *ALSAP*-genetically engineered durum wheat lines had direct effects on the gas exchange rate, water-use efficiency index, grain filling, and yield. Thus, our results indicated that the K9.3 and K21.3 durum wheat lines had greater gas exchange parameters in terms of their photosynthetic rate, stomatal conductance, transpiration rate, and intracellular CO₂ concentration under water-deficit conditions than did their WT counterparts. Additionally, although it was more pronounced in the K21.3 lines that highly expressed the *ALSAP* gene, the *ALSAP*-overexpressing durum wheat lines exhibited a better water-use efficiency than did their WT counterparts under water deficit conditions. In rice, *ALSAP*-overexpressing plants showed enhanced transpiration efficiency and maintained higher CO₂ concentrations than did Nipponbare control plants when subjected to severe drought stress [32]. Similarly, Ghneim-Herrera et al. [33] recorded significant differences in the photosynthetic parameters, leaf water potential, and antioxidant accumulation in the flag leaves of *ALSAP*-rice lines that were subjected to drought at the early tillering stage during greenhouse dry-down experiments. In contrast, under open-field conditions and drought stress applications during the flowering stage, Ghneim-Herrera et al. [33] did not observe significant differences in the aforementioned traits of *ALSAP*-rice lines compared to their Nipponbare counterpart plants.

There was a strong correlation between the leaf gas exchange rate and leaf CT [49]. Thus, CT is a useful indicator of plant water status, as well as the effectiveness of the transpiration process in protecting from warm-up of the leaves [37]. The K9.3 and K21.3 lines exhibited lower CT than did the WT plants under water-deficit conditions, which was probably a result of the enhanced transpiration rate in *ALSAP*-lines under such conditions. Previously, Gonzalez-Ribot et al. [50] reported that the morphological and physiological traits are strongly associated with the yield performance of durum wheat plants. Thus, our findings revealed that no significant differences in terms of SN, SL, TKW, and GY between the WT plants and the *ALSAP*-durum wheat lines under well-watered conditions. However, these aforementioned traits decreased under water-deficit conditions for the WT plants and even the *ALSAP*-durum wheat lines. The effect of water-deficit stress was very pronounced in WT plants, with 13, 28, 26, and 55 % decreases in their SN, NSS, TKW, and GY, respectively, compared to their records under well-watered conditions. Interestingly, although the decline by 7, 8, 8, and 39 % in the SN, NSS, TKW, and GY records, respectively, of the K21.3 *ALSAP*-line due to water-deficit stress, the *ALSAP*-durum wheat line was able to maintain a 25 % surplus of GY compared to WT plants under such conditions. These findings suggest that the expression of *ALSAP* gene in durum wheat lines enhances their ability to maintain flag leaf area index, WUE index, and gas exchange rate under water-deficit conditions, which positively impacts their performance in terms of NSS, TKW, and GY.

The significant correlations between evaluated traits denote overlap with others [51], but relying on these correlations alone is insufficient, and for greater accuracy, we used other statistical analyses, such as multiple regression [stepwise multiple linear regression (SMLRA)]. The SMLR results (Table S2) denoted that the traits TKW, Gs, TW, LEWT, and CT were only directly related to the GY trait, which varied according to genotype, but at least it will relate to one trait [51,52]. Therefore, we realized that TKW, Gs, TW, LEWT, and CT are reliable traits for forecasting yield performance (Table S2). As shown by the equations of the model (GY, Table S2), certain traits might display a positive correlation, whereas others may exhibit a negative correlation. To compute the plant growth and development performance, the TKW, Gs, TW, LEWT, and CT traits were the most effective traits [53,54]. The CT trait is closely related to with several photosynthetic processes involved in plant drought stress responses [55,56], and it is a robust physiological trait that should be taken into consideration in wheat breeding programs [55–57]. The genotype that has a high capacity to maintain low CT and gas exchange is extremely coveted due to its leaf-cooling response under stress [52,58,59].

5. Conclusions

In summary, this study constitutes the first on-farm evaluation of *ALSAP*-genetically engineered durum wheat lines (K9.3 and K21.3). The expression of *ALSAP* gene in marker-free genetically engineered durum wheat lines penalized neither yield nor plant growth under well-watered conditions and was associated with increased GY under water-deficit conditions. This performance is linked to a greater vegetative biomass, leaf area, photosynthetic rate, and water-use efficiency index under water-deficit stress. These findings are promising for the assessment of abiotic stress tolerance potential under open-field conditions and the integration of these *ALSAP* lines in durum wheat water-deficit tolerance breeding programs.

Data availability statement

All data is contained within the article or supplementary material.

CRedit authorship contribution statement

Walid Ben Romdhane: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ibrahim Al-Ashkar:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Abdullah Ibrahim:** Investigation, Formal analysis, Data curation. **Mohammed Sallam:** Investigation, Formal analysis, Data curation. **Abdullah Al-Doss:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Afif Hassairi:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e30933>.

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