The impact of drastic changes in soil moisture showcasing the genotypic variation in the adaptation of two wheat genotypes (*Triticum durum* Desf.)

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Abstract

Although the breeding programs have enhanced the yield of cereal crops over the course of the last decades, the climate change effect is more and more challenging yield stability and is threatening farmer's livelihood. Among abiotic stresses, drought is the most devastating environmental factor causing yield loss. The objective of this research is to study the effect of water stress among two genotypes of durum wheat (*Triticum durum Desf*): the local variety "Bidi" and the commercialized variety "Om Rabiaa" grown in a pot experiment under greenhouse conditions. We focused on water status, morpho-physiological and biochemical parameters. For this purpose, a severe water deficit was induced by withholding irrigation for 15 days on the 35thday after sowing. Concerning the water status, it was assessed through the relative water content (RWC) after 14 days of treatment. There was a reduction of 37% for Bidi and 23.26% for Om Rabiaa. Only the treatment (T) had a significant effect ($P<0.01$). As for the morphological parameters, the green leaf number (GLN) was affected by 58.27% for Om Rabiaa and 38.97% for Bidi after 14 days of withholding irrigation. The treatment (T) and the genotype (G) had a very significant effect ($P<0.01$). On the other hand, the plant height was reduced by 23.06 % for Om Rabiaa and only by 13.17% for Bidi after 14 days. A very highly significant effect ($P<0.0001$) of (T) was observed. Concerning the physiological parameters, the genotype had a highly significant effect ($P<0.01$) on SPAD values. At the same time, the net assimilation rate (A) has plummeted by 78.26% for Om Rabiaa. Whereas, we recorded a reduction of 55.25% for Bidi after 14 days and the (T) was very significant ($P<0.01$). The transpiration rate (E) was affected by 82% on average. Similarly, the stomatal conductance (gs) dropped clearly after 7 days of treatment, particularly for Bidi. The photochemical efficiency of PSII (Fv/Fm) was notably reduced for Om Rabiaa. The above-ground biomass accumulation was reduced in both genotypes and the (T) was highly significant ($P<0.01$). For root biomass, Bidi showed to be less affected by drought and the reduction was only about 19% compared to 48% for Om Rabiaa. Eventually, osmoregulation was important in both genotypes and high proline contents were recorded after 14 days of treatment.

1. Introduction

The vulnerability of modern crops to abiotic stresses causes wide annual yield fluctuations between bad years and good years. And there is a major global food deficit as the demand for food is higher than what is being produced. By 2025, the world farmers would have to produce about 3.0 billion tons of cereals to feed the earth's population of nearly 8.0 billion people. This means that worldwide, an average cereal yield of 4 t/ha is to be achieved and sustained (Nagarajan and Nagarajan, 2010). Cereals represent a major component of the human diet worldwide, either directly as baked goods derived from flour, or indirectly as components of animal feed (grain, brans, straws, and other residues). Global cereal production and trade are dominated by wheat and maize (Coombs and Hall, 1997). Average global yields increased from 1.4 t h $^{-1}$ during the 1970s to more than 2 t ha^{-1} in recent years, leading to a great increase in total production. However, global production was curtailed in 2005 due to lower plantings in the major European producing countries combined with a severe drought affecting growing areas in the Mediterranean Basin (Royo et al., 2009). In Tunisia, yield decreased from 1.69 t/ha in 2004 to 1.53 t/ha in 2005 (USDA, 2005). Short periods of very high temperature (>35°C) are a common occurrence in many wheat-growing areas of North Africa. Actually, wheat is mainly grown under rainfed conditions, characterized by unpredictable rainfall and a large incidence of abiotic stresses. Drought and heat during the grain filling period, nutrient deficiencies, and soil problems are the main yield constraints. Tunisian Durum wheat landraces were later increasingly replaced by improved varieties. The introduction of productive varieties resulted in the abandon of the genetically diverse, locally well-adapted but unimproved landraces, and the extinction of genetic variability at the farm scale except for some limited clusters throughout the country (Royo et al., 2009).

Crop response to heat and drought stresses depends on the intensity and the duration of these stresses and the phenological stage of the crop at which they occur. Therefore, the selection of physiological traits for drought tolerance requires a comprehensive understanding of the nature of the trait and its contribution to yield as well as its response to the environment (Ludlow and Muchow, 1990) (Sheshshayee and Bindumadhava, 2003). When drought stress was imposed during the reproductive growth stage of wheat, pollen fertility was most affected. The most sensitive stage of wheat yield to drought stress is in the early spikelet development (Praba et al., 2009). Drought stress decreased the relative leaf water content, and the transpiration rate and concomitantly increased the leaf temperature in wheat and rice (Farooq et al., 2009; Siddique et al., 2001). The stomatal closure and the decrease in stomatal conductance and transpiration rate under drought stress have been related to higher water-use efficiency (the ratio of dry matter produced to water consumed) in wheat (Abbate et al., 2004). In the case of severe drought stress where plant growth and biomass accumulation are greatly diminished, the water-use efficiency is also reduced (Costa et al., 1997). Research focusing on the osmotic adjustment in wheat plants indicated that the osmotic adjustment was greater at the tillering stage than at the heading stage. It was not clear; however, whether the differences among cultivars in the allocation of biomass to grain under water stress were due to osmotic adjustment (Moustafa et al., 1996). Therefore, knowledge of morpho-physiological mechanisms involved in response to soil moisture depletion may contribute to a better selection of varieties adapted to different agro-climatic conditions. This study aimed to assess characteristics related to plant water use in durum wheat genotypes with contrasting yield performance under drought stress. Specifically, the work aimed at (i) assessing the morphophysiological response in local and enhanced wheat varieties and (ii) investigating how soil moisture depletion showcases drought tolerance traits.

2. Material and Methods

2.1. Plant Material and Growth Conditions

Pot experiments were conducted in greenhouse semi-controlled conditions in a completely randomized design during the 2017/18 growing season at the Regional Center of Agricultural Research located in Sidi Bouzid, Tunisia. Plant materials consisted of two durum wheat (*Triticum durum* Desf.) genotypes Om Rabiaa (enhanced variety) and Bidi (local variety): Om Rabiaa: drought tolerant and subscribed in Tunisia in 1996. Issued of the cross of L0589 realized at ICARDA/Syria and introduced in Tunisia in 1987 (Deghais et al., 2007); Bidi: its multiplication for commercial purposes started in 1913/1914. Nowadays, it almost disappeared from field crop areas except for some clusters in central Tunisia (Deghais et al., 2007).

The pots were reshuffled weekly to minimize the position effect. Pots were filled with 5Kg of topsoil. Each pot was supplied with ample nutrition mainly comprising Potassium (K), Nitrogen (N), and Phosphorus (P) at 120-120-150. Sowing was performed at an average density of 6 seeds per pot spaced approximately 3 inches from all sides. Later on, replicates were thinned to 4 seedlings per pot. Genotypes were grown under well-watered and drought treatments using six replicates each.

2.2 Water treatments

Irrigated pots were watered after the appearance of seedlings, at the stem elongation, anthesis, and grain filling stages.

The plants were kept well-watered initially. The pots were weighed at 100% of field capacity regularly and the amount of lost water was added to maintain the required field capacity. Water-stressed pots were not watered starting from the beginning of tillering stage (stage 13, 21 Zadoks et al., 1974). After that, a drought was given for 14 days. Eventually, two different treatments were applied i.e., well-watered (C) and water-stressed plants (S).

2.3. Measurements

2.3.1. Relative water content

The flag leaf relative water content (RWC) was determined gravimetrically. The RWC was calculated using the following formula: RWC (%) = (FW-DW)/ (TW-DW) x 100, where FW-fresh mass, DW-dry mass, TW-turgid mass. The measurements of relative water content were conducted simultaneously to gas exchange parameters.

2.3.2. Gas exchange parameters

Gas exchange parameters (net assimilation rate: A, stomatal conductance: gs, and transpiration rate: E) were measured using the CI-340 handheld photosynthesis system. Measurements were carried out between 10:00 and 12:00 a.m. Data logging started after 45 s of the insertion of leaves into the chamber.

2.3.3. Chlorophyll content estimation

SPAD measurements were determined on flag leaf using a chlorophyll meter (Minolta-502).

2.3.4. Growth parameters

Plant height was measured in cm at 14 days after treatment with a ruler. The green leaf number (GLN) was determined. The fresh weights of the aboveground and root biomass were measured by harvesting four plants that were randomly selected from replicate pots for each water treatment. Finally, plants were harvested, and the dry weights of the above-ground and root biomass were determined.

2.3.5. Proline content

Proline content was determined following the method of Bates et al. (1973), with few modifications. About 0.5 g of leaves were homogenized in a pre-chilled pestle and mortar with 5 ml of 3% sulphosalicylic acid. Then, the homogenate was centrifuged at 3500 g for 15 min at 4°C. The supernatant (0.2 ml) was transferred to a plastic tube containing 3% ninhydrin (0.4 ml), and 0.2 ml of 96% acetic acid and 0.2 ml of 3% sulphosalicylic acid were added. Tubes were incubated for 1 h at 96°C in a water bath and 2 ml of toluene was added to each tube, then stirred, and centrifuged at 3500 g for 15 min at 4°C. The absorbance of the upper phase was measured at 520 nm. The determination of the proline was carried out with a calibration curve.

2.4. Statistical analysis

All the recorded variables were tested by applying a two-way analysis of variance (ANOVA). Then, Duncan's multiple range test (DMRT) was further applied for each of the variables to test the differences among the means of the treatments (Duncan, 1955). Data were analyzed on SPSS 16.0. Value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Effect of water stress on plant water status

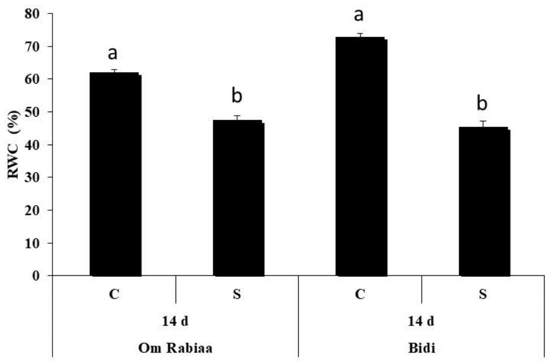

Figure 1. The relative water content (RWC) of two wheat varieties during waterlogging (S) and the well-watered treatment (C). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments.

Data showed that after 14 days of withholding irrigation, the highest values of relative water content (RWC) were recorded in control plants (Figure 1). RWC was about 72. 8% in control plants of var. Bidi. However, in stressed plants values were about 45.33% with a reduction of 37% compared to control. However, the RWC registered in control plants of Om Rabiaa was about 62%. While, stressed plants recorded 43.33%. A slight drop of 23.66% was observed. Statistical analysis using ANOVA test showed that the treatment had a highly significant effect ($P<0.01$) on the variation of the relative water content.

3.2. Effect of water stress on growth parameters

3.2.1. Plant height

After 14 days of withholding irrigation, height decreased by 23.06% for Om Rabiaa reaching 31.16 cm compared to the control 40.5 cm. For Bidi, the reduction was only about 7.05 %. (Figure 2A) Statistical analysis using the ANOVA test showed that the treatment and the genotype had a very highly significant effect ($P<0.001$) on the variation of the height after 14 days of treatment.

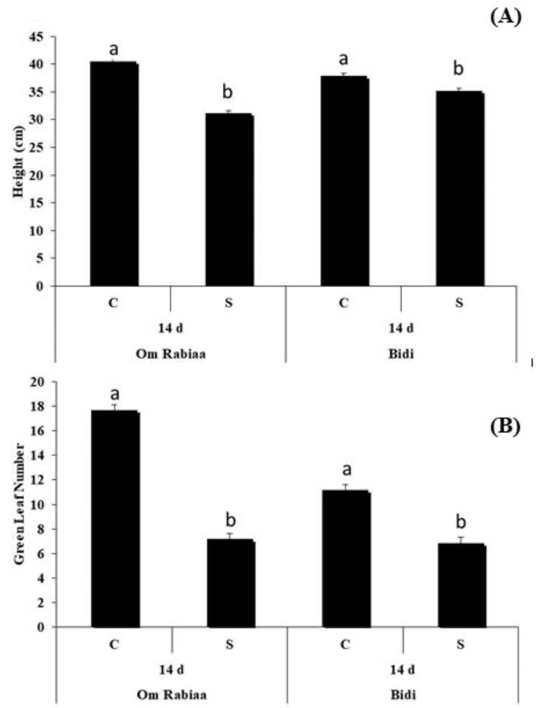

Figure 2. The plant height (A) and green leaf number (B) of two wheat varieties during waterlogging (S) and the well-watered treatment (C). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments.

3.2.2. The green leaf number

Following 14 days of waterlogging, the green leaf number (GLN) recorded 17.66 in control plants of var. Om Rabiaa. The stressed plants recorded a pronounced decrease of 59.45%. For var. Bidi, the GLN was reduced by 38.97% in stressed plants (Figure 2B). Statistical analysis using the ANOVA test showed that the treatment and the genotype had a highly significant effect ($P<0.01$) on the variation of the green leaf number after 14 days of treatment.

3.3. Effect of water stress on gas exchange parameters

3.3.1. The net assimilation rate

After 14 days of treatment, the net assimilation rate in control plants of Om Rabiaa was about 14.95 µmole m^{-2} s^{-1}. While stressed plants recorded 3.28 µmole m^{-2} s^{-1} on the 14th day after treatment with a decrease of 78% compared to control (Figure 3A). For var. Bidi, A dropped to 7.49 µmole m^{-2} s^{-1} on the 14th day of treatment with a reduction of 55.25% compared to control. Statistical analysis showed that the genotype and the treatment had a highly significant effect ($P<0.01$) on the variation of the net assimilation rate.

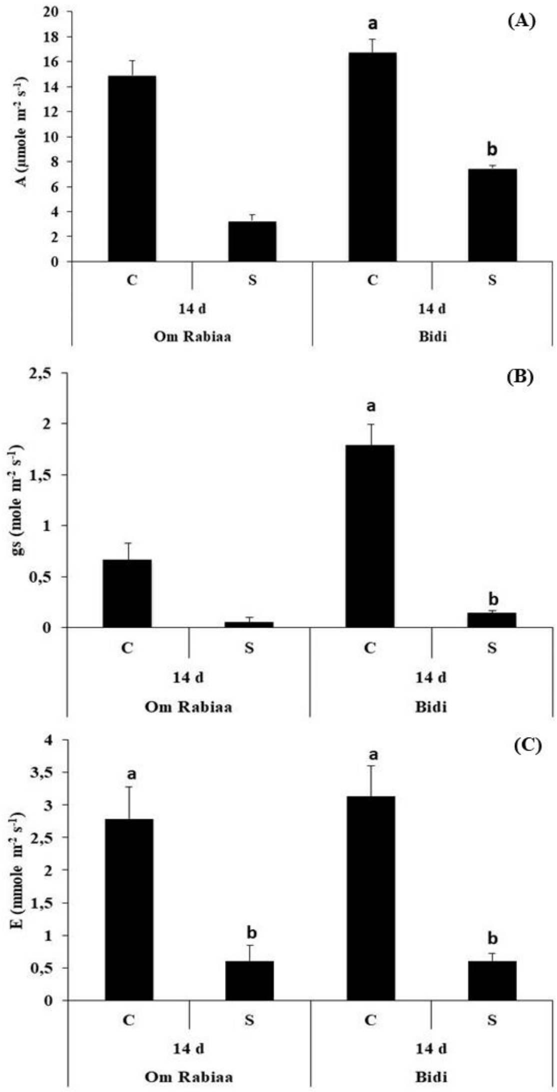

Figure 3. The net assimilation rate (A), stomatal conductance (B), and transpiration rate (C) of two wheat varieties during waterlogging (S) and the well-watered treatment (C). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments.

3.3.2. The stomatal conductance

After 14 days of treatment, stomatal conductance registered 0.31 mmole m^{-2} s^{-1} in control plants of var. Om Rabiaa. Values were reduced to 0.05 mmole m^{-2} s^{-1}(Figure 3B). For var. Bidi, gs was about 1.7 mmole m^{-2} s^{-1} in control plants. In stressed plants, gs dropped to 0.14 mmole m^{-2} s^{-1} after 14 days of treatment. The genotype and treatment had a highly significant effect ($P<0.01$) on stomatal conductance.

3.3.3. The transpiration rate

After 14 days after treatment, the recorded values of transpiration rate were about 2.59 mmole m^{-2} s^{-1} s in control plants of var. Om Rabiaa. While in stressed plants, E was reduced to 0.61 mmole m^{-2} s^{-1} with a reduction of 78.13% (Figure 3C). The var. Bidi, recorded 3.13 mmole m^{-2} s^{-1} in control plants. While in stressed plants E values were reduced to 0.61 with a reduction of 80.51% compared to the respective control. The treatment had a significant effect ($P<0.05$) on the variation of transpiration rate.

3.4. Effect of water stress on PSII photochemical efficiency

After 14 days after treatment (DAT), the PSII photochemical efficiency (Fv/Fm) was about 0.74 in control of var. Om Rabiaa. While stressed plants registered 0.51 with a reduction of 32% compared to control. For var. Bidi, Fv/Fm recorded 0.66 in control (Figure 4). In stressed plants, values of 0.54 were recorded and the reduction was about 17% compared to control. The treatment had a significant effect on var. Om Rabiaa.

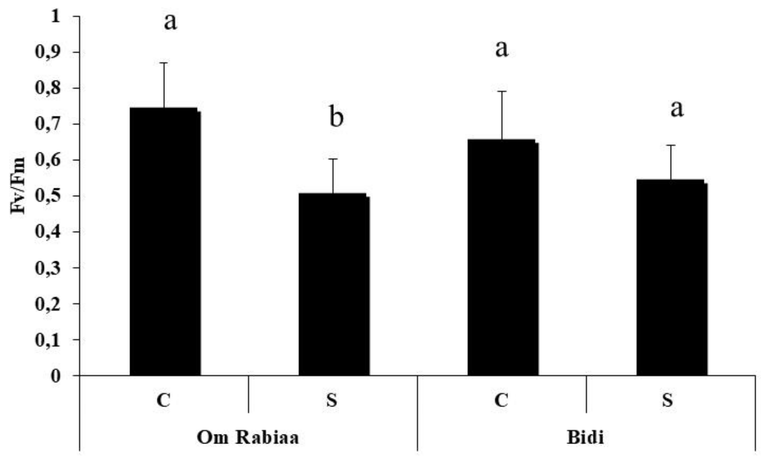

Figure 4. The photochemical efficiency of PSII (Fv/Fm) of two wheat varieties during waterlogging (S) and the well-watered treatment (C). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments.

3.5. Effect of water stress on the biomass accumulation

After 14 DAT, the fresh weight of the aboveground biomass (AGFW) recorded 1.89 g in stressed plants of var. Om Rabiaa with a reduction of 54.45% compared to control. For var. Bidi, the fresh weight was 3.11g in control plants and 1.94 g in stressed plants with a reduction of 37.62% (Figure 5A). Concerning the dry weight (AGDW), the highest values were about 0.68g and 0.57g respectively for var. Bidi and var. Om Rabiaa with the respective reductions of 30.61% and 38.7% compared to control.

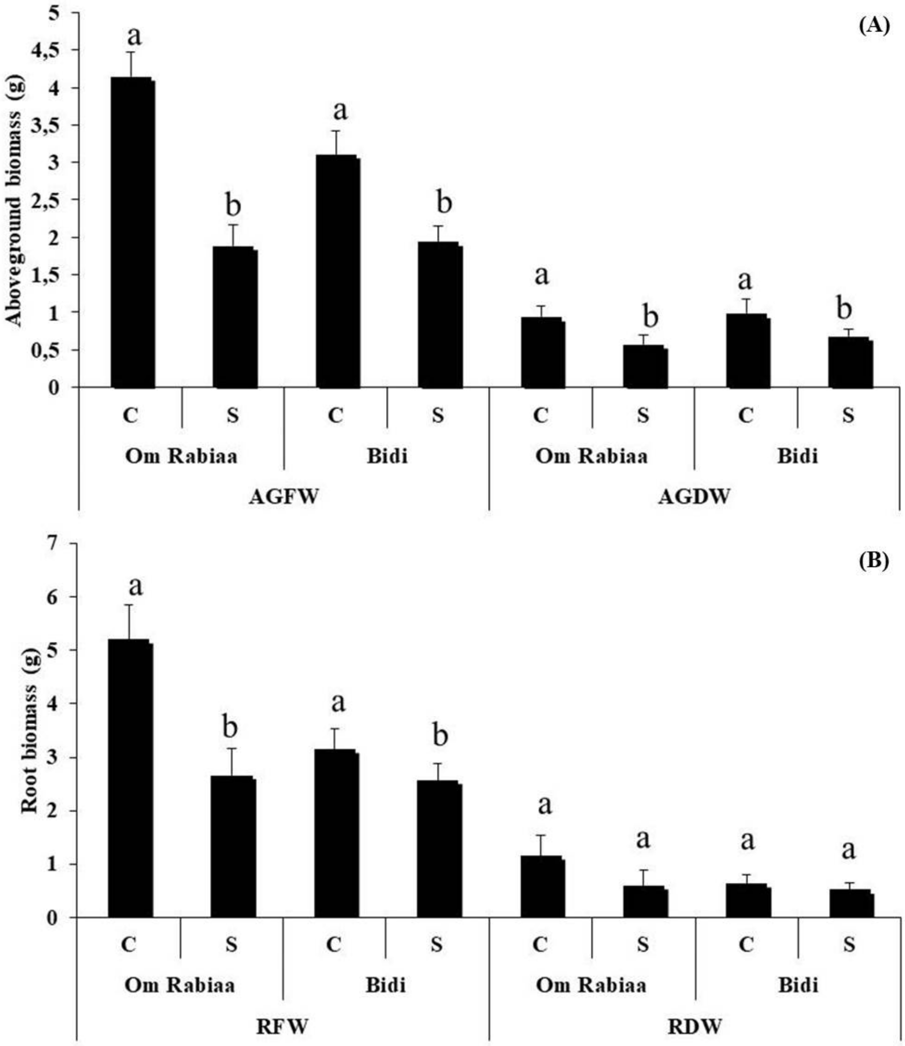

Figure 5. The aboveground biomass (A) and root biomass (B) of two wheat varieties during waterlogging (S) and the well-watered treatment (C). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments.

In stressed plants, root fresh weight (RFW) was about 2.56 g with a reduction of 50.76% in var. Om Rabiaa. While in var. Bidi the RFW was about 3.14g in control plants compared to 2.56 g in stressed plants with a reduction of 18.47% (Figure 5B). For var. Om Rabiaa, root dry weight was about 1.15g in control compared to 0.59g in stressed plants with a reduction of 48.69%. However, the reduction RDW was about 19.04% in var. Bidi with control plants recorded 0.63g and 0.51g in stressed plants. Statistical analysis showed that the treatment and the genotype had a highly significant effect ($P<0.01$) on the variation of the RFW. No significant effect was observed for RDW.

3.6. Effect of water stress on the SPAD values

After 14 DAT, SPAD values were slightly reduced for var. Om Rabiaa (30.31 in control and 30.16 in stressed plants) with a reduction of 0.49% (Figure 6). For var. Bidi, a reduction of 0.5% was observed compared to control after 14 days after treatment. The genotype had a highly significant effect ($P<0.01$) on the variation of the SPAD values.

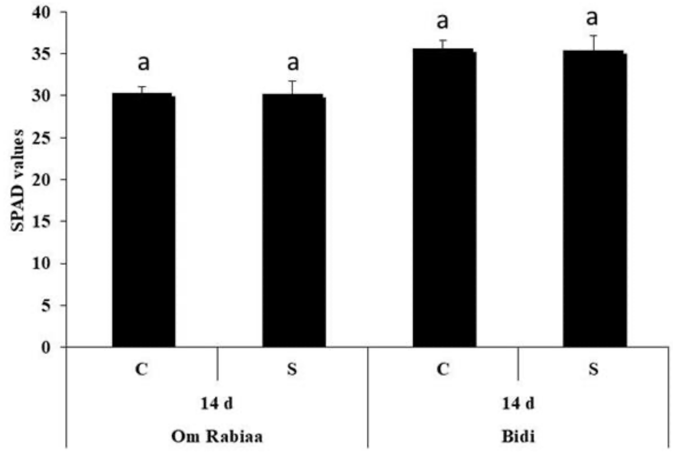

Figure 6. The SPAD values of two wheat varieties during waterlogging (S) and the well-watered treatment (C). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments.

3.7. Effect of water stress on the proline content

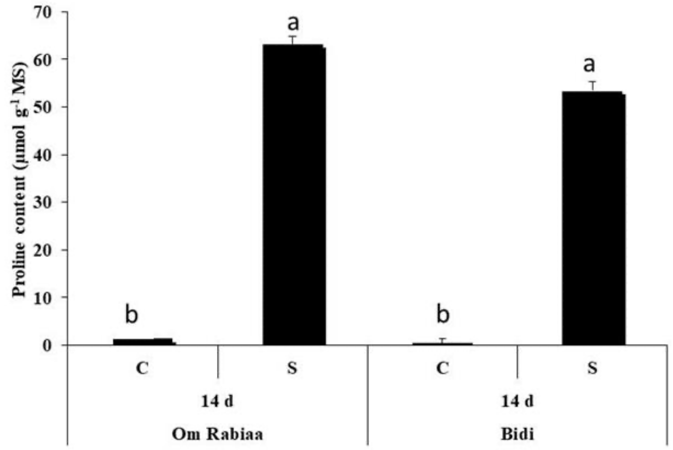

Figure 7. The proline accumulation of two wheat varieties during waterlogging (S) and the well-watered treatment (C). Bars indicate the standard deviation of three replicates, and different letters represent the significant difference between the treatments.

The highest proline content was recorded in stressed plants. The values in control plants for var. Om Rabiaa were about 1.38 µmol g^{-1}MS. (Figure 7). While in stressed plants values were about 63.26 µmol g^{-1}MS with an increase of 97.94% compared to control. On the other hand, for var. Bidi values were about 0.4 µmol g^{-1}MS in control plants. However, in stressed plants, values were about 53.48µmol g^{-1}MS with an increase of 99.26% compared to control. The treatment had a significant effect on proline content.

4. Discussion

The decrease in RWC was more pronounced in var. Bidi. Our results are similar to the findings of Houasli et al., 2014 showing that the relative water content was reduced under drought conditions. Thus, the RWC was rapidly reduced in susceptible genotypes (Thameur et al., (2012). Therefore, this reduction was directly correlated to the decrease in soil water content (Albouchi et al., 2000; Bajji et al., 2001).

Drought affected growth parameters substantially in var. Om Rabiaa. Similar results were obtained by Ferryra et al., (2004) who showed that morphological parameters were clearly affected. In barley, the reduction of green leaf number and leaf appearance rate (Thameur et al., 2011; Thameur et al., 2012) were recorded.

The decrease in leaf area might be due to the decrease in leaf expansion and/or accelerated leaf senescence (El Azeb et al., 2012; Hacini, 2014).

Ali Dib et al., (1992) and Elfakhri et al., (2008) showed that plant height is more affected in susceptible genotypes. In fact, this confirms an adaptation mechanism to reduce water loss by transpiration and maintain the water status of the whole plant (Boudjabi et al., 2017). SPAD values are used as an indicator to evaluate the integrity of the photosynthetic apparatus under drought conditions (Thapa et al., 2018). According to Hikosaka et al., (2006), the chlorophyll content can be influenced by the leaf age, leaf position, and environmental factors such as light, temperature, and water availability. Bousba et al., (2009) indicated that the stomata closure in stressed plants is a strategy to avoid water loss. Yet, it can cause the reduction of chlorophyll content. Siakhène, (1984) showed that the increase in chlorophyll content under drought is due to a reduction in cell size which leads to an increase in its content. This was observed in the genotype Bidi. According to Yuping Li et al., (2017) and Thapa et al., (2018), gas exchange parameters were notably affected by prolonged drought. According to Ali Dib et al., (1992) and Bousba et al., (2009), the drop in (A) was related to the decrease in chlorophyll content particularly in Om Rabiaa, and the increase of stomatal resistance to CO_2 penetration (a drop of gs) so as to provide the protection to plant water status through a decrease in transpiration rate (El Azab et al., 2012);

According to Bousba et al., 2009, the important reduction of the transpiration in both studied genotypes after 14 days of drought is related to stomata closure which is the preliminary strategy used to cope with water deficit. Hence, reducing water loss by transpiration through a prompt stomata closure is an efficient adaptation mechanism to water stress (Djekoun and Planchon, 1992). However, a firm stomata closure can cause disorders at the water status level.

Analysis of photochemical efficiency of PSII (Fv/Fm) showed that the decrease of this parameter was more important in var. Om Rabiaa. According to Jagtap et al., (1998), water deficit leads to a sharp decrease in the ratio (Fv/Fm) in five varieties of *Sorghum bicolor* L recording a more pronounced rate in susceptible genotypes. Accordingly, the analysis of chlorophyll fluorescence and its photochemical and non-photochemical components under drought showed a disturbance in the photochemical reactions of photosynthesis resulting in the blocking of electron transfer between LHC II et PS II (O'Neil et al., 2006).

Ali Dib et al., (1992) showed that a slight decrease in the chlorophyll fluorescence intensity in wheat varieties is due to an inhibition of the chloroplast photochemical activity which might be a result of the inhibition of the Calvin cycle (CO_2 fixation).

Accumulation of the root biomass was clearly affected by drought, particularly for Om Rabiaa which recorded a notable decrease. The variety Bidi was able to produce more root dry weight than Om Rabiaa after 14 days of drought.

According to Labdelli, (2011) and Thapa et al., (2018), the dry weight is one of the efficient indicators of the effect of water stress. In fact, drought reduced the elaboration of the AGDW more than RDW in durum wheat and bread wheat. Nevertheless, Boudjabi et al., (2017) reported that RDW accumulation is more important in stressed plants which is a way to produce new roots to enhance water uptake. The decrease of the above-ground biomass accumulation is more important in the genotype Om Rabiaa than in Bidi. These results are in accordance with those reported by Sassi et al., (2012) who showed that the wheat varieties replied similarly to drought with a reduction of the dry and fresh aboveground biomass with differences among varieties recording the highest accumulations in tolerant varieties.

Many authors showed that the increase of proline content is related directly to water deficit treatment application (Cechin et al., 2006; Mouellef, 2010). This increase is attributed to the degree, the duration of the applied stress, and the behavior of the genotype (Chaib et al., 2015). Other authors found that the increase of proline concentration is also considered an adaptation mechanism in certain varieties (Delauny and Verma, 1993; Hare and Cress, 1998); however, other authors proposed it as a breeding technique for the selection of drought-tolerant barley genotypes (Bellinger et al., 1991). Tahri et al., (1997) showed that the increase in proline content in wheat is negatively correlated with chlorophyll a and b content. This correlation is due to a competition between these two components to the common precursor which is glutamate. The latest becomes insufficient for the synthesis of both components due to the inhibition of the activity of the glutamate synthetase under drought.

5. Conclusion

The results of this study showed that genotypes with contrasting yield performance under drought stress differ significantly in their response to soil moisture variation. Drought tolerant genotype Bidi had a lower reduction rate for gas exchange parameters and photochemical efficiency, after 14 days of withholding irrigation as compared to Om Rabiaa. In addition, this resulted in lower biomass accumulation in Om Rabiaa where proline accumulation showed less increase compared to Bidi upon progressive exposure to water deficit. The tolerant genotype Bidi was able to extract water from dryer soil better than Om Rabiaa. This study suggests that the variety Bidi is more recommended in regions subjected to drastic changes in soil water availability.

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