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# Effect of water deficit stress on physiological traits of some Algerian barley genotypes

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## ABSTRACT

Recently, drought stress became more frequent and this presents a serious challenge for plant breeders to create tolerant barley genotypes with good stability under water deficit conditions. The present study aims to evaluate the effects of water deficit stress with 20% of maximum soil water capacity at heading stage on the physiological traits of some barley genotypes and to set recommendations on their possible use in drought tolerance breeding programs. The results revealed significant differences between genotypes in all tested traits. In this regard, Rahma and Tissa maintained a high relative water content and cell membrane stability under water deficit stress, respectively. These characteristics present effective mechanisms to face drought stress in semi-arid regions. Jaidor was the most stay-green genotype characterized by maintaining a high level of chlorophyll content after water deficit stress treatment. Moreover, Acsad176 accumulated high soluble sugars content as a response to water deficit stress. These genotypes could be considered as potential sources of genes for selection of drought tolerant barley varieties.

**Keywords:** barley, genotypes, heading stage, physiological traits, water stress

## INTRODUCTION

Barley (*Hordeum vulgare* L.) as one of the most important cereal crops in the world is severely limited by drought in many production areas (Kosova et al., 2014). Under drought stress, the growth and development of plants are restricted by a decrease of net photosynthetic rate, leaf osmoregulation ability, and cell membrane stability. As a result, it leads to grain yield reduction (Li et al., 2016).

The barley final yield is dependent on water supply, and it is more adversely affected when drought is imposed at the pollination and flowering stages (Ceccarelli et al., 2007). Therefore, post anthesis drought conditions induce physiological changes and affect barley grain yield (Al Ajlouni et al., 2016).

From a physiological point of view, drought is an imbalance in the plant water regime resulting in an excessive evapotranspiration by shoot compared to the water uptake by root (Reynolds et al., 2005). The plant response to stress is determined by several morpho-physiological traits, which interact and differ in their individual response according to the intensity and duration of water deficit (Witcombe, 2008).

The accumulation of osmolytes represent the first line of defense against drought to reduce cell water loss and to maintain tissue turgor (Kaczmarek et al., 2017). Proline protects cellular structures during dehydration, and it is essential for osmotic adjustments (Zadebagheri et al., 2014). According to Wu et al. (2017), the increasing accumulation of osmoprotectants like proline

is an important adaptive strategy of barley to survive under terminate drought stress, especially for sensitive genotypes.

Furthermore, increased storage of soluble sugars is described as physiological plant response to drought in many studies (Blum, 1996; Passioura, 2012; Dbira et al., 2018) allowing plants to limit evapotranspiration and accentuate the water absorption from the soil (Gluten and Eris, 2004).

The relative water content (RWC) expresses the amount of water in the tissue relative to the total amount of water present in a fully turgid tissue. Therefore, it is a useful measurement of plant water status and the degree of drought tolerance (Cornic and Massacci, 1996), and represents one of the most reliable indicators for defining both the sensitivity and the tolerance of plants to water deficit (Sanchez-Rodriguez et al., 2010).

At the onset of stress, cell membranes are one of the first components affected, and their integrity under stressed conditions represents a major indicator of drought tolerance in plants (Blum and Ebercon, 1981). Measurement of solute leakage from the plant tissue was used to estimate the damage to the cell membrane caused by drought (Premachandra and Shimada, 1987; Tardieu et al., 2012) and subsequently selects tolerant genotypes (Öztürk et al., 2016). Moreover, reduction of chlorophyll a and b contents was reported in different drought-affected plant species, such as wheat (Chakraborty and Pradhan, 2012) which conduct to advanced leaf senescence (Sánchez-Díaz at al., 2002) The objective of this study was to investigate the effect of water deficit stress on physiological characteristics of the tested barley genotypes and to assess their response to drought stress. These results will be important for evaluating the variation for tolerance and to understand mechanisms of drought tolerance to select the genotypes and exploit them in future breeding programs. In this context, physiological approaches can be used in the attempt to identify traits conferring drought tolerance in each genotype.

## MATERIAL AND METHODS

### *Plant materials and growth conditions*

The experiment was carried out on 17 barley genotypes and cultivars as described in Table 1.

**Table 1.** Description of plant materials used in the experiment for drought tolerance assessment

| Genotype name | Description |
|---|---|
| Tichedrett<br>Saida | 6-row type Algerian cultivars and genotypes |
| Rihane03<br>Barberousse/Chorokhod<br>Plaisant/Charan01 | 6-row type varieties and advances breeding lines from the Icarda breeding program |
| Soufara'S<br>Rahma<br>Tissa | 2-row type varieties and advances breeding lines from the Icarda breeding program |
| Acsad176<br>El Fouara | 6-row type Syrian released cultivars |
| El Bahia | selected 6-row cultivar by ITGC of Setif |
| Barberousse<br>Jaidor<br>Express<br>Plaisant | 6-row type French varieties |
| Tina<br>Begonia | 6-row type Spanish cultivars |

Grains were surface sterilized with 2% sodium hypochlorite (Javel) during 4 min and then double rinsed with distillated water. Sterilized seeds were germinated in petri dishes for 72 h at room temperature in the dark, and after three days seedlings were transferred to pots of 14 L filled with a mixture of sand, peat and soil (1:1:1; v/v). Each pot contained 15 plants. They were grown in a greenhouse in the High national school of agronomy of Algeria during the growth season 2017/2018 and maintained at field capacity by weighing pots and supplying tap water daily.

The experiment was conducted in split plot design with two treatments (well-watered and drought stressed) and in four replications. Drought stress was applied by withholding irrigation of the stressed treatment at heading stage (GS 51; Zadoks et al.,1974). Each pot has been weighted daily to maintain soil water content at 20% of the maximum soil water capacity, while the control was kept at 85% of the maximum soil water capacity.

The pots were watered and weighed every day to maintain soil moisture of the stressed treatment and the control at 20% and 85% of the maximum soil water capacity respectively for 15 days.

The soil used for the preparation of the mixture was clay loam type with 0.7% nitrogen content, 32.52 ppm of phosphor content, 9.60 pH and 214 µs of EC. The soil contained 2.21% of total limestone, 3.81% of carbon and 6.59% of organic matter.

Sufficient level of nutrients was maintained by applying 70 kg/ha of phosphor and 87.5 kg/ha of potassium at transplanting of seedlings; 56 kg/ha and 15 kg/ha of nitrogen were applied at tillering and stem elongation stages respectively (GS 22 and GS 32; Zadokset al, 1974). Moreover, leaf spraying of fertilizers was applied to ensure sufficient supply of nutrients as shown in table 2 (GS 37; Zadoks et al., 1974).

**Table 2.** Leaf spraying of fertilizers applied to the tested genotypes during the experiment

| Nutrient | Quantity | Nutrient | Quantity |
|---|---|---|---|
| N | 1 kg/ha | Cu | 0.06 kg/ha |
| $P_2O_5$ | 1.865 kg/ha | Fe | 0.016 kg/ha |
| $K_2O$ | 1.03 kg/ha | Mn | 0.083 kg/ha |
| $MgO_2$ | 0.1 kg/ha | Mo | 0.0003 kg/ha |
| $SO_3$ | 0.625 kg/ha | Zn | 0.057 kg/ha |
| B | 0.002 kg/ha | | |

Temperature in the greenhouse during the growing season is presented in Table 3. Relative humidity varied from 54 to 80%. The greenhouse was not additionally lightened nor was it shadowed. Samples from control and stressed plants were collected and directly subjected to physiological analyses.

***Relative water content (RWC)***

Leaf samples were collected and immediately weighted to determine fresh weight (FW), and then hydrated to full turgidity by floating on distilled water for 24 h in the dark to determine turgid weigh (TW).

**Table 3.** Temperature of the greenhouse during the growing season

| Climatic characters/ Months 2017/2018 | Mean °T (°C) | Min °T (°C) | Max °T (°C) |
|---|---|---|---|
| December | 13.1 | 8.2 | 18.9 |
| January | 13.6 | 7.7 | 20.4 |
| February | 12.3 | 7.3 | 18 |
| March | 16.1 | 11.4 | 21.3 |
| April | 18.1 | 12.6 | 23.9 |
| May | 19.6 | 14.1 | 24.7 |
| June | 24 | 17.1 | 30.2 |

The leaves were then oven dried at 80 °C for 72 h and weighed to determine dry weigh (DW). Relative water content (RWC) was calculated using the equation of Barrs and Weatherley (1968) as follows:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

***Cell membrane stability***

The three leaves sampled were washed three times with distilled water according to Blum and Ebercon (1981) to remove the electrolytes that adhere to their surfaces, then cut into 1 cm long segments. The leaf segments were incubated in the dark for 24 hours at room temperature in test tubes containing 10 ml of distilled water. The first measurement was determined by conductivity meter (HI 2314); then test tubes containing the segments were autoclaved at 110 °C for 15 minutes to kill plant tissue and liberate all the electrolytes. After cooling, the second measurement was recorded and cell membrane stability was calculated as follow:

$$CMS\ (\%) = [(1-(T1/T2))/(1-(C1/C2))] \times 100$$

where T1 and T2 refer to first and second conductivity measurement of the treatment respectively, and C1 and C2 are first and second conductivity measurement of control respectively.

***Chlorophyll a+b content***

Total chlorophyll content was determined using spectrophotometer as described by Arnon (1949) from

100 mg of fresh leaf grinded in 10 ml of acetone 80%. The extract was centrifuged at 4000 revolutions per minute for 10 min, then supernatant containing the pigments was taken. The optical densities were read at wavelengths 663 and 645 nm respectively to calculate total chlorophyll content as follows:

Chl a+b = 8.02 (OD 645) + 20.20 (OD 663)

The photosynthetic pigments were expressed as mg/g dry weight (DW).

***Proline content***

Proline content was determined as described by Troll and Lindsley (1955) and modified by Bates et al. (1973) from 100 mg of fresh weigh (FW) ground in 2 ml of 40% methanol. The mixture was heated in a water bath at 85 °C for one hour. After cooling, 1 ml of the extract was taken, to which 1 ml of acetic acid and then 1 ml of the ninhydrin reagent were added. The tubes were then homogenized and placed in a water bath at 95 °C for 30 minutes. After the solution turned red, the tube containing the reaction medium was cooled before adding 5 ml of toluene. After vortexing, the upper phase containing proline was taken and then sodium sulphate ($Na_2SO_4$) was added to remove the water. The optical density was read using a spectrophotometer at a wavelength of 520 nm and the concentration was determined from a standard curve and expressed in µg/100mg dry weight.

***Total soluble sugars***

Content of total soluble sugars was determined following the method devised by Dubois et al. (1956), where 100 mg of fresh leaves was cut into segments and macerated for 48 hours in a test tube filled to 2/3 with ethanol at 80 °C. The extract obtained after evaporation of the ethanol was diluted with 20 ml of distilled water from which 2 ml were taken and placed in a test tube. A volume of 4 ml of anthrone reagent was then added. After stirring, the tube was placed in a bath at 90 °C for 8 minutes. Then the tubes were put in the dark to avoid oxidation of the sugars and cooled in ice for 30 minutes. The optical density was read using a spectrophotometer at a wavelength of 585 nm. The concentration was determined from a standard curve and expressed in mg/100mg dry weight.

***Statistical analysis***

The results were subjected to analysis of variance (ANOVA) using R 3.5.3 statistical software to examine differences between the barley genotypes under control and drought conditions for all studied parameters. Graphs have been performed on the base of the mean of each genotype for both regimes with standard deviation.

## RESULTS

The results of the present study revealed significant differences between genotypes, treatments and the interaction genotype × treatment in all physiological traits tested when they were subjected to water deficit stress at 20% of maximum soil water capacity at heading stage (Table 4).

***Relative water content***

Under control conditions, the relative water content of leaves in all genotypes was kept at relative turgidity, while all genotypes indicated a decrease in RWC under water stress conditions (Figure 1).

The cultivar Rahma showed with 77.57% the highest level of RWC under water stress condition. The lowest level of RWC was recorded by Begonia with 53.86%. However, RWC of Begonia was reduced by 41.89%, followed by Soufara'S with 36.32%, which indicates their sensibility to drought stress. After imposing drought stress for 15 days, RWC of Rahma and El Fouara were reduced only by 19.08% and 21.23%, respectively.

***Cell membrane stability***

As shown in Figure 2, Tissa had 86.96% of cell membrane stability and was the least affected by water stress. On the other hand, Begonia was the genotype which was the most affected by water stress with cell membrane stability of 56.43%.

**Table 4.** Analysis of variance for physiological traits in barley genotypes grown under water deficit stress and normal conditions

| Source of variation | MS | | | | | |
|---|---|---|---|---|---|---|
| | DF | RWC | CMS | Chl a+b | PC | TSS |
| Genotype | 16 | 66.7* | 200.262** | 164.7*** | 115.8*** | 0.24437** |
| Treatment | 1 | 26612.5*** | / | 26010.0*** | 9308.2*** | 1.33010*** |
| Genotype × Treatment | 16 | 61.1* | / | 105.7** | 96.4*** | 0.24759** |
| Error | 48 | 28.2 | 67.801 | 39.7 | 19.1 | 0.07816 |

MS: Mean square, DF: Degree free, RWC: Relative water content, CMS: Cell membrane stability, Chl a+b: Chlorophyll a+b, PC: Proline content, TSS: Total soluble sugars

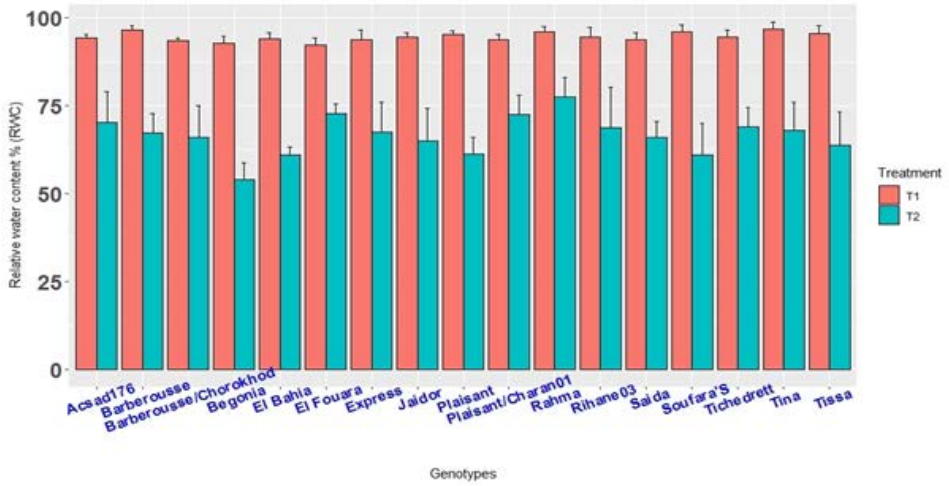


**Figure 1.** Relative water content (%) of barley genotypes under controlled conditions (T1) and after 15 days of water deficit at 20% of maximum soil water capacity (T2)

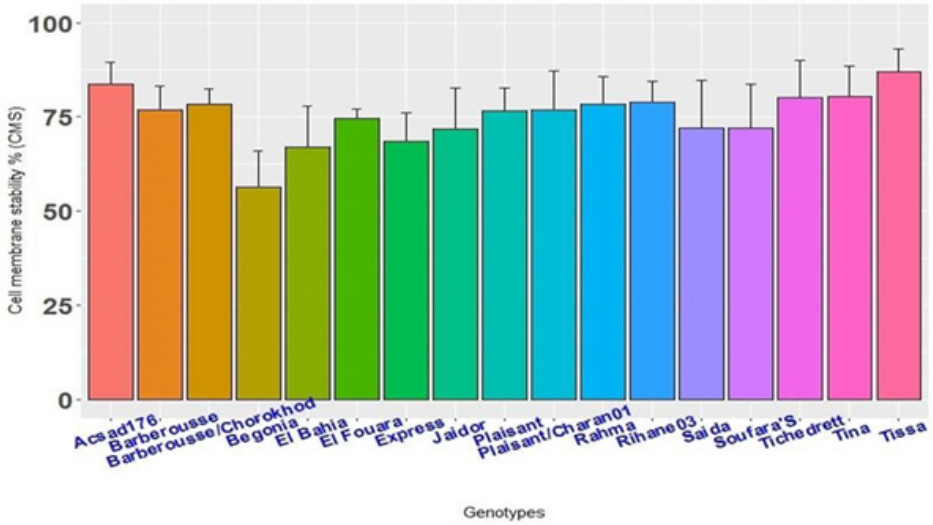


**Figure 2.** Cell membrane stability (%) of barley genotypes after 15 days of water stress at 20% of maximum soil water capacity

***Chlorophyll a+b content***

Under water stress condition, all genotypes and cultivars indicated a decrease in chlorophyll a+b content. Therefore, Jaidor showed higher level of total chlorophyll a+b with 31.52 mg/g DW, while El Bahia and Plaisant showed only 18.29 mg/g DW and 19.01mg/g DW of chlorophyll a+b under water deficit stress, respectively. After 15 days of drought stress, chlorophyll a+b content was reduced by 67.11% in Plaisant and by 62.68% in Barberousse/Chorokhod leaves making these genotypes more affected by water stress among all the tested genotypes. Chlorophyll a+b content was lower by 31.04% under water stress condition in Acsad176 leaves compared to the control group (Figure 3).

***Proline content***

Barberousse/Chorokhod showed significantly higher level of proline content 43.25 µg/100mg DW under water stress conditions with an increase of 318.03% compared to the control. Furthermore, Plaisant recorded the lowest proline content by 18.94 µg/100mg DW with an increase of 31.69% when they were subjected to water stress (Figure 4).

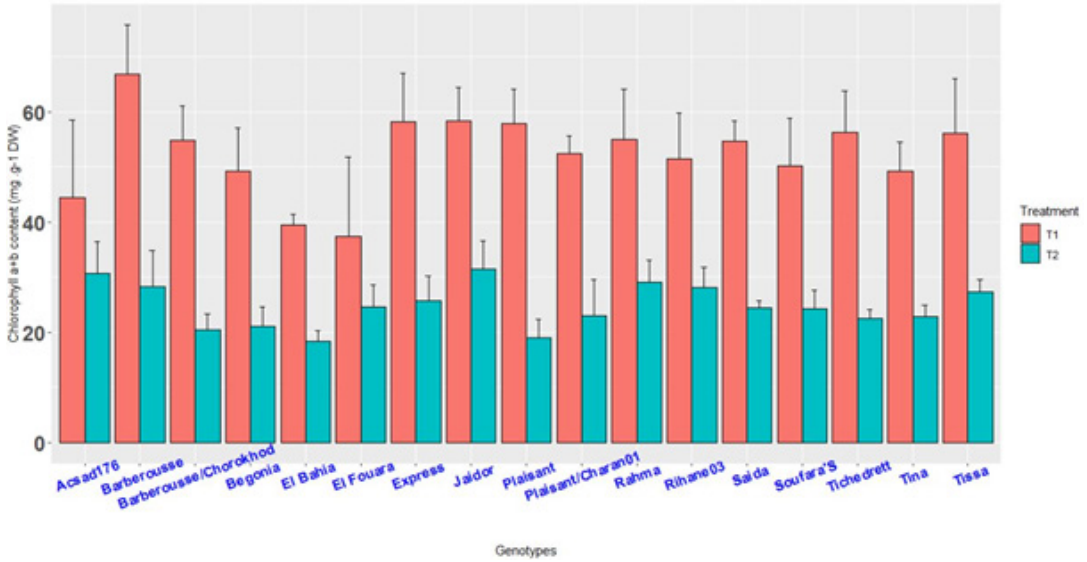


**Figure 3.** Chlorophyll a+b content of barley genotypes under control condition (T1) and after 15 days of water deficit at 20% of maximum soil water capacity (T2)

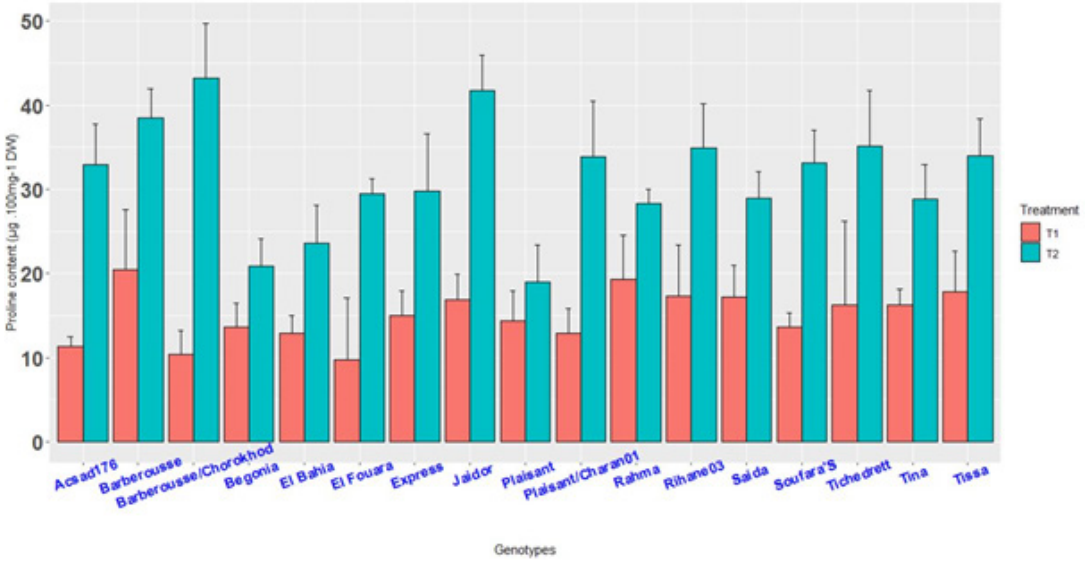


**Figure 4.** Proline content of barley genotypes under control conditions (T1) and after 15 days of water deficit at 20% of maximum soil water capacity (T2)

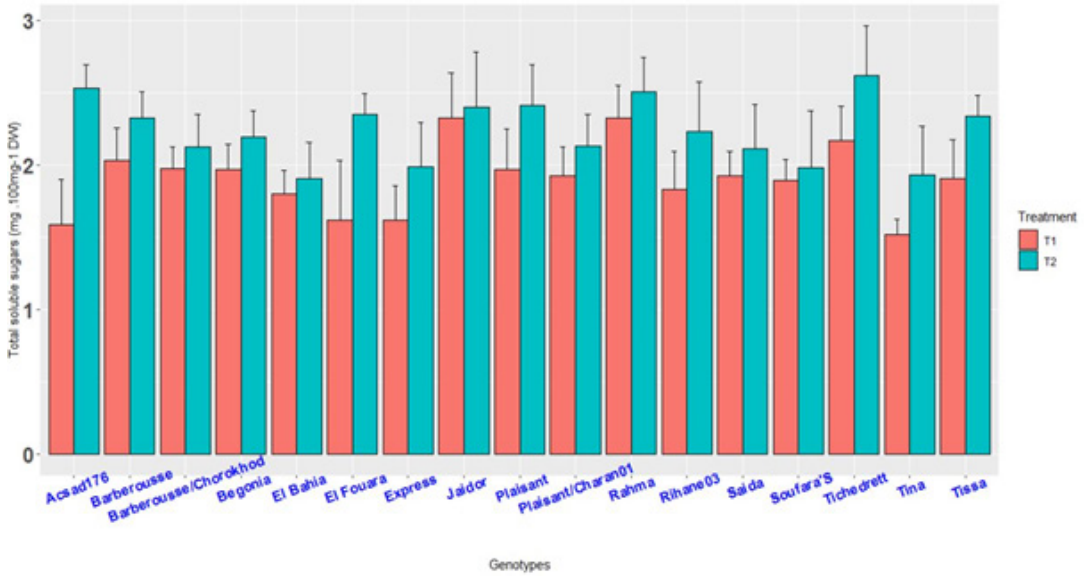


**Figure 5.** Total soluble sugars of barley genotypes under control condition (T1) and after 15 days of water deficit at 20% of maximum soil water capacity (T2)

***Total soluble sugars***

Total soluble sugars accumulation has been reported in this study, whereas Tichedrett recorded 2.62 mg/100mg DW with an increase of 20.82% compared to the control group. This value was the highest among genotypes under water stress condition (Figure 5).

The lowest content of total soluble sugars content is attributed to El Bahia (1.91 mg 100 m/g DW) with an increase of 5.87% compared to the control. Furthermore, Jaidor showed an increase of total soluble sugars content only by 3.38% compared to the control. Acsad176 recorded a high-level increase of total soluble sugars content with 59.31% compared to the control.

## DISCUSSION

The development of new genotypes adapted to harsh environments where stresses are affecting crop production is an important goal in plant breeding, especially in areas where drought stress became more frequent in the recent years.

The integrity and stability of plasma membrane constitute a physiological mechanism of tolerance to drought, which allow plants to continue its physiological process and maintain grain yield in such conditions. Genotypes which showed high levels of cell membrane stability can be described as survivors from drought injury. This can cause severe damage in plant cells and break its photosynthetic manufacturing. Previous research has found that cell membrane stability (Rehman et al., 2016) and chlorophyll contents (Ghotbi-Ravandi et al., 2014) are valuable indicators of plant response towards abiotic stresses.

Many researchers have used cell membrane stability as a criterion to study and measure drought tolerance in genotypes (Premachandra et al., 1990; Bajji et al., 2001; El Basyoni et al., 2017). In our experiment, the cell membrane stability of Begonia was reduced to 56.43% after 15 days of water deficit, which makes it the most susceptible genotype to drought injury. As a winter genotype, Begonia may tolerate colder than drought conditions due to its genetic structure. Tissa, a two-row genotype, was the least affected by water stress. That allows it to maintain many physiological processes and can therefore be selected in future breeding programs.

In the present study, RWC and Chlorophyll a+b content was reduced by 29.61% and by 34.42% respectively after 15 days of water stress at the heading stage. At this stage, drought stress mostly causes significant reduction of grain yield in semi-arid areas. Rahma with 77.57% showed a higher level of RWC under water stress condition and reduction by 19.08% from the control group. This genotype is considered as a genotype with the lowest

water loss and therefore it can be highly recommended for semi-arid areas. On the other hand, water stress reduced RWC of Begonia by 41.89% compared to control. The lowest mean of RWC (53.86%) was recorded in this genotype. This is in line with the results on cell membrane stability. Aboughadareh et al. (2013) affirmed that water deficit conditions cause water loss in the plant and result in relative reduction of water content.

Keeping high level of chlorophyll a+b content can be a useful indicator for tolerance to drought stress. In this regard, Jaidor had low susceptibility to loss of chlorophyll pigment with 31.52 mg/g DW. Reduction of the content of chlorophyll a+b under water stress was the highest in leaves of Plaisant (67.11%) and Barberousse/Chorokhod (62.68%). Acsad176 stayed more green than other genotypes. This enables to pursue photosynthesis and keep a stable yield in water stress conditions. Rong-Hua et al. (2006) concluded that chlorophyll content could be considered as a reliable indicator in screening barley genotypes for drought tolerance. According to De-Mezer et al. (2014), variation in physiological traits, including relative water and chlorophyll content, are closely associated with barley's response to drought stress.

Barberousse/Chorokhodand Jaidor accumulated high content of proline under water stress to protect their subcellular structures including membranes. These results affirm those showed in the section of cell membrane stability. In such conditions, proline can serve as a nitrogen or carbon source (Verbruggen et al.,1996). Plaisant showed the lowest proline content (18.94 µg/100 mg) when it was subjected to water stress. This genotype is a winter barley. It expressed lower susceptibility to drought stress than Begonia, which was also characterized by a low proline content (20.87 µg/100 mg) at heading stage.

Many studies reported a strong accumulation of total soluble sugar content under water stress conditions (Perez-Lopez et al., 2010; Ali Fayez and Ali Bazaid, 2014). Genotypes are forced to accumulate soluble sugars as osmoprotectant to avoid cell damages. In this regard, Tichedrett accumulated high total soluble sugars content. Low content of total soluble sugarswas attributed to El Bahia genotype which also expressed high reduction of chlorophyll a+b content during exposition to water stress at heading stage. Therefore, it is suggested that stay-green traits helpful for plants which are grown under water deficit conditions by means of accumulation of more soluble sugars. This in turn protects its physiological processes and maintains yield stability.

Genotypes showed different behavior across treatments. Under well-watered conditions genotypes presented high level of leaves water content, good cell membrane stability and high chlorophyll a+b content. They also synthetized low proline and accumulate low levels of soluble sugars.

On the other hand, under water deficit stress, plants lose water. However, some genotypes may retain more water in their leaves compared to others; some can retain their cell membrane stability more efficiently by synthetizing proline or soluble sugars that allow them to avoid cell damages caused by drought stress. In many cases, stay-green trait is an alternative for plants to pursue their photosynthesis process. All these mechanisms are important for breeding strategies which should be directed towards a stable genetic structure of new genotypes. In this context, the present study constituted the first step to achieve cited goals and to improve local land races and production.

## CONCLUSION

Results of the present study revealed significant differences between genotypes in all the tested traits when they were subjected to drought stress. This indicates that the magnitude of differences in genotypes was sufficient to select them to be better adapted to drought conditions. Some genotypes were less affected by water loss, drought injury and reduction of chlorophyll content. Rahma and Tissa, two row genotypes of barley, can be selected as tolerant to water stress. Moreover, Jaidor kept high content of chlorophyll after water stress. This trait at heading stage facilitates photosynthesis for grain filling and has a suitable consequence on grain yield. Accumulation of proline and soluble sugars is associated

with drought response in barley. Therefore, Tichedrett showed high accumulation of total soluble sugar under water stress, which may play an important role in drought response mostly in semi-arid conditions.

It is clear that for breeders and physiologists, there is no prefect genotype that contains all mechanisms to tolerate drought stress. Therefore, choosing genotypes that contain suitable traits in breeding programs is of utmost importance.

## ACKNOWLEDGMENTS

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