

Response of two barley accessions “Ardhaoui” to deficit irrigation using saline water in southern Tunisia.

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Abstract - To examine drought tolerance of two barley accessions “Ardhaoui” under deficit irrigation using saline water in southern Tunisia, Three treatments were used: with control T₀ (100%ETc) and stressed T₁ (75%ETc), T₂ (50%ETc). Proline and soluble sugar contents increased significantly under water stress between accessions compared to control and varies at growth stages. The increasing of Ca²⁺ concentration enhanced the absorption of Na⁺ ion, consequently K⁺/Na⁺ decreased significantly between accessions, which suggested that Karkeni was tolerant to water stress and more productive than Bengardeni under this strategy of irrigation in arid region of Tunisia.

Keywords: water stress, proline, soluble sugar, minerals, yield and barley.

1. Introduction

In most African countries, food production was threatened by water scarcity for agricultural crops. Drought is the most serious abiotic stress at global scale (Pennisi, 2008; Salvatore, 2010) and its frequency is expected to increase as a consequence of climate changes (CECCARELLI et al., 2010). Drought and salinity are the two major abiotic efforts limiting crop growth and productivity (Wang et al., 2003). In nature, crops are subjected to the combination of different types of stress, such as water stress and salinity. Also, soil salinity in large areas has been observed, especially in arid areas (Rabhi et al., 2007; Yousfi et al., 2007). Moreover, drought and salinity also weaken the metabolic mechanisms involved in the assimilation of CO₂ and the combined effects of stomatal closure and metabolic deficiency ultimately reduce the biomass accumulation (Flexas et al., 2004). Turgor phenomena in the plant cell are generally maintained during drought conditions by osmotic adjustment and this requires the synthesis and transport of compatible solutes and osmolytes, such as soluble sugars, polyols, proline and glycine betaine (Foyer et al., 1998; Morison et al., 2008).

Barley is one of the most important cereal crops in Asia, Europe, Middle East and in North and South Africa, even where eventually water scarcity and drought affect crops yielding (CECCARELLI et al., 2007). Ceccarelli et al., (2004) and Shakhathreh et al., (2001), as indicated that barley productivity depends not essentially on water supplies, and it is threatened when the water stress is imposed at the pollination and flowering stages, rather than in the vegetative or seed filling stages (CECCARELLI et al., 2007). In Tunisia, barley is usually grown in semi-arid and arid regions, this plant is widely cultivated in the region of production of cereals and pasture (Oueslati et al., 2005).

Barely is among the most tolerant cereal crops to salinity. It has the ability (i) to produce osmotic adjustments by toxic salt ions in vacuoles, which keeps a favorable K⁺/Na⁺ ratio in the cytoplasm at high leaf Na⁺ concentrations, (ii) to cause the transport of Ca²⁺ ions to the shoot; (iii) to distribute ions between mesophyll and epidermal cells; and (iiii) to trigger antioxidant metabolism (Fricke et al., 1996; Munns et al., 2006; Pérez-López et al., 2010b; Pérez-López et al., 2009). However, at higher concentration than 8 dS.m⁻¹, salt stress reduces barley growth causing changes to alterations in water and nutrient uptake rates and consequently decreases photosynthesis (Pérez-López et al., 2013a; Pérez-López et al., 2012; Pérez-López et al., 2010a; Pérez-López et al., 2013b). Nevertheless, despite much effort has been devoted to correlating these changes with the degree of salt adapter in plants, no agreement has been reached among researches (Chen et al., 2007; Genc et al., 2007). To maintain their growth, plants have developed several strategies when water availability is limited or unpredictable; an



adaptation to the lack of water by improving their absorption, accumulation of compatible solutes and mineral nutrients during to maintain turgor, metabolic activity and absorption of water in case of fall soil water potential (Hummel et al., 2010). Moreover, synthesis of osmo-regulators such as soluble sugars and proline in the event of severe stresses (Ashraf & Foolad, 2007; Delauney & Verma, 1993). To select adapted plant to Mediterranean-type climates, it is mandatory to study the phenological, morphological, physiological and agronomic parameters (González & Ayerbe, 2010; González et al., 1999) in order to improve plant adaptation to drought. Barley is one of suitable and profitable crops in these conditions (González et al., 2007).

The objectives of this work is (i) to evaluate the biochemical and agronomic performances of two barley accessions subjected to deficit irrigation at different growth stages in dryland, (ii) to assess the responses of this material to water stress as tool of selection and (iii) to determine the irrigation strategy to improve water productivity of barley in southern Tunisia.

2. Materials and Methods

2.1. Experimental conditions

The experiment was conducted at the experimental site of the Institute of Arid Lands of Medenine (IRA: 33°29'3''N, 10°38'46''E, Altitude 184m in the South-East of Tunisia). The climate is Mediterranean, with hot, dry summers and mild winters an average annual rainfall of 125 mm. Minimum temperatures recorded during the months of November to May, are included between 3.5 and 15.7 °C, while maximum temperatures are between 16 and 39.8 °C in the same period.

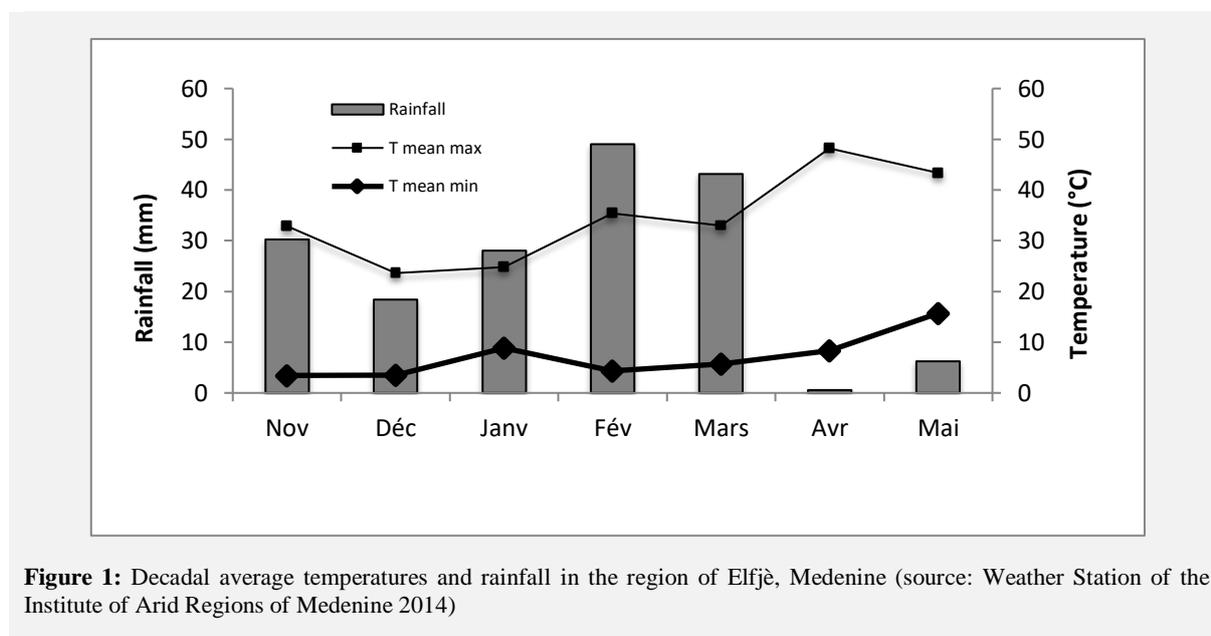


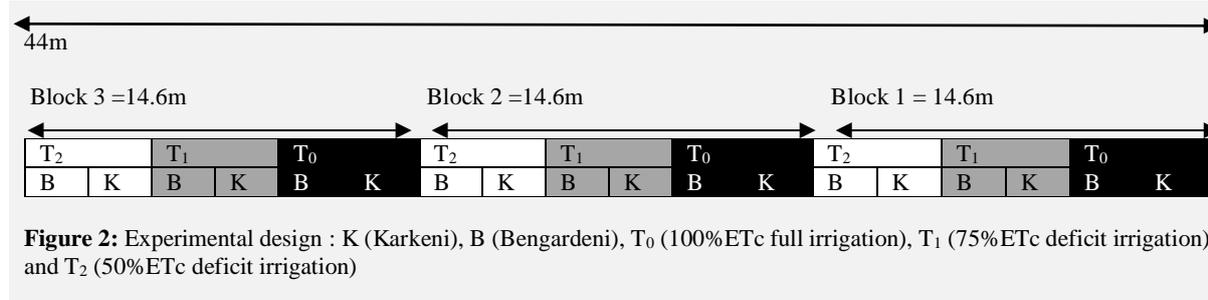
Figure 1: Decadal average temperatures and rainfall in the region of Elfjè, Medenine (source: Weather Station of the Institute of Arid Regions of Medenine 2014)

2.2. Plant material

Two barley accessions cv. Ardhaoui (six rows) were used in this study. Seeds were collected from two different regions in southern Tunisia (“karkeni” from Karkenah and “Bengardeni” from Bengardene) to compare their behavior when grown under conditions of water stress.

2.3. Experimental design

The experimental plan consisted of 3 blocks with an area of 352 m². Each block (117.3 m²) was divided into three equal plots of 39.1 m² (T₀ (100%ETc), T₁ (75%ETc) and T₂ (50%ETc)) irrigation treatments, each treatment was divided into two sub-plot contain the two accessions Karkeni and Bengardeni, each sub-plot contains 6 rows spaced 40 cm (in total there will be 108 lines) (Figure 2), the distance between the emitters is of 16 cm. Before sowing, 175kg.ha⁻¹ N, 100kg.ha⁻¹ P and 125kg.ha⁻¹ K were equally distributed for all rows. The seeds were sown on depth of 2 to 3 cm, the planting density is 230 plants per m². All blocks were drip irrigated with water from a well having an EC_i of 10.8 dS.m⁻¹. Each dripper had a 2 l/h flow rate. A control mini-valve in the lateral permits use or non-use of the dripper line.



3. Measurement

3.1. Proline and soluble sugars determination

The technique used is that of Troll and Lindsley (1955). The quantification of the reaction by ninhydrin proline spectrophotometric measurement. Reacting is characterized by the appearance of the red color due to the proline-ninhydrin complex. Of each accession and each treatment, 100 mg of fresh material are removed from the leaves of the flourished 3rd row from the top. These samples were cut into small pieces and placed in test tubes containing screwed 2 ml Methanol (40%) and heated to boiling in a water bath at 85°C for 60 min. After cooling samples, 1 ml of the extract of each was removed and placed into new tubes to which was added 1 ml of acetic acid and 1 ml of a solution containing 120 ml H₂O, 300 ml acetic acid, 80 ml ortho-phosphoric acid "H₃ PO₄, density 1.7" and 25 mg of ninhydrin. The mixture is heated to boiling for 30 min. The solution turns to red. After cooling, was added 5 ml of toluene in each tube with stirring for two phases, then removed the upper phase in which a pinch of Na₂SO₄ was added to removing water and the optical density was measured by a spectrophotometer thereafter (Bio-Rad Smart Spec™ 3000) at the wavelength of 528 nm.

The content of soluble sugars, 100 mg of fresh material is taken to which is added 5 ml of 80% methanol and the samples were heated in a water bath at 70°C for 30 minutes. The soluble sugar content was determined by the phenol-sulfuric acid method (Roby and White, 1987). 1 ml is removed from the extract which was added 1 ml of phenol 5% and 5 ml of concentrated sulfuric acid. After stirring and cooling, determine the absorbance in a spectrophotometer at 640 nm. The calibration is performed by glucose solutions with concentrations of 0.05 to 0.3 mg.ml⁻¹.

Concentration of soluble sugars (μmol/mg MF) = α * Abs * 1 / Re

With α = slope of the calibration curve, Re = m / v = 0.1 / 5, Abs = read.

3.2. Yield and yield components

The measured yield components agronomic were: Plant height (PH), dry matter (DM), spike length (SL), tillers number per m² (TN), spikes number per m² (SN), grain number per spike (GN), 1000 grains weight (TWG) and grain yield (GY).

Table1: Unit, time of measurement, replication and protocol of yield and yield components.

Parameters	Unit	Time of measurement	Replication number	Protocol
PH	cm	In every growth stage	7	A simple measure
DM	g	At maturity	7	A simple measure
SL	cm	At maturity	7	A simple measure
TN	number	At maturity	7	simple manual counting
SN	number	At maturity	7	simple manual counting
GN	number	At maturity	7	simple manual counting
TWG	g	At maturity	7	With automatic counter grains CONTADOR
GY	q/ha	At maturity	7	GY=TWG*SN*GN

3.3. Minerals analysis

Samples were totally dried at 100±5°C to constant weight. Then, 1g of each dry sample was incinerated during 4 h at 550°C. Ashes were mixed with 4 ml of ultra-pure water and 1 ml of concentrated Hcl. The solution was heated until boiling, then filtered and adjusted to 100 ml with ultra-pure water. This solution

will be used for mineral analysis. All minerals contents were determined with an Atomic Absorption Spectrometry (Thermo SCIENTIFIC iCE 3000 AA Spectrometer). The contents of sodium (Na⁺), potassium (K⁺) and calcium (Ca²⁺) in the dry matter were calculated as:

$$C \text{ (mg/100g)} = c \text{ (mg/l)} * 10^{-3} * 50 * 100 * DF$$

Where C is the concentration of mineral (mg/100 g DM), “c” is the concentration of mineral (mg/l), DF is the factor of dilution.

3.4. Statistical analysis

All data presented were mean values of each treatments and were taken on three (sugars, proline and minerals) and seven (yield and yield components) replicates. Analyses of variance (ANOVA: General Linear Model) was carried out using the statistical package SPSS v 20. Relationships between yield and yield components were determined using Pearson’s simple correlation test (Pearson).

4. Results

4.1. Soil salinity and humidity

The final average EC_i values (0-60 cm soil depth) under different treatments was presented in Figure 3. Initial soil salinity determined at the time of planting was 11.23 dS.m⁻¹. However, soil salinity decrease in deficit irrigation treatments (T1 and T2) than full irrigation (T0). EC_i values were in a decreasing order 50%ET_c > 75%ET_c > 100%ET_c, also, soil salinity varies between growth stages due to the sourcing of rainfall (24, 48 and 49 mm) (Figure 1) which causes increased soil moisture and consequently decreased salinity.

The values of EC_i varies between growth stages, soil salinity has the highest values at tillering and booting stages, this is because of excessive irrigation. At maturation stage, soil salinity decreases with decreasing irrigation.

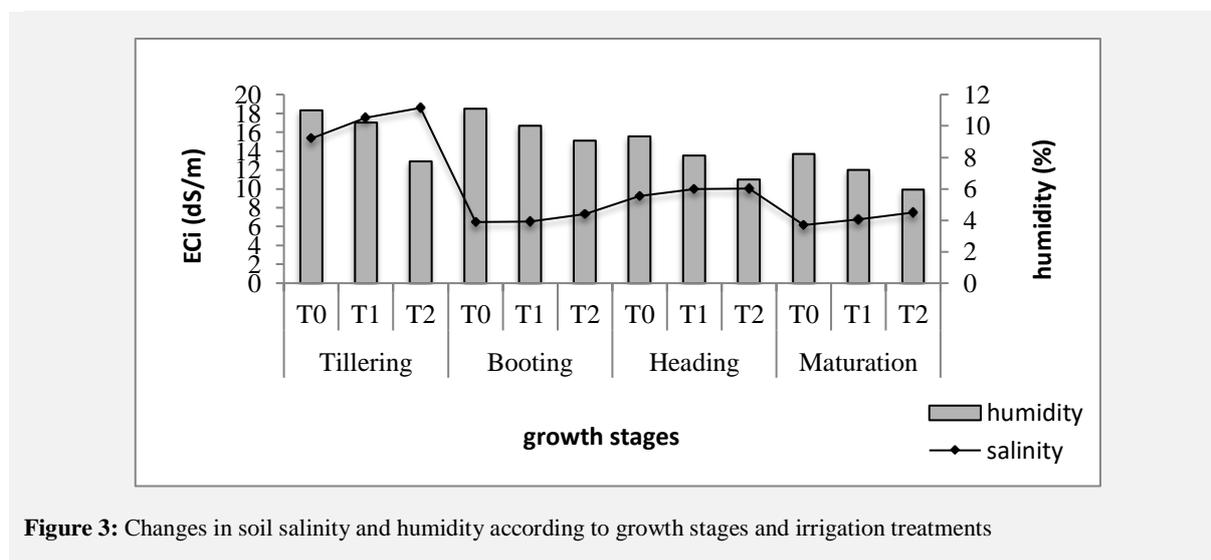


Figure 3: Changes in soil salinity and humidity according to growth stages and irrigation treatments

4.2. Determination of proline content

Analysis of variance showed significant difference between the irrigation treatments at p<0.01. Water stress increase significantly proline content for both T₁ (75%ET_c), T₂ (50%ET_c) treatments compared to control T₀ (100%ET_c) for the two accessions (Table 2). Furthermore, Tillering stage present the high proline levels which increase from 0.085 to 0.1 mg.g⁻¹ for “Karkeni” and from 0.063 to 0.091 mg.g⁻¹ for “Bengardeni” respectively for T₀ and T₂ treatments (Figure 4).

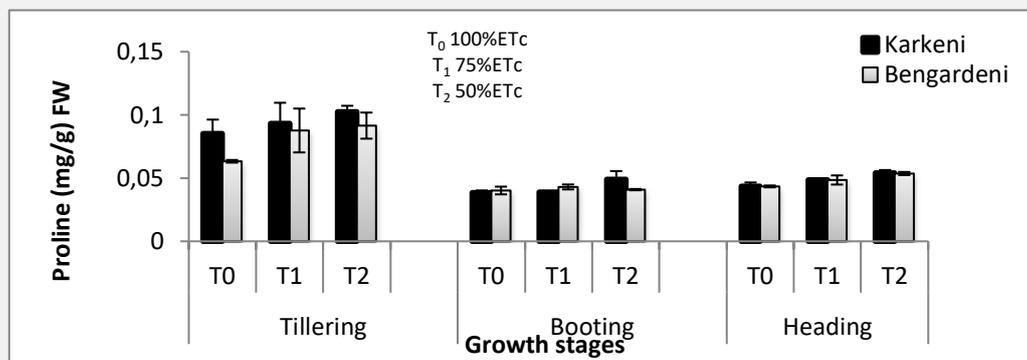


Figure 4: variation of proline content in the tow barley accessions with the irrigation treatments during the growth stages

For all treatments and during the growth stages the difference between accessions showed a highly significant at ($p < 0.001$). The interaction between treatments, accessions and growth stages (T^*A , T^*GS , A^*GS and T^*A^*GS) showed also a highly significant differences at ($p < 0.001$) (Table2).

4.3. Determination of soluble sugar content

Soluble sugar content increase significantly between the irrigation treatments for the tow barley accessions ($p < 0.01$). In fact, in the Tillering stage, the soluble sugar content increase by 16% and by 19% respectively for “Karkeni” and “Bengardeni” for the treatments T_1 (75%ETc) and T_2 (50%ETc) (Figure 5).

The difference between accessions is not significant (Table 2). Moreover, at Booting and Heading stages the difference between accessions is significantly at ($p < 0.01$). However, at the Booting stage, soluble sugar content reduce significantly ($p < 0.01$) for the Karkeni compared to Bengardeni. Our results showed a highly significant ($p < 0.001$) between growth stages. Their interaction T^*A no significant, GS^*A , GS^*T and T^*A^*GS showed a significant difference at ($p < 0.1$, $p < 0.001$ and $p < 0.01$) respectively.

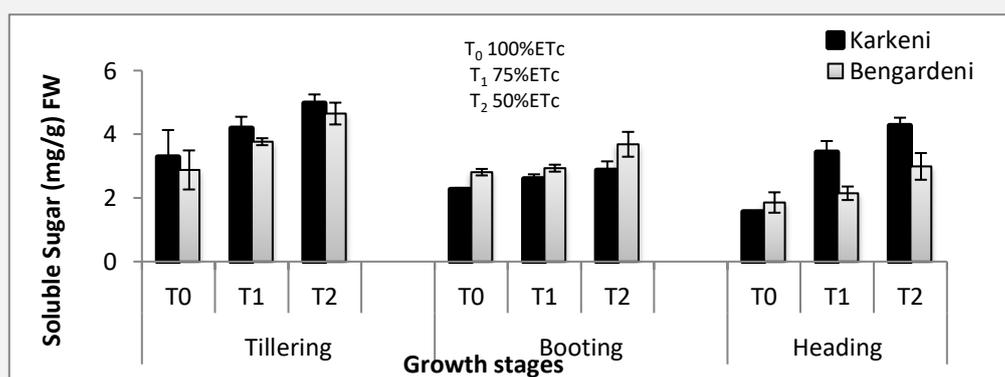


Figure 5: variation of soluble sugar content in the tow barley accessions with the irrigation treatments during the growth stages

Table 2: Effect of accessions, treatments and growth stages on soluble sugar and proline and their interaction (T^*A , GS^*A , T^*GS and T^*GS^*A)

ANOVA	ddl	Soluble sugar	Proline
Treatment (T)	2	*	**
Accession (A)	1	ns	***
Growth Stages (GS)	2	***	***
T^*A	2	ns	***
GS^*A	2	*	***
GS^*T	2	***	***
T^*GS^*A	4	*	***

*, **, *** significant at $p < 0.05$; $p < 0.01$, $p < 0.001$ respectively and ns: non significant

4.4. Minerals composition

Water stress (T_1 and T_2) and growth stages caused a highly significant ($p < 0.001$) increases in Na^+ and Ca^{2+} contents (from 2182.65 to 2529.3 mg/100g DW) for Karkeni and (from 8.77 to 11.57 mg/100g DW) for Bengardeni respectively compared and affected significantly K^+/Na^+ selectivity ($p < 0.001$).

Table 3: Effect of the treatments, accessions and growth stages on mineral composition and their interactions (T*A, A*GS, T*GS and T*GS*A)

ANOVA	Ca^{2+}	K^+	Na^+	K^+/Na^+
Treatment (T)	***	***	***	***
Accession (A)	**	ns	**	**
Growth Stages (GS)	***	***	***	***
T*A	ns	ns	ns	ns
A*GS	***	**	***	*
T*GS	***	ns	ns	ns
T*GS*A	ns	ns	ns	ns

*, **, *** significant at $p < 0.05$; $p < 0.01$ and $p < 0.001$ respectively; ns: non-significant

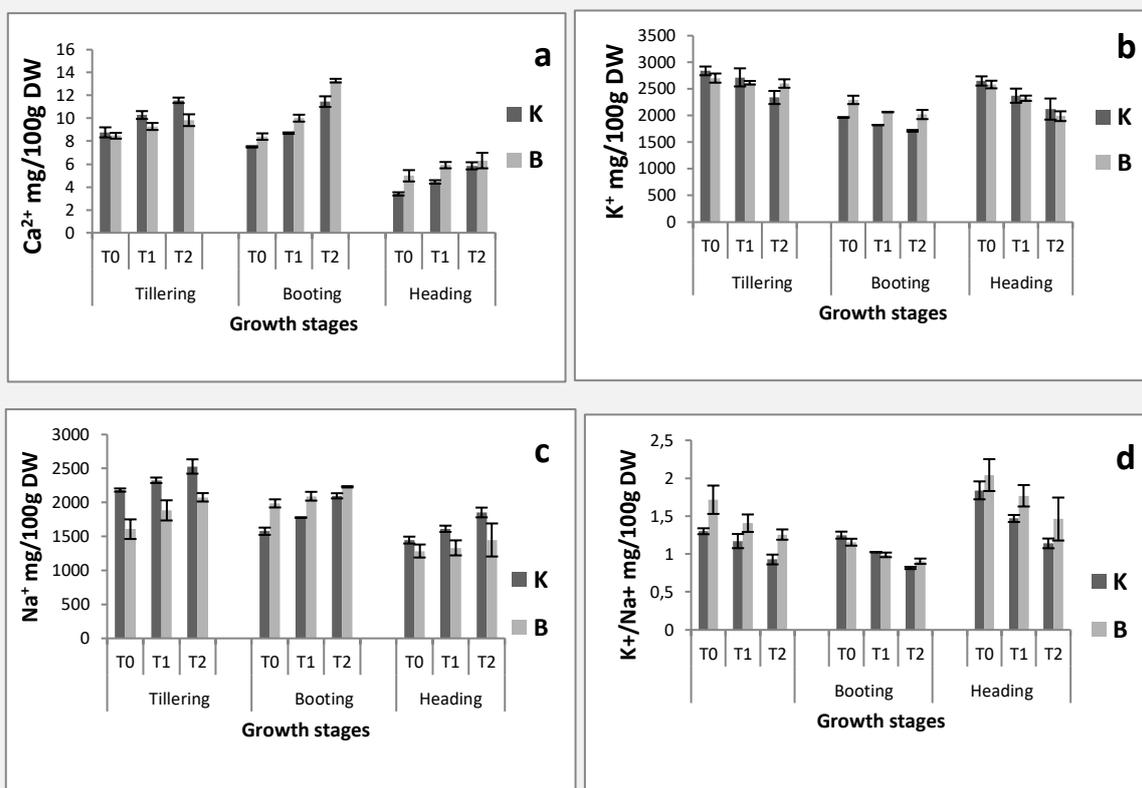


Figure 6: Ca^{2+} , K^+ and Na^+ contents and K^+/Na^+ selectivity under irrigation treatments (T₀ 100%ETc, T₁ 75%ETc and T₂ 50%ETc) during the growth stages.

For all growth stages and under water stress, K^+ content decrease significantly at $p < 0.001$ for the two accessions. In fact, at Tillering stage, K^+ content decreased from 2838.79 to 2339.79 mg/100g DW in Karkeni and from 2703.46 to 2601.52 in Bengardeni. With water stress T_1 and T_2 K^+/Na^+ ratio decreased (from 1.17 to 0.92 in Karkeni and from 1.4 to 1.25 mg/100g DW in Bengardeni) in the same stage (Figure 6d). At Booting stage, Bengardeni represented the high levels compared to Karkeni for the Na^+ contents.

4.5. Yield and yield components

To analyze the effect of full and deficit irrigation treatments on the final yield, eight parameters were retained: plant height, tiller number, spike length, total dry matter, grain yield, spike number / m², seed number / spike and 1000 grains weight. The data are showed in Figures 7a, 7b, 7c, 7d, 7e, 7f, 7g and 7h. Plant height was significantly in the T₁ and T₂ than T₀ treatments (Figure 7a) also between accessions p<0.001. However, dry matter production was not significantly affected by irrigation treatments, a highly significant difference between the accessions (p<0.001). Therefore, water stress caused a significantly reduction in the tiller number and a subsequently reduction on the number of spike per plant.

Spike length, grains number per spike and 1000 grains weight decreased significantly between the two accessions in the T₁ and T₂ treatments compared to T₀. Consequently, grain yield showed a highly significant between all irrigation treatments p<0.001 (Figure 7b, 7d, 7f and 7h; Table 2).

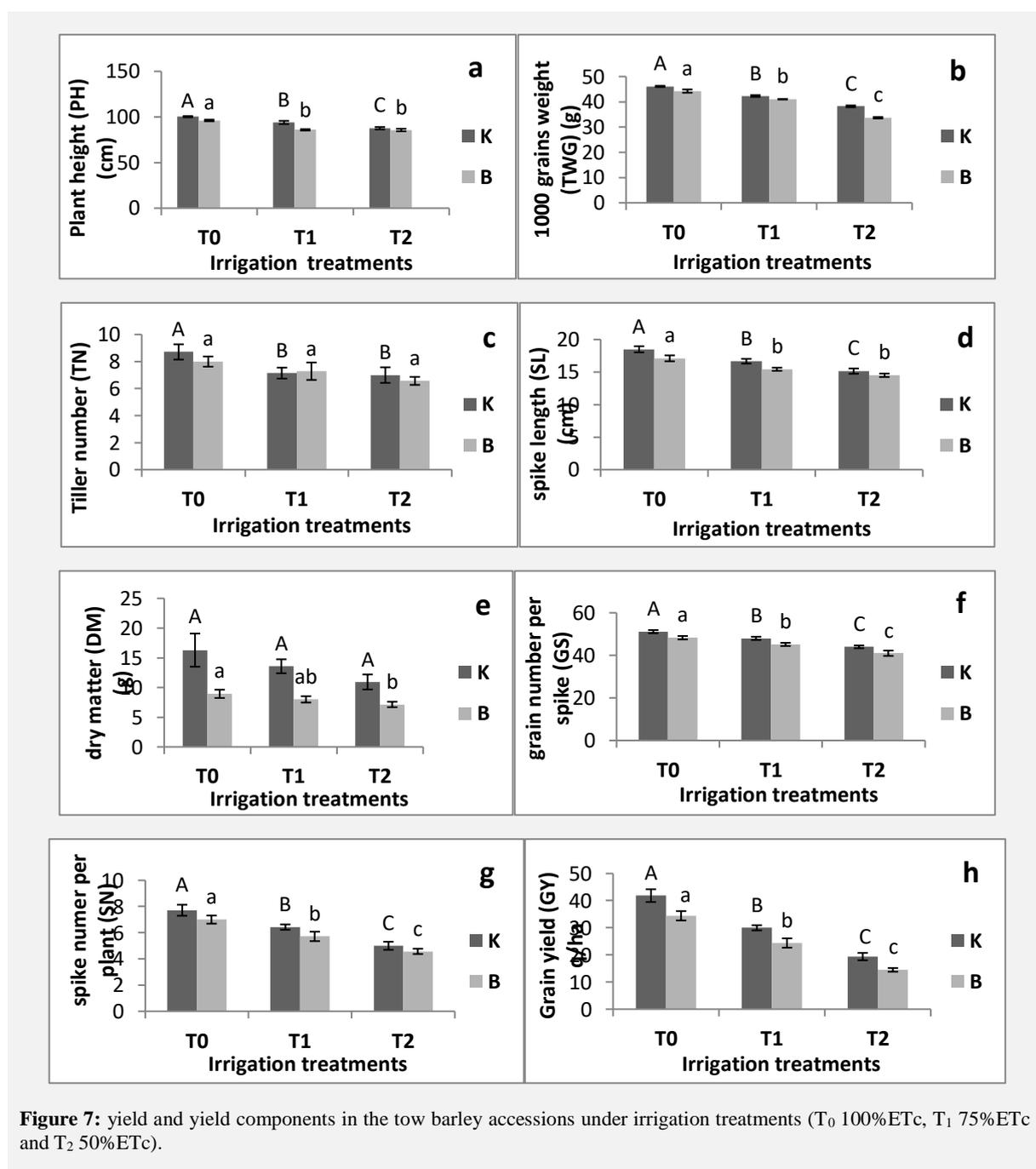


Figure 7: yield and yield components in the tow barley accessions under irrigation treatments (T₀ 100%ETc, T₁ 75%ETc and T₂ 50%ETc).

Furthermore, we noticed that grain yield was positively correlated to spike length ($r= 0.784^{**}$), grain number per spike ($r= 0.836^{**}$) and 1000 grains weight ($r= 0.881^{**}$) at $p<0.01$ (Table 4).

Table 4: Effect of the treatments, accession on yield and yield components and their interaction (T*A).

ANOVA	ddl	PH	TN	SN	SL	DM	TWG	GS	GY
Treatment	2	***	**	***	***	ns	***	***	***
Accession	1	***	ns	*	**	***	***	***	***
T*A	2	*	ns	ns	ns	ns	***	ns	ns

*, **, *** significant at $p<0.05$; $p<0.01$ and $p<0.001$ respectively; ns: non-significant

In fact, a highly significant decrease on plant height, dry matter, grains per spike and 1000 grains weight in the two barley accessions. A significant positive correlation observed between tiller number ($r= 0.561^{**}$), number of spikes per plant ($r= 0.960^{**}$), spike length ($r= 0.784^{**}$), number of grain per spike ($r= 0.881^{**}$) and grain yield.

Table 5: Correlation analysis of yield components with the grain yield GY (q/ha).

	PH	TN	SN	SL	DM	TWG	GS
GY	0.800**	0.561**	0.960**	0.784**	0.427**	0.881**	0.836**

*, ** significantly at $p<0.05$ and $p<0.01$ respectively.

5. Discussion

In our study, in addition to water deficit, the experiment was conducted in field and in saline conditions (initial soil salinity $EC_i = 11.23 \text{ dS.m}^{-1}$, salinity of irrigation water $EC_i = 10.8 \text{ dS.m}^{-1}$), so the plants that grow on this type of soils are often subjected to salinity (Abbas et al., 2015; Biswas & Biswas, 2014). Indeed, results show that the water deficit responses triggers biochemical and agronomic changes on barley plants and at the same time promotes adaptations mechanisms to ensure the survival and productivity of plants in serious conditions (Bartels & Sunkar, 2005). However, EC_i values increases under drought (T1 and T2) than control T0 in all growth stages in an order $50\%ET_c > 75\%ET_c > 100\%ET_c$, this increasing it may be attributed to little leaching of the soil expected under drought (Schoups et al., 2005). Accumulation of osmo-regulators such as proline and soluble sugars facilitate osmo-regulation under drought and salinity has been observed in plants (Trovato et al., 2008). In present study, proline and soluble sugars increases under water stress (T₁ and T₂) and varies between accessions during the growth stages, this augmentation enable plants to tolerate drought (Kocsy et al., 2005). Proline content present the higher level at Tillering than Booting and Heading stages; he is from 0.1 to 0.049 for Karkeni respectively at Tillering and Booting stages and from 0.91 to 0.04 for Bengardeni in the same stages ant treatments (T₀ to T₂) (50%) (Figure 4). This difference for this trait probably results from a different genetic potential among the varieties studied, which is widely observed also (Shao et al., 2006a; Geravandi et al., 2011). Soluble sugar increased in the same way as the proline. This variation can be explained by rainfall (Figure 3) and by physiological mechanisms of plants, (Szira et al., 2008). Highly correlation between proline and soluble sugar are observed. Similar results for (Morant-Manceau et al., 2004) and (Navarro and Rubio, 2006).

Water stress increase significantly Ca^{2+} content between barley accessions and irrigation treatments, it may be assumed that maintaining higher translocation of Ca^{2+} is an important way to reduce water stress or beneficial to improve plant tolerance to drought. Increase in Na^+ ion and decrease in K^+ ion content uptake interrupted ionic balance as observed in the tow accessions under all treatments (Ahmed et al., 2013; Qiu et al., 2011).

The accumulation of Na^+ ion was the expense of the K^+ absorption ($r= -0.013$), K^+/Na^+ ratio decreased significantly ($p<0.01$) between barley accessions, it is more important in Bengardeni accession than Karkeni under water treatments. The varieties used accumulate more K^+ than Na^+ , other works found that under saline conditions the plant accumulate more Na^+ than K^+ MP. Lindsay et al., (2004) on durum

wheat and Munns *et al.*, (2006) on wheat and barley. The high K^+/Na^+ ratio indicate that the Karkeni accession is less susceptible than Bengardeni to water stress under saline conditions. These results are similar find that by (Ahmed *et al.*, 2013; Wu *et al.*, 2011) working on Tibetan wild and cultivated barley. In addition, water stress showed a highly significant difference between barley accessions in yield and yield components ($p < 0.001$).

6. Conclusion

Karkeni is less sensitive to water stress than Bengardeni, as shown by their relatively high proline and soluble sugars contents. Moreover, drought tolerance of Karkeni was found to be associated with a lower selectivity K^+/Na^+ ratio. Karkeni is the less sensitive to water stress and the most productive than Bengardeni under three water regimes. In addition, it can be concluded that the full irrigation (T_0 100%ETc) and (T_1 75%ETc) strategies offer significant advantage for yield and reduce the build-up of salinity compared to the (T_2 50%ETc) irrigation practices in barley production under arid conditions.

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