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Markers of water stress in straw cereals (*Triticum* and *Hordeum*) at different phenological stages

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Abstract
The study focused on six varieties of straw cereals (*Triticum* and *Hordeum*) subjected to a single water stress mode WHD (With Hydric Deficit) at 40% of the field capacity during three phenological stages. Five parameters were measured: morpho physiological parameters relative water content (RWC), chlorophyll content (Chl), leaf area (LA) and accumulation of osmolytes (proline and soluble sugars). The wheat varieties retain their relative high water content in the middle and at the end of the cycle. They accumulate large amounts of osmolytes proline and soluble sugars, maintaining a considerable leaf area. The two genotypes of barley exhibit two distinct behaviors. Saida marks the strong accumulation of proline 15 times the control and maintains his RWC, While Manel does not react to stress. The first genotype tolerates stress and the second resists. Wheat and barley exhibits physiological and biochemical adaptation lines under deficient conditions (markers).

Keywords: Wheat (*Tritium*), barley (*Hordeum*), water stress, phenological stages, markers, adaptation.

Introduction
Cereals have traditionally constituted an important part of food resources and economic exchanges, but their production remains highly dependent on climatic conditions, particularly rainfall. Environmental stresses, including water stress, severely limit plant growth and plant productivity (Wang et al., 2003). The Algerian climate is characterized by periods of water deficit and high temperatures which can appear, progressively or brutally at first; in the middle and at the end of the season (Chaib, 1998). Therefore, production of rain fed crops and particularly cereals will be in question. However, research into an understanding of the different resistance patterns developed by plants is needed to identify selection criteria that can be used in varietal improvement programs (Turner, 1979).

The purpose of this study is to test varieties of cereals for human consumption (durum and Hordeum) from different local origins and introduce the relation of their capacity to resist water shortages and to assess the effect of water stress on their development by Morphological (identification of biometric characteristics), physiological (evolution of parameters of water status: relative water content, chlorophyll content), and biochemical (evaluation of the accumulative capacity of compatible osmolytes: proline and soluble sugars) In order to reflect on a better conservation strategy (collection and storage) and valorization of these genetic resources by the selection of a material adapted to abiotic constraints.

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Materials and methods

Vegetal material

Two genotypes were studied: two of durum wheat (*Tritium durum*: Bidi17, Djnah El Ketayfa (DK)), two of Common wheat (*Triticum aestivum*: Florence aurore (FA), Mexipake) and two of barley (*Hordeum vulgare*: Manel and Saida 183) (Table 1).

Table 1. Origin and characteristics of the genotypes studied.

Genotype		Origin	Characteristics
Durum wheat	Bidi 17	Algeria., Guelma 1936	Average Productivity, Semi-late, Low tiller, Average height
	Djnah El Ketayfa(DK)	Tunisia	Disease-sensitive, semi late, fairly tolerant to drought, medium tiller.
Soft wheat	Florence aurore 8193(FA)	Tunisia-Algeria	Improve wheat, sensitive to diseases and cold.
	Mexipake	Mexico, Pakistan 1973 breeders CIMMYT	Moderately tolerant to drought, good yield, good quality, moderately resistant to diseases.
Barle y	Manel	Tunisia	High yield potential, disease resistance.
	Saida 183	Algeria	Good productivity, semi-early cycle with medium tiller, visibly susceptible to diseases.

Conduct of experience

In the first stage, we evaluated the ability of these genotypes to grow under water stress conditions. The second step concerns the study of the morpho-physiological and biochemical behavior of plants under these deficient conditions.

The test is carried out in the laboratory of Development and Valorization of Plant Genetic Resources (L.D.V.R.P.) Chaâbat Erssas, in glasshouse under semi-controlled conditions. The temperature varies at night between 9 ° and 15 ° and day between 24 ° and 42 °. The humidity varies between 75% and 100%. The pots are placed in a greenhouse at the bio pole at Chaâbat Erssas at the University of Constantine, according to a randomized Fisher block system with four repetitions for each variety. The total is 24 pots sorted by row according to the experimental arrangement. Seeding is carried out manually in 7 kg pots on clay loam soil at a density of 14 grains / pot to a surface area 26 cm long and 18 cm wide, given the need for biomass in the experiences application. The seeds used are supplied by the D.V.R.P. Plant watering is undertaken regularly once a week (300 ml / pot) during the initial stages, twice a week at the stages of up-swelling, and three times a week from the stage of heading to 600 ml / pot . The test is also regularly maintained by manual weeding and by a raise in the addition of organic fertilizer to the surface of the pots at the tilling stage. Water stress is applied at only one level, which is 40% of the field capacity (F.C.) on three different phases of the plant development cycle according to the early maturity of the varieties. The stages are successively: 1st stage: end of tilling, beginning of stage 2, stage 2: end of swelling, 3rd stage: beginning of heading for durum wheat, total heading for barley, and beginning of flowering for soft wheat.

The experiment is divided into two batches: the first batch contains the controls (plants without water deficit WIHD at 75% FC, or the plants are normally watered throughout the life cycle of the plant.) Plants with water deficit (WHD) spread over three stages applied to stress. Water stress is maintained by stopping irrigation until the desired stress level (40%) is reached (Avery Berkel FX 220, Max30kg, Min100g, Error = 5g). The leaf samples of the control and stressed seedlings were subjected to several Analytical measurements and maintained due to three replicates per variety for both WHD and WIHD treatments.

Measured parameters

The parameters measured are physiological, biochemical (osmotic) and morphological (adaptation characteristics): Physiological parameters are the relative water content (RWC) and the determination of chlorophyll pigments (Chl).

The relative water content is measured according to the method of Barrs (1968). The fresh cut leaves (fresh weight FW) are directly prolonged in test tubes filled with distilled water. The tubes are placed in the dark in a cool place. After 24 h, the saturated leaves are again weighed (TW turgor pressure weight), and then the sample is dried in an oven at 85 ° C and weighed one last time after 48 h, for regular time intervals, to obtain constant weights (Dry weight - DW). The water content is estimated in (%) relative to the fresh weight. The calculation is made using the formula of Clarke & McCaig (1982):

$$RWC(\%) = \frac{FW - DW}{TW - DW}$$

DW, dry weight of the leaf; TW, Turgor pressure weight (after 24 hours in water); FW, fresh weight of leaf excised.

The chlorophyll pigment determination technique (Chl) is measured by the method of French (1970), consists in taking 100 mg of fresh material for each sample, adding 10 ml of a solution prepared from (75% acetone and 25% ethanol, keeping them in closed black boxes for 48 hours at 30 C °, in the dark. The optical density is measured at 663 nm for chlorophyll a and at 645 nm for chlorophyll b. The concentrations of chlorophyll a and b expressed in mlmol / mgMF are given by the formulas established by Maclachlan & Zalik (1963):

$$Chl\ (a)(\ mg/g\ FW) = \frac{12.3OD\ (663) - 0.86OD\ (645)}{10}$$

$$Chl\ (b)\ (mg/g\ FW) = \frac{9.3OD\ (645)\ - 3.6OD\ (663)}{10}$$

The chlorophyll (a + b) content is calculated by the formula "Chl (a + b) = Chl (a) + Chl (b)" and also calculation of the "Chl (a) / Chl (b)" ratio.

The biochemical parameters (osmotic) consist in measuring the quantities of constituents of biological organs in general soluble sugars, amino acids (proline). Proline or pyrrolidine-2-carboxylic acid is one of the 20 main amino acids that enter into the constitution of proteins and is easily oxidized by ninydrine. The method followed is Trolls & Lindsey (1955), simplified and developed by Dreier & Göring (1974). The optical densities are converted to proline by the equation (Benlaribi, 1990):

$$Y = \frac{0.62\ \times\ OD\ (528)}{DW}$$

OD, optical density; DW, Dry Weight (mg); Y, proline content µmol / mg DW.

The total soluble sugars (sucrose, glucose, fructose, their methyl derivatives and polysaccharides) are determined by the phenol method (Dubois et al., 1956). The soluble sugar content is calculated according to the following equation:

$$Suc = \frac{1.65\ \times\ OD\ (490)}{MS}$$

OD, optical density; DW, Dry Weight (mg); Suc, sugar content µmol / mg DW.

Morphological parameters (adaptation characteristics) characterized by leaf area (LA). It concerns the flagship sheet, which consists of taking the surface of the plant leaf on tracing paper and then copying it over black paper, then cutting it and moving it to the planimeter (Area Meter AM 200) which displays the surface area in mm² as soon as it receives the sheet image, and then converts the area to cm².

Statistical analysis

The results obtained during this experiment are treated by an analysis of variance (Anova two factors) carried out using the software XL-Stat.2010. The Newman-Keuils test (SNK) allowed to give the averages and to classify the significance level at 5%.

Results and Discussion

Relative water content

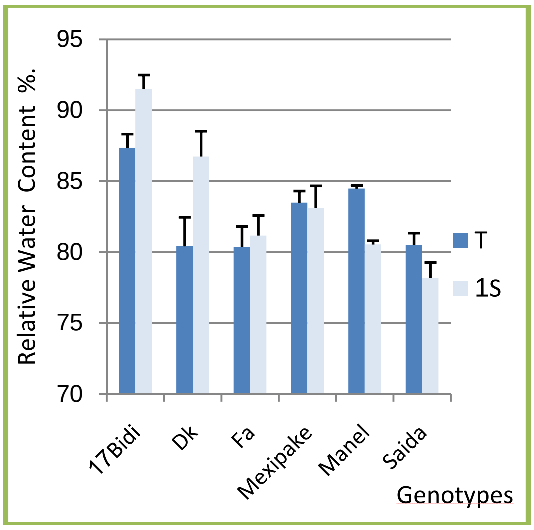
The analysis of the relative water content allows describing in a global way the hydric status in response to water stress, and to evaluate the ability to achieve good osmoregulation and to maintain cellular turgor pressure (El Jaafari et al., 2000).

At the End tiller –start upstream stage, the relative water content in WIHD plants ranged from 80.36 ± 1.46% to 87.36 ± 0.96%. The minimum value is recorded in the FA genotype, while the maximum value is recorded in Bidi17 durum wheat (Figure 1A). The RWC in WHD plants ranged from 78.19 ± 1.09 to 91.51 ± 0.98% in the genotypes studied. The minimum value is recorded in the Saida barley genotype, while the maximum value is recorded in the Bidi17 wheat variety in both durum varieties, the water content increases slightly in the WHD batch compared to the WIHD batch. In both wheat varieties, both varieties retain the same values for both control and stress treatments. The order of increase is 1.05 times, 1.08 times, 1.01 times and 1.00 times respectively. This proves that wheat leaves are not affected by lack of water. The estimated results seem to confirm those of (Nouri, 2002), which show that wheat genotypes that maintain a high RWC in the presence of water stress are tolerant genotypes. The variance analysis of the results obtained reveals the existence of an insignificant difference between the two WIHD and WHD tests and highly significant between the varieties studied and even for the interaction between the two factors. The Newman Keuils test (SNK) at the 5% threshold classifies the test factor into a single group:

S1 = T ⇔ 83.50 ∠82.77 and classifies the factor varieties into three groups: Bidi17 > Dk; Mexipake; Manel> Fa; Saida. Each variety has a certain behavior towards the stress and the applied stage. The Bidi 17 variety feels the stress and stores the highest value by prediction, compared to the other varieties. While the two Fa and Saida varieties react weakly to stress and score a minimal value. At the end stage, at the end of the run-start swelling, the WHD plants recorded a sharp decrease in the order of half compared to the WIHD plants, with the exception of the Manel variety, which retains the same content in the two batches. Their water contents fluctuated from 56.46 ± 0.69 to 50.81 ± 2.87% in Saida and Mexipake respectively (Figure 1B). This means that the more the intensity of water stress increases and the plant develops, the lower the relative moisture content will be, while keeping relatively high values in comparison with the control. This reduction would be due to the phenomenon of dehydration for varieties but not for others. The SNK test at the 5% threshold classifies the test factor into two different groups: T > S2 ⇔ 82.52 > 58.12. The same test regroups the varieties studied into five groups: Manel > Bidi17 > Mexipake > Dk; Fa > Saida. The first presents the Manel variety of barley which maintains its ability to store water in its leaves unlike the variety Saida (last group) which loses its content by sweating. Maintaining the moisture content is higher or lower compared to the control; would probably be due to an active osmoregulation, following the establishment of a mechanism of tolerance to water stress, namely osmotic adjustment. Indeed, the same type of work carried out by (Nouri, 2002) on different types of plants subjected to water stresses has confirmed that the implementation of certain mechanisms of drought tolerance has been noted.

In the third stage, the content in WIHD plants at the end of the cycle is very high compared with the previous stages, in the middle and at the beginning of the cycle. It varies from 85.14 ± 1.35% to 90.62 ± 2.16% in Bidi17, and Mexipake successively. WHD plants reported a remarkable drop, accounting for half of the majority of the varieties studied. Their grades fluctuate between 47.91 ± 3.39% and 52.46 ± 2.42%. Only the Mexipake genotype shows an optimal 90.62 ± 2.61% in WIHD and a 2.5-fold decrease in WHD. This is explained by its sensitivity to water shortage (Figure 1C). The SNK test at the 5% threshold classifies the test factor into two different groups: T> S3 ⇔ 88.07 > 48.12 and subdivides the variety factor into four different groups: Dk> Manel; Fa> Saida; Bidi17> Mexipake.

The DK variety shows the first group with the highest water content of an average of 71.05%. While the Mexipake variety does not react in the same way and marks the average of 63.80%.



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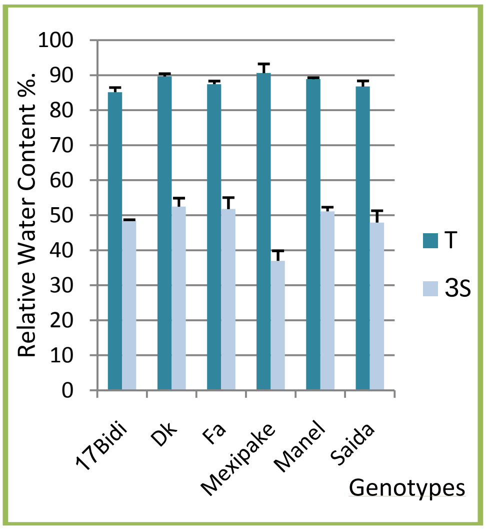

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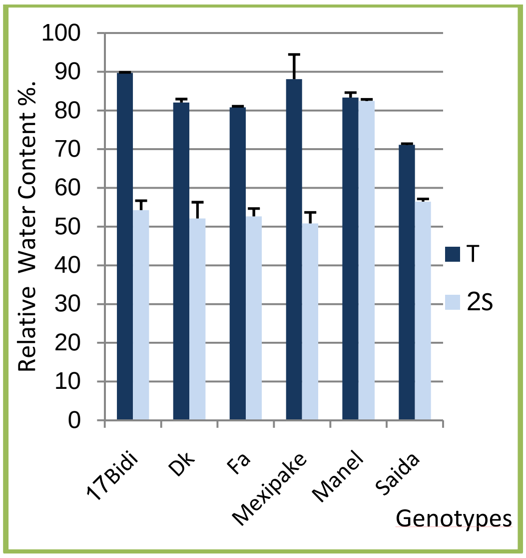

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Figure 1. Water content in the six genotypes studied in phases. **A**, End tiller-start upstream; **B,** End upstream-start swelling; **C**, Early heading (durum wheat), End heading (barley), and early flowering (soft wheat).

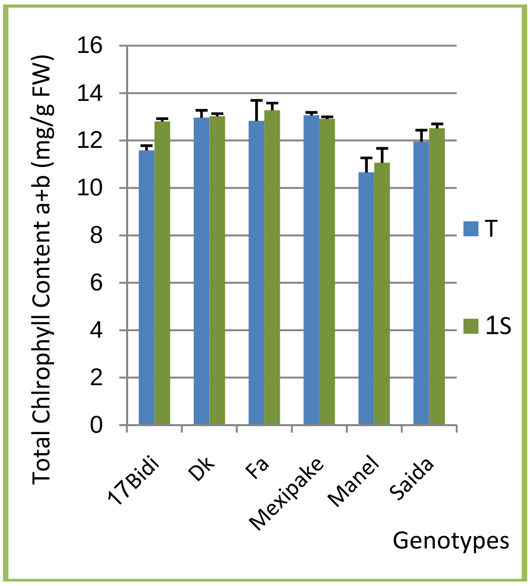
By comparing the evolution of the relative water content of the six varieties during the three stages, all the genotypes of the WIHD batch maintain almost the same water content except the Mexipake variety which increases its water content in the two last stages in the middle and at the end of the cycle. The WHD batch in the middle of the cycle markedly decreased half of its relative water content in the majority of varieties compared to the End tiller-Start Upstream, with the exception of the barley variety Manel which retains the same content in WHD Plants than WIHD. The WHD plants at the end of the cycle also mark a slight decrease compared to the WHD plants of the End upstream -start swelling stage. Only the three varieties of wheat (Bidi 17, DK and Fa) retain the same levels at the last two stages. It should be noted that these varieties are considered to be tolerant during the End tiller-Start upstream and End upstream-Start swelling phases, as the water content of the durum wheat leaves decreases proportionally with the reduction of water in the soil, which agrees with the findings of (Bajji et al., 2001), who note that this decrease in RWC is more rapid in susceptible varieties than in resistant varieties. Barley Manel does not influence during the End tiller – Star upstream stage and End upstream-start swelling. It will affect the early flowering stage. The Mexipake variety does not record changes during the upstream / swelling stage but loses the largest percentages during the last two stages end swelling and start flowering. Water stress causes a drop in the water percentage; this drop depends on the genotype and its behavior towards the lack of water. According to Nourri (2002), the relative water content varies according to soil moisture and to the degree of water retention in the plant tissue. It also depends on the balance between absorption and sweating.

Total Chlorophyll content

Photosynthesis or chlorophyll assimilation is the physiological process by which autotrophic plants are able to use solar energy to ensure their nutrition exclusively using mineral foods, the determination of total chlorophyll (a + b) is established in our study.

At the End tiller -start upstream stage, water stress induced a very slight increase of the order of 1.11 times, 1.03 times, 1.04 times and 1.05 times in the four genotypes Bidi17, FA, Manel and Saida respectively to WIHD plants. Both Mexipake and Dk appear to be the least affected by stress. They maintain the same analogues to the two WIHD and WHD treatments (Figure 2A). The statistical study revealed the existence of significant difference between the two WIHD and WHD tests and between the varieties studied and even for the interaction between the two factors. The SNK test at the 5% threshold classifies the stress test factor into two groups, which are difficult to distinguish: T > S1 ⇔ 12.60 >12.17 and classifies the variety factor into three groups: Fa; Dk; Mexipake> Saida; Bidi17> Manel. Between WHD and WIHD plants, the three FA, DK and Mexipake genotypes mark the highest averages and form the first group. The last group contained only the Manel variety of barley with an average of 10.86 mg/g FW.

At the stage, End upstream-start swelling, the content in WIHD plants varies from 8.39 ± 0.05 to 8.93 ± 0.10 mg/g FW. The minimum value is recorded in the Saida genotype, while the maximum value is recorded in Mexipake soft wheat. A slight increase in both hard wheat (Bidi17 and Dk) and barley Saida is of the order of 1.02 times, 1.03 times and 1.02 times respectively compared to the control. For FA, Mexipake and Manel genotypes, the decreases were similar 0.97 times, 0.99 times and 0.95 times respectively to the control (Figure 2B). The variance analysis of the results obtained reveals the existence of a non-significant difference between the stress levels and a significant difference between the varieties and the interaction between the two factors. The SNK test at the 5% threshold classifies the test factor into two similar groups: T; S2 ⇔ 8.71; 8.67, and classifies the variety factor into two different groups: Mexipake; Bidi17; Dk ; Fa > Saida ; Manel. The first group includes all wheat with an average of 8.74 to 8.90 mg/g FW. While the two barley, Saida and Manel belong to the second group with an average of 8.4 mg/g FW. In the third stage, WIHD plants have similar values, fluctuating between 13.50 ± 0.05 mg/g FW in DK and 14.35 ± 0.24 mg/g FW in Mexipake (Figure 2C). The order of decrease in WHD plants varies from 0.92 times to 0.98 times in all genotypes relative to WIHD plants. They mark convergent values that fluctuate between 12.52 ± 0.87 mg/g FW in Bidi17 and 13.43 ± 0.23 mg/g FW in Mexipake respectively. Anova with two factors reveals the existence of a significant difference between the two tests (stressed and control) and between the varieties and even the interaction between the two factors.



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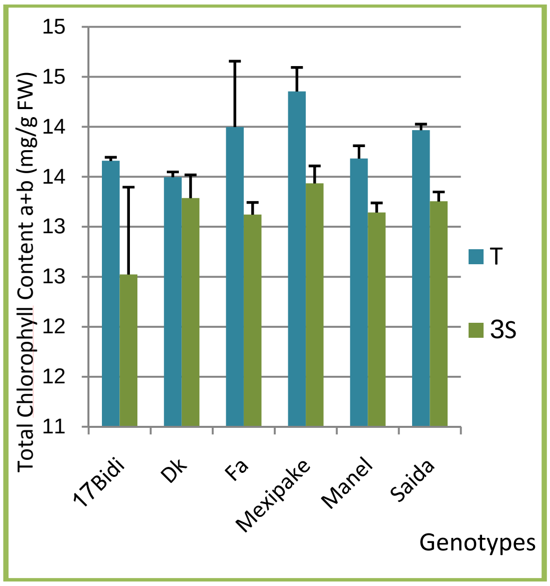

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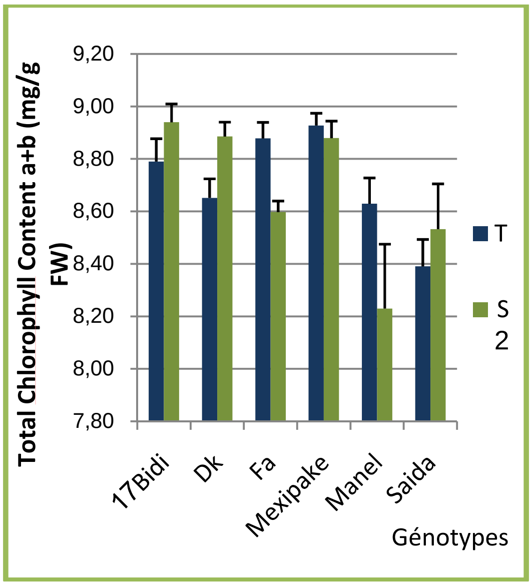

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Figure 2. Chlorophyll content in the six genotypes studied in phases. **A**, End tiller-Start upstream; **B**, End upstream-Start swelling; **C**, Start heading (Durum wheat), End heading (barley), and Start flowering (soft wheat).

The SNK test classifies the test factor into two different groups> S3 ⇔ T ⇔ 13.86 > 13.12. And classifies the variety factor into five different groups: Mexipake > Saida; Fa > Manel > Dk > Bidi17. The four varieties of soft wheat and barley belong to the first three groups, which interact with one another. On the other hand, the two hard wheats belong to the last two groups. These are the most affected by stress. At this stage, plants react to water stress by decreasing their chlorophyll content. The amounts of chlorophyll at the Start upstream-End swelling stage decrease considerably compared to the End tiller-start upstream stage. This decrease may be due to the increase in temperature in the greenhouse during the experimental period. Thus, total chlorophyll synthesis is inhibited by about 70% in etiolated cucumber seedlings exposed to light in a 42°C culture chamber (Nouri, 2002). On the other hand, at the last stage of heading and start flowering, the six genotypes recorded levels close to those of the first End tiller-Start upstream stage. The increase in total chlorophyll levels is a consequence of the reduction in foliar cell size under the effect of a water stress that generates a higher concentration (Siakhène, 1984). On the other hand, the drop in chlorophyll levels is the consequence of the reduction in stomata opening to limit water losses by evapotranspiration and by increasing the resistance to atmospheric CO_2 input required for photosynthesis (Bousba et al., 2009). The amount of leaf chlorophyll can be influenced by many factors such as leaf age, leaf position (Hikosaka et al., 2006).

According to the kinetics of the evolution of the chlorophyll content (a + b) during the course of our experiment, it is noted that the chlorophyll (a + b) content differs from one stage of life to another irrigated plants or stressed plants. It is high at the beginning and at the end of the cycle and lower at the middle of the cycle in both cereals (*Triticum* and *Hordeum*). The amounts of chlorophyll at the End upstream -early swelling stage have decreased considerably compared to the End tiller-Start upstream stage. This decrease may be due to an increase in the temperature of the greenhouse during the experimental period. Thus, total chlorophyll synthesis is inhibited by about 70% in etiolated cucumber seedlings exposed to light in a 42 C ° culture chamber (Nouri, 2002). On the other hand, at the heading stage and at the beginning of the flowering period, the six genotypes recorded levels close to those of the first End tiller-Start upstream stage. This return to the same content is explained by Hireche (2006): plants located in hot zones have sensitivity to temperature changes much stronger than those in regions where the climate is cooler. The results also show that in the first stage the FA variety was most stressed, in the second it is the Bidi 17 durum wheat and in the third stage the Mexipake variety to which belongs the largest quantities of the total chlorophyll provides during the three stages.

Proline

Proline is known to be widely present in plants and normally accumulates in large quantities in response to environmental stress, both as a result of increased production and reduced degradation (Roeder, 2006). Given previous experiments, accumulation begins only at the level of 40% of the field’s capacity where the plant feels the lack of water (Chaib, 1998; Redjamia, 2006; Zarafa, 2006). Hence the estimated content is around 2 µmol / mg DW (Benlaribi & Monneveux, 1988) under non-limiting irrigation conditions. In our three phases of stress, the proline content is estimated from the 40% degree of the F.C.

Both cereals accumulate less proline at the beginning of the cycle, End upstream- start swelling, were the plant does not feel stress and behaves normally and gives better yields than irrigated plants. The content in WHD plants ranged from 22.08 ± 1.25 to 3.15 ± 0.14 µmol / mg DW in the genotypes studied. The minimum value is recorded in the barley genotype, while the maximum value is recorded in Saida barley. The other genotypes show fluctuating levels from 5.76 ± 1.46 to 12.12 ± 1.06 µmol / mg DW (Figure 3A). At WIHD (75% F.C.), the four varieties of soft wheat and barley show values below 2µmol / mg DW. While the two varieties of durum wheat have a value greater than 2 µmol / mgDW (Benlaribi & Monneveux, 1988), and only the Saida variety shows a strong accumulation, which is more than 15 times the initial value of the control.

The variance analysis of the results obtained reveals the existence of very highly significant differences between the two WIHD and WHD treatments and between the studied varieties and the interaction between the two factors. The

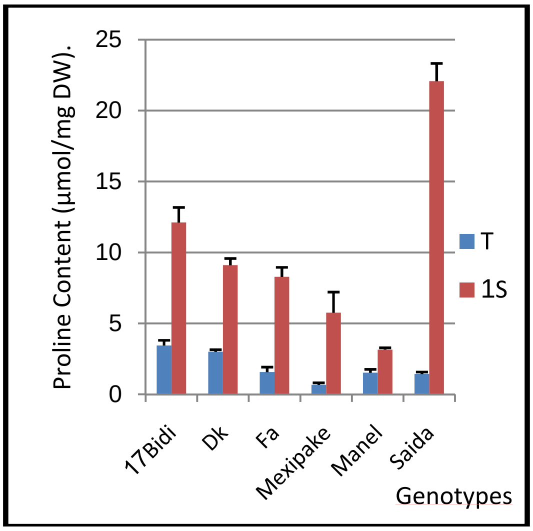
SNK test classifies the treatment factor into two different groups: S1 >T ⇔ 10.08 > 1.94 and classifies each distinguished variety of another:

Saida > Bidi17 > Dk > Fa > Mexipake > Manel. In the middle of the cycle, End upstream-start swelling, the four varieties feel stress and resist by a strong accumulation for wheat varieties. While the barley varieties Saida and Manel remain stable with the same grade of Start stage cycle. The content in WHD plants ranged from 8.75 ± 0.43 to 29.22 ± 1.11 µmol / mg DW in the genotypes studied. The minimum value is recorded in the Manel barley genotype, while the maximum value is recorded in durum wheat Dk. The other genotypes show fluctuating levels of 14.74 ± 1.44 to 26.36 ± 3.76µmol / mg DW (Figure 3B). The rate of increase of proline in stressed plants compared to control plants was not more than double in the majority of the studied genotypes. Which reflects that the plants at this stage of development do not react strongly to water shortage, and exert another mechanism of resistance to stress, through the avoidance voice and shortening of their life cycle, Or the evasion by maintaining a satisfactory internal hydric state in the presence of a hydric constraint. This high water potential can be obtained by increasing the root absorption of water (El hassani and Persoons, 1994), reducing water losses (Levitt, 1980) and stomatal regulation (Leclerc, 1999). Very significant differences between stress levels and between varieties and even the interaction between the two factors are established. The SNK test at the 5% threshold classifies the test factor into two different groups: S2 > T⇔ 20.85 > 12.36 and groups the varieties into four different groups: Dk > Mexipake ; Fa ; Bidi17 > Saida > Manel. At the end of the cycle, a critical stage of development, from heading to flowering, varieties of wheat have a slight content. While the both varieties of barley behave in the same way. The content in WIHD plants varies from 1.89 ± 0.28 to 6.25 ± 0.26 µmol / mg DW. The minimum value is recorded in the barley Saida genotype, while the maximum value is recorded in soft wheat, Mexipake. At this level, the amount of proline accumulated by the six genotypes decreases compared to the previous stage, but the order of increase also developed by comparing stressed (WIHD) to controls (WHD). It is about 5.16 times, 6.07 times, 4.46 times, 2.24 times, 3.97 times and 7.51 times in Bidi 17, Dk, Fa, Mexipake, Manel and Saida respectively compared to the control (Figure. 3C).

Very significant differences are revealed between the two tests (stressed and control) and between the six varieties and even the interaction between the two factors. The SNK test classifies the test factor into two different groups: S3 > T ⇔ 13.69 > 3.23 and classifies the variety factor into five different groups: Mexipake; Bidi17 > Manel > Saida > Fa > Dk.

By comparing the proline content in the different genotypes according to different phases of stress applied. We distinguish that the two cereals accumulate less proline at the beginning of cycle S1 (end tiller-start upstream) or the plant does not feel stress and behaves normally and gives better yields than irrigated plants.

In the middle of the End upstream-Start swelling cycle, the four varieties feel stress and resist it by a strong accumulation for wheat varieties and barley variety Saida. While the Manel variety remains stable with the same grade of start cycle stage. On the other hand, at the end of the cycle, a critical stage of development (heading and flowering), the varieties of wheat have a slight content. While the both varieties of barley behave in the same way. Thus, we conclude that: the proline content increases positively and progressively, but it depends on the stage, the climatic conditions and the genotypes influenced. The variance analysis of the results obtained reveals a very highly significant difference between the stress levels according to these stages. Both varieties of durum wheat accumulate the highest amounts of proline. The Bidi 17 genotype accumulates more proline during the three phases, but the most critical stress applied is the second because the amount of proline is higher compared to the other two stages 26.24 ± 1.43> 16.28 ± 0.60> 12.12 ± 1.06 and increase order 3.52 times, 2.81times and 5.16 times respectively according to the stages. The genotype Djenah El Khatéyfa also accumulates quantities as large as those of Bidi 17. For soft wheat, the Mexipake variety marks a higher proportion of proline than that of Florence dawn during the three stages. The Saida barley genotype is strongly influenced at the 1st stage. It accumulates proline of 15.44 times compared to the control. On the other hand, the Manel variety marks the smallest amounts of proline and almost stable in relation to the control during all the phases studied. This allows us to suggest the proline test as a biochemical indicator (osmotic) of water deficiency.



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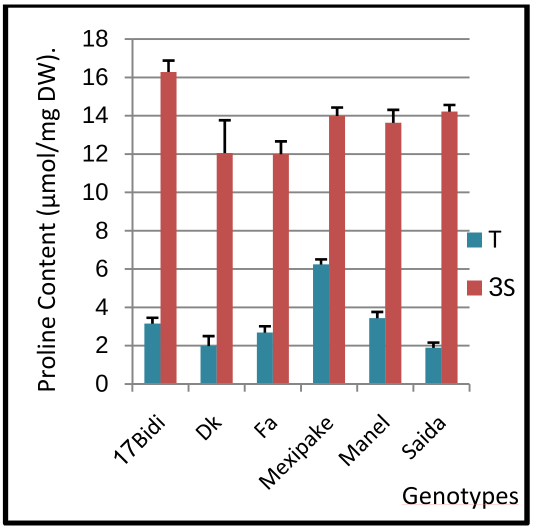

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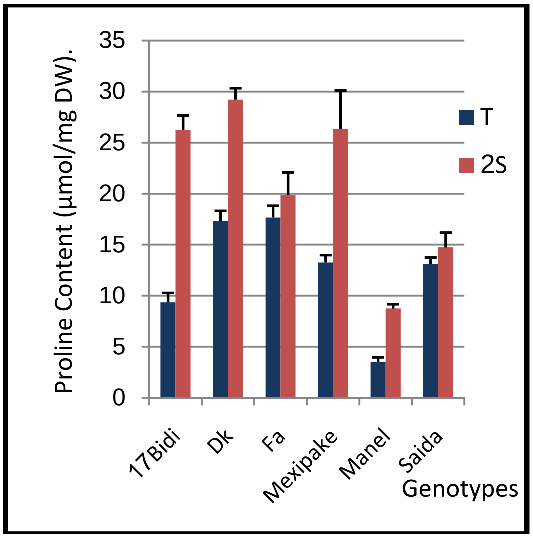

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Figure 3. Proline content in the six genotypes studied in phases. **A**, End tiller-Start upstream; **B**, End upstream-Start swelling; **C**, Start heading (durum wheat), End heading (barley), and Start flowering (soft wheat).

In stressed plants, an increase in proline content is underlined, and this accumulation is a direct consequence of water deficit (Benhassir and Boulguergour, 2010). Depending on the evolution of foliar levels in proline during the vegetative cycle, the dynamics of accumulation were independent of the stage of development and, on the contrary, very closely related to rainfall (Monneveux & This, 1997). Positive correlations between accumulated proline and water deficiency tolerance were observed in both barley and wheat (Salma et al., 2005). This could be explained by the ability of some genotypes to accumulate proline more rapidly (but not necessarily in larger amounts) during a water stress, which made the dosage result strongly dependent on the delay between the beginnings of the water stress of the sampling date (Benlaribi & Monneveux, 1988). The accumulation of proline also constitutes a true mechanism of tolerance to drought. Indeed, the proline content is higher in the case of water deficiency and, in particular, in the genotypes most resistant to drought. The variety, which accumulates more proline in its various organs and particularly at the level of the flag leaf under water deficit conditions, gives the highest yield and has the largest grain filling capacity. On the other hand, the lower yielding variety and the less bulky grains accumulate less proline (Salma, 2002). The existence of an intra-specific variation in cereals for the accumulation of proline due to water deficiency suggests the possibility of selection on the basis of this character some genotypes that have a good ability to Survival and a stable grain yield under limiting water conditions (Bergareche et al., 1993). For this raison Bellinger et al. (1991) have proposed the accumulation of proline as a technique of selection for barley cultivars resistant to drought.

Soluble Sugar content

At the stage End tiller-Start upstream, the stressed batch (WHD), mark values fluctuate between 3.51 ± 0.63 to 11.94 ± 1.79 µmol / mg DW. The maximum is attributed to Saida, and lower at Mexipake. It should be noted that Saida barley has the highest concentration in the control and stressed batch, and Mexipake wheat also has the lowest grade in both tests (Figure 4A). Note that only the two varieties of barley synthesize a higher concentration to 40% F.C. The rate of increase is equal to 1.20 times and 1.12 times in Manel and Saida respectively compared to the control. While the three genotypes of wheat (Bidi 17, Dk and Mexipake) report half. Only the Fa variety remains approximately equal in the two WIHD and WHD tests (6.20 ± 0.76 and 5.69 ± 0.70).

This decrease explains why the tiller wheat uses more energy, including soluble sugars, and this is the reason why this low rate is noticed, which validates the work of (Peeters, 2004) who proposed an explanation of the reduction of soluble sugars at this stage by the constitution of an extremely branched system and aggregated at its base at the level of a tiller plateau which very quickly produces a large leaf area. Each branch or thallus comprises a series of very elongated leaves set in one another and retains, during the vegetative part of the cycle, short internodes and a very low localization of the apexes and the zones of growth of the leaves near the plateau of tiller (Peeters, 2004).

The variance analysis of the results obtained reveals a very highly significant difference between the two WIHD and WHD treatments and between the varieties studied and the interaction between the two factors. The test (SNK) classifies the treatment factor into two different groups: T > S1 ⇔ 7.68 > 6.54 and classifies the variety factor into four groups: Saida > Manel > Dk ; Fa ; Bidi17 > Mexipake.

At the stage End upstream-start swelling, all genotypes of the WIHD batch generally mark a slight increase compared to the previous stage, with the exception that the two genotypes Mexipake and Manel decreased their contents by 1/5 and 1/8 Respectively from the previous stage. The contents range from 1.52 ± 0.38 to 21.23 ± 1.27 µmol / mg DW. The minimum value is recorded in the Mexipake genotype, and the maximum value is always recorded in Saida barley

(Figure 4B). For the WHD batch, grades fluctuate between 11.42 ± 0.98 and 18.58 ± 0.63 µmol / mg DW, Mexipake soft wheat still has the lowest value at 40% F.C., while Whereas the maximum value is recorded this time in soft wheat Florence aurora. Only the two Mexipake and Manel genotypes show a high rate of increase compared to the control. It is 7.53 times and 6.02 times. The other genotypes showed increases in the order of 1.59times, 1.28times, 2.40times, 3.97times in Bidi 17, Dk, Fa, respectively, compared to the control. Barley Saida marked a decrease rate of 0.70 times compared to the control.

The statistical study reveals a very highly significant difference between the two tests (stressed and control) and between the varieties and their interaction. The SNK test shows that at this stage the stressed lot has reacted unlike the previous stage: S2 > T ⇔ 14.34 > 8.80 . And classifies the variety factor in five different groups: Saida> Bidi17> Fa> Dk> Manel; Mexipake

At the last stage, the contents increase considerably and show higher values compared to the two previous stages in the two WIHD and WHD batches. They vary widely, fluctuating between 57.41 ± 2.25 and 31.34 ± 1.15, the maximum value of which is accounted for by durum wheat Dk, but the minimum value is recorded for barley Saida at the level of Stressed samples (Figure 4C). At WIHD Plants, soluble sugars increase in most of the genotypes studied except for Saida barley, which retains the same value of the stage End upstream-Start swelling. The amplitudes of the fluctuations are between 16.52 ± 2.48 and 35.35 ± 3.38 µmol / mg DW in the two genotypes Bidi 17 and Florence Aurora respectively. This implies that the content of soluble sugars increases correlatively with the course of the stage in all the studied genotypes.

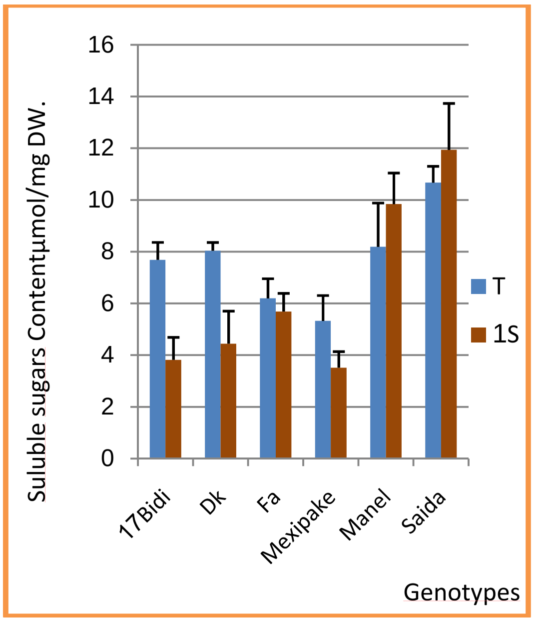
The statistical study shows that there is a very significant difference between the two tests, between the varieties and even their interaction. The SNK statistical test reaffirms that the stressed batch provided double the soluble sugars than the controlled batch: S2 > T ⇔ 43.26 > 21.85. The SNK group shows the presence of four groups: Fa > Dk > Manel > Saida; Mexipake ; Bidi17.

Stressed plants react by increasing amounts of sugars in their cells. This increase is actually a biochemical marker of adaptation to the hydric stress condition (Apel et al., 2004; Chen et al., 2004; Chaib et al., 2006).

Depending on the kinetics of the soluble sugar content in the studied genotypes in the three stages studied, we conclude that, at the End tiller- Start up Stream stage, the two varieties of barley Saida and Manel have large amounts of soluble sugars. This accumulation may result from another type of thermal stress, for example. At the second stage End upstream-Start swelling, both varieties of Bidi 17 and Florence dawn seem to be tolerant and show values of 18.27 ± 0.19 and 18.58 ± 0.63 µmol / mg DW respectively. At the last stage, End heading and Start flowering, all varieties express their strong accumulations; mainly the three genotypes Djenah El Ketayfa, Fa and Manel which are highly influenced by water stress. They accumulate more proline to confront the lack of water at this critical time, phase of the formation of the reproductive organs or the metabolism of the plant requires a lot of energy. In wheat, many studies show that high levels of K +, proline, soluble sugars at different stages of growth are responsible for good resistance to drought (Apel et al., 2004; Chen et al., 2004; Chaib et al., 2008).

Leaf area

The flag leaf is the main donor organ of the photosynthesis necessary for the development of the grain of wheat. The lifetime of this leaf is estimated by its green surface, which appears as a developer of the functioning level of the photosynthetic apparatus in the presence of water deficit (Gate et al., 1990). The leaf



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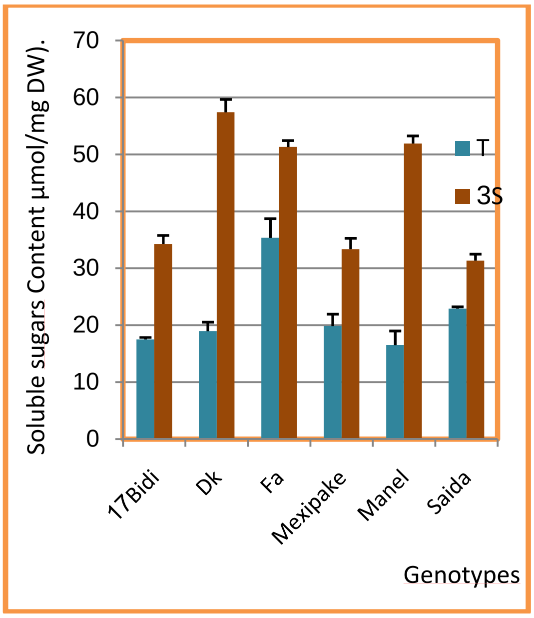

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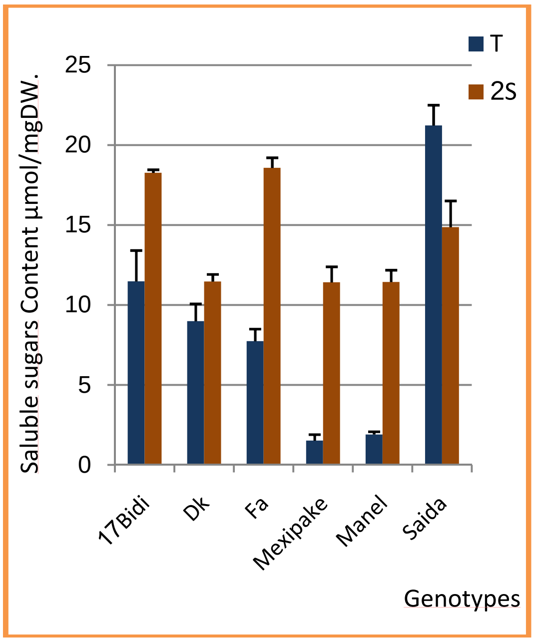

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Figure 4. Soluble sugar content in the six genotypes studied in phases. **A**, End tiller-Start up Stream; **B**, End upstream-Start swelling; **C**, Start heading (durum wheat), End heading (barely), and start flowering (soft wheat).

area is determined by phenology, stem morphology, the average leaf emergence and leaf water potential level (Blum, 1996). The latter, adds that the variation of the leaf area is an important mean, stressed plants tend to maintain control of the use of water.

At the stage End tiller-Start upstream, the leaf area in the three varieties Bidi17, FA and Manel increases by 1.16 times and 1.08 times, 1.34 times respectively compared to the control, whereas the varieties which mark a decrease in their leaf area are both wheat Dk and Mexipake with a decrease rate of 0.78 times and 0.86 times respectively. However, barley Saida showed no change with 21.61 ± 2.50 cm² and 21.05 ± 6.26 cm² on both treatments respectively (Figure 5A). For WHD plants, the values were 16.76 ± 5.16, 27.67 ± 5.81cm² respectively for the two FA and DK genotypes. Authors suggest that a reduced leaf area may be beneficial because it effectively reduces the total water loss of the plant (in Gummuluru et al., 1989).

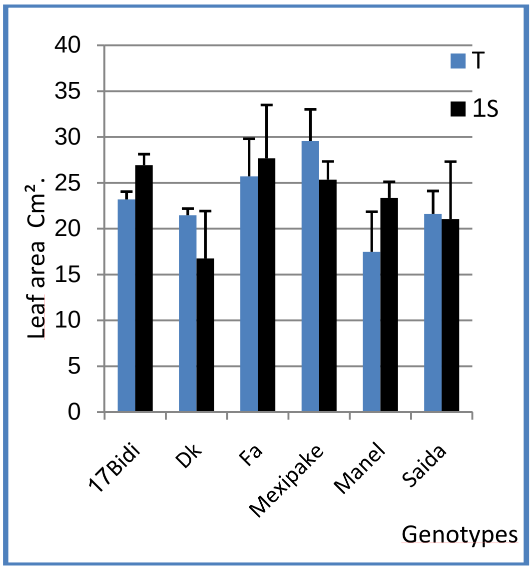
Whereas Johanson et al. (1983) conclude that plants with larger leaf area can tolerate dehydration by maintaining high water potential. The variance analysis of the results obtained reveals no significant difference between the two treatments: S1 =T ⇔ 23.51= 23.17. But the SNK test separates the varieties into three groups: Mexipake; Fa; Bidi17> Saida; Manel> Dk.

At the stage End upstream-start swelling, an increase in leaf area is observed at this stage compared to the previous stage, either for WIHD or WHD plants, due to a morphological modification of the aerial biomass following its development. The order of decrease in the five genotypes is almost similar; 0.93times, 0.83times, 0.88times, 0.73times and 0.87 times in Bidi 17, Dk, FA, Mexipake and Manel respectively. But for Saida barley no change is observed (Figure 5B).

Our results agree with those of Salma et al. (2005), which conclude that the plant's response to drought takes many forms, among which the most visible is the reduction of the leaf area as a consequence to a decrease in Number and size of leaves. The analysis of the variance reveals the existence of a significant difference between the levels of stress and between the varieties and their interactions. The SNK test classifies the test factor into two different groups: T > S2⇔ 33.69 > 29.29 and distinguish the varieties each of which a group except the two varieties of barley falls into the same group: Fa > Mexipake > Bidi17 > Dk> Manel; Saida

According to Granier and Tardieu (2000), during a water deficit, the plant reacts by the reduction of its aerial biomass, especially the surface of its last leaf; While Salma et al. (2005) suggest that larger plants can tolerate dehydration and can maintain high water potential. In the final stage, WIHD plants ranged from 19.43 ± 2.45 cm² in Saida barley to 37.99 ± 3.23 cm² in Mexipake soft wheat (Figure 5C). For the WHD batch the valuesfluctuate between 17.66 ± 0.20 and 21.93 ± 1.33) cm², the minimum is marked by FA, while the maximum by Bidi 17. The statistical study reveals a very high difference between the two tests (stressed and control) and between the varieties and their interactions. The SNK test classifies the test factor into two different groups: T > S3 ⇔ 28.79 > 19.69 and classifies the variety factor into three different groups: Mexipake ; Dk ; Fa > Bidi17 > Manel; Saida.

By comparing the evolution of the leaf area of the six varieties during the three stages, we conclude that the leaf area increases in the WIHD irrigated plants according to the stage of development of the life cycle (Start tiller to start swelling), then begins to shrink into heading stage and start flowering. This can be explained by the phenomenon of the protection of the reproductive organs. Only the Dk variety retains a surface similar to the last two stages. Overall, there is a reduction in foliar area in WHD plants compared to WIHD plants. But, it does not exceed half. At the end of the study, wheat leaves (Bidi 17, Dk, FA and Mexipake) are almost the same size (long and thin) compared to the leaves of both barley (Manel and Saida) which are small and large (Granier & Tradieu, 2000) reported that leaves of water-deficient plants usually reach smaller apparent final sizes. This was observed in barley Saida, which did not record any change during the three stages. The effect of drought may result, according to the adaptive strategy of each species or variety, by morphological changes to increase the absorption of water and / or to reduce transpiration and the competition between the organs for the assimilated. These changes affect the aerial or subterranean part: reduction of leaf area and number of sizes, leaf winding (Salma et al., 2005).



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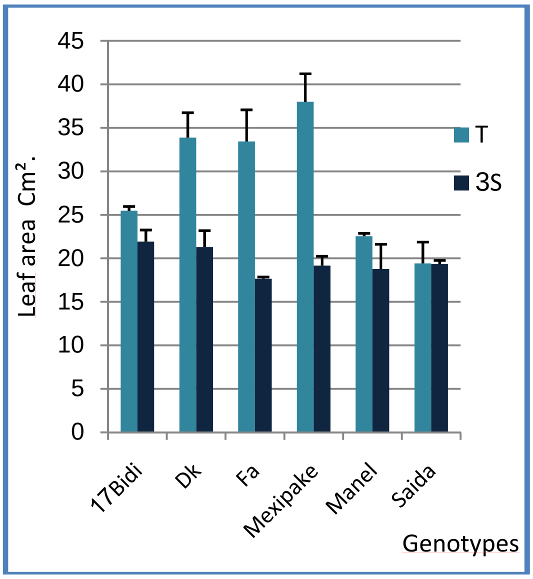

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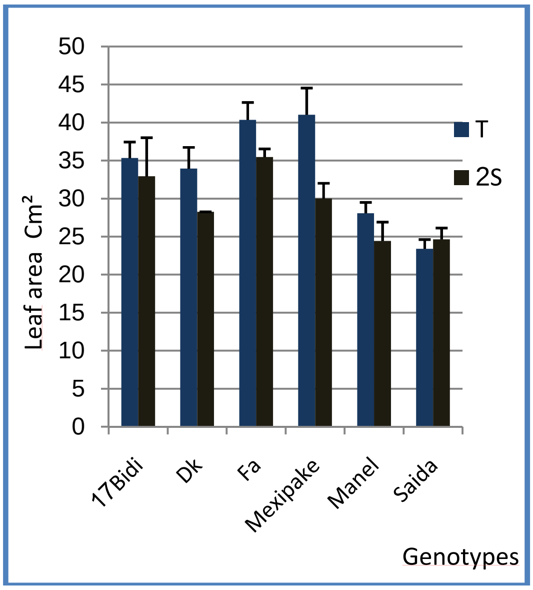

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Figure 5. Leaf area in the six genotypes studied in phases. **A**, End tiller-Start upstream; **B**, End upstream-Start swelling; **C**, start heading (durum wheat), End heading (barley), and Start flowering (soft wheat).

Conclusion

The interest of this work is to evaluate the impact of a water deficit applied at different stages of development in two types of cereals (Wheat and Barley). The aim is to characterize the effect of this stress on the morpho-physiological and biochemical behavior of plants under deficient conditions. The results seem to vary between the different stages and reveal variability between the different varieties. In our study, we found only the proline (good marker) level that was significantly higher than the control indicating some metabolic disturbance that follows the intensity of the water stress and the stage at which it is applied. The estimated results show that genotypes of durum wheat are the varieties that tolerate this lack of water. They retain a relatively high water content during the three stresses, their total chlorophyll content differs from one stage to another, but the Bidi17 variety is influenced by this marker rather than the DK variety. These two varieties of hard wheat feel the stress when it is installed and begins to accumulate proline (the highest level in Bidi17 during all three stages). The effect of the stress of these two genotypes on the soluble sugars was not remarkable, Bidi17 had accumulated sugars during the second stress, whereas the Dk variety at the heading stage. A slight decrease in leaf area in these two genotypes but the Dk variety is marked by this parameter more than Bidi17. The two varieties of soft wheat recorded a significant decrease in relative water content, especially for the Mexipake variety, and were marked by the total chlorophyll marker (Fa mainly). The latter was markedly stressed and accumulated proline during the First and last stage. They synthesize soluble sugars but in average quantities compared to other varieties. The leaf area is marked by a strong decrease in flowering stage it may be due to a leaf rolling noticed in these tender wheats. For the barley, the two varieties indicate a simple fall in water content, this decrease is clear in Saida during the last two stresses. The total chlorophyll labeling only influenced the last stage. A strong accumulation of proline in the Saida variety which exceeds 15 times the initial value of the control during the onset of stress, and the heading stage seems most marked for the two varieties which accumulate proline in large quantities. A strong accumulation of sugars is also recorded at the same stage. And the only marker that has not changed anything for barley is the leaf area (no change at this level). So it is concluded that durum wheat varieties and Saida barley seem to be the most affected by this stress during all three stages. The phenological stage, the most affected by a marked modification for all the parameters and especially the genotypes studied, is the last stage (heading-flowering). It seems to be the most critical. Consequently, the plant reacts adequately to applied hydric stress and controls its metabolic functions as a result of the governing conditions in the culture medium in order to tolerate the stress of water deficit.

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