Comparative Study of the Biochemical and Physiological Mechanisms of Two Varieties of Durum Wheat (Triticum durum L.) Subject to Salt Stress

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Abstract

Background: To study the effect salt stress on two varieties of durum wheat (Triticum durum Desf.) "GTA dur" and "Simeto" variety. **Methods:** Seedlings of each variety were subjected to 0, 25, 50, 75, 100, 250 mMNaCl. Germination rate, leaves and roots length, protein contents, Relative Water Contents (RWC), sugars and proline concentrations were determined for seedlings treated with salt after 3, 6 and 9 days. **Findings:** Salt stress has caused morphological and physiological changes in leaves and roots. With the increase of salt concentrations and processing time, the germination rate, contents of total proteins, leaf and roots length and relative water content in the leaves and root of durum wheat were reduced. Sugars and proline levels in the leaves had shown an accumulation with the increase of salt concentrations and processing time, the germination in high concentrations of NaCl solution (100 mM). **Improvements:** Wheat seedlings when subjected to salt stress have developed a set of adaptive mechanisms such as morphological, physiological and biochemical changes, which enable them to perceive and respond specifically to different constraints.

Keywords: Germination, Proline, Salt Stress, Soluble Sugars, Triticum durum

1. Introduction

Wheat is an important food crop world-wide. Soil and water salinity have been considered as a major environmental stresses affecting the performance of many crop plants and limiting factor to crop production in arid and semiarid regions of the world¹. In Algeria, there approximately 3.2 million ha are currently threatened by salinity. Because of the developing area of salt-affected land, salinity becomes an everlasting challenge to agriculture and food supply².

Unfortunately, most of crop plants cannot grow in high salt concentrations³. The consequence of all these ultimately leads to inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis and disturbs nucleic acid metabolism in wheat⁴.

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Salinity effects on plants include two distinct types of stress: water stress, caused by the greater difficulty of water absorption, and ionic stress, related to the sodium ion effect on the diverse cellular functions, decreased nutrient absorption, enzyme activities, photosynthesis and metabolism⁵⁻⁹.

Decreased photosynthetic rates may result from the closure of stomata and decreased mesophyll conductance, induced by osmotic stress, or from salt-induced damage to the photosynthetic apparatus¹⁰.Physiologically, salinity can be considered as a complex case of stress, comprising osmotic and salt-specific stresses².In plant cells chloroplasts, mitochondria and peroxisomes are important intracellular generators of ROS¹¹. It is now widely accepted that reactive oxygen species (ROS) are responsible for various stress-induced damage to

macromolecules and ultimately to cellular structure^{12,13}, and needs to be scavenged for maintenance of normal growth.

Wheat is a major food in the most countries where salinity problems exist or might develop. Large areas of formerly arable land are being removed from crop production every year due to increasing soil salinity. Also, most of the crop plants like wheat are glycophytes, which are sensitive to even low salt concentrations.

The objective of this work is to evaluate the sensitivity and tolerance at the germination first, and then at the vegetative stage of seedlings of two durum wheat varieties including "GTA dur " and " Semito " cultivated in Algeria , to determine their behavior in response to increasing salt stress in order to improve their productivity in areas where they are cultivated.

2. Materials and Methods

The experiment is conducted on grains of durum wheat (*Triticum durum* Desf.). These two durum wheat genotypes. "Semito" and "GTA dur" were provided by the Algerian Office Inter Cereals (AOIC) El Hadjar Annaba, Algeria. Grains of each variety were disinfected with bleach to 3% for a few minutes and rinsed with distilled water to remove all traces of chlorine. After 24 hours of soaking in distilled water, the seeds are put in germination in Petri dishes on sterile Gases (10 seeds per dish), soaked in 10 ml of irrigation solutions. The application of saline treatment begins at 2-3 leaf stage, with different doses of NaCl: 25, 50, 75 and 100 mM for 3, 6 and 9 days.

2.1 Germination Percentage

The counting of germinated seeds and the cumulative percentages of germination were performed every 3 days for 10 days. A seed is considered germinated when the radicle becomes visible¹⁴. Thus, the rate of germination (% G) at a given time is expressed by the ratio of the number of germinated seeds on the total number of seeds sown.

n = number of germinated seeds and N = total number of seeds sown

2.2 Leaves and Roots Length

At the end of the treatment, the measurement of the length of the aerial part and root of the seedlings is carried out with a scale.

2.3 Determination of Soluble Proteins

Determination of Soluble Proteins The method of ¹⁵, was used to determine the concentration of soluble proteins in wheat leaves with BSA as standard. Absorbance was recorded at 595 nm. Soluble proteins were expressed as mg.g⁻¹FW.

2.4 Determination of Relative Water Content (RWC)

The RWC was determined in fresh leaf discs of 2 cm² diameter; discs were weighed quickly and immediately floated on distilled water in Petri dishes to saturate them with water for the next 24h, in dark. The adhering water of discs was blotted and tugor mass was noted. Dry mass of the discs was recorded after dehydrating them at 70°C for 48h¹⁶. RWC was calculated by using the following formula:

2.5 Determination of Soluble Sugars

The total soluble sugars (sucrose, glucose, fructose, their methyl derivatives and polysaccharides) are assayed by the method¹⁷. The absorbance was read at a wavelength of 485 nm.

2.6 Determination of Proline Content

The method of¹⁸, was used to determine the concentration of proline in wheat leaves. Absorbance was measured at 528 nm by spectrophotometer Jenway 3600. The proline concentration in the sample was determined from a standard curve using analytical grade proline and calculated on fresh weight basis (mg.g⁻¹FW).

2.7 Statistical Analysis

The statistical analysis and Analysis of variance (ANOVA) was performed using GraphPad prism (V5.0) software, followed by multiple comparison test Student-Newman-Keul. Asterisks indicate that differences from control values were statistically significant at p<0,05.

3. Results

3.1 Germination Rates (%)

The results' analysis shows that the concentration of NaCl in the medium affects the germination rate of both studied species" GTA dur " and " Semito ". The results



Figure 1. Germination rate of two hard wheat variety "GTA dur " and " Semito " Under salt stress. Asterisks indicate that differences from control values were statistically siniicantatp<0,05.



Figure 2. Length of leaves and roots of the variety of durum wheat «GTA dur " under salt stress. Asterisks indicate that differences from control values were statistically siniicantatp<0,05.

are shown in Figure 1. This influence is confirmed by the analysis of variance with one classification criterion whose difference-in-mean is highly significant (p< 0.001) for both durum wheat varieties "GTA dur" and "Semito ". Compared with the control, we see that the germination rate decreases significantly at the 50 mM dose. For the treatment with the highest concentration (100 mM), the germination rate goes from 93.33 % for the control to 26.66 %, and from 90% to 13.33% for "GTA dur" and "Semito" respectively. The seeds germination rate significantly decreases as alt concentration increases.

3.2 Leaves and Roots Length

Figure 2 shows the change in the length of the leaves of two varieties of Durum wheat subjected to salt stress. The analysis of variance (ANOVA) with one classification criteria, shows a very highly significant decrease (p <0.001) for both wheat varieties.

The NaCl application at low doses (25 mM), gave an insignificant effect for the "Semito" variety. Whereas for GTA hard" variety», the treatment shows a highly significant result compared to the control, there has been a difference of 1.67 cm treatment with the same dose (25 mM).Under the effect of salt stress at the highest concentrations (75 mM and 100 mM) there was a very highly significant decrease (p <0.001) for both durum



Figure 3. Rates of protein in the leaves and roots of two varieties of durum wheat "GTA dur " and " Semito " under salt stress after 3, 6 and 9 days of treatment.

wheat varieties. Leaf length decreases from 16.53 cm and 15.86 cm, average value of the unstressed control at 3.56cm and 2.76 cm, average value measured under stress 100 mM NaCl, for GTA hard and Semito variety respectively.

If salt stress influences the growth in length of the aerial parts of "Semito" and "GTA dur", it also affects their underground parts. The results are illustrated in Figures 2; this fact is confirmed by the analysis of variance ANOVA one whose difference very highly significant (p < 0.001).

Our results emphasize that the root length growth for the seeds treated with a low concentration of NaCl (25 mM), seems indifferent to salt stress and shows no significant difference vis-a-vis the low level of salinity. Whereas when increasing the level of salinity, a decrease in root length is observed for both varieties compared to the control. Root length shifts from 15.66 cm and 14.6 cm (control) to 3.2 cm and 3.63 cm with 75 mM dose, and 1.06 cm and 2.76 cm with 100 mM dose in GTA dur and Semito varieties respectively.

3.3 Soluble Proteins

Figures 3 show the protein content of aerial and underground parts of two durum wheat varieties "GTA dur" and "Semito" after 3, 6, 9 days of treatment. we can observe that the salt stress causes a decrease in protein content in leaves and roots of the two varieties but the underground parts rate is lower than the upper parts.

In the "GTA dur" variety, the rate of proteins in the leaves (Figure 3) after 3 days of treatment, increases slightly compared to the control in a very highly significant way (p <0.001) with the saline treatment of 25 mM. However, salt stress reduces proteins content for high concentrations.

The analysis of variance of two classification criteria (Dose x Time) shows that there is no significant difference (p> 0.05) for the rate of proteins in the leaves between the 3rd and 6th day of treatment, but it is very highly significant (P < 0.001) between the 6th and 9th day.

According to the result acquired in the Figure 3, the protein content among the "GTA dur" variety subjected to salt stress, was 3.42 mg.g⁻¹, 3.24 mg.g⁻¹ and 1.77 mg.g⁻¹ for 3, 6 and 9 days of treatment with 100 mM.

Among "Semito" variety, the analysis of variance of only one classification criteria ANOVA shows that there is no significant difference (p> 0.05) in the rate of protein in leaves compared with the control for the 3rd day. After 6 and 9 days, the ANOVA shows a very highly significant decrease (P <0.001).

According to the results in Figure 3, the protein rate varies from 6.10 mg.g⁻¹ for control of 4.76 mg.g⁻¹, and 3.46 mg.g⁻¹ for treatment with 75 and 100 mM, respectively, after 3 days of treatment. While it varies from 6.68 mg.g⁻¹



Figure 4. Relative water content of leaves and roots for both durum wheat varieties " GTA dur " and " Semito " after 3 , 6, 9 days of treatment.

¹ in the control of 1.01 mg.g⁻¹, and 1.14 mg.g⁻¹ for the treatment with 75 and 100 mM respectively, in the 9th day.

Salt stress affects the protein rate of "GTA dur" and "Semito" underground parts in the same way it affected their aerial parts (Figure 3B). The analysis of variance with two classification criteria (dose x time) to showed a very highly significant decrease (p <0.001) for the "GTA dur" variety, and anon-significant decrease (p> 0.05) for the "Semito" variety.

In the "GTA dur" variety, variance analysis with single classification criteria is significant for the dose 50 mM. Roots protein levels revealed a highly significant result for 75 mM and very highly significant for the 100 mM dose. For the 9th day treatment, there is a highly significant result for the 100 mM dose.

The obtained result indicates that the protein content decreases by increasing the dose and the treatment time. 5.34 mg.g^{-1} , 5.47 mg.g^{-1} and 4.76 mg.g^{-1} is recorded for the control which reaches 3.24 mg.g^{-1} , 2.97 mg.g^{-1} and 1.28 mg.g^{-1} for 100 mM dose , after 3, 6 and 9 days of treatment, respectively.

Whereas the analysis of variance with one classification criterion for Semito variety is not significant compared to the control for the 3rd day of treatment, is significant for the 6th day, and the 9th day very highly significant. According to the result acquired in Figure (3B), the rate of proteins registered in the control of Semito variety is 4.13 mg.g⁻¹, 4.22 mg.g⁻¹ and 4.36 mg.g⁻¹, which reaches 3.11 mg.g⁻¹, 2.48 mg.g⁻¹ and 2.66 mg.g⁻¹ under salt stress with the highest concentration (100 mM) for 3, 6 and 9 days respectively.

3.4 Relative Water Content (RWC)

Figure 4 shows that the relative content of both durum wheat varieties "GTA dur" and "Semito" after 3. 6 and 9 days of treatment in aerial and underground parts. It varies by decreasing under the effect of salinity.

After 3 days of treatment the results of relative water content in leaves shows that in the GTA variety, it increased a highly significantly, it passes from 59.57% to 81.2% for the saline treatment which means an increase of 36.31 %. From 50 mM dose on, analysis of variance shows a non-significant result. For the 100 mM dose, the stress intensity seems more aggressive, and ANOVA shows a very highly significant decrease, where a relative water content of 39.97%, 32.23% and 30.41% is recorded compared to the control after 3, 6 and 9 days of treatment.

The same results are recorded in the "Semito" variety. After 6 and 9 days of treatment with the lowest dose (25 mM) the analysis of variance is not significant. The water relative content is 82.89% and 86.8% for the control and 85.71% and 86.24% for treatment with 25 mM after 6 and 9 days of treatment. This value regresses a very highly significantly from the 50 mM dose. After nine days of treatment, it goes from 86.81% in the control to 74.85%, 50.04 % and 23.92 %, with the doses 50, 75 and 100 mM, respectively, representing a reduction of 13.77 %, 42.35 % and 72.44%.

3.5 Rate of Total Sugars

Figures 5 shows the content of total sugars of aerial and underground parts of two varieties of durum wheat "GTA dur" and "Semito" after 3, 6, 9 days of treatment.

In the "GTA dur" variety, sugars level in the leaves (Figure 5A) with 25 mM salt treatment was not significant after 3, 6, 9 days of treatment. However, there is a very highly significant increase from the dose of 50 mM for the three studied times. According to the result acquired in the figure, the sugar rate in the control of the "GTA dur" variety records 0.0684 mg.g⁻¹, 0.0745 mg.g⁻¹ and 0.0741 mg.g⁻¹, and reaches 0.16 mg.g⁻¹, 0.21 mg.g⁻¹, 0.19 mg.g⁻¹ with 100 mM treatment after 3, 6 and 9 days respectively.

For roots, we notice an increase from the 25 mM dose, where 0,051 mg.g⁻¹ is recorded, after 3 days of treatment to reach 0,062 mg.g⁻¹ after 6 days of treatment. Also there is a very highly significant increase for 75 mM and 100 mM doses. The sugars rate goes from 0.060 mg.g⁻¹ to 0,072 mg.g⁻¹ with 75 mM dose, and from 0.092 mg.g⁻¹ to 0,187 mg.g⁻¹ with 100 mM dose for 6 and 9 days respectively.

Among the Semito variety the sugars level in the leaves reveals a non significant result compared to the control after 3 days of treatment. After 6 and 9 days we notice a very highly significant increase for the analysis of variance with a single criterion. We notice that the control, passes from 0,071 mg.g⁻¹, 0,069 mg.g⁻¹ to reach 0,093 mg.g⁻¹, 0,116 mg.g⁻¹ and 0,102 mg.g⁻¹ 0,105 mg.g⁻¹ in treatment with 75 mM and 100 mM doses after 6 and 9 days of treatment respectively. After a treatment of 3 days, a very highly significant decrease in sugars rate is noted with 25 mM dose in roots, where it passes from 0.067 mg.g⁻¹ of control to 0.055 mg.g⁻¹ and continues until the 9th day.

3.6 Proline Rate

The Figure 6 shows the proline content of aerial and underground parts of two durum wheat varieties "GTA dur" and "Semito" after 3, 6, 9 days of treatment.

The analysis of variance with one criterion for proline level in the leaves in the "GTA dur" variety (Figure 6A), showed a very highly significant reduction compared to control (p < 0.001). After 3 days of treatment with 25 mM NaCl, proline rate passes from 7.19 mg.g⁻¹ of control to



Figure 5. Rate of total sugars in the leaves and roots for both durum wheat varieties under salt stress after 3, 6, 9 days of treatment.



Figure 6. Proline levels in leaves and roots of two varieties of durum wheat under salt stress after 3, 6, 9 days of treatment.

reach 4.66 mg.g⁻¹. However, for the highest concentration of salt stress, there is a very highly significant increase in proline level where, 10.51 mg.g⁻¹ of proline is recorded and accumulated in the leaves.

For roots, analysis of variance shows a decrease of proline rate for the 3rd day, but after 09 days of treatment we notice a highly significant increase for the three treatment times. The proline level is more remarkable in the leaves than in the roots in the "GTA dur" variety.

Among the variety "Semito", there is a very high reduction of proline level in the leaves after 3 days of treatment. 11.75 mg.g⁻¹ is recorded in the control which reaches 4.55 mg.g⁻¹, 8.30 mg.g⁻¹ and 8.38 mg.g⁻¹ for doses 25, 50 and 75 mM respectively. For the 100 mM dose, the result is not significant. But after 9 days of treatment there is a remarkable increase with this dose. Is recorded a rate of 16.92 mg.g⁻¹ with respect to control of 13.66 mg.g⁻¹.

The proline level in the roots is shown in Figure (6B). We notice a very highly significant increase from the 50 mM dose after 3 days of treatment. 7.65 mg.g⁻¹ is recorded for the control which passes to 10.18 mg.g⁻¹. This increase continues in a very highly significant way for the other doses and for the treatment time, where there is an increase that reaches 11.02 mg.g⁻¹, 12.531 mg.g⁻¹, 12.91 mg.g⁻¹ for the treatment time of 3, 6, 9 days with 100 mM dose compared to the control which records 7.65 mg.g⁻¹, 7.95 mg.g⁻¹, 8.70 mg.g⁻¹ respectively.

We note that the "GTA dur" variety accumulates more proline in its leaves and its roots than the Semito variety.

4. Discussion

Salt stress Limit and slows plant growth through its various parameters: germination, root length and stem, fresh and dry weight of plants by reducing water activity, specific ion toxicity (sodium and chlorine) and the decrease the essential nutrients availability¹⁹. Each treatment applies a heterogeneous effect on *Triticum durum* L seed germination. Germination rates are calculated to better analyze and compare these effects and the influence of these treatments on this parameter.

Germination is an important and vulnerable stage in the life cycle of higher plants. It determines the state of seedlings and plants growth. One of the most widely used methods in the study of salt tolerance of plants is the determination of germination percentage²⁰.

Our results clearly show that the seeds of the species durum wheat (*Triticum durum*) germinate better in the absence of salt or an environment enriched with a low concentration of NaCl (25 mM). Many studies on the germination of salt stress conditions indicate that the seeds of most plant species reach a maximum level of germination in distilled water^{21, 22}.

When the salt concentration increases, a decrease in sprouts rate occurs in the concentration of 50 mM NaCl. However, high salt doses (75 and 100 mM NaCl) produce a strong decrease in the number of germinated seeds (sprouts). This inhibition is more pronounced from 50 mM NaCl for seeds of "GTA dur" variety, compared to seeds of Semito variety that experienced a relatively less inhibition and slowing germination. This well shows that the germination of seeds in the presence of salt stress varies from one species to another.

The decrease of germinated seeds rate subjected to salt stress is related to an osmotic dormancy process developed under these conditions. In addition, the salt retards germination; this delay may be due to the alteration of the seed' hormones and enzymes²³. The enzyme most involved in the plants' germination process is α -amylase, it is inhibited by salt stress by altering its structure²⁴. In *Abelmoschus esculents* L. for example, the final germination percentage is reduced to 20% in the presence of high salt concentrations²⁵.

Azam²⁶, reported that NaCl had a lesser effect on the germination and seedling growth of *Kochia Scoparia*. The drop in the rate of water uptake by the seeds of *K. scoparia* when they were soaked in NaCl solution is probably caused by the decrease in water potential gradient between the seeds and their surrounding media^{26, 27}.

The decrease in germination could be linked to salinity by inducing an imbalance of metabolic processes leading to the formation of phenolic compounds²⁸. In saline conditions, competition and interaction between ions causes nutritional imbalance. The Na⁺ ions reduce ions Ca^{+ 2} and K⁺ availability as well as their transport to different parts of the plant, which affects the structure and composition of the vegetative and reproductive organs²⁹.

The presence of NaCl around the roots causes degradation of certain specific proteins involved in the germination and growth of roots and stems³⁰.

The protein content evolution in the aerial part among both varieties had a variable response in seedlings of both varieties ; if "Semito" displays a non-significant reduction depending on the stress intensity after 3 days of treatment, there is however a very highly significant increase in proteins in "GTA dur". The reduction of the soluble protein content under the effect of salt stress is shown by several authors including³¹, in their work on two varieties of ogre (*Afza*l and EMB2-12), and³², in their work on the tomato variety (*Shirazy*). These authors reported that the salinity induces the decrease of some soluble proteins and that this variation of the protein content does not necessarily confer to the plant a tolerance to salt stress.

To adapt to salt stress, the plant can avoid damages by reducing growth³³. This is the most common effect of abiotic stresses on plants physiology; reducing growth is an adaptive capacity necessary for the survival of a plant exposed to an abiotic stress. Indeed, this delayed development allows the plant to accumulate energy and resources to combat stress before the imbalance between the inside and outside of the organism increases to a threshold where the damages will be irreversible. The growth is inversely correlated with resistance to salt stress of a species / variety³⁴.

Salinity affects all the physiological processes of the plant. Its effect is reflected in particular in reduced height growth of the two varieties of durum wheat. The "GTA dur" variety shows a reduction in the length of the leaves more remarkable than that of Semito variety. The effect of salinity on the growth of leaves in height shows that irrigation with a salt concentrated water causes the shortening of the leaves. These findings were similar to those made by³⁵ on citrus in salt stress conditions, and those shown by³⁶ on cereals. The measurements of the lengths of rods made at the end of the experiment show that the salt stress, even moderate (25 mM) in the "GTA dur" variety, reduces the height growth.

According to³⁷, the depressant action of the salt is manifested by reduced plants height³⁸. The reduction of the seedling growth of both varieties is attributed to a combination of the osmotic effect and the specific effect of Na ⁺ and Cl⁻ ions^{39,40}.

In our results, salinity further reduced the growth of aerial parts of the two varieties of durum wheat compared to that of roots. Similar results were reported by⁴¹. This resistance of the root system of clover to salt stress may be due to a decrease of carbon allocation for leaf growth for the benefit of root growth⁴². The reduction of the growth may also be related to disturbances of growth regulators rates (abscisic acid and cytokinins) induced by salt^{43,44} sometimes to a reduction of the photosynthetic capacity due to a decrease in the stomatal conductance of CO₂ under saline stress⁴⁵.

The relative water content (RWC) or leaf turgidity is a genotypic feature that is related to the ability of the plant to maintain a water level in the leaf which is to guarantee the continuity of the metabolic activity including, among others, the photosynthesis. This ability is related to the possibility for the plant to constantly take up water (root system), to control of water losses by evaporating surfaces (number and diameters of the stomata, stomatal resistance to steam outlet water), and the osmotic adjustment⁴⁶. Several studies show that the water deficit among cereals, in particular durum wheat, affects both stomatal and non-stomatal phenomena of photosynthesis⁴⁷.

All metabolic reactions occur in the aqueous phase. Water participates in metabolic transformations. It is also essential for the functioning of proteins. Besides, living organisms are distinguished by their high water content: it can go up to 90% of the mass or even more in higher plants⁴⁸. Scofield⁴⁹ note that the water content decreases as the stress increases, but it decreases faster among sensitive varieties than among resistant varieties. The (RWC), in addition to its relationship with cell volume, more accurately reflects the balance between the water which is available in the leaf and transpiration rate, osmotic potential and⁵⁰. Akbari et al.⁵¹, Indicate that, high amount of Na+ accumulation and drastic reduction in RWC was found in salt sensitive cultivars of wheat. Tolerance to stress condition defined as an ability of plants to grow in low water potential and in this way, high RWC is one of tolerance mechanisms to stress condition^{51, 52}.

With regard to the soluble sugars, the two varieties confronted salt stress by a strong accumulation of leaf soluble sugars compared to the root. This higher accumulation among "GTA dur", gives a relative performance compared to "Semito" because several authors as⁵³, had shown that soluble sugars accumulate in sunflower varieties that differ in their degree of salinity tolerance; but they also found that tolerant varieties accumulate larger proportions of sugars than sensitive varieties. Kamalraj et al.54, found that sugar contents of leaves decreased in tolerant genotypes of wheat under NaCl stress which had linked to stress tolerance. The increase of sugars level is due to their storage in a complex form reserves of substance, and the reduction in production caused by water deficit causes a sugar levels increase.

Proline, usually having a low rate in plants' tissues that are cultivated on salt-free environment and therefore not binding on the water level, is dramatically accumulated in response to salt stress. Several authors among which^{55–57}, had mentioned that this amino acid is one of osmoticums that plants synthesize when exposed to water or salt stress. Its role is necessary for osmotic adjustment to balance the osmotic potential of the soil in accordance with what has been shown by other studies including those of ^{58,59}. However, a strong accumulation of this amino acid is a sign of metabolic disturbance⁶⁰. The Increase of the soluble sugars content in the leaves at the beginning of the water stress phase is due to the use of these carbohydrates in the proline synthesis through the NADPH consumption. The proline synthesis stimulation by light is due to energy rich components NAD(P)H of photosynthesis. Two (02) molecules of NAD(P)H were used for the synthesis of a proline molecule from glutamic acid⁶¹.

5. Conclusion

The different organs of plant (roots or leaves) are considered as if they all had the same properties, despite the fact that they form an age-structured population and thus are likely to present differences in their functioning and in their reaction to alterations of the environment. In salt treated wheat, the accumulation and remobilization of the major solutes contributing to the osmotic adjustment were shown to be affected by leaf and root tissue senescence.

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7. References

- 1. Denden M, Bettaieb T, Salhi A, Mathlouthi M. Effet de la salinité sur la fluorescence chlorophyllienne, la teneur en proline et la production florale de trois espèces ornementales. Tropicultura. 2005; 23(4):220–5.
- 2. Flowers TJ. Improving corp salt tolerance. Journal of Experimental Botany. 2004; 55(396):307–19.
- 3. Läuchli A, Grattan SR. Plant growth and development under salinity stress. Advances in molecular-breeding towards salinity and drought tolerance. Jenks MA, Hasegawa PA, Jain SM, editors; Springer-Verlag; 2007.
- 4. Levine RL, Garland D, Oliver C. Determination of carboxyl content in oxidatively modified proteins. Methods in Enzy-mology. 1990; 186:464–78.

- Zhu JK. Plant salt tolerance. Trends in Plant Science. 2001; 6:66–71.
- 6. Ashraf M, Shahbaz M. Assessment of genotypic variation in salt tolerance of early CIMMYT hexaploid wheat germplasm using photosynthetic capacity and water relations as selection criteria. Photosynthetica. 2003; 41:273–80.
- 7. Sayed OH. Chlorophyll fluorescence as a tool in cereal crop research.Photosynthetica. 2003; 41:321–30.
- 8. Kao W Y, Tsai T T, Tsai H C, Shih C N. Response of three Glycine species to salt stress. Environmental and Experimental Botany. 2006; 56:120–5.
- Hajlaouia H, Denden M, Bouslama M. Effet du chlorure de sodium sur les critères morpho-physiologiques et productifs du pois chiche (Cicer arietinum L.). Ann. INRGREF. 2006; 8:171–87.
- 10. Flexas J, Bota F, Loreto F, Cornic G, Sharkey TD. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biology. 2004; 6:269–79.
- 11. Rich PR, Bonner WD. The sites of superoxide anion generation in higher plant mitochondria. Archives of Biochemistry and Biophysics. 1978; 188:206–13.
- 12. Moftah AH, Michel BE. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiology. 1987; 83:238–40.
- Kandpal RP, Vaidyanathan CS, Udaykumar M, Krishnasastry KS, Appaji-Rao N. Alternation in the activities of the enzyme of proline metabolism in ragi (Eleusine coracana) leaves during water stress. Journal of Biosciences. 1981; 3:361–9.
- 14. Mbaye N, Diop AT, Guèye M, Diallo AT, Sall CE, Samb PI. Etude du comportement germinatif et essais de levée de l'inhibition tégumentaire des graines de Zornia glochidiata Reichb. Ex DC., légumineuse fourragère. Revue Élev. Méd. vét. Pays trop. 2002; 55(1):47–52.
- 15. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976; 72:248–54.
- Clarke JM, Mc Caïg TN. Excised-leaf water retention capability as an indicator of drought resistance of triticum genotypes. Canadian Journal of Plant Science. 1982; 62:751–8.
- 17. Dubois M, Gilles KA, Hamilton JK. Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956; 28:350–6.
- Troll W, Lindsley J. A photometric method for the determination of proline. Journal of Biological Chemistry. 1955; 215:655–60.
- 19. Carpici E, Clik B, Necmettin B, Bayrame G, Asik B. The effects of salt stress on the growth, biochemical parameter and mineral element content of some maize (Zea mays L.) cultivars. African Journal of Biotechnology. 2010; 9:6937–42.
- 20. Dantas BF, Ribeiro LD, Aragao CA. Physiological response of cowpea seeds to salinity stress. Revista Brasileira de Sementes. 2005; 27:144–8.
- 21. Ghoulam C and Fares K. Effect of salinity on seed germina-

tion and early seedling growth of sugar beet (Beta vulgaris L.). Seed Science and Technology. 2001; 29:357–64.

- 22. Alatar AA. Effect of temperature and salinity on germination of Achillea fragrantissima and Moringa peregrina from Saudi Arabia. African Journal of Biotechnology. 2011; 10(17):3393–8.
- 23. Botia P, Carvajal M, Cerda A, Martinez V. Response of eight Cucumismelo cultivars to salinity during germination and early vegetative growth. Agronomie. 1998; 18:503–13.
- 24. Saboury AA, Karbassi F. Thermodynamic studies on the interaction of calcium ion with alpha-amylase-thermochemical. Actinomycetol. 2000; 362:121–29.
- Dkhil BB, Denden M. Salt stress induced changes in germination, sugars, starch and enzyme of carbohydrate metabolisme in Abelmoschus esculentus L. (Moench) seeds. African Journal of Agricultural Research. 2010; 5(12):1412–18.
- Borzouei A. Partitioning water potential and specific salt effects on seed germination of Kochia scoparia. Indian Journal of Science and Technology. 2012; 5 (1):1907-1909. doi: 10.17485/ijst/2012/v5i1/30954.
- 27. Katembe WJ, Ungar IA and Mitchell JP. Effect of salinity on germination and seedling growth of two Atriplex species (Chenopodiaceae). Annals of Botany. 1998; 82:167–75.
- 28. Ayaz FA, Kadioglu A, Turgut R. Water stress affects on the content of low molecular weight carbohydrates and phenolic acids in Ctenanthe setosa (Rose.) Eichler. Canadian Journal of Plant Science. 2000; 80:373–78.
- 29. Cerda A, Martinez V. Nitrogen fertilization under saline conditions in tomato and cucumber plants. Journal of Horticultural Sciences. 1988; 63:451–58.
- Khan MA, Gulzar S. Germination responses of Sporobolus ioclados : a saline desert grass. Journal of Arid Environments. 2003; 53:387–94.
- Khosravinejad F, Heydary R, Farboodnia T. Effect of salinity on organic solutes contents in barley. Pakistan Journal of Biological Sciences. 2009; 12(2):158–62.
- 32. Amini F, Ehsanpour AA, Hoang QT, Shin JS. Protein pattern changes in tomato under in vitro salt stress. Russian Journal of Plant Physiology. 2007; 54(4):464–71.
- 33. Lamzeri H. Réponses écophysiologiques de trois espèces forestières du genre Acacia, Eucalyptus et Schinus (A. cyanophylla, E. gomphocephala et S. mölle) soumises à un stress salin. Thèse de magistère en Ecologie et Environnement. Option : Ecologie végétale; Université Mentouri Constantine; 2007.
- 34. Bois G. Ecophysiologie de semis de coniféres ectomycorhizés en milieu salin et soclique. Thèse de doctorat; 2005.
- 35. Fattoum B M. Evaluation de la tolérance au stress salin de certains porte-greffes de citrus. Mémoire de diplôme des études approfondie de l'INAT; Tunis; 2003.
- Naceur B M, Rahmoune C, Sdiri H, Meddahi ML, Selmi M. Effet du stress salin sur la croissance et la production en grains de quelques variétés maghrébines de blé. Sécheresse. 2001; 12:167–74.
- 37. Ahmed BH, Arafet M, Zid E. Tolérance à la salinité d'une Poaceae à cycle court: la sétaire (Setaria verticillata L.). C.R.

Biologies. 2008; 331:164-70.

- Singh A, Prasad R. Salt stress growth and cell bound enzumes in Arachis hypogea L. seedling. International Journal of Integrative Biology. 2009; 7(2):107–23.
- 39. Turan MA, Turkmen N, Taban N. Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. Journal of Agronomy and Crop Science. 2007; 6(2):378–81.
- 40. Taffouo VD, Wamba FO, Youmbi E, Nono G, Amougou A. Growth, yield, water status and ionic distribution response of three bambara groundnut (Vigna subterranea L.) landraces grown under saline conditions. International Journal of Botany. 2010; 6(1):53–8.
- 41. Dubey RS, Singh AK. Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolising enzymes in rice plants. Biologia Plantarum. 1999; 42:233–9.
- 42. Brungnoli E, Bjorkman O. Growth of cotton under continuous salinity stress: Influence on allocation pattern, stomatal and non stomatal components of photosynthesis and dissipation of exes light energy. Planta. 1992; 187:335–47.
- 43. Kuiper D, Schuit J, Kuiper PJC. Actual cytokinin concentrations in plant tissue as indicator for salt resistance in cereals. El Bassam N. et al.,editors, Genetic Aspects of Plant Mineral Nutrition; 1990.
- 44. Termaat A, Passora JB, Munns R. Shoot turgor does not limit shoot growth of NaCl affected wheat and barley. Plant Physiology. 1985; 77:869–72.
- 45. Santiago LS, Lau TS, Melcher PJ, Steele OC, Goldstein G. Morphological and physiological responses of Hawaiian Hibiscus tiliaceus populations to light and salinity. International Journal of Plant Sciences. 2000; 161:99–106.
- 46. Araus JL, Alegre L, Ali Dib T, Benlaribi M, Monneveux P. Epidermal and stomatal conductance in seedings of durum wheat landraces and varieties. In physiology Breeding of Winter Cereals for Stressed Mediterranean Environments. INRA Montpellier ed., les colloques. 1991; 55:225–31.
- 47. Ykhlef N. Photosynthèse, activité photochimique et tolérance au déficit hydrique chez le blé dur (Triticum durum Desf.). Thèse de doctorat d'Etat; Université Mentouri Constantine; 2001.
- Richter G. Métabolisme des végétaux. Physiologie et biochimie. Pressepolytechniques et universitaires romandes. 5^{ème}édition; 1993.
- 49. Scofield T, Evans J, Cook MG, Wardlaw IF. Factors influencing the rate and duration of grain filling in wheat. Australian Journal of Plant. 1988; 4:785–97.

- 50. Nouri L. Ajustement osmotique et maintien de l'activité photosyntétique chez le blé dur (Triticum durum Desf.), en conditions de déficit hydrique. Thèse de magister en Biologie Végétale; 2002.
- Ghogdi A , Izadi-Darbandi A and Borzouei A. Effects of salinity on some physiological traits in wheat (Triticum aestivum L.) cultivars E. Indian Journal of Science and Technology. 2012; 5(1):1901–6. doi: 10.17485/ijst/2012/ v5i1/30953.
- 52. Sinclair TR and Ludlow MM. Who thought plants thermodynamics? The unfulfilled potential of plant water potential. Australian Journal of Plant Physiology. 1985; 12:213–17.
- 53. Ashraf M, Tufail M. Variation in salinity tolerance in sunflower (Helianthus annuus L.). Journal of Agronomy and Soil Science. 1995; 174:351–62.
- 54. Kamalraj S, Sridevi S, Gangadevi V, Venkatesan A, Muthumary J. Effect of NaCl on biochemical changes and endophytic fungal assemblages in the leaves of a mangrove, Ceriops roxburghiana Arn. Indian Journal of Science and Technology. 2008; 1(4):1–7. doi: 10.17485/ijst/2008/ v1i4/29233.
- 55. Khedr AH, Abbas MA, Amal AW, Quick P, Gaber MA. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of Pancratium maritimum L. to salt stress. Journal of Experimental Botany. 2003; 54(392):2553–62.
- 56. Claussen W. Proline as a measure of stress in tomato plant. Plant Science. 2005; 168:241–48.
- 57. Debnath M. Responses of Bacopa monnieri to salinity and drought stress in vitro. Journal of Medicinal Plants Research. 2008; 2(11):347–51.
- Gadallah MA. Effects of proline and glycine betaine on Vicia faba responses to salt stress. Biologia Plantarum. 1999; 42:249–57.
- 59. Demir Y. Growth and proline content of germinating wheat genotypes under ultraviolet light. Turkish Journal of Botany. 2000; 24:67–70.
- M'hamed HC, Abdellaoui R, Kadri KM, Naceur B, Belhadj S. Evaluation de la tolérance au stress salin de quelques accessions d'orge (Hordium vulgare L.) cultivées en Tunisie: Approche physiologique. Sci. Tech. 2008; 28:30–7.
- 61. Adams E, Frank L. Metabolism of proline and hydroxyproline. Annual review of Biochemistry. 1980; 49(1):1005–62.