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Some Physiological and Biochemical Effects of NaCl Salinity on Durum Wheat (*Triticum durum* Desf.)

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Abstract: The main objective of this study was to examine the effects of NaCl on the leaf growth and some physiological and biochemical characteristics of an Algerian durum wheat (var. Waha) landrace. NaCl (150 mM), as compared to control plants, significantly reduced all the leaf-growth parameters as leaf area (33%), leaf dry weight (41%) and leaf fresh weight (32%), but did not induce any change in the leaf succulence and, consequently, in the leaf RWC. On the other hand, NaCl salinity induced the accumulation of proline (2.25 fold, $P<0.05$) and soluble sugars (60%, $P<0.01$) in the leaves of durum wheat seedlings. However, while photosynthetic pigments content do not undergo important change, total carotenoids content decreased (14%) under salt stress conditions. NaCl treatment enhanced significantly the accumulation of H2O2 (4.0-fold, $P<0.01$) and led to lipid peroxidation.

All of these parameters and the interaction among them were analyzed using ANOVA and multiple correlation analysis. The importance of the interactions between osmolytes and antioxidants in determining the salt tolerance of durum wheat was discussed.

Key words: Salt stress % Durum wheat % Proline % Carotenoids % Hydrogen peroxide % Lipid peroxidation

INTRODUCTION

The history of man has been intimately linked to that of plants and more particularly that of cereal straw, which he very soon learned to domesticate, to cultivate and to select [1]. Cereals have great importance in agricultural research programs worldwide [2]. While wheat and barley are the main cereal crops in Algeria, durum wheat is by far the most cultivated grain. However, wheat crop often confront abiotic stresses such as drought, salinity, which are among the most important strength-limiting factors of wheat production particularly in arid and semi-arid areas [3]. Taking into consideration low grain yields, maintaining production has become a key objective of the national strategy for improving grain. This stability could be achieved by identifying mechanisms of tolerance of cereals to various abiotic stresses. A better understanding of the physiological and biochemical mechanisms involved in resistance to salt stress, is essential for improving the salt tolerance of crops.

The presence of salt in the culture medium is likely to reduce the percentage of germination and growth of crops [4, 5]. However, crops do not exhibit the same degree of sensitivity or tolerance to salinity [6]. Many researches have focused on studying the impact of salinity on metabolic processes of plants and several biochemical mechanisms of tolerance were discussed. Indeed, under conditions of stress, plants adjust osmotically their cellular content by synthesizing amino acids such as proline [7]. The active accumulation of compatible solutes such as amino acids, polyamines and sugars appears to be an effective mechanism of stress tolerance [8].

Recently, several studies have reported that salinity cause also reactive oxygen species up-production, which themselves can lead to secondary signals [9]. For instance, salt stress significantly induced the amount of hydrogen peroxide (H_2O_2), which is a signaling molecule involved in many processes of growth and development [10]. To overcome the toxicity of ROS, a complex antioxidant defense system, including enzymatic and non enzymatic compounds, is present in all plant cells [11]. The decrease in the content and the activity of various antioxidants in response to salt stress has been reported in several species [3, 12, 13] and regarded as one of the mechanisms explaining, at least in part, the deleterious effects of salinity on crops. Plants use two systems to defend against and repair damage caused by oxidizing agents. First, the enzymatic antioxidant system which is mainly represented by superoxide dismutase (SOD), peroxidase (PRX), catalase (CAT) and ascorbate

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peroxidase (APX) and then, the non enzymatic system represented by water or fat-soluble low molecular weight compounds such as vitamin C, alpha-tocopherol and glutathione, etc.) [14]. Several studies have demonstrated the existence of a close positive correlation between the rate and extent of the increase in antioxidant activity and plant salt tolerance [15]. It has also been highlighted that improving abiotic stresses tolerance in crops is possible through genetic improvement of its antioxidant systems or following exogenous addition of antioxidants [10].

The purpose of this study was to provide additional information about the effect of NaCl salinity on the growth of leaves and on some physiological and biochemical changes in durum wheat and, also, about the interaction between accumulation of osmolytes and ROS scavenging metabolism.

MATERIALS AND METHODS

This experiment was carried out in semi-controlled chamber, with a photoperiod of 16h, relative humidity of 65%-75% and with ambient room temperature of 20-25°C. After disinfection with sodium hypochlorite (1%) for 10 minutes, an extensive rinse with distilled water and drying, the seeds of durum wheat (var. Waha) were planted in plastic pots (1kg) at 2 cm depth (10 seeds per pot). After the onset of the third leaf, experiment was divided into two groups (n= six) representing two treatments: CG: the control group - irrigated with tap water, SG: the stressed group: irrigated with salt water (150mM NaCl). Two weeks after the application of stress, plants were harvested and the following parameters were measured.

Leaf Area and Water Content: Leaf area (LA) was measured using an area meter (LI-COR, model LI-3000). Relative water content (RWC) was determined according to Barrs [16] cited by Kingsbury *et al.* [17]: *RWC = (FW - DW) x100 / (TW - DW),* with FW: fresh weight, DW: dry weight and TW: fresh weight at full turgidity.

Leaf Succulence (LS), defined as water content per unit area and was calculated by the following formula: (FW – DW)/LA [18].

Chlorophylls and Carotenoids: The content of the chlorophyll a, b and total carotenoids were determined following Higazy *et al*. [19] as previously described [3].

Proline and Water Soluble Carbohydrates Assay: Quantitative determination of free proline content was performed according to Bates *et al*. [20]. However, Water soluble carbohydrates (WSC) were determined according to Dubois *et al.* [21].

Hydrogen Peroxide Assay: The content of H_2O_2 was determined according to Velikova *et al.* [22] cited by Wahid *et al.* [23]. Fresh leaves (0.1 g) was homogenized in 5 ml of 0.1% Trichloro-acetic acid (TCA) and centrifuged at 12,000 rpm for 15 minutes. 0.5 ml of the supernatant is then mixed with 0.5 ml of buffer (Potassium phosphate 10mM pH7) and 1ml of 1M KI. The absorbance reading was taken at 390 nm.

Lipid Peroxidation: LPO was estimated as thiobarbituric acid reactive substances (TBARS) [24]. A fresh sample (0.5 g) was homogenized in 10 ml of 0.1% TCA and the homogenate was centrifuged at 15,000 rpm for 15 min. To a 1.0-ml aliquot of the supernatant, 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA were added. The mixture was heated at 95°C for 30 min and then cooled in an ice bath. After centrifugation at 10,000 rpm for 10 min, the absorbance of the supernatant was recorded at 532 nm in a spectrophotometer. The TBARS content was calculated according to its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed in units (U). One 'U' is defined as µmole of MDA formed min^{-1} mg^{-1} protein.

Statistical Analysis: Data were subjected to analysis of variance, using the ANOVA procedure (P equal 5%) by the means of STATISTICA.6 software. Furthermore, multiple correlation analysis was performed using STATGRAPHICS Centurion XV (following the linear regression models). Data were presented as arithmetic mean ± standard deviation (n = 3).

RESULTS AND DISCUSSION

Growth Parameters: As shown in Table 1, salt stress significantly reduced all leaf growth parameters. In comparison with control plants, leaf fresh weight (LFW), leaf dry weight (LDW) and leaf area (LA) were decreased by about 32%, 41% and 33%, respectively. In contrast, leaf succulence (LS) doesn't undergo any change, which reflects an effective cytoplasmic resistance to salt stress. These results were consistent with other studies conducted about the effect of salt stress on wheat and other cereal crops [25]. On the other hand, it seems that relative water content undergo a slight increase, but not significant, in the presence of NaCl (150 mM) (Table 1). This result is in contradiction with those obtained by Sairam *et al.* [15] and Brini *et al.* [26].

Table 1: Effects of NaCl on the leaf growth parameters, leaf succulence and leaf Relative Water Content

Treatment	LFW (mg)	LDW (mg)	LA (cm^2)	LS (mg/cm^2)	RWC %
T (0 mM)	123.03±15.3	10.97±1.67	8.39±0.50	13.3±0.8	89.39±5.47
NaCl (150 mM)	83.80±24.0	6.5±0.17	5.56±0.28	13.6±1.8	92.40±1.13
F	n.s	21.12 **	68.14 **	n.s	n.s

** highly significant 1%; n.s. non significant

Table 2: Effects of NaCl on some biochemical characteristics of durum wheat

			Chlorophyll (mg/g FW)				

Treatment	Proline (mg/g FW)	WSC (mg/g FW)	a	b	TCC mg/g FW	MDA µM/min*mg	H_2O_2 mM
T (0 mM)	0.51±0.11	45.92±0.85	10.79±1.14	2.89±0.2	183.99±11.73	0.51±0.03	5.46±1.71
NaCl(150 mM)	1.15±0.33	73.82±11.82	11.14±1.43	2.8±0.47	158.62±34.77	0.56±0.04	22.4±6.57
F	10.31*	16.22*	n.s	n.s	n.s	n.s	18.65**

* significant 5%, ** highly significant 1%; n.s. non significant

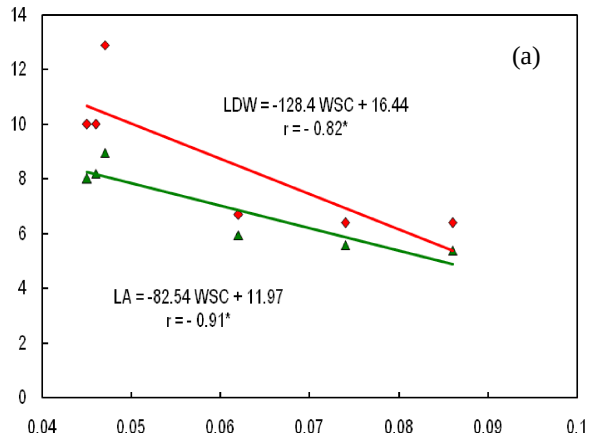
It is widely accepted that exposure to high concentrations of NaCl induced reduction in plant growth and development [27, 28]. The inhibition of leaf growth or leaf elongation appears to be one of the first effects of salt stress [29]. However, physiological and biochemical mechanisms underlying NaCl-induced inhibition of leaf growth are still poorly understood [28, 30].

Leaves, which are the main organs for photosynthesis and transpiration, play an important role in the growth and development of higher plants, especially under stress conditions [28]. Thus, the leaf elongation rate and final leaf size seems to be a key element in plant tolerance to abiotic stresses [31, 32]. Decline in leaf growth under salt stress is due, least in part, to an inhibition of H+-pumping activity and increase in apoplastic pH of leaves [33], or may be attributed to the changes in plant water relations due to stomatal closure, which also induced reduction in enzymatic activity, cell wall expansion as well as leaf expansion [34, 35]. Furthermore, high salinity is known to induce ionic stress (excessive accumulation of toxic ions including Na + and Cl-), which causes senescence and abscission of old leaves, thus reducing the available photosynthetic area [28]. The reduction in leaf area can be caused either by reduced cell division or expansion, since both of them are equally sensitive to environmental stresses [36].

Biochemical Parameters:

Proline and Water Soluble Carbohydrates Content: Proline content was significantly (P#0.05) increased in plants exposed to salt stress, in which it became 2.25 times higher (Table 2). Analysis of correlation prove the existence of a positive and highly significant (P #0.01) correlation (r = 0,964**) between leaf proline and hydrogen peroxide content (Fig. 1b). Similarly, in our experience, as shown in Table 2, NaCl induced a highly significant (P #0.01) increase of WSC in leaves of stressed plants, in which case the sugars content suffered an increase of 60% compared to control plants. Similar results were reported by Baka *et al.* [37] on wheat, Garg *et al.* [38] on rice and Khelil *et al.* [39] on tomato. Furthermore, correlation analysis showed (Fig. 1a) the existence of significant negative correlations between the content of soluble sugars and leaf growth parameters (FW, DW, LA) with coefficients of correlation of -0,88*, -0,82* and -0,91* respectively.

Osmotic homeostasis is one of the mechanisms adopted by plants to overcome salt stress [40]. To achieve this, plants must maintain a sufficient supply of water to the leaves (Table 1) through several processes such as stomatal closure, osmotic adjustment, changes in tissue elasticity and increase in abscisic acid (ABA) [40]. Osmotic adjustment has been widely proposed as a plant attribute that confers adaptation to salt stress [41]. To do this, plants use inorganic ions and/or organic compounds, the so-called compatible solute [42, 43]. Investigating the mechanisms by which wheat physiologically adapts to salinity showed that varieties of wheat which are able to maintain photosynthesis and growth at low soil Rw often display a relatively greater capacity for leaf osmotic adjustment [44]. From the results obtained here and those of previous studies, it appears that this variety has relatively greater capacity for leaf osmotic adjustment which allows it to tolerate salt stress conditions quite well [37, 44].



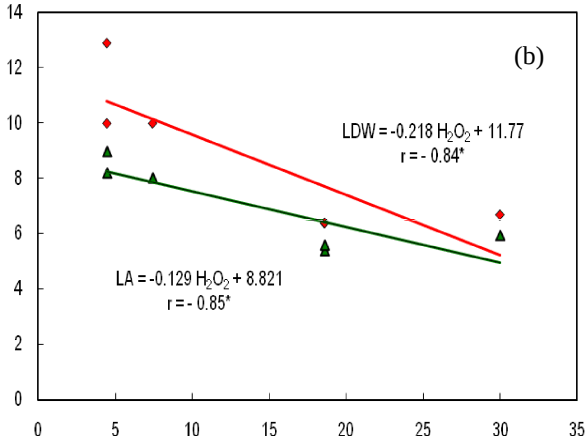


Fig. 1a: Correlation between WSC and LA/LDW
Fig. 1b: Correlation between H_2O_2 and LA/LDW

Photosynthetic Pigments

Chlorophyll a and b: As it appears from the results, NaCl stress, even at this concentration of 150 mM, which can be regarded as little more than the threshold of salt tolerance in durum wheat [45], had no great impact on chlorophyll content (Table 2). Lacerda *et al.* [46] reported similar results on sorghum. However, several studies reported that NaCl at high concentrations induced chlorophyll degradation [15]. Chlorophyll synthesis and chloroplast protection against oxidative damage seem to be an important character for plant living under salt stress conditions [47, 48]. Although both the chlorophyll and proline biosynthetic pathways use glutamate as a common precursor [49], low chlorophyll degradation could not alone explain the accumulation of proline observed in this cultivar. Chlorophyll degradation could occur during salt stress due to high proteolytic activity [50]. For example, it was reported that NaCl mild stress stimulates chlorophyllase activity [51]. However, the decrease in chlorophyll content of leaves may be seen as an adaptive strategy to avoid oxidative stress by reducing the amount of light intercepted and therefore reducing the amount of ROS generated by chloroplasts [52].

Total Carotenoids Content (TCC): As accessory pigments, carotenoids participate in photoinduced electron transfer processes and protect chlorophyll photoxidative damage [48]. In our experience, total carotenoids content of the leaves decreased by ~14% with NaCl treatment (Table 2). ANOVA indicates that this decrease was not significant (P#0.05). However, correlation analysis indicate the existence of a negative relationships between carotenoids content and proline (-0.64) as well as H_2O_2 content (-0.67). Several previous studies have provided similar information [53]. The decrease of total carotenoids content (TCC) may be explained as a result of either a low synthesis rate or enhanced degradation induced by reactive oxygen species (ROS), as has been proposed by many authors [48, 54]. Furthermore, the relative importance of carotenoids in the plant antioxidant defense systems is evident, so one can believe that salt stress may induce oxidative stress through reduction of both level and activity of antioxidant enzymatic and non enzymatic systems [55].

Hydrogen Peroxide (H_2O_2) and Lipid Peroxidation: H_2O_2 content was significantly increased by about 4-fold compared to control plants (Table 2). The analysis of correlations shows that there are significant negative correlations between leaf H_2O_2 content and all of leaf growth parameters. Hydrogen peroxide, an extremely toxic by-product of certain metabolic pathways, is rapidly split into water and oxygen. However, recent works have shown that hydrogen peroxide is very helpful to plants and, as signaling molecule, it plays a pivotal role in many metabolic processes [56]. Under stress the production of H_2O_2 becomes more significant, which led to suggest that H_2O_2 may be a key intermediate in stress tolerance [56, 57]. Furthermore, leaf senescence is accompanied by an increase in H_2O_2 content, indicating the involvement of this molecule in the process of pigment and membrane degradation, etc., which characterize this event [58].

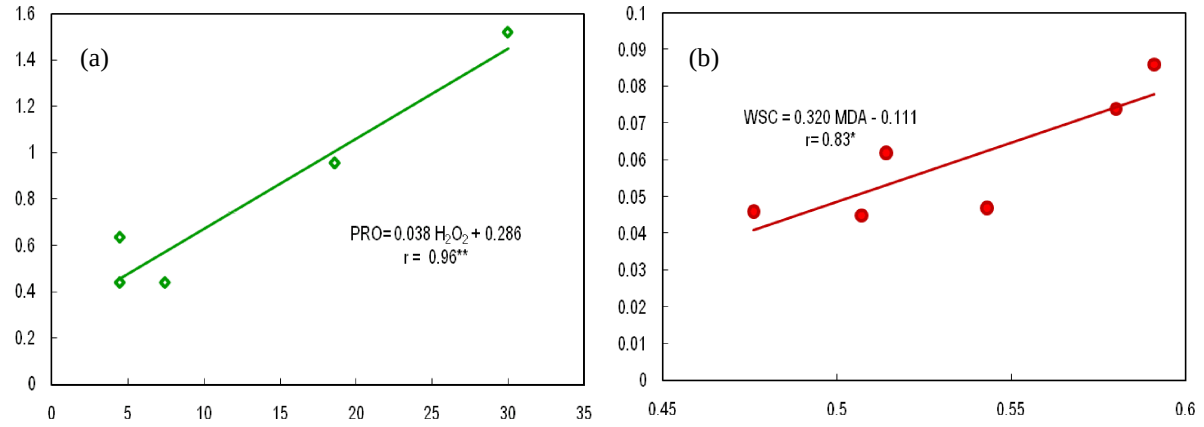


Fig. 2a: Correlation between H_2O_2 and Proline
Fig. 2b: Correlation between MDA and WSC

As seen in Fig. 1b, the highly negative correlation between the accumulation of hydrogen peroxide, on one hand and LDW (-0.836*) and LA (-0.853*), on the other, indicate the existence of a close relationship between oxidative stress and inhibition of leaf growth. Similar results were obtained in other studies [10, 59]. However, highly positive correlation between H_2O_2 and proline (0.96**) (Fig. 2a) suggests that proline has probably a role in the detoxification of ROS, namely H_2O_2. Siripornadulsil *et al.* [60] reported that proline plays an intermediary role in the neutralization of ROS.

Several studies on wheat and other species have found out the negative effect of osmotic stress on enzymatic and non-enzymatic oxidative status [15, 61]. It now seems to be clear that at least some of the harmful effects of salt stress on plant growth and productivity can be explained by oxidative damage due to an imbalance between prooxidants and antioxidants in favor of the former [15]. In the case of leaves, so it was suggested that among the factors influencing the growth of leaves under salt stress is the production and accumulation of ROS (H_2O_2 and singlet oxygen) [11]. This assumption is very plausible, because chloroplasts are known as the main source of ROS in plants [62].

On the other hand and may be a consequence of H_2O_2 accumulation, wheat leaves showed (Table 2) a small increase in the rate of lipid peroxidation indicating the higher oxidative damage limiting capacity. Similar results occurred in salt tolerant barley cultivars [63] and tall oat grass [64]. However, salt sensitive rice varieties had higher MDA content in response to salt stress [65].

Multiple correlation analysis indicate the existence of a significantly (5%) positive correlation (0,88*) (Fig. 2b) between the rate of MDA accumulation and the level of WSC accumulated in the leaves which suggest the involvement of water soluble carbohydrates in membrane protection against oxidative-degradation.

Sairam *et al*. [15] reported similar results on four cultivars of wheat (*T. aestivum* L.) which respond to salt stress in contrasting ways. Comparing our results with those of Sairam *et al.* [15] we found that our cultivar "Waha" has shown a behavior similar to cultivar "Kharchia", the best tolerant one in their experience.

CONCLUSION

Taken together, our result show that the salt tolerance in durum wheat depend greatly on the osmotic adjustment (proline, soluble sugars), keeping reactive oxygen species under control (H_2O_2, carotenoids) and the interactions between these two processes. As a result, the identification of the main actors implicated in regulation of the interaction between these two processes may be useful for improving the salt stress tolerance of durum wheat.

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