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Biochemical and Physiological Behavior Against Salt Stress Effect on Two Quinoa Accessions (Chenopodium Quinoa Willd.)

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***Abstract**: Chenopodium quinoa Willd., is a halophyte plant, showing a great variability of response to salt stress. To better understand quinoa response to salinity, an open field experiment was carried out by subjecting two different origins quinoa accessions (27 GR and Line 0291) irrigated with different NaCl concentrations (50, 100, 150 and 200 mM). Photosythetic parameters (stomatic conductance and photosynthetic activity) biochemical parameters (proline, total sugars, total proteins), dry matter, water content and total grain yield were determined at fruiting stage. Results showed that increasing NaCl concentration induce a stimulation of dry matter and water content of different accessions, which can be explained by a well osmotic adjustment. İn addition, in 150 and 200mM of NaCl, proline synthesis in leaves was stimulated of 50% and 54% for 27 GR and of 29% and 87% for Line 0291 respectively. Similary, sugar content seemed to be stimulated by increased NaCl concentrations. However, salt stress induces for total protein content, a stimulatory action for Line 0291 of 70.6%, 69.8% and 14.4%, but an inhibitory one for 27 GR of 15.54%, 35.65% and 51.79% respectively under 100, 150 and 200mM treatments. Correlation analysis showed that proline contents were positively and significantly (P<0.05) correlated with water contents in accession 27 GR leaves (r=0.761). While in Line 0291 accession, water content was significantly correlated with protein content (r=0.893) and dry matter (r=0.768). A significant decrease was noted for photosynthetic parameters and total grain yield which the most affected accession was 27 GR. İn fact, Line 0291 accession has the highest ability to absorb more water under stress compared to the control, this is due to its capacity to accumulate more osmolytes in their cells. Therefore, it has a more important*

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photosynthetic activity. This accession has also the highest dry matter production and total grain yield.

Keywords: Chenopodium Quinoa Willd, Salt Stress, Osmolytes, Photosynthetic Parameters, Total Grain Yield.

1. INTRODUCTION

Soil salinization is a fundamental problem affecting soil's structure and reduce **[1].** Indeed, 20% of agricultural land currently used in the world is affected by salt **[2].** This percentage increasing every year due to excessive fertilization, illogical crop irrigation practices and excessive tillage, besides natural causes such as salt intrusion in coastal areas **[3].** Currently, due to plants sensitivity to salt, it would be exceedingly difficult to plant crops on saline land, this has a dramatic consequence for population. Therefore, the challenge is to find a solution to feed population of the world, which is expected to reach to 9 billion by 2050 [4]. These forces agricultural production in saline soils and which can be possible through salinity tolerant crops selection [5] [6].

One of these crops is quinoa (*Chenopodium quinoa* Willd), which has become an ideal crop with great adaptability and ability to grow in harsh climatic conditions characterized by elevated temperatures, poor soil and bad water [7][8]. However, quinoa is a pseudo cereal halophyte and native to Andean South America [9]. It has an important nutritional value, in fact that its seeds are rich in vitamins and necessary amino acids [10]. Therefore, quinoa is a thorough source of calcium and is suitable for consumers who are lactose intolerant and allergic to gluten [10]. In addition, this plant leaves can be also consumed by humans as a leafy green vegetable or by animals as a highly nutritious food. Currently, quinoa is grown in more than 90 countries where 80% of production comes from Bolivia and Peru, while all other countries produce the remaining 20% [7]. In many countries, quinoa cultivation is still in the "experimental phase" despite this rapid exposure.

Currently, quinoa is successfully planted in a range of harsh environments [11]. Indeed, it was concluded that quinoa can resist drought and salinity due to its ability to osmotically adjust their internal environment and can give yields similar to normal conditions [12]**.** Indeed, that in elevated NaCl levels in soil, salt disrupts plant's ability to absorb water [13]**.** Therefore, to escape cellular dehydration caused by osmotic effect, plant reduces cellular expansion rate in growing tissues and stomatal opening degree in leaves. This reaction alters photosynthetic machinery and reduces plant's ability to utilize light absorbed by photosynthetic pigments [14]**.**

In this study an open field experiment of two different origins quinoa accessions (27 GR, Line 0291) cultivated in NaCl stress, was carried out. These accessions were irrigated with different NaCl concentrations (50, 100, 150 and 200 mM) and their biochemical and physiological behavior against salt stress effect is described.

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2. MATERIALS AND METHODS

a. Materials

The name, origin, and source of two studied quinoa accessions were mentioned in **Table I.**

Table I: Name, origin, and source of the two studied quinoa accessions

Name	Accession	Origin	Ecotype group	Source
27 GR	Ames 13728	USA, New Mexico	Lowland	USDA-NPGS
Line 0291	PI 665274	Bolivia- LaPaz	Northern Highland	USDA-NPGS

USDA-NPGS, United States Department of Agriculture - North Central Regional Plant Introduction Station of the US National Plant Germplasm System.

b. Methods

These accessions were grown in open fields in the center west of Tunisia (Sidi Bouzid) whose soil parameters were indicated in Table II. These accessions were irrigated by drip with 50 (control), 100, 150 and 200 mM of NaCl. Salt stress was applied at panicle stage, and different parameters were determined at fruiting.

Table II: Pedological parameters of experimental parcel soil.

Apparent density	3,02 g/cm3
Texture	Sandy-loam (Clay=6.66; Loam=15.66; Sand=77.68)
Electrical conductivity	1.33 ms/cm
Salinity	0.931g/100g soil
pH	8.24
Total limestone ($CaCO_3$)	1.73%
Total carbon	0.106%
Organic material	0.18%
Potassium (K_2O)	280ppm
Available phosphate (P_2O_5)	* 15 ppm in the first 20 centimeters * 3.87 ppm in the rest.

Remark: Saturated paste is the method used to measure conductivity soil. The pH is measured using a pH meter with a conventional soil/solution ratio (1/2.5).

c. Statistical analysis

All data were subjected to variance analysis using SPSS 26.0 software and differences between means were compared by Duncan tests ($p < 0.05$). Averages followed by the same letters are not significantly different at $p < 0.05$.

Measured parameters

d. Soluble sugars assay

Schields and Burnett Method [15] which is based on condensation of degradation products of neutral sugars was used. Absorbances were measured using a spectrophotometer at 585 nm.

e. Proline assay

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This amino acid was assayed by Monneveux and Nemmar method [16]. Leaves were treated by methanol (40%) and then heated in a water bath at 85° C. 1 ml of extract is added to a mixture of distilled water, acetic acid and ninhydrin. Optical density was read at 528 nm.

f. Protein assay

Total soluble protein content was measured using Bradford method [17] which is based on bovine serum albumin use as protein standard. Absorbances were measured at 595 nm.

g. **Dry matter production**

Fresh plants collected at the end of their vegetative cycle were dried in an oven at 80°C for 72 hours then dry matter (DM) was determined.

3. RESULTS

1.1. Salt stress effect on photosynthetic activity and stomatic conductance

Salt stress effect study on photosynthetic parameters showed a significant inhibition of photosynthetic activity and stomatic conductance of two studied accessions.

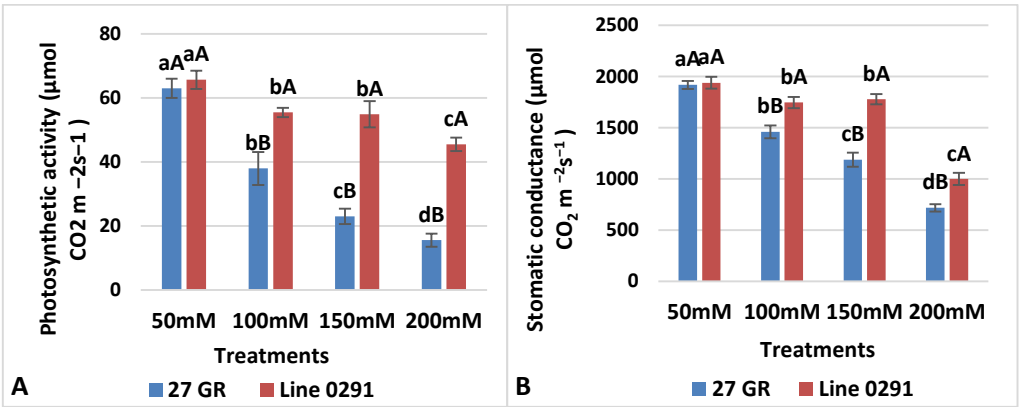

Fig. 1 NaCl treatments (50, 100, 150 or 200 mM) effect on photosynthetic activity (A) and stomatic conductance (B) of quinoa accessions.

Means comparison based on Duncan test was calculated between accession (capitals letters) and between treatments (small letters) (a, b, c, A, B) Averages followed by the same letters are not significantly different at $p < 0.05$ according to the Duncan test (indication is valuable for all figures and tables).

Indeed, for photosynthetic activity, a decrease compared to the control (50 mM) about -40, -64 and -75 was observed for 27 GR and -15, -16 and -30% for Line 0291 respectively under 100, 150 and 200 mM (Figure 1 A). Similarly, stomatic conductance decreases from 1918 and 1939 µmol CO_2 m $^{-2}$ s^{-1} to 718 and 998 µmol CO_2 m $^{-2}s^{-1}$ respectively for 27 GR and Line 0291 (Figure 1 B).

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1.2. Salt stress effect on dry matter

Statistical analysis of salt stress effect on dry matter showed that salinity stimulated significantly dry matter production. Additionally, regardless of treatment, Line 0291 accession showed a maximum biomass compared to Line 0291 (Fig 2).

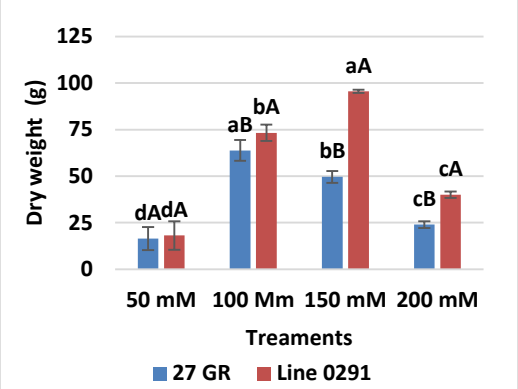

Fig. 2 NaCl treatments (50, 100, 150 or 200 mM) effect on dry matter of quinoa accessions.

1.3. Salt stress effect on water content

Figure 3 showed that under control conditions, 27 GR and Line 0291 accessions explain water contents ranging respectively from 21,8% and 45%. However, increasing salt stress severity stimulated significantly foliar tissues hydration of two studied accessions.

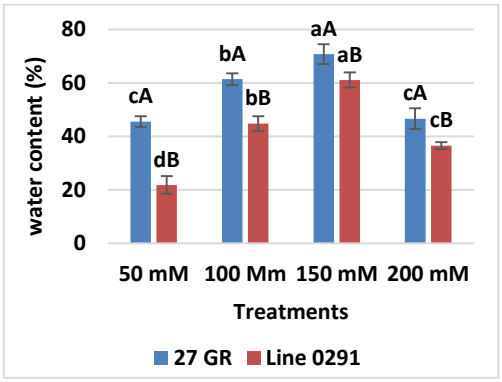

Fig. 3 NaCl treatments (50, 100, 150 or 200 mM) effect on water contents of quinoa accessions.

Stimulation percentages under 100 and 150 mM treatments were respectively in order of +35% and +55% for 27 GR and +105% and +179% for Line 0291 accession. Moreover, at 200mM treatment, salt stress resulted in a tissue hydration statistically similar to the control in 27 GR accession. On the contrary, a significant stimulation with +67% was noted in Line 0291.

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1.4. salt stress effect on sugar and proline content

In response to quinoa plants exposure to salinity, a significant stimulation of total sugars levels was noted in two accessions leaves. Indeed, stimulation percentages under 100, 150 and 200 mM treatments were respectively in order of +13%, +18% and +47% for 27 GR and + 66%, +46% and +86% for Line 0291 (Figure 4 B).

Similarly, a significant increase of proline content was noted under 150 and 200 mM with stimulation percentages respectively in order of +50% and +54% for 27 GR and +29% and +87% for Line 0291 (Figure 4 A).

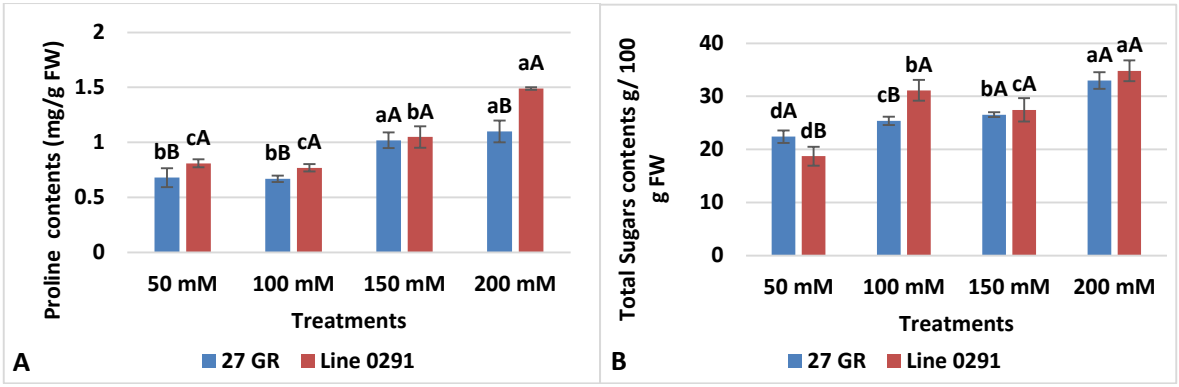

Fig. 4 NaCl treatments (50, 100, 150 or 200 mM) effect on proline (A) and sugar contents (B) of quinoa accessions.

1.5 Salt stress effect on protein content

Statistical analysis of protein content in quinoa plants leaves irrigated by NaCl showed the existence of two different behaviors (Figure 5). In fact, for 27 GR, protein contents were decreased in parallel with the increase of stress intensity. Indeed, inhibition percentages were in order of -16%, -35% and -52% compared to the control respectively under 100, 150 and 200 mM. Furthermore, a significant stimulation was noted for Line 0291. These stimulation percentages were in order of +71%, +69% and +14% **(Figure 5).**

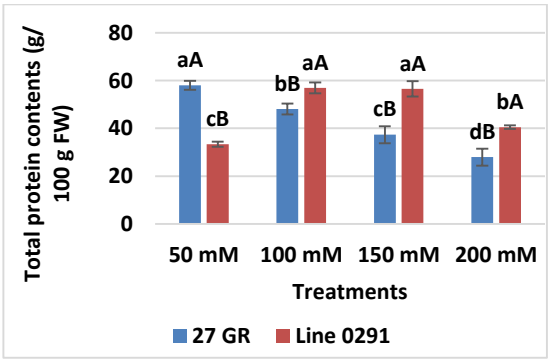

Fig. 5. NaCl treatments (50, 100, 150 or 200 mM) effect on protein contents of quinoa accessions.

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1.6 Salt stress effect on total grain yield

Total grain yield was gradually reduced with increase salt stress intensity, which 27 GR accession is the most affected. Reduction percentages are in order of -16, -47 and -63.8% for 27 GR and -12, -17 and -32% for Line 0291 respectively under 100, 150 and 200 mM (**Table III).**

Table III: NaCl treatments (50, 100, 150 or 200 mM) effect on total grain yield of quinoa accessions

	50 mM	**100 mM**	**150 mM**	**200 mM**
27 GR	68.1±1.5 aB	56.9±2.4 bB	35.5±2.1 cB	24.6±1.5 dB
Line 0291	91.9±1.1 aA	80.8±2 bA	75.6±0.8 cA	62.4±1 dA

4. DISCUSSION

Salinity is one of the main factors affecting yields and productivity agricultural. Therefore, one of the challenges of current research in plant ecophysiology is to produce plants varieties of agronomic interest exhibiting tolerance to salt stress. This study was carried out to determine tolerance level of two quinoa accessions (27 GR, R-132 and Line 0291) exposed to different NaCl concentrations (50, 150, 150 and 200 mM).

Statistical analysis of physiological attributes showed that stomatic conductance and photosynthetic activity decrease under salinity. This decrease could be explained by the fact that under osmotic stress resulting from salinity exposure, plant tries to regulate their stomatal conductance by stimulating ABA synthesis in roots and subsequent transport to leaves as a regulatory signal. Stomatal closure reduces water loss but also CO_2 uptake, thereby inhibiting photosynthesis [18].

Various experiments were carried out under salinity treatments confirming our results. Indeed, [19] showed that the exposure of two varieties of quinoa 'Utusaya' and 'Titicaca' to 400 mM of NaCl inhibited CO_2 assimilation respectively by 25% and 67%. Similarly [20] found that increasing water salinity to 250 mM NaCl reduced photosynthetic net rate uptake from 30 µmol CO_2 m^{-2} s^{-1} to 10 µmol CO_2 m $^{-2}s^{-1}$.

Another experiment that was performed by [21] on valley variety "Hualhuas" showed that photosynthesis rate reduced by 70% when quinoa plants were grown under a salinity level of 500 mM NaCl. Similarly, [22] showed that increasing water salinity from 100 to 400 mM NaCl reduced photosynthesis assimilation by 48%. However, contradictory results were found by [2] confirming that stomatal conductance was not decreased on saline soil in all the four genotypes (A1, A7, Puno, Vikinga) which confirming their salt-loving nature.

The study of proline content variation under salinity showed that the gradual increase of salt stress intensity stimulated proline synthesis of studied accessions. This Osmoprotectant synthesis is dependent on several factors including accession and treatments. Our results were consistent with those of [23] who suggested that proline contents in leaves stimulated with increasing salt levels for each studied quinoa cultivar. The same reaction has been observed in other species by [24] who showed that salt stress caused an increase in proline levels in two varieties of durum wheat and that this accumulation is variable from one variety to another.

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Concerning sugar content, a stimulatory action of NaCl was noted for these accessions. This increase was found by [25] [26] who showed that quinoa plants reduced the adverse effects of salt stress through increased accumulation of osmolytes such soluble sugars. The same reaction has been mentioned in quinoa under drought and frost stress [27] [28]. This carbohydrates overproduction has been attributed to sugars role in carbon storage, ROS neutralization, cell osmotic adjustment and protein structure protection [29]. In contrast, an inhibitory action was detected in quinoa plants exposed to salinity by [30].

In addition to proline and sugar levels, quinoa plants could confront salt stress by stimulating protein synthesis, this stimulation was observed especially in Line 0291 accession. While, for 27 GR, a gradually decrease was noted. Undoubtedly, under salinity conditions, plants maintain their osmotic potential by compatible organic solutes accumulation in their cytoplasm [31]. Furthermore, osmolytes stimulation (for example proline, sucre and protein) plays a crucial role not only in osmotic potential reduction of plant but it also acts as osmoprotectors, which protects cells and helps to maintain cell membrane integrity [32].

In addition, the study of salt stress effect on dry plant matter production showed that the gradual increase of NaCl concentrations (100 and 150 mM) stimulated plant biomass in both studied accessions. An experiment that was carried out by [2] on four quinoa genotypes (A1, A7, Puno, Vikinga) having different origins exposed to salinity suggested that biomass production was not much affected in all genotypes except for Vikinga as compared to non-saline soil.

Concerning the study of salt stress effect on water content, the statistical analysis suggested the stimulation of plant hydration except Line 0291 accession where under the most severe treatment (200 mM) plant accumulates water contents like that recorded under control conditions. So, these genotypes have a relatively better tolerance under these treatments. Indeed, a high-water content allows plants to have a good cellular turgescence necessary for expansion. The ability of plants to maintain high water levels under stress could be explained by the fact that under a stress exerted by high NaCl concentration, plant tries to absorb more water by osmotically adjustment of cytoplasm, this is possible by organic solutes accumulation inside cells [32]. Under salt stress conditions, and to maintain good tissue hydration, the plant osmotically adjusts its tissue content by accumulating organic and inorganic matter [33]. Therefore, like a halophyte, quinoa tries to maintain a critical level of inorganic ions to avoid the negative impact of salinity. Thus, maintaining a high-water content in growing and expanding leaves under salt stress indicates the effectiveness of osmotic adjustment, which counterbalances the decrease in water potential.

Concerning total grain yield of two quinoa accessions, a significant decrease was confirmed by the statistical analysis of data. The same results were found by [22] showing that the gradual increase of salinity intensity from 100 to 400 mM in irrigation water decreased "Titicaca" variety seed yield with 72%. Contradictory results were found by [11] suggesting that showed no yield reduction when ‘Titicaca’ variety was grown in the field under 22 dS m^{-1}.

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5. CONCLUSION

Nowadays, the ever-increasing global demand for nutritious, beneficial and healthy foods has prompted scientists to seek alternative crops especially for marginal areas where plant production is low due to climatic conditions unfavorable, poor soils and lack of good quality irrigation water. In many countries scientists are experimenting with the production of quinoa because it is rich in nutrients, tolerant of salinity and uses much less water than other crops.
In this context, this study focused on evaluating the response of two different quinoa accessions (27 GR and Line 0291) to saline soil conditions under field conditions to identify the most tolerant variety. The physiological and biochemical responses of two quinoa accessions (27 GR and Line 0291) exposed to salinity suggested the ability of Line 0291 accession originated from the Northern Highland to overcome stress better than the other accession coming from lowland. Indeed, it has the highest ability to accumulate more osmolytes in their tissues, this accumulation allowing them to absorb more water under stress compared to the control. Therefore, it has a more important photosynthetic activity. This accession has also the highest dry matter production and total grain yield compared to 27 GR.

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