

Germination and Biochemical parameters of the *Triticum aestivum* varieties (Pirsabak and Ata Habib) in response to NaCl stress.

Abstract

Abiotic stress is an important ecological problem limiting crop growth and productivity of other important substances like Carbohydrates, protein, and Chlorophyll 'a' and 'b' contents in plants. The objective of the present study was to investigate the effects of various concentrations of NaCl, 20ppm, 40ppm, 60ppm, 80ppm, 100ppm and non-saline concentration on two varieties, Ata Habib and Pirsabak of *Triticum aestivum*. Effects of NaCl were observed on Biochemicals contents (Carbohydrates, protein, Chlorophyll a and b) and germination percentage, seedling shoot length, seedling root length, seedling fresh and dry biomass, root numbering and leaf numbering. The result showed that germination and seedling growth reduced with the increasing concentration of NaCl while total carbohydrates and chl 'a' and 'b' increased with increasing salt application on both varieties but protein contents decrease significantly on a high level of sodium chloride. The overall comparison of both varieties in morphologically and biochemically parameters under sodium chloride showed that Pirsabak was more affected by NaCl compared to Ata Habib indicating that the species can tolerate NaCl laden soils. We recommend that the experiment be repeated with more varieties of *Triticum aestivum* under field conditions to compare results and to obtain more NaCl tolerant varieties.

Keywords: Sodium chloride, *Triticum aestivum*, Biochemical contents

Introduction

Abiotic stress, like drought, cold, heat nutrient deficiency, and excess of salt or toxic metals in the soil and the survival of plants under adverse environmental conditions relies on the incorporation of stress adaptive metabolic and structural changes into endogenous developmental programs. Abiotic stress affects plant productivity and development thus causing serious agricultural yield losses and intimidate food security. The adversarial effects of these abiotic stresses are exacerbated by climate change, which has been predicted to result in an increased frequency of extreme weather. [1]; [2]; [3]; [4]; [5]. Soil salinity is a universal problem that affects approx. 20 % of irrigated land and reduces crop yields significantly [6]. Excessive salinization of soils is a major agronomical and ecological problem, in particular in arid and semi-arid areas and Mediterranean ecosystems. Increased soil salinity restrains the

cultivation of crops in many semi-arid and arid regions of the world and is often caused by non-adapted irrigation practices, and some natural cases such as water around the coasts, salty rain, contamination from the parental rocks, and oceanic salt have worsened the concentration of salts in the rhizosphere soil [7]; [8]; [9]; [10]; [11]. In such way salinization arable land is becoming widespread throughout the world, and assessments say that salinization of arable land will result in 30% land loss within next 25 years and up to 50% in next 50 years [12]; [13]. Salt damages can decrease chlorophyll and nitrate content, inhibit carbonic anhydrase and nitrate reductase activities, destroy the PSII reaction center, induce ion deficiencies, disrupt electron transport in the photosynthetic apparatus, reduce the net photosynthetic rate stomatal conductance, affect membrane stability index and reduce the relative water content [14]; [15]; [16]. As salinity causes ion disequilibrium and hyperosmotic stress, thereby disabling the vital cellular functions of a plant. Plants try to withstand these stresses either by tolerating it or by adopting a dormant stage, or some molecular, physiological mechanism. These mechanisms are interconnected and finely tuned to create specific responses that lead to the plant's acclimation to changing environments. The maintenance of biological membrane potential, the activities of several enzymes and appropriate osmolyte concentration to cope with cell volume regulation are all dependent on ion homeostasis and, more specifically, on Na⁺ and K⁺ homeostasis [17]; [18]. NaCl stress is an effective strategy to improve secondary metabolites in the plant. It could improve glucosinolates levels and nutritional value of radish sprouts [19] and broccoli florets [20]. NaCl stress also influenced phenolic compounds in buckwheat sprout [21] and anthocyanin in tomato [22]. However, little is known about the effect of NaCl stress on the nutritional quality of the sprouts of different broccoli cultivars.

Wheat (*Triticum aestivum* L.) considered being the second most important cereal in the world. In 2014–2015 9.12 million ha were cultivated with a total production of 25.48 million tons in Pakistan [23] and member of Poaceae family. The production of wheat is estimated to about 691 million tons per year [24]. Wheat crop occupying about 17% of the world's cropped land and contributing 35% of the staple food production will have a crucial bearing on food security and the global economy in the coming decades [25].

Material and Method

Site

To determine the effects of different salinity levels on germination and seedling growth, an experiment was carried out in the Botanical Garden of Botany, Bacha Khan University Charsadda, Pakistan.

Plant materials:

Triticum aestivum seeds were collected from NIFA Research Institute, Peshawar KP. *Triticum aestivum* varieties seeds were surface sterilized with 70% ethanol for 30 seconds and then washed with distilled water.

The research was carried out in randomized pots with three replicate. The NaCl salt was applied on two varieties of Wheat (Pirsabak and Ata Habib) in different concentrations, 0 was taken as control and 20 40 60 80 and 100ppm sodium chloride solution were used. After 45 days of seed germination, germination percentage, shoot, and root lengths, fresh and dry weight, chlorophyll a chlorophyll-b, protein content, and carbohydrate were recorded. The germination percentage was calculated with the following formula:

$$\text{Germination percentage(\%)} = \frac{\text{Number of germinated seeds} \times 100}{\text{Number of total seeds}}$$

Biochemical Analysis

- i. Chlorophyll content of leaves was determined by the method of [26].
- ii. The protein content of leaves was measured by the method of [27] using BSA as standard.
- iii. Sugar estimation of fresh leaves was done following the method of [28]

Statistical analysis

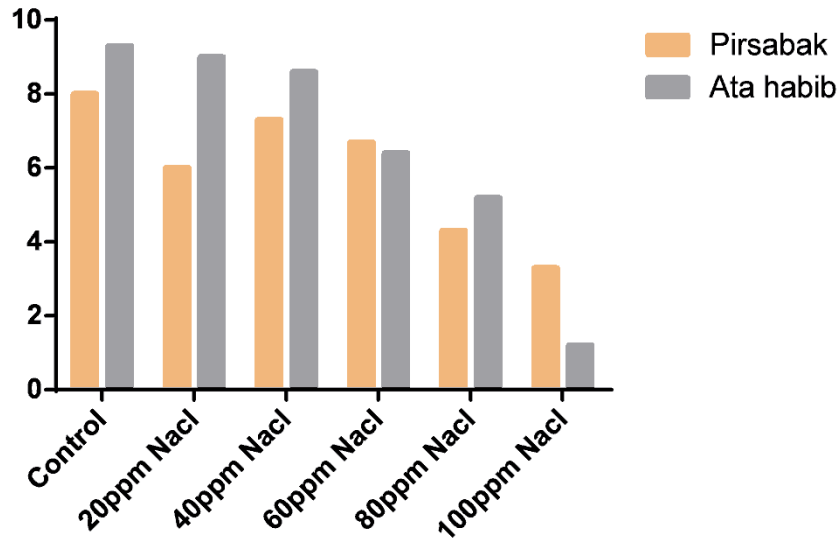
The data collected was transformed to normalize before analyzed using analysis of variance and the average mean comparison using the Newman-Keuls test ($P < 0.05$). Statistically, an analysis was conducted using Statistical Analysis System STATISTICA 10.1 version (Stat Soft, Inc. France).

Results and Discussion

Salinity is the major abiotic stress and significant factor affecting crop growth and production all over the world and especially in the semi-arid and arid regions. Seed germination was toxically affected by salinity stresses (Zhu, 2002). The two cultivars of *Triticum aestivum* (Pirsabak and Ata Habib) treated with different concentrations (control, 20ppm, 40ppm,

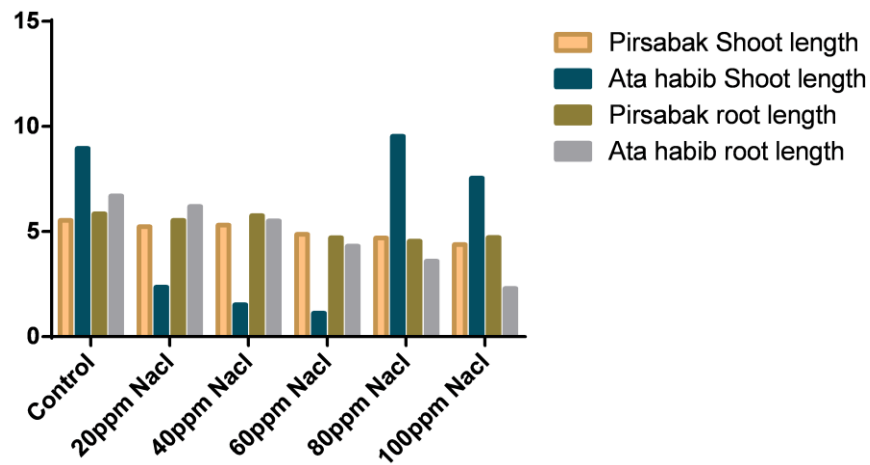
60ppm, 80ppm, and 100ppm) of NaCl. Our results show a reduction in germination under salinity as compared to their non-saline media in both varieties but much reduction occurred in Pirsabak compared to Ata Habib (Fig: 1). This decrease in germination percentage is earlier findings on cereals by [29], spinach [30], sunflower [25], chickpea [31] and barley [32]. Similar results were also reported in mustard, by [33]. Seeds of the *Triticum* cultivars treated with NaCl concentrations which significantly reduce the shoot and root length. But shoot length of the cultivars Ata Habib showed increasing on high salinity (60ppm and 80ppm) which is significantly similar to non-saline result (Fig: 2). Significant reduction in plumule growth with increasing salinity in the present study compared with the findings [34]; [35] who also reported a significant reduction in plumule growth at 10 dS/m and higher salinity concentration. [36] reported the same result that salinity decreases the absorption of water and growth of shoot and root. It's also compared to our results that, root growth speed and nutrient absorption is significantly decreased by salinity [37]. [38] suggested that salinity decreased root and shoot growth and when to increase salinity level, the amount of reduction will also increase. Biomass of *Triticum* (Fresh weight and dry weight) of both varieties significantly reduce under salinity stress as compared to non-saline stress called control (Fig: 3). This is strongly supported by [39] who study that salinity reduced fresh, dry weight and shoot length of *Zea mays* seedlings. The same results were also reported for Corn [40], cucumber and pepper [41]. Many researchers also reported that dry and fresh weights of plant cotton might be affected under saline conditions [42]; [43]. A similar investigation has also been recorded in *Solanum Lycopersicum* [44] and *Zea mays* [45]. our plant's Root and leaf number showed negative effect under sodium chloride of both wheat varieties but the leaf of Pirsabak showed maximum result on high concentration(80ppm) while minimum result on (40ppm) while the other parameters i.e leaf number of Ata Habib, root number of Pirsabak and Ata Habib showed significantly reduction when increasing NaCl concentration (Fig: 4). Additionally, this type of salinity stress produces changes in leaf number which leads to the deterioration of the leaves and thus to inhibition of photosynthesis. The reduction of the plant in the growth of under salt stress can lead to the death of the plant [38]. similarly result also reported by [43] and [46]. Chlorophyll generally known as photosynthetic efficiency depends on photosynthetic pigments such as chlorophyll 'a' and chlorophyll 'b' which play a key role in the reactions of photosynthesis [47]. Total chlorophyll 'a' and chlorophyll 'b' data in (Fig: 5) showed that plant promote Chl 'a' and 'b' contents under high salinity as compared to control plants. The differences in chlorophyll content in the leaf may be due to a reduction in biosynthesis or increased degradation of total chlorophyll under saline

conditions. Comparison of both varieties under salinity the chlorophyll b contents in Ata Habib show more reduction when compared to Pirsabak variety of wheat. It is also similar that in salt-stressed plants, breakdown of the ultrastructure of chloroplasts includes thylakoids of plastid envelop, [48], and photosynthetic substances may result due to direct Na⁺ toxicity or salt-induced oxidative decline [49]. Organic compound especially carbohydrates are the main solutes involved in osmotic adjustment in some plants. Currently, the study of carbohydrates showed increased on the high concentration of sodium chloride, the comparison showed that sugars contents in Pirsabak are more increased than Ata Habib. The maximum value of sugar in Ata Habib show on (80ppm) while minimum on (20ppm) and Pirsabak maximum sugar contents on (100ppm) while minimum on control (Fig: 6) These results are in support with the findings of [50] on maize plant. According to his investigation increase in total sugars and sucrose in the plant, soya bean may indicate that more stimulation in the enzymes of sugar hydrolysis. Similarly, the result also examines by [51] they study Under NaCl salinity, starch and soluble carbohydrates accumulated in plants. Data presented in(Fig:) protein contents showed a reduction in wheat varieties when treated with NaCl salt. Maximum protein reduction showed in pirsabak when compared to Ata Habib cultivars of Triticum. The high value of protein observed in Pirsabak on (20ppm) and in Ata Habib on non-saline(control) while the minimum result reported in current observation on (100ppm) in both Pirsabak and Ata Habib. A different researcher has also reported a reduction in protein content by salt stress [50]; [52]; and [53]. [54] noticed the promotion of protein content of the tomato plant *Lycopersicon* under salinity.



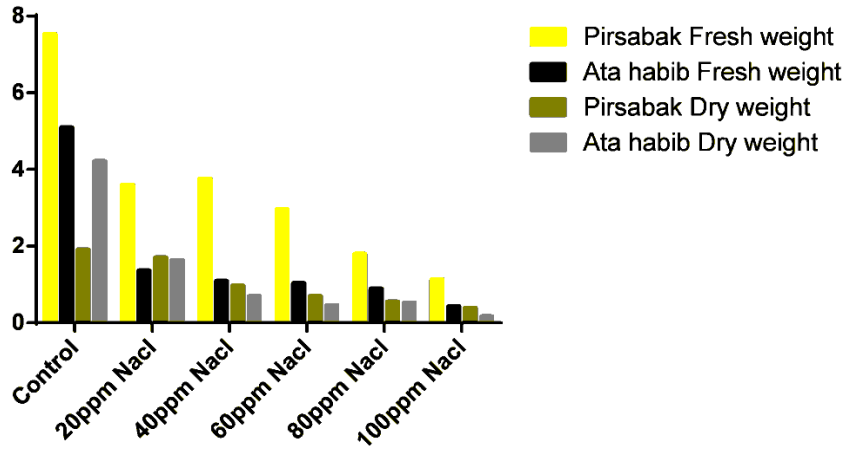
Effect of different concentration of NaCl on Germination of Pirsabak and Ata Habib

Figure 1. Effects of different concentration of NaCl on germination of Pirsabak and Ata Habib



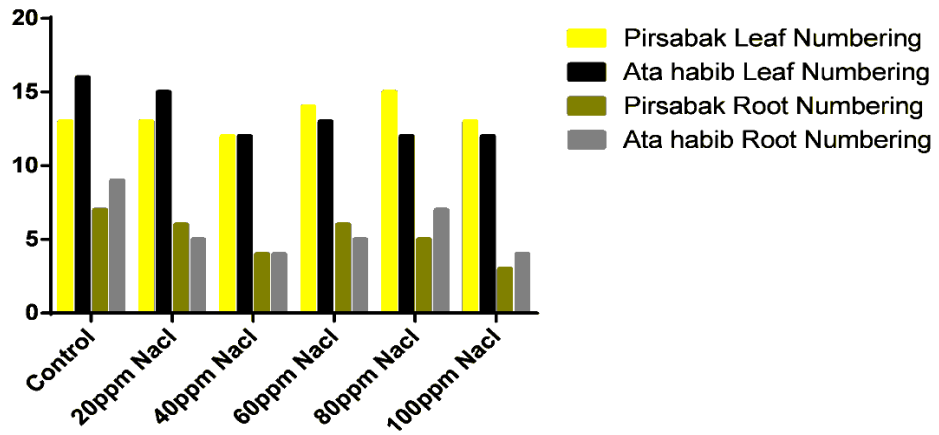
Effect of different concentration of NaCl on Plumule and Radicle length of Pirsabak and Ata Habib

Figure 2. Effect of different concentration of NaCl on plumule and Radicle length of Pirsabak and Ata Habib



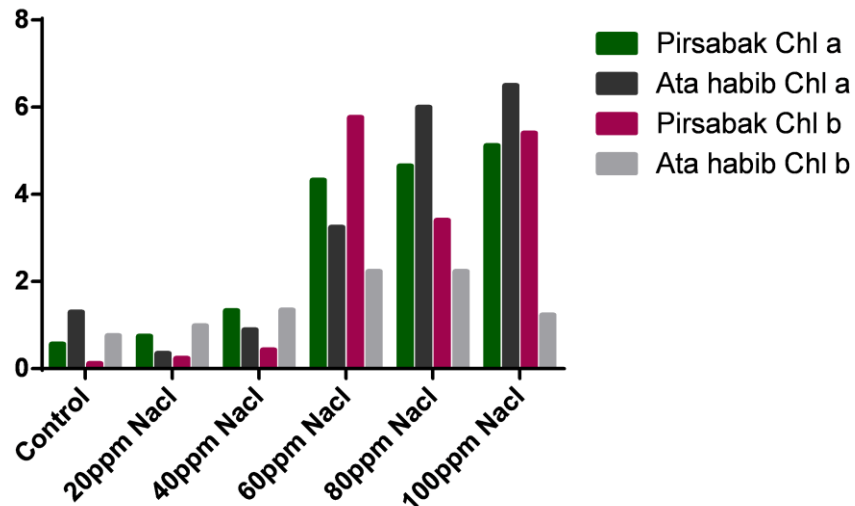
Effect of different concentration of NaCl on Fresh and Dry weight of Pirsabak and Ata habib

Figure 3. Effects of different concentration of NaCl on fresh and Dry weight of Pirsabak and Ata habib



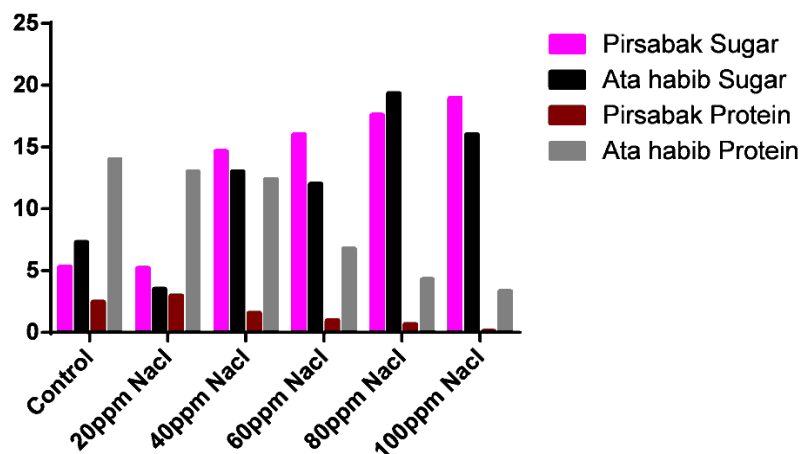
Effect of different concentration of NaCl on Leaf and root numbering of Pirsabak and Ata habib

Figure 4. Effects of different concentration of NaCl on leaf and root numbering of Pirsabak and Ata habib



Effect of different concentration of NaCl on Total chl ,a, and ,b, contents of Pirsabak and Ata Habib

Figure 5. Effects of different concentration of NaCl on total chla, and b, contents of Pirsabak and Ata Habib



Effect of different concentration of NaCl on Total sugar and Protein contents of Pirsabak and Ata Habib

Figure 6. Effect of different concentration of NaCl on total sugar and protein contents of pirsabak and Ata Habib

CONCLUSION

From the current study, it is concluded that salinity showed an adverse effect on seeds germination, seedling growth and Biochemicals contents of wheat plants. This study was carried out to test the seed tolerance of Ata Habib and Pirsabak wheat varieties to NaCl, the germination stage, the results show that the salt has a negative effect on the morphological and protein contents while the positive effect on Sugar and Chlorophyll a and b contents. We recommend that the experiment be repeated with more varieties of *Triticum aestivum* under field conditions to compare results and to obtain more NaCl tolerant varieties. It is also

recommended that the species be applied in phytoremediation of NaCl pollutes soil since they have shown tolerance.

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