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# Morphophysiological Responses of Oat (*Avena sativa* L.) Genotypes from Pakistan's Semiarid Regions to Salt Stress

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#### ABSTRACT

Soil salinity is a major constraint to modern agriculture, with around 20% of the previously irrigated area becoming salt affected. Identifying suitable salt stress-tolerant genotypes based on their agronomic and physiological traits remains a herculean challenge in forage-type Oat (Avena sativa L.) breeding. The present study was designed to investigate the response of oat crop plants against the salt (NaCl) stress in Mardan, Pakistan. The experiment was carried out in complete randomized design (CRD) with two factors trail comprising of the performance of four different genotypes of oat (NARC oat, PARC oat, Green Gold and Islamabad oat) in response to four levels of saline stress (0, 25, 50 and 75 mmol L-1 NaCl). Plant growth and physiological parameters including germination (G, %); fresh shoot weight (FSW, g); fresh root weight (FRW, g); chlorophyll-a, chlorophyll-b, total chlorophyll, and total carotenoids were analyzed for identifying salt tolerance. Germination (%) of oat genotypes was negatively affected by higher salt stress. Mean values showed that maximum germination (57.5%) was recorded for control while minimum germination (48.75%) was recorded for 25 mmol L<sup>-1</sup> NaCl and that maximum germination (58%) was recorded for PARC oat. The root and shoot fresh weight of all genotypes declined with increasing salt stress, while NARC and Green Gold oat showed considerably higher values than the other genotypes. Although chlorophyll and carotenoids were found to be negatively affected by increasing salt concentrations, NARC and Green Gold oat genotypes performed considerably better at 75 mmol L-1 NaCl when compared to the other genotypes. Based on the mean shoot dry weight ratio ± one standard error, the four Oat genotypes were categorized as salt-tolerant (Green Gold), moderately tolerant (PARC and NARC), and salt-sensitive (Islamabad). The more salt-tolerant genotype (Green Gold) demonstrated relatively high salinity tolerance and may be useful for developing high-yielding oat hybrids in future breeding programs under salt stress conditions.

**KEYWORDS:** NaCl, Osmotic stress, Germination capability, Chlorophyll photobleaching, Climate change, Food security, Salt resistance

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## **INTRODUCTION**

World population growth is projected to increase to 9 billion inhabitants within the next generation, which means meeting global food demands will require agricultural output to increase almost two-fold by 2050. At the same time, Soil salinity affects around 831 million hectares of land worldwide (FAO, 2014). Indeed, soil salinity is a major constraint to modern agriculture, with approximately 20% of once-irrigated land worldwide presently salt-contaminated (Shrivastava & Kumar, 2015). Its accumulation in the root zone is the main cause of reduced crop growth because salinity deteriorates soil health by disturbing the rhizosphere ecology and inducing ion toxicities in the soil (Zhang *et al.*, 2010).

For crop plants that are salt sensitive, elevated sodium ions  $(Na^+)$  impose toxic effects on a cellular level by perturbing potassium  $(K^+)$ -dependent processes, inducing deleterious protein

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conformations, activating osmotic stress that all cause growth inhibition and ultimately cell death. Soil salinity is an acute issue for both irrigated and non-irrigated crops (Zhang *et al.*, 2010, 2013), inducing osmotic stress, water deficit, reduced leaf expansion, and stomata closure; these physiological responses in turn, reduce photosynthesis and biomass accumulation (Rahnama *et al.*, 2010; James *et al.*, 2011).

The most critical stage in seedling establishment, which affects crop production performance on salt-affected soils, is seed germination. Salinity retards seedling germination, reduces seedling growth, and dispersion of germination events, and reduces seedling metabolism, resulting in decreased plant growth and crop productivity (Choudhary et al., 2021). Saline soil can inhibit seed germination by causing osmotic stress, which prevents water uptake, or by specific ion toxicity, which also inhibits the processes of dividing and expanding cells, as well as altering the activity of some important enzymes, resulting in a reduction in seed reserve utilization (Jacob et al., 2020). Thus, salt damaged the germination process by changing the normal germination mechanism, resulting in lower growth and development and, eventually, decreased economic output. Furthermore, the increased occurrence of salt stress on arable land implies an urgent need for a greater knowledge of plant tolerance mechanisms in order to preserve agricultural production through optimal crop growth conditions management (Zia et al., 2022).

Many researchers have attempted to screen salt-tolerant cultivars on diverse species during crop seedling growth (Ahmad *et al.*, 2022; Zia *et al.*, 2022). Although salt stress affects all plant growth stages, seed germination and seedling growth stages are considered to be more sensitive in most plant species (Dehnavi *et al.*, 2020; Mbarki *et al.*, 2020).

Oat (Avena sativa L.) is an important worldwide food and feed crop. In terms of yield and nutritive value, it is also ideal for animals. It is a highly nutritious food, rich in energy, carbohydrates, fat, vitamins, and minerals (Hussain et al., 2002). Oats are grown for use as grain as well as forage and fodder, straw for bedding, hay, haylage, silage and chaff (Islam et al., 2022). It is a fast-growing plant with an average yield of 45-55 tons of green fodder or 2.5-3.0 tons of grain per hectare (Alemayehu, 1997). The importance of this crop is increasing worldwide for both the purposes of its ease of cultivation and profitability. Oats can be grown in problematic conditions such as drought, poor soil fertility, high salinity and alkalinity. It is also considered medium salt tolerant (100 mM) and may be utilized as a pioneer crop with high ecological value in saline-alkali areas (Wang & Song, 2006). Oat germinated well at low salinity (50 mM), and the root length and root number of oat seedlings were significantly improved (Wu et al., 2009; Luo et al., 2012). However, when the salt level was raised to 150~200 mM, the germination and vigor index declined dramatically (Luo et al., 2012).

As saline soil is distributed in over 100 countries worldwide, it has become one of the world's environmental problems (Zaman *et al.*, 2018). In Pakistan, salinity damages around 10 million ha of land, accounting for 12.9% of the country's land (FAO, 2008).

Salinity has a deleterious effect on crop germination and early growth stages (Jacob *et al.*, 2020). Although there have been many studies in the context of Oat grain yield, there was a scarcity of data on Oat's response, particularly in early growing stages under saline conditions. Given ever increasing demand for oats, it becomes critical to undertake a high-throughput method to understand the extent of salt tolerance of this crop. This form of evaluation would assure its widespread cultivation in this country. Considering these perspectives, this study employed four distinct Oat genotypes to evaluate the different responses and adaptations to salt stress. The genotypes were tested to see how salt affected some key physiological and morphological traits.

#### MATERIALS AND METHODS

#### Plant Material and Growth Conditions

Four Oats (Avena sativa L.) genotypes (NARC oat, PARC oat, Green Gold and Islamabad oat) were selected that have been popularly cultivated as forage crops in recent years across Pakistan. These cultivars were obtained from NARC Islamabad. The experiment was conducted in a greenhouse at the Agronomy research laboratory, AMKC Mardan, starting on 22 December 2021. In a controlled temperature chamber, Oat seeds were soaked in distilled water in Petri dishes at 4 °C. The seeds were planted one week later in 30 cm  $\times$  30 cm  $\times$ 30 cm plastic containers (with holes at the bottom) filled with well-washed sandy clay combined with vermiculite. In this experiment, a randomized block design (RBD) was adopted (four genotypes  $\times$  four replicates per genotype  $\times$  four salinity levels) (n = 64). After emergence, five plants were kept in each pot. After germination, a half-strength Hoagland solution was applied for one week before being raised to full strength until harvest. The same amount of Hoagland solution was provided to each container following each full irrigation.

After a month of growth, when the plants had reached the tillering stage, four different salinity treatments were imposed:

- T1 (control medium, irrigated with normal Hoagland solution);
- T2 (mild NaCl saline conditions, irrigated with saline Hoagland solution at 25 mmol L<sup>-1</sup> NaCl);
- T3 (moderate saline conditions, irrigated with saline Hoagland solution at 50 mmol L<sup>-1</sup> NaCl);
- T4 (high saline conditions, irrigated with saline Hoagland solution at 75 mmol L<sup>-1</sup>NaCl).

The salinity concentration was increased progressively over 10 days by adding NaCl to the nutrient solution. Overall, the final saline treatments were applied for 40 days, or until the plants reached the heading stage, at which point the high salt conditions were too stressful for growth, and then the whole shoot and root biomass was harvested.

#### **Growth Physiological Parameters**

For germination (G, %), counting began immediately when the first seedling appeared, and measurements were taken on a daily

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basis. The total number of visible seedlings was counted. The measurement was repeated until there was no further change. When the visible radicle length reached 2 mm, the seed was marked as germinated.

Before all of the plants were harvested, three fresh leaf samples from each container were taken and the fresh weight shoot (FSW, g) and root (FRW, g) were assessed immediately.

Chlorophyll-*a*, chlorophyll-*b*, chlorophyll a+b, and total carotenoids content were determined spectrophotometrically using 200 mg FW of leaf material immersed in 8 mL of acetone 80% solution (v/v) and were kept at 40 °C in dark for 48 hours. The absorbance of the extract was read at 663.6, 646.6, and 440.5 nm and pigment concentrations were calculated according to (Lichtenthaler, 1987) and were expressed in mg per g of FW.

### Determination of Relatively Sensitive and Tolerant Genotypes Groups

The genotypes were classified as salt tolerant based on the percentage decreases in growth characteristics under high salty conditions (i.e., 75 mmol  $L^{-1}$  NaCl) compared to the control medium, which was watered with a normal Hoagland solution. The salinity tolerance of four genotypes was evaluated using the fresh weight ratio (FSW) of the 75 mmol  $L^{-1}$  NaCl and control treatments: genotypes with a mean fresh weight ratio that was more than one standard error above or below the median value were categorized as salt-tolerant or salt-sensitive (Damon *et al.*, 2007).

### **Statistical Analysis**

Data were subjected to single factor analysis of variance using a randomized block design (RBD), and mean separation was performed by the Turkey test if the F test was significant at  $P \le 0.05$ . Statistical analyses were performed using software Statistica PL, version 10. The data for all the traits were transformed to bar graphs having  $\pm$  SE bars.

### **RESULTS AND DISCUSSION**

# Germination Percentage of Oat Seeds Under Salinity Stress

Seed germination is the most crucial phase in seedling establishment and affects crop production and performance on salt-affected soils. In general, salt stress during germination delays germination, reduces the rate, and increases the dispersion of germination events (Khajeh-Hosseini *et al.*, 2003; Janmohammadi *et al.*, 2008). In the present work, significant reductions in germination percentage were observed among all of four Oat genotypes under salt stress. Data regarding the germination percentage of oat genotypes as affected by various salinity levels is presented in Figure 1. Statistical analysis of the data showed that the germination % of oat genotypes was significantly affected by salt stress. Mean values shows that maximum germination (57.5%) was recorded for control while



**Figure 1:** Seed germination (%) of Oat genotypes as affected by different salinity levels. Bar chart with different letters is significantly different at  $\alpha = 0.05$  (Tukey test). Data represent averages of four biological replicates per genotype and conditions ( $n = 4 \pm SE$ )

minimum germination (48.75%) was recorded for 25 mmol L<sup>-1</sup> NaCl treatments. Mean values shows that among genotypes maximum germination (58 %) was recorded for oat variety PARC while minimum germination (51 %) was recorded for oat rest of the oat genotypes. It is generally known that plant growth responses to salt stress vary depending on the extent and duration of stress, plant variety or species, and developmental stage. It is noted that PARC performed better than NARC oat and other studied genotypes. Our findings reveal that when salt stress intensity increases, germination capabilities decrease, which is consistent with the findings of earlier researchers (Jacob et al., 2020; Masuda et al., 2021; Ahmad et al., 2022; Zia et al., 2022). Our results are in harmony with the results of Iqbal et al. (2022). They also reported that the osmotic action of salts in the growth medium reduced germination percentages. Salt stress affects seed germination largely by reducing the osmotic potential of the soil solution sufficiently to inhibit seed water absorption, producing sodium and/or chloride toxicity to the embryo, or altering protein synthesis (Iqbal et al., 2020). The osmotic potential, delays the uptake of water needed for nutrient mobilization and causes germination to be reduced as a result of salt stress. According to Zia et al. (2022), the osmotic impact of salts contained in the growing medium gradually reduced the germination percentage.

### Shoot-root Fresh Weight of Oat Under Salinity Stress

Data regarding shoot fresh weight (g) of oat genotypes as affected by various salinity levels is presented in Figure 2a. Statistical analysis of the data shows that shoot fresh weight (g) of oat genotypes was significantly affected by salt stress. Mean values shows that maximum shoot fresh weight (0.725 g) was recorded for control while minimum root fresh weight (0.31 g) was recorded for 50 mmol L<sup>-1</sup> NaCl treatments. Mean values shows that among genotypes maximum root fresh weight (0.705 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight (0.775 g) was recorded for oat variety Green Gold while minimum root fresh weight



**Figure 2:** Fresh shoot weight (a) and fresh root weight (b) of Oat genotypes as affected by different salinity levels. Bar chart with different letters is significantly different at  $\alpha = 0.05$  (Tukey test). Data represent averages of four biological replicates per genotype and conditions ( $n = 4 \pm SE$ )

genotypes NARC. It is noted that with an increase in salt stress root dry weight decreases, but the preference of both genotypes is different as Green Gold performed better than NARC oat and other genotypes. Sodium chloride content may inhibit early seedling development owing to osmotic stressors; shoot fresh weight declined substantially as salt concentration increased (EL Sabagh *et al.*, 2020).

Data regarding root fresh weight (g) of oat genotypes as affected by various salinity levels is presented in Figure 2b. Statistical analysis of the data shows that root fresh weight (g) of oat genotypes was significantly affected by salt stress. Mean values shows that maximum root fresh weight (1.87 g) was recorded for control while minimum root fresh weight (1.02 g) was recorded for 50 mmol L<sup>-1</sup> NaCl treatments. Mean values shows that among genotypes maximum root fresh weight (1.72%) was recorded for oat variety Green Gold while minimum root fresh weight (1.35) was recorded for oat genotypes NARC. It is noted that with an increase in salt stress root dry weight decreases, but the preference of both genotypes is different as Green Gold performed better than NARC oat and other genotypes. Similar results were reported for wheat by Sarwar *et al.* (2004), for cotton (Meloni *et al.*, 2001), and for maize (Zia *et al.*, 2022). Higher NaCl concentrations may inhibit early seedling development and Root fresh weight decreased as salinity stress increases (Wang *et al.*, 2020; Tabassum *et al.*, 2021). Fresh root growth was similarly reduced as root length and root mass decreased with increasing salt. It was also claimed that the increased salt content hurt the root's fresh weight (Ahmad *et al.*, 2020; Fatima *et al.*, 2021).

# Chlorophyll Content of Oat Genotypes as Affected by Salt Stress

Data regarding chlorophyll-a content of oat genotypes as affected by various salinity levels are presented in Figure 3a. Statistical analysis of the data shows that chlorophyll-a of oat genotypes was significantly affected by salt stress. Mean values shows that maximum chlorophyll-a (2.1275 mg  $g^{-1}$  FW) was recorded for control while minimum chlorophyll (0.6825 mg g<sup>-1</sup> FW) was recorded for 75 mmol NaCl L<sup>-1</sup> treatments. Mean values shows that among genotypes maximum chlorophyll (1.5325 mg g<sup>-1</sup> FW) was recorded for oat variety PARC oat while minimum chlorophyll-a (1.155 mg g<sup>-1</sup> FW) was recorded for oat rest of oat genotypes. It is noted that with an increase in salt stress, chlorophyll-a decreases but the preference of both genotypes is different as PARC performed better than NARC oat and other genotypes. Our results are in line with the finding of Javed et al. (2021), who reported a linear reduction in chlorophyll-a content in maize with increased salt stress.

Data regarding to chlorophyll-b content of oat genotypes as affected by various salinity levels is presented in Figure 3b. Statistical analysis of the data shows that chlorophyll-b of oat genotypes was significantly affected by salt stress. Mean values shows that maximum chlorophyll-b  $(2.6975 \text{ mg g}^{-1} \text{ FW})$  was recorded for control while minimum chlorophyll (1.005 mg g<sup>-1</sup> FW) was recorded for 75 mmol L<sup>-1</sup> NaCl treatments. Mean values shows that among genotypes maximum chlorophyll-b (2.875 mg g<sup>-1</sup> FW) was recorded for oat variety PARC oat while minimum chlorophyll-b (1.5125 mg g<sup>-1</sup> FW) was recorded for oat rest of oat genotypes. It is noted that with an increase in salt stress chlorophyll-b decreases but the preference of both genotypes is different as PARC performed better than NARC oat and other genotypes. These findings are corroborated by Ahmad et al. (2022) and Zia et al. (2022), they demonstrated a linear decrease in chlorophyll-*b* concentration with increasing salt stress.

Data regarding the total chlorophyll content of Oat genotypes as affected by various salinity levels is presented in Figure 3c. Statistical analysis revealed that salt levels significantly affected the chlorophyll a+b content of different Oat genotypes. Mean values shows that maximum chlorophyll a+b (5.14 mg g<sup>-1</sup> FW) was recorded for control while minimum chlorophyll a+b(1.45 mg g<sup>-1</sup> FW) was recorded for 75 mmol L<sup>-1</sup> NaCl treatments. Mean values show that among genotypes, maximum chlorophyll a+b (3.30 mg g<sup>-1</sup> FW) was recorded for Green Gold oat while minimum chlorophyll a+b (2.92 mg g<sup>-1</sup> FW) was recorded in the rest of the oat genotypes. The significant variation in



**Figure 3:** Chlorophyll-*a* (a), Chlorophyll-*b* (b), Chlorophyll *a*+*b* (c), and total Carotenoids (d) contents of Oat genotypes as affected by different salinity levels. Bar chart with different letters is significantly different at  $\alpha = 0.05$  (Tukey test). Data represent averages of four biological replicates per genotype and conditions (*n* = 4 ± SE)

the chlorophyll index could be due to the genetic potential of the cultivar. Total chlorophyll contents of Oat genotypes also decreased with applied salt stress and decreased the most in PARC oat (77.5%) closely followed by Islamabad (74%), and least in NARC (65%) and Green Gold (69.6%), at 75 mmol L<sup>-1</sup> NaCl, when compared to the corresponding control. It is noted that with an increase in salt stress, chlorophyll decreases but the preference of both genotypes is different as PARC performed better than NARC oat and other varieties. These findings are also supported by Fatima *et al.* (2021) and Javed *et al.* (2021); they observed a reduction in chlorophyll *a+b* contents with increased salt stress.

# Carotenoids Contents of Oat Genotypes as Affected by Salt Stress

Data regarding Carotenoids of oat genotypes as affected by various salinity levels is presented in Figure 3d. Statistical analysis of the data showed that Carotenoids of oat genotypes were significantly affected by salt stress. Mean values shows that maximum chlorophyll (13.75 mg g<sup>-1</sup> FW) was recorded for control while minimum Carotenoids (8.75 mg g<sup>-1</sup> FW) were recorded for 75 mmol L-1 NaCl treatments. Mean values shows that among genotypes maximum Carotenoids (11.895 mg g<sup>-1</sup> FW) were recorded for oat variety NARC oat while minimum Carotenoids (10.98 mg g<sup>-1</sup> FW) was recorded for oat variety PARC oat. A linear reduction in carotenoid contents with increased salt stress was also reported by Cha-Um and Kirdmanee (2009). It is noted that with an increase in salt stress, chlorophyll decreases but the preference of both genotypes is different as PARC performed better than NARC oat and other genotypes.



**Figure 4:** Salt tolerance variation (shoot dry weight ratio of 50 mmol NaCl L<sup>-1</sup> and the control) for 15 genotypes grown for 30 days separated at one standard error of the genotype effect above and below the median

#### Salt Tolerance Among Genotypes

Salt tolerance evaluated using the ratio of shoot fresh weight at 75 mmol L<sup>-1</sup> NaCl to shoot fresh weight in the non-saline controls, differed among four Oat genotypes (ranged from 0.106 to 1.643), with PARC and NARC regarded as moderately tolerant genotypes; Green Gold was the most salt-tolerant genotype, while Islamabad was the most salt-sensitive (Figure 4).

#### CONCLUSIONS

Salinity has an obvious detrimental effect on crop germination and growth, and variation in seedling morpho-physiological characteristics is critical for determining the extent of salt tolerance in plants. The current research examined the salt tolerance of typical Oat genotypes at the seedling stage under a series of salt concentrations. Clear variation in salt tolerance was observed at the germination and seedling stages. Although increasing salt concentrations were found to be detrimental to seed germination, chlorophyll, and carotenoids, NARC and Green Gold oat genotypes performed much better than the other genotypes at high salinity levels. The salt-tolerant genotype (Green Gold) showed relatively high salinity tolerance and may be relevant for further investigations into salt-tolerance in crops cultivated in Pakistan's semiarid regions.

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