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# Comparative study on germination and seedling growth of wheat cultivars under salt stress regimes

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**Abstract**

Salinity interferes with germination and hampers the growth of wheat especially at seedling stage which necessitates determining of salt tolerant cultivars. Based upon the current situation a controlled experiment was carried out at the Laboratory of Horticulture, Faculty of Agriculture, Cukurova University, Turkey to comparatively evaluate wheat varieties response to imposed salt stress. Germination and seedling growth properties under salt stress were taken as response variables. The seeds of five bread wheat genotypes ('Wafia', 'Lucilla', 'Envoy', 'Lok1'and 'RSP- 561') were placed in Petri dishes with salinity doses (Control (0), 4, 8 and 12 dS.m$^{-1}$ NaCl) which were applied at germination and subsequent early seedling phases under laboratory conditions. The results revealed that root growth was highly sensitive to salt stress and the varieties of Envoy and Lucilla remained relatively tolerant to salt stress than other cultivars. The biochemical analysis revealed that proline content spiked with increasing salinity level, 'RSP-561' under 8 dS.m$^{-1}$and 12 dS.m$^{-1}$ recorded the maximum proline content. Salt stress boosted leaf proline content of salt sensitive wheat genotypes ('Wafia' and 'Lok1'), whereas declined proline level was observed for salt tolerant cultivars. In addition, salt-sensitive genotypes showed a reduction in chlorophyll content *a*, *b*, total chlorophyll and carotenoid while, 'Wafia' and 'RSP-561' recorded the minimum Chlorophylls and Carotenoid contents. Further investigations are needed, however, to enhance understanding of the salt stress effects during the whole growing cycle of wheat.

**Keywords:** Biochemical analysis, Proline content, Salt tolerance, Chlorophyll, Wheat seedling

## Introduction

Globally, soil salinity has emerged as the leading challenge to modern farming systems which reduces crops yield by damaging the different cellular functions of plants. Gradually, accumulation of salt in upper soil horizons leads to destruction of soil structure and texture (Hassan et al., 2018). Among abiotic stresses, soil salinity has posed a serious threat all over the world to global food security by affecting over 7% of the land area across the globe (Hasan et al., 2015). Soil salinity has emerged as a drastic constraint that adversely affect the growth and development of the wheat crop globally (Kizilgeci and Yildirim, 2014). Exogenous salt accumulation drastically affects seed germination, lowers water retention and ion imbalance leading to ion toxicity and osmotic imbalance (Khan and Panda, 2008). Moreover, it inhibits the growth and development of seedling by reducing photosynthesis rate and negatively effecting other vital physiological processes such as respiration and protein synthesis (Pal et al., 2004).

Wheat (*Triticum avestivum* L.) is considered as a major cereal crop and staple food worldwide. Wheat meets the increasing food requirements of a lot of country. To ensure the food and nutritional security of the skyrocketing population,

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its yield needs to be multiplied as owing to the limitation in increasing its acerage (Hossain et al., 2018; Jahan et al., 2019). In addition, wheat becomes a source of providence to million of growers across the globe and thus contributes to national grass domestic production (GDP) of agrarian-based economies (Barutcular et al., 2017; Yildirim et al., 2018)

Seed germination along with early seedling growth phases are the most critical stages at which salt stress may impart drastic effects on growth of crop plants (Almansouri and Lutts, 2001; Oral et al., 2019; Kizilgeci et al., 2020) caused by salt ions toxicity. Wakeel et al. (2011) reported that some plants tend to be salt sensitive at shoot growth stage because of nascent salinity tolerance mechanism. Root and shoot length is one of the dominant factors against salt stress because roots are in direct touch in soil and they uptake the water and minerals from the soil and supply it to other parts of the plant (Bhutto et al., 2018). Root and shoots traits are important indicators for assessing plants tolerance or susceptibility to salt stress (Almodares et al., 2007). Moreover, these parameters provide valuable clues for selections of genotypes in future breeding programs (Khan et al., 2008).

The germination vigour and salt tolerance mechanism usually vary among different crops and even different cultivars respond differently to salt stress owing to varying genetic makeup (Shalhevet, 1995). Throughout the life cycle of a plant, its susceptibility to salinity at the time of seed germination as well as early seedling stages are much higher than the subsequent growth stages (Ashraf et al., 1986). Thus, it was hypothesized that wheat cultivars might respond differently to salt stress leading to screening and identification of the most salt tolerant cultivar. Therefore, the current trial was executed with dual objectives to evaluate germination and seedling response of five wheat varieties to induced levels of salinity and identify the superior cultivars for salt tolerance.

**Material and Methods**

**Location and duration**

The experiment was conducted at the Laboratory of Horticulture, Faculty of Agriculture, Cukurova University, Turkey.

**Plant materials, experimental treatments and design**

Factorial experiment using a completely randomized design (CRD) with three replications was used for current study. Five bread wheat genotypes viz. 'Wafia', 'Lucilla', 'Envoy', 'Lok1' and 'RSP-561' were used in this experiment. Four levels of salinity viz., control (0), 4, 8 and 12 $dS.m^{-1}$ NaCl were applied as salt treatments on wheat genotypes from germination to early seedling stage under laboratory conditions.

**Experimental procedure**

Seeds were subjected to surface sterilization using sodium hypochlorite (5% v/v) for 10 minutes and then washed thrice with distilled water. The seeds were placed Petri-dishes containing filter paper, each petri-dish consisted of 20 seeds. Day and night lengths were 18/6 h, with 24±1 °C at growth chamber. Salt solutions were prepared by using a calculated amount of NaCl for 4, 8 and 12 $dS.m^{-1}$concentration, and NaCl solution applied in each petri-dish according to the treatment specification.

**Data collection**

The experiment was complated by harvesting seedlings after 7 days and measured the seed vigour, shoot length, root length, coleoptile length, seedling fresh weight, root fresh weight. After measuring the fresh weight, the seedlings were then placed into an oven at 70 °C temperature for 48 h to measure root dry weight and seedling dry weight.

The relative water content (RWC) was determined by using the following formula

$$RWC = (FW - DW) x100 / (TW - DW)$$

Where, FW: Fresh weight, DW: Dry weight and TW: Fresh weight at full turgidity.

Proline content was determined as suggested by Bates et al. (1973). The solution's optical density was noted on a spectrophotometer using 528 nm wavelength, while proline's quantification was done with the help of a calibration curve which was obtained by plotting the intensity rate increment as a function of L-proline concentration (0-5µg $ml^{-1}$).

Chlorophyll *a, b* and total chlorophyll along with carotenoids were determined by following the methodology of Lichtenthaler and Wellburn (1983). The Absorbance was determined with the help of spectrophotometer using 470, 646 and 663 nm wavelengths.

**Statistical analysis**

Statistical analyses of recorded data were performed by employing LSD (Least Significant Difference) test at 5% level of probability using 'JMP 13.0'Command program.

**Results and Discussion**

**Coleoptile length (cm):** The coleoptile length of different wheat varieties showed different results under varying levels of salt stress (Figure 1). The highest coleoptile length was observed at 4 $dS.m^{-1}$ doses, followed by 8 $dS.m^{-1}$ and 12 $dS.m^{-1}$. The highest coleoptile length was produced in the 'RSP-561' variety (3.21 cm) at 8 $dS.m^{-1}$salt application. Similar to our studies, Kizilgeci et al. (2010) reported that the highest coleoptile length value had a high salt dose compared to control conditions. It was recorded that the decrease in root length in response to higher salt stress and noted that the response of roots was very low in both saline and sodic soils due to higher ionic toxicity (Leishman and Westob, 1994). Previously, it has been reported that salt stress drastically reduced germination rate, while seedling growth was also significantly reduced (Valadyani et al., 2007).

**Root length (cm)**

Wheat varieties recorded a significant reduction in the length of roots under varying levels of salinity (Figure 2). However, the highest decline in root length was recorded for salinity level of 12 $dSm^{-1}$ for all the varieties. Among wheat cultivars, the highest root length was assessed for Lucilla followed by 'RSP-561' and 'Wafia'. Salinity adversely affects the shoot growth of plants by altering water relations due to salt accumulation in intercellular spaces (Khayatnezhad et al., 2010). The seedling establishment at early growth stages of plants is crucial for getting the maximum shoot length and consequently higher yield (Khatkar and Kuhad, 2000). The salinity stress induced root length reduction might be attributed to NaCl's growth inhibitory effect which drastically hampered root growth to a greater extent compared to shoot growth (Rahman et al., 2001). Previously, root length and seedling growth of wheat cultivars were drastically reduced with increasing NaCl stress (Khatun et al., 2013). Yildirim et al. (2015) noted that root length could serve as reliable criteria for determining the salinity tolerance of crops genotypes, as roots have direct contact with soil and are responsible for absorption of nutrients and water.

**Seedling length (cm)**

Seedling length of wheat varieties significantly affected by different levels of NaCl concentration (Figure 3). The highest seedling length was produced by 'Lucilla'and 'RSP-561'. The reduction of seedling length is a common phenomenon of several crops grown under saline environments (Amin et al., 1996). High salinity may inhibit the root and shoot elongation due to slowing down the water uptake by the plant (Bayuelo-Jiménez et al., 2002). Similar findings were reported by Qu et al. (2012), who observed that salinity accelerated leaf abscission along with reducing inter-nodal development which resulted in stunted shoot growth. It was inferred that reduction in root and shoot growth might be attributed to sensitivity of cultivars to slight increment in salinity level in the growth medium (Hassan et al., 2018).

**Root fresh weight (mg)**

Root fresh weight (mg) was significantly influenced by genotype and salinity levels interaction (Figure 4). The root fresh weight was found significantly higher at control, However, The heaviest root fresh weight was obtained in Lucilla although the slightest was obtained in 'RSP-561'. It was recorded that the lowest root fresh weight (1 g) was noted at 16 $dSm^{-1}$ NaCl level (Mansour & Salama, 1996). Shahzad et al. (2012) observed a decrease in root fresh weight as salt concentration increases and noted that this trait may be used for salt stress tolerance.

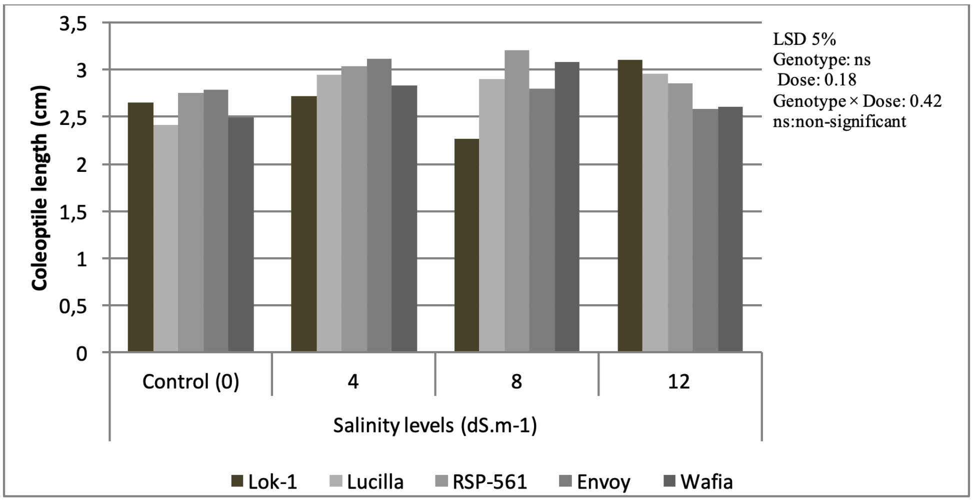


Figure 1. Coleoptile length (cm) of bread wheat genotypes grown under different salinity levels.

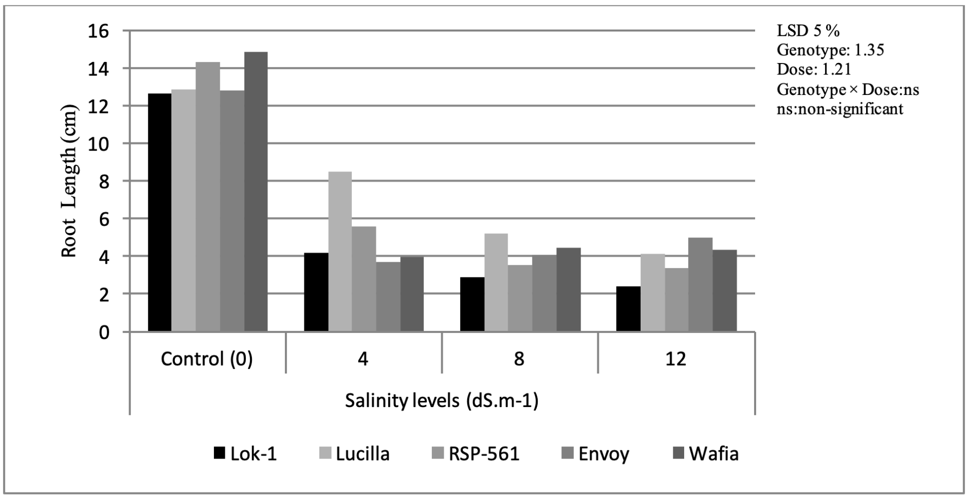


Figure 2. Root length (cm) of bread wheat genotypes grown at different salinity levels

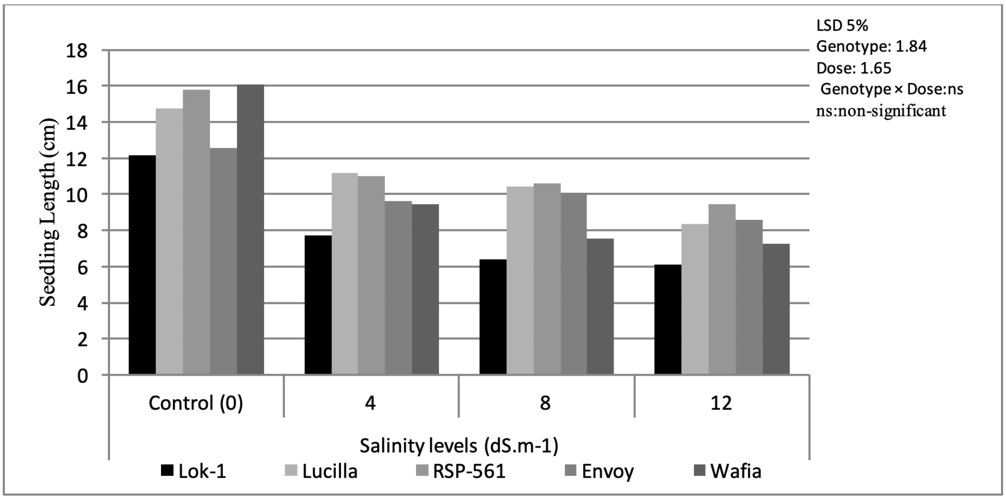


Figure 3. Seedling length (cm) of bread wheat genotypes grown at different salinity levels

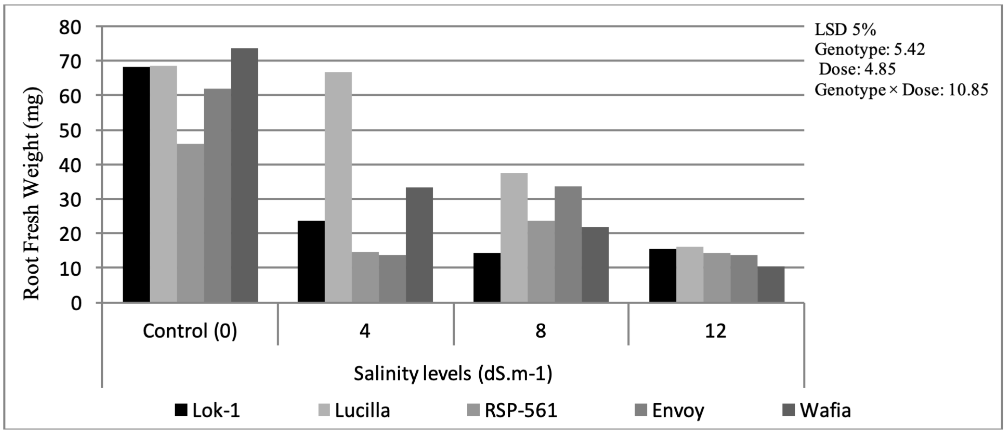


Figure 4. Root fresh weight (mg) of bread wheat genotypes grown at different salinity levels

**Seedling fresh weight (mg)**

The results related to seedling fresh weight indicated significant ($P<0.05$) response of wheat cultivars to salt stress. The decreased seedling fresh weight was recorded at an increasing level of NaCl concentration (Figure 5). The maximum seedling fresh weight was recorded in 'Lucilla' and 'RSP-561'. while the lowest was recorded in 'Envoy'. Studies have found that salt stress affects plant growth at different levels in some wheat varieties and causes a reduction in vegetative organs, in the fresh weights of plants in general. In their studies investigating the effects of salt stress on wheat, Kizilgeci et al. (2010) reported that salt stress caused losses in fresh weight of seedlings. The shoot fresh weight was induced by salinity stress Karaki (1998), also noted that the root fresh weight was not affected by salt stress. However, seedling fresh weight was significantly affected by salinity.

**Root dry weight (mg)**

Root dry weight was affected by the different concentrations of NaCl levels (Figure 6), among various NaCl levels, the maximum root dry weight was recorded under control condition, While, minimum dry weight was observed under highest salinity level of 12 $dSm^{-1}$. In the case of varieties, the highest root dry weight was obtained from 'Lucilla' and 'Envoy', followed by 'RSP-561' and 'Wafia', while the lowest value was observed in 'Lok-1'. It was found the salinity had a significant adverse effect on root dry weight (Baalbaki et al., 1999).This is in confirmation to previous reports which concluded that salinity level increment reduced growth and development of cereals including rice (Masood et al., 2005). It was also inferred that shoot dry weight remained prone to salinity than root dry weight (Essa, 2002). Similarly, different cultivars of bread wheat recorded a significant reduction in shoot dry weights under increasing levels of salt concentration (Kizilgeci et al., 2010).

**Seedling dry weight (mg)**

The results related to the seedling dry weight of wheat varieties was influenced by various NaCl levels (Figure 7). The highest seedling dry weight value was obtained under control conditions and the lowest was under 8 $dSm^{-1}$ and 12 $dSm^{-1}$ conditions. Whereas the highest seedling dry weight value was obtained in the wafia variety under control conditions, the lowest value was obtained in the 'Lok-1' varieties in the 12 $dSm^{-1}$ salt application. These finding are in line with previously reported conclusions whereby varying seedling dry weights of wheat genotypes were taken as indication of varying salt sensitivity (Khatun et al., 2013).

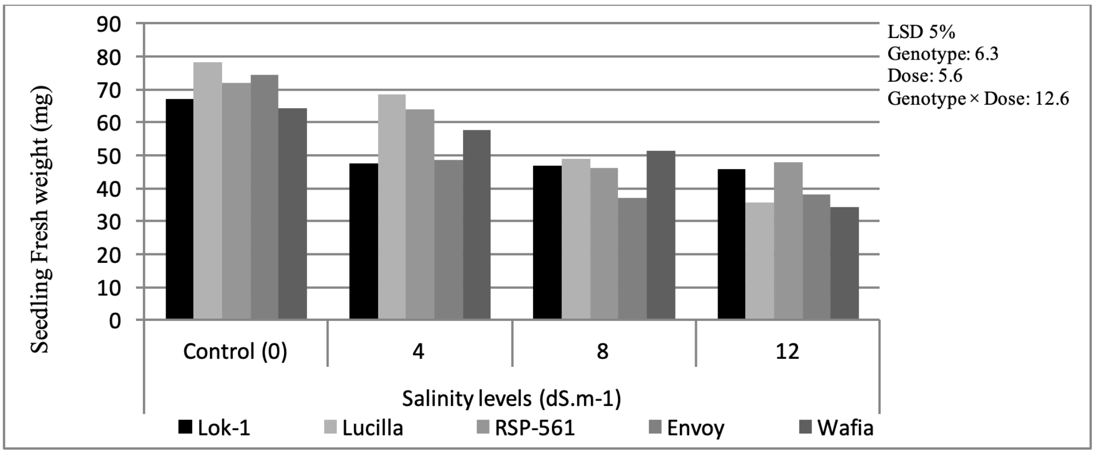


Figure 5. Seedling fresh weight (mg) of bread wheat genotypes grown at different salinity levels

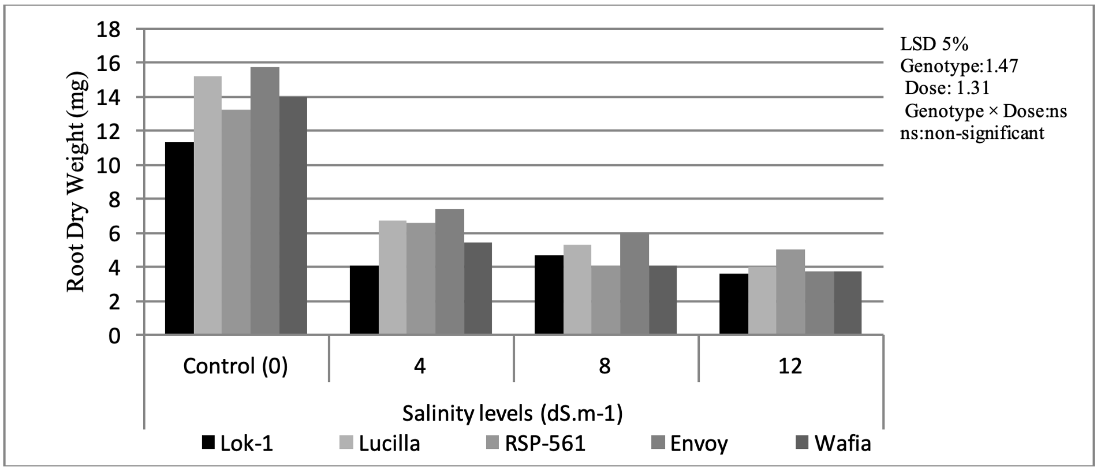


Figure 6. Root dry weight (mg) of bread wheat genotypes grown at different salinity levels

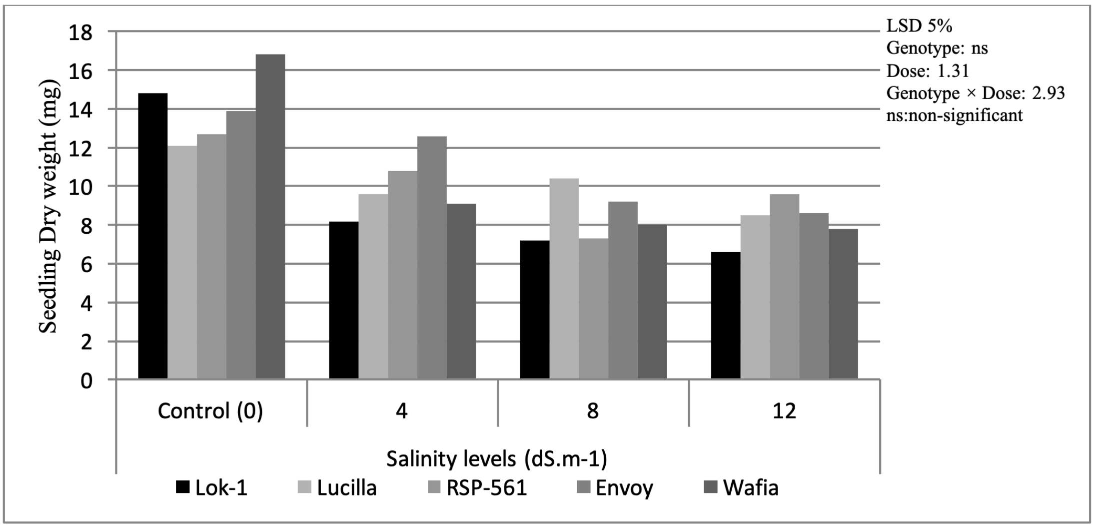


Figure 7. Seedling dry weight (mg) of bread wheat genotypes grown at different salinity levels

**Relative water content**

The data related to relative water content revealed a highly significant response of wheat varieties to various salinity levels. The interaction between varieties and NaCl levels was also significant (Figure 8). The maximum relative water content was recorded under control conditions, while the lowest relative water content (RWC) was determined under 8 dSm$^{-1}$ conditions.due to drought with PEG and salt-water content in the shoot reduced significantly (Bajji et al., 2000) and salt stress conditions lead to drought stress in root due to less uptake of water by roots.

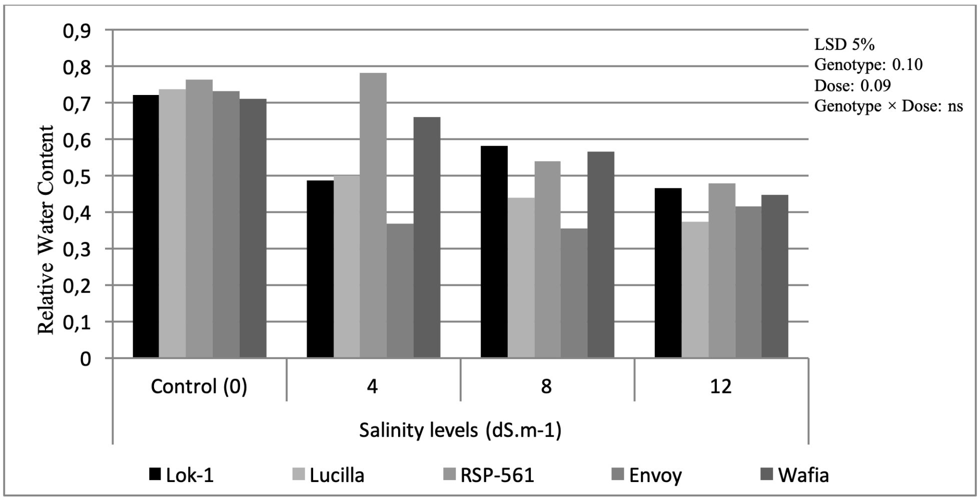


Figure 8: Relative water content of different wheat genotypes grown at different salinity levels

**Seed vigour (%)**

Salinity level interacted significantly wheat genotypes to influence seed vigour which expressed the speed of germination (Figure 9). Germination vigour index was found higher at control, with a range from 90.83% in 'Wafia' to 65.42% in 'Lok-1'. The drastic reduction in seed germination might be attributed viability loss caused by imposed salinity as reported by Gulzar et al. (2001).

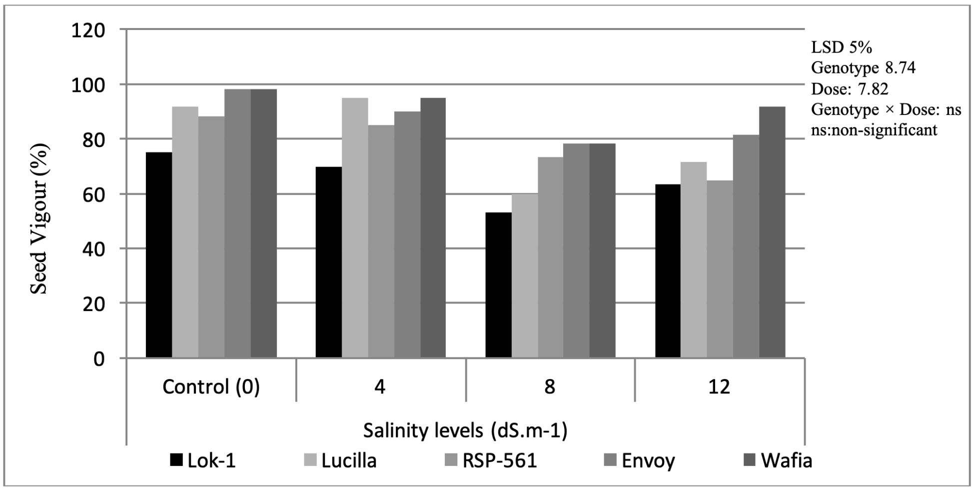


Figure 9. Seed vigour (%) bread wheat genotypes grown at different salinity levels

**Proline content (micromol g$^{-1}$)**

Proline content varies significantly between varieties and salinity stress. Salinity stress enhanced proline accumulation in leaves of varieties, but the increase in 'Wafia' leaves was higher than that in other leaves under 4 dS.m$^{-1}$ treatment, compared to control (Figure10). The maximum proline was determined at 4 and 8 dSm$^{-1}$, while the minimum value was determined at 12 dSm$^{-1}$. Among the genotypes, 'Wafia' and 'RSP-561' had the maximum proline levels and the minimum was 'Lok-1'. The highest proline levels were recorded in wafia cultivars at 4 dS.m$^{-1}$ salt dose, while the lowest proline levels were recorded in 'Lok-1' and 'Lucilla' varieties at 12 dS.m$^{-1}$salt dose. Enhanced proline concentration in the leaves might be due to its rapid synthesis and the breakdown of proline rich proteins during stress (Greenway and Munns, 1980). It has also been described as an adaptive physiological characteristic of plants for ensuring their survival under salt stress (Datta et al., 2009). These findings are in line with other studies which concluded that proline wasa physiological marker of increasing salinity level which got increased in wheat genotypes which were salt-tolerant compared to the salinity sensitive genotypes (Hasan et al., 2015). Accumulation of solutes (proline, glycinebetaine and sugars) have been reported as reliable indicators to quanitify and predict the drastic impact of salt stress (Qasim et al., 2003). There was some evidence which supported the role of proline accumulation in imparting salinity tolerance to crops under moderate to severe salt stress (Khan et al., 2009). It has been reported that in flag leaf of salt-tolerant wheat genotypes, ıncreased proline levels could have a protective function (Hasan et al., 2015).

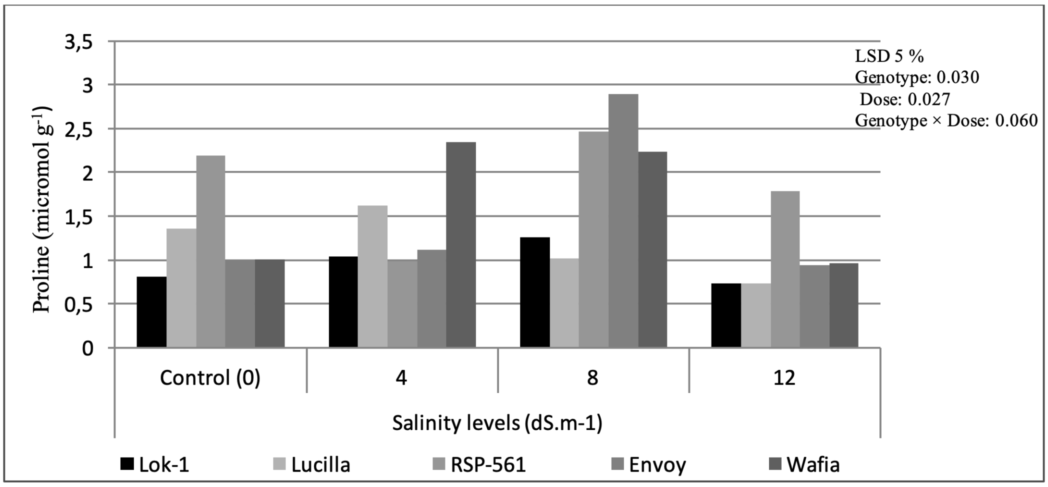


Figure10. Proline (micromol $g^{-1}$) of bread wheat genotypes grown at different salinity levels

**Chlorophyll *a* (mg $g^{-1}$)**

The results revealed that salinity regimes reduced chlorophyll *a* of all measured wheat cultivars. However, these had varied responses to the increased levels of salinity (Figure 11). The maximum chlorophyll *a* was documented under control condition, whereas the minimum value was determined at 8 and 12 $dSm^{-1}$. Among wheat cultivars, 'Lok-1' had the maximum chlorophyll- while 'RSP-561' and 'Wafia' varieties at 12 $dSm^{-1}$ salt concentration recorded the minimum values. The photosynthetic rates were drastically reduced under salt stress resulting in significant reduction of seedling dry weight (Netondo et al., 2004). The contrasting findings were also reported where six genotypes of rice had higher chlorophyll content under salt stress (Alamgir and Ali, 1999). However, contradictory results inferred that chlorophyll tend to decline significantly under salt stress (Hasan et al., 2015). It was also concluded that cholorophyll content declined under stress owing to membrane instability which led to degradation of pigment along with ceased its synthesis (Ashraf et al., 2005).

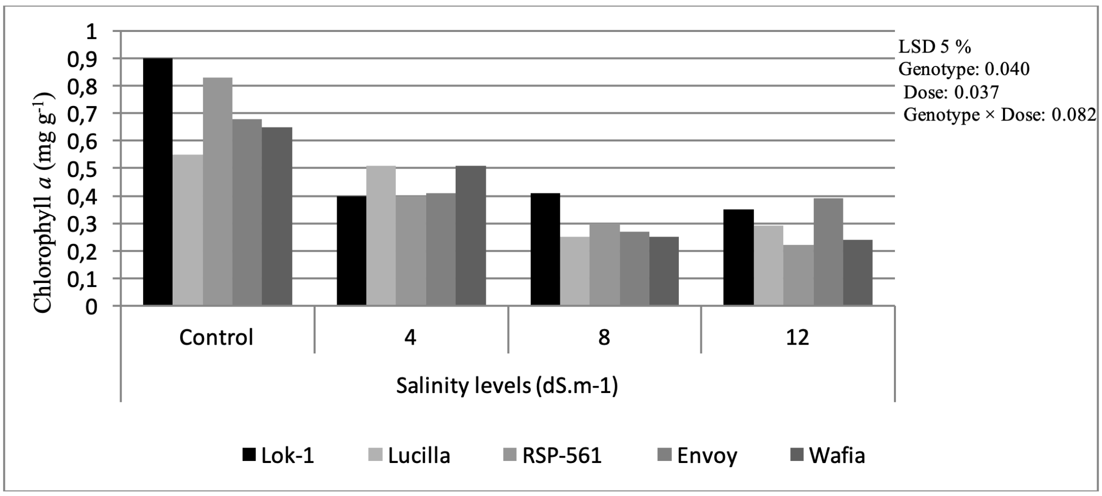


Figure 11. Chlorophyll *a* (mg $g^{-1}$) of bread wheat genotypes grown at different salinity levels

**Chlorophyll *b* (mg $g^{-1}$)**

The chlorophyll *b* of wheat varieties was affected significantly by salinity (Figure 12). Statistically, significant differences were found between genotype, salt applications and Genotype × Dose applications for chlorophyll *b*. The highest chlorophyll *b* was determined under control condition, while the minimum value was determined at 8 and 12 $dSm^{-1}$. 'Envoy' had the highest chlorophyll *b*, whereas 'Lucilla' and 'Wafia' had the lowest chlorophyll *b*. The highest chlorophyll *b* was recorded in 'Lok-1' cultivar at control, whereas the lowest chlorophyll *b* was recorded in 'Wafia' variety at 8 $dS.m^{-1}$salt dose. It might be inferred that salt ions toxicity ions coupled with physiological water deficit in the flag leaves tend to delay synthesis of chlorophyll along with triggering degradation of previously synthesized chlorophyll (Hasan et al., 2015; Zheng et al., 2008).

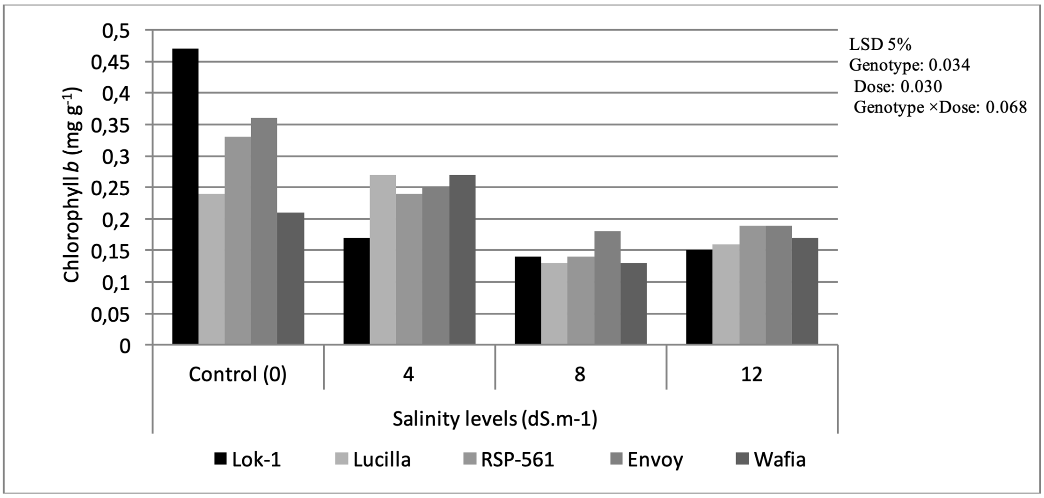


Figure 12. Chlorophyll-b (mg $g^{-1}$) of bread wheat genotypes grown at different salinity levels

**Total chlorophyll (mg $g^{-1}$)**

With increasing NaCl concentration, total chlorophyll content decreased sharply of all wheat varieties. Statistically, there were significant differences between the genotype, salt applications and Genotype × Dose for total chlorophyll. The highest total chlorophyll was recorded under control condition, while the minimum value was recorded at 8 $dSm^{-1}$and 12 $dSm^{-1}$.'Lok-1' had the highest total chlorophyll, whereas 'Lucilla' and 'Wafia' had the lowest total chlorophyll (Figure 13). The highest total chlorophyll was recorded in 'Lok-1' cultivar at control, whereas the lowest total chlorophyll was recorded in 'Wafia' and 'Lucilla' varieties at 8 $dSm^{-1}$ salt dose.

The chloroplastids cell membrane deterioration under salt stress might be responsible for significant reduction in total chlorophyll content leading to lesser photosynthetic efficiency (Seeman et al., 1985; Datta et al., 2009; Ashraf et al. 2005; Iqbal et al. 2006; Khan et al., 2009; Hasan et al., 2015). It has also been suggested that chlorophyll might serve as a key parameter to indicate salt tolerance or sensitivity in crop plants.

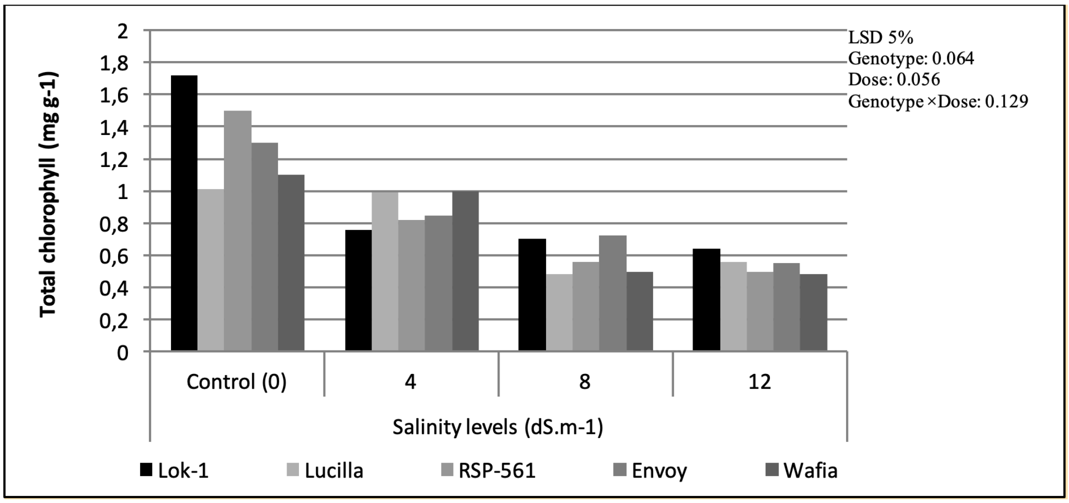


Figure 13. Total chlorophyll (mg $g^{-1}$) of bread wheat genotypes grown at different salinity levels

**Carotenoid (mg $g^{-1}$)**

It was observed that a significant variation in carotenoid concentrationresulted in all wheat varieties under varying salinity regimes (Figure 14). The highest carotenoid was recorded under control condition, while the minimum value was recorded at 8 and 12 $dSm^{-1}$.'Lok-1'had the highest carotenoid, whereas 'Lucilla' had the lowest carotenoid. The highest carotenoid was recorded in 'Lok-1' cultivar at control, whereas the lowest carotenoid was recorded in 'RSP-561' variety at 12 $dSm^{-1}$ salt dose. These findings are in agreement to previous researches which inferred that salt stress altered and interfered with the vital morphological developments, physiological processes as well as metabolic activities within crop plants (Rhoades, 1993).

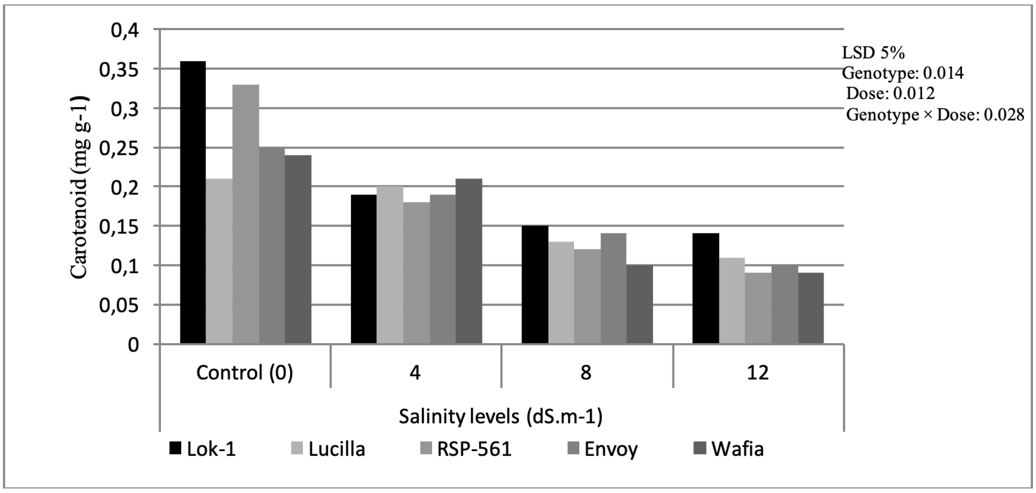


Figure 14. Carotenoid (mg $g^{-1}$) of bread wheat genotypes grown at different salinity levels

**Conclusion**

Result of current study indicated that wheat cultivars 'Lucilla' and 'Envoy' performed better based on seedling traits, while 'Envoy' and 'RSP-561' remained superior for biochemical characteristics under salt stress condtions. Accordingly, Envoy might be suggested as planting material to conduct further indepth investigations on drastic impacts imparted by salinity on growth and biochemical processes taking place at different phonological phases of crop plants. In addition, findings of this study might be used as reference point to conduct further field investigations in order to understand physiological and biochemical changes during vegetative and reproductive developmental processes of wheat under varying salinity regimes.

**Compliance with Ethical Standards**

**Conflict of interest**

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

**Author contribution**

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

**Ethical approval**

Not applicable.

**Funding**

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**Data availability**

Not applicable.

**Consent for publication**

Not applicable.

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