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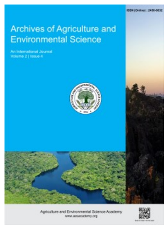

ORIGINAL RESEARCH ARTICLE

Seed germination and seedling development of spring wheat (*Triticum aestivum* L.) landraces under elevated salinity conditions

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ABSTRACT

Due to osmotic stress, ion toxicity, and nutrient imbalances, salinity is a common environmental problem that affects about 20.87% of agricultural land. It severely limits crop production, including wheat, by making it harder for seeds to germinate and for seedlings to grow. To study the effect of salinity stress on wheat landraces, we aimed to examine the seedling characteristics of wheat landraces under salinity stress. The study followed a two-factor Completely Randomized Design (CRD) with four replications. Four different concentrations of NaCl (0 mM, 25 mM, 50 mM, 75 mM) and five landraces (Bhartale, Jhuse, Rato Gahun, Mudule, and Aadhikhole) were used for investigation during the germination and early seedling stages of wheat landraces. The highest germination percentage (GP) (97.25%) and seed vigour index (SVI) (92886) were observed in Jhuse, whereas the lowest GP (60.50%) and SVI (11494) were observed in Rato Gahun. Rato gahun had the highest mean germination time (5.99 days), and Mudule had the lowest mean germination time (4.82 days). The maximum fresh weight (0.12 g) and dry weight (DW) (0.042 g) were recorded by Aadhikhole and Jhuse, and the minimum fresh weight (DW) (0.10 g) and DW (0.032 g) were observed in Mudule. The maximum shoot and root lengths were observed in Bhartale (3.05 cm) and Jhuse (3.54 cm), whereas the minimum shoot length (SL) (2.07 cm) and root length (RL) (2.41 cm) were observed in Rato Gahun. Above all, salinity stress negatively impacts wheat seedling characteristics, and Mudule shows some promising stress tolerance compared to others.

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INTRODUCTION

Salinity is widely acknowledged as one of the most common hazards to the environment, limiting the production of crops, mostly in arid parts of the world. Osmotic strains, ion specificity/toxicity, and nutritional imbalances caused by a high number of ions in the root medium reduce growth and yield (Wahid *et al.*, 2007). Salinity is a worldwide problem; approximately 20.87% of agricultural land is affected by salinity, which is considered the oldest abiotic stress (Hopmans *et al.*, 2021). Salt stress is the most common problem in semi-arid and arid regions, and it affects plant growth and development (Munns & Tester, 2008). Soil salinity affects water, mineral, and microbial contents, which makes sensitive crops less able to germinate and grow (Hasanuzzaman *et al.*, 2019). Various physiological, biochemical, and morphological processes in plants, including nutrient uptake, seedling growth, and water uptake, are affected by salinity (Akbarimoghaddam *et al.*, 2011). Salinity affects various phases of plant growth, such as germination, seedling stage, and vegetative growth phases, up to maturity (Uçarlı, 2021). Salt stress decreases the nutrient uptake capacity of plants, along with water intake, affecting the germination and seedling stages of wheat. Seed germination is negatively affected by stressful conditions during the germination phase of most crops, and germination is also considered the most critical stage of plant growth (Tabatabaei, 2014). Salt affects the imbibition of water through the seed coat and delays germination (Ehtaiwesh & Rashed, 2019). Salt also affects the root, shoot, and ultimately the seedling length of plants because it also affects cells and cell walls (de la Reguera *et al.*, 2020). Salt stress also affects fresh weight because of NaCl; the fresh weight of roots and shoots is also affected by salt ions during the growth phase of plants. Under salt stress, the fresh weight also decreases (Zhang *et al.*, 2020). Excessive Na+ and CL- ion toxicity adversely affects water and nutrient uptake by plants, causing a reduction in growth and development, leading to a reduction in dry matter as well (Khare *et al.*, 2015).

Wheat is the third most grown staple food in Nepal, with a total cultivated land of 716,978 ha and a production of 2,144,568 tons in 2078/79 (MoALD, 2023). Wheat landraces are traditional crop varieties established by farmers through natural and human selection and are adapted to specific environments (Jaradat, 2013). Seed priming is a simple procedure to enhance germination at the initial stage (Abbasdokht *et al.*, 2010). Seed priming improves the pre-germination metabolic process, which starts with water soaking and breaks down dormancy, also enhancing the seed vigor index and seed germination (Adhikari *et al.*, 2021). Hydropriming is a common, effective, and low-cost priming technique that enhances germination aiding, pre-germination and metabolic processes (Singh & Rakesh Singh, 2017). Developing a genotype with potential salt tolerance is a useful strategy to combat salinity stress (Pervaiz *et al.*, 2007). To identify whether there is a genetic basis for selection and breeding goals, as well as whether there are valuable genotypes or novel genes for resistance to salt stress, testing for salt-tolerant wheat germplasms is vital (Muhammad & Hussain, 2012). Investigating inter-cultivar genetic variation in salt tolerance is also key to boosting salt tolerance (Noreen *et al.*, 2007). Additionally, wheat is less susceptible to salinity stress than other crops (Munns *et al.*, 2006), but the ability to tolerate may vary according to salinity level and genotypes. Thus, this study was conducted to examine the effects of varied levels of salinity stress on landrace wheat genotypes, which are domesticated but commercially unavailable.

MATERIALS AND METHODS

Experimental framework

This study aimed to provide a thorough understanding of the dynamics of post-hydropriming salinity in several wheat landraces during germination and early seedling growth. Using a comprehensive experimental design, we employed a two-factor completely randomized design (CRD) with various NaCl concentrations (0, 25, 50, and 75 mM) and distinct wheat landraces (Bhartale, Jhuse, Rato ghahun, Mudule, and Aadhikhole) in the physiological laboratory of Gokuleshwor Agriculture and Animal Science College.

Hydropriming and petri dishes preparation

Before the experiment, all wheat seeds were hydroprimed for 12 h and then dried for 25 min in a shade. This pretreatment entailed submerging the seeds in tap water to create a balanced environment for the germination process (Sarlach *et al.*, 2013). Careful preparation of the Petri dishes was essential to maintaining the quality of the experiment. To ensure clean and aseptic conditions, the petri dishes were autoclaved for 30 min at 121 °C (Rutala & Weber, 2014). Each Petri dish containing twenty-five seeds on blotting paper was allocated exclusively to the various treatments.

NaCl induced salinity levels

The NaCl concentrations were applied at four levels (0 as a control, 25 mM, 50 mM, and 75 mM) to the respective Petri dishes, with a uniform volume of 15 ml in each petri dish.

Germination traits

Germination counts started once the seeds began to germinate. The count was obtained when the plumule length exceeded 2 mm until 10th day. Mean germination time (MGT) was computed by multiplying the number of newly germinated seeds at each time point (represented by the letter "n") by the corresponding number of days since the germination test began (represented by the letter "D").

$$MGT = \sum n.D / N \qquad \text{Eq. 1}$$

Where N represents the number of final germinated seeds (Demir *et al.*, 2008).

The germination percentage (G%) was expressed as follows:

$$G\% = (NSG/TNSS) \times 100 \qquad \text{Eq. 2}$$

NSG is a representation of all seeds that emerged at the end of the experiment. TNSS represents the number of seeds sown overall (Nasri *et al.*, 2011).

Seedling growth

To measure morphological traits, such as seedling length, shoot and root length, and fresh and dry weight, five seedlings were randomly selected for each Petri dish. Ten days after the seedlings were sown, the lengths of the shoots and roots were measured, and a weighing balance was used to record the fresh weights of the roots and shoots. The shoot and root dry weights were obtained on the eleventh day after sowing. To ensure scientific uniformity, seedlings were carefully dried for 12 h at 70 °C in a hot-air oven; this process was repeated twice for methodological consistency (Yusufe *et al.*, 2017). The seed vigor index (SVI) was calculated using morphological traits. This formula was used to calculate the SVI (Abdoli *et al.*, 2013).

$$\text{Seedling length} = \text{root length} + \text{shoot length} \qquad \text{Eq. 3}$$

$$SVI = (\text{Seedlings length} \times \text{Germination percentage}) \times 100 \qquad \text{Eq. 4}$$

Statistical Analysis

Data were entered into Microsoft Excel, and statistical analyses were performed using RStudio version 4.3.2. Two-way analysis of variance (ANOVA) was conducted to study the effects of salinity level, landraces, and their interactions. Post-hoc analysis (Tukey) was done on R-studio version 4.3.3.

RESULTS AND DISCUSSION

Germination percentage (GP)

During this study, ANOVA demonstrated significant results among the various landraces, the concentration of salt (P<0.001), and the interaction between the landraces and salt concentration (P<0.01). Mudule recorded the highest GP (97.25%) among the different landraces, followed by Jhuse (95.75%), Bartale (91.75%), and Aadhikhole (76.75%). The lowest GP was recorded in Rato gahun (60.50%). Concentration means displayed the highest GP was observed at 0 mM (91%) followed by 25 mM (85.4%) and 50 mM (82%). 75 mM showed the lowest germination rate (79.2%). The interaction between landraces and concentration showed the highest germination by Jhuse (99%) at 0 mM, which was statistically at par with Mudule (99%) at 0 mM, 25 mM, 50 mM, 75 mM, and Jhuse at 25 mM, 50 mM. Rato gahun showed the lowest germination was shown by Rato gahun (53%) at 75 mM (Figure 1). The germination percentage of the different landraces decreased with increasing salt concentration. Alharbi *et al.* (2022) reported the same result of decreased germination with increasing salt concentrations. The decrease in germination with increasing salt concentration may be due to the combined effect of osmotic pressure and salt toxicity or it may create an environment for the entry of ions that may be harmful to embryos or early seedlings (Uçarlı, 2021).

Mean germination time (MGT)

Data analysis of ANOVA revealed that the mean germination time varied significantly among various landraces (P<0.001) but not significantly among different salinity levels. However, no significant differences were observed between these interactions. Of all the landraces, Rato gahu had the highest MGT (5.99 days), which was statistically at par with Aadhikhole (5.92 days). Furthermore, the lowest MGT was shown by Mudule (4.82 days), which was statistically at par with Jhuse (4.86 days) and Bhartale (5.00 days). Based on the ANOVA, there was no statistically significant variation in the concentration and interaction between concentration and landrace. The highest mean concentration was observed at 75 mM (5.44 days), while the lowest was at 0 mM (5.24 days). In the interaction between concentration and landraces, Rato gahun showed the highest mean germination time under 75 mM (6.36 days), and the lowest mean germination time was observed in Mudule under 0 mM (4.67 days). Increasing concentrations of salt also increased the mean germination time in all landraces, except Jhuse. There was an increase in mean germination time with increasing salt concentration (Figure 2). Bhartale demonstrated a decrease in the mean germination time at 50 mM; however, at 75 mM, the mean germination time exceeded 0 mM. This finding also aligns with those of Biswas *et al.* (2023), who reported that the mean germination time increases with increasing salinity levels. A higher salinity level could create an osmotic barrier that prevents water from absorbing, affecting the mean germination time but not the final germination rate (Irik & Bikmaz, 2024).

Seed vigor index

ANOVA revealed that the seed vigor index varied significantly among salinity levels and various landraces (P<0.001); however, no significant differences were found between their interactions. The highest seed vigor index was shown by Jhuse (92886), which was statistically similar to Mudule (85445), followed by Bhartale (74124) and Aadhikhole (64972). Rato gahun exhibited the lowest seed vigor index, which was exhibited by Rato gahun (11494). The concentration means revealed that the highest seed vigor index was at 0 mM (74818.4), followed by 25 mM (54460.8), and 50 mM (42408.8). The lowest seed vigor index was observed at 75 mM (2536.6). The interaction between landrace and concentration showed no significant differences. The maximum seed vigor index was shown by Mudule at 0 mM (92800), and the minimum seed vigor index was observed with Rato gahun at 75 mM (17024) in the interaction between landrace and concentration (Figure 3). There was a lower seed vigor index with a high concentration of NaCl in all landraces, except for Jhuse, although at 25 mM. This result is also supported by (Cheng *et al.*, 2023). R *et al.* (2024) also reported a decreased seed vigor index with increased salinity levels in Macrotyloma uniflorum and Vigna mungo. In that study, the highest seed vigor index was observed in the control and the lowest at 12 dsm^{-1}.

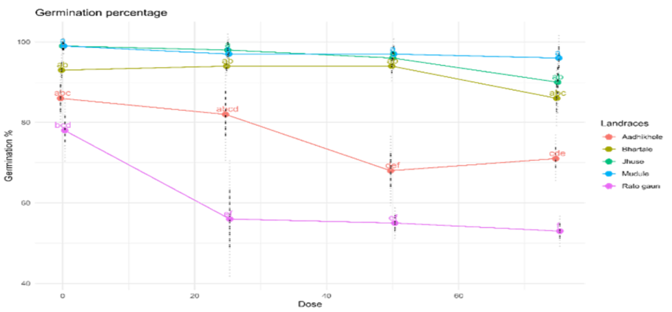

Figure 1. *Effect of salinity stress on germination percentage of wheat landraces.*

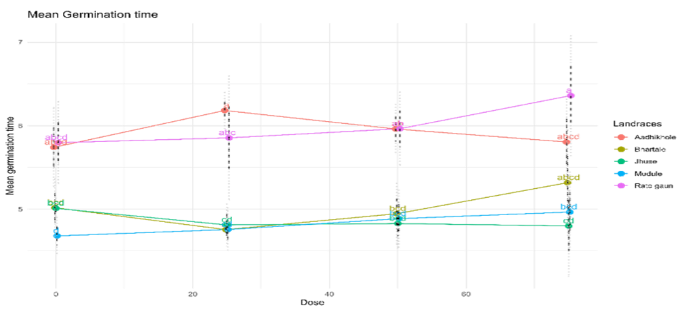

Figure 2. *Effect of salinity on mean germination time of wheat landraces.*

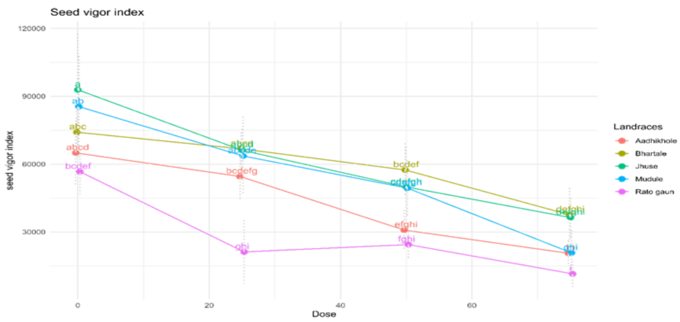

Figure 3. *Effect of salinity on seed vigor index of wheat landraces.*

Shoot length

The maximum shoot length was recorded for Bhartale (3.05 cm), followed by Jhuse (2.81 cm). The minimum shoot length was observed in Rato Gahun (2.07 cm), followed by Mudule (2.61 cm), which is similar to that of Aadhikhole (2.65 cm) in landraces. ANOVA revealed that shoot length varied significantly among salinity levels and various landraces (P<0.001); however, no significant differences were found between their interactions. The maximum concentration mean was shown by 0 mM (3.96 cm), followed by 25 mM (2.961 cm). The mean minimum concentration was recorded as 75 mM (1.37 cm), followed by 50 mm (2.26 cm). As there was no significant difference in the interaction between landraces and concentration, the maximum shoot length was observed on Mudule at 0 mm (4.41 cm). The minimum shoot length was observed in Rato gahun at 75 mm (0.97 cm) (Figure 4).

Root length

The highest root length was observed in Jhuse (3.54 cm), followed by Aadhikhole (3.06 cm). The lowest root length was observed in Rato Gahun (2.41 cm), followed by Mudule (3.20 cm), which is statistically similar to Bhartale (3.32 cm) in terms of landrace mean. The greatest root length was observed at 0 mM (4.70 cm), followed by 25 mM (3.27 cm). ANOVA revealed that shoot length varied significantly among salinity levels, various landraces (P<0.001) and interaction between salinity level and landraces (P<0.05). The shortest root length was observed at 75 mm (1.76 cm), followed by 50 mM (2.68 cm). As there was no significant difference in the interaction between salt concentrations, maximum root length was observed in Jhuse (5.43 cm). The lowest root length was observed in Rato gahun (1.16 cm) (Figure 5).

Seedling length

The shortest seedling length was observed in Rato gahun (4.48 cm) based on the landrace mean. Similarly, the greatest seedling length was observed at 0 mm (8.67 cm), followed by 25 mM (6.24 cm). The lowest seedling length was shown at 75 mM (3.14 cm), followed by 50 mM NaCl (4.95 cm). According to the ANOVA, there was no significant difference in the interaction. ANOVA revealed that seedling length varied significantly among salinity levels and various landraces (P<0.001); however, no significant differences were found between their interactions. The highest seedling length was shown by Bhartale (6.38 cm), which was statistically at par with Jhuse (6.36 cm), Mudule (5.81 cm), and Aadhikhole (5.72 cm). The highest among all landraces was observed for Mudule at 0 mM (9.69 cm). The lowest value was recorded on Mudule at 75 mM (2.37 cm) (Figure 6). The results showed an increase in shoot and root lengths with a decrease in NaCl concentration. The highest shoot, root, and seedling lengths were observed in Mudule at 0 mm (4.41 cm), Jhuse (5.43 cm), and Mudule at 0 mM (9.69 cm), respectively (Figures 4 and 5). Similar results were also obtained by Adilu & Gebre (2021) for shoot length, Liu *et al.* (2022) for root length, and Song *et al.* (2023) for seedling length. Root and shoot growth may be hindered by the detrimental effects of NaCl as well as the unequal absorption of nutrients by seedlings (Mbarki *et al.*, 2020). Root length is more affected by salinity level than shoot length, which is in line with the findings of Zou *et al.* (2022).

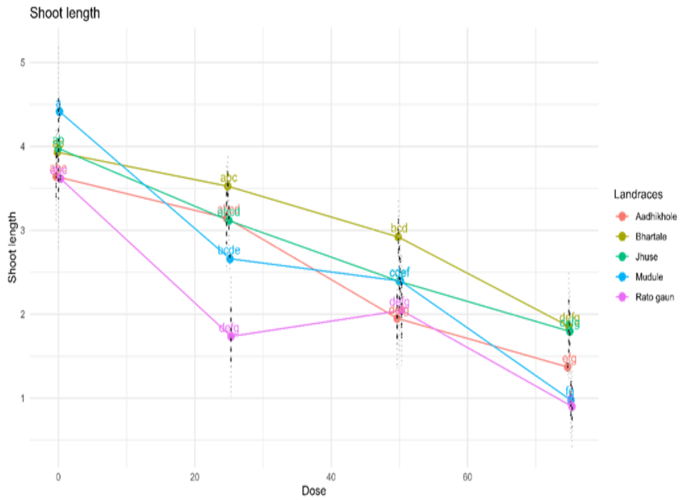

Figure 4. *Effect of salinity stress on shoot length of wheat landraces.*

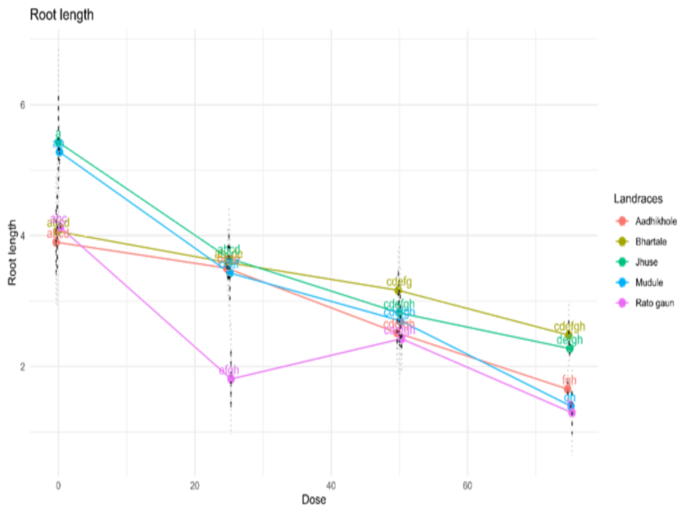

Figure 5. *Effect of salinity stress on root length of wheat landraces.*

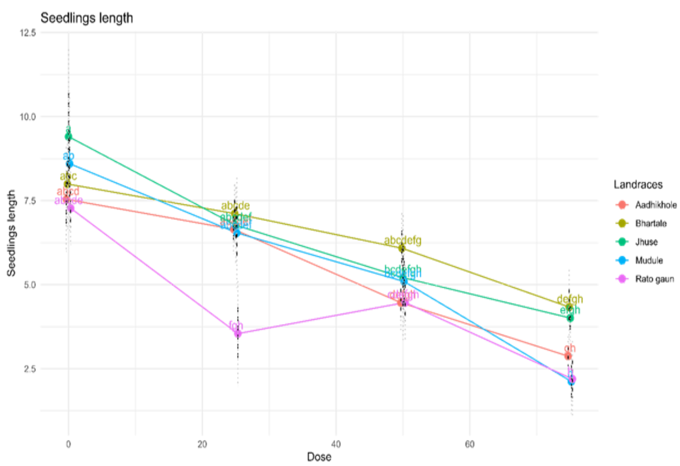

Figure 6. *Effect of salinity stress on seedling length on wheat landraces.*

Fresh weight

ANOVA revealed that fresh weight varied significantly among salinity levels and various landraces (P<0.001); however, no significant differences were found between their interactions. The maximum fresh weight was recorded by Aadhikhole (0.12 g), which was statistically at par with Jhuse (0.12 g), and the minimum fresh weight was recorded on Mudule (0.10 g), which was statistically at par with Bhartale (0.10 g) and Rato Gahun (0.10 g). The mean maximum fresh weight was recorded at 0 mM (0.12 g), followed by 25 mM (0.11 g). The minimum fresh weight recorded was 75mM (0.09 g), which was statistically similar to 50mM (0.10 g). No significant difference was observed in the interaction between landrace and concentration. The maximum fresh weight was recorded by Bhartale at 0 mM (0.14 g), and the minimum fresh weight was recorded by Mudule at 75 mM (0.07 g). The fresh weight declined with an increase in the concentration of NaCl in all landraces. However, in Jhuse there was almost constant fresh weight excluding at 25 mM (Figure 7). This is analogous to the results of Gola (2018) for the four vegetables. Excessive salt concentration in the soil around the roots affects water absorption, which in turn alters cell metabolism and ultimately causes a decrease in plant growth and development and reduces fresh weight (Devi *et al.*, 2021).

Dry weight

Jhuse (0.042 g) had the highest dry weight among landraces, whereas Mudule (0.032 g) had the lowest dry weight. At 75 mM (0.038 g), the highest dry weight was obtained. The lowest dry weight was obtained at 0 mM (0.034 g). ANOVA revealed that dry weight varied significantly among various landraces (P<0.001) and non-significantly among salinity levels; however, no significant differences were found between their interactions. The interaction between landraces and concentration showed that the highest dry weight was obtained by Jhuse at 50 mM (0.05 g), and the lowest was obtained by Bhartale at 25 mM (0.03 g) (Figure 8). The results show that with an increase in salt concentration, the dry weight of all landraces decreased a lower weight. Chen *et al.* (2022) noticed that the dry weight of the seedlings decreased as the salt concentration increased in barley and wheat, which is not consistent with our study.

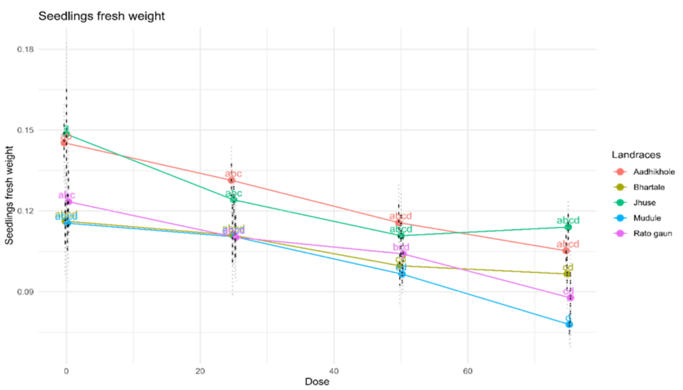

Figure 7. *Effect of salinity stress on seedling fresh weight in five wheat landraces.*

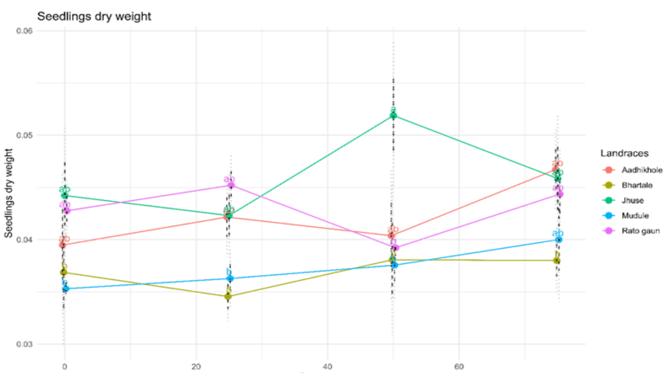

Figure 8. *Effect of salinity stress on seedling dry weight in five wheat landraces.*

Conclusion

The purpose of this study was to examine, under controlled settings, the effects of different salt levels on the germination, growth, and vigor of five distinct wheat landraces: Jhuse, Aadhikhole, Mudule, Rato gahun, and Bharatale. The outcomes showed that the landraces differed significantly in how they responded to salt stress, with fresh weight, shoot length, root length, germination percentage, and seedling vigor all declining as salinity increased. Jhuse and Mudule were the two landraces that were superior at withstanding salt stress; even at high NaCl concentrations, they demonstrated greater germination rates, seedling vigor, and growth characteristics. The results imply that wheat's capacity to withstand salt is genotype-dependent, with some landraces such as Jhuse and Mudule showing greater resistance to salinity. In order to preserve agricultural yield in saline-prone locations, it is imperative to choose and breed wheat varieties with higher salt tolerance, which is a practical consequence of our results. The study also emphasizes how crucial seed priming methods like hydropriming are for enhancing early seedling development and germination in stressful environments. All things considered, this study advances our knowledge of how wheat landraces respond to salt stress and lays the groundwork for upcoming breeding initiatives that will produce salt-tolerant wheat cultivars, which will be crucial for maintaining food security in areas where soil salinity is a problem. Under conditions of salt stress, it also showed early seedling and germination properties. Our research suggests that Mudule is a promising landrace for further study and deployment. However, it is important to note that a single experiment may not be sufficient to obtain reliable results. Therefore, large-scale experiments should be conducted at farmers' fields to further bolster the commercialization potential.

DECLARATIONS

Author contribution

Conceptualization: P.C.; Methodology: P.C.; Software and validation: P.C.; Formal analysis and investigation: P.C.; Resources: P.C. and D.R.B..; Writing—original draft preparation: P.C., A.K., L.K., and D.R.B.; Writing—review and editing: P.C.; Visualization: P.C.; Supervision: P.C. All authors have read and agreed to the published version of the manuscript.

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Supplementary data: Not available.

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