

EVALUATION OF PANT CHARACTERISTICS AND PHYSIOLOGICAL PERFORMANCE OF MUNGBEAN (*VIGNA RADIATA* (L.) WILCZEK) GENOTYPES UNDER SALT STRESS

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Abstract

Mungbean is a delicious popular pulse crop whose yield is hampered by biotic and abiotic factors. Salinity is considered serious abiotic stress that hinders growth and yield drastically. To find out the response features of salinity tolerance in mungbean, a series of experiments were carried out in the Department of Crop Botany of BSMRAU. The experiment was performed in the greenhouse of the Crop Botany department using nutrient solution under hydroponics conditions. Initially, fifty-two mungbean genotypes were used in the experiment to screen out a susceptible and tolerant genotype. The results indicated that salinity affected the plants at various morphological characteristics namely plant height, and dry matter of root, stem, and fruit. The genotypes were placed in four groups based on their performance in salinity. A higher quantity of proline with a lower amount of Malon-dialdehyde was observed with the increase in salinity. Chlorophyll content increased initially and after that declined sharply. The susceptible genotype resulted in a sharp decline of chlorophyll and increased proline content which reflected the ¹accumulation of root and shoot dry matter, and consequently, the total dry matter content compared to that of the tolerant genotype.

Key words Mungbean, Salinity, Resistant, Susceptible, Dry matter

Introduction

The coastal region of Bangladesh comprises nearly 30% of the cultivable land. Around 1.056 million ha of land in 2086 million ha of coastal and off-shore lands are subject to a wide range of salinity (Moslehuddin *et al.*, 2015). Due to the presence of brackish soil, most of the lands remain fallow during the dry period (February-May) because of the scarcity of quality fresh water for irrigation and the poor drainage system (Karim *et al.*, 1990; SRDI, 2000). Saline-tolerant pulse crops would have a good perspective to be

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cultivated in this area during winter. Among the pulses, mungbean is a vital crop of Bangladesh with multipurpose usages. It has the clear benefit of having a small life span and can be grown in different types of soil and environment (Rao *et al.*, 2016). A high-value crop like mungbean is a potential candidate for this area. However, it is sensitive to salinity, and salt stress harms plant growth and development as saline stress leads to low osmotic potential (osmotic stress), specific ion impacts (ionic stress), and nutritional imbalance (Parida and Das, 2005).

The morphology of salt-induced plants is severely affected, thereby changing the tissue structures and system. High salt stress harms plants, while moderate to low levels negatively influence the plant development rate and sequentially show symptoms related to morphological, physiological, or biochemical change (Hasegawa, 2013).

The altered anatomical features change the physiological, namely photosynthetic pigments, such as, chlorophyll, and amino acid synthesis is hampered by saline conditions (Rahman *et al.*, 2002). Anatomical and physiological strategies could foster the development and endurance of the plant under stressful conditions. Deposition of Na^+ and Cl^- in plant tissues can lead to plant growth reduction under high saline conditions. At the seedling stage, plant growth is severely affected by the distribution of Na^+ , Cl^- , and K^+ in root and shoot. In saline conditions, Na^+ and Cl^- concentrations increased in roots and shoots, but this escalation is less in tolerant than sensitive ones (Singh *et al.*, 2017). One of the most important salinity tolerance strategies of the crop is thought to be the dilution of the excessive amounts of deposited Na^+ and Cl^- ions from the plant body (Sultana *et al.*, 2021).

A high-yielding saline-tolerant mungbean variety can find a place in the existing cropping pattern in the salty soil, which is yet to be developed. To fit this crop in the existing cropping pattern, it is imperative to develop a saline-tolerant mungbean variety. Aiming to this criterion, the department of Crop Botany of BSMRAU has conducted a series of experiments to study the salinity-induced responses in different plants, including Mungbean. And in this sequence, fifty-two mungbean genotypes were procured from the Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI), Gazipur. They were used in the quest of evaluating the response of mungbean plants in saline conditions.

Materials and Methods

A glass house and laboratory experiments were carried out at the Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.

Initially, the procured genotypes were screened against salinity, and later screened genotypes were evaluated from different physiological perspectives.

Screening of genotypes against salinity: Two treatments, namely salinity of 8 dSm⁻¹ and control, were imposed in the experiment. The NaCl salt was applied in the container through irrigation water starting at 7 days old seedlings continued up to 40 days.

The collected genotypes were used for screening against salt stress. Watering on the pot was performed regularly, maintaining a scheduled routine during the experiment period. Plants were kept in plastic pots, which were put in a bigger plastic container (10 L) containing Hoagland nutrient solution (Hoagland and Arnon, 1950). Intercultural operations were performed regularly to ensure the typical growth and development of the mungbean plant.

Plants were harvested 45 days after germination. Roots and shoots were separated, scrubbed, and weighed after drying in an oven at 81° C for 72 hours. Data were recorded on common growth parameters (plant height, root and shoot length, root and shoot dry weight). The characters were studied in percent relative values for a proper understanding of the salinity tolerance in mungbean genotypes. The following formula was used to calculate the relative value:

$$\text{Relative value} = \frac{\text{Value of saline treated genotype}}{\text{Value of control genotype}} \times 100$$

The mungbean genotypes were grouped based on their shoot dry matter. A tolerance scale was made to categorize the genotypes in different group orders based on dry shoot matter (Ashraf and Wahid, 2000).

Physiological and Yield Attributes of Salinity Tolerance in Mungbean: After initial screening, the selected two genotypes were tested in terms of physiological and yield response. The experiment was planned in a factorial, a completely randomized design where factor one is mungbean genotypes (BD 6895 and BD 6905) and factor two is salinity levels (control, 6, 8, 10, and 12 dSm⁻¹).

Estimation of Proline Content: To estimate the proline, the top-most completely extended leaf samples were used according to the process of Bates *et al.* (1973). The standard curve was used to determine proline concentration and calculated on a fresh weight basis as follows:

$$\text{Proline content } (\mu\text{mole/g fresh wt.}) = \{ \mu\text{g proline/ml} \times \text{vol. of extra. sol. (ml)} \times \text{toluene used (ml)} \} / \{ 115.13 \mu\text{g/mole} \times \text{g sample} \}$$

Estimation of Chlorophyll Content: Chlorophyll contents were appraised using the youngest and top-most completely extended leaf samples with the following method described by Porra *et al.* (1989). The formula for measuring the chlorophyll a, b, and total chlorophyll are:

$$\text{Chlorophyll a (mg}^{-1} \text{ fresh weight)} = [12.21 (A_{663}) - 2.81 (A_{646})] \times [V/1000 \times W]$$

$$\text{Chlorophyll b (mg}^{-1} \text{ fresh weight)} = [20.13 (A_{646}) - 5.03 (A_{663})] \times [V/1000 \times W]$$

$$\text{Total Chlorophyll (mg}^{-1} \text{ fresh weight)} = [20.2 (D_{646}) + 8.02 (D_{663})] \times [V/1000 \times W]$$

Where, V = Volume of acetone used (mL) W = Weight of fresh leaf sample in (g).

Determination of lipid peroxidation: The thio-barbituric acid (TBA) method described by Tayebi-meigooni *et al.* (2012) was used to determine the level of Malon-dialdehyde (MDA). An extinction coefficient of $155 \text{ nm}^{-1} \text{ cm}^{-1}$ was adapted to calculate the MDA concentrations using the following formula:

$$\text{MDA (}\mu\text{molg}^{-1} \text{ fresh weight)} = [(A_{532} - A_{600})/155] \times 10^3 \times \text{Dilution factor}$$

The data were analyzed using statistical software (Statistix10) and comparisons with *P*-values < 0.05 were considered significantly different by using honestly significant difference (HSD) values (Tukey's Test).

Results and Discussion

Screening of genotypes against salinity: Fifty-two mungbean genotypes were used for screening against one salt stress (8 dSm^{-1}). The characters were studied in percent relative values for a proper understanding of the salinity tolerance in mungbean genotypes. Relative plant height ranged from 45.6 to 82.0, with a mean of 63.3. In mungbean genotypes, there was remarkable variation in root dry weight which ranged from 14.5 to 99.0 with a mean of 56.4. Relative shoot dry matter of mungbean genotypes fluctuated from 27.3 to 100.0 with a mean of 58.1, which were significantly different among the genotypes with a corresponding mean of 39.16.

Table 1. Variation in quantitative plant characters of 52 mungbean genotypes under salinity stress.

| Plant characters | Minimum | Maximum | Mean | LSD | CV (%) |
|---------------------------|---------|---------|-------|-------|--------|
| Relative plant height | 45.6 | 82.0 | 63.32 | 17.01 | 13.4 |
| Relative root dry weight | 14.5 | 99.0 | 56.43 | 17.58 | 15.5 |
| Relative shoot dry weight | 27.3 | 100.0 | 58.10 | 39.16 | 33.5 |

The genotypes BD-6888, BD-6895, BD-6906, BD-10028, and BD-10585 performed better in relative plant height in salinity-stressed conditions (Fig. 1). Salinity-induced stunted plant growth and necrosis of leaf were observed among the different pulse crop varieties where the growth of aerial shoot was more affected compared to that of the root. Sehrawat et al. (2015) reported a reduction in height and other growth parameters in mungbean in response to salinity stress. Greenway and Gibbs (2003) mentioned the destruction of energy in a saline condition caused growth retardation in the plant.

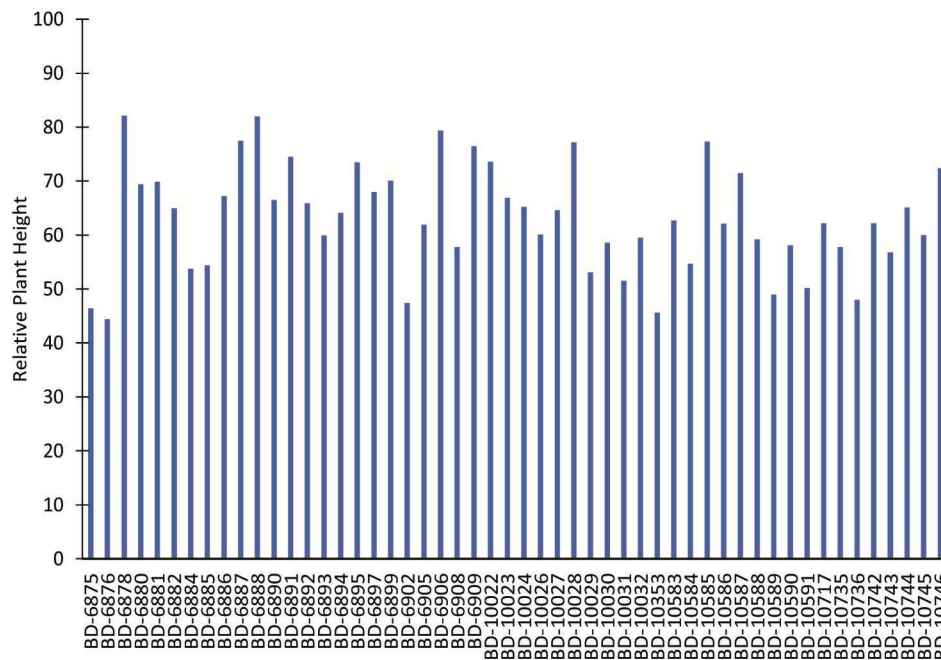


Fig. 1. Relative plant height (%) of 52 mungbean genotypes under salt stress at 8 dSm⁻¹

Relative root dry matter

The genotypes cv. BD-6878, BD-6887, BD-6888, BD-6897, and BD-6908 performed better in salinity-stressed conditions (Fig. 2). The increase in salt concentration has been reported to reduce the partitioning of dry matter significantly in *Glycine max* and *Phaseolus vulgaris* (Taffouo et al., 2009). Dai et al. (2009) reported that in perennial ryegrass, root dry matter is reduced with increasing salinity. The findings of the present results are also similar to some researchers' reports claiming that root weight decreased as increment with salt concentration for some plants (Kaya et al., 2005).

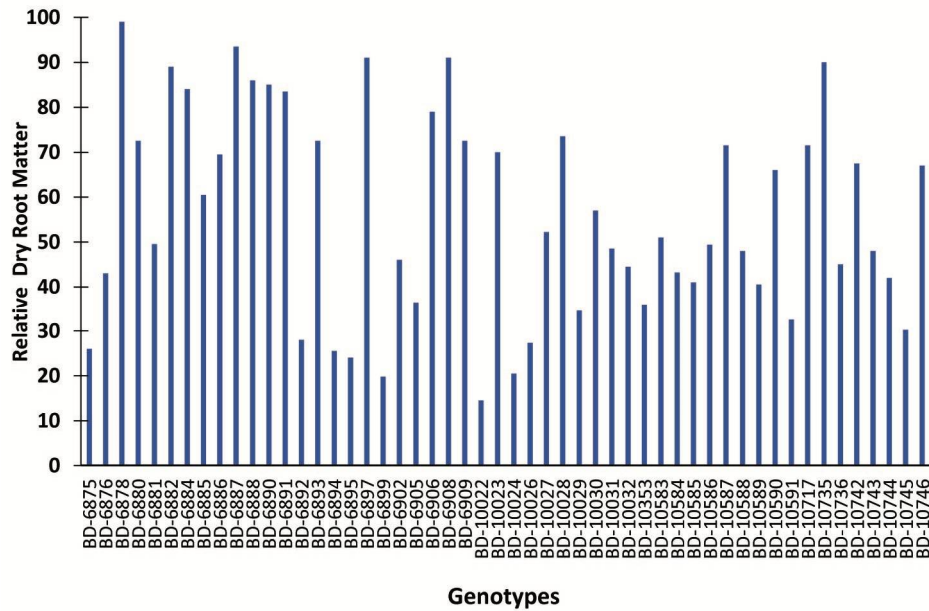


Fig. 2. Relative dry root matter (%) of 52 mungbean genotypes under salt stress at 8 dSm⁻¹

Relative shoot dry matter

Relative shoot dry matter (%) was severely affected by the salinity in 52 mungbean genotypes under saline conditions (Fig. 3). However, some genotypes cv. BD-6878, BD-6885, BD-6887, BD-6888, BD-6895, BD-6906, BD-10585, BD-10587, BD-10588, and BD-10717 performed better under saline stress conditions compared to that of the others.

The plant height of mungbean was reported to be reduced drastically in saline conditions (Ullah *et al.*, 2016). Salt stress was reported to reduce the growth of most legumes, as well as mungbean (Kabir *et al.*, 2004). These reductions in growth often resulted in reductions in tissue water potential, eventually reducing water availability to the cells (Garg and Bhandari, 2016), which leads to stomatal closing, less photosynthesis, and ultimately stunted growth (Garg and Manchanda, 2009).

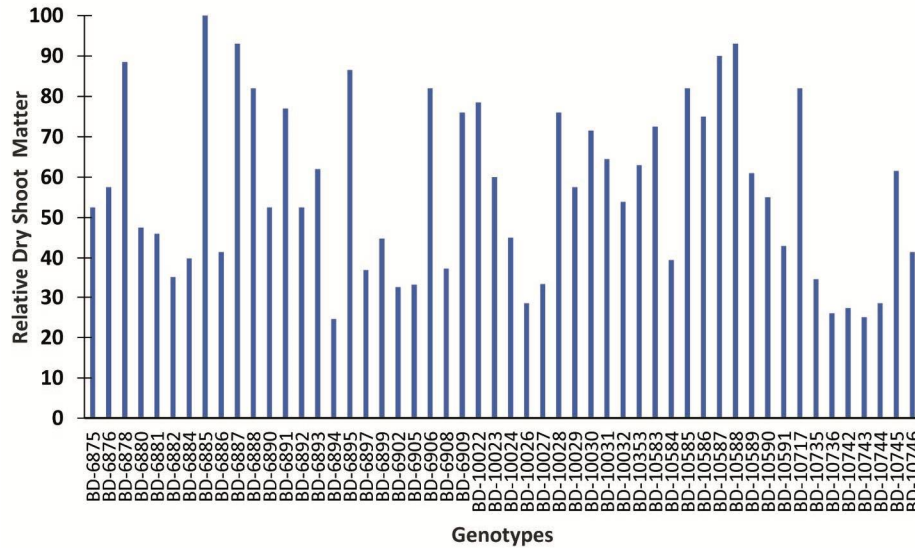


Fig. 3. Relative shoot dry matter (%) of 52 mungbean genotypes under salt stress at 8 dSm⁻¹

Grouping of the mungbean genotypes based on dry shoot matter

Grouping of the mungbean genotypes based on dry shoot matter under salinity stress (Ashraf and Wahid, 2000) (Table 2).

Table 2. Grouping of mungbean genotypes based on dry shoot matter under salinity stress.

| Scale | RSDW (%) | Frequency | Genotypes |
|-----------|----------|-----------|--|
| Group I | >80 | 10 | BD-6878, BD-6885, BD-6887, BD-6888, BD-6895, BD-6906, BD-10585, BD-10587, BD-10588, BD-10717 |
| Group II | 60-80 | 13 | BD-6891, BD-6893, BD-6909, BD-10022, BD-10023, BD-10028, BD-10030, BD-10031, BD-10353, BD-10583, BD-10586, BD-10589, BD-10745 |
| Group III | 40-60 | 14 | BD-6875, BD-6876, BD-6881, BD-6886, BD-6890, BD-6892, BD-6899, BD-10024, BD-10029, BD-10032, BD-10590, BD-10591, BD-10746, BD-6880 |
| Group IV | < 40 | 15 | BD-6882, BD-6884, BD- 6894, BD- 6897, BD-6902, BD-6905, BD-BD-6108, BD-10026, BD-10027, BD-10584, BD-10735, BD-10736, BD-10742, BD-10743, BD-10744 |

Genotypes were categorized into four groups, viz. group I, group II, group III, and group IV. Group I included ten genotypes viz. BD-6878, BD-6885, BD-6887, BD-6888, BD-6895, BD-6906, BD-10585, BD-10587, BD-10588 and BD-10717 in which RSDM more than 80%. This group produced the highest relative shoot dry matter.

Thirteen genotypes were found in group II namely, BD-6891, BD-6893, BD-6909, BD-10022, BD-10023, BD-10028, BD-10030, BD-10031, BD-10353, BD-10583, BD-10586, BD-10589, and BD-10745 with an RSDM range from 60 to 80%. This group was the second-highest shoot dry matter producer than other related traits. Fourteen genotypes viz. BD-6875, BD-6876, BD-6881, BD-6886, BD-6890, BD-6892, BD-6899, BD-10024, BD-10029, BD-10032, BD-10590, BD-10591, BD-10746, and BD-6880 were grouped in group III which was the (40-60) % RSDM. Fifteen genotypes BD-6882, BD-6884, BD-6894, BD-6897, BD-6902, BD-6905, BD-6908, BD-10026, BD-10027, BD-10584, BD-10735, BD-10736, BD-10742, BD-10743, and BD-10744 were grouped in group IV which was less than 40% of relative shoot dry matter.

Among the genotypes, one tolerant (BD-6895) and one susceptible (BD-6905) were selected for physiological evaluation. The selection was based on this screening and another experiment conducted in the department of Crop Botany (Khan *et al.*, 2022).

Screening and grouping based on responses were done at the seedling stages in chickpeas (Mustafa *et al.*, 2020). The shoot biomass under saline conditions and the shoot biomass production ratio under salt stress to that of the control showed notable differences at all sampling stages when 41 chickpea genotypes were screened against salinity stress (Serraj *et al.*, 2004). Generally, stunted growth of the plant is one of the most common salinity effects. A gradual reduction in plant height, root and shoot length, and root and stem dry matter with a progressive increase in salinity were found in several reports (Khan *et al.*, 2010).

Physiological and Yield Attributes of Salinity Tolerance in Mungbean

Chlorophyll content was increased with lower doses of salinity up to 6 dSm⁻¹, and after that declined with the increase of salinity (Fig. 4). The chlorophyll content of genotype BD-6905 declined sharply after 6 dSm⁻¹ (Fig. 4 A). In the case of chlorophyll b, the genotype BD-6895 showed an increasing trend up to 8 dSm⁻¹, and after that declined sharply. On the other hand, genotype BD-6905 gradually declined after 6 dSm⁻¹ (Fig. 4 B). In the case of total chlorophyll, both genotypes showed an initial increasing trend, and after that declined. However, the genotype BD-6895 showed better performance in total chlorophyll content.

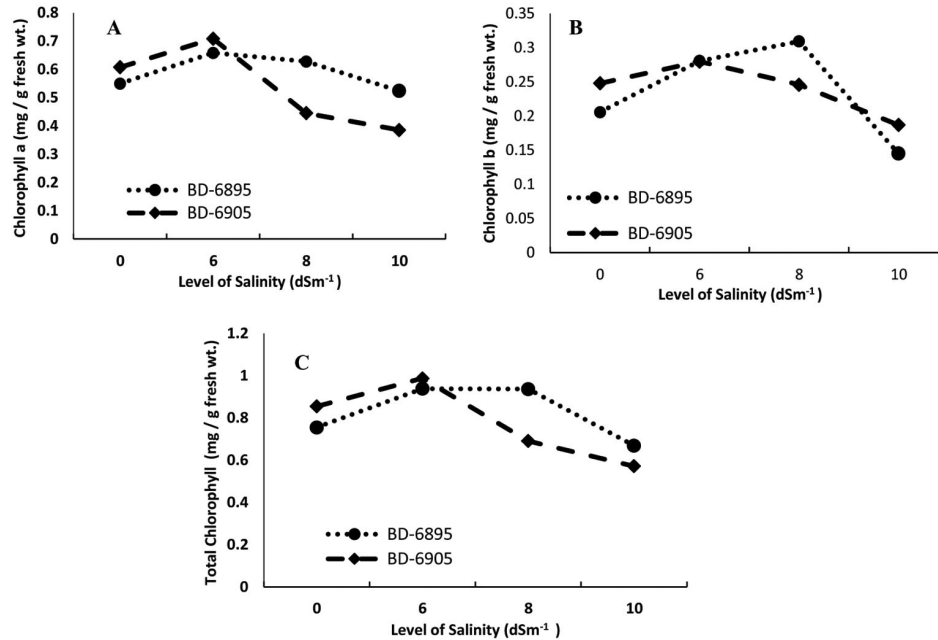


Fig. 4. Effect of varying salinity levels on mungbean genotypes' chlorophyll content (A-Chlorophyll a, B-Chlorophyll b, and C-Total chlorophyll content).

The reduction of chlorophyll content in mungbean genotypes in saline conditions was reported by Sehrawat *et al.* (2015). Salinity-induced reduction in chlorophyll level of the leaf was reported up to three-fold of control (Subashree *et al.*, 2021). With the increase in the salinity, Roychoudhury and Ghosh (2013) have reported a similar drop in the chlorophyll content.

Lipid Peroxidase (MDA) Activity

The MDA (Malon-dialdehyde) contents of both the genotypes showed a decreasing trend with the increase of salinity levels where BD-6905 showed higher values (Fig. 5). Lipid peroxidation is an important membrane-damaging agent under abiotic stresses (Yang *et al.*, 2010) and MDA act as a marker to observe the ROS (reactive oxygen species)-induced membrane injury under stresses (Gong *et al.*, 2008). Oxidative stress markers such as malondialdehyde (MDA) contents are amplified due to a continuous upsurge in salt stress (Ghosh *et al.*, 2015). The salt tolerance mechanism can be achieved by decreasing malondialdehyde levels (Mahmood *et al.*, 2022).

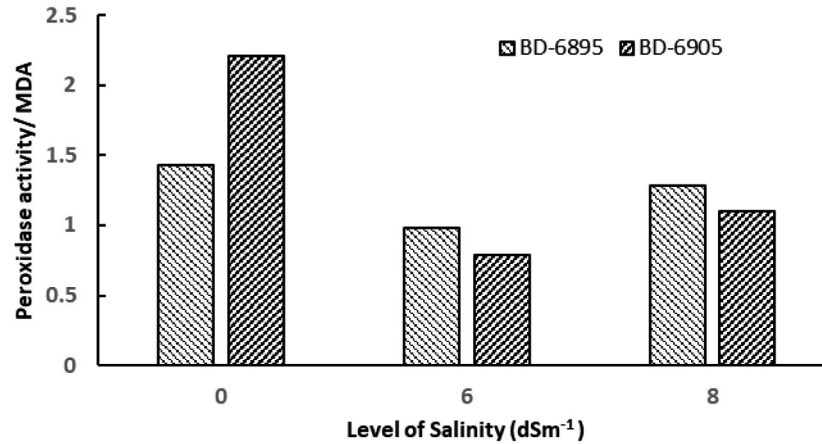


Fig. 5. Effect of varying salinity levels on the lipid peroxidase (MDA) accumulation in mungbean.

Proline Content

Higher proline was accumulated in both genotypes with the upsurge of salt levels. In this experiment, clear proline augmentation was observed with the increase in salinity. The genotype BD-6895 showed higher accumulation than BD-6905 (Fig. 6).

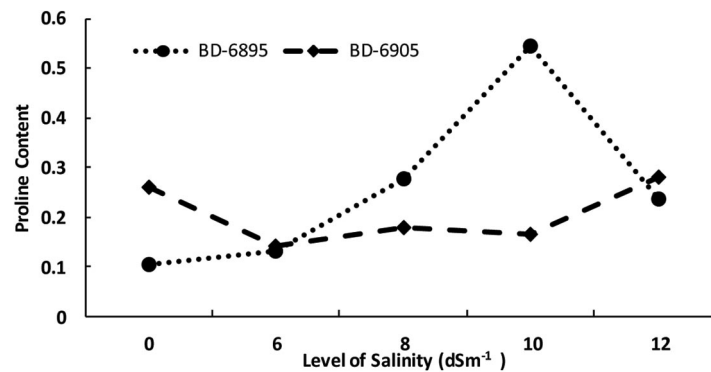


Fig. 6. Effect of salinity on the accumulation of proline in mungbean

Different osmolytes, like proline, induces physiological process favorably by maintaining the cellular equilibrium under salinity stress through the regulation of diffusion (Yang and Guo, 2018). The present experiment revealed that the leaf proline compound

increased with the salinity. A similarly increased accumulation of proline content under salt stress in mungbean was reported by Ghosh *et al.* (2015). Reddy *et al.* (2015) suggested that a higher proline accumulation may reduce saline-imposed stress and shield photosynthetic and antioxidant enzyme activities.

Dry Matter Accumulation

Shoot dry matters were seriously affected due to the imposition of salinity, where BD-6895 showed better performance than BD-6905 (Fig. 7). Similar trend was detected for the root dry matter and finally, the whole plant dry matter. Dry matter is one of the key aspects to evaluate plant performance. The highest dry matter was recorded in the control condition, and dry weight was reduced with the increasing salinity treatment (Benlioglu and Ozkan, 2020).

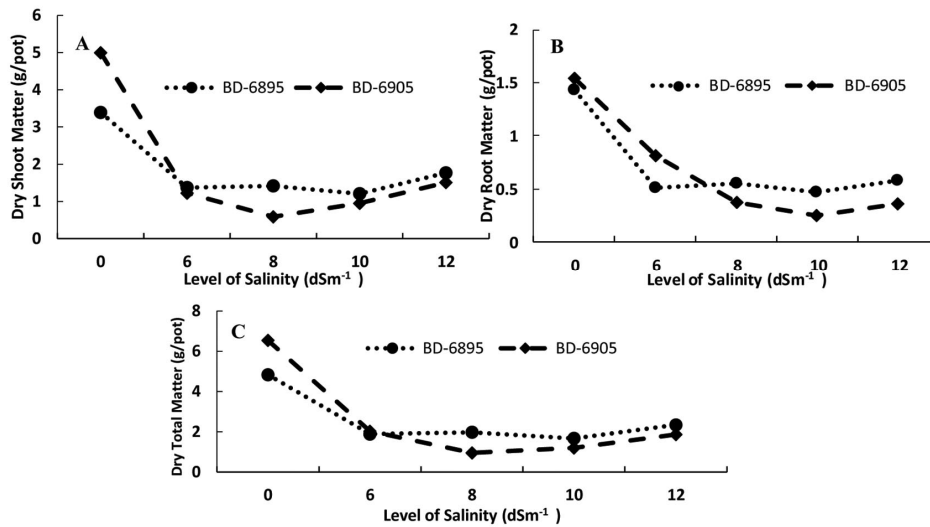


Fig. 7. Effect of varying levels of salinities on the dry matter accumulation in mungbean (A- Dry Shoot Matter, B-Dry Root Matter, C- Dry Total Matter).

Ercan (2008), in an experiment on lentils, reported a significant reduction in plant dry weights under saline stress and reported that reduction as a sign of susceptibility. Drought stress in sunflowers resulted in a drop in the whole plant's fresh and dry weight (Manivannan *et al.*, 2007). Several reports which showed decreases in the fresh and dry

weight of plants in salt shock (Baloglu *et al.*, 2012; Munir and Aftab, 2009; Mohammed, 2007) are similar to the inferences of the current experiment.

Among the legumes, mungbean bears an acceptable genetically inherent tolerance system, and many physiological traits are yet to be explored. It has the clear benefit of being a short-duration crop and can be grown in different types of soils and environments (Rao *et al.*, 2016). The current series of the experiment focuses on the saline stress and the ability of mungbean to find a place in the saline coastal area of Bangladesh as a fill crop (mungbean-rice-wheat to replace fallow-rice-fallow) or a relay crop in the existing cropping pattern. Among the procured genotypes, BD-6895 was screened as tolerant, and BD-6905 as susceptible from the initial screening and findings of a previous experiment (Khan *et al.*, 2022). Considering the physiological parameters, mungbean genotypes showed high proline and low MDA deposition in tissues under salt stress. Chlorophyll content also increased initially and declined sharply with the increase in the saline treatment. Of the two selected genotypes, the susceptible genotype BD-6905 resulted in a sharp deterioration of chlorophyll content and augmentation of the proline content compared to that of the tolerant one BD-6895. And that change is eventually reflected in the accumulation of root and shoot dry matter content, and ultimately, the total dry matter content of the mungbean plant.

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