

Original Research

Morpho-Biochemical Characterization and Genetic Analyses of the Traits of Mungbean Genotypes Confined with Yield Attributing Traits and Salinity Stress Tolerance

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Abstract

Low phenotypic and genotypic variability for yield-attributing traits and susceptibility to salinity stress are constraints of mungbean productivity. The objectives of the study are to phenotype a set of exotic mungbean genotypes for yield-attributing traits and salinity tolerance, as well as to conduct genetic analysis of these traits for efficient selection. Three consecutive experiments were conducted to fulfill these objectives. In the first experiment,



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26 mungbean genotypes were evaluated for yield traits under a randomized complete block design (RCBD) with three replications. Significant variation was found for all studied traits. Genotypes Durdona, BARI Mung-6, Binamoog-8, and BMX 1137 were high-yielding, while Binamoog-8, Binamoog-9, BMX 11140, BMX 11154, and BMX 11157 were categorized as early maturing. Traits including plant height, primary branches, pods per plant (PPP), pod length (PL), 100-seed weight (HSW), and yield per plant exhibited high heritability and genetic advance as a percentage of the mean. Yield per plant showed a significant positive correlation with PPP, PL, and HSW. In experiment II, the salinity tolerance of nine high-yielding mungbean genotypes was evaluated at the seedling stage using a completely randomized design with two treatments: control and salt stress (EC = 8 dS/m). Fourteen-day-old seedlings were subjected to salt stress for 7 days. Data on various root and shoot traits were recorded along with biochemical characteristics, including chlorophyll content, root and shoot Na⁺/K⁺ ratio, lipid peroxidation, H₂O₂, and proline content. These characteristics led to the identification of the genotypes Durdona and BMX 1131 as salt-tolerant at the seedling stage. The third experiment assessed the reproductive-stage salinity tolerance in pot culture under 8 dS/m salt stress using an RCBD with three replications. Saline stress was imposed after opening the first flower and continued for three weeks. Salt stress significantly reduced yield and related traits in most genotypes. BMX 1141 showed the most significant yield reduction (74.73%), whereas BMX 11111, BMX 11122, BMX 11170, BMX 1131, and Durdona exhibited lower declines. Stress tolerance indices classified Durdona as salt-tolerant at the reproductive stage. These findings underscore the potential for breeding salt-tolerant mungbean genotypes using Durdona to enhance mungbean productivity.

Keywords

Mungbean; evaluation; yield; heritability; salinity stress; seedling stage; reproductive stage; stress tolerance indices

1. Introduction

Mungbean (*Vigna radiata* L.) is a vital pulse crop, valued for its high-quality protein, essential amino acids, fatty acids, fibers, minerals, and vitamins [1-3]. Its short life cycle and nitrogen-fixing ability make it particularly valuable in sustainable agriculture and diverse cropping systems [4]. Mungbean is esteemed among the entire pulse species because it is an easily digestible pulse. It is a predominant and essential pulse crop in Bangladesh, but its productivity remains low compared to other mungbean-producing countries. Its true yield potential has yet to be achieved owing to several constraints, and one of the significant constraints is its susceptibility to several abiotic and biotic stresses [5]. Mungbean improvement programs have been limited due to low genetic variability in the gene pool [6]. Phenotypic diversity evaluation, through characterizing morphological and agronomical traits of the available germplasm, plays a crucial role in the selection of appropriate parents for genetic improvement and also for maximizing genetic gain [7]. The extent of genetic variation and the importance of heritability of desirable traits determine the success of crop breeding improvement. Therefore, evaluation of exotic germplasm under control and salt

stress conditions is essential to breed varieties for higher yield under these conditions and also for sustainable agricultural production.

In Bangladesh, over 30% of the net cultivable land is situated in coastal regions, with approximately 53% of these areas affected by varying salinity levels [8]. Both primary (natural) and secondary (irrigation-induced) salinity are accelerating the salinization of arable land at a rate of 1-3% annually, which could result in a 50% loss of arable land by 2050 [9]. This alarming trend has compelled nearly one-third of coastal farmers to adopt mono-cropping, predominantly cultivating Aman rice during the monsoon season, leaving most of the land to fallow during other seasons due to high salinity. Consequently, cropping intensity in these coastal regions is only around 133, significantly lower than the national average of 200. The overreliance on mono-cropping, coupled with the imbalanced use of inorganic fertilizers, pesticides, and intensive land use without organic amendments, has led to a decline in soil quality and fertility [10]. Diversification of cropping systems, particularly through the inclusion of pulses, offers a sustainable solution to these challenges. Short-duration pulses can not only diversify rice-based systems but also enhance overall system productivity, especially in southern Bangladesh. Therefore, the addition of short-duration, high-yielding, and salt-tolerant mungbeans in cropping patterns of coastal areas offers a great promise to avert food insecurity, improve the nutrition of poor households, and improve soil health by reducing soil salinity.

One of the primary limitations of mungbean cultivation is its vulnerability to abiotic stresses, particularly salinity. Salinity stress affects key developmental stages of mungbean, including germination, vegetative growth, and reproductive development. It significantly impacts plant growth by reducing osmotic potential, which hinders water uptake and induces the accumulation of salts in plant tissues, severely disrupting physiological processes. This dual effect contributes to yield losses that can exceed 60% under moderate salinity conditions [11, 12]. Beyond ionic and osmotic stress, salinity exacerbates biochemical disruptions by promoting the excessive production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), and hydroxyl radicals ($\bullet OH$), leading to oxidative stress [13-16]. ROS accumulation disrupts cellular homeostasis, causing oxidative damage to proteins, lipids, and nucleic acids. This damage inhibits photosynthesis, induces metabolic dysfunction, and compromises cellular structures, ultimately leading to growth impairments, reduced fertility, and premature senescence in plants [17, 18]. To cope with salt stress, plant adopts a variety of strategies to overcome salt stress. Na^+ exclusion from the shoot is considered necessary for a plant to counteract the detrimental effects of increased salinity, and a significant portion of Na^+ exclusion (>98%) in plants is accomplished by restricting net Na^+ uptake at the soil-root interface and net xylem loading in roots. Furthermore, the K^+/Na^+ ratio is considered the key feature conferring salinity stress tolerance in plants, which is often considered a potential screening tool for plant breeders [11, 19, 20]. In addition, accumulation of proline is a common phenomenon of plants in response to abiotic stresses, including salinity stress [21]. It is well established that excessive production of ROS is a general response of plants to stressors, including salinity [22]. Though ROS are most often regarded as a toxic molecule, inhibiting growth and development processes in plants, at low concentrations, they act as important signaling molecules that, through signal transduction pathways, modulate the expression of stress-responsive genes [23].

Mungbean exhibits moderate salinity tolerance during germination, with tolerance up to 5-6 dS/m causing minimal reduction in emergence and seedling vigor [5, 24-26]. However, the

reproductive stage is significantly more vulnerable to salinity, as it disrupts flowering, pod set, and seed development processes directly linked to final yield and poorly compensated under stress [27, 28]. For salinity screening in mungbean, 8 dS/m has been widely adopted because this level ensures sufficient physiological and biochemical alterations without completely killing the plant, making it suitable for phenotyping both at the seedling and the reproductive stages [9, 27, 28].

Over the years, systematic breeding efforts have led to the development of numerous improved varieties in Bangladesh; however, we have no record of a salt-tolerant mungbean variety, although a few genotypes showed moderately salinity-tolerant reactions [9]. Therefore, addressing salinity stress is essential for improving mungbean productivity and ensuring sustainable cultivation practices. Several efforts over the past few decades have aimed to develop short-duration, high-yielding, and salt-tolerant mungbean varieties, but with limited success [2, 5]. This is mainly due to the complex nature of salt tolerance (a polygenic characteristic that varies with genotype and growth stage) and the lack of precise morpho-physiological and biochemical biomarkers related to salinity stress tolerance at various growth phases, as well as low phenotypic and genetic variability in the available mungbean germplasm. In the case of rice, a very poor correlation exists between tolerance at the seedling and reproductive stages, suggesting that tolerance at these two stages is regulated by a different set of genes. However, no information is available on mungbean regarding the different sets of genes that regulate salt tolerance at the seedling and reproductive stages. Therefore, the evaluation of exotic genotypes based on yield-attributing traits and their biometrical analyses, and the identification of morpho-physiological and biochemical markers related to salt-tolerance at various stages of plant growth are essential for the breeding and development of climate-resilient mungbean varieties. In light of these challenges, the present research was undertaken with the following objectives: (i) to characterize exotic advanced breeding lines of mungbean for yield attributes traits and their genetic analyses, (ii) to study the salinity tolerance of selected lines at the seedling and reproductive phases of growth, and to identify key determinants contributing to salt tolerance.

2. Materials and Methods

2.1 Experiment 1: Characterization of Mungbean Genotypes for Earliness and Yield

2.1.1 Site Description, Plant Materials, and Experimental Design

The experiment was conducted at the Farm Research Laboratory, Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh, during the Kharif-2 season (August-November 2022). Twenty-six mungbean genotypes, including four cultivated varieties viz., Binamoog-5, Binamoog-8, Binamoog-9, BARI Mung-6 developed by Bangladesh Institute of Nuclear Agriculture and Bangladesh Agricultural Research Institute, twenty-one exotic lines viz., BMX 1131, BMX 1137, BMX 1141, BMX 11106, BMX 11107, BMX 11108, BMX 11111, BMX 11116, BMX 11122, BMX 11140, BMX 11148, BMX 11153, BMX 11154, BMX 11155, BMX 11157, BMX 11159, BMX 11165, BMX 11170, BMX 11176, BMX 11249, and BMX 11276 collected from Asian Vegetable Research and Development Center; and one salt tolerant variety 'Durdona' collected from Uzbekistan were used as plant materials. The experiment followed a randomized complete block design (RCBD) with three replications. Each block comprised 26 plots, measuring 3 m² each, with plants spaced at 30 × 10 cm and a 60 cm gap between the plots.

2.1.2 Land Preparation, Sowing, and Fertilizer Application

The experimental field was prepared by ploughing and cross-ploughing with a power tiller, followed by removing weeds and debris. Laddering was done to achieve proper soil tilth and leveling. Mungbean seeds were directly sown using the line sowing method on 14 August 2022, and irrigation was applied immediately after sowing to ensure optimal growth conditions. Fertilizers and manures, including Cow dung, Urea, Triple Super Phosphate (TSP), and Muriate of Potash (MP), were applied to the field at 2500, 40, 80, and 45 kg/ha, respectively.

2.1.3 Data Collection

Seven randomly selected plants from each genotype's replication were used to collect data on ten yield-attributing traits, such as days to first flowering (DFF), days to first harvest (DFH), days to last harvest (DLH), plant height (PH), number of primary branches per plant (NBP), number of pods per plant (PPP), pod length (PL), number of seeds per pod (SPP), 100-seeds weight (HSW), and yield per plant (YPP).

2.1.4 Statistical Analysis

Data were analyzed using R software (version 3.4.1) [29]. Analysis of variance (ANOVA) was performed using the *Doebio* research package to detect significant differences among genotypes, followed by mean separation using the Least Significant Difference (LSD) test. Correlation analysis was conducted using the *Metan* package. Principal component analysis (PCA) was performed with the *FactoMineR* and *Factoextra* packages to assess trait relationships and genetic diversity.

2.1.5 Estimation of Genetic Parameters

Equations proposed by biometricians Allard [30] and Johnson et al. [31] were used to calculate genetic parameters, such as phenotypic and genotypic variance, heritability in the broad sense, phenotypic and genotypic coefficient of variations, genetic advance, and genetic advance as a percentage of mean, for each plant type individually. According to Deshmukh et al. [32], the PCV and GCV estimates are categorized as low, <10%, moderate, 10-20%, and high, >20%. Johnson et al. [31] classified the heritability in the broad sense (h^2b) as low, 0-30%, medium, 31-60%, and high, >60%, and the GA% as low <10%, moderate, 10-20%, and high, >20%.

2.2 Experiment 2: Hydroponic Screening for Salt Tolerance at the Seedling Stage

2.2.1 Plant Materials and Growth Conditions

The experiment took place at the GPB department's growth chamber at BAU, Mymensingh-2202. Plant material was derived from nine different mungbean genotypes, including the high-yielding variety Binamoog-8, BARI Mung-6, Durdona, and six exotic lines BMX 1131, BMX 1141, BMX 11111, BMX 11122, BMX 11148, and BMX 11170. To encourage sprouting, seeds of the chosen genotypes were washed, disinfected with 70% ethanol, and then placed on wet filter paper in Petri dishes for 24 hours. They were then left in the dark for three to four days. A line of sprouted seeds was then placed into the holes of a sheet of styrofoam that was floating in a rectangular plastic tray filled with

distilled water. The tray's measurements were 32.50 cm × 28.50 cm × 13.00 cm in length, width, and breadth. Peters® Professional (Geldermalsen, Netherlands) solution was used to replenish the water in the trays after the seedlings had grown for four days. The temperature was kept at 25 ± 2°C (with 16 hours of light and 8 hours of darkness) during the photoperiod. A continuous supply of nutrients to the plants was ensured by stirring the fluid with an air pump. Additionally, the nutrient solution was changed every seven days.

2.2.2 Experimental Design and Salt Stress

In hydroponic circumstances, the study was conducted using a completely randomized design (CRD) with three independent replications. The treatments included salt stress (EC = 8 ds/m) and control (C). The selected salinity level was based on previous studies where EC = 8 dS/m was effectively used to evaluate salt tolerance in mungbean genotypes [9, 27, 28]. Saline solution was added to the nutrient solution to expose 14-day-old mungbean seedlings to salt stress (8 dS/m). By varying the EC of the nutrient solution, the salinity level was kept constant. Using an EC meter (Hanna HI 4321, Weilheim, Germany), the EC of the nutritional solution was tracked. Plants were grown in a nutrient solution devoid of salt in the control condition. The pH of the nutrient solution was kept between 7.0 and 7.1.

2.2.3 Data Collection

Different morphological traits (such as Root length, RL; Shoot length, SL; Root fresh weight, RFW; Shoot fresh weight, SFW; Root dry weight, RDW; Shoot dry weight, SDW) and biochemical traits (such as Na⁺/K⁺ ratio for root, Na⁺/K⁺ ratio for shoot) of each genotype were recorded after 7 days of salt stress treatments from ten randomly selected seedlings. Malondialdehyde (MDA), hydrogen peroxide (H₂O₂), and proline content were measured from leaf tissues.

Determination of Na⁺/K⁺. After harvesting, mung bean plants were separated into roots and shoots and then dried in an oven at 60°C for 3 days to achieve a constant weight. The oven-dried samples were ground using a mortar and pestle and stored in plastic sample collection bags. The finely powdered plant material was digested using a micro-Kjeldahl digestion system [33]. The digested samples were then analyzed for sodium (Na⁺) and potassium (K⁺) using a flame photometer (Model PERKIN-ELMER, 2380). The Na⁺/K⁺ ratio was calculated based on the concentrations of Na⁺ and K⁺ in the plant tissues.

Determination of MDA Content. The determination of MDA content follows Heath and Packer [34]. Fresh leaf samples (0.1 g) were homogenized with 1 mL of 0.1% TCA, and the homogenate was centrifuged at 12,000 rpm for 15 minutes. The supernatant was mixed with 4 mL of 20% TCA containing 0.5% TBA. This mixture was boiled at 95°C for 15 minutes and then quickly cooled on ice. After cooling, the mixture was centrifuged again at 12,000 rpm for 5 minutes, and the supernatant was transferred to a new tube. The absorbance of the resulting solution was measured at 532 nm using a spectrophotometer to determine the MDA content. All steps, except for the absorbance measurement, were performed at 4°C.

Determination of H₂O₂ Content. The determination of H₂O₂ content in fresh leaf samples was conducted following the method of Velikova et al. [35]. Fresh leaf samples (0.1 g) were homogenized

with 1 mL of 0.1% TCA using a mortar and pestle, and the homogenate was then centrifuged at 12,000 rpm for 15 minutes. The supernatant was collected and mixed with a 10 mM phosphate buffer (pH 7.0) and 1 M potassium iodide. This mixture was kept in the dark for 1 hour. Finally, the absorbance of the resulting solution was measured at 390 nm using a spectrophotometer. All steps, except for the absorbance measurement, were performed at 4°C to ensure the stability of the samples.

Determination of Proline Content. The proline content in fresh leaf samples was determined using the Ninhydrin assay as described by Bates et al. [36]. Fresh leaf samples (0.05 g) were homogenized with 1 mL of 0.1% TCA using a mortar and pestle, followed by the addition of 3 mL of 3% sulfosalicylic acid (SSA). The mixture was then centrifuged at 4,000 rpm for 10 minutes, and the supernatant was collected. This supernatant was mixed with ninhydrin reagent and glacial acetic acid and incubated at 95°C. After incubation, the absorbance of the solution was measured at 520 nm using a spectrophotometer. This method quantifies the proline content by forming a colored complex with ninhydrin that can be detected spectrophotometrically.

2.2.4 Statistical Analysis

The same as section 2.1.4.

2.3 Experiment 3: Screening for Salt Tolerance at the Reproductive Stage

2.3.1 Experimental Materials

The same as experiment 2.

2.3.2 Site, Design, and Treatments

The Farm Research Laboratory of the Department of GPB, BAU, Mymensingh-2202, was the site of the experiment. Two treatments—control and salt stress (EC = 8 ds/m)—and three replications were used in the experiment, which was conducted after an RCBD.

2.3.3 Preparation of Soil for the Tray and Seed Sowing

The experiment was conducted in a small-sized earthen pot filled with field soil. The pot was 20 cm in depth with a diameter of 27 cm. The soil was prepared by puddling the soil and mixing the recommended doses of Cow dung, Urea, TSP, and MOP at 2500, 135, 240, and 120 kg/ha, respectively. Finally, each pot was filled with six kg of soil mixture. Mungbean seeds were directly sown into the experimental pots. Following sowing, the seeds were irrigated to provide an optimal growth environment.

2.3.4 Imposition of Salt Stress

For each genotype, salt stress was applied during the reproductive stage (just before the first flower opening) using saline irrigation water (EC = 8 dS/m) while preserving 100% field capacity. This process continued for three weeks as it covered the critical flowering and early pod development phases, which are highly sensitive to salinity stress and greatly influence final yield [9]. The plants

were grown according to standard cultural procedures and harvested when they reached maturity following a three-week treatment with salt stress.

2.3.5 Data Collection

Data on eight quantitative traits, such as days to maturity (DM), PH, NBP, PPP, PL, SPP, HSW, and YPP, were recorded from seven randomly selected plants from each replication.

2.3.6 Statistical Analysis

The same as section 2.1.4.

2.3.7 Stress Tolerance Indices

Stress tolerance indices were calculated in grain yield per plant using the following equations:

Mean Productivity (MP) = $(Y_s + Y_p)/2$ [37].

Geometric Mean Productivity (GMP) = $\sqrt{Y_p \times Y_s}$ [38].

Stress Susceptibility Index (SSI) = $\frac{1 - Y_s/Y_p}{1 - \frac{Y_s}{Y_p}}$ [39].

Tolerance Index (TOL) = $Y_p - Y_s$ [37].

Stress Tolerance Index (STI) = $\frac{(Y_s)(Y_p)}{(Y_p^2)}$ [38].

Yield Stability Index (YSI) = $\frac{Y_s}{\bar{Y}_s}$ [40].

3. Results

3.1 Experiment 1: Characterization of Mungbean Genotypes for Yield-Attributing Traits and their Genetic Analyses

3.1.1 Analysis of Variance and Mean Performance Analysis

All of the traits under study had extremely significant ($p \leq 0.01$) variation, according to the genotypes' analysis of variance results (Table S1). Table 1 shows the average performance of ten traits for 26 genotypes. Among the studied genotypes, Binamoog-8, BARI Mung-6, BMX 1131, BMX 11122, BMX 11140, BMX 11148, BMX 11153, and BMX 11157 were the early flowering genotypes (34.33 days), while BMX 11155, BMX 11176, and BMX 11276 required the maximum number of days (38.33 days) for flowering. Based on the first harvest, early-maturing genotypes included Binamoog-5, Binamoog-8, Binamoog-9, BARI Mung-6, and Durдона, which took an average of 56.33 days to reach maturity. In contrast, BMX 11176 (60.00 days) and BMX 11122 (60.67 days) required the longest duration to harvest the first mature pods. The maximum days to harvest the last mature pods were recorded in BMX 11116 (82.00 days) and BMX 11276 (80.67 days), while Binamoog-8 and BMX 11154 required the least (70.33 days). The highest plant height was recorded at BMX 11249 (96.90 cm), and the lowest was recorded in BMX 11108 (49.97 cm). For NBP, BMX 11165 had the highest value (4.33), while BMX 11108 (1.93) and BMX 11140 (2.00) had the lowest. BARI Mung-6 recorded the highest PPP (27.96), whereas BMX 11155 had the lowest (5.63) PPP. The longest PL was found in BMX 11153 (10.87 cm), and the shortest PL was found in BMX 11159 (6.26 cm). The

highest SPP was observed in BMX 11140 and BMX 1137 (12.57), while BMX 11107 had the lowest (8.57) SPP. Durdona had the highest HSW (5.84 g), and BMX 11159 had the lowest (1.93 g). Importantly, Durdona also showed the highest YPP (13.99 g), while the lowest (1.88 g) was recorded in BMX 11249 (Table 1).

Table 1 Mean performance of ten quantitative traits of twenty-six genotypes of mungbean grown under field condition.

Genotypes	DFF	DFH	DLH	PH	NBP	PPP	PL	SPP	HSW	YPP
Binamoog-5	35.33 de	56.33 d	72.33 e-g	86.30 bc	3.33 b-i	13.41 fg	8.58 c	11.08 c-f	3.78 g-j	5.60 c-i
Binamoog-8	34.33 e	56.33 d	70.33 g	77.40 gh	2.63 f-j	23.04 b	8.15 c-f	11.00 c-g	4.15 d-h	10.86 b
Binamoog-9	34.67 de	56.33 d	70.67 fg	69.17 j	3.47 a-h	11.50 h-j	8.32 c-f	10.65 d-j	4.64 cd	5.63 c-h
BARI Mung-6	34.33 e	56.33 d	72.33 e-g	85.53 cd	3.73 a-d	27.96 a	8.62 c	9.76 f-k	4.24 d-g	11.66 b
Durdona	34.67 de	56.33 d	76.00 b-d	60.47 m	3.13 d-i	22.84 b	9.68 b	10.32 e-j	5.84 a	13.99 a
BMX 1131	34.33 e	56.67 cd	72.67 e-g	75.77 hi	2.73 e-j	9.60 k-m	8.37 c-e	10.27 e-j	4.55 de	4.38 g-k
BMX 1137	35.67 c-e	56.67 cd	71.00 e-g	78.87 fg	2.80 d-j	10.70 h-k	8.21 c-f	12.57 a	5.15 bc	6.57 c
BMX 1141	37.33 a-c	57.00 b-d	72.33 e-g	88.80 b	4.13 a-c	14.88 ef	8.54 cd	10.31 e-j	4.06 e-i	6.29 cd
BMX 11106	35.00 de	56.67 cd	71.67 e-g	81.10 ef	3.07 d-i	10.06 j-m	8.32 c-f	10.18 e-j	3.91 f-j	3.78 j-l
BMX 11107	34.67 de	56.33 d	73.33 d-f	62.23 lm	2.47 ij	14.43 ef	6.54 j-l	8.57 k	3.20 k-m	4.10 h-k
BMX 11108	37.67 ab	59.67 ab	76.67 b	49.97 p	1.93 j	10.37 i-l	6.47 k-l	10.78 d-i	2.86 mn	3.25 j-m
BMX 11111	35.33 de	58.33 a-d	71.00 e-g	65.20 k	3.27 c-i	20.53 cd	7.24 i-j	9.63 h-k	2.82 mn	5.91 c-g
BMX 11116	36.33 b-d	59.33 a-c	82.00 a	74.57 i	2.60 g-j	8.68 l-n	7.98 c-h	9.57 i-k	3.93 f-j	2.92 k-m
BMX 11122	34.33 e	60.67 a	76.33 bc	66.00 k	2.53 h-j	12.20 g-i	7.63 f-i	9.97 e-j	3.67 h-k	4.65 e-j
BMX 11140	34.33 e	56.67 cd	70.67 fg	54.00 o	2.00 j	8.54 l-n	7.78 e-i	12.57 a	3.91 f-j	3.81 j-l
BMX 11148	34.33 e	56.33 d	71.67 e-g	71.57 j	3.60 a-f	12.37 gh	7.81 e-i	10.51 d-j	4.42 d-f	6.22 c-e
BMX 11153	34.33 e	57.00 b-d	75.67 b-d	85.13 cd	3.60 a-f	7.57 no	10.87 a	11.72 a-d	5.31 ab	4.58 f-j
BMX 11154	34.67 de	56.33 d	70.33 g	83.27 de	2.67 f-j	15.93 e	8.08 c-g	11.21 b-e	3.66 h-l	5.87 c-g
BMX 11155	38.33 a	59.33 a-c	75.67 b-d	57.50 n	2.40 ij	5.63 p	7.43 g-i	11.09 c-f	3.43 j-l	2.27 lm
BMX 11157	34.33 e	56.33 d	70.67 fg	74.53 i	2.67 f-j	8.70 l-n	8.39 c-e	12.50 ab	4.13 d-h	4.03 i-k
BMX 11159	35.33 de	57.00 b-d	71.33 e-g	76.17 g-i	2.73 e-j	21.80 bc	6.26 l	10.94 d-h	1.93 o	4.75 d-j
BMX 11165	38.00 ab	59.00 a-d	76.67 b	75.87 hi	4.33 a	8.23 m-o	7.79 e-i	9.39 jk	3.83 g-j	3.19 j-m
BMX 11170	35.33 de	57.00 b-d	73.67 c-e	64.67 kl	3.53 a-g	19.23 d	7.18 i-k	11.75 a-d	2.63 n	6.03 c-f
BMX 11176	38.33 a	60.00 a	77.00 b	70.70 j	3.67 a-e	11.63 g-j	7.86 d-i	11.09 c-e	3.51 i-l	4.33 g-k
BMX 11249	37.67 ab	59.00 a-d	77.00 b	96.90 a	4.27 ab	6.63 op	7.32 h-i	12.30 a-c	2.76 mn	1.88 m
BMX 11276	38.33 a	56.33 d	80.67 a	63.73 kl	2.87 d-j	7.70 no	7.36 g-i	9.70 g-k	3.10 l-n	2.38 lm

Here, different letters in the same column are significant at 5% level of significance following Tukey's method. Here, DFF = Days to first flowering, DFH = Days to first harvest, DLH = Days to last harvest, PH = Plant height (cm), NBP = Number of branches per plant, PPP = Pods per plant, PL = Pod length, SPP = Seeds per pod, HSW = 100-seed weight (g), YPP = Yield per plant (g).

3.1.2 Estimation of Genetic Parameters

Table 2 displays the Phenotypic variance (σ^2P), genotypic variance (σ^2G), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in a broad sense (h^2b), genetic advance (GA), and genetic advance as a percentage of the mean (GA%). The characters which showed very high σ^2G and σ^2P were PH (129.71 and 130.49), followed by PPP (34.96 and 35.31), DLH (10.19 and 11.05), PPP (34.96 and 35.31) and YPP (8.16 and 8.42), whereas the rest of the characters showed the low magnitude. The magnitude of GCV and PCV were recorded high (>20%) for the traits YPP (53.45% and 54.30%), PPP (44.64% and 44.89%), HSW (22.82% and 23.30%), and NBP (20.29% and 22.73%) while moderate estimates (10-20%) were observed for the traits PL (11.90% and 12.25%), and PH (15.62% and 15.67%). Low GCV and PCV (<10%) were recorded for traits such as DFF (4.12% and 4.46%), DFH (2.29% and 2.79%), and DLH (4.32% and 4.50%). However, GCV was low for SPP, while PCV value was moderate (9.50% and 10.31%). High h^2b (>60%) was recorded for all the traits with PH (99.40%), PPP (99.03%), YPP (96.92%), HSW (95.90%), PL (94.35%), DLH (92.22%), SPP (85.00%), DFF (85.65%), NBP (79.67%), and DFH (67.26%). GA was highest for PH (23.39), followed by PPP (12.12), DLH (6.31), and YPP (5.79). The GA% was notably high (>20%) for traits such as YPP (108.40%), PPP (91.57%), HSW (46.03%), NBP (37.30%), and PH (32.09%). Moderate GA% estimates (10-20%) were recorded for traits such as PL (23.81%), SPP (18.04%), and DLH (8.55%). Low GA% estimates (<10%) were recorded for traits such as DFF (7.86%) and DFH (3.86%) (Table 2).

Table 2 Genetic parameters of studied traits of twenty-six mungbean genotypes.

Traits	GV (σ^2g)	PV (σ^2p)	GCV (%)	PCV (%)	h^2b (%)	GA	GA (%)
DFF	2.16	2.53	4.12	4.46	85.65	2.80	7.86
DFH	1.73	2.57	2.29	2.79	67.26	2.22	3.86
DLH	10.19	11.05	4.32	4.50	92.22	6.31	8.55
PH	129.71	130.49	15.62	15.67	99.40	23.39	32.09
NBP	0.39	0.49	20.29	22.73	79.67	1.15	37.30
PPP	34.96	35.31	44.67	44.89	99.03	12.12	91.57
PL	0.90	0.95	11.90	12.25	94.35	1.89	23.81
SPP	1.04	1.23	9.50	10.31	85.00	1.94	18.04
HSW	0.76	0.79	22.82	23.30	95.90	1.76	46.03
YPP	8.16	8.42	53.45	54.30	96.92	5.79	108.40

Here, DFF = Days to first flowering, DFH = Days to first harvest, DLH = Days to last harvest, PH = Plant height (cm), NBP = Number of branches per plant, PPP = Pods per plant, PL = Pod Length, SPP = Seeds per pod, HSW = 100-seed weight (g), YPP = Yield per plant (g), GV = genotype variances, PV = Phenotypic variances, h^2b = Broad-sense heritability, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, GA = Genetic advance and GA% = Genetic advance in percentage of mean, respectively.

3.1.3 Phenotypic Correlation Coefficient among the Studied Traits

The phenotypic correlation coefficients among the ten traits of mungbean genotypes are shown in Figure 1. YPP showed significant positive correlations with PPP (0.82***), HSW (0.49***), and PL

(0.40***), and significant negative correlations with DFF (-0.46***), DFH (-0.39***), and DLH (-0.38***). Among other yield-contributing traits, the highest positive correlation was observed between HSW and PL (0.83***), followed by NBP with PH (0.58***), and PL (0.29*), and DFF with DFH (0.52***), and DLH (0.57***), and DFH with DLH (0.52***). On the other hand, significant negative correlations were also found between DLH and PPP (-0.38**), and PPP with both DFF (-0.39***), and DLH (-0.38***).

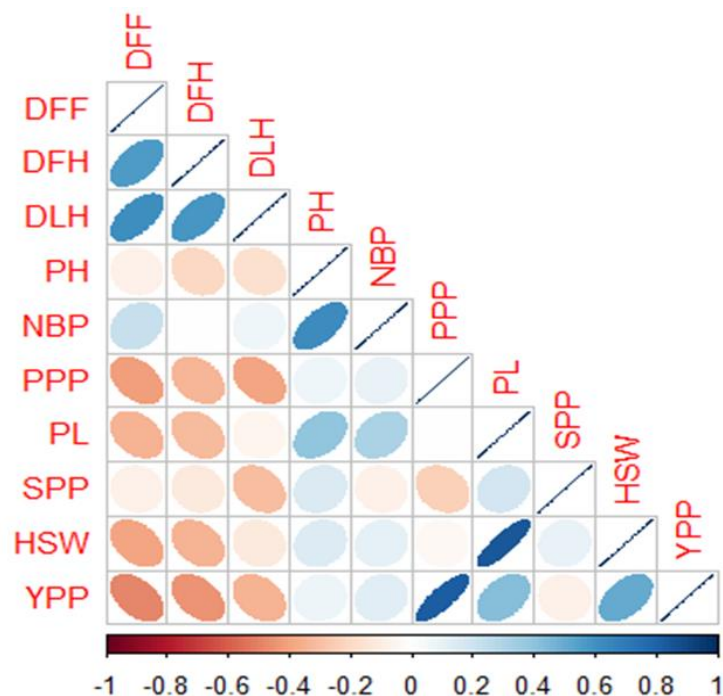


Figure 1 Correlation coefficients among traits of mungbean genotypes. Here, DFF = Days to first flowering, DFH = Days to first harvest, DLH = Days to last harvest, PH = Plant height (cm), NBP = Number of branches per plant, PPP = Pods per plant, PL = Pod length, SPP = Seeds per pod, HSW = 100-seed weight (g), YPP = Yield per plant (g).

3.1.4 Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) of twenty-six mung bean genotypes identified four principal components (PCs) that together accounted for 78.6% of the total variance in yield and yield-related traits (Table S2). PC1, explaining 32.8% and PC2, accounting for 19.5% of the variance, Biplot analysis illustrated positive correlations between PL, PPP, and HSW with YPP, evidenced by their close positioning in the plot and the formation of small angles. Additionally, YPP demonstrates negative correlations with DFF, DFH, and DLH as they diverge and form a large angle (close to 180°), indicating a negative correlation. Genotypes such as Durdona and BARI Mung-6 display similar directional trends as YPP in the biplot (Figure 2).

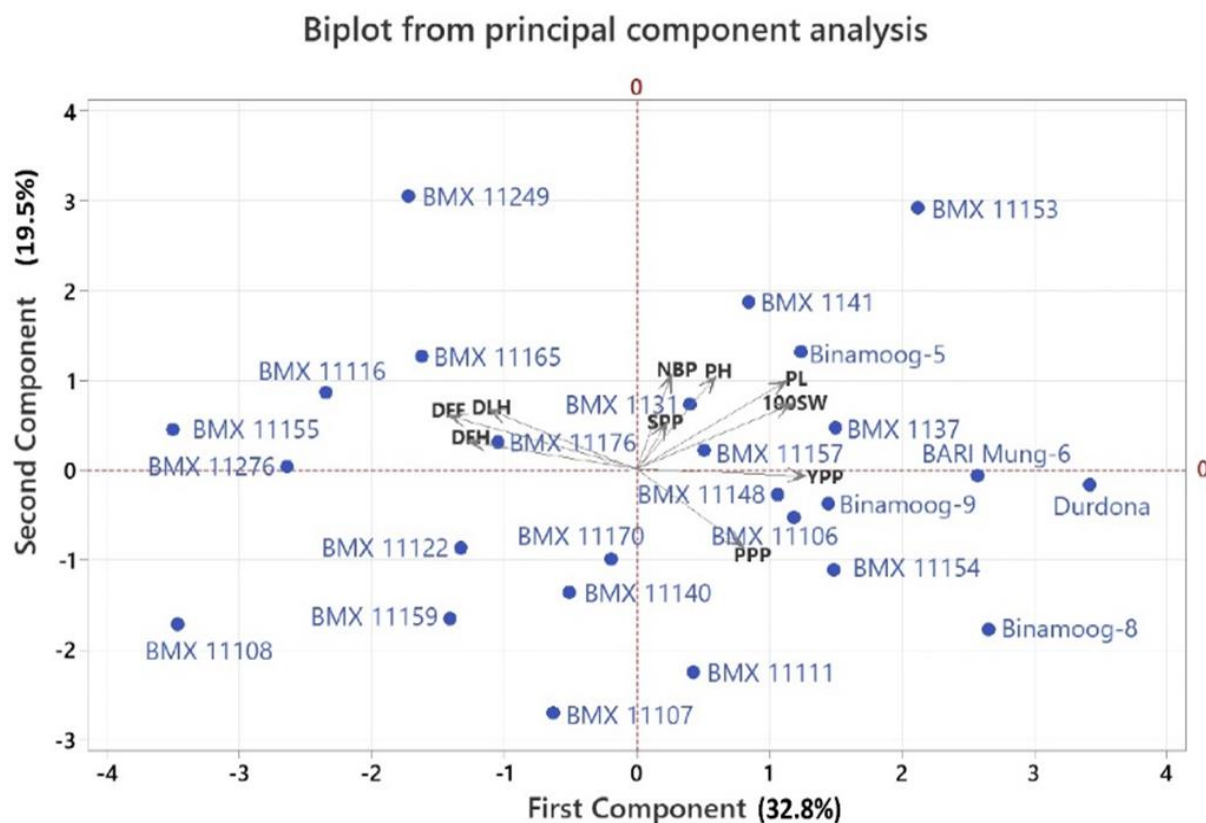


Figure 2 Biplot from principal component analysis of morphological traits of twenty-six mungbean genotypes grown at Kharif -2 season. Here, DFF = Days to first flowering, DFH = Days to first harvest, DLH = Days to last harvest, PH = Plant height (cm), NBP = Number of branches per plant, PPP = Pods per plant, PL = Pod length, SPP = Seeds per pod, HSW = 100-seed weight (g), YPP = Yield per plant (g).

3.2 Experiment 2: Screening of Mungbean Genotypes for Salt Tolerance at the Seedling Stage under Hydroponic Conditions

3.2.1 Analysis of Variance and Mean Performance Analysis

The results of the analysis of variances for the genotypes (G), treatment (T), and $G \times T$ showed a highly significant variation for all the studied traits (Table S3). The mean performance of ten traits across twenty-six genotypes is presented in Table 3. Under salt stress, mungbean genotypes showed significant reductions in morphological and biochemical characteristics at the seedling stage. RL decreased significantly across all genotypes, with the highest reduction in BMX 11111 (32.38%) and the lowest in Durdonia (10.96%). SL also declined markedly, with Binamoog-8 exhibiting the most significant reduction (37.34%) and Durdonia the least (11.85%). BMX 11122 recorded the most significant decreases in RFW and RDW (33.20% and 43.12%, respectively), while Binamoog-8 showed the highest reductions in SFW and SDW (31.24% and 35.53%, respectively). The most significant decrease in SPAD was observed in BMX 11170 (33.98%), whereas Durdonia and BMX 11131 had the least declines (5.50% and 3.02%, respectively). Salt stress significantly increased the Na^+/K^+ in all the studied genotypes; the highest increase was recorded in Binamoog-8 for both root (95.22%) and shoot (87.15%), whereas the lowest shoot Na^+/K^+ was recorded in Durdonia (60.52%) and BMX 11131 (45.07%). MDA increased sharply in Binamoog-8 (59.85%), whereas the lowest was recorded

in BMX 1131 (37.05%). The highest increase in H_2O_2 content was recorded for Binamoog-8 (58.08%). However, the lowest increase was found in BMX 1131 (31.16%). Proline content significantly increased in all genotypes, with BMX 11122 showing the highest induction (93.49%), but the lowest increase was found in BMX 11170 (51.29%). The phenological appearance of the seedling goes well with the alteration of the morphological and biochemical traits in response to salt stress (Figure 3).



Figure 3 Phenological appearance of mungbean seedlings grown under salt stress (8 dS/m) in hydroponic solution after seven days.

Table 3 Mean performances of nine mungbean genotypes based on different morphological and biochemical traits under control and salt conditions (8 dS/m) at the seedling stage.

Genotypes	Treatments	RL (cm)	SL (cm)	RFW (mg)	SFW (mg)	RDW (mg)	SDW (mg)	SPAD Value	Na ⁺ /K ⁺ R	Na ⁺ /K ⁺ S	MDA (nmol/g)	H ₂ O ₂ (nmol/g)	Proline (ug/g)
BARI Mung-6	Control	10.86 a	14.57 c	96.64 ab	378.77 c	29.29 ab	126.26 c	40.62 ab	0.17 h	0.09 h	28.33 gh	21.54 j	2.64 ij
	Salt	7.68 e-g	9.14 h	67.67 d-f	267.58 g	17.37 e-g	83.62 h	37.80 bc	0.69 f	0.41 e	47.59 e	40.16 ef	28.37 b
Binamoog-8	Control	9.45 b-d	12.60 d	75.33 c-e	327.69 e	22.83 cd	109.23 d	39.34 ab	0.15 h	0.18 gh	24.22 i	22.53 ij	3.80 hi
	Salt	7.67 e-g	7.90 i	62.33 d-f	225.33 i	15.86 e-g	70.42 ij	31.00 de	3.05 ab	1.37 b	60.34 ab	53.75 ab	12.90 e
Durdona	Control	9.94 a-c	15.55 b	91.67 ab	404.27 b	27.78 ab	134.76 b	41.60 ab	0.20 h	0.12 gh	25.72 g-i	23.66 h-j	2.32 ij
	Salt	8.85 c-f	12.09 e	76.75 cd	334.47 de	20.23 de	104.52 e	39.31 ab	1.48 d	0.31 ef	45.51 ef	37.28 fg	33.54 a
BMX 1131	Control	11.05 a	16.37 a	102.70 a	425.58 a	31.12 a	141.86 a	41.37 ab	0.18 h	0.18 gh	27.44 g-i	25.25 hi	2.24 ij
	Salt	9.26 b-d	12.37 de	75.84 cd	341.73 d	19.95 de	106.79 de	40.12 ab	1.08 e	0.33 ef	43.59 f	36.68 g	30.29 b
BMX 1141	Control	10.31 ab	14.30 c	93.67 ab	371.80 c	28.38 ab	123.93 c	40.17 ab	0.26 gh	0.22 fg	24.01 i	25.36 hi	3.57 hi
	Salt	8.96 c-e	10.44 g	74.26 c-e	291.46 f	19.47 d-f	91.08 g	32.78 de	0.46 g	1.03 c	55.00 cd	45.17 d	26.34 c
BMX 11111	Control	7.65 fg	7.67 ij	53.17 fg	199.29 j	16.11 e-g	66.43 j	41.13 ab	0.24 gh	0.22 fg	29.46 g	26.67 h	4.10 g-i
	Salt	5.18 h	6.32 j	44.83 g	184.40 k	10.55 h	57.62 k	29.68 ef	3.28 a	1.57 a	63.35 a	55.21 a	10.37 f
BMX 11122	Control	10.83 a	11.32 f	100.80 ab	294.34 f	30.55 ab	98.11 f	42.42 a	0.22 h	0.12 gh	28.14 gh	24.47 h-j	1.13 j
	Salt	8.72 c-f	8.18 i	67.33 d-f	232.75 hi	17.37 e-g	72.73 i	35.07 cd	1.12 e	0.80 d	55.27 cd	49.32 c	17.37 d
BMX 11148	Control	10.34 ab	10.85 fg	87.77 bc	293.04 f	26.60 bc	97.68 f	37.58 bc	0.18 h	0.15 gh	25.54 hi	23.52 h-j	4.87 gh
	Salt	7.84 e-g	9.16 h	60.69 ef	258.07 g	15.36 fg	80.65 h	32.84 de	2.21 c	0.88 d	52.50 d	42.42 de	24.57 c
BMX 11170	Control	8.30 d-g	9.41 h	44.80 g	244.55 h	13.58 gh	81.52 h	40.59 ab	0.16 h	0.19 gh	29.33 gh	26.40 h	5.97 g
	Salt	7.05 g	8.29 i	42.06 g	225.57 i	9.71 h	70.49 ij	26.80 f	2.95 b	1.38 b	57.36 bc	51.57 bc	12.26 ef

Notes: Different letters in the same column are significant at a 5% level of significance following Tukey's method. Here, RL = Root length, SL = Shoot length, RFW = Root fresh weight, SFW = Shoot fresh weight, RDW = Root dry weight, SDW = Shoot dry weight, Na⁺/K⁺ R = Na⁺ - K⁺ ratio for root, Na⁺/K⁺ S = Na⁺ - K⁺ ratio for shoot, MDA = Malondialdehyde content, H₂O₂ = Hydrogen peroxide content.

3.2.2 Correlation Coefficient

The correlation analysis under control and stress conditions is presented in Figure 4a, b. Under both conditions, RL was positively correlated with SL (0.72***, control; 0.79***, stress), RFW (0.88***, control; 0.83***, stress), SFW (0.73***, control; 0.79***, stress), RDW (0.88***, control; 0.83***, stress), SDW (0.73***, control; 0.79***, stress), and proline (0.69**, stress), whereas it showed a negative correlation with $\text{Na}^+/\text{K}^+ \text{ S}$ (-0.51**, control; -0.71***, stress) and H_2O_2 (-0.55**, control; -0.67***, stress) (Figure 4a, b). SL showed positive correlations with RFW (0.76***, control; 0.80***, stress), SFW (0.99***, control; 0.99***, stress), RDW (0.76***, control; 0.80***, stress), and SDW (0.99***, control; 0.99***, stress), but it showed a negative correlation with MDA (-0.41*, control; -0.43*, stress), H_2O_2 (-0.43*, control; -0.43*, stress), $\text{Na}^+/\text{K}^+ \text{ R}$ (-0.11, control; -0.32, stress), and $\text{Na}^+/\text{K}^+ \text{ S}$ (-0.32, control; -0.41*, stress).

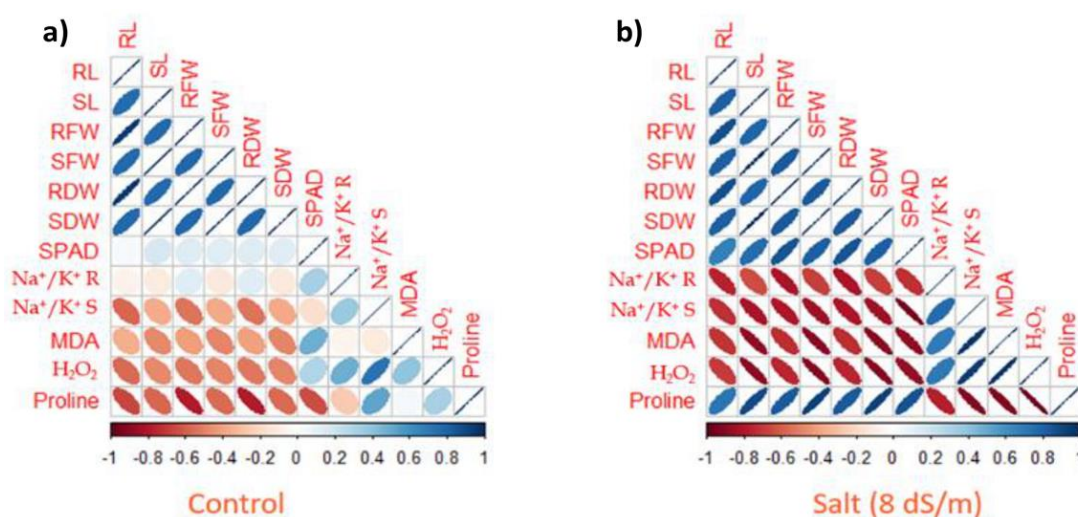


Figure 4 Correlation coefficient among twelve characters of mungbean genotypes under control (a) and salinity stress condition (b); Here, RL = Root length, SL = Shoot length, RFW = Root fresh weight, SFW = Shoot fresh weight, RDW = Root dry weight, SDW = Shoot dry weight, $\text{Na}^+/\text{K}^+ \text{ R}$ = Na^+/K^+ ratio for root, $\text{Na}^+/\text{K}^+ \text{ S}$ = Na^+/K^+ ratio for shoot, MDA = Malondialdehyde content, H_2O_2 = Hydrogen peroxide.

RFW was positively correlated with SFW (0.93***, control; 0.94***, stress), RDW (0.93***, control; 0.94***, stress), and SDW (0.94***, control; 0.94***, stress), and negatively with $\text{Na}^+/\text{K}^+ \text{ R}$ (-0.39*, control; -0.45*, stress) and $\text{Na}^+/\text{K}^+ \text{ S}$ (-0.56**, control; -0.74***, stress). SFW also showed positive correlations with RDW (0.94***, control; 0.94***, stress) and SDW (0.94***, control; 0.94***, stress), and negative correlations with $\text{Na}^+/\text{K}^+ \text{ R}$ (-0.43*, control; -0.55**, stress) and $\text{Na}^+/\text{K}^+ \text{ S}$ (-0.53**, control; -0.73***, stress). RDW was positively correlated with SDW (0.99***, control; 0.99***, stress), and negatively with $\text{Na}^+/\text{K}^+ \text{ R}$ (-0.48*, control; -0.66***, stress) and $\text{Na}^+/\text{K}^+ \text{ S}$ (-0.55**, control; -0.73***, stress). SDW also showed negative correlations with $\text{Na}^+/\text{K}^+ \text{ R}$ (-0.44*, control; -0.62***, stress) and $\text{Na}^+/\text{K}^+ \text{ S}$ (-0.53**, control; -0.73***, stress).

$\text{Na}^+/\text{K}^+ \text{ R}$ and $\text{Na}^+/\text{K}^+ \text{ S}$ were positively correlated under control (0.76***) and negatively under stress (-0.91**). MDA was positively correlated with H_2O_2 (0.41*, control; 0.47**, stress) and negatively with proline (-0.60**, control; -0.75***, stress). H_2O_2 was also negatively correlated with

proline (-0.60**, control; -0.69**, stress). Proline showed a positive correlation with Na^+/K^+ S under stress (0.69**) (Figure 4a, b).

3.2.3 Principal Component Analysis (PCA)

PCA of twelve traits in nine mung bean genotypes at the seedling stage identified two principal components (PC1 and PC2) that explain 74% and 12% of the total variance, respectively, with a cumulative variance of 86% (Figure 5). The biplots reveal distinct patterns in the relationships between morphological and biochemical traits (Figure 5). PC1 tends to separate the position of genotypes under control conditions on the right side, whereas genotypes under stress conditions are on the left side. The right-side positioning indicates the genotypes have higher values for SL, SDW, RFW, RDW, RL, SFW, and SPAD in commonly treated conditions. In contrast, the left-side positioning indicates the genotypes have higher values for MDA, H_2O_2 , Proline, and Na^+/K^+ ratio under stress conditions. Interestingly, Genotypes Durdona and BMX 1131 under stress conditions are positioned on the right side (Figure 5).

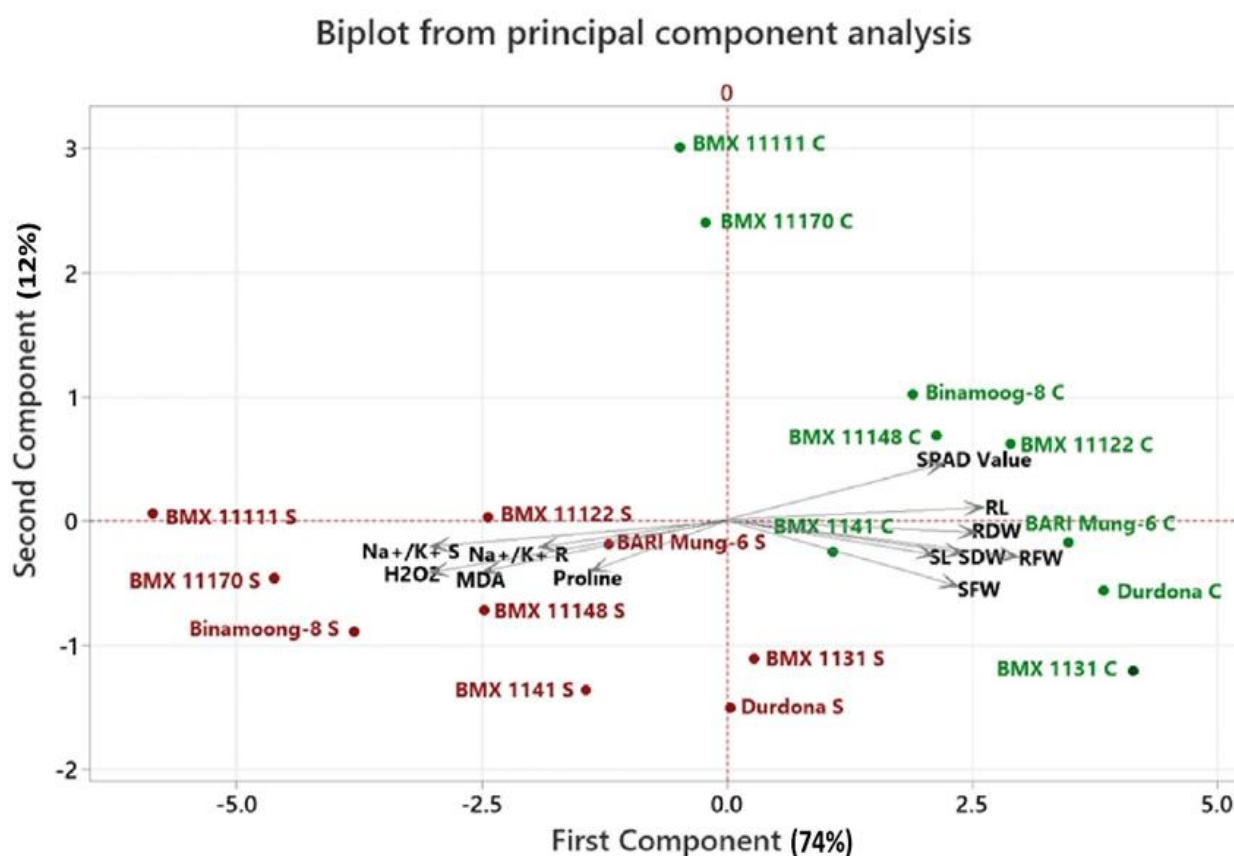


Figure 5 Biplot from principal component analysis for twelve different traits in nine mung bean genotypes at the seedling stage. Here, RL = Root length, SL = Shoot length, RFW = Root fresh weight, SFW = Shoot fresh weight, RDW = Root dry weight, SDW = Shoot dry weight, Na^+/K^+ R = Na^+/K^+ ratio for root, Na^+/K^+ S = Na^+/K^+ ratio for shoot, MDA = Malondialdehyde content, H_2O_2 = Hydrogen peroxide.

3.3 Experiment 3: Screening for Salt Tolerance at the Reproductive Stage

3.3.1 Analysis of Variance and Mean Performance Analysis

The analysis of variance revealed highly significant ($p \leq 0.001$) variation for G and T across all traits (DM, PH, NPB, PPP, PL, SPP, HSW, and YPP). Significant $G \times T$ interaction effects were observed for all traits at 0.1%, whereas NPB was at the 1% level (Table S4). The mean performance of the studied traits is presented in Table 4. The results indicated that the characteristics were significantly affected by salt stress, with percentage increases and decreases shown in Table S5. Due to salinity, a considerable increase in the trait DM was found, with the highest growth in BARI Mung-6 (8.72%) and the least in Durdona (1.79%) under stressed conditions. The most pronounced reduction for the trait PH was found in BARI Mung-6 (61.32%) and the lowest in BMX 11122 (10.22%). The maximum decrease in NPB was observed in BMX 11170 (51.66%), and the minimum in BMX 11111 (15.9%). The most substantial reduction in PPP was recorded in Binamoog-8 (62.55%), with the lowest reduction in BMX 11111 (23.64%). For PL, the highest reduction was found in BMX 11148 (22.86%), and the lowest was found in BMX 11122 (4.9%). For SPP, the maximum reduction was observed in BMX 11148 (43.91%), and the minimum reduction was recorded in BMX 11122 (0.61%). The most pronounced decrease in HSW was found in BMX 11148 (38.96%), followed by BMX 11111 (26.44%), BMX 11170 (20.6%), BMX 1141 (20.21%), BARI Mung-6 (20.07%), BMX 11122 (17.62%), Binamoog-8 (16.01%), Durdona (11.98%), and BMX 1131 (8.43%). Notably, the highest reduction in YPP was recorded in BMX 1141 (74.73%), followed by BMX 11148 (72.8%), BARI Mung-6 (67.19%), Binamoog-8 (59.47%), Durdona (55.37%), BMX 1131 (46.53%), BMX 11170 (45.87%), BMX 11122 (37.45%), and BMX 11111 (32.53%) (Table 4).

Table 4 Mean performances of yield-related traits of nine mungbean genotypes grown under control and salinity stress (8 dS/m) conditions.

Genotypes	Treatment	DM	PH	NPB	PPP	PL	SPP	HSW	YPP
BARI Mung-6	Control	57.33 ij	39 a	2.25 b-d	8.37 cd	6.78 b-e	6.73 fg	4.37 gh	3.87 b
	Salt	62.33 cd	15.08 j	1.73 ef	3.26 jk	5.92 fg	5.54 h	3.49 kl	1.27 g-i
Binamoog-8	Control	66.66 b	26.66 c	3.5 a	9.4 b	6.81 b-e	7.62 de	5.04 e	2.86 d
	Salt	69.33 a	22.5 f-h	1.88 d-f	3.52 i-k	5.52 gh	6.73 fg	4.23 hi	1.15 h-j
Durdona	Control	55.66 kl	24.31 d-f	2.12 c-e	10.89 a	7.86 a	8.2 b-d	7.25 a	4.37 a
	Salt	56.66 jk	20.5 h	1.8 d-f	5.8 fg	6.27 ef	6.42 fg	6.38 bc	1.95 e
BMX 1141	Control	58.33 hi	32.16 b	2.58 bc	9.02 bc	7.29 a-c	7.55 de	6.11 c	3.01 cd
	Salt	61.33 de	26.16 cd	2.22 b-e	4.37 hi	6.81 b-e	6.06 gh	4.88 ef	0.76 k
BMX 1131	Control	58.66 gh	26.8 c	2.12 c-e	4.95 gh	7.88 a	9.5 a	6.13 c	1.53 fg
	Salt	61.66 cd	21.83 gh	1.4 f	2.8 k	6.41 d-f	6.50 fg	5.61 d	0.81 k
BMX 11170	Control	55.33 l	24 ef	3.2 a	7.25 e	7.37 ab	7.76 c-e	4.13 h-j	1.72 ef
	Salt	58.66 gh	17.6 i	1.54 f	3.40 jk	6.75 c-e	7.05 ef	3.27 lm	0.93 jk
BMX 11148	Control	60.33 ef	32.33 b	2.5 bc	8.58 b-d	6.88 b-e	8.44 bc	6.58 b	3.29 c
	Salt	62.66 c	26.02 c-e	1.78 d-f	3.77 ij	5.30 h	4.73 i	4.02 h-j	0.89 jk
BMX 11122	Control	58.66 gh	25.1 c-e	2.25 b-d	5.74 fg	5.98 fg	6.26 gh	4.62 fg	1.42 f-h
	Salt	62.33 cd	22.53 f-h	1.60 f	3.89 ij	6.27 ef	6.30 f-h	3.81 jk	0.89 jk
BMX 11111	Control	55.66 kl	22.98 fg	3.17 a	7.72 de	6.95 b-d	8.55 b	3.94 ij	1.58 fg
	Salt	59.66 fg	15.39 j	2.66 b	5.9 f	5.5 gh	6.43 fg	2.90 m	1.06 i-k
Mean		60.07	24.49	2.24	6.03	6.58	7.02	4.82	1.85
CV (%)		1.05	5.08	13.35	8.52	5.58	6.71	4.82	10.30
LSD Value		1.05	2.06	0.49	0.85	0.61	0.78	0.38	0.31

Here, DM = Days to maturity, PH = Plant height (cm), NPB = No. of primary branches per plant, PPP = Pods per plant, PL = Pod length (cm), SPP = Seeds per pod, HSW = 100-Seed weight (g), YPP = Yield per plant (g).

3.3.2 Principal Component Analysis

In PCA analysis, PC1 explained 53.6% of the total variance, and PC2 accounted for 13.71% of the total variation. PC1 revealed distinct positioning in the biplot based on treatment (Figure 6). Genotypes from control conditions were predominantly on the right, correlating with high positive trait coefficients of YPP, PL, PPP, SPP, PH, HSW, and NBP, except for BMX 11122. Saline-treated genotypes are positioned on the left, indicating a higher coefficient of DM. Interestingly, Durdona under saline conditions tends to be placed at the right side compared to other genotypes under saline conditions (Figure 6). This spatial arrangement highlighted the differentiation between genotypes and their trait associations under varying conditions.

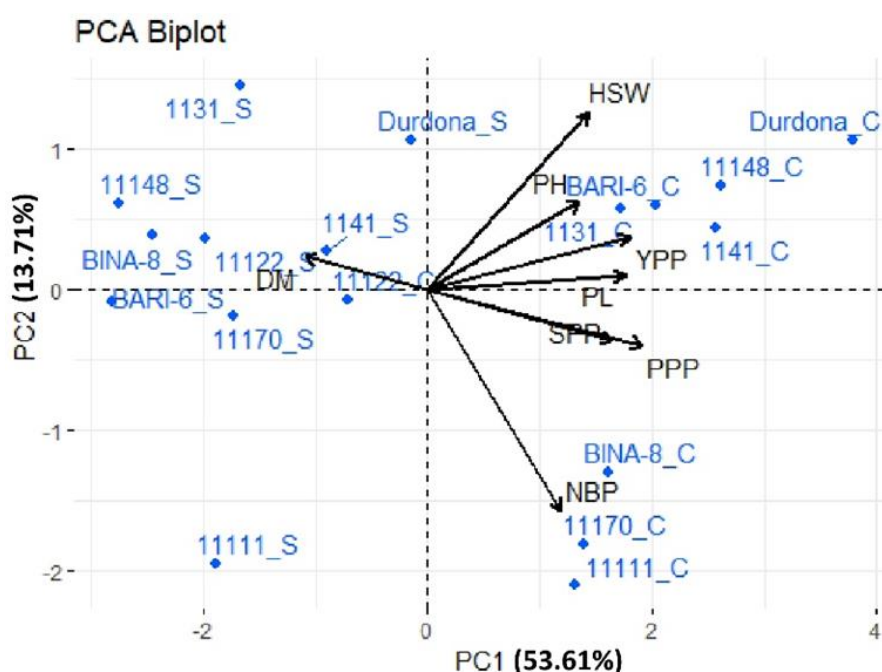


Figure 6 Principal component analysis of yield and yield attributing traits of nine mungbean genotypes under control and salt-stressed conditions. Here, DM = Days to maturity, PH = Plant height (cm), NBP = Number of branches per plant, PPP = Pods per plant, PL = Pod length (cm), SPP = Seeds per pod, HSW = 100-seed weight (g), YPP = Yield per plant (g).

3.3.3 Stress Tolerance Indices

Six stress tolerance indices (MP, GMP, SSI, TOL, STI, and YSI) estimated from YPP under control and salt stress conditions for nine mungbean genotypes formed three distinct clusters in a heatmap and hierarchical clustering (Figure 7; Table S6). In this heatmap and hierarchical clustering, cluster "a", consisting only of Durdona, exhibited the highest MP (3.16), GMP (2.92), STI (1.23), moderate YSI (0.44), and SSI (0.94). Cluster "b", comprising BARI Mung-6, Binamoog-8, BMX 1141, and BMX 11148, showed MP values of 2.57, 2.00, 1.88, and 2.09, respectively, and GMP values of 2.21, 1.82, 1.51, and 1.72, respectively. This cluster had high SSI (1.14, 1.01, 1.27, and 1.23, respectively) and low YSI (0.32, 0.4, 0.25, and 0.27, respectively). Cluster "c", including BMX 1131, BMX 11170, BMX 11111, and BMX 11122, displayed the lowest MP (1.15, 1.17, 1.32, and 1.32, respectively), GMP

(1.11, 1.12, 1.26, and 1.3, respectively), STI (0.18, 0.183, 0.23, and 0.24, respectively), and SSI (0.63, 0.79, 0.77, and 0.55, respectively). Genotypes of this cluster also exhibited the highest YSI (0.67, 0.62, 0.54, and 0.53, respectively).

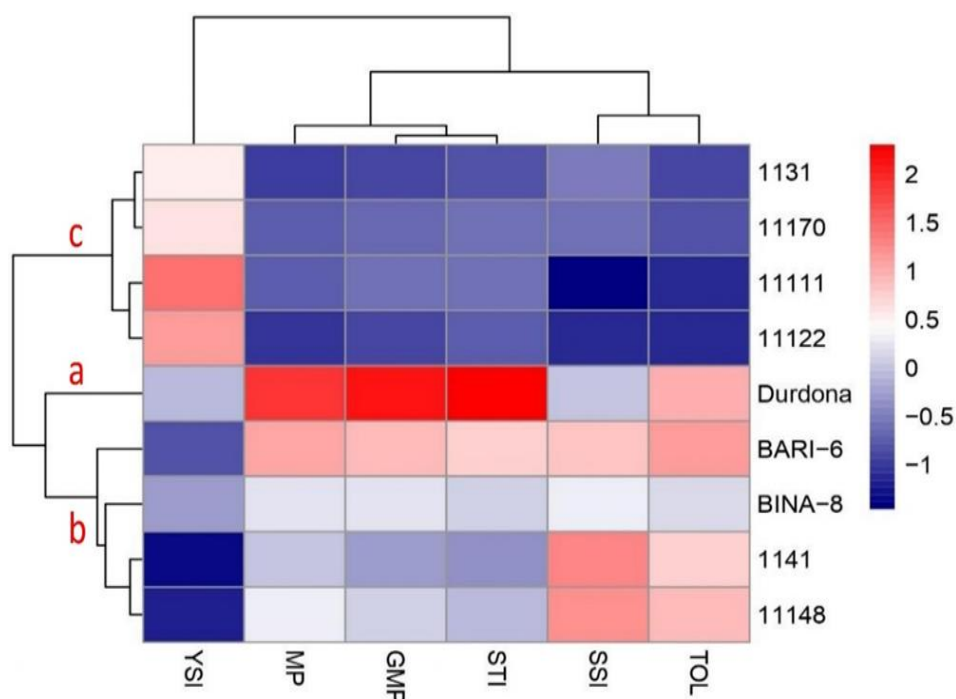


Figure 7 Heatmap showing stress tolerance indices among nine mungbean genotypes for yield per plant. Here, MP = Mean productivity; GMP = Geometric mean productivity; SSI = Stress Susceptibility Index; TOL = Tolerance Index; STI = Stress Tolerance Index; YSI = Yield stability index.

4. Discussion

The accelerating pace of climate change poses significant challenges to global food security, necessitating urgent efforts to enhance crop resilience. Mungbean, recognized for its nutritional and agronomic significance, is vital in advancing sustainable agriculture. Therefore, having early-maturing, high-yielding, and salt-tolerant mungbean varieties is imperative to ensure food security and support climate-resilient cropping systems. While a few previous studies have already examined salinity tolerance at multiple growth stages such as Masud [41], who evaluated salinity effects on mungbean growth and yield at seedling and reproductive stages, Iqbal et al. [42] investigated morpho-physiological responses and ion accumulation at vegetative and maturity stages and these works have significantly advanced the understanding of salinity impacts on mungbean. However, a notable gap remains in the comprehensive and integrated evaluation of diverse exotic advanced breeding lines, both at the seedling and reproductive stages of growth within a single study utilizing morpho-physiological and biochemical traits as well as stress tolerance indices. To the best of our knowledge, this is the first comprehensive study integrating multi-stage phenotypic and biochemical data to identify robust salt-tolerant mungbean genotypes integrating genetic analyses and stress tolerance indices.

4.1 Experiment 1: Characterization of Mungbean Genotypes for Earliness and Yield

4.1.1 Analysis of Variance and Mean Performance Analysis

Genetic variation within germplasm is a fundamental prerequisite for the success of any breeding program. The present study also showed significant variation among ten vital morphological traits of twenty-six genotypes, as shown by the ANOVA results. Each genotype showed distinct variation, highlighting their diversity and potential for crop enhancement, which is desirable to the breeder for developing suitable high-yielding genotypes [43]. The presence of significant variability in yield and yield-attributing traits in mungbean genotypes was also reported by other researchers [44, 45].

Genotypes like Binamoog-8, BARI Mung-6, BMX 1131, BMX 11122, BMX 11140, BMX 11148, BMX 11153, and BMX 11157 emerged as early-flowering genotypes, whereas BMX 11155, BMX 11176, and BMX 11276 required maximum time to flower (Table 1). These results are consistent with earlier research [46], which similarly highlighted differences in flowering times among the mungbean genotypes studied. Early-maturing genotypes such as Binamoog-5, Binamoog-8, Binamoog-9, BARI Mung-6, and Durdona show substantial promise for developing early-maturing mungbean varieties. These genotypes are particularly advantageous for regions affected by drought or those with shorter growing seasons, as they enable multiple cropping cycles within a year. In this study, DLH also varied, with BMX 11116 and BMX 11276 taking the most prolonged duration and Binamoog-8 and BMX 11154 being the shortest. Optimizing the harvest window duration is crucial for enhancing harvest efficiency, which impacts overall yield and seed quality [47, 48]. PH is positively correlated with yield-attributing traits, which is another essential characteristic from an agronomic perspective [49]. Taller plants like BMX 11249 may enhance light interception and productivity. At the same time, genotypes with short stature, BMX 11108, could offer advantages in terms of lodging resistance and better adaptability to intensive cultivation systems. Higher NBP could contribute to increasing pod production. In this study, NBP also varied significantly, with a high in BMX 11165. These plants' architectures are fundamental in determining the yield potential of mungbean genotypes. Recent studies also highlighted the role of plant architecture in determining yield potential [50-52]. Regarding PPP, BARI Mung-6 has the most and BMX 11155 the fewest, directly influencing the yield potential of these genotypes. Studies by Christian et al. [53] and Islam et al. [54] also emphasize the importance of higher pod numbers as a critical determinant of mungbean yield. Pod traits like PL and SPP are crucial for yield and seed quality in legumes. Longer pods correlate with higher seed counts, boosting productivity, while more significant seed counts enhance market value and economic appeal [55]. BMX 11153 exhibited longer pods with higher seed counts, whereas BMX 11107 had shorter pods with fewer seeds. Durdona demonstrated superior performance regarding HSW. YPP ranged from 1.88 to 13.99 g. Durdona was the high-yielding genotype followed by BARI Mung-6 and Binamoog-8, highlighting their suitability for commercial cultivation and potential contributions to food security and economic prosperity. These findings underscore the genetic variability within mungbean germplasm and the potential for targeted breeding efforts to develop cultivars with improved agronomic performance and resilience to biotic and abiotic stresses.

4.1.2 Genetic Parameters Analysis

The success of crop breeding depends on genetic diversity and the inheritance of desirable traits. Breeders must analyze genetic variation to establish effective strategies and selection criteria to enhance target traits [56, 57]. Phenotypic variance typically exceeds genotypic variance because it incorporates both genotypic and environmental variance [58]. The study reveals PCV values were higher than GCV values for all traits, suggesting a more substantial ecological influence. This finding is consistent with the findings of Wang et al. [49] and Kim et al. [59]. Broad-sense heritability estimates (>90%) for traits like PH, PPP, and YPP imply strong genetic control and potential for genetic improvement, as supported by [60, 61]. High heritability reflects a strong genetic influence on a trait but does not guarantee significant genetic gains. However, when coupled with high genetic advances, it indicates better potential for genetic improvement and effective selection in breeding programs. Understanding genetic advances helps breeders plan effective selection strategies [62]. Traits with higher genetic advances, such as PH, PPP, DLH, and YPP, respond well to selection pressure. High heritability, along with high GA%, was found for traits such as PH, NBP, PPP, PL, HSW, and YPP, indicating significant potential improvement through selection [63, 64].

4.1.3 Relationship between Yield and Yield Contributing Traits

Yield is a complex quantitative trait governed by multiple genes and influenced by various interrelated morphological traits. Correlation analysis quantifies these relationships, offering critical insights into trait interactions and guiding the selection of optimal trait combinations for yield improvement in breeding programs [65]. In this study, YPP was positively correlated with PPP, HSW, and PL, indicating that higher pod numbers, heavier seeds, and longer pods contribute to greater yield. This aligns with the findings of previous studies [66, 67]. However, YPP showed negative correlations with DFF, DFH, and DLH, suggesting that earlier flowering and maturity might limit yield due to reduced time for pod and seed formation, as highlighted by Azam et al. [52] and Geetika et al. [68]. A strong positive correlation between HSW and PL indicates that longer pods generally lead to heavier seeds, consistent with findings by Ullah et al. [69]. NBP was positively correlated with both PH and PL, suggesting that taller plants with more branches may also have longer pods, consistent with previous studies [70, 71]. Significant positive correlations between DFF, DFH, and DLH indicate that earlier flowering leads to earlier maturity, consistent with Ullah [72]. Negative correlations between DLH and PPP, SPP, and YPP, along with negative correlations of PPP with DFF and DLH, suggest that higher pod numbers may be associated with delayed maturity, similar to the observations of Geetika et al. [68]. These findings highlight the trade-offs between early maturity and yield, providing valuable insights for breeding strategies targeting high-yielding, early-maturing cultivars. Further studies are needed to validate these correlations and understand the genetic mechanisms controlling trait expression. Integrating genetic analyses, such as QTL mapping and GWAS, along with field trials under diverse conditions, can aid in developing superior mung bean cultivars [73, 74].

4.1.4 Principal Component Analysis (PCA)

PCA is a multivariate technique that reduces the complexity of data by analyzing multiple traits simultaneously, providing valuable insights for parental selection, breeding strategies, and the

development of enhanced cultivars [75]. In this study, the first four PCs were selected based on their eigenvalues greater than 1, explaining 78.6% of the total variance in yield and yield-related variables, which is comparable with the findings of Ajaykumar et al. [76].

Further biplot analysis was conducted to get valuable insights on the relationships between agronomic traits and yield per plant, aligning with principles of multivariate analysis in plant breeding [77]. Positive correlations between PL, PPP, and HSW with YPP resonate with studies emphasizing the importance of these traits in determining crop productivity. For instance, longer pods and higher pod numbers are associated with increased yield potential due to enhanced reproductive efficiency, while higher seed weight contributes to overall yield and seed quality. Conversely, negative correlations between YPP and DFF, DFH, and DLH underscore the significance of timely management practices in crop production. Early flowering and harvest dates are often associated with higher yields, reflecting efficient resource utilization and reduced susceptibility to biotic and abiotic stresses [78]. Genotypes like Durdona, BARI Mung-6, Binamoog-8, and BMX 1137 exhibited similar directional trends as YPP, which suggests their potential for high productivity (Figure 2). These genotypes can serve as valuable genetic resources for breeding programs to maximize yield and resilience in target environments.

4.2 Experiment 2: Screening of Nine Mungbean Genotypes for Salinity Tolerance at the Seedling Stage using Hydroponics Media

4.2.1 Analysis of Variance and Mean Performance Analysis

In this experiment, the ANOVA results revealed significant variations among the studied genotypes for all traits at the genotype, treatment, and their interaction levels. Plants adapt their morpho-physiological systems to environmental changes under stress to ensure survival. Specifically, salt stress affects the root and shoot systems and induces morphological and physiological adjustments in the root system to enhance nutrient absorption [79]. Salt stress lowers extracellular water potential and reduces water bioavailability in the root zone, decreasing water and nutrient uptake. This inhibits plant growth and shoot biomass production [80]. The present study found that salt stress significantly reduced root and shoot traits across all genotypes, with Durdona showing the lowest reductions in RL, BMX11170 in SL, BMX11170 in RFW, BMX11111 in SFW, Durdona in RDW, and BMX11111 in SDW (Table 3). These findings align with previous researchers [2, 20, 81-83]. This highlighted the detrimental effects of salt stress on root and shoot growth as well as fresh and dry biomass. However, the reduction was more pronounced in shoot than in root growth. This aligns with observations that shoots are more affected by salt stress due to the superior osmotic adjustment capability of roots under such conditions [84, 85].

Salt stress significantly reduced chlorophyll content (SPAD value) across all genotypes, disrupting photosynthesis and affecting growth. This decline is consistent with findings in other researchers [86, 87]. This is due to membrane swelling in chloroplasts and/or excess Na^+ and Cl^- ions in the leaves. The accumulation of ions results in excess ROS production, reducing photosynthesis [88, 89]. Genotypes Durdona and BMX 1131 experienced comparatively lower reduction than other genotypes, indicating their potential for showing tolerance.

Na^+/K^+ ratios are a key indicator of stress tolerance, as excessive Na^+ in the soil leads to ion toxicity and hyperosmotic and oxidative stress [20, 90]. In most plants, sodium is non-essential, and high Na^+ concentrations can disrupt ionic homeostasis, competing with K^+ uptake and resulting in a

higher Na^+/K^+ ratio [91, 92]. This imbalance can damage membrane integrity and affect cellular structures [93]. In this study, salt stress significantly increased the Na^+/K^+ ratio in the roots, reflecting the impact of salt stress on ionic homeostasis. The genotypes BMX 1141, Durdona, and BMX 1131 exhibited superior salt tolerance, with BMX 1141 showing the lowest increase in root Na^+/K^+ ratio, indicating effective ionic balance in the roots. In contrast, Durdona and BMX 1131 had lower increases in shoot Na^+/K^+ ratio, suggesting better prevention of Na^+ movement to the aerial parts, thus protecting photosynthesis and shoot health [20]. The lower shoot Na^+/K^+ ratio in these genotypes may be attributed to enhanced Na^+ efflux from the roots to the rhizosphere through the SOS1-dependent exclusion system, as well as efficient Na^+ loading and unloading at the xylem, which helps limit Na^+ accumulation in the shoots [11].

The analysis of MDA, H_2O_2 , and proline levels provided key insights into oxidative stress and osmotic regulation under salt stress. Elevated MDA and H_2O_2 levels indicated oxidative damage caused by lipid peroxidation and cellular component disruption due to ROS production, while increased proline levels suggested osmotic adjustment mechanisms (Table 3). These findings align with studies highlighting the role of MDA and H_2O_2 as markers of oxidative stress and proline as a protective osmolyte that stabilizes membranes, proteins, and water balance during stress [14, 94]. In this study, BMX 1131 and Durdona exhibited minimal induction of MDA and H_2O_2 , indicating reduced oxidative damage, while producing higher amounts of proline, suggesting superior osmotic adjustment capacity under salt stress. This highlights their potential for salt tolerance by effectively balancing oxidative stress and osmotic regulation [95]. The variability in these markers among mungbean genotypes emphasizes the need to identify cultivars with enhanced antioxidant capacity and osmotic adjustment for breeding salt-tolerant varieties that sustain productivity under saline conditions.

4.2.2 Correlation Coefficient

Under salt stress, root and shoot traits showed strong positive correlations, indicating that improved root growth supports enhanced shoot development. RL was positively correlated with SL, RFW, SFW, RDW, and SDW, suggesting that better root growth contributes to overall biomass. These results align with findings by other researchers in wheat and alfalfa [96, 97]. RL showing negative correlations with NaK_S and H_2O_2 suggests that longer roots are linked to better ion balance and reduced oxidative stress under stress conditions. SL had negative correlations with MDA and H_2O_2 , suggesting that the high production of MDA and H_2O_2 under saline conditions, which indicate oxidative stress, inhibits shoot growth, leading to shorter shoots. RL and SL both showed positive correlations with proline, suggesting that proline accumulation plays a key role in mitigating salinity stress by acting as an osmoprotectant. Proline helps maintain cellular turgor and stability under stress conditions by balancing osmotic pressure, stabilizing proteins and membranes, and scavenging ROS. These contribute to enhanced root and shoot growth, enabling better stress tolerance under saline conditions [91]. SPAD was positively correlated with shoot growth traits, highlighting the role of chlorophyll content in plant health under stress, which is in line with previous studies [87, 98].

The negative correlations of Na^+/K^+ S and Na^+/K^+ R with biomass-related traits suggest that a lower K^+ to higher Na^+ ratio impairs plant growth under salt stress. Elevated Na^+ levels disrupt cellular processes and reduce the efficiency of potassium-dependent metabolic functions, such as

enzyme activity and osmoregulation. This imbalance reduces plant biomass and stunts growth, as plants struggle to maintain proper ion homeostasis under salt stress [92, 99]. The positive correlation between MDA and H_2O_2 suggests that as lipid peroxidation increases, there is a corresponding rise in ROS production. This is because H_2O_2 is a major ROS that accumulates in plant cells under stress, leading to oxidative damage. The degradation of membrane lipids, reflected by higher MDA levels, is a consequence of ROS activity [94]. The negative correlation between MDA and proline suggests that plants with lower oxidative damage may also accumulate less proline. Proline, an important osmolyte and ROS scavenger, helps plants manage stress. This reduced reliance on proline under lower oxidative damage indicates a more efficient stress response, where plants maintain cellular integrity without excessive osmolyte accumulation [94, 95]. These findings highlight the importance of maintaining a balance between growth and ion homeostasis for improving salt tolerance.

4.2.3 Principal Component Analysis (PCA)

In PCA analysis, PC1 explains 73.8% of the variation, capturing traits like root and shoot length, fresh and dry weights, and chlorophyll content. The biplot analysis differentiates genotypes based on their response to control and stress conditions. Genotypes on the right side, indicating better performance under control conditions, exhibit higher growth values for SL, SDW, RFW, RDW, RL, SFW, and SPAD. In contrast, genotypes on the left side show higher stress markers such as MDA, H_2O_2 , Proline, and Na^+/K^+ ratio under stress. Higher PC1 scores indicate robust growth and vigor, while lower scores suggest susceptibility to stress, as shown by negative loadings of stress indicators like Na^+/K^+ ratio, MDA, H_2O_2 , and proline [100, 101]. Interestingly, despite being under stress, the genotypes Durdona and BMX 1131 were positioned on the right side of the biplot, indicating their superior tolerance. This suggests that these genotypes may possess mechanisms that enable them to mitigate the adverse effects of salt stress, possibly through effective ion regulation or osmolyte accumulation, contributing to better overall performance (Table 3).

4.3 Experiment 3: Screening for Salt Tolerance at the Reproductive Stage

4.3.1 Analysis of Variance and Mean Performance Analysis

According to the ANOVA analysis, significant variation was observed across all traits due to salt stress (Table S4). This highlights the strong influence of salt stress on the genotype's performance. Jahan et al. [9] and Mittal et al. [88] also reported significant variation in the traits that they studied under salt stress at the reproductive stage.

In this study, significant variation in DM was observed among mungbean genotypes under salt stress, with BARI Mung-6 showing the most substantial increase (8.72%), followed by genotypes BMX 11111 and BMX 11122, while Durdona exhibited less induction (Table 4). The extended maturity duration may serve as an adaptive response, allowing genotypes to prolong vegetative growth to recover from stress. Ahmed [102] similarly reported delayed maturity in mungbean under salt stress. For PH, the most pronounced decrease was recorded in BARI Mung-6, which showed a reduction of 61.32%. In contrast, genotypes Durdona, Binamoog-8, and BMX 11122 experienced comparatively lower reductions. Previous researchers also reported significant reductions in PH due to salt stress in mungbean genotypes [9, 103]. Under salt stress, a notable reduction in NBP was

recorded, with the most substantial decrease occurring in genotype BMX 11170, which experienced a drop of 51.66%. In contrast, genotypes BMX 1141 and Durdona exhibited lower reductions. This highlights the adverse impact of salt stress on branch development. Disruption of hormonal regulation under salt stress, particularly involving auxins and cytokinins, critically impairs the processes necessary for branch formation and growth. Salinity stress significantly decreased SPP. Notably, genotype Binamoog-8 exhibited the most substantial reduction in SPP, with a decrease of 62.55%. In contrast, genotypes BMX 1131 and BMX 11122 showed comparatively lower reductions in SPP. The reduction in SPP can be attributed to decreased flower numbers and impaired pollen production, which are negatively impacted by salt stress [104]. The most significant reduction of PL was seen in genotype BMX 11148 (22.86%), while BARI Mung-6 and BMX 11170 showed smaller reductions, indicating better tolerance. Due to increased osmotic stress, turgor pressure is reduced, which is essential for cell expansion and pod elongation. Salinity stress significantly reduced SPP across all genotypes compared to the control. The most considerable reduction was recorded in genotype BMX 11148 (43.91%), whereas BARI Mung-6 and Binamoog-8 exhibited comparatively minor reductions. The reduction in seed number can be attributed to the adverse effects of salt stress on reproductive processes, leading to the formation of shriveled seeds and subsequent yield loss [102, 103]. For the 100-SW, BMX 11148 exhibited the highest decrease of 38.96%, while Binamoog-8, Durdona, and BMX 1131 showed comparatively lower reductions. These findings are consistent with previous research [102, 105], which indicates that high salinity impairs pod setting and reduces grain weight and yield [106].

YPP, a crucial indicator of overall productivity in mungbean, reflects the plant's ability to convert resources into harvestable seed. Exposure to salt stress resulted in a notable reduction in YPP, with genotype BMX 1141 experiencing the most substantial decrease of 74.73%. Susceptible lines often produce shriveled seeds under salinity stress, compromising the pod setting, which results in fewer pods and lower yields [107]. In contrast, BMX 11111 and BMX 11122 showed lower yield reductions, indicating better yield stability to salinity stress. Enhanced osmotic adjustment, improved ion homeostasis, and more efficient nutrient uptake mechanisms might be the reasons for the lower reduction in YPP under stress.

4.3.2 Principal Component Analysis

The biplot effectively visualizes the trait-specific genotypic relationships among mungbean genotypes under different treatment conditions, providing insights into their performance (Figure 6). The right-side positioning of genotypes under non-saline conditions indicates superior growth potential, supported by higher positive coefficients for key yield-related traits (SPP, YPP, PL, HSW, PH, and NBP). This suggests these genotypes can better utilize resources and exhibit improved overall vigour in optimal conditions. Comparable findings were also reported by Yimram et al. [44] and Akter et al. [108]. Conversely, the left-side clustering of saline-treated genotypes reveals compromised performance and highlights their stress-induced limitations. Notably, Durdona's spatial positioning emphasizes its potential as a salt-tolerant mungbean genotype, as it is located on the right side of the biplot under both normal and stress conditions.

4.3.3 Stress Tolerance Indices

For the identification of stress-tolerant cultivars, several researchers have suggested many stress tolerance indices. Several researchers usually employed these stress tolerance indices to separate the stress-tolerant and susceptible genotypes under stressed conditions [99, 109]. In this study, MP, GMP, SSI, TOI, STI, and YSI were used to evaluate the stress tolerance of nine mungbean genotypes, which were grouped into three clusters through heat map analysis, highlighting their diversity in stress tolerance (Figure 7; Table S5).

Cluster “a” consisted solely of Durdona, which exhibited the highest values for MP, GMP, and STI, indicating strong performance under stress and normal conditions. Selecting genotypes based on MP and GMP could enhance yields in stress and non-stress environments [37]. STI is a more reliable parameter, and genotypes with higher STI values exhibit superior performance in both normal and stressed environments. This makes Durdona a prime candidate for stress-resilient breeding. Cluster “b”, included BARI Mung-6, Binamoog-8, BMX 1141, and BMX 11148, characterized by low YSI, suggesting lower yield stability under stressed conditions and higher SSI and TOL, indicating greater susceptibility to salt stress. Cluster “c”, with genotypes BMX 1131, BMX 11170, BMX 11111, and BMX 11122, had the highest YSI but the lowest values for all other indices. These genotypes showed relative tolerance due to their higher yield stability and lower SSI and TOL values. However, their lower STI, MP, and GMP performance indicated limited overall yield potential under stress and non-stress conditions. This suggests that while these genotypes exhibit stress tolerance, their overall productivity may lag compared to other genotypes [99].

The findings highlight the importance of multiple indices in identifying stress-tolerant genotypes. Durdona, with high yield and stress tolerance, is a valuable asset for breeding, supported by seedling stage results. In contrast, BMX 1131 exhibited tolerance at the seedling stage but showed poor performance during the reproductive phase, indicating possible growth-stage-specific tolerance mechanisms. This suggests that tolerance traits may be regulated differently at early (seedling and vegetative) and later (reproductive) stages. Similar patterns have been reported in mungbean, where it was stated that early-stage tolerance does not necessarily correlate with tolerance at later stages. Sehrawat et al. [103] observed minimal effects of salinity during germination and seedling growth, but significantly greater impacts during flowering and pod development. Van et al. [110] also emphasized that the reproductive stages in mungbean are more susceptible to salinity than the early stages. Evidence was also found in other crops, such as rice and chickpea, which have shown that salinity tolerance at the seedling and vegetative stages is not necessarily correlated with tolerance at the reproductive stages [111, 112]. Therefore, the contrasting performance of Durdona and BMX 1131 reinforces the need for comprehensive, multi-stage evaluations to identify stress-resilient genotypes for reliable breeding.

While the present study provides valuable insights into salinity tolerance in mungbean, it was conducted under controlled conditions using a single salinity level. This limits the ability to account for genotype $\times$ environment ($G \times E$) interactions, which are known to influence stress response consistency significantly. Therefore, to confirm the stability and practical relevance of the identified tolerant genotypes, future work should incorporate multi-location field trials under diverse environmental and salinity conditions.

5. Conclusions

This study reveals significant phenotypic and genotypic variability among the studied mungbean genotypes for yield-attributing traits. Based on the findings of the first experiment, we have identified a few potential early (Binamoog-8, Binamoog-9, BMX 11140, BMX 11154, and BMX 11157) and high-yielding mungbean genotypes (Durdona, BARI Mung-6, Binamoog-8, and BMX 1137) that can be grown under the agro-climatic conditions of Bangladesh. The genotypic and phenotypic coefficients of variation of the studied traits showed negligible differences, suggesting that the environment had less impact on the expression of the characteristics. High genetic advance coupled with high heritability was seen for plant height, number of primary branches, number of pods per plant, pod length, 100-seed weight, and seed yield per plant, which offers ample opportunities for selection for maximizing genetic gain. Based on the findings of the second and third experiments, Durdona and BMX 1131 were classified as the seedling-stage saline-tolerant genotypes, whereas Durdona was categorized as the reproductive-stage salt-tolerant genotype. Apart from morphological traits, emphasis should also be given to the biochemical characteristics, such as lipid peroxidation and H_2O_2 , as they showed greater variation in the tolerant and susceptible genotypes. The identified biomarkers can be utilized to isolate salt-tolerant and susceptible genotypes through the assessment of germplasms at the early stages of crop growth. However, further studies should be done with the selected lines under direct field conditions to get a clear picture of the salt tolerance of the studied germplasm.

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Author Contributions

Study conception and design: Mumtarim Haque Mim, Biswajit Das, Sadia Akter; Data collection: Mumtarim Haque Mim, Biswajit Das, Sadia Akter, Sheikh Mahfuja Khatun; Analysis and interpretation of results: Mumtarim Haque Mim, Biswajit Das, Sadia Akter, Sheikh Mahfuja Khatun, Jannatul Naim; Draft manuscript preparation: Mumtarim Haque Mim, Biswajit Das, Sadia Akter; Writing-review & editing: Mohammad Anwar Hossain, Mohammad Pessarakli, Mohammad Anwar Hossain.

Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Table S1: Mean square value of different phenotypic traits of twenty-six mungbean genotypes.
2. Table S2: Principal components (PCs) for morphological traits in twenty-six mungbean genotypes from principal component analysis (PCA).

3. Table S3: Analysis of variance (ANOVA) of morphological and biochemical characters of nine mungbean genotypes grown under control and salt stress conditions at the seedling stage.
4. Table S4: Analysis of variance on yield and yield-related traits of nine mungbean genotypes grown under control and salinity stress (8 dS/m) conditions.
5. Table S5: Percent increase/decrease due to salt stress for different yield-attributing traits of nine mungbean genotypes.
6. Table S6: Stress tolerance indices in mungbean genotypes, estimated from yield per plant obtained in control and salinity stress conditions.

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