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RESEARCH ARTICLE

Salinity sensitivity in mungbean: tissue ion accumulation in relation to growth and yield in contrasting genotypes

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Abstract
Background and aims Salinity poses a significant challenge to agricultural production, and mungbean (*Vigna radiata* L.) is among the more salt-sensitive food legumes. This study evaluated salinity tolerance in four contrasting mungbean genotypes by analysing their morpho-physiological responses at the vegetative stage and at maturity.
Methods Plants were grown in soil-filled pots subjected to five salinity treatments (0, 25, 50, 75 and 125 mM NaCl) in a temperature-controlled glasshouse. Salinity was imposed 15 days after sowing (DAS) and plants were harvested at 38 DAS and 56 DAS.
Results Genotypes varied in their sensitivity to salinity measured as various morpho-physiological traits including foliar injury, SPAD, nodulation, growth, yield and yield components. Growth reduction was similar across genotypes at the vegetative stage but was more pronounced in sensitive than tolerant genotypes at maturity. High seed yield in salt tolerant genotypes was associated with large seeds, more flowers and pods, and number of seeds per plant in saline soil. Salinity stress decresead leaf osmotic potential while increasing leaf water content in all genotypes. Salinity stress increased leaf Na^+, Cl^- and also leaf K^+ in all genotypes for maintaining the charge balance as Na^+ was only about 14% of Cl^- in leaf tissues. Tolerant genotypes accumulated less leaf Na^+ and Cl^- and maintained higher leaf K^+/Na^+ than sensitive genotypes, however, Cl^- concentrations were increased to 260–395 mM in all genotypes.
Conclusions Salinity sensitivity results from toxic concentrations of Na^+ and Cl^- in leaves, leading to reduced chlorophyll content, growth and yield. These findings demonstrate that the ability of controlling Na^+ and Cl^- accumulation in leaves, tissue tolerance to high Cl^- and maintaining high leaf K^+/Na^+ ratio may contribute to salinity tolerance in mungbean.

Keywords Na^+ and Cl^- exclusion · Mungbean · Osmotic adjustments · Salinity tolerance · Reproductive growth · Vegetative growth

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Introduction

Mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*] is an important warm-season food legume. The crop has a short life span and is grown for edible seeds and sprouts, forage, and soil fertility enhancement. Mungbean seeds are rich in protein, dietary fibre, vitamins, and minerals, thus contributing significantly to a plant-based balanced diet (Hou et al. 2019). Domesticated in South Asia, mungbean cultivation has expanded to Southeast Asia, Australia and East Africa (Nair and Schreinemachers 2020). Globally, mungbean is cultivated on ~ 7.3 million hectares, accounting for nearly 12% of total pulse production. About 56% of the global area of mungbean is in South Asia, including India, Pakistan and Bangladesh (Nair and Schreinemachers 2020). Mungbean is predominantly grown during the dry period in tropical and subtropical rainfed rice-based farming systems where abiotic stresses such as soil salinity, drought and transient waterlogging (due to unseasonal heavy rainfall) threaten crop production. In this region, mungbean faces elevated soil salinity levels during the reproductive stage due to the capillary rise of salt from groundwater during the dry period, leading to severe constraints on yield (Rengasamy 2002; Haque 2006; James et al. 2023).

Soil salinity adversely affects plant growth and development, reducing crop yield. Plant responses to salinity stress differ depending on the plant's developmental stage, salt concentration and duration of salt exposure (Munns and Tester 2008). Typical of most legumes, mungbean is considered extremely sensitive to salinity (Sehrawat et al. 2015). Soil salinity adversely affects most growth stages of mungbean from seed germination and seedling establishment (Breria et al. 2020; Liu et al. 2022) to vegetative growth (Alharby et al. 2019), flowering and pod development (Manasa et al. 2017). Flowering and pod-filling are considered the most salinity stress sensitive stages in mungbean (Sehrawat et al. 2014; Le et al. 2021). Salinity stress results in reduced leaf chlorophyll content, leaf numbers, root and shoot lengths and biomass, pod numbers, seed size and seed yield in mungbean (Wahid et al. 2004; Syeed and Fatma 2011; Manasa et al. 2017). The deleterious impacts on growth and yield result from both osmotic and ionic toxicity stress (James et al. 2002; Munns and Tester 2008). Salinity stress leads to high Na^+ and Cl^- accumulation in leaves, adversely affecting photosynthesis, vegetative growth and seed yield (Wahid et al. 2004; Syeed and Fatma 2011; Le et al. 2021). Potentially toxic Na^+ and Cl^- levels in reproductive organs have also been suggested to impact crucial reproductive processes like pollen or ovule fertilisation in chickpea (Samineni et al. 2011; Turner et al. 2013). However, in mungbean the effect of salinity stress on reproductive development is largely unexplored.

Salinity tolerance in plants is associated with three main mechanisms; osmotic stress tolerance, ion exclusion and tissue tolerance (Munns and Tester 2008; Roy et al. 2014; Zhao et al. 2020). Plants accumulate Na^+ and Cl^- in vacuoles and organic solutes in the cytoplasm for osmotic adjustment in response to the high negative osmotic potential of NaCl in the root zone (Greenway and Munns 1983; Yeo 1983; Munns et al. 2016). Ion exclusion involves restricting the entry of salt ions into the leaves and preventing concentrations from reaching toxic levels (Roy et al. 2014) Tissue tolerance involves the capacity of plants to compartmentalise Na^+ and Cl^- in vacuoles of the leaves and maintain low concentrations of these ions in the cytoplasm and organelles (Flowers 1972; Greenway and Osmond 1972; Munns et al. 2016). In mungbean, tolerant genotypes accumulated low Na^+ and Cl^- and maintained high K^+ in leaves (Wahid et al. 2004; Syeed and Fatma 2011). Salinity tolerance in mungbean was also associated with high proline, glycine betaine and total soluble sugar content in leaves (Syeed and Fatma 2011; Alharby et al. 2019). In a recent study, Cl^- toxicity in leaf tissues primarily reduced the growth and yield in mungbean, where an osmotic treatment of –0.43 MPa (equivalent to 100 mM NaCl) and 100 mM Na^+ salts (without Cl^-) affected growth and yield less than Cl^- salts (without Na^+) (Le et al. 2021). Mungbean also maintained lower Na^+ in leaves than roots while accumulating more Cl^- in leaves than roots (Le et al. 2021). However, this study used only a single putatively tolerant genotype.

Understanding salinity tolerance mechanisms in mungbean will facilitate identifying traits for developing salt-tolerant varieties. This study evaluated the effect of various NaCl concentrations on contrasting mungbean genotypes grown in soil-filled pots to investigate salinity tolerance mechanisms in mungbean at the vegetative stage and maturity. The specific objectives were to (1) understand differing salinity stress responses and identify key traits associated with salinity tolerance in mungbean and (2) investigate whether the effect of salinity stress in mungbean is related to ion accumulation in specific tissues. This is the first study in mungbean to investigate accumulation of Na^+, K^+ and Cl^- in different tissues in relation to various morpho-physiological traits and reproductive success expressed as production of flowers, matured pods and seeds. This is also the first study in mungbean to explore how increased inorganic ions (Na^+, K^+ and Cl^-) and organic solutes contribute to osmotic adjustment under salinity stress. Thus, this study fills an important gap in understanding the salinity sensitivity of mungbean and associated mechanisms of salinity tolerance.

Materials and methods

The study comprised two experimental sets to evaluate the effect of salinity stress at the vegetative and maturity stages: Set I for sampling at the vegetative stage and Set II for sampling at maturity.

Plant materials and growth conditions

The four mungbean genotypes were selected for this study which comprised two commercial varieties: Jade AU from Australia and BARI Mung-6 from Bangladesh (they were the best-performing released varieties in Australia and Bangladesh at the time of the experiment) and two genotypes contrasting in salinity tolerance, putative salt tolerant V02211 (origin-Philippines) and putative salt sensitive V01317 (origin-Pakistan), chosen based on salinity screening by the World Vegetable Center, Taiwan (source: personal communication with Ramakrishnan Nair). Seeds of the latter two genotypes were kindly provided by the Department of Agriculture and Fisheries, Queensland, Australia.

The experiment was conducted from August to October 2020 in a controlled temperature glasshouse at The University of Western Australia, Perth, WA, Australia (31°57´S, 115°47´E) under 30 ± 3/24 ± 2 °C day/night temperatures with 11–13 h day length and maximum PAR (photosynthetically active radiation) of 1400–1650 $\mu mol\ m^{-2}\ s^{-1}$. Plants were grown in free-draining plastic pots (180 mm in diameter and 180 mm in height for Set I; 200 mm in diameter and 190 mm in height for Set II) lined with polybags to prevent water, salt and nutrient leaching. Pots were filled with 3.15 kg (Set I) or 4.75 kg (Set II) of 2 mm-sieved oven-dried red-brown sandy clay loam soil (pH 8.79, electrical conductivity 0.28 $dS\ m^{-1}$ in 1:5 soil: water extract) collected from Mukinbudin (30°78′S, 118°31′E), Western Australia. This soil type was previously used as a substrate in salinity studies (Kotula et al. 2015). The water content (w/w) at field capacity was 19.7%. The soil was fertilised based on soil analysis. Nutrients were added to the soil as (g kg^{-1} soil): 0.129 K_2SO_4, 0.225 $CaSO_4.2H_2O$, 0.191 KH_2PO_4, 0.025 $MgSO_4.7H_2O$, and 0.70 mL kg^{-1} soil of half-strength Hoagland solution micronutrients (Kotula et al. 2019b). The nutrients were added with deionised water in a sufficient solution volume to wet the soil to 80% field capacity before sowing. Prior to sowing, seeds were surface sterilised with 1% commercial bleach (active ingredients NaOCl 40 mg L^{-1}) for one min, rinsed with deionised water for four min and then germinated on Whatman filter paper moistened with deionised water in 50 mm Petri dishes for 12 h in darkness. For each pot, six seeds were sown at 30 mm depth along with peat-based Group I mungbean Rhizobium (*Bradyrhizobium* spp.) strain CB 1015 (5.25 g pot^{-1}; Group N, New Edge Microbials Pty Ltd, Albury NSW, Australia). Pots were watered with deionised water to maintain 80% field capacity on alternate days. Seedlings were thinned seven days after sowing to three plants per pot in Set I and two plants per pot in Set II.

Experimental design and treatments

Five salinity treatments were used: 0 (non-saline control), 25, 50, 75 and 125 mM NaCl. The experiments had a randomised complete block design with two factors (4 genotypes × 5 salinity treatments) and four replications. Eighty experimental pots for each set were established, with an equal number of pots assigned to each treatment and an additional 16 pots per set for an initial harvest (4 genotypes × 4 replicates). Pots were re-randomised weekly to reduce positional effects in the glasshouse. Salinity treatments were imposed 15 days after sowing (DAS) for both sets when most plants were approximately at the V1 stage (unifoliate leaves attached to the first node are fully expanded and flat as the first trifoliate leaf attached to the upper node starts to unroll; Pookpakdi et al. 1992). The NaCl treatments were stepped up by the addition of 25 mM NaCl (0.288 g NaCl kg^{-1} soil) progressively each day until they reached their final concentration. NaCl was applied to pots in a sufficient solution volume to wet the soil to 80% field capacity, with the equivalent volume of deionised water added to non-saline control pots. Pots were weighed on alternate days and watered with deionised water to 80% field capacity throughout the experimental period. As we used polybags to prevent water and salt leaching and most plant exclude more than 90% of the Na^+ and Cl^- in the soil solution, with the remaining transported to shoots via the xylem (Munns 2005). The salt concentrations remained, to a good approximation, constant throughout the experiments.

Harvests

Three plant harvests were made: (i) an initial harvest from Set I and Set II at the commencement of salinity treatments (15 DAS); (ii) a second harvest from Set I at the vegetative stage (38 DAS) which was 23 days after the first addition of 25 mM NaCl (appearance of distinct foliar injury symptoms under different salinity stress levels); (iii) final harvest from Set II at maturity (56 DAS) which was 41 days after the first addition of 25 mM NaCl (~ 80% of pods of all genotypes and treatments were mature and turned black). At each harvest, plant parts were separated into youngest fully expanded leaves (YFEL) and other green leaves (laminas), dead leaves (> 20% chlorotic / necrotic leaf area), stems (with petioles) and roots. Dead leaves were combined with leaves that had fallen from the plants during the experiment. Shoots were cut at the soil surface, and roots were washed carefully with flowing tap water on a sieve with a 2 mm mesh size and blotted dry with a paper towel.

Measurements

At the vegetative stage (38 DAS) and maturity (56 DAS), foliar injury was assessed visually based on a 1–10 scale where: 1, no injury symptoms; 2, leaf with slight chlorosis or beginning to yellow; 3, chlorosis or overall yellowing on 25% of plant; 4, necrosis beginning on 25% of plant; 5, necrosis on 25% of plant or chlorosis on 50% of plant; 6, necrosis more than 50% of plant or chlorosis on 75% of plant; 7, necrosis more than 75% of plant or chlorosis on whole plant; 8, necrosis on whole plant and very youngest leaves still green; 9, only stem and shoot tips green, 10 = plant dead, no green parts (adapted from Maliro et al. 2008). No foliar injury was observed at the initial harvest. The chlorophyll content of the YFEL was measured using a SPAD meter (Minolta, Osaka, Japan) before harvesting in the vegetative stage (38 DAS) and maturity (56 DAS). The SPAD values were measured three times from each of the three leaflets of the YFEL and averaged. For each leaf, measurements were taken from the same part of the leaf. Plant height (cm) from the base of the stem to the apical meristem was recorded at each harvest. The YFEL (three leaflets) were harvested from each plant in the pot, fresh weight was recorded, and leaves were stored to analyse osmotic potential, ions and osmolytes (described below). Rhizobial nodulation was scored using the 0–8 scale developed by Yates et al. (2016) where 0 (absent) = no nodules, 0.5 (ineffective) = white ineffective nodules, 1 (rare effective) = 0–5 small pink nodules/plant, 2 (scarce) = 6–10 small pink nodules/plant, 3 (moderate) = 11–20 small pink and/or 1–2 large pink nodules/plant, 4 (adequate) = 21–40 small pink and/or 3–4 large pink nodules/plant, 5 (ample) = < 40 small pink and/or 5–9 large pink nodules/plant, 6 (abundant) = ≤ 10 large pink nodules/plant, 7 (very abundant) = crown nodulation < 5 mm diameter and 8 (extremely abundant) = crown nodulation ≥ 5 mm diameter. Shoot and root fresh weights were recorded immediately after harvest and oven-dried at 65 °C for 72 h prior to recording dry weight. Shoot relative growth rate (RGR) was calculated as follows: $RGR = \frac{\ln W2 - \ln W1}{t2 - t1}$ where W1 and W2 are shoot dry mass at time t1 (initial harvest) and t2 (either second or third harvest) (Hunt 1982).

Time to 50% flowering and time to 50% pod maturity were recorded for each pot. New flowers and new pods were counted daily to maturity. Dropped flowers and green pods were also collected on a pot basis. Harvested pods were combined with dropped green pods, counted and then grouped as filled and empty pods. Empty pods comprised dropped green pods, infertile pods, and pods with immature seeds. The number of mature seeds/pod and seeds/plant were counted. All mature seeds/plant were pooled, weighed, and 100-seed weight calculated using the weight of mature seeds/number of mature seeds. The remaining samples, including pod walls and immature seeds, were weighed and included in shoot mass measurements. Flower abortion percentage was calculated as [(total number of flowers produced – total number of pods (filled + empty)) / total number of flowers produced × 100]. The percentage of empty pods was calculated as [(total number of pods produced – total number of filled pods) / total number of pods produced × 100].

Tissue ion analysis

Three leaflets of the YFELs (one sample per plant per pot) were collected at the vegetative stage (38 DAS) and maturity (56 DAS), weighed, wrapped in aluminium foil, immediately frozen in liquid N_2, and stored at –80 °C until freeze-drying. The freeze-dried YFELs and oven-dried samples of different tissues (older green leaves, stems with petioles, seeds and pod walls) were pulverised separately (2010 Geno/Grinder®, SPEX SamplePrep, Metuchen, NJ, USA) and analysed for Na^+, K^+ and Cl^- following the method described in Munns et al. (2010). Briefly, ~ 100 mg tissue was weighed and extracted in 10 mL of 0.5 M HNO_3 by shaking for 72 h in the dark at room temperature. The extracts were diluted in Milli Q water as required and analysed for Na^+ and K^+ using a flame photometer (PFP7, Jenway, Dunmow, UK) and for Cl^- using a chloridometer (Model 50CL, SLAMED ING, GmbH, Frankfurt, Germany). The reliability of the analyses was confirmed by taking a reference tissue (broccoli, ASPAC no. 85) with known ion concentrations through the same procedures. Na^+, K^+ and Cl^- concentrations were calculated both at dry mass basis (μmol g^{-1} dry mass) and tissue water basis (mM).

Leaf osmotic potential and water content

Two or three leaflets of the YFELs (one sample per plant per pot) were collected at vegetative stage (38 DAS) and maturity (56 DAS), weighed, collected in an air-tight cryo-vials, frozen in liquid N_2, and stored at –20 °C. Leaf tissues were thawed in the vials and then crushed using a manual press to obtain a sample of tissue sap. The osmotic potential of 20 μL of sap was measured using a freezing point depression osmometer (Fiske Associates, Model One-Ten, MA, USA). Leaf water content was calculated as: fresh mass of YFEL—dry mass of YFEL and expressed as mL g^{-1} dry mass.

Determination of organic solutes

Three leaflets of the YFELs (one sample per plant per pot) were collected at the vegetative stage (38 DAS), weighed, frozen in liquid N_2, freeze-dried and stored at –20 °C until required. Organic solutes were determined for the 0 and 75 mM NaCl treatments as described by Fan et al. (1993) and Colmer et al. (2000). In brief, organic solutes were extracted twice from 0.1 g ground leaf tissue with 3 mL of 5% (w/v) ice-cold perchloric acid. The supernatants from each extraction were combined and neutralised to pH 3.0–3.5 using K_2CO_3. The neutralised samples were centrifuged at 15,000 rpm for 30 min at 4 °C, and the supernatants collected. The neutralised extracts were analysed using a high-performance liquid chromatography (HPLC) system detector (Waters, Milford MA, USA) with a 600 E pump, 717 plus auto-injector, and a 996 photodiode-array detector (PDA) with an evaporative light-scattering detector (ELSD 3300) (Alltech, Deerfield, IL, USA). Samples were analysed on two different columns (Prevail ES carbohydrate and Sugar-Pak) with three different gradients used with the Prevail column and one gradient for Sugar-Pak (Slimestad and Vågen 2006). Identification and purification of sample organic solutes were confirmed using the peak PDA spectral data (195 nm) and retention times of standards.

Statistical analysis

Data are presented as mean ± standard error (SE). Statistical analyses were performed using Genstat Software 20th Edition (VSN International Ltd. Hemel Hempstead, UK) and R software (R-4.0.3). Two-way ANOVA was used to assess the effects of genotypes, salinity treatments and genotype × treatment interaction. Means were compared for significant differences using LSD at the 5% probability level.

Results

Leaf damage

Plants exposed to salinity stress developed foliar injury symptoms that were initially characterised by leaf yellowing or chlorosis of leaflet margins and subsequently extending chlorosis to the entire leaflet and finally complete necrosis and senescence. Initial foliar injury symptoms were first observed on BARI Mung-6 at 26 DAS in the 125 mM NaCl treatment followed by VO1317 (30 DAS), Jade AU (31 DAS) and VO2211 (32 DAS) (data not shown). At the vegetative stage (38 DAS), there was a significant ($P < 0.05$) genotype × treatment interaction for foliar injury (Fig. 1a). No symptoms of foliar injury were observed in pots of the non-saline control treatment at vegetative stage. Combining the observations of the 25, 50 and 75 mM NaCl treatments, Jade AU displayed less foliar injury symptoms with an average score of 2, VO221 and VO1317 had a score of 3, and BARI Mung-6 had a score of 4. In the 125 mM NaCl treatment, the severity of foliar injury symptoms also varied across genotypes, with scores of 7 in BARI Mung-6 and VO1317, a score of 5 in VO2211 and a score of 4 for Jade AU. At maturity (56 DAS), all genotypes demonstrated similar foliar injury across treatments (i.e., end of the experiment) (Fig. 1b).

Vegetative growth

Salinity stress reduced shoot dry mass compared to non-saline control treatment in all genotypes at both vegetative stage and maturity, but genotypic differences were evident only during the final harvest at maturity (significant genotype × treatment interaction at $P < 0.05$ at maturity; Fig. 2a, b). At the vegetative stage, shoot (leaves with petioles and stems) dry mass did not differ among genotypes in the non-saline treatment with an average of 1.25 g plant^{-1} (Fig. 2a). Shoot dry mass decreased in all genotypes with the increase of salinity stress; however, the reduction in shoot dry mass (compared to non-saline control) was greatest in BARI Mung-6. The 75 mM NaCl treatment significantly reduced shoot dry mass (% of non-saline controls) to 53% in BARI Mung-6, 65% in Jade AU and VO1317 and 74% in VO2211.

At maturity, shoot (leaves, stems, pod walls and immature seeds) dry mass differed among the four genotypes grown in non-saline conditions, ranging from 1.45 g plant^{-1} in Jade AU to 2.17 g plant^{-1} in VO1317 (Fig. 2b). Similar to the vegetative stage, shoot dry mass decreased with the increase of salinity stress at maturity and the reduction was highest in BARI Mung-6. In the 75 and 125 mM NaCl treatments, shoot dry mass significantly decreased in all genotypes, less so in Jade AU (64% and 46% of non-saline controls, respectively) and VO2211 (65% and 32% of non-saline controls, respectively) than BARI Mung-6 (30% and 27% of non-saline controls, respectively) and VO1317 (52% and 32% of non-saline controls, respectively).

Salinity stress severely affected root dry mass in all genotypes, but there was no significant genotype × treatment interaction at $P < 0.05$ at either vegetative stage or maturity (Fig. 2c, d).

Salinity stress reduced the plant height of all genotypes at both vegetative stage and maturity (Table S1). However, significant genotype × salinity

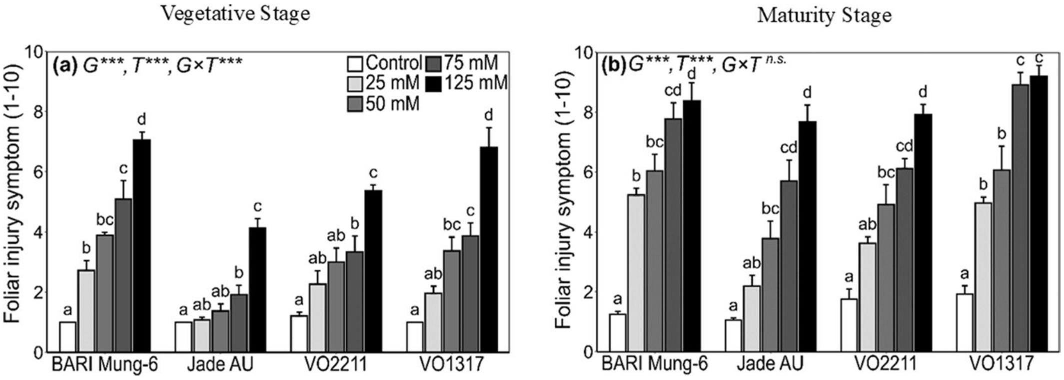

Fig. 1 Foliar injury symptoms of four mungbean genotypes at the (**a**) vegetative stage (38 DAS) and (**b**) maturity (56 DAS). Plants were grown in soil with 0 (non-saline control), 25, 50, 75 and 125 mM NaCl treatments imposed on 15 DAS. Data are means ± SE of four replicates. Significant differences between salinity treatment means within each genotype are indicated by different letters ($P \leq 0.05$). Two-way ANOVA was used to compare genotype (G), salinity treatment (T) and genotype × treatment (G × T) effects. Significant differences for each G, T and G × T effect are provided (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., non-significant)

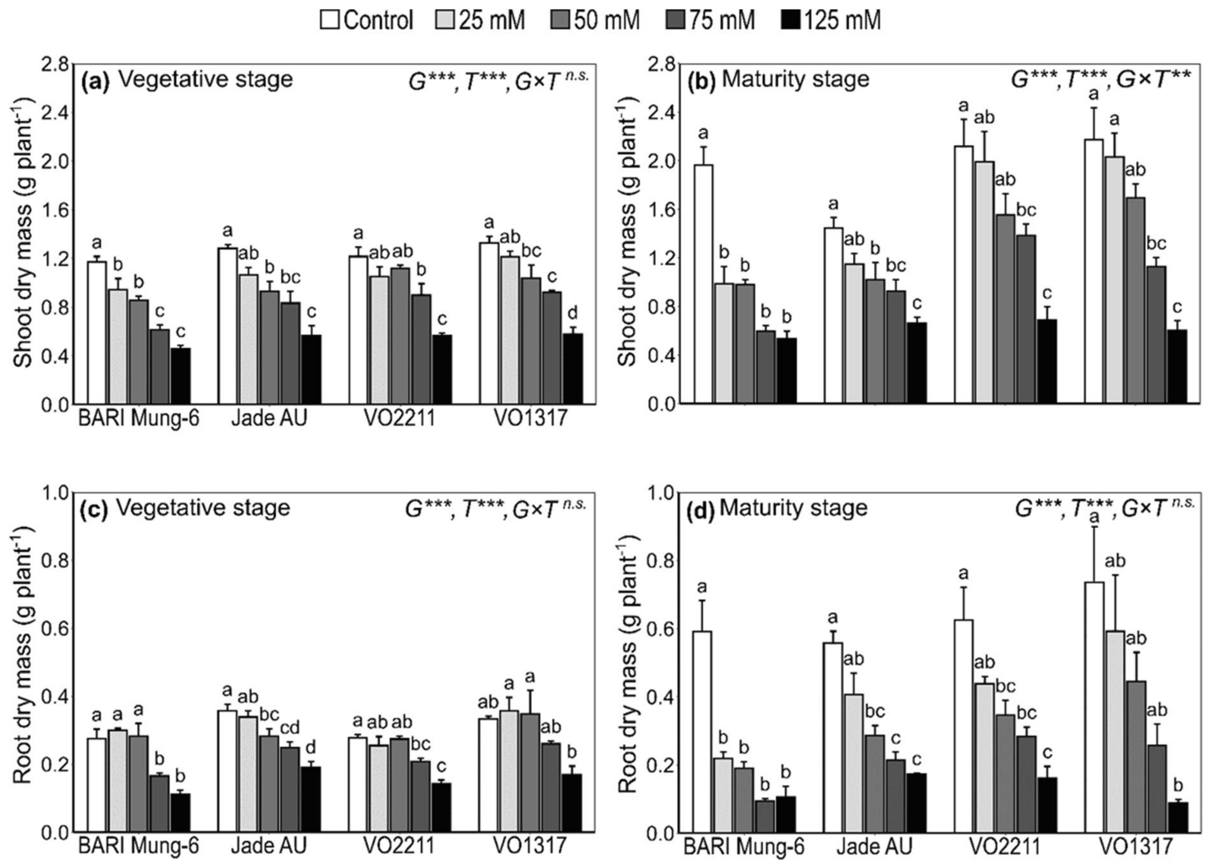

Fig. 2 Shoot dry mass (**a**, **b**) and root dry mass (**c**, **d**) of four mungbean genotypes at the vegetative stage (38 DAS; **a**, **c**) and maturity (56 DAS; **b**, **d**). Plants were grown in soil with 0 (non-saline control), 25, 50, 75 and 125 mM NaCl treatments imposed on 15 DAS. Data are means ± SE of four replicates. Significant differences between salinity treatment means within each genotype are indicated by different letters ($P \leq 0.05$). Two-way ANOVA was used to compare genotype (G), salinity treatment (T) and genotype × treatment (G × T) effects. Significant differences for each G, T and G × T effect are provided (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., non-significant)

treatment interaction ($P < 0.05$) was only found during the final harvest at maturity, when VO2211 and VO1317 were significantly taller than BARI Mung-6 and Jade AU. Plant heights of BARI Mung-6 and VO1317 were more severely affected by salinity stress than the other two genotypes. Salinity stress reduced shoot relative growth at similar rates for all genotypes at the vegetative stage and maturity, with no significant genotype × salinity treatment interaction at $P < 0.05$ (Table S1).

Nodulation

Nodulation was assessed as a score and the non-saline controls had an average nodulation score of 6 at vegetative stage and maturity (Table S1). Salinity stress reduced nodule formation in all genotypes at vegetative stage and maturity, but significant genotypic differences were only observed during the final harvest at maturity (significant genotype × treatment interaction at $P < 0.05$). At maturity, the 25 mM

NaCl treatment severely reduced nodulation in BARI Mung-6 (2.6) and Jade AU (3.4), whereas nodulation in VO1317 was affected by 50 mM (4.5) and in VO2211 by 125 mM NaCl (1.7). Overall, at higher salinity stress (75 and 125 mM NaCl), VO221 and Jade AU had higher nodulation than BARI Mung-6 and VO1317.

Reproductive attributes

Flower production and flower abortion

A non-significant genotype × salinity treatment interaction occurred for time to 50% flowering (Fig. S1a). In the non-saline controls, the first flowers opened at 32, 34, 35 and 36 DAS on BARI Mung-6, Jade AU, VO1317 and VO2211, respectively. The 75 and 125 mM NaCl treatments delayed time to 50% flowering in VO2211 to 39 and 43 DAS, respectively, but had no significant effect on time to flowering in the other genotypes. For flower number/plant, a significant ($P < 0.05$) genotype × salinity treatment interaction occurred (Fig. 3a). In non-saline conditions, BARI Mung-6 and VO1317 produced the highest number of flowers/plant (10), while Jade AU had the lowest (7). In BARI Mung-6, flower number/plant decreased to ~ 63% of non-saline control under 25 and 50 mM NaCl treatments, while in the other three genotypes, it was only decreased in 125 mM NaCl treatment. Salinity treatments did not differentially affect flower abortion among genotypes with no significant genotype × salinity treatment interactions for flower abortion percentage (Fig. S1b).

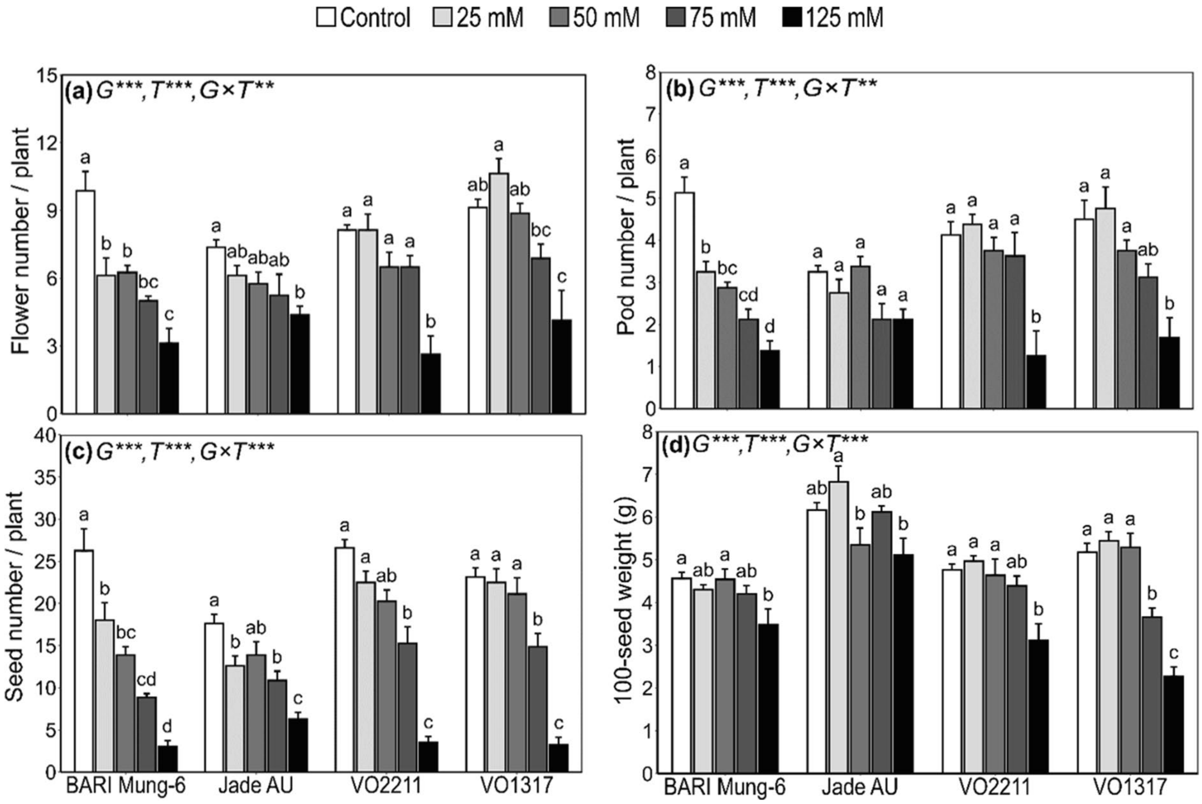

Fig. 3 Flower number/plant (**a**), pod number/plant (**b**), seed number/plant (**c**) and 100-seed weight (g) (**d**) of four mungbean genotypes. Plants were grown in soil with 0 (non-saline control), 25, 50, 75 and 125 mM NaCl treatments imposed on 15 DAS. Data are means ± SE of four replicates. Significant differences between salinity treatment means within each genotype are indicated by different letters ($P \leq 0.05$). Two-way ANOVA was used to compare genotype (G), salinity treatment (T) and genotype × treatment (G × T) effects. Significant differences for each G, T and G × T effect are provided (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., non-significant)

Pod production and pod abortion

A non-significant genotype × salinity treatment interaction was found for time to 50% pod maturity (Fig. S1c). In the non-saline controls, the genotypes had similar times to 50% pod maturity (49–52 DAS). While the salinity treatments did not significantly affect time to 50% pod maturity for BARI Mung-6 and Jade AU, the 125 mM NaCl treatment delayed time to 50% pod maturity by 3 and 4 days in VO1317 and VO2211, respectively, compared to non-saline controls. For pod number/plant, a significant ($P < 0.05$) genotype × salinity treatment interaction was found (Fig. 3b). Pod number/plant in non-saline conditions varied among genotypes from 5.1 in BARI Mung-6 to 3.2 in Jade AU. The pod-bearing ability of BARI Mung-6 decreased as salt stress increased more than in other genotypes being 63% of the non-saline control at 25 mM NaCl and 26% at 125 mM NaCl. While salinity treatment increased the percentage of empty pods in all genotypes, there were no genotypic differences for the trait (non-significant genotype × salinity treatment interaction at $P < 0.05$; Fig. S2d).

Seed yield

A significant genotype × salinity treatment interaction at $P < 0.05$ occurred for seed number/plant, 100-seed weight and seed yield (g plant^{-1}), but non-significant genotype × salinity treatment interaction was observed for seed number/pod (Figs. 3c, d and 4a, b). In the non-saline conditions, seed yield components (seed number/plant, seed number/pod and 100-seed weight) differed among the genotypes, however, all the genotypes had similar seed yield (~ 1.2 g plant^{-1}). Under control conditions, BARI Mung-6 and VO2211 produced the highest number of seeds/plant (26) while Jade AU produced the lowest number of seeds/plant (18). Jade AU had the largest 100-seed weight (6.2 g) and BARI Mung-6 had the smallest (4.5 g). Seed number/pod ranged from 6 to 8 in the non-saline controls for all genotypes. Seed number/plant and seed yield decreased as salt stress increased in all genotypes. The 75 mM NaCl treatment decreased seed yield and seed number/per plant, respectively, to 31% and 33% in BARI Mung-6, 46% and 64% in VO1317, 53% and 57% in VO2211, and 61% and 62% in Jade AU compared to non-saline controls. The 125 mM NaCl treatment decreased seed yield to about 10% of controls in the three genotypes and 29% of the non-saline control in Jade AU. Similarly, Jade AU grown under 125 mM NaCl produced more seeds/plant (36% of non-saline control) than the other three genotypes (11–14% of controls). The 25, 50 and 75 mM NaCl treatments did not affect seed size (100-seed weight) in most genotypes, except for Jade AU at 50 mM NaCl and VO1317 at 75 mM NaCl. The 125 mM NaCl decreased seed size to 83% in Jade AU, 76% in BARI Mung-6, 65% in VO2211 and 44% in VO1317 compared to non-saline controls. Only the 125 mM NaCl treatment decreased seed number/pod in all genotypes (average 4).

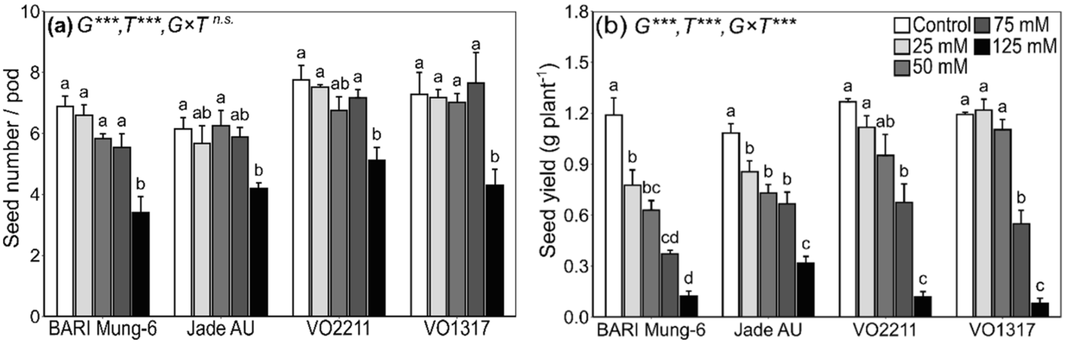

Fig. 4 Seed number/pod (**a**) and seed yield (g plant^{-1}) (**b**) of four mungbean genotypes. Plants were grown in soil with 0 (non-saline control), 25, 50, 75 and 125 mM NaCl treatments imposed on 15 DAS. Data are mean ± SE of four replicates. Significant differences between salinity treatment mean within each genotype are indicated by different letters ($P \leq 0.05$). Two-way ANOVA was used to compare genotype (G), salinity treatment (T) and genotype × treatment (G × T) effects. Significant differences for each G, T and G × T effect are provided (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., non-significant)

Tissue ion concentrations

Salinity stress increased Na^+, K^+ and Cl^- concentrations in all tissues (YFEL, petioles and stem, and older leaves) for all genotypes at vegetative and maturity stages, except for stem K^+ concentration which tended to decrease at both stages (Fig. 5, S2 and S3). Salinity stress also increased seed and pod wall Na^+, K^+ and Cl^- concentrations in all genotypes (Fig. S4).

Na^+ concentration in different tissues

For Na^+ concentrations in YFEL, petioles and stem, and older leaves at the vegetative stage, there was a significant ($P<0.05$) genotype × salinity treatment interaction (Figs. 5a, e, i). In the non-saline controls, the genotypes had similar YFEL Na^+ concentrations (44 µmol g^{-1} dry mass) (Fig. 5a). The 25, 50 and 75 mM NaCl treatments did not significantly affect YFEL Na^+ concentrations compared to non-saline controls in most genotypes

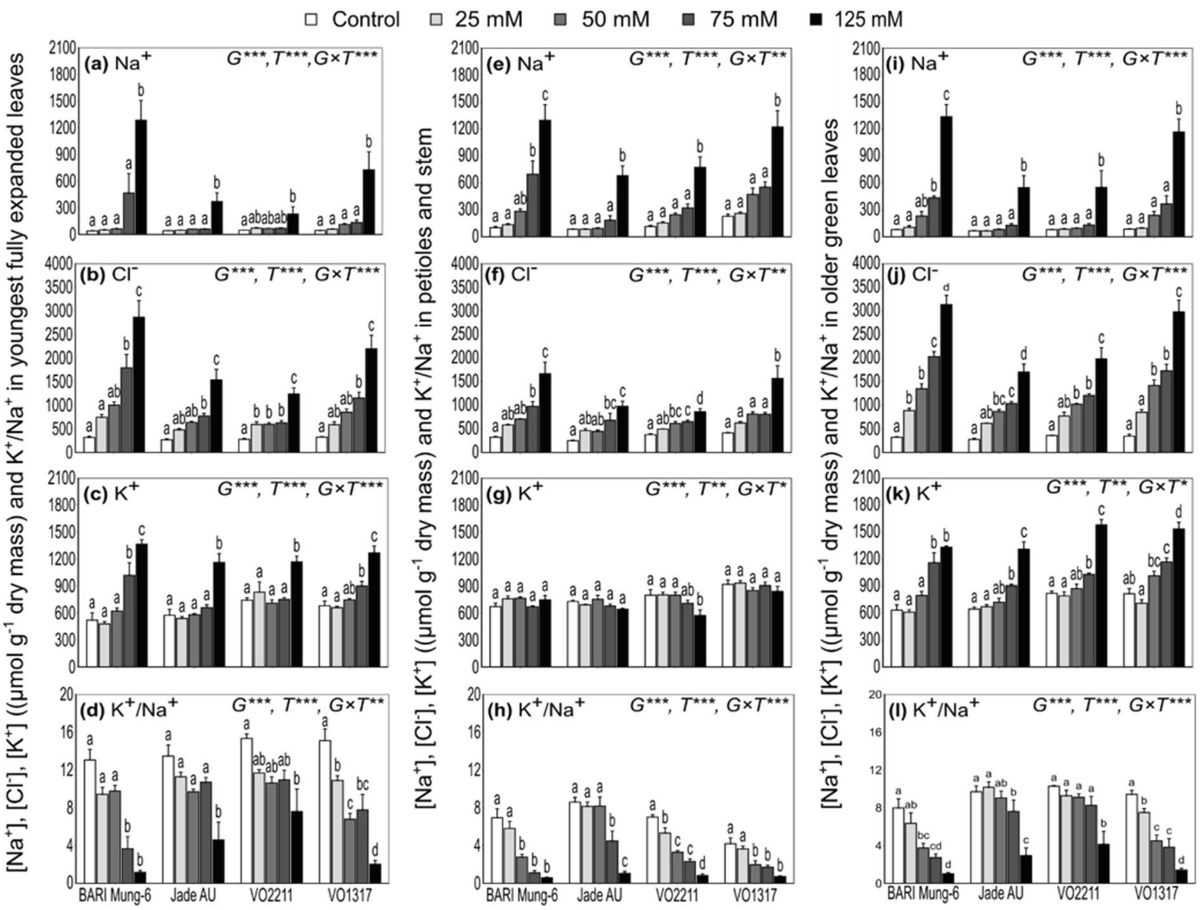

Fig. 5 Na^+, Cl^- and K^+ concentrations (µmol g^{-1} dry mass) and K^+/Na^+ in youngest fully expanded leaves (**a**–**d**), petioles and stem (**e**–**h**) and older green leaves (**i**–**l**) of four mungbean genotypes at the vegetative stage (38 DAS; **a**, **c**, **e**). Concentrations on tissue water basis (mM) for YFEL are presented in Fig. S2. Plants were grown in soil with 0 (non-saline control), 25, 50, 75 and 125 mM NaCl treatments imposed on 15 DAS. Data are means ± SE of four replicates. Significant differences between salinity treatment means within each genotype are indicated by different letters ($P \leq 0.05$). Two-way ANOVA was used to compare genotype (G), salinity treatment (T) and genotype × treatment (G×T) effects. Significant differences for each G, T and G×T effect are provided (* $P<0.05$; ** $P<0.01$; *** $P<0.001$; n.s., non-significant). Note different scales for Na^+ and K^+ , and Cl^- concentrations

except BARI Mung-6, where the 75 mM treatment increased YFEL Na^+ concentration by 12-fold. The 125 mM NaCl treatment dramatically increased YFEL Na^+ concentration by 32.6-fold (1284 μmol g^{-1} dry mass) in BARI Mung-6, 16-fold (653 μmol g^{-1} dry mass) in VO1317, 8.7-fold (370 μmol g^{-1} dry mass) in Jade AU and 4.7-fold (230 μmol g^{-1} dry mass) in VO2211 compared to non-saline controls. The Na^+ concentration on tissue wataer basis (mM) in YFEL at vegetative stage at 125 mM NaCl treatment was the lowest in VO2211 (46 mM), it was 71 mM in Jade AU, and it rose to 118 mM in VO1317 and 177 mM in BARI Mung-6 (Fig. S2a). Similarly, BARI Mung-6 and VO1317 had higher Na^+ concentrations in petioles and stem, and older leaves than Jade AU and VO2211 in the 75 and 125 mM NaCl treatments (Fig. 5e, i). Overall, Jade AU and VO2211 accumulated the highest Na^+ concentration in petioles and stem followed by older leaves and YFEL; there were significant genotype × tissue interaction ($P < 0.05$).

The Na^+ concentrations in YFELs, petioles and stem, older leaves, seeds and pod walls at maturity are presented in Fig. S3a, e, i and S4a, e. Overall, BARI Mung-6 and VO1317 accumulated higher Na^+ concentrations than VO2211 and Jade AU in different tissues in the 75 and 125 mM NaCl treatments.

Cl^- concentration in different tissues

A significant ($P < 0.05$) genotype × salinity treatment interaction was found for Cl^- concentrations in YFEL, petioles and stem, and older leaves at the vegetative stage (Fig. 5b, f, j). In the non-saline controls, YFEL Cl^- concentrations ranged from 286 μmol g^{-1} dry mass in Jade AU to 326 μmol g^{-1} dry mass in VO1317 (Fig. 5b). Overall, there was a gradual increase in Cl^- concentrations in YFEL with increasing salinity treatment concentration in all genotypes. Yet YFEL Cl^- concentrations increased to higher values in BARI Mung-6 (1795 and 2862 μmol g^{-1} dry mass) and VO1317 (1155 and 2194 μmol g^{-1} dry mass) than Jade AU (774 and 1539 μmol g^{-1} dry mass) and VO2211 (629 and 1240 μmol g^{-1} dry mass) at 75 and 125 mM NaCl treatments, respectively. The Cl^- concentration (mM) on tissue water basis in YFEL at vegetative stage at 125 mM NaCl treatment was 260 mM in VO2211, 303 mM in Jade AU, 363 mM in VO1317 and 395 mM in BARI Mung-6 (Fig. S2b). Similarly, BARI Mung-6 and VO1317 had higher Cl^- concentrations in petioles and stem, and older leaves than Jade AU and VO2211 in the 75 and 125 mM NaCl treatments (Fig. 5f, j). Overall, all genotypes accumulated higher Cl^- concentration in older leaves and YFELs than petioles and stem (significant genotype × tissue interaction; $P < 0.05$).

The Cl^- concentrations in YFELs, petioles and stem, older leaves, seeds and pod walls at maturity are presented in Figs. S3b, f, j and S4b, f. Overall, BARI Mung-6 and VO1317 accumulated higher Cl^- concentrations than VO2211 and Jade AU in different tissues in the 75 and 125 mM NaCl treatments.

K^+ concentrations in different tissues

A significant ($P < 0.05$) genotype × salinity treatment interaction was found for K^+ concentrations in YFEL, petioles and stem, and older leaves at the vegetative stage (Fig. 5c, g, k). In the non-saline controls, YFEL K^+ concentrations ranged from 521 to 743 μmol g^{-1} dry mass (Fig. 5c). The 25 and 50 mM NaCl treatments did not affect K^+ concentration in YFEL of all genotypes, but 75 mM NaCl treatment increased YFEL K^+ concentration by 2-fold in BARI Mung-6 and 1.3-fold in VO1317, whereas it remained unaffected in Jade AU and VO2211 compared to non-saline controls. The 125 mM NaCl treatment increased YFEL K^+ concentrations to on average 1241 μmol g^{-1} dry mass in all genotypes. The K^+ concentration on tissue water basis (mM) at the vegetative stage showed that salinity stress did not affect YFEL K^+ concentration in most genotypes and it varied from 126 to 248 mM (no significant genotype × treatment interaction; Fig. S2c). A similar pattern of increase in K^+ concentrations was found at the 75 and 125 mM NaCl treatments in the older leaves of all genotypes (Fig. 5k). In contrast, the salinity treatments did not affect K^+ concentrations in petioles and stem, except for VO2211 under 125 mM NaCl, which decreased to 70% (574 μmol g^{-1} dry mass) of the non-saline control (Fig. 5g). Overall, all genotypes had higher K^+ concentration in YFELs and older leaves than petioles and stem (significant genotype × tissue interaction; $P < 0.05$).

The K^+ concentrations in YFELs, petioles and stem, older leaves, seeds and pod walls at maturity are presented in Figs. S3c, g, k and S4c, g. Similar to the vegetative stage, salinity stress affected K^+ concentrations more in BARI Mung-6 and VO1317 than VO2211 and Jade AU.

K^+/Na^+ ratio in different tissues

For K^+/Na^+ ratio, there was a significant ($P < 0.05$) genotype × salinity treatment interaction in YFEL, petioles and stem, and older leaves at the vegetative stage (Fig. 5d, h, l). In the non-saline controls, all genotypes maintained a similar K^+/Na^+ ratio in YFEL (average 14.2; Fig. 5d). YFEL K^+/Na^+ ratio decreased with the increase of salinity stress in BARI Mung-6 and VO1317 while only 125 mM NaCl treatment decreased YFEL K^+/Na^+ ratio in Jade AU and VO2211. The 125 NaCl treatment decreased the K^+/Na^+ ratio in YFEL to 1.2 in BARI Mung-6, 2.0 in VO1317, 4.6 in Jade AU and 7.6 in VO2211. Similarly, the 75 and 125 mM NaCl treatments decreased the K^+/Na^+ ratio of petioles and stem, and older leaves more in BARI Mung-6 and VO1317 than Jade AU and VO2211 (Fig. 5h, l). Overall, all genotypes maintained a higher K^+/Na^+ ratio in YFELs than petioles and stem, and older leaves (significant genotype × tissue interaction; $P < 0.05$).

The K^+/Na^+ ratio in YFELs, petioles and stem, older leaves, seeds and pod walls at maturity are presented in Figs. S3d, h, l and S4d, h. Overall, BARI Mung-6 and VO1317 had lower K^+/Na^+ ratios than VO2211 and Jade AU in different tissues in the 75 and 125 mM NaCl treatments.

Leaf chlorophyll contents (SPAD value)

Salinity stress decreased chlorophyll contents (SPAD value) in YFEL compared to the non-saline control and a significant ($P < 0.05$) genotype × salinity treatment interaction was found at both vegetative stage and maturity (Fig. 6a, b). In the non-saline controls, chlorophyll content did not differ among genotypes, with average SPAD values of 49.8 at the vegetative stage and 40.5 at maturity. The magnitude of decline of SPAD values was greatest in BARI Mung-6, with decreasing to 68 and 53% of controls in the 75 and 125 mM NaCl treatments, respectively, at the vegetative stage. In Jade AU, SPAD values decreased to 88% in the 75 mM treatment and 72% in the 125 mM NaCl treatment compared to non-saline controls. In VO2211 and VO1317, 75 mM NaCl did not significantly affect SPAD values compared to non-saline controls, but 125 mM NaCl reduced SPAD values to 87% in VO2211 and 67% in VO1317 (Fig. 6a). However, at maturity, salinity stress (75 mM NaCl treatment) reduced SPAD values in BARI Mung-6 and Jade AU whereas it was unaffected in VO2211 (Fig. 6b). VO1317 had no green leaves at 75 and 125 mM NaCl treatments for SPAD measurements, as BARI Mung-6 at 125 NaCl treatment.

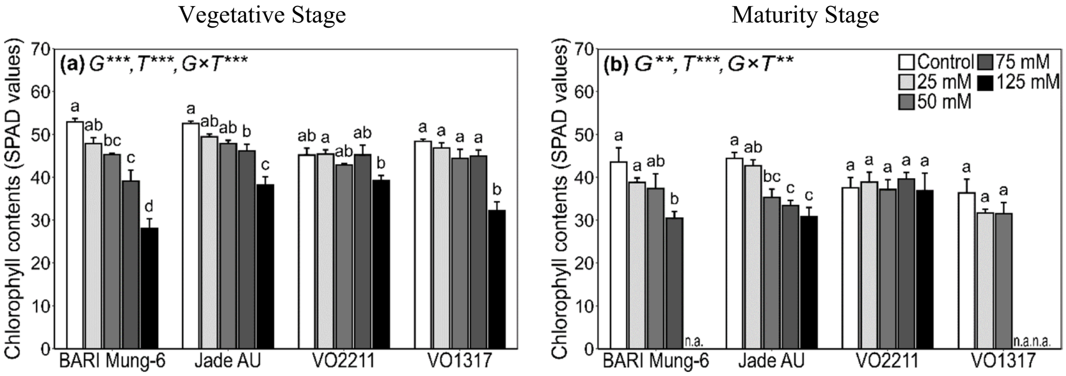

Fig. 6 Chlorophyll contents (SPAD values) in youngest fully expanded leaves of four mungbean genotypes at the (**a**) vegetative stage (38 DAS) and (**b**) maturity (56 DAS). Plants were grown in soil with 0 (non-saline control), 25, 50, 75 and 125 mM NaCl treatments imposed on 15 DAS. Data are means ± SE of four replicates. Significant differences between salinity treatment means within each genotype are indicated by different letters ($P \leq 0.05$). Two-way ANOVA was used to compare genotype (G), salinity treatment (T) and genotype × treatment (G × T) effects. Significant differences for each G, T and G × T effect are provided (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., non-significant). For BARI Mung-6 at 125 mM NaCl and VO1317 at 75 and 125 mM NaCl, there were no enough green leaf lamina for SPAD measurmemts, as indicated by n.a. (data not available)

Leaf osmotic potential, organic solutes and osmotic adjustments

Leaf osmotic potential (Ψ_s) decreased with increasing salinity stress in all genotypes; however, significant genotype × salinity treatment interaction at $P < 0.05$ occurred only at the vegetative stage (Table S2). At the vegetative stage, leaf osmotic potential was the highest in BARI Mung-6 (–0.99 MPa) and lowest in VO1317 (–1.32 MPa) in non-saline controls. The 75 mM NaCl treatments decreased Ψ_s by 1.6-fold in BARI Mung-6 (–1.58 MPa), 1.3-fold in Jade AU (–1.39 MPa), 1.2-fold in VO2211 (–1.38 MPa) and VO1317 (–1.54 MPa) compared to non-saline controls (Table 1).

Salinity stress increased leaf water content in all genotypes and a significant genotype × salinity treatment interaction at $P < 0.05$ occurred at both vegetative stage and maturity (Table S2). At the vegetative stage, leaf water content was, on average, 3.14 mL g^{-1} dry mass in all genotypes in non-saline controls. The 75 mM NaCl treatment increased leaf water content by 2-fold in BARI Mung-6 and 1.4-fold in VO1317 but did not affect leaf water content in Jade AU and VO2211 compared to non-saline controls (Table S2).

There was a significant genotype × salinity treatment interaction at $P < 0.05$ for sucrose and glucose content (Table S3). In the non-saline-controls, VO1317 accumulated more sucrose (19.4 mg g^{-1} dry mass) and glucose (6.3 mg g^{-1} dry mass) compared to the other three genotypes (average 6.7 mg g^{-1} dry mass sucrose and 2.6 mg g^{-1} dry mass glucose). The 75 mM NaCl treatment increased sucrose accumulation in YFEL by 2.9-fold in VO2211, 2.7-fold in Jade AU and 1.7-fold in BARI Mung-6, but did not affect sucrose accumulation in VO1317 compared to non-saline controls. Conversely, BARI Mung-6, Jade AU and VO2211 produced the same amount of glucose in the 75 mM NaCl treatment compared to non-saline soil, whereas VO1317 decreased glucose concentration to 57% of the control). No significant genotype × salinity treatment interaction at $P < 0.05$ occurred for fructose and proline in YFEL (Table S3). The contribution of organic solutes (sucrose, glucose, fructose and proline) to osmotic potential in BARI Mung-6 and VO1317 was higher for non-saline controls (average 4.9%) than the 75 mM NaCl treatment

Table 1 Estimated contribution of organic solutes (combined glucose, fructose, sucrose and proline) and inorganic ions to leaf osmotic potential (Ψ_s) of four mungbean genotypes at the vegetative stage (38 DAS) grown in soil with 0 (non-saline control) and 75 mM NaCl treatments imposed on 15 DAS

Genotype	Treatment	Leaf Ψ_s (MPa)	Estimated contribution of ions and organic solutes to leaf osmotic potential, Ψ_s (%)					
			% Na^+	% K^+	% Cl^-	% Inorganic ions	% Organic solutes	% Total
BARI Mung-6	Control	–0.99 ± 0.05	3.1 ± 0.2	41.5 ± 5.8	25.5 ± 1.4	70.1 ± 6.1	2.8 ± 0.8	72.9 ± 6.0
	75 mM	–1.58 ± 0.12	9.7 ± 2.3	25.2 ± 1.4	44.1 ± 2.9	79.0 ± 3.9	1.4 ± 0.5	80.5 ± 4.0
Jade AU	Control	–1.07 ± 0.01	2.9 ± 0.2	43.8 ± 4.1	20.4 ± 0.78	67.1 ± 4.4	3.8 ± 0.3	70.9 ± 4.1
	75 mM	–1.39 ± 0.05	3.3 ± 0.2	30.7 ± 1.7	35.9 ± 1.6	69.9 ± 3.3	4.1 ± 0.4	73.9 ± 3.6
V02211	Control	–1.17 ± 0.04	3.2 ± 0.3	48.8 ± 3.9	18.2 ± 0.9	70.2 ± 4.5	3.0 ± 0.7	73.2 ± 4.6
	75 mM	–1.38 ± 0.03	3.4 ± 0.2	36.8 ± 1.1	31.1 ± 3.0	71.3 ± 4.0	4.3 ± 1.0	75.5 ± 4.2
V01317	Control	–1.32 ± 0.12	2.9 ± 0.4	42.2 ± 2.9	20.5 ± 2.2	65.6 ± 5.0	6.9 ± 1.9	72.5 ± 6.3
	75 mM	–1.54 ± 0.06	4.9 ± 1.1	32.9 ± 2.5	41.6 ± 3.0	79.5 ± 3.3	2.7 ± 0.2	82.1 ± 3.4
LSD (0.05)	G	0.15*	2.4*	6.4*	4.2***	n.s	1.8*	n.s
	T	0.11***	1.7*	4.6***	3.0***	6.1*	n.s	n.s
	G×T	0.22*	3.5*	n.s	n.s	n.s	2.6*	n.s

Van't Hoff equation Ψ_s (milli osmol L^{-1}) = $-$csRT, where cs = osmolality (mmol L^{-1}), R = 0.0083143 L MPa mol^{-1} K^{-1}, and T = 301 K were considered. Contribution = (Ψ_s calculated/ Ψ_s measured) × 100

The contribution of individual ions to measured Ψ_s was determined using the van't Hoff equation, where the calculated Ψ_s was based on ion concentration on a fresh weight basis. The percentage value is based on the measured value of leaf Ψ_s (MPa). Data are means ± SE of four replicates. Two-way ANOVA was used to compare genotype (G), salinity treatment (T) and genotype × treatment (G×T) effects. Significant differences for each G, T and G×T effect are provided (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., non-significant)

(2%), whereas for Jade AU and VO2211, the contribution of organic solutes was higher in the 75 mM NaCl treatment (4.2%) than in the non-saline control (3.4%) (Table 1). The contribution from inorganic ions (Na^+, K^+ and $Cl^–$) ranged from 65.6% in VO1317 to 70.2% in VO2211 in the non-saline control and from 69.9% in Jade AU to 79.5% in VO1317 in the 75 mM NaCl treatment (Table 1). In the non-saline controls, K^+ contributed the most to osmotic potential (41.5–48.8%), whereas $Cl^–$ contributed the most under saline conditions (31.1–44.1%), followed by K^+ (25.2–36.8%) and Na^+ (3.3–6.3%) (Table 1).

Relationships between leaf ion concentrations and morpho-physiological parameters

Correlation analysis was undertaken to assess possible relationships between seed yield, foliar injury symptoms, SPAD and salt ion concentration in YFELs at the vegetative stage (Table 2). A strong negative correlation was found for seed yield with concentrations of Na^+ and $Cl^–$ in YFELs. Seed yield was not significantly correlated with YFELs K^+ concentration but had a strong positive correlation with K^+/Na^+ ratio in YFELs. Foliar leaf injury and SPAD were strongly correlated with Na^+ and $Cl^–$ concentrations and K^+/Na^+ ratio in YFELs.

Discussion

Mungbean—an important legume crop—is affected by soil salinity in various parts of the world. The stress adversely affects its growth and yield, and genotypes respond differently to salinity stress, although the mechanisms involved are not yet fully understood. This study assessed the variation in responses to different NaCl treatments among four diverse mungbean genotypes at the vegetative stage and maturity and investigated morphological and physiological traits associated with salinity tolerance. Mungbean responses to salinity depended on the salinity level, and significant genotypic variation in salinity tolerance exists in mungbean. The ranking of Jade AU as tolerant, VO2211 as moderately tolerant, VO1317 as moderately sensitive and BARI Mung-6 as sensitive was evident as higher shoot dry mass and seed yield when grown in different NaCl treatments. Higher seed yield in salt tolerant than salt sensitive genotypes was associated with larger seed size and better maintenance of flower production, and numbers of pods and seeds per plant in saline soil. Tolerant genotypes better controlled Na^+ and $Cl^–$ accumulation and maintained higher K^+/Na^+ ratio in leaves compared with the sensitive genotypes. These key findings are discussed below.

Leaf Na^+ and $Cl^–$ exclusion and high K^+/Na^+ ratio contribute to salinity tolerance in mungbean

Tolerant genotypes had lower Na^+ and $Cl^–$ concentrations and higher K^+/Na^+ ratios in YFELs than the sensitive genotypes. Tolerant genotypes had higher Na^+ concentration in petioles and stem, and older leaves compared to YFELs. So, a possible mechanism of controlling Na^+ accumulation in YFELs in tolerant genotypes may be preferential partitioning of Na^+ into petioles and stem. Similarly, Le et al. (2021) found higher Na^+ content in roots and stems than in the young leaves in the mungbean genotype Jade AU. $Cl^–$ concentrations in YFELs of all genotypes increased to 260–395 mM; however, tolerant

Table 2 Correlation coefficients (r) between pairs of various mopho-physiological traits at vegetative stage and seed yield

Traits	Seed yield (g $plant^{-1}$)	Foliar injury symptom	SPAD	YFEL Na^+ (mM)	YFEL $Cl^–$ (mM)	YFEL K^+ (mM)
Foliar injury symptom	−0.86***					
SPAD	0.79***	−0.92***				
YFEL Na^+ (mM)	−0.73***	0.84***	−0.92***			
YFEL $Cl^–$ (mM)	−0.89***	0.94***	−0.91***	0.86***		
YFEL K^+ (mM)	−0.05	0.17	−0.32	0.18	0.09	
YFEL K^+/Na^+ (mM)	0.84***	−0.91***	0.86***	−0.84***	−0.97***	0.01

* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$

genotypes had significantly lower Cl^- contents than the sensitive genotypes. Unlike Na^+, all genotypes had higher Cl^- contents in YFELs and older leaves than in petioles and stem; similarly, Le et al. (2021) found higher Cl^- contents in YFELs than in roots and stems. In a recent study, Iqbal et al. (2024) found salt exclusion from leaves was due to better control of salt ions (Na^+ and Cl^-) transport in roots in salt tolerant mungbean genotype Jade AU, as compared to salt sensitive BARI Mung-6. At tissue concentrations nearing 100 mM, both Na^+ and Cl^- can inhibit the activity of most enzymes (Greenway and Osmond 1972; Flowers and Dalmond 1992; Munns and Tester 2008). High levels of Na^+ and Cl^- in leaves inhibits metabolism, damages chloroplasts, alters leaf anatomy and reduces photosynthesis (Khan et al. 2015; Bose et al. 2017; Kotula et al. 2019a; Iqbal et al. 2024). In the present study, sensitive genotypes showed significantly greater foliar injury as a result of leaf chlorophyll degradation (indicated by low SPAD value) compared to tolerant genotypes and the injury had a strong correlation with Na^+ and Cl^- concentration in YFELs. These results indicate that higher concentrations of Na^+ and Cl^- in leaves lead to reduction in photosynthesis, the total area available for photosynthesis, dry matter production and ultimately affect seed yield in sensitive genotypes. Similarly, salinity tolerance was associated with Na^+ and/ or Cl^- toxicity in mungbean (Salim and Pitman 1988; Le et al. 2021; Iqbal et al. 2024), chickpea (Dua 1998; Khan et al. 2015), soybean (Luo et al. 2005; Le et al. 2023) and faba bean (Tavakkoli et al. 2010). In the present study, YFEL Cl^- concentrations in all saline treatments were substantially higher than the threshold toxicity level of 332 μmol g^{-1} dry mass or 100 mM, even in tolerant genotypes (Reuter and Robinson 1997; Munns and Tester 2008). Plants can tolerate high Na^+ and Cl^- in leaves at the cellular level by preferential accumulation in epidermal cells, partitioning in vacuoles and restricting accumulation in the cytosol or chloroplasts of mesophyll cells and sustain photosynthesis (James et al. 2006; Munns et al. 2016; Oi et al. 2022). Iqbal et al. (2024) found tolerant mungbean genotype Jade AU better regulated Na^+ and Cl^- accumulation in chloroplast and maintained higher photosynthesis than sensitive BARI Mung-6. Therefore, in addition to controlling Na^+ and Cl^- accumulation in YFELs, tissue tolerance to high Cl^- may contribute to the salinity tolerance of tolerant genotypes.

Salinity stress increased K^+ in YFELs of all genotypes in the 125 mM treatment and did not alter YFEL K^+ concentrations in any genotypes in the other NaCl treatments except for BARI Mung-6 under 75 mM NaCl. These results contradict the generalised hypothesis that salinity stress reduces K^+ in plants (Greenway and Munns 1980; Colmer et al. 1995; Shabala and Pottosin 2014). Similar increased K^+ ions in leaves after salinity stress have been reported in mungbean (Salim and Pitman 1988), where the authors suggested that increased K^+ ions could benefit osmotic adjustments. Increased K^+ in leaves after salinity stress has also been reported in chickpea (Kotula et al. 2015) and durum wheat (Dvořak et al. 1994). In our study, we found that salinity stress decreased osmotic potential and increased leaf water content, similarly, salinity stress increased leaf water content in mungbean (Le et al. 2021; Iqbal et al. 2024), cowpea, soybean and common bean (Le et al. 2021) and halophytes like *Suaeda fruticosa* (L.) (Khan et al. 2000). Increased of leaf water status indicated that mungbean adjusted their water potential and osmotic potential to more negative levels as salinity increased. Salinity stress increased leaf succulence (measured as water per unit leaf area) in dicotyledonous halophytes and also in many dicot non-halophytes (reviewed in Munns et al. 2016) including in mungbean (Iqbal et al. 2024) which leads to larger cells with a resultant dilution of salt. Plants accumulate inorganic and organic solutes to adjust to the high negative osmotic potential of NaCl in the root zone (Munns et al. 2016). In our study, salinity stress significantly increased the accumulation of sucrose and proline in tolerant genotypes and the concentration of sucrose was significantly higer in tolerant as compared to sensitive genotypes under saline conditions. However, organic solutes contributed only 1–4% to osmotic adjustment in the four mungbean genotypes in the 75 mM NaCl treatment. During salinity stress, inorganic ions, Na^+, K^+ and Cl^-, accumulation played a major role (70–80%) in leaf osmotic adjustment. In comparison to Na^+ uptake in leaves, Cl^- uptake was considerably higher in all genotypes. Consequently, K^+ would need to increase in YFEL to maintain the charge balance, as Na^+ was only about 14% of Cl^- in leaf tissues (Oi et al. 2022). Although K^+ increased in YFELs of all genotypes, the tolerant genotypes maintained higher leaf K^+/Na^+ ratio than the sensitive genotypes which may be important for higher salinity tolerance (Shabala and Cuin 2008). These findings suggest that

Na^+ and Cl^- exclusion from leaves, Cl^- tissue tolerance and maintaining high K^+/Na^+ are possible explanations for the variation in salinity tolerance among the four mungbean genotypes.

Growth and yield components

Salinity stress reduced the growth of all genotypes, however significant genotypic differences in growth were only found at maturity (no significant genotype × treatment interaction for shoot dry mass at the vegetative stage). This delay in genotypic differences aligns with a two-phase plant response to salinity model (Munns 1993; Munns and Tester 2008). Variations in dry matter production can be attributed to variations in the net carbon assimilation rate, which can be caused by differences in photosynthesis per unit leaf area or total photosynthesising area (James et al. 2002; Iqbal et al. 2024). These variations became apparent with increasing leaf injury due to toxic concentrations of Na^+ and Cl^- in leaves more in sensitive genotypes than tolerant genotypes. Salinity stress reduced number of nodules in all genotypes, however, tolerant genotypes had higher nodulation score than sensitive genotypes. Salinity stress-induced reduction in nodulation affects the plant's nitrogen acquisition and overall growth (Abd-Alla et al. 2019). In the present study, we observed that mild salinity stress (25 mM NaCl) reduced nodulation in tolerant genotype Jade AU, however, it did not affect plant growth. Similarly, Chakraborty et al. (2021) found that moderate salt stress severely affected nodulation but had a modest effect on root and shoot growth in *Medicago truncatula* L. These authors found that moderate salt stress increased the responsiveness of early symbiotic genes induced by rhizobium which increased ABA levels and alleviated the salt stress effects. Higher seed yields in tolerant genotypes under salinity stress were associated with higher flower, pod and seed numbers per plant, greater biomass and nodulation than in sensitive genotypes of mungbean (current study, Ahmad et al. 2005; Sehrawat et al. 2014; Manasa et al. 2017). The percentage of flower abortion was unaffected in all genotypes under all salinity treatments, and salinity stress increased the percentage of empty pods similarly in all genotypes; thus, reduced flower and pod numbers in sensitive genotypes were not associated with the percentage of flower abortion and empty pod numbers, with similar results reported in chickpea (Vadez et al. 2012). Tolerant genotype Jade AU had larger seeds (100-seed weight) than the other three genotypes under both non-saline and saline soils, indicating that large seeds contributed to high seed yield in Jade AU under salinity stress. We observed higher Na^+ and Cl^- in seed and pod walls in sensitive genotypes compared to tolerant genotypes. This suggests that reduced seed yield in salt-stressed mungbean might be due to increased Na^+ or Cl^- accumulation in reproductive structures. Elevated levels of Na^+ and Cl^- in flowers, seeds and pod walls affected reproductive processes like pollen development and fertilisation in chickpea (Samineni et al. 2011; Turner et al. 2013). However, Kotula et al. (2015) did not find any significant differences in Na^+ and Cl^- in reproductive structure between salt sensitive and tolerant chickpea genotypes. In addition, the reduced flower, pod and seed numbers and seed size in sensitive genotypes under salinity treatments could be due to limited carbon supply caused by reduced photosynthesis (Khan et al. 2017; Iqbal et al. 2024) or hormonal changes (Albacete et al. 2014). Tolerant genotypes had less chlorosis and necrosis leaf symptoms and higher chlorophyll content compared to sensitive genotypes which may result in higher photosynthesis (James et al. 2002; Iqbal et al. 2024). Thus, continued assimilate supply could produce higher number of reproductive sites, and consequently greater flower and seed production in tolerant genotypes. Moreover, mungbean is an indeterminate species, which means that young vegetative tissue may directly compete with reproductive development for carbon supply throughout the reproductive period. This competition may be greater in sensitive genotypes than in tolerant genotypes. The above results collectively support earlier findings that the mungbean reproductive stage particularly the flower initiation period is the most sensitive to salinity stress (Sehrawat et al. 2014; Le et al. 2021).

Prospects for screening mungbean germplasms for salinity tolerance

Mungbean genotypes varied in their level of tolerance at different NaCl concentration treatments. Similar observations at various salinity doses have been reported for mungbean (Hafeez et al. 1988), faba bean (Tavakkoli et al. 2012) and chickpea (Kotula et al. 2015). In the present study, the most significant variation among genotypes were observed at the 75 mM NaCl treatment suggesting that 75 mM NaCl can be used as effective concentration to screen mungbean germplasm for variation in salinity tolerance. Salinity tolerance also depends on the duration of exposure to salinity stress and it varies genotypes to genotypes (Negrão et al. 2017). In the present study, we observed that among the sensitive genotypes, VO1317 continued to grow similarly to the non-saline control until 30 DAS, at which point it abruptly displayed signs of foliar damage and thereafter drastically decreased growth, whereas BARI Mung-6 gradually decreased its growth following salt treatment (data not shown, obersarvations during the experiment). Significant genotypic differences in growth were only found at maturity. These results suggest that screening at early growth stage exposed to salinity stress for short period of time may not accurately predict salinity tolerance at whole plant growth stages without considering other factors like salinity's impact on morpho-physiological traits, growth, and yield. The significant relationship between Na^+ and Cl^- exclusion, K^+/Na^+ ratio and seed yield suggests that the trait controlling low leaf Na^+ and Cl^- accumulation might be useful for improving salinity tolerance in mungbean. The high correlation of leaf Na^+ and Cl^- accumulation with foliar injury symptoms and SPAD values demonstrates the feasibility of employing leaf injury symptoms and SPAD values as a quick trait to screen mungbean genotypes for salinity tolerance at the vegetative stage (James et al. 2002). Systematic screening of a wide range of mungbean germplasm collections focusing on the key salinity tolerance traits like osmotic stress tolerance, salt ion exclusion or tissue tolerance would be useful for understanding salinity tolerance mechanisms and breeding salt tolerant mungbean varieties.

Conclusion

This study revealed the physiological mechanisms of salinity tolerance among different mungbean genotypes. It was found that the importance of associated traits varied with the severity of salinity stress and growth stages. Higher seed yield in salt tolerant than salt sensitive mungbean genotypes in saline soil is associated with high reproductive success including the maintenance of a high number of flowers, pods and seed numbers/ plant and larger seed size. Ion accumulation in plant tissues may play a crucial role in mungbean salinity tolerance. Tolerant genotypes excluded Na^+ and Cl^- in leaves compared to sensitive genotypes; however, Cl^- concentrations exceed the critical toxicity levels. The study proposed Na^+ and Cl^- 'ion exclusion' and Cl^- 'tissue tolerance' capacity to explain differences in salinity tolerance among genotypes. Tolerant genotypes also maintained higher K^+/Na^+ ratio than sensitive genotypes. The study has highlighted that the selection of genotypes with high flowers, pods and seed numbers that accumulate low leaf Na^+ and Cl^- and maintain high K^+/Na^+ would be useful for breeding high-yielding salt tolerant mungbean varieties.

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Authors' contributions MSI designed the study in consultation with LK, AIM and WE. MSI conducted the experiments, analysed the data, and wrote the first draft of the manuscript. All authors contributed to the manuscript revision and editing and approved the submitted version of the manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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