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SALICYLIC ACID SEED PRIMING IMPROVED DRY BIOMASS AND IONIC EFFICIENCY OF MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] UNDER SALT STRESS CONDITIONS

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Abstract

Salinity is a major constraint for mungbean seedlings establishment. Salicylic acid (SA) is an endogenously synthesized signaling compound contributes to regulate physiological processes in plants as well as biotic and abiotic stress responses. Effect of SA seed priming of mungbean in developing resistance under salt stress was investigated. The seeds of mungbean varieties (NM-2016 and NM-20-21) were soaked in 0.01% and 0.02% of SA while, no priming and hydropriming was taken as control. Seeds were sown in washed river sand with two salinity (0, 80 mM NaCl) levels. The results showed that priming with 0.01% SA improved plant height and dry biomass as compared with hydropriming and non-priming plants under salt stress. Meanwhile, 80 mM salt stress notably decreased the concentrations of Ca^{2+}, N, P, and K^+ while Na^+ and Cl^- contents increased in leaves as compared to control plants. But 0.01% SA improved the concentrations of almost all nutrients except Mg^{2+} under salt stress as compared to hydropriming and control. Collectively, NM-20-21 variety performed better than that of NM-2016 and seed priming with 0.01 % SA was found more effective in improving growth and nutrient uptake of mungbean seedlings.

Key words: Word; Salinity; Salicylic acid; Seed priming; Mungbean genotypes.

Introduction

Salinity is considered as one of the major agricultural constraints mainly reducing crop productivity (Sehrawat *et al*., 2019; Shahrasbi *et al*., 2021). Research studies showed that 50% of cropland and 20% of the world cultivated land has been badly affected by salt stress (Naveed *et al*., 2020). In Pakistan 6.63 million ha of lands are affected by salinity (Akram *et al*., 2010). Higher salt concentration in soil limits root proliferation, seed germination, uptake of nutrients and water to plants which ultimately retards the plant growth and photosynthetic activity, reducing cell expansion, leaf area ultimately cause cell death (Vasantha *et al*., 2017). Salinity induces the formation of reactive oxygen species and minimizes the metabolic functions of plants (Das & Roychoudhury, 2014). Saline stress also damages the cell membrane, induces DNA mutation, and disrupts biochemical functions (Ahmed *et al*., 2019; Kamran *et al*., 2020). To survive with the stress of saline environment, plants have developed different physiological strategies like accumulation of compatible solutes including soluble sugars and proline (Hasegawa *et al*., 2000; Liu *et al*., 2013).

Some plant growth regulators have been well described as signaling molecules that participate in plant defense responses and enhance growth both under optimum and stress conditions. Specifically, Salicylic acid(SA), a phenolic compound produces inside the cell system of plants and acts as a water-soluble plant growth hormone to overcome biotic and abiotic stresses (Miura & Tada, 2014; Khan *et al*., 2015). SA is also involved in the regulation of photosynthesis, as a precursor for the biosynthesis of other growth regulators and in nitrogen metabolism (Khan *et al*., 2012; Miura & Tada, 2014; Nazar *et al*., 2015). Apart from, basic mineral nutrients like nitrogen (N), phosphorous (P) and potassium (P), other nutrients such as iron (Fe), magnesium (Mg), sulfur (S) are considered vital for plant growth and development processes as these nutrients work as activators or co-factors in different metabolic enzymes. These nutrients also contribute in the production of antioxidants to improve abiotic stress tolerance in plants (Wang *et al*., 2011). SA interacts with different nutrients both synergistically and antagonistically as well as it controls various growth-related characters under stress conditions. In this regard, Theerakulpisut *et al*., (2016) described that seed priming with SA improved the Na/K ratio and membrane integrity in rice seedlings under saline conditions. Further, SA seed priming in vegetables increased the protein contents, pigments, osmotic solute contents and antioxidant enzyme activities under salt stress conditions (Ghoohestani *et al*., 2012; Rafique *et al*., 2011; Azooz, 2009). In a recent study, SA primed carrot seeds exhibited higher antioxidants and total phenolic contents (Rehman *et al*., 2020). However, limited literature exists on SA seed priming effects specifically in leguminous crops.

Mungbean (*Vigna radiata* L.) is a common legume crop consumed all around the world and cultivated more than six million ha area (8.5% of the total area of pulses) globally (Hou *et al*., 2019). It is an important nutrient-rich crop being an important source of protein (25-31%), dietary fibers, carbohydrates, vitamins, and essential micronutrients (Ashraf *et al*., 2015; Alharby *et al*., 2019). Mungbean is mostly cultivated and consumed in Asian countries where its production is badly affected by several abiotic conditions such as saline and sodic soil (Sehrawat *et al*., 2019). Salt stress negatively correlates with seed germination process of Vigna spp by inhibiting germination and growth indices as well as reduction in chlorophyll stability index, chlorophyll a/b ratio and chlorophyll contents (Jabeen *et al*., 2003). Many reports have been documented so far, which describes the effects of salt stress on different growth stages of mungbean as germination attributes, vegetative and reproductive growth stages, particularly in flowering and pod-filling stages (Sehrawat *et al*., 2013, 2014a, 2015). Specifically, during seed germination the accumulation of Na and Cl ions decreased the activity of hydrolyzing enzymes due to an outside increase of osmotic potential (Sehrawat *et al*., 2014b).

Therefore, current work was designed to investigate the impact of SA on nutrients uptake in mungbean seedlings grown under salt stress as well as to investigate whether the exogenous application of SA can minimize the adverse effects of salinity on growth and productivity of mungbean under salt stress conditions.

Material and Method

Plant materials and treatments: A pot experiment was conducted in 2019 in the greenhouse of the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan. Two mungbean varieties, NM-2016 and NM-20-21 were purchased from the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan and sown on 15th February 2019 in saline and non-saline conditions. All seeds were germinated within one week. Sampling was performed 30 days after sowing. Before sowing, both mungbean varieties (NM-2016 and NM-20-21) were primed with water and with four levels of salicylic acid (control (0), hydropriming in distilled water, 0.01% SA and 0.02% SA for 4 hours. Two levels of salt (0, 80 mM NaCl) were applied. Each treatment was replicated thrice. One-month old plants were harvested and determined their growth parameters such as plant height (cm) and dry weight per plant (g).

Mineral analyses: To further calculate the mineral contents in mungbean leaves, samples were dried in the oven at 70°C for 2 days and extracted by acid digestion method given by Lowther (1980). Sodium and potassium contents were determined using Flame-Photometer (Jenway PFP-7). Calcium and magnesium were determined by method as described by Hide (1954**).** Nitrogen was estimated by micro-Kjeldahl method (Bremner, 1960). Phosphorus (P) was determined by a spectrophotometer (Dynamica, Halo SB-10, UV-VIS single beam) (Chang & Jackson, 1957) and concentration of chloride was determined with chloride analyzer (Corning-920, Germany).

Statistical analysis

All data were statistically analyzed by using analysis of variance (ANNOVA) technique and Statistica 3.5 Computer Program. To assess the differences among mean values, the Least Significant Difference test at 5% probability level was used.

Results

Growth attributes of mungbean in response to salicylic acid priming under salt stress: Data for plant dry weight and plant height differed significantly in response to different levels of salicylic acid (SA) and salinity in both mungbean varieties (Fig. 1). Both parameters displayed a declining pattern under salt stress. Seed priming with SA significantly increased the dry biomass and plant height as compared to control. Specifically, 0.01% SA application increased the plant height by 15% and 4% in NM-2016 and NM-20-21 respectively, as compared to NaCl treated plants (Fig. 1a). Whereas, under non-saline conditions, SA application did not show a significant change in plant height. Overall, the mungbean variety NM-20-21 showed greater height both under saline and non-saline conditions than variety NM-2016.

Similarly, 0.01% SA application improved the dry biomass by 6% and 18% in NM-2016 and NM-20-21 plants as compared to their respective NaCl treated plants (Fig. 1b). Under non-saline conditions, 0.01% SA priming, increased the dry weight by 6% in NM-20-21 while no significant increase was observed in NM-2016 plants. The data of dry weight showed better performance of NM-20-21 variety both under saline and non-saline conditions.

Effect of salicylic acid on mineral concentrations: Both mungbean verities grown with 80 mM NaCl concentration, exhibited a significant decrease in P and K contents and a non-significant decline was observed in N contents compared to the control (Fig. 2). Specifically, 0.01% SA application showed more pronounced effects to increase the N contents by 14% and 9% in NM-2016 and NM-20-21 as compared to NaCl and hydropriming plants. Whereas, under non-saline condition, NM-2016 displayed 4% increase in N content as compared to control but NM-20-21 did not exhibit any change in N contents as a result of SA application. Similarly, 0.01% SA application increased the P contents in mungbean leaves by 24% and 11% in NM-2016 and NM-20-21 respectively as compared to NaCl and hydropriming plants. Whereas, 11% and 12% increase was observed due to 0.01% SA priming in NM-2016 and NM-20-21 respectively, as compared to NaCl treated plants.

Plants grown with 80 mM NaCl displayed 3- and 2-fold increase of Na^+ contents in Nm-2016 and NM-20-21 respectively as compared to non-treated plants while Cl^- contents displayed 1- and 2.5-fold increase as compared to plants under the non-saline condition (Fig. 3 a, b). However, 0.01% SA application reversed the effect of salinity on the Na^+ and Cl^- contents as compared to respective NaCl-treated plants and hydro-priming plants. Specifically, seed priming with 0.01% SA along with 80mM salt stress decreased the Na contents by 25% and 19% and Cl contents by 12% and 30% in NM-2016 and NM-20-21 respectively as compared to only NaCl treatment.

The salt stress significantly decreased the Ca^{2+} contents (2-fold) in mungbean leaves as compared to untreated plants but 0.01% SA primed plants under saline condition showed 49% and 16% increase in Ca^{2+} contents as compared to salinity alone in both NM-2016 and NM-20-21 varieties respectively (Fig 3c). The Mg^{2+} contents did not show any significant increasing pattern both under stressed and non-stressed conditions with 0.01% SA and 0.02% SA application (Fig 3d). But, in comparison of only NaCl treated plants, 0.01% SA + 80 mM NaCl displayed higher values of Ca^{2+} contents. These results describe the positive role of SA for improving the ionic content of mungbean leaves under salt stress conditions. The Na/K and Na/Mg ratio were less for the seed priming with SA under the salinity. The 01% SA treated plants displayed lowest Na/K ratio and Na/Mg ratio under salinity (Fig. 3e and 3f).

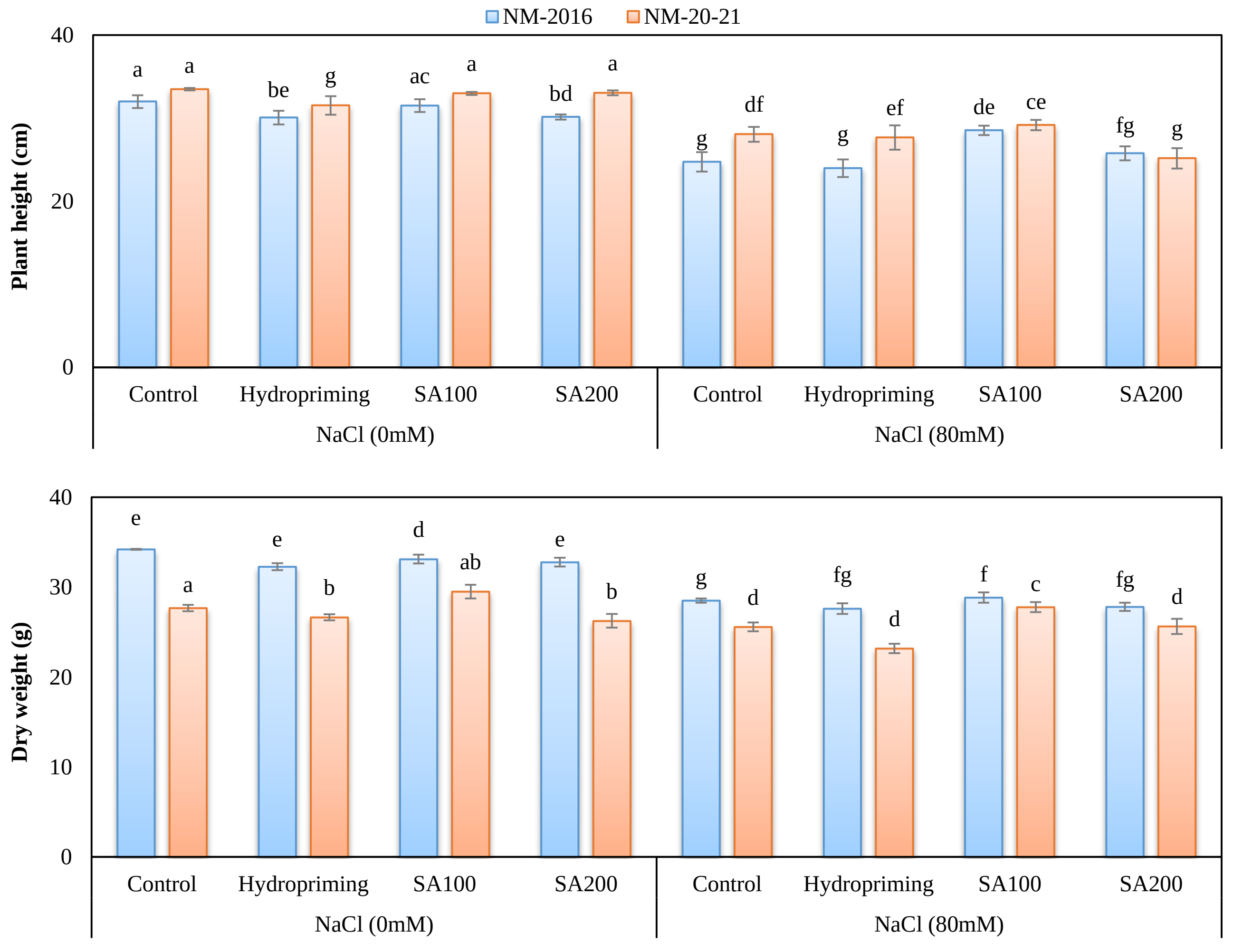

Fig. 1. The effect of salicylic acid seed priming on (a) plant height and (b) plant dry biomass under salt stress. The vertical bars denoted standard error of three replicates (n=3). Different lettering on bars indicate significant differences (p<0.05, LSD) among treatments.

Correlation among the growth and ionic contents of mungbean: To understand the growth and mineral traits, which describe salinity tolerance in mungbean, a relationship among all parameters was analysed (Fig. 4). Plant height and dry biomass were positively and significantly correlated with K, Ca, N, and P while negatively correlated with plant Na and Cl contents. Additionally, Na is negatively correlated with K, Ca, N, Mg, and P. These results showed that salt tolerance in mungbean was related to Ca, K, Na, and P mineral concentrations.

Discussion

Salicylic acid is an autogenous hormone which participates in multiple activities of plants such as seed and fruit yield and quality, seedling vigour, lipid peroxidation, nitrogen metabolism as well as in the defence system of plant for efficient quenching of ROS via antioxidant enzymes (Singh *et al*., 2016; Nazari *et al*., 2020). Salicylic acid enhances the ability of plants to tolerate different abiotic stresses by alleviating the development and growth of the plant. Its usage is safe and practical in activating plant defence systems; moreover, it also acts as growth regulator (Koo *et al*., 2020). Previously, some studies have reported positive regulatory influences of salicylic acid in seed priming on germination indices, in the growth of the plant and its development, in toleratingsbiotic and abiotic stresses, in antioxidative metabolism and nutrient contents (Mahesh *et al*., 2017; Shaikh-Abol-hasani & Roshandel, 2019; Galviz-Fajardo *et al*., 2020). The present study was directed to understand the effect of SA priming on plant dry biomass production and nutrient uptake of mungbean plants grown under both saline and non-saline conditions. Shaikh-Abol-hasani & Roshandel (2019) described a positive regulatory role of SA priming on carrot seeds in terms of germination indices and plant dry biomass production under saline conditions, these findings are in agreement with our studies.

In our study, mungbean plants raised from 0.01% SA primed seeds displayed in increase in N, P and K content rather than the seeds only treated with NaCl while no promising increase was observed in nutrient contents under non-saline conditions. Sheteiwy and his colleagues (2019) have reported the effect of SA seed priming on *Oryza sativa* seedlings and showed an improved uptake of as K^{+}, Ca^{+2}, and Mg^{+2} nutrients while reduction in Na^{+}/K^{+} ratios in both leaves and roots of the plants grown under salt stress condition. This increase in nutrient uptake and reduction in Na^{+}/K^{+} ratio might be due to the regulation of K^{+} and Na^{+} transporters and H^{+} pumps which cause a main driving force for uptake of nutrients. Further, Nazar *et al*., (2011) also reported the interface of SA with nutrients both synergistically and antagonistically which ultimately regulates the growth of the plant and other developmental responses under optimum and stress environment.

Nitrogen, P, and K are considered among basic mineral nutrients due to their diverse functions such as N, Mg and P are significant structural parts of chlorophyll and nucleic acid respectively, while K contributes in several physiological processes, environmental stresses, enzymes activation under stress environment (Waraich *et al*., 2011; Guo *et al*., 2016; Ahanger & Agarwal, 2017).

Salicylic acid (SA) mitigate the destructive effects of salinity by inducing N enzymes which suggest a compulsory role of SA and its crosstalk with N assimilation mechanisms under salt stress (Nazar *et al*., 2011). Improved K content in mungbean leaves could be an indicator of SA mediated better growth, yield, gas exchange attributes, and salinity resistance (Tufail *et al*., 2013). Similarly, P being an essential part of sugars, lipids and nucleic acids could be involved in different developmental processes at both cellular and organismic level of palnt. Balanced Na^+/K^+ ions ratio mainly contributes to salt tolerance mechanisms of plants as K^+ ions are involved to reduce the Na uptake (Wu *et al*., 2010; Zheng *et al*., 2014). In our study, plants primed with 0.01% SA showed decreased levels of Na^+ and Cl^- ions in saline condition rather than the plants treated with NaCl only. It might be SA which reduced the Na uptake by increasing the accumulation of other plant nutrients such as Ca^{2+}, Mg^{2+}, K^+, and N, increased dry matter and low membrane damage takes place in plants (Yildirim *et al*., 2008). This is the reason that mungbean plants with SA priming displayed better height and increased dry matter under stress conditions. El-Hendawy and his coworkers (2009) proposed that lower level of Na^+ in plant leaves indicates salt tolerance under both field and greenhouse conditions.

Intracellular Ca^{2+} actively intricate the regulation of plant responses to drought and salinity being involving in different transduction pathways and osmoregulation processes (Bartels & Sunkar, 2005; Ayub *et al*., 2021; Ozturk *et al*., 2021). We also observed increased levels of Ca^{2+} contents in mungbean leaves treated with 0.01% SA both under saline and non-saline environments in comparison to control plants and with hydro-priming treatments. Our findings are in agreement with the fact that Ca^{2+} ions play an important part as a second messenger in various biological processes which suggests that activation of Ca^{2+} mediated signal transduction system further leads towards adjustment of high salt conditions (Hasegawa *et al*., 2000; White & Broadley, 2003). In the cytosol calcium ions increases by the activation of immune receptors through calcium channels. This increase in calcium ions is decoded by sensor proteins, such as calmodulin (CaM) and calcium dependent protein kinases (CDPKs) (Dodd *et al*., 2010; Schulz *et al*., 2013). For binding of CaM protein, a transcription factor Calmodulin Binding Protein 60g (CBP60g) is required for the ICS1 expression and salicylic acid accumulation during immunity (Wan *et al*., 2012). Salicylic acid (SA) is a phytohormone and a small phenolic compound that functions as important signalling molecule during plant immunity (Seyfferth & Tsuda, 2014). There is a close link between SA and Ca (and Ca-signaling) in stressed plants (Chen *et al*., 2011). The application of salicylic acid was affective in improving the growth and calcium uptake in plants. Furthermore, interaction between SA and Ca^{2+} signalling resulted in maintenance of K^+/Na^- ion selectivity and in defense responses induced by stress (Hayat *et al*., 2010; Akhtar *et al*., 2013). SA revealed a strong interaction with Ca^{2+} signaling (Du *et al*., 2009; Al-Whaibi *et al*., 2012; Yücel & Heybet, 2016). These authors have been reported elevated proline levels in plants under the combine treatment of Ca^{2+} and SA proves that SA application along with Ca^{2+} could be beneficial to mitigate delitrious effects of salinity via enhanced activities of antioxidant enzymes and reduction in oxidative stress. These processes ultimately protect photosynthetic pigments and improve growth under salt stressed environment. Besides, SA can induce the expression of calmodulin-binding proteins and Ca^{2+}-dependent protein kinases which are ultimately involved in abiotic stress responses (Yang *et al*., 2013).

Previously, SA has been reported to raise the intake and level of Mg^{2+} contents in roots and shoots of *Triticum aestivum* under drought stress conditions (El Tayeb & Ahmed, 2010). However, we did not observe any significant changes in Mg^{2+} contents due to the SA application under both saline and non-saline conditions. In accordance to our results, Hussain and his colleagues (2003) and Thu and his coworkers (2017) reported that Mg^{2+} concentration in leaves of rice plants was not significantly affected under salinity stress whereas significant variations were reported in roots and sheaths of rice. We also found that 0.01% SA treated plants displayed lower Na/Mg ratio as compared to only NaCl treated plants both under saline and non-saline conditions (Fig. 3f) especially in NM-20-21 variety, which is a positive indicator of salt tolerance in mungbean plants. Furthermore, our correlations analysis data described a strong positive correlation among growth parameters such as height and dry weight of mungbean plants and K, Ca, N and P minerals except Mg. On the other hand, Na and Cl contents displayed a negative correlation with these minerals. It is concluded from the above data that SA interacts with different mineral nutrients to direct several plant responses under salt stress conditions to alleviate its harmful effects. Salinity reduced the growth and nutrient uptake in both mungbean varieties; however, NM-20-21 performed better than NM-2016 in terms of growth and biomass. Results also showed that seed priming with 0.01% SA was effective in improving growth and nutrient uptake at the seedling level.

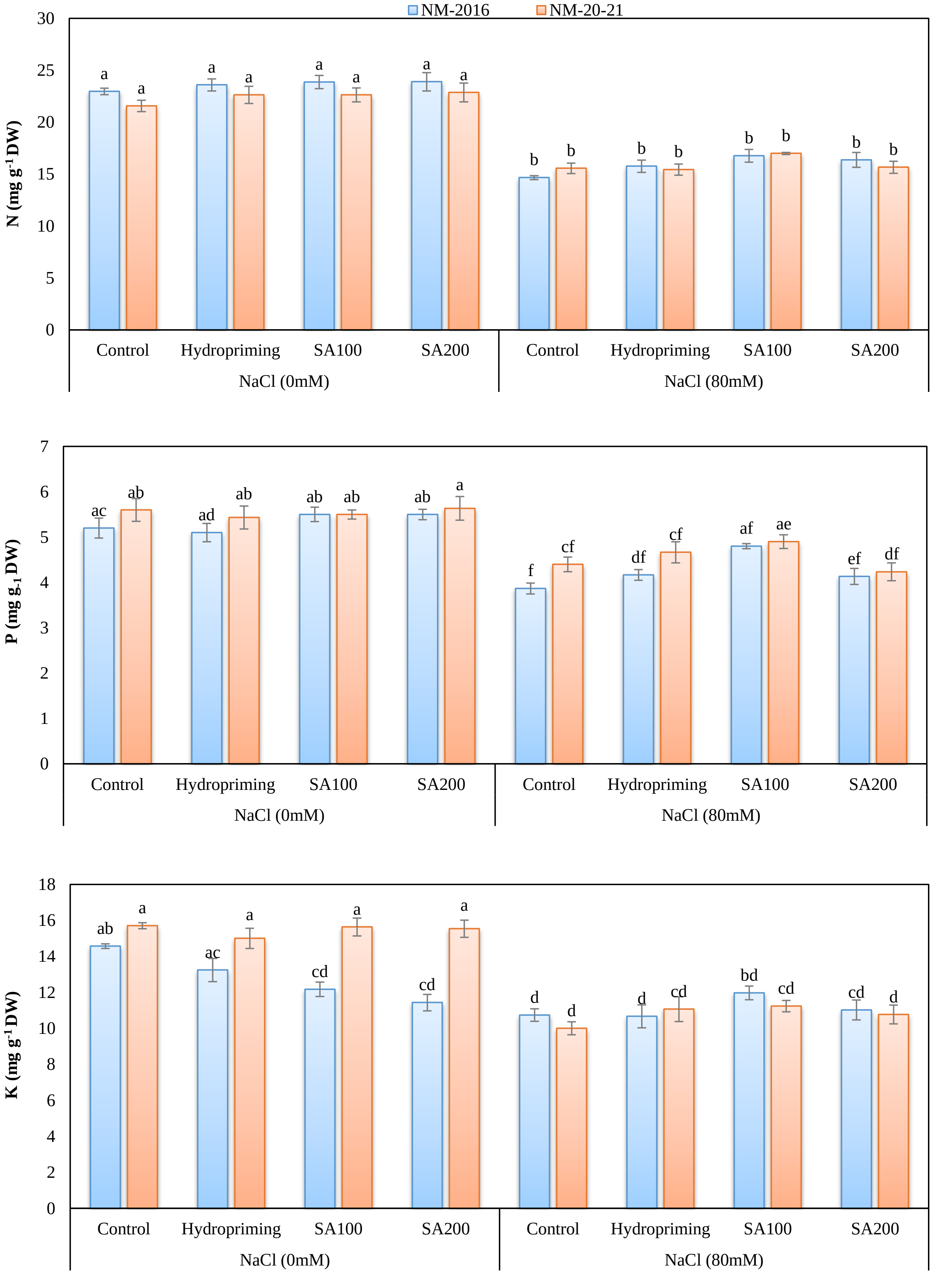

Fig. 2. The effect of salicylic acid seed priming on (a) nitrogen (b) phosphorus, and (c) potassium contents of mungbean varieties under salt stress. The vertical bars denoted standard error of three replicates (n=3). Different letters on bars indicate significant differences (p<0.05, LSD) among treatments.

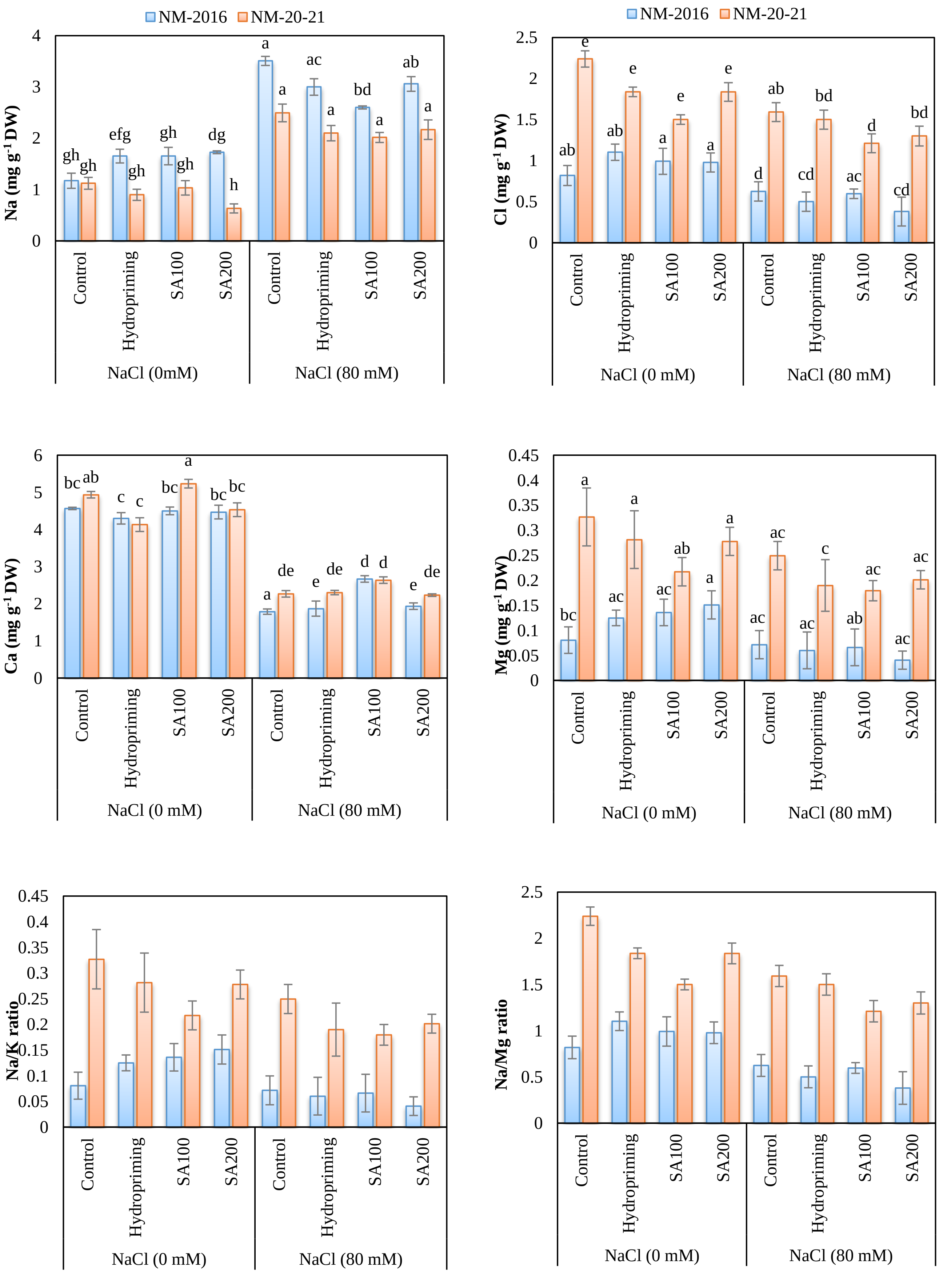

Fig. 3. The effect of salicylic acid seed priming on (a) sodium (b) chloride (c) calcium (d) magnesium (e) Na^+/K^+ ratio (f) $Na+/Mg^{2+}$ ratio of mungbean varieties under salt stress. The vertical bars denoted standard error of three replicates (n=3). Different letters on bars indicate significant differences (p<0.05, LSD) among treatments.

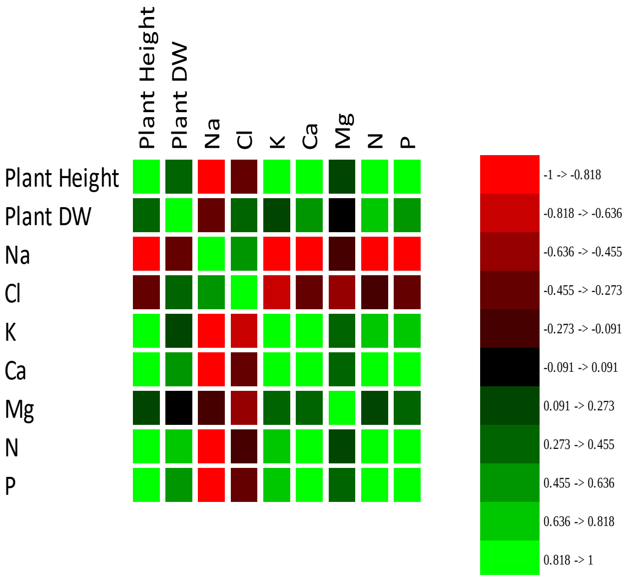

Fig. 4. The Pearson correlation matrix of nutrient and growth attributes of mungbean seedlings in response to 80mM salt stress by using computer based software Statistix 8.1.

Conclusion

From the results of present investigation it can be concluded that salinity reduced the growth and nutrient uptake in both mungbean varieties, however, seed priming with salicylic acid @ 0.01% (100 mg/L) was effective in improving growth and nutrient uptake in saline condition. Mungbean variety NM-20-21 performed better than NM-2016 under saline conditions.

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