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The differences in response of *Vigna sinensis* and *Phaseolus vulgaris* to varied salt stress levels

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Abstract: Crop response to salinity may varies among species, might be due to ions accumulation and yield components contribution in yield. This study was conducted to evaluate the effects of varying salinity (0, 1.5, 3.0, 4.5 and 6.0 dS m^{-1}) on cowpea (*Vigna sinensis*) and common bean (*Phaseolus vulgaris*) to distinguish their salinity tolerance thresholds. Although salinity reduced growth, yield, shoot and root potassium content as well as increased shoot and root sodium content in both crops; these alterations were lower in cowpea than common bean. So that the highest salinity level caused 60% and 50% reduction in grain yield and 59% and 49% reduction in dry matter in common bean and cowpea, respectively. Harvest index and leaf number could be independently of salinity stress. Multivariate regression analysis showed that grain number plus dry matter and thousand grain weigh plus dry matter were the most effective traits on grain yield of common bean and cowpea, respectively. Since thousand grains weight was found to be less sensitive trait to salinity, so cowpea was less sensitive under salinity stress compared to common bean. The same argument was followed in salinity threshold analysis, where salinity tolerance threshold was 3.62 for common bean and 4.36 dS m^{-1} for cowpea. The results of this study indicated that more Na accumulation in root and less translocation into shoot might be responsible for higher salinity tolerance in cowpea crop.

Key words: Ion distribution; Potassium; Pulse; Sodium; Thresholds

1. Introduction

As two members of grain legumes, common bean (*phaseolus vulgaris L.*) and cowpea (*Vigna unguiculata)* are major source of human dietary protein, minerals, vitamins, and represents nearly half of the consumed grain legumes worldwide (Ashraf and Waheed, 1993; Bayuelo-Jimenes et al., 2002). Legume crops are also vital in agriculture as it forms root nodules via symbiotic associations with nitrogen fixing bacteria (Murillo-Amador et al., 2002; Tavakoli et al., 2010). Nearly 250`000 tones beans has been produced in 2012 growing season in Iran (FAO, 2012); where the soil salinity (up to 2–4 dS m^{-1}) is one of the most factor limiting plant growth and yield (Pakniyat et al., 2003). However, it is known that even at 1 dS m^{-1} salinity level, the productivity of cowpea and common bean can be reduced up to 20% (Maas and Hoffman, 1977).

Soil salinity is one of the most severe abiotic stress factors limiting the productivity of agriculture worldwide. Soil salinity reduces the growth of crops by a combination of osmotic stress and ion-specific toxicity mechanisms. Plants have evolved various mechanisms to adapt to salt stress (Munns, 2002), including regulation of ion uptake and transport to the leaves (generally referred to as ion exclusion), managing the effects of water deficits in the plant tissues induced by the high salt concentration in the soil (osmotic adjustment) and intracellular compartmentation of Na^+ and Cl^- into the vacuoles (related to tissue tolerance) and salt excretion (Davenport et al., 2005; Attia and Nouaili, 2009). Improving salt tolerance in cowpea and common bean may therefore significantly increase the agronomic potential of legumes and will thus contribute to improve food security for an expanding population. However, few studies have been published on their response to salinity and there is little information available on their genotypic variation and physiological tolerance mechanisms in response to salt stress.

There are several researches that conducted to evaluate different response of bean cultivars to salinity (Bayuelo-Jimenes et al., 2002; Gama et al., 2007). Traits such as grain yield, survival, vigor, leaf damage and plant height, have been the most common criteria for identifying salinity tolerance (Maas and Hoffman, 1977; Longstreth and Nobel, 1979). Gama et al. (2007) showed that salinity adversely affected not only on the biomass yield and relative growth rate, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root weight ratio of two common bean cultivars. In a research it was shown that salinity increased epidermal and mesophyll thickness, palisade cell length and palisade diameter

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as well as spongy cell diameter in leaves of bean (Longstreth and Nobel, 1979).

Plants differ greatly in their tolerance of salinity, as reflected in their different growth responses, which this variation in dicotyledonous species is greater than in monocotyledonous species (Munns, 2002). Pulses, such as cowpea (*Vigna sinensis*) and common bean (*Phaseolus vulgaris* L.), are among the most sensitive crops to salinity stress (Maas and Hoffman, 1977). The differences between these two species will be highlighted by their response to varied salt rang. Understanding the mechanisms of tolerance to salinity may ultimately help to improve yield on saline lands, however there are low information about salinity threshold of cowpea and common bean and variations in their response and/or their mechanisms. Since these two crops are widely cultivated in arid and semiarid regions, where salinity is a widespread problem in these areas; this study was conducted to evaluate the effects of varying salinity levels on growth and ion accumulation of cowpea and common bean crops. It was expected that the result of this study revealed which crops might be more salt tolerance and why? To contribute to the understanding of the physiological mechanisms involved in salinity tolerance, the main aims of the present study were to analyse the growth and ion relations of cowpea and common bean in contrasting species under different levels of salinity stress.

2. Materials and methods

This study was carried out as a factorial experiment based on completely randomized design (CRD) with four replicates at Research Greenhouse of Shiraz University at 2013. The pots (5 L size) were filled with field soil (Soil classification: Fine, mixed, mesic, Cacixerollic Xerochrepts), sand and humus as 2:1:1 ratio. The used soil texture was sandy clay with pH = 7.05 and EC= 0.63 dS m^{-1}.

Uniform seeds of cowpea (29005 cv.) and common bean (Daneshkade cv.) were surface-sterilized for 5 min in sodium hypochlorite solution and then in 96% ethanol for 30 seconds. Ten seeds of cowpea and common bean were sown in 3-4 cm depth in each pot and after seedling emergence they were thinned to six. Each pot was considered an experimental unit. Minimum and maximum temperatures in the greenhouse were 15 and 26 °C, respectively, and relative humidity was 60-65 %. The plants were exposed to 14 h illumination daily.

The cowpea and common bean plants were exposed to five salinity treatments (ST) via irrigation after 21 days after sowing (complete establishment), using 2:1 weight ratio of NaCl: $CaCl_2$. Before this time the pots were irrigated to attain field capacity (+ 25% leaching fraction) every week. Salinity treatments included 0 as control (ST_0), 1.5 (ST_1), 3.0 (ST_2), 4.5 (ST_3) and 6.0 dS m^{-1} (ST_4). Electrical conductivity of irrigation water and drainage were controlled by a portable EC meter (Model 2052 digital USA) in each irrigation. The salinity levels in each pot were developed by the application of saline water at 2 subsequent irrigations.

Measured characters included plant height (Ht), dry matter (DM), pod number per plant (PN), grain number per pod (GN), thousand grain weight (TGW) and grain yield (GY), as well as, sodium (Na) and potassium (K) concentration of root and shoot. All six plants in each were harvested at physiological ripening. Plant height, pod number and grain number were determined in all plant and their average were considers as data of that pot. Thereafter, the samples were oven dried at 70°C for 48 h, and thousand grain weight, dry matter and grain yield were measured. To assay the ions concentrations of whole shoots and roots, they were digested in 40 mL of 4% HNO_3 at 95°C for 6 hours in a 54-well HotBlock (Environmental Express, Mt Pleasant, South Carolina, USA). The concentrations of Na^+ and K^+ in the digested samples were determined using a flame photometer (SL-CC-102 India).

Collected data were subjected to analysis of variance (ANOVA) and multivariate regression using SAS *v*. 9.1 software. Significant differences were determined using the least significant difference (LSD) test at $P \leq 0.05$. Threshold values based was estimated based on Van Genuchtan and Hoffman (1984) method using SAS *v*. 9.1 software.

3. Results and discussion

for Salinity had only decreasing effect on plant height of both crops at higher salinity levels (Fig. 1A), so that salt stress at 6.0 dS m^{-1} was associated with 32.6 and 24.3% reduction in common bean and cow pea, respectively. Furthermore, there was no significant difference between plant height under ST_0, ST_1 and ST_2 treatment, and both crops responded to salinity levels similarly. Difference in plant height between common bean and cowpea was higher under non stress than stressful conditions (Fig. 1A). Our results are in agreement with those Tavakoli et al., (2010), who reported a reduction in plant height as well as dry weight in bean due to salinity. Although dry matter of both crops was decreased upon salinity stress; dry matter reduction in cowpea was less and occurred at more severe salinity stress (Fig. 1B). The highest salinity levels (ST_4) resulted in 59 and 49% reduction in in common bean and cow pea dry matter, respectively. Pirasteh-Anosheh et al., (2014) also showed that the highest plant height and dry matter were achieved in control treatments (i.e. no salinity stress) and the lowest at highest salinity level. Our results also indicated that the amount of reduction was associated with stress severity. These reductions in plant height and dry matter might be due to effect of salinity on resource use efficiency, such as water and nutrition. Salt stress reduces the ability of plants to utilize such resources as water and results in a reduction in growth (Munns, 2002; Gama et al., 2007). As well, high concentrations of Na^+ in a result of salinity can cause deterioration in the soil structure and may exacerbate the effects of salinity by impeding drainage as well as affecting the availability of water as the soil dries (Ashraf and Harris, 2004). Tavakoli et al. (2010) also attributed reductions in plant height and dry matter to changes in plant metabolic processes. Leaf number per plant in both crops was not affected by salinity (data not shown); indeed sensitivity of different plant organ to salinity differs as also being noted by other researchers (Munns, 2002). In present investigation it was found that number of leaves per plants, in comparison to other traits, could be independently of salinity stress.

Salinity reduced pod number per plant (Fig. 1C) and grain number per pod (Fig. 1D) in both crops; so that the lowest pod and grain number were obtained under ST_4 treatment (i.e. the highest salinity level). There was no significant difference in pod as well as grain number for both crops between non stress (ST_0) and light salt stress (ST_1) conditions. Grain number in ST_1, ST_2, ST_3 and ST_4 was lower as 1.7, 25.0, 35.7 and 64.2% in common bean and 0.9, 20.0, 25.4 and 34.5% in cow pea respectively, compared to ST_0. These amounts were 0.0, 20.0, 36.0 and 56% for pod number of common bean and 0.0, 8.3, 13.8 and 27.7% for pod number of cow pea. Common bean responded to salinity faster than cowpea, thus the reduction in pod number and grain number due salt stress was lower in cowpea (Fig. 1C and 1D). The most reduction percentage due to salinity was found in grain number in common bean (64.2%). Salt stress is reported to be responsible for both inhibition and delay in seed germination and seedling establishment (Almansouri et al., 2001), and these effects might be involved for lower plant growth and consequently lower pod and grain number in present study. Under salinity conditions, a decrease in water uptake during seed imbibition might result to excessive uptake of ions (Murillo-Amador et al., 2002).

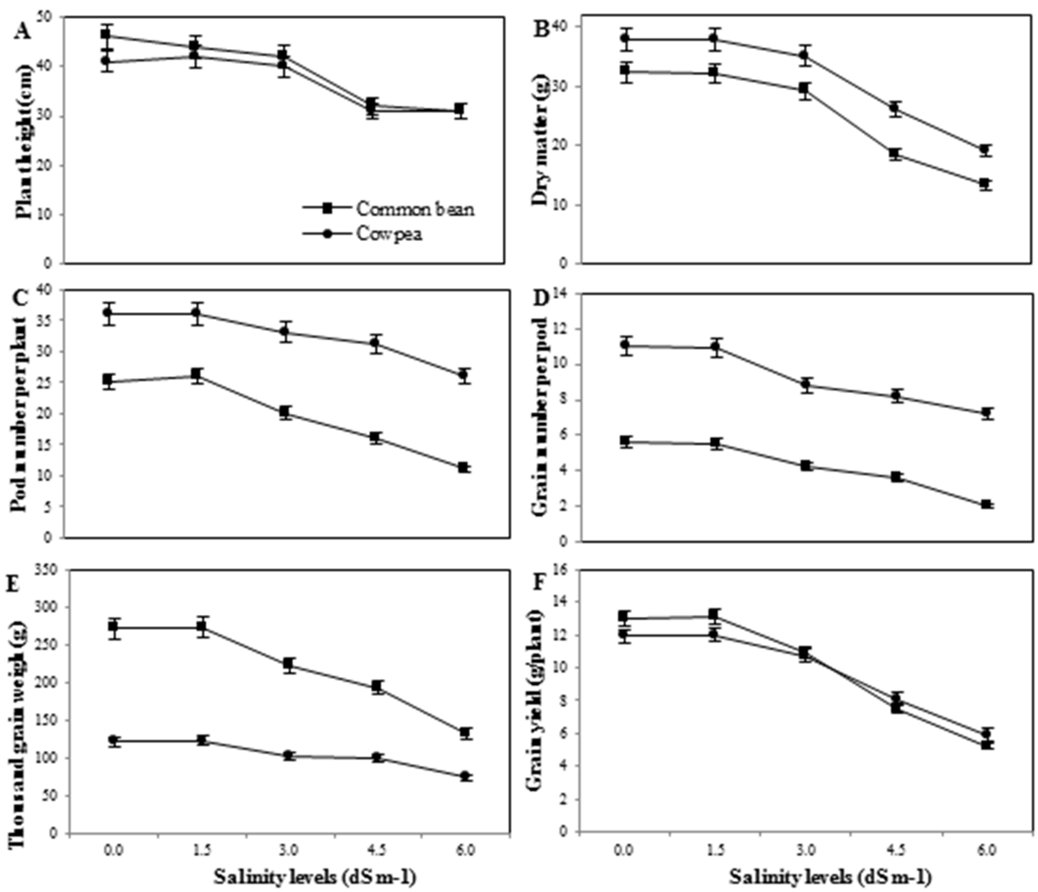

Fig. 1: Variations growth, yield and yield components (±SE) of common bean and cowpea under different salinity levels

Salinity had negative effect on 1000 grain weigh in both crops and as salinity was increased the reduction in 1000 grain weigh was enhanced (Fig. 1E). There was no significant difference between grain weight in both crops under non stress and ST_1 treatments. Although grain weight of both crops was reduced under salinity treatments, such reduction was considerably lower in cowpea (38.8%) compared to common bean (51.3%). So that difference between grain weight of these two crops was the highest under non stress (151 g) and was the lowest in ST_4 (58 g) conditions (Fig. 1E). Reduction in 1000 grain weight under salt stress conditions could be attributed to lower leaf production, expansion, photosynthesis, as well as, accelerated leaf senescence (Munns, 2002; Ahmed, 2009). These effects finally results to reduce assimilate availability for grain filling. A well document for this argument is finding of Ashraf and Waheed (1993), who reported that in tolerant chickpea (*Cicer arietinum*) genotypes there was a positive significant correlation between salt tolerance at the early and later growth stages, and these genotypes finally produced greater seed yield.

On average, dry matter and grain yield were found to be the most sensitive traits to salt stress (Fig. 1F and 2). Salt stress at ST1, ST2, ST3 and ST4 caused 0.0, 16.1, 42.3 and 60.0% in grain yield for common bean and 0.0, 10.0, 31.9 and 50.4 for cow pea, respectively. Grain yield, compared to other traits, showed a different trend in both crops in response to salinity stress. Salt stress of 3 dS m^{-1} and greater created a significant reduction in grain yield in two crops.

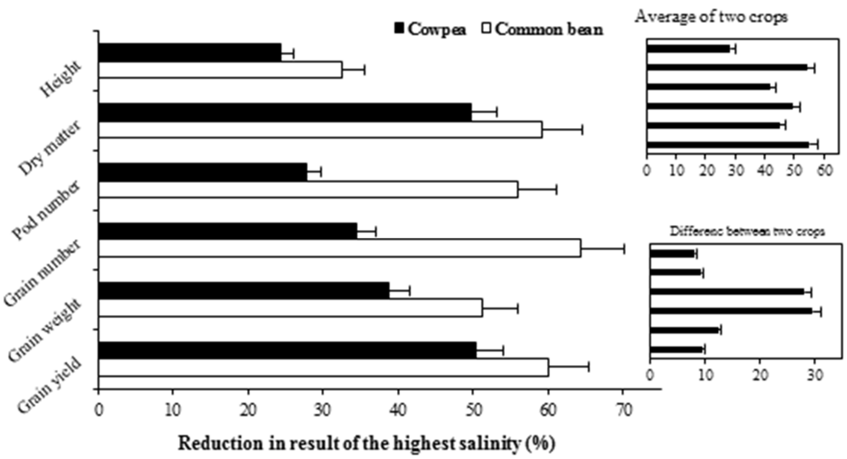

Fig. 2: Salt sensitivity of the different organs in common bean and cowpea under the highest salt stress (6 dS m-1) than non-stress

Although common bean had higher grain yield in non-stress and light stress; due to lower reduction of grain yield in cowpea, grain yield of cowpea under severe salinity stress was greater than common bean (Fig. 1F). Harvest index of common bean was greater than cowpea in all salinity treatments and it was not significantly affected by salinity (data not shown). It is reported that in common bean, NaCl concentration at 50mM has reduced grain yield due to salt-induced reduction in photosynthesis (Brugnoli and Lauteri, 1991). The yield reduction under salinity stress might be the results of several physiological responses, such as modification of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency as well as utilization and allocation of carbon (Ahmed, 2009; Munns, 2002). In present study, reduced yield might be the results of reductions in pod number (Fig. 1C), grain number (Fig. 1D) and thousand grain weight (Fig. 1E). Ahmed (2009) indicated that reduced grain yield in bean has been associated with reduced yield components under saline conditions. Reduced grain yield also can be contributed to lower nitrogen fixation in saline conditions, as Balasubramanian and Sinha (1976) reported that there was a considerable fall in the nitrogen fixation efficiency of beans under saline environment.

Threshold analysis showed that cowpea had a higher value than common bean, so that salinity tolerance thresholds of these crops were 3.62 and 4.36 dS m^{-1}, respectively. Maas and Hoffman (1977) indicated tolerance thresholds are used in genotypes selection for higher resistance to salinity. Both common bean and cowpea has been grouped into sensitive crops that have lower salinity tolerance than most field crops such as wheat and barley (Maas Hoffman, 1977). In this study, it appeared that common bean was a more sensitive crop to salinity than cowpea. Common bean also showed lower reduction for all measured traits under salinity stress, as it is shown in Fig. 2. Other studies have shown differences in tolerance thresholds among pulses. Cowpea has been reported to be less sensitive pulse than others (Balasubramanian and Sinha, 1976; Maas and Hoffman, 1977; Katerji and Hoorn, 2003; Attia and Nouaili, 2009).

Multivariate regression analysis showed that grain yield in both crops was significantly correlated with some traits. Grain number and dry matter were found to be the most effective traits on common bean grain yield; while in cowpea there were thousand grain weight and dry matter which significantly correlated with grain yield (Table 1). Bearing in mind that thousand grain weights has been reported to be the less sensitive trait for salinity conditions (Fig. 2), thus cowpea could be proposed to be less sensitive to salinity. This argument was also followed in threshold analysis. It has been known that salinity reduce not only grain yield, but also leaf area, grain number as well as shoot and root weights in several legumes such as such as chickpea (Ashraf and Waheed, 1993), faba bean (Zahran and Sprent, 1986; Tavakoli et al., 2010), soybean (*Glycine max*) (Grattan and Maas, 1988), and bean (Serraz et al., 2001; Gama et al. 2007). Since, salt sensitivity of different organs in a plant is varied (Munns, 2002); therefore the morhpho-physiological traits play a crucial role in reduction of plant efficiency that lead to decrease crop yield (Ahmed, 2009).

Table 1: Multivariate regression analysis for identifying the most effectiveness traits on grain yield

	Source of variance	df	Mean square	P value
Common bean	Regression	2	14.057	0.005
	Error	2	0.006	
	Total	4		
	Suggested model: Y= -0.825+ 0.182 GN + 0.282 DM			
Cowpea	Regression	2	24.334	0.004
	Error	2	0.011	
	Total	4		
	Suggested model: Y= -0.569+ 0.255 TGW + 0.219 DM			

GN: Grain number, DM: Dry matter, TGW: Thousand grain weigh

Salinity more than 1.5 dS m^{-1} increased sodium accumulation (Na^+) in both shoot and root of cowpea and common bean (Fig. 3A and 3B), which there was some difference between them. Increase in Na^+ accumulation was associated to salinity severity, as the highest Na^+ was observed in ST_5.

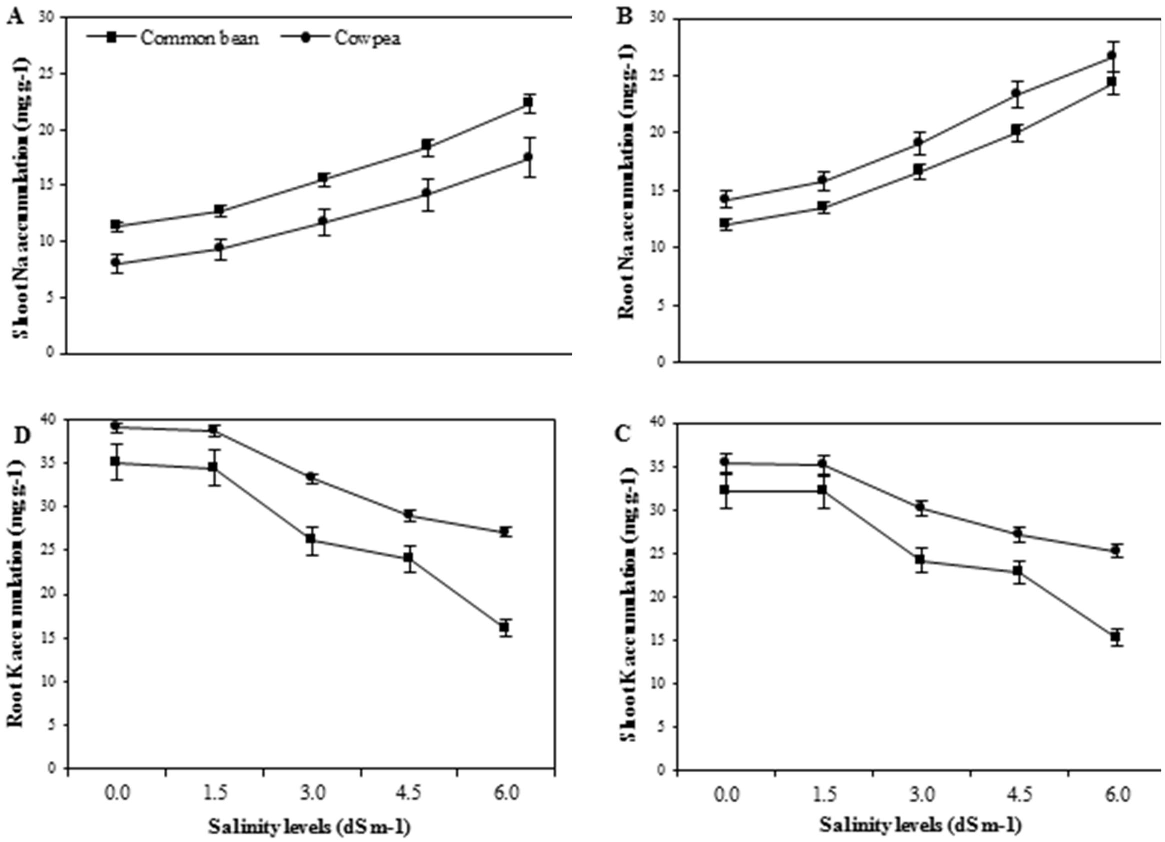

Fig. 3: Ions accumulation (±SE) in shoot and root of common bean and cowpea under different salinity levels

On average, salinity levels at ST_1, ST_2, ST_3 and ST_4 were associated with 13.9, 40.9, 68.9 and 106.2% increase in shoot Na and 11.8, 36.2, 65.6, 94.2% increase in root Na, respectively. Monovalent ions such as Na^+ could have different roles in crops biochemical process (Ashraf et al., 2010) that it has been reported to have adverse effects on crops due to its toxic impacts (Ouerghi et al., 2000). Ashraf and Waheed (1993) in check pea, Pakniyat et al. (2003), and Pirasteh-Anosheh et al. (2014) in barley also reported an increasing trend in Na^+ accumulation under salinity conditions. Furthermore, Na^+ accumulation in cowpea shoot was found to be significantly lower than common bean (Fig. 3A). In the other side, the greater Na accumulation level was found in cowpea root (Fig. 3B). In the other words, Na accumulation in root to shoot ratio was greater in cowpea than common bean. Salt tolerance in crop plants is reported generally to be associated with low uptake and accumulation of Na^+ (Ashraf and Harris, 2004; Pirasteh-Anosheh et al., 2011). Therefore, Na^+ accumulation in plant tissues could be used as an important indicator for salinity tolerance (Ashraf and Waheed, 1993). More Na accumulation in roots and lower transformation to shoot is a mechanism to higher salinity tolerance of plants (Blumwald et al., 2000); which might be a reason for more tolerance of cowpea. Davenport et al. (2005) indicated reduced Na^+ loading into the xylem is one of the main mechanisms of salinity tolerance and it is often considered one of the most crucial features of restricting Na+ accumulation in plant tissues. In our study, this also reflected in different of reduction in dry matter of two crops.

As salinity increased Na^+ accumulation, potassium ion accumulation (K^+) in shoot and root of both crops were decreased due to salinity, and this reduction was enhanced with increase in salt stress (Fig. 3C and 3D). On average of two crops, ST_1, ST_2, ST_3 and ST_4 caused 0.6, 19.5, 26.0 and 40% decrease in shoot K^+ and 1.3, 19.9, 28.4, 41.7% decrease in root K^+, respectively. Indeed, shoot K^+ accumulation of cowpea was more than common bean in all conditions; however the difference between them was the higher in higher salinity levels (Fig. 3C). The similar pattern was observed for accumulation of K^+ in root (Fig. 3D). Salinity stress is known to alter the ion equilibrium in plant tissues (Pirasteh-Anosheh et al., 2014) and resultantly, some important ions could be effectively used as important selection criteria for salt tolerance. For example, K^+ concentration is believed to be an index of salinity tolerance in most crop species (Ashraf and Harris, 2004.). Pakniyat et al. (2003) also noted that higher K^+ concentration was associated with salt tolerance of barley.

4. Conclusions

Salinity reduced growth and productivity of cowpea and common bean, might via reduced K+ as well as enhanced Na+ content in both shoot and root. First, these changes were closely being associated to salt stress levels; so that the variations in growth, yield and ion accumulation were increased as salinity was intensified. Second, the reductions in traits were not similar; dry matter and grain yield were reduced more than others traits. However, plant height was reduced less than other traits, whereas leaf number and harvest index were independently of salt stress. Therefore, sensitivity of plant organs to salinity found to be differed. Third, higher tolerance of cowpea to salinity have been reflected in greater Na+ accumulation in root and its lower transformation into shoot as a probability mechanism for salt tolerance. Cowpea had also higher K+ accumulation in both shoot and root tissues. The higher salinity tolerance in cowpea also might be related to higher regression of grain yield with thousand grain weight, as a less sensitive trait.

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