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SALT STRESS RESPONSES OF PIGEON PEA (*CAJANUS CAJAN*) ON GROWTH, YIELD AND SOME BIOCHEMICAL ATTRIBUTES

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Abstract

Growth responses of leguminous plants to salinity vary considerably among species. Pigeon pea (*Cajanus cajan* (L.) Millsp.) is a sub-tropical crop, grown worldwide particularly in South Asia for edible and fodder purposes, while little is known about its salinity tolerance. In order to investigate the effect of salinity, plants were established at six different levels of sea salt concentrations i.e. 0.5, 1.6, 2.8, 3.5, 3.8 and 4.3 (EC_e $dS.m^{-1}$). Plant growth was measured using vegetative [height, fresh and dry biomass, moisture, relative growth rate (RGR) and specific shoot length (SSL)], reproductive (number of flowers, pods, seeds and seed weight) and some biochemical parameters (chlorophylls, carotenoids, sugars and proteins). Pigeon pea showed a salt sensitive growth response, however, it survived up to 3.5 (EC_e $dS.m^{-1}$) sea salt salinity. Plant height, biomass, SSL and RGR linearly decreased under saline conditions. Leaf pigments increased (chlorophylls) or maintained (carotenoids) at 1.6 $dS.m^{-1}$ and subsequently decreased in higher salinity. Low moisture content and succulence along with more accumulation of soluble sugars and proteins may be attributed to leaf osmotic adjustments at low salinity. Salinity adversely affect reproductive growth of *C. cajan* where production of flowers, pods, number of seeds and seed weight were significantly reduced. Present study provides basic information related to plant growth, seed yield and some biochemical attributes, which suggest *C. cajan* as a salt sensitive leguminous crop. However, detailed information is required to understand the eco-physiological responses of this plant under field and green house conditions.

Keywords: Edible, Fodder, Marginal lands, Saline agriculture, Salt tolerance.

Introduction

Soil salinity impose serious threats to plant growth, agricultural production and soil fertility particularly in arid/semiarid areas. About one-third of cultivated land is salt affected throughout the globe, which makes salinity a major limiting factor for food/ fodder production. Declining fresh water supply, high evapotranspiration, water table rise, increasing salt load in irrigation water and agricultural malpractices are considered foremost factors that further intensify this problem. Salinity decreased agricultural production up to 35% (Koyro *et al*., 2013), creating shortage of food for human and animal consumption and this situation is aggravating with time (Turkan & Demiral, 2009).

Nature and magnitude of salt stress affect growth of both halophytes and glycophytes. However, in latter, growth is usually reduced even at low salt concentration particularly in crop plants (Munns, 2002). Among glycophytes a large variability exists, ranging from highly sensitive to tolerant species (Greenway & Munns, 1980). Leguminous plants are perhaps more susceptible to salt stress and are unable to survive in saline soils due to insufficient tolerance mechanisms (Ahmad & Sandhu, 1988). Growth and reproduction of these plants adversely affected at various levels from morphology to genetics which ultimately challenge their survival in saline substrates (Munns, 2002). Individual and synergistic effects of osmotic, ionic and nutritional imbalances could be accounted as the possible weapons of salt attack (Gupta & Huang, 2014).

In general, a negative connection is observed between plant height and salt concentration (Taffouo *et al*., 2009). Similarly, salt adversely affect leaf number, size, fresh and/or dry plant biomass, succulence and growth rate (Taffouo *et al*., 2010; Memon *et al*., 2010). In response plants usually accumulate high amount of compatible solutes to maintain water status. Thus sugars, proteins, amino acids and quaternary ammonium compounds are synthesized at the cost of growth (Qin *et al*., 2009). Many studies showed inhibitory effects of salinity on photosynthesis which can be observed by measuring leaf pigments (Parida *et al*., 2002). Results of specific studies clearly indicated that salinity altered the chlorophyll and carotenoid contents in salt treated plants (Taffouo *et al*., 2010; Azeem & Ahmad, 2011).

Leguminous crops are considered suitable to increase bio-productivity and reclaiming marginal soils. Pigeon pea (*Cajanus cajan* (L.) Millsp) is one of the important leguminous crops, consumed as split pulse. Its green pods are cooked as vegetable and leaves are used for animal fodder. The food legume now become common in more than 50 countries of Africa, Latin America, Caribbean and parts of Asia, however, it is mainly cultivated (> 90%) in Indian subcontinent (Waheed *et al*., 2006). Beside its edible importance, Pigeon pea is famous for several medicinal properties (Pal *et al*., 2011) and used successfully along with several other herbs in traditional system of medicine (Abideen *et al*., 2015; Qasim *et al*., 2010, 2011, 2014; 2016). Plant is used in the treatment of diabetes, stomach ailments, pain, ulcers, inflammation and induce lactation when applied over breast (Lans, 2007; Ganesan, 2008; Upadhyay *et al*., 2010). A large number of bioactive compounds have been identified from this plant with antibacterial, antiplasmodial, antiinflammatory, antioxidant, hypocholesterolemic, hepatoprotective and anthelmintic activities (Luo *et al*., 2008; Ezike *et al*., 2010; Nicholson *et al*., 2010; Pal *et al*., 2011). Furthermore, it provides organic matter to the soil in the form of green manure or through cover cropping (Kone, 2012).

Besides its high nutritional and economic value Pigeon pea has an ability to withstand drought (Subbarao *et al*., 2000) and improve soil fertility in nitrogen deficient conditions due to its symbiotic relation with nitrogen fixing organisms (Fujita *et al*., 2004). Such properties making this crop a good candidate to bringing new areas under cultivation particularly in arid/semiarid region (Odeny, 2007). Recently, studies have been focused to investigate effect of different abiotic stresses on germination, growth and yield of Pigeon pea alone and with some companion intercrops at field and controlled conditions (Tayyab *et al*., 2015; Ghosh *et al*., 2006; Waheed *et al*., 2006). However, little is known about the salinity tolerance of this crop. Few reports are available in this context, however those were more focused on vegetative growth or on legume-rhizobium symbiotic relations (Manchanda & Garg, 2011). Therefore, present investigation was aimed to study the effect of sea salt salinity on vegetative and reproductive growth of Pigeon pea using drum pot culture.

Materials and Methods

Growth conditions and salinity treatment: A modified drum pot culture (lysimeter) proposed by Ahmad & Abdullah (1982) was used in present experiment. Each drum pot filled with 200 kg of sandy loam containing cow-dung manure (9:1) having 28% water holding capacity. Drum pots were fixed at cemented platform at slanting position with basal hole to ensure rapid drain. Over irrigation was practiced to avoid salt accumulation in the root zone. Experiment was carried out at Biosaline research field (Department of Botany, University of Karachi) using six different drum pot sets (each in triplicate) with variable sea salt concentrations having EC_{iw} 0.4, 0.9 1.6, 2.4, 2.8 and 3.2 $dS.m^{-1}$. Surface sterilized (0.1% sodium hypochlorite) seeds (10) were sowed in each drum pot. After two weeks of establishment in their respective sea salt solutions plants were thinned to three healthy and equal size seedlings. Each drum pot irrigated (15 L) at weekly intervals. Soil electrical conductivity was measured by EC meter (Jenway 4510, made UK) using saturated soil paste. The average electrical conductivity was maintained at EC_e 0.5, 1.6, 2.8, 3.5, 3.8 and 4.3 $dS.m^{-1}$. These ECe values are further used for data representation. Experiment was conducted for a period of 18 weeks (July to November 2009) during which environmental data (Fig. 1) including average humidity (midnight 76% and noon 54%), temperature (low 23^{o}C and high 33^{o}C), wind velocity (14 kmph) and rainfall (~4 cm) was recorded (data provided by Pakistan Meteorological Department, Karachi).

Growth measurements at vegetative and reproductive phase: Shoot height was measured fortnightly after seedling establishment. Fresh weight, dry weight and moisture content of shoot were recorded initially after two weeks and at final harvest. Reproductive data in terms of number of flowers, number of pods, number of seeds and seed weight per plant was recorded.

Leaf succulence (Abideen *et al*., 2014), specific shoot length (SSL; Panuccio *et al*., 2014) and relative growth rate (RGR; Moinuddin *et al*., 2014) were measured using following equations:

$$\text{Succulence } (\text{g } H_2O \text{ g}^{-1} \text{ DW}) = (FW - DW) / DW$$

$$SSL = \text{shoot length} / \text{shoot dry weight}$$

$$RGR \ (\text{g g}^{-1} \text{ day}^{-1}) = (ln W_2 - ln W_1) / (t_2 - t_1)$$

where, FW fresh weight; DW dry weight; W_1 and W_2 initial and final dry weights and t_1 and t_2 initial and final time of harvest in days.

Biochemical estimations: Biochemical analysis was carried out after 18 weeks of salt treatment. Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophylls, chlorophyll *a*/*b* ratio and carotenoid contents) were estimated according to Arnon (1949). Total soluble and insoluble sugars were estimated by the method of Yemm & Willis (1954) and total soluble proteins by Bradford (1976) method. Optical density was measured by spectrophotometer (Jenway 6305, UV visible made UK).

Statistical analysis: Statistical analysis was carried out using SPSS (version 14). Graphs were plotted using Sigma plot (version 11). ANOVA and the significant differences among means were examined by least significant difference (LSD) post-hoc test according to Zar (2010).

Results

Effect of sea salt on vegetative growth including height, fresh and dry weight of *C. cajan* is presented in figure 2 & 3. Comparative analysis showed that plant growth was significantly increased with time ($p<0.001$), however, it was linearly decreased ($p<0.001$) with increasing salinity. Figure 4 shows the percent change in moisture, succulence, relative growth rate (RGR) and specific shoot length (SSL) of *C. cajan*. Under saline conditions, all these parameters were significantly reduced in comparison to control, however, SSL showed decline after 3.8 dSm^{-1} (EC_e). Growth reduction was more pronounced at 3.8 and 4.3 $dS.m^{-1}$ (EC_e) at which plants died before reaching the reproductive maturity (Fig. 2). Therefore, further analysis was carried out in plants grown up to 3.5 $dS.m^{-1}$ (EC_e) sea salt concentrations.

Salinity significantly reduced ($p<0.001$) reproductive parameters including number of flowers, pods, seeds and seed weight of *C. cajan* (Fig. 5). Among all treatments, highest reduction was observed in 3.5 $dS.m^{-1}$ (EC_e) at which number of flowers and pods were reduced up to 72% and 70%, respectively. Similar trend was observed in total number and weight of seeds which showed 80% and 88% reduction, respectively.

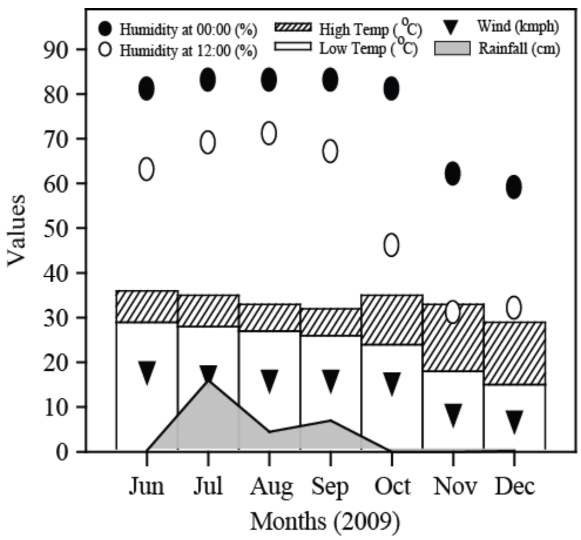

Fig. 1. Environmental data of study area during experimental period (July-November 2009).

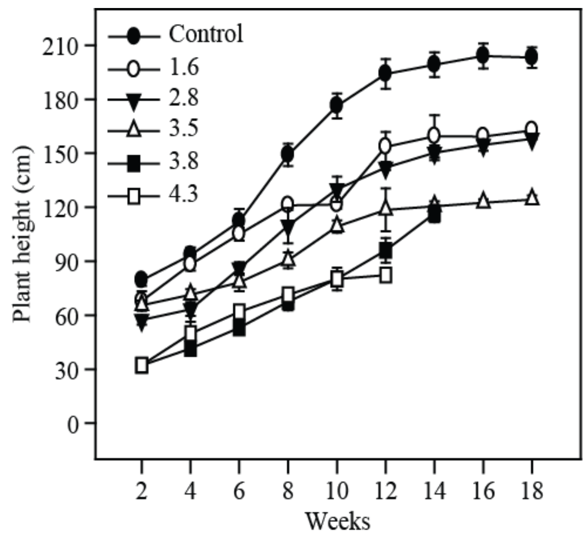

Fig. 2. Effect of salinity on height of *Cajanus cajan* during 18 weeks of salt treatment (EC_e control, 1.6, 2.8, 3.5, 3.8 and 4.3 dSm^{-1}).

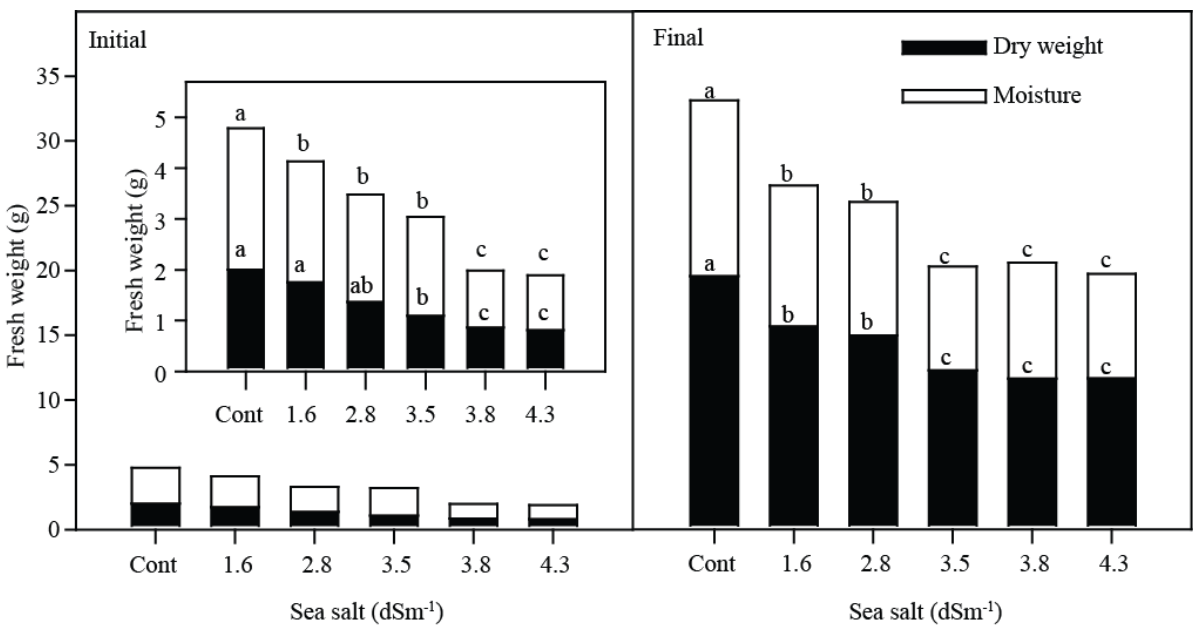

Fig. 3. Effect of salinity on initial and final biomass (fresh and dry) of *Cajanus cajan.* Different letters represent significant differences at p<0.05.

Figure 6 shows the effect of salinity on pigments (chlorophyll *a*, *b*, *a*/*b* ratio and carotenoids) of *C. cajan* leaves. A slight increase in total chlorophyll (18.28%) and chlorophyll *a*/*b* ratio (12.15%) was observed at low salinity (EC_e 1.6 $dS.m^{-1}$), however, these parameters were significantly reduced (41.25 and 36.30% respectively) at high salt treatment ($p< 0.001$). Chlorophyll *a* was found higher than chlorophyll *b* in all treatments, however, chlorophyll *b* was throughout un-affected. At high salinity (EC_e 3.5 $dS.m^{-1}$) chlorophyll *a*, total chlorophylls and *a*/*b* ratio was decreased by 50.5%, 41.2% and 36.30%, respectively. Carotenoid content was un-changed at low salinity (EC_e 1.6 $dS.m^{-1}$) however, it decreased with further increase in salinity.

Total leaf sugars were remained un-affected at 1.6 $dS.m^{-1}$ (EC_e) and subsequently decreased with further increase in salinity (Fig. 7). Although, total sugars were decreased at 2.8 and 3.5 $dS.m^{-1}$ (EC_e), a significant increase (~25%) of soluble sugars was observed at higher salinities. However, this increase was accounted for decrease (50.4 %) in insoluble sugars at respective salinities.

An increase in leaf protein content was found at lower salinity (EC_e 1.6 $dS.m^{-1}$) which was followed by significant reduction with further increase in salinity (Fig. 7). This decline was 20.4% at 2.8 $dS.m^{-1}$ (EC_e) and 56.46% at 3.5 $dS.m^{-1}$ (EC_e).

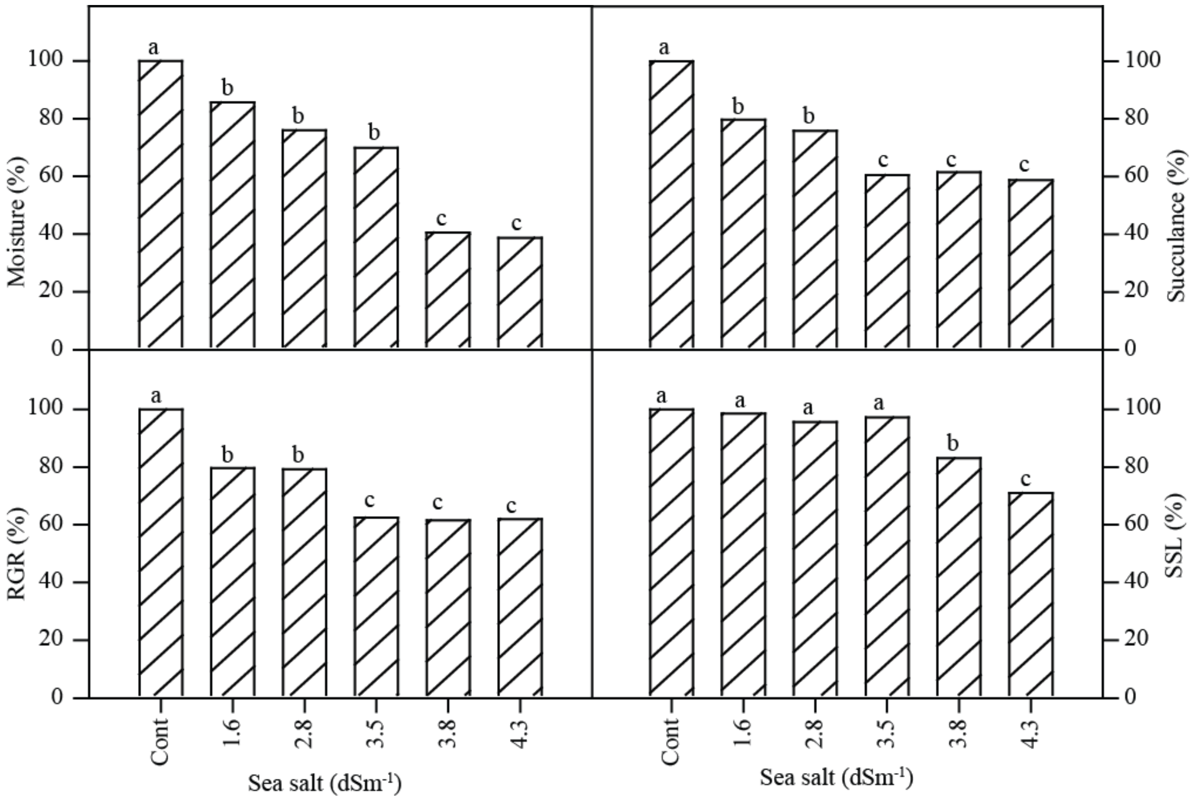

Fig. 4. Percent change in moisture, succulence, relative growth rate (RGR) and specific shoot length (SSL) of *Cajanus cajan* under increasing salinity treatments. Different letters represent significant differences at $p<0.05$.

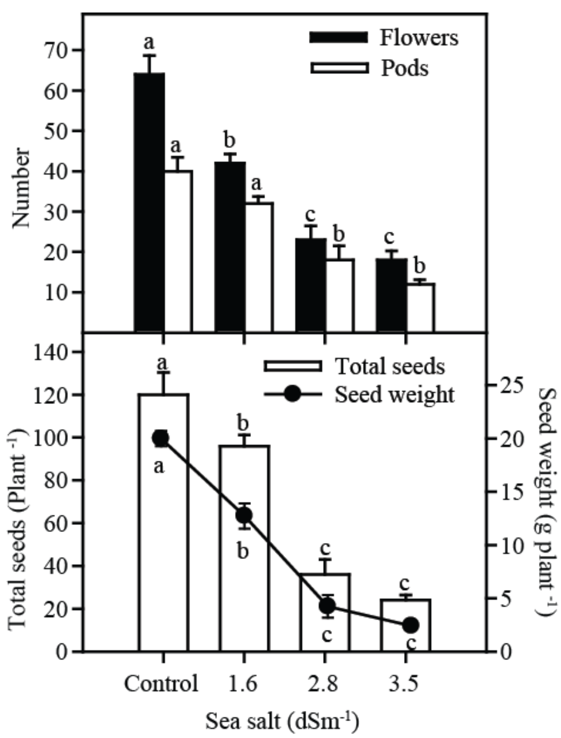

Fig. 5. Reproductive growth parameters including number of flowers, pod, seeds and seed weight of *Cajanus cajan* under increasing salinity treatments. Different letters represent significant differences at $p<0.05$.

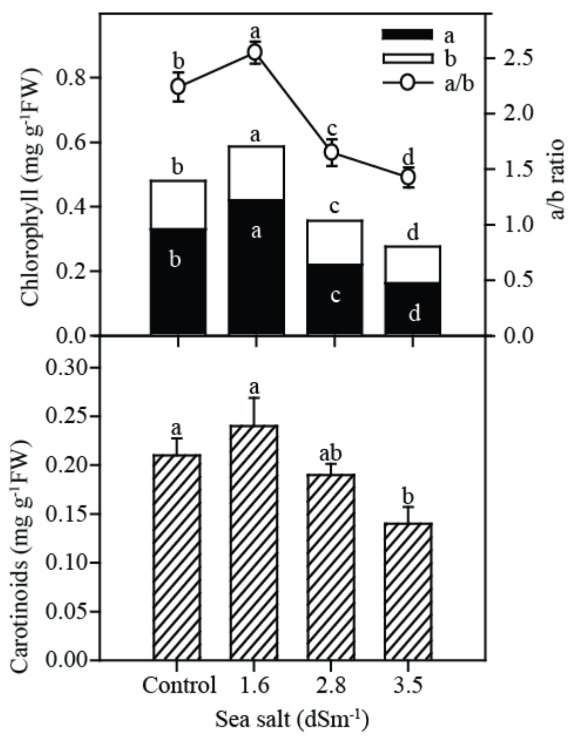

Fig. 6. Leaf pigments including chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of *Cajanus cajan* under increasing salinity treatments. Different letters represent significant differences at $p<0.05$.

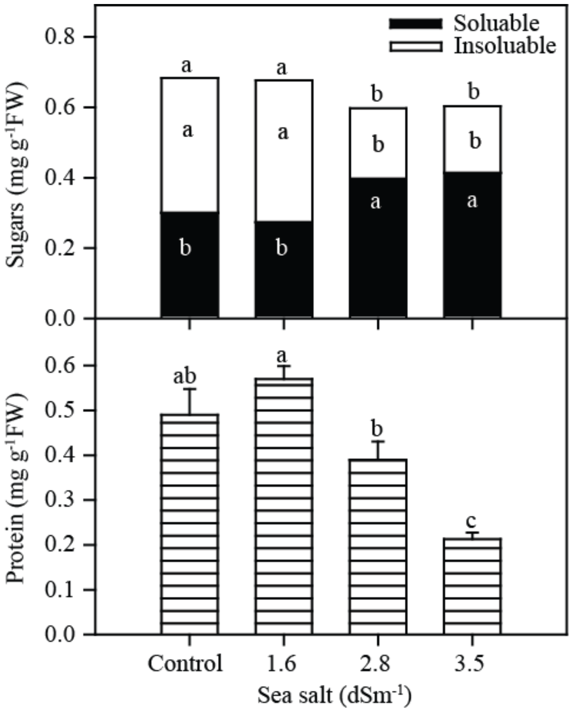

Fig. 7. Total proteins, soluble, insoluble and total sugars of *Cajanus cajan* under increasing salinity treatments. Different letters represent significant differences at $p<0.05$.

Discussion

Soil salinity is a major limiting factor for plant growth and yield production particularly for leguminous plants (Guasch-Vidal *et al*., 2013). In this study plant height, RGR, fresh and dry biomass was severely reduced with increasing salinity and mortality of plants was noticed after 14 weeks at higher salinities (EC_e 3.8 and 4.3 dS.m^{-1}). This growth inhibition may be accounted for individual and synergistic effect of water deficit, nutrient imbalance and specific ion toxicities (Hasegawa *et al*., 2000; Silveira *et al*., 2001). Salt induced ion imbalance results in lower osmotic potential which alter physiological, biochemical and other metabolic processes leading to overall growth reduction (Del Amor *et al*., 2001). Excessive amount of salt in cytoplasm challenge the compartmentalization capacity of vacuole and disrupts cell division, cell elongation and other growth processes (Munns, 2005; Munns *et al*., 2006). Our results are parallel with some other studies in which significant growth inhibition of peas, chickpea, faba and Mungbean beans was reported under salt stress (Elsheikh & Wood, 1990; Delgado *et al*., 1994, Akhtar *et al*., 2013). Singla & Garg (2005) also observed a similar salt sensitive growth response in *Cicer arietinum*. In our study, the fresh and dry biomass of *C. cajan* decreased with increasing salinity. Other researchers also found similar reduction in legumes biomass e.g. pea plant, common bean (Hernandez *et al*. (1999) and sesbania (Mahmood *et al*., 2008) when grown in saline mediums.

Salinity also impose deleterious effects on reproductive growth of *C. cajan*. Production of flowers and pods were significantly decreased in response to salinity. Increase in flower shedding leading to decreased number of pods, indicating salt sensitivity of plant at reproductive phase which was more pronounced at high salinity (Vadez *et al*., 2007). Furthermore, seed production and weight of seeds per plant was also linearly decreased. Salt induced reduction of reproductive growth was also found in mungbean in which 60% and 12% less pods and seeds were produced at 0.6% saline solution, respectively (Qados, 2010). Similar, results were also reported in faba bean (De Pascale & Barbieri, 1997), tomato (Scholberg & Locascio, 1999), sunflower (Katerji *et al*., 1996) and watermelon (Colla *et al*., 2006). Salinity reduced reproductive growth by inhibiting growth of flowers, pollen grains and embryo which leads to inappropriate ovule fertilization and less number of seeds and fruits (Torabi *et al.*, 2013).

In this study, total chlorophyll and chlorophyll *a*/*b* ratio were increased in low salinity whereas; higher salinity had adverse effect on all chlorophyll measurements which indicate its breakdown due to high Na^+ accumulation in cytosol (Li *et al*., 2010; Yang *et al*., 2011). In this study, chlorophyll *a* was found to be more sensitive than chlorophyll *b*. In line with our results, several studies suggested that under salt stress, chlorophyll *b* may be converted into chlorophyll *a* and decrease in total chlorophyll is mainly attributed to the reduction of chlorophyll *a* (Fang *et al*., 1998; Eckardt, 2009). This diminution could be due to the destruction of enzymes responsible for green pigments synthesis (Strogonov, 1973) and/or increased chlorophyllase activity (Sudhakar *et al*., 1997). Thus, insipid leaf was a visible indicator of salt induced chlorophyll damage which was well correlated with quantified values as reported in other legume species (Soussi *et al*., 1998; Al-Khanjari *et al*., 2002). Garg (2004) also found similar reduction in leaf pigments (*a*, *b* and total chlorophyll) in chickpea cultivars under salinity stress.

At low salinity (1.6 dSm^{-1}), unaffected level of carotenoids along with increased chlorophyll content, may suggest a role of carotenoids in protection of photosynthetic machinery (Sharma *et al*., 2012). Similar response was found in *Cajanus indicus* and *Sesamum indicum* (Rao & Rao, 1981), however, Sivasankaramoorthy (2013) and Ramanjulu *et al*., (1993) reported slight increase of leaf carotenoids in *Zea maiz* and mulberry when exposed to NaCl. High salinity was destructive for both leaf pigments (chlorophyll and carotenoids) of *C. cajan* as reported in some other salt sensitive crops (Reddy & Vora, 1985). Under high salinity, sometimes total carotenoids decreased because plant converts carotene (beta-carotene, in particular) to Zeaxanthin which protects from photoinhibition (Sharma & Hall, 1991).

In present study, moisture content and succulence of *C. cajan* were significantly reduced with increasing salinity, which indicated a loss of turgor. It may suggested that, *C. cajan* decrease water content to achieve low osmotic potential when exposed to increasing salinity, which is in agreement with findings of Parida & Das, (2005) and Abideen *et al*. (2014). In addition, increased production and accumulation of organic substances is also necessary to sustain osmotic pressure which provide potential gradient to absorb water from saline medium (Hasegawa *et al*., 2000; Cha-um *et al*., 2004). Compatible solutes including carbohydrates, amino acids, proteins and ammonium compounds play important role in plant water relations and cell stabilization (Ashraf & Harris, 2013). In this study, *C. cajan* accumulated more soluble sugars which is considered as a typical plant response under saline conditions (Murakeozy *et al*., 2003). Sugars serve as organic osmotica and their available concentration is related to the degree of salt stress and plant's tolerance (Ashraf, 1994; Murakeozy *et al*., 2003). Sugars are also involved in osmoprotection, osmoregulation, carbon storage and radical scavenging activities (Parvaiz & Satyawati, 2008). On the other hand, insoluble and total sugars were reduced in higher salinity which is supported by Parida *et al*. (2002) and Gadallah (1999) who found similar results in *Bruguiera parviflora* and *Vicia faba*.

Total soluble proteins of *C. cajan* were reduced due to deleterious effects of salinity. Accumulation of Na^+ in cytosol disrupts protein and nucleic acid synthesis (Bewley & Black, 1985). Gill & Sharma (1993) and Muthukumarasamy & Panneerselvam (1997) also reported decreased protein content in *C. cajan* seedlings with increasing salinity. Similar results were found in *Lycopersicon esculentum* (Azeem & Ahmad, 2011), *Zingiber officinale* (Ahmad *et al.,* 2009) and some genotypes of *Sorghum bicolor* (Ali *et al.,* 2013; Kausar *et al*., 2014) when grown under variable salt concentrations.

Cajanus cajan showed a salt sensitive growth response, however its growth was not reduced by 50% at 3.5 $dS.m^{-1}$ sea salt salinity (EC_e). Plant could survive salinity by slow down RGR, keeping low water content and leaf succulence and investing more energy towards the synthesis of organic osmolytes. Production of flowers, pods and seeds were compromised with salinity, however, unaffected legume production under low saline medium highlights the potential of *C. cajan* to be grown at marginal soils. In addition, this plant could provide protein rich edible seeds and nutritious green fodder which could also help to improve socio-economic condition of poor farmers from theoretically unproductive soils of particularly arid world.

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