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REGULAR ARTICLE

Salt Stress induced changes in growth, pigments and protein contents in two horse gram [*Macrotyloma uniflorum* (Lam.) Verdc] varieties

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Abstract

Growth parameters and photosynthetic pigments changes in horse gram were investigated under salinity of different concentrations (0, 40, 80. 120mM). The two horse gram varieties Paiyur-2 and CO-1 were sampling was done in young and fully matured leaves were taken from control and salinity treated plants on 15th Days After Treatment (DAT) and 30th DAT. Treatments were planted in pots. Growth parameters such as plant height, lea area, fresh and dry weight of the whole plants decreased in both varieties under salinity stressed condition. Photosynthetic pigments such as total chlorophyll, chlorophyll 'a' chlorophyll 'b' were significantly reduced in the salinity stressed leaves. Quantitative differences with response to salinity. Were also noticed in the content of soluble protein in two horse gram varieties. Our data revealed that Paiyur-2 maintained lower reduction of growth and higher contents of photosynthetic pigments as well as soluble protein content when compared to variety CO-1 during the adverse effect of salinity stress.

Key words: NaCl, Photosynthetic pigments, Growth, Protein, Horse gram

Introduction

Salinity is one of the major abiotic stresses in arid and semi-arid regions but salt-affected soils have been recorded in practically all the climatic regions where more than 800 million hectares of agricultural and or over 6% of the world surfaces are salt affected. Sodium chloride is the most soluble, pervasive, and superabundant salt in the world (FAO, 2000; Munns and Tester, 2008). Rapid population growth and subsequent food shortage especially in Asia and Africa and advancing salinity in arable land due to climate change have increased the importance of finding salt tolerant genotypes (Blumwald et al., 2004). Growth and potential yield are the main factors affected by salinity (Khalida and Da Silva, 2010). Plant growth will be reduced with the onset of salinity due to osmotic stress due to lowering of the water potential or specific ions interaction on metabolic processes (Munns, 2002). The metabolic imbalances due to ionic toxicity, osmotic stress and nutritional deficiency may lead to oxidative stress (Hasaneen et al., 2009; Baatour et al., 2010). The major plant metabolisms like photosynthesis, protein synthesis, lipid and energy metabolism are being affected by salinity (Parida and Das, 2005; Desingh et al., 2006; Desingh and Kanagaraj, 2007).

Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc] is a popular pulse, locally known as Gaheth belongs to the family Fabaceae that still remain an under exploited legume crop. Horsegram is one among the nutritious pulses and have many medicinal values. In India, horsegram is commonly known as *Kollu*

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(Tamil). Seeds are rich in protein and consumed in majority by poorest section of the society. It is native to the old world tropics. It was probably domesticated in India where its cultivation known since prehistoric times. It is one of the most important food legume being grown in almost all over the world including temperate and sub-tropical regions (Durga, 2012; Krishna, 2010). In India it is the most extensively grown pulse in south India. Apart from the nutritional components it contains iron, molybdenum and calcium (Bhokre, 2012). Seed contains carbohydrate (57%), protein (22%), dietary fibre (5%), fat (0.50%), calcium (287mg), phosphorous (311mg), iron (6.77mg), calories (321 Kcal) as well as vitamins like thiamine (0.4mg), riboflavin (0.2mg) and niacin (1.5mg) per 100g of dry matter (Gopalan et al., 2000; Bolbhat and Dhumal, 2012; Bhartiya et al., 2015). It also helps in lowering cholesterol levels and has a role as it contains antioxidants. Horse gram is famous for its medicinal uses because different parts of the plant are used for the treatment of heart disease, Asthma, bronchitis urinary discharges and eliminating kidney stones and water is prescribed for treating jaundice (Ghani, 2003).

In India, horse gram has a wide geographic distribution extending over a range of environmental conditions. However, as other crops in India, horse gram is also subjected to environmental stresses, particularly salinity. Although much information is available on the agronomics aspects of horse gram, very little is known about the effects of salinity on physiological and biochemical aspects of horse gram. The present study was undertaken to evaluate the salinity responses of two horse gram varieties [*Macrotyloma uniflorum* (Lam.) Verdc] usually used for cultivation.

Materials and methods

Plant material and growth conditions

The certified Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc] seeds (Variety: CO–1, PAIYUR–2) were procured from Tamilnadu Agriculture University, Coimbatore and Paiyur. Seeds with inform size were selected and the plants were raised in pots containing red and clay soil and pH of the soil was 7.2 with EC of 0.2 dsm^{-1}. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. Plants were grown under natural climatic conditions. The maximum irradiance (PAR, 400-700nm) available during growth was 1800-2000 μmol m^{-2}s^{-1} on a clear day. Daily maximum and minimum temperatures were 29-33^{o}C and 20-22^{o}C, respectively. Plants were watered for the first 20 days after germination.

Salinity treatments

The seedlings were divided into four groups. One group of seedlings was maintained under non-salinized conditions which served as control plants. The watering solution for control plants consists of tap water and one-fourth strength of Hoagland nutrients (Hoagland and Arnon, 1950). Other three groups were salinized by irrigation daily to soil capacity (500 ml d^{-1}) with the nutrient medium containing 40 mM, 80 mM and 120 mM NaCl. 40mM consider as a low salinity level, 80mM consider as a medium salinity level and 120mM salinity consider as a high salinity level. All the plants used in this study were of comparable size.

Sodium chloride used in this study was Laboratory AR grade Assay 99.8%, (Universal Laboratories Pvt. Ltd. Mumbai). Salt treatment was continued until each plant received the required mM NaCl. Care was taken for individual plants in each group received the pre-calculated concentrations of NaCl in full. Additional pots with plants were also maintained for control, as well as each salinity treatment for need of plant material.

Sampling days

Young and fully matured leaves were taken from control and salinity treated plants on 15th Days After Treatment (DAT) and 30th (DAT) for all the experiments described below.

Growth components

Height of the plant

The height of the plant was measured with a measuring tap on 15th DAT and 30th DAT.

Leaf area

The leaf area was calculated multiplying the length and breadth of the broadest regions of the leaf.

$$\text{Leaf area} = \text{length} \times \text{breadth}$$

Fresh weight of the whole plant

Mature plants were carefully uprooted. The roots were washed, blotted and whole plant was weighed.

Dry weight of the whole plant

Mature plants were carefully uprooted and the roots were washed, blotted and the whole plant was dried in an oven at 75-80° for 40 hours until a constant weight was obtained.

Photosynthetic pigments

Total chlorophyll

The total chlorophyll content of the leaves was estimated according to Arnon, (1949).

Protein content

Total leaf protein content was estimated by Lowry's method (1951) using Folin-Ciocalteu reagent.

Statistical analysis

Data for each parameter analyzed by Two-Way ANOVA and significant differences between treatment mean and varieties were determined by using SPSS (version 15.0, SPSS, Chicago, IL, USA). Data are presented as the mean ± SE of five independent determinations and significance was determined at the 95% confidence ($P \leq 0.05$) limits.

Results and discussion

Plant height

Plant height was decreased with increasing salinity levels (40mM, 80mM and 120mM) in all two horsegram varieties on all the sampling days (15thDAT (Days After Treatment) and 30th DAT) and it was shown in Fig. 1. Maximum plant height was recorded in the variety PAIYUR-2 (32.51 cm) under high salinity (120mM) on 30th DAT relative to control plants (60.46 cm respectively) while minimum plant height was recorded in CO-1 (29.13 cm) over the control plants (51.35 cm respectively). Currently, there are about 20 % of the world's cultivated land are affected by salinity (Zhu, 2001). Hence, salt stress is the major hindering factor for crop productivity (Munns, 2002; Perez-Tornero et al., 2009). In the present study, the values of plant height was lowered by increasing salinity and were more pronounced using the highest concentration of NaCl (120 mM) compared to untreated control plants of horsegram varieties (Fig. 1). However, lower reduction in plant height was observed PAIYUR-2 with high salinity on all the sampling days (15th DAT and 30th DAT), while significantly higher reduction was recorded in CO-1 on all the sampling days under salinity stress. Reduction in growth under salinity has been reported in various plant species e.g. rice (Demiral and Turkan, 2006), tomato (Kaya et al., 2001), cotton (Kanagaraj and Desingh, 2009), Finger millet (Manikandan and Desingh, 2009a). The reduced plant height exposed to saline medium might be due to the continued effect of decreased shoot and root length, leaf number and leaf area (Maggio et al., 2007; Baatour et al., 2010). Our data on plant height suggest that variety PAIYUR-2 maintained its better height on all the sampling days under varying salinity levels compared to other varieties, indicating substantial salt tolerance.

Leaf area

Salt stress affects the leaf area by reducing the leaf expansion (Abbruzzese et al., 2009; Wang and Nil, 2000; Cramer, 2003). This reduced leaf area minimizes the light interception and thereby photosynthesis (Parida and Das, 2005). In our study, all varieties of horsegram plants showed reduction in surface area of the leaves on exposure to salinity. Among the two varieties, PAIYUR-2 exhibited lower reduction of leaf area under salinity stress even on 30th DAT relative to control plants, while comparatively higher reduction of leaf area was observed in CO-1 under salinity stress. Cell expansion and division processes are affected by Salinity, which in turn results in the reduction in leaf area (Ticha, 1982; Curtis and Lauchli, 1987) and cell division (Hasegawa et al., 2000). Leaf area was measured in salinity treated and control plants of two horsegram varieties on two sampling days (Fig. 2). On 30th DAT, significantly higher reduction of leaf area was measured in the variety CO-1 (6.14 cm^2) over the control plants (21.58 cm^2 respectively) with 120mM salinity stress, while lower reduction of leaf area was observed in the variety PAIYUR-2 (8.38 cm^2) compared to control plants (26.23 cm^2 respectively). The results on leaf area clearly indicated that under all salinity levels, PAIYUR-2 recorded higher leaf area in the plants on all the sampling days, which is relative to higher photosynthetic rates.

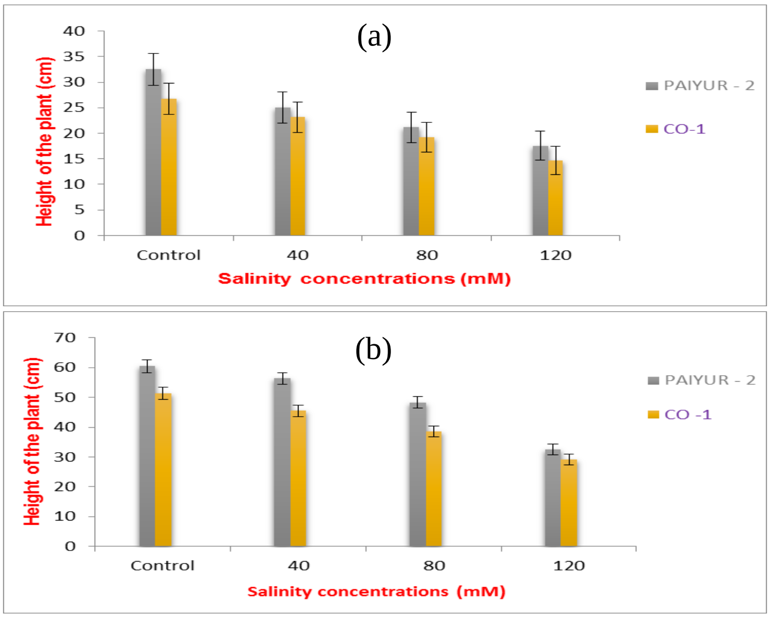

Figure 1. Effect of varying levels of salinity on plant height of two horsegram varieties on 15th DAT(a), 30th DAT(b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

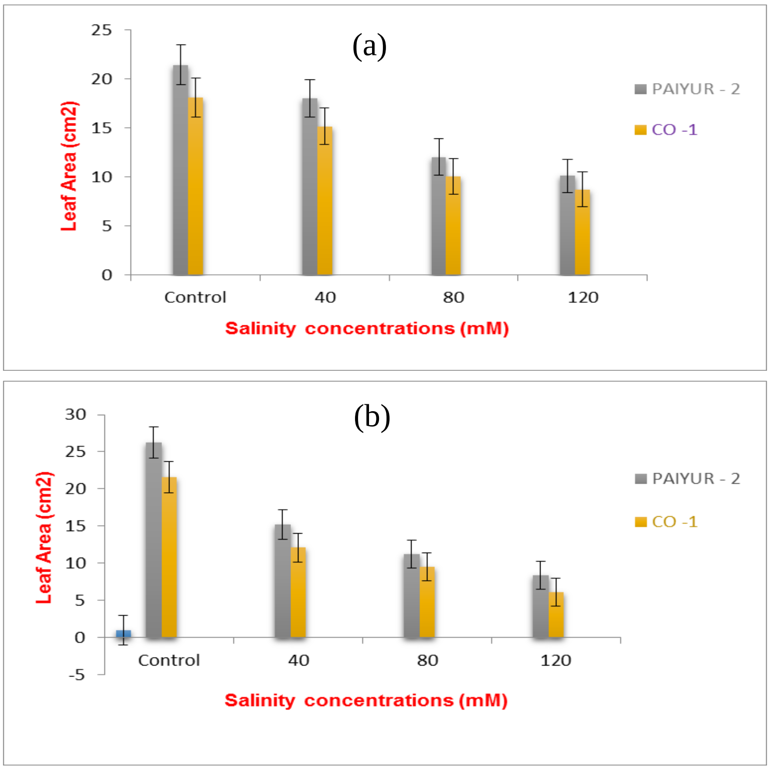

Figure 2. Variation of leaf area of two horse gram varieties on 15th DAT(a), 30th DAT(b) under varying levels salinity. Each value represents mean ± SE of five independent determinations ($p<0.05$).

Fresh weight of the whole plant

The lowest fresh and dry weight of the whole plant was noted in CO-1 under all the levels of salt stress. The main reason for this is the reduced absorption and translocation with reduced biochemical metabolism under salinity (Parida and Das, 2005). Fresh weight of the whole plant was decreased with increasing salinity concentrations on all the sampling days in two horsegram varieties (Fig. 3). On 30th DAT, the significantly higher decrease in fresh weight of the whole plant was observed in CO-1 by 38% (8.12gram) with 120mM salinity relative to control plants (20.85gram respectively), while lowest decrease in fresh weight of the whole plant was recorded in PAIYUR-2 by 24% (10.43gram) over the control plants (26.65gram respectively). The present data on biomass revealed that horse gram variety PAIYUR-2 recorded higher biomass even under higher salinity level compared to other varieties which is directly related to growth and yield of the plant.

Dry weight of the whole plant

The dry mass is mainly affected by salinity, the main reason stands in the reduction of leaf growth and decline in the rates of net photosynthesis and CO_2 assimilation (Sharkey, 1985; Aragao et al., 2005). Reduced growth under salt stress is due to osmotic reduction in availability of water and accumulation of ions (Marschner, 1995). Fig. 4 showed variation of the dry weight of the whole plant under salinity stress and it was decreased with increasing salinity levels on all the sampling days. Maximum reduction of dry weight of the whole plant was recorded in the variety CO-1 and it was (3.45gram respectively) compared to controls (5.73gram respectively) on 30th DAT with 120mM salinity, while minimum reduction of dry weight of the whole plant was noticed in the variety PAIYUR-2 and it was 3.86 gram respectively relative to control (6.25 gram respectively). The reduction in plant dry weight can be attributed to the reduced photosynthetic capacity of the leaves under salinity stressed conditions (Sanchez-Rodriguez et al., 1999).

Total chlorophyll

In the current study, the total chlorophyll content of leaves averaged over two varieties indicated that it decreased significantly with the increase in salt concentration. Effect of salinity on total chlorophyll content was studied in two horsegram varieties and it was decreased with increasing salinity levels on all the sampling days as shown in Fig. 5. On 30th DAT highest total chlorophyll content was recorded in the variety PAIYUR-2 (0.68 mg/gfw) over to control plants (1.28 mg/gfw, respectively) under 120mM salinity stress, whereas low total chlorophyll content was observed in the variety CO-1 (0.57 mg/gfw) relative to controls (1.20 mg/gfw, respectively). Varieties differed significantly under salt stress treatments. However, highest total chlorophyll content under salinity stress was recorded in PAIYUR-2 on all the sampling days (15th DAT and 30th DAT) even at high salinity concentrations, whereas low level of total chlorophyll content was observed in CO-1 under salinity stress. Chlorophyll content is one of the major component of salt tolerance in crop plants (Srivastava et al., 1988). According to Hernandez et al., (1995) the elevation in chlorophyll degradation in salt sensitive pea cultivar was more when compared to tolerant one. NaCl reduces chlorophyll content in crop plants such as broad bean (Gadallah, 1999), cotton (Boyer, 1965) and rice (Sultana et al., 1999). The earlier reports in cucumber (Kaya et al., 2003) and tomato (Agong et al., 2003; Doganlar et al., 2010) supported our results. Chlorophyll reduction is correlated with increased production of chlorophyll-degrading enzyme, chlorophyllase and ion accumulation in leaves (Sultana et al., 1999).

Chlorophyll 'a' and 'b'

On all the sampling days, Chlorophyll 'a' and 'b' content was decreased with increasing salinity levels in all the horsegram varieties (Figs. 6 & 7). On 30th DAT, with 120mM salinity treatment, lowest chl 'a' and chl 'b' content was observed in the variety CO-1 and it was 0.20 mg/gfw and 0.37 mg/gfw, respectively, over the controls (0.43 mg/gfw and 0.77 mg/gfw, respectively), while highest chl 'a' and chl 'b' content was recorded in the variety PAIYUR-2 and it was 0.23 mg/gfw, 0.45 mg/gfw, respectively, compared to control plants (0.45 mg/gfw and 0.83 mg/gfw, respectively). Photosynthetic pigments like chlorophylls 'a' and 'b' have major role in the photosynthetic efficiency (Taiz and Zeiger, 2006). In our study, salinity stress led to a decrease in chlorophyll 'a' and 'b' on all the sampling days (15th DAT and 30th DAT) and this effect increased consistently with increasing salinity levels as compared to non-stressed treatment. However, higher reduction of chlorophyll 'a' and 'b' was observed in CO-1 and lowest reduction was noticed in the variety PAIYUR-2 on all the sampling days with varying salinity levels. Similar results were reported in tomato (Mohammad et al., 1998), cotton (Meloni et al., 2001) under salinity stress. The present study on the pigment composition clearly showed that the variety PAIYUR-2 maintained high pigment content on all the sampling days than other horse gram varieties when subjected to salt stress.

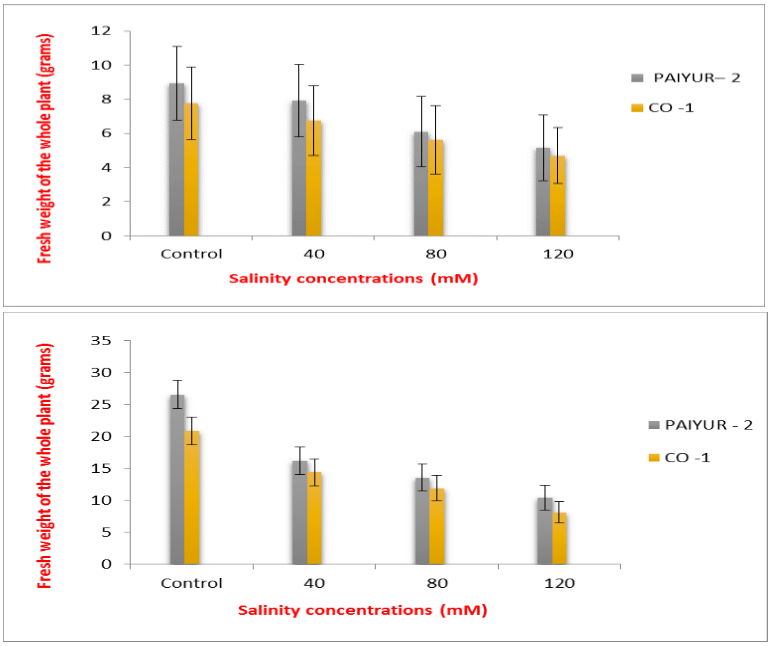

Figure 3. Influence of varying levels of salinity on fresh weight of the whole plant of horse gram varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations (p<0.05).

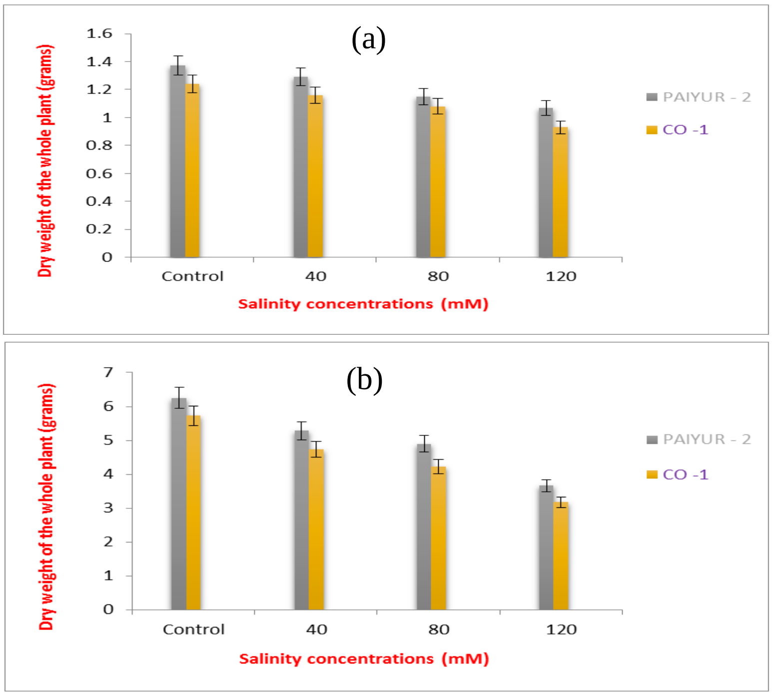

Figure 4. Salinity stress effects on dry weight of the whole plant of horse gram varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations (p<0.05).

Protein content

In this investigation, when NaCl concentration increased, a soluble protein in two horse gram variety was significantly changed. Comparatively, lower decrease of soluble protein content was observed in the leaves of horse gram variety PAIYUR-2 on all the sampling days even with high salinity levels as compared to controls. More decrease of soluble protein content was observed in the variety CO-1, on all the sampling days compared to control plants. High temperature, salinity and drought stress can cause denaturation and dysfunction of many proteins (Vinocur and Altman, 2005). Salinity treatment caused a depletion of protein from, shoot and root tissues of lentil (Misra and saxena, 2009) and *Phaseolus aureus* (Misra and Dwived, 1990). Protein content was decreased in leaves of all the two horse gram varieties with increasing salinity level on all the sampling days (Fig. 8). Under 120mM salinity stress, on 30th DAT, protein content was highly decreased in CO-1 by 50% (29.73 mg/gfw) over the control plants (58.57 mg/gfw, respectively), while low decrease of protein content was recorded in the variety Paiyur-2 by 26% (32.68 mg/gfw) relative to control plants (60.51 mg/gfw, respectively). The decrease in soluble proteins in leaves by salinity indicated that salinity might have promoted hydrolysis of protein resulting in an accumulation of proline particularly at high concentration of NaCl and or inhibited protein synthesis. The results in this study clearly indicates that even though the protein synthetic machinery of the horse gram varieties are affected by the salinity stress, variety Paiyur-2 maintained higher protein content on all the sampling days compared to other varieties. There will be difference in the response of plant species to salinity and even different cultivars within the same species (Munns, 2002; Borsani et al., 2003). Under saline conditions, there is a change in the pattern of gene expression and both qualitative and quantitative changes in protein synthesis (Amini and Ehsanpour, 2005; Xu et al., 2010).

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Author contributions

All authors contributed equally in the study and preparation of article. All authors approved the final version of the manuscript for publication.

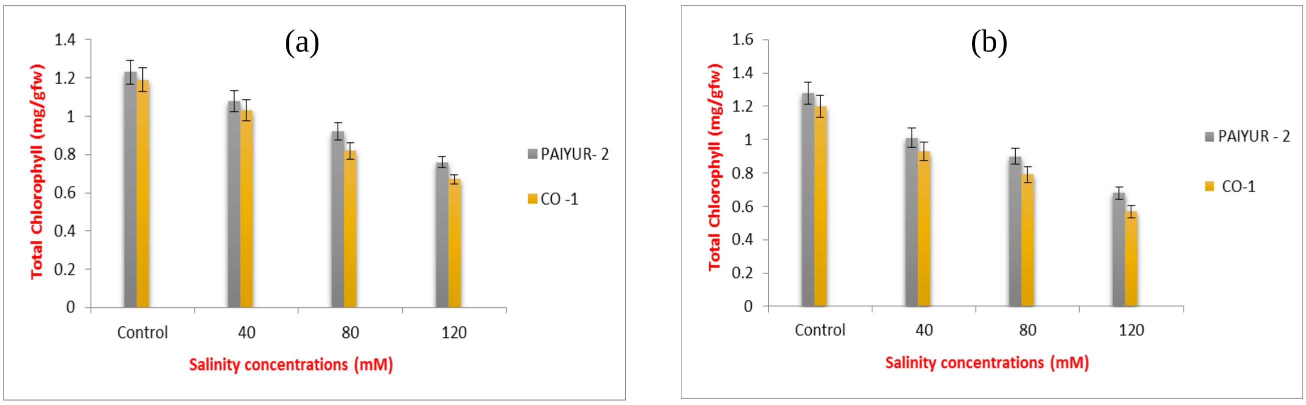

Figure 5. Changes of total chlorophyll content in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b) under varying levels of salinity.
Each value represents mean ± SE of five independent determinations ($p<0.05$).

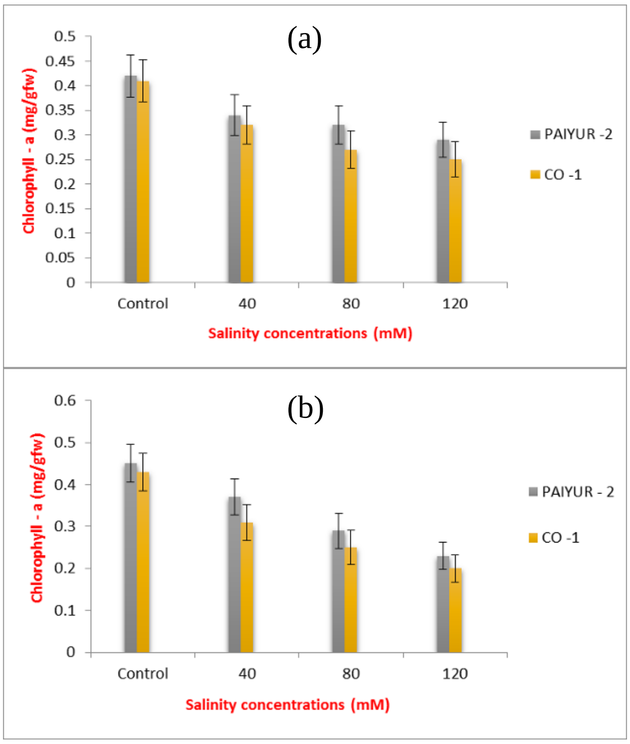

Figure 6. Influence of varying levels of salinity on chlorophyll 'a' content in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

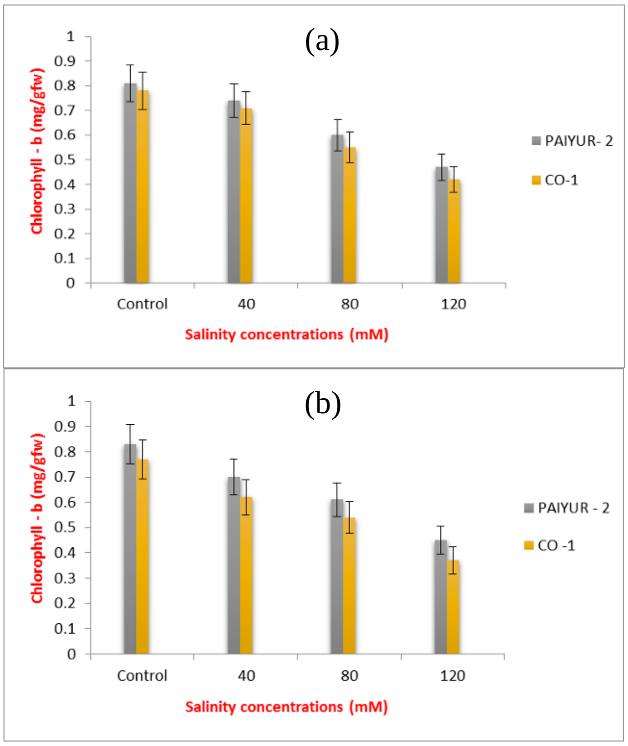

Figure 7. Effect of varying levels of salinity on chlorophyll 'b' content in leaves of horse gram varieties on 15th DAT (a) 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

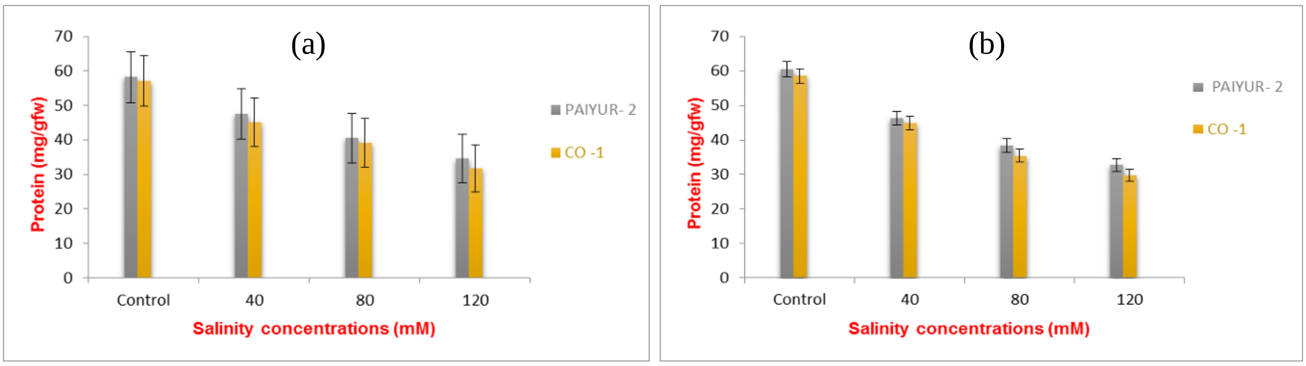

Figure 8. Effect of different salinity levels on soluble protein content in the leaf extracts of horse gram varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

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