Journal of Scientific Agriculture 2017, 1: 37-45
doi: 10.25081/jsa.2017.v1i0.30
http://updatepublishing.com/journals/index.php/jsa

REGULAR ARTICLE

Effect of salinity stress on carbohydrate, lipid peroxidation and proline contents of two horse gram [*Macrotyloma uniflorum* (Lam.) Verdc] varieties

G. Kanagaraj* and C. Sathish

PG and Research Department of Botany, Government Arts College for Men, Krishnagiri 635 001, Tamil Nadu, India.

Abstract

This study was conducted to investigate the effects of different concentrations (0, 40, 80 and 120mM) of salinity on horse gram [*Macrotyloma uniflorum* (Lam.) Verdc] plants grown in pots. The two horse gram varieties PAIYUR-2 and CO-1 were used for the study. Sampling was done on 15th Days After Treatment (DAT) and 30th DAT from control and salinity treated plants. The response of the horse gram plants to salinity stress was analysed by estimating the levels of carbohydrates, starch, lipid peroxidation, proline and glycine betaine. Higher salinity markedly reduced sugar concentration in both horsegram varieties, while starch content showed reverse trend. Lipid peroxidation (estimated by MDA content) significantly increased under salinity in both varieties but the rate of increment was higher in CO-1. The concentration of proline and glycine betaine were also altered under salinity. From the results of this investigation, it may be concluded that plants of variety PAIYUR-2 have high adaptive potential under salinity when compared to variety CO-1.

Key words: Salinity stress, sugars, starch, proline, horse gram, carbohydrates, osmolytes

Introduction

Throughout the world salt stress is one among the major abiotic stresses. If the quantity of salt is high in the soil it will diminish crop growth and productivity. Almost 20% of the world's cultivated area is affected by salinity (Kaya et al., 2002). The plant response to salt and such other abiotic a stress is complex and involves a large number of events. Many studies reported the complex phenomenon that controls the salinity tolerance in plants through morphological, developmental, physiological and biochemical processes (Desingh and Kanagaraj, 2007; Zhang et al., 2010; Manivannan et al., 2017). In glycophytes, the salinity declines the productivity in all aspects (Hasegawa et al., 2000; Kanagaraj et al., 2009). The secondary metabolite accumulation like compatible solutes is the main metabolic change that supports stress protection in plants. Osmolytic compounds like proline, glycine betaine helps in stress protection and can be found in almost all abiotic constraints in plants (Hasegawa et al., 2000). Together with antioxidant enzymes, they can deactivate or scavenge the Reactive Oxygen Scavengers as well (Hellman et al., 2000; Ashraf and Foolad, 2007).

In plants, the salt stress conditions hinder the basic metabolism like photosynthesis, photorespiration, amino acid and carbohydrate synthesis (Sengupta and Majumdar, 2009; Ahuja, 2001; Murakeozy et al., 2003). Carbohydrates can play a role in osmoprotection, osmotic adjustment, carbon

Received 02 April 2017; Revised 05 May 2017; Accepted 07 May 2017; Published 07 May 2017

*Corresponding Author

Dr. G. Kanagaraj
PG and Research Department of Botany, Government Arts College for Men, Krishnagiri 635 001, Tamil Nadu, India.

Email: kanagabot15@gmail.com

storage, and radical scavenging during salt stress conditions. Oxidative stress induced by ROS is the major issue during abiotic stress like salinity, and if not scavenged, it can lead to impaired metabolism (Mittler, 2002).

Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc] is one among the highly nuttitious pulses crop, belongs to the family Fabaceae. This crop can adapt harsh environmental conditions (Kumar, 2006). Apart from that, this is still remaining an under exploited legume crop. Horsegram is having many ethno-medicinal values in India (Pati and Bhattacharjee, 2013). In India it is the most extensively grown pulse in south India, the maximum area being in Andhra Parades, Karnataka and Tamil Nadu. PAIYUR-2 and CO-1 are two important horse gram varieties grown in India. Elucidation of the biochemical and molecular mechanisms by which horse gram plants tolerate environmental stresses is necessary for breeding and genetic engineering approaches to improve horse gram performance under stress. Even though the role of salinity in limiting the productivity of the crop plants has been studied, limited information regarding the response of the horse gram plants to salinity stress is available. Recently, we reported the effect of salinity on growth and photosynthetic contents of horse gram plants (Kanagaraj and Sathish, 2017). The present study has been undertaken to compare the salinity stress adaptations in two different horse gram cultivars, which are commonly cultivated in India, in order to identify with a better performance even in saline soils.

Materials and methods

plant material and growth conditions

The certified Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc] seeds (Variety: CO–1, PAIYUR–2) were procured from Tamil Nadu Agriculture University Coimbatore and Paiyur (Krishnagiri district). 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 $\mu mol\ m^{-2}s^{-1}$ on a clear day. Daily maximum and minimum temperatures were 29-33^oC and 20-22^oC, 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.

Carbohydrate analysis

The total sugar concentration was estimated by following the method of DuBois et al. (1956) and expressed as mg/gdw. Reducing sugar was estimated by following Somogy's method as modified by Nelson, (1944). The content of the reducing sugar was calculated using glucose as the standard and expressed as mg/gdw. Non-reducing sugar content was calculated by subtracting the amount of reducing sugar from total sugar content. Starch content was estimated according to McCready et al. (1950) and expressed as mg/gdw.

Lipid peroxidation

Analysis of lipid peroxidation was done by measuring the malondialdehyde equivalents by following the method of Hodges et al. (1999). Lipid oxidation rate equivalents (nmol malondialdehyde ml^{-1}) were calculated.

Proline and glycinebetaine

Proline (Bates et al., 1973) and glycinebetaine (Storey et al., 1977) contents were estimated from the leaf extracts and expressed as mg/gfw.

Statistical analysis

Two-Way ANOVA was used for data analysis 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 replicates and significance was determined at the 95% confidence ($P \leq 0.05$) limits.

Results and discussion

Total sugar

Total sugar content was decreased with increasing salinity level on all the sampling days (15DAT and 30DAT) in leaves of two horse gram varieties (Fig. 1). On 30th DAT, with 120mM salinity stress, PAIYUR-2 recorded higher total sugar content (8.13mg/gdw, respectively) relative to control plants (16.12 mg/gdw, respectively), while lowest sugar content was observed in the variety CO-1 (7.08 mg/gdw, respectively) compared to controls (14.80 mg/gdw, respectively). In the current study, higher sugar content was maintained in PAIYUR-2 whereas low sugar content was recorded in CO-1 on all the sampling days with varying salinity concentrations (40, 80 and 120mM) (Fig. 1). Change in soluble sugar content under salt stress has already been reported for a number of species. For example, Ashraf and Tufail, (1995) reported high sugar content in sunflower varieties with high rate of salt tolerance. As already discussed, salinity causes reduced CO_2 assimilation rates which intent might affect the total carbohydrate content in the leaves. It was hypothesized that due to limitation supply of structural and non-structural carbohydrate in horsegram plant growth will be significantly reduced due to limited supply of energy and carbon skeletons during various stages of growth (Poljakoff-Mayber and Lerner, 1994). Our data showed that higher amount of total sugars in variety PAIYUR-2 might be responsible for higher salt tolerance.

The relationship between salinity stress and carbohydrate accumulation patterns in green leaves is very interesting. Soluble carbohydrates have been mentioned as important compounds in osmoregulation in plants under water and salt stresses (Silva-Ortega et al., 2008) and also needs for cell growth, are supplied mainly through the process of photosynthesis and photosynthetic rates are usually lower in plants exposed to salinity especially to NaCl (Kanagaraj et al., 2009). The general trend was that carbohydrate content was reduced under salinity stressed plants. This study provides evidence that the synthesis and accumulation of structural and non-structural carbohydrates is dependent upon salinity stress.

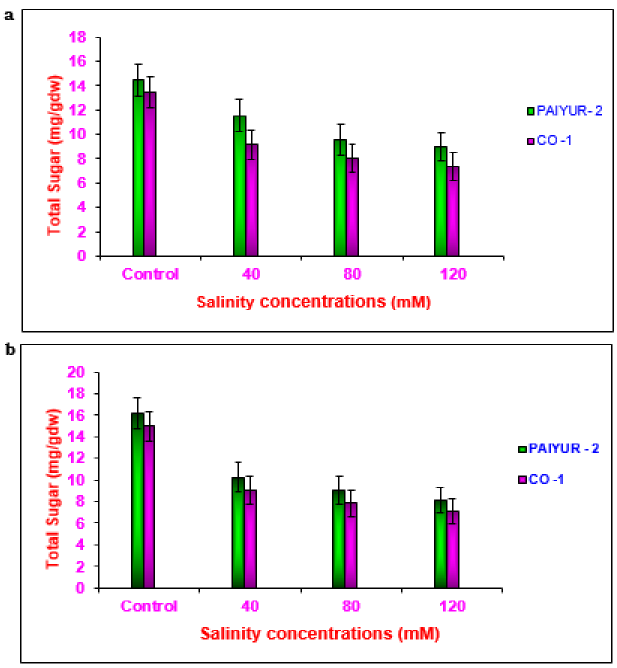

Fig. 1. Changes of total sugar content in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b) under varying levels of salinity. Each value represents mean ± SE of five independent determinations ($p<0.05$).

Reducing and non-reducing sugar

Under salinity stress, reducing and non-reducing sugar content was studied in leaves of two horse gram varieties and it was shown in Figs. 2 & 3. With 120mM salinity stress, on 30th DAT, higher level of reducing and non-reducing sugar content was observed in the variety PAIYUR-2 and it was 7.12 mg/gdw and 1.01mg/gdw, respectively, over the controls (12.90 mg/gfw and 3.22 mg/gfw, respectively), while lower level of reducing and non-reducing sugar content was recorded in the variety CO-1 and it was 6.43mg/gdw and 0.61 mg/gdw, respectively, relative to control plants (11.82 mg/gfw and 2.98 mg/gfw, respectively). Similar pattern of response was observed in the estimation of reducing and non-reducing sugars in horse gram varieties under salinity regime with different sampling days (Figs. 2,3). Adaptation of plants to salinity conditions is achieved by the reduction in the cell growth and homeostasis leading to the accumulation of compatible osmolytes such as ions, amino acids and soluble sugars (Farouk, 2011). Sugars are thought to help control key metabolic processes such as photosynthesis (Krapp et al., 1993; Hasaneen et al., 2009) and starch synthesis and breakdown (Koch, 1996). The sink systems of the plant compete for the limited carbon supplies under salinity which affect the overall plant growth and yield (Munns and Tester, 2008; Daie, 1996).

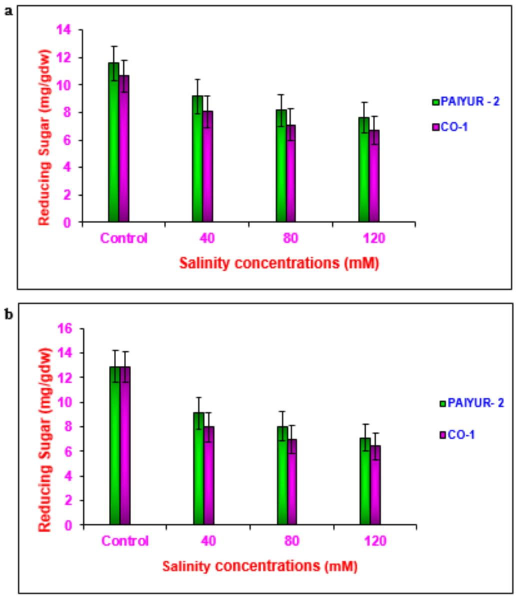

Fig. 2. Changes of reducing sugar content in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b) under varying levels of salinity. Each value represents mean ± SE of five independent determinations ($p<0.05$).

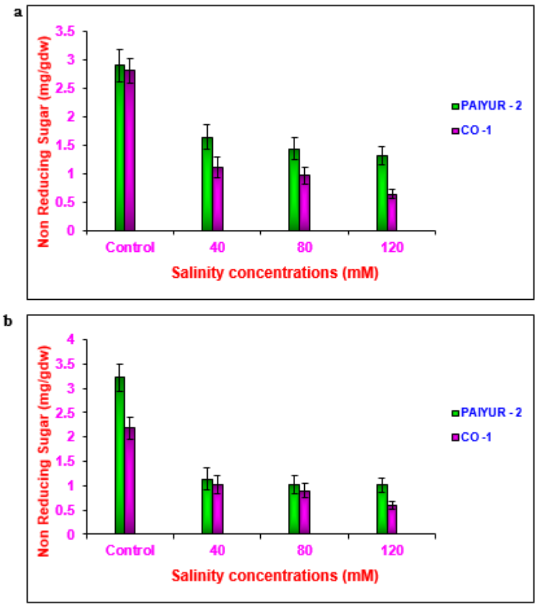

Fig. 3. Effect of varying levels of salinity on non-reducing sugar in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

Starch content

It is surprising to observe that starch content was inversely related to salinity. The reducing levels of starch in control and low salinity concentration (40mM) treated plants indicate that the export of carbohydrates to various organs is at a faster rate compared to those with high salinity treated plants. In our study, starch content was increased with increasing salinity levels on all the sampling days (Fig. 4). Among the varieties, lower increase was observed in the variety PAIYUR-2 while higher increase was noticed CO-1 under salinity stress with different sampling days. The data indicate that starch accumulation as well as its degradation and mobilization is an important physiological criterion in determining the rates of carbon fixation. Starch accumulation is also an important criterion to understand carbohydrate partitioning efficiency. It is presumed that unless the accumulated starch is degraded and mobilized into various regions of plants, further carbon assimilation would not be effective and feedback inhibition of photosynthesis becomes operational. Starch content was increased under salinity stress on all the sampling days in the leaves of horsegram varieties (Fig. 4). Among the varieties, significantly higher starch content was observed in the variety CO-1 (44.88 mg/gdw) over the controls (26.74 mg/gdw respectively) on 30th DAT with 120mM salinity stress, while lower starch content was recorded in the variety PAIYUR-2 (42.84 mg/gdw) relative to control plants (26.18 mg/gdw, respectively). The study also suggests that salinity stress causes significant accumulation of starch in the leaves which might ultimately reduce the CO_2 assimilation patterns in the intact leaves. Salinity stress might alter the export of photo assimilates to the growing regions, thus affecting the overall growth and development of horse gram plants. The results also show that starch accumulation in leaves under salinity stressed conditions of all horsegram varieties, might be due to decreased capacity to metabolize starch, producing during photosynthesis. The regulation of carbon allocation and partitioning would have an important influence in the maintenance of growth rate and yield (Balibrea et al., 1999; Geigenberger, 2011). However, physiological basis, this type of starch accumulation and its metabolism in salinity stressed crop is not clear and would be interesting to follow the effect of salinity on starch metabolism. Our study on carbohydrates clearly indicates that PAIYUR-2 had an effective carbohydrate partitioning mechanism than other horsegram varieties which might contribute for efficient photosynthesis.

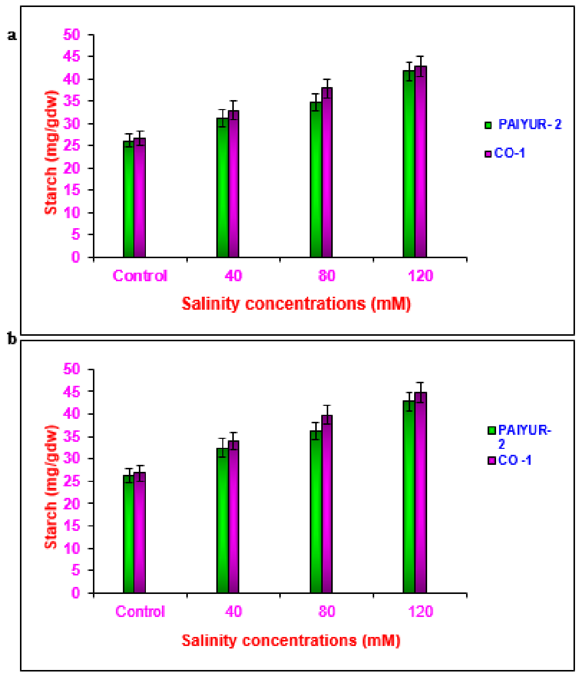

Fig. 4. Levels of starch content in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b) under salinity stress. Each value represents mean ± SE of five independent determinations ($p<0.05$).

Lipid peroxidation

The electrolytic leakage and lipid peroxidation rates were observed in two horsegram varieties such as PAIYUR-2 and CO-1 in control and treated plants of different salinity concentrations (40mM, 80mM, 120mM). Electrolytic leakage and lipid peroxidation was more in varieties CO-1 compared to PAIYUR-2. Lipid peroxidation is a destructive chain reaction and it can directly damage the structure of membrane (Desingh and Ramachandra Reddy, 2005; Koca et al., 2006). Our data also indicate that the degree of cell membrane injury and levels of membrane lipid were relatively less in PAIYUR-2 than CO-1 under salinity stressing conditions. Malondialdehyde content was measured in the leaves of both the horsegram varieties PAIYUR-2 and CO-1 (Fig. 5). Salinity stress has resulted increased content of malondialdehyde with increasing salinity. In PAIYUR-2 at 120 mM salinity malondialdehyde content was increased by 35% compared to control plants. At 120 mM salinity in CO-1 malondialdehyde content was increased by 55% (3.87 nmol/ml) when compared to control plants (2.14 nmol/ml). Cell membrane are the first target of many plant stresses and therefore, if the plant can keep the integrity of the membrane, it will successfully overcome the stress (Bajji et al., 2002; Bor et al., 2003).

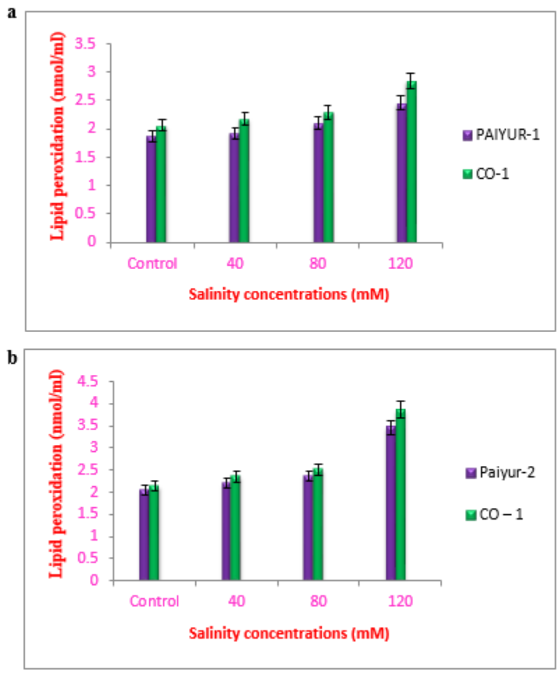

Fig. 5. Salinity stress effects on lipid peroxidation in the leaves of two horse gram varieties on 15th DAT (a), 30th DAT (b) under salinity stress. Each value represents mean ± s.e. of five independent determinations.

Proline (PRO) and glycinebetaine (GB)

In the current study, all the sesame varieties exhibited increased PRO and GB (Fig. 6,7) accumulation in leaves with increasing salinity level compared to the control. We observed a substantial increase in proline level in PAIYUR-2 with all salinity treatments on all the sampling days, which might be attributed to the strategies adapted by plants to cope up with stress conditions. Under salinity stress, PRO content was increased on all the sampling days in the leaves of two horsegram varieties as shown in Fig. 6. Under high salinity (120mM), on 30th DAT, significantly higher enhancement of PRO content was recorded in the variety PAIYUR-2 by 65% (2.48 mg/gfw), over the control plants (6.91 mg/gfw, respectively), while lower increase of PRO content was observed in the variety CO-1 by 50% (3.86 mg/gfw) relative to control plants (1.85 mg/gfw, respectively). PRO always showed high concentration during various stresses especially salt stress and mainly due to their role in stress resistance (Smirnoff and Combes, 1989) and PRO has multiple functions, such as osmotic pressure regulation, protection of membrane integrity, stabilization of enzymes/proteins, maintaining appropriate $NADP^+$, NADPH ratios and scavenger of free radicals (Misra and Saxena et al., 2009; Tiwari et al., 2010). Accumulation of PRO under stress conditions such as high salinity, in plants, has been correlated with stress tolerance (Amini and Ehsanpour, 2005). We observed a substantial increase in pro level in PAIYUR-2 with all salinity treatments on all the sampling days, which might be attributed to the strategies adapted by plants to cope up with stress conditions.

The concentration of GB is always reported high in various stresses mainly salt stress (Carillo et al., 2011). Its biosynthesis in rice is important for increased salt tolerance (Sakamoto and Murata, 1998). It had been reported that the accumulation of GB was found to the high in salt tolerant cultivar (Misra and Gupta, 2005). From our data, it has been noticed that a higher accumulation GB was found in leaves of PAIYUR-2 under salinity stress on all the sampling days. The contributing role of pro and GB to osmotic adjustments under salt stress was confirmed by several researches (Meloni et al., 2001; Khan et al., 2017). The accumulations of PRO and GB under salt stress protect the cell by balancing the osmotic level of cytosol with that of vacuole and external environment (Misra and Saxena et al., 2009; Smirnoff and Cumbes, 1989). We observed a substantial increase in GB level in PAIYUR-2 with all salinity treatments on all the sampling days, which might be attributed to the strategies adapted by plants to cope up with stress conditions. Under salinity stress, GB content was increased on all the sampling days in the leaves of two horsegram varieties as shown in Fig. 7. Under high salinity (120mM), on 30th DAT, significantly higher enhancement of GB content was recorded in the variety PAIYUR-2 by 65% (6.67 mg/gfw), over the control plants (2.27 mg/gfw, respectively), while lower increase of GB content was observed in the variety CO-1 by 50% (3.54 mg/gfw) relative to control plants (1.76 mg/gfw, respectively). It has been reported that higher osmolyte concentration helps in the maintenance of comparatively higher relative water content and antioxidant enzyme activity (Smirnoff and Cumbes, 1989). In cucumber (Tiwari et al., 2010) and in melon (Villora et al., 1998) similar trends of increase in PRO and GB with increase in salt concentration were reported. The present study revealed that the higher osmolyte (PRO and GB) concentration in PAIYUR-2 under salinity stress helped to maintain the structure and function of cellular macromolecules and in favor to better growth with yield.

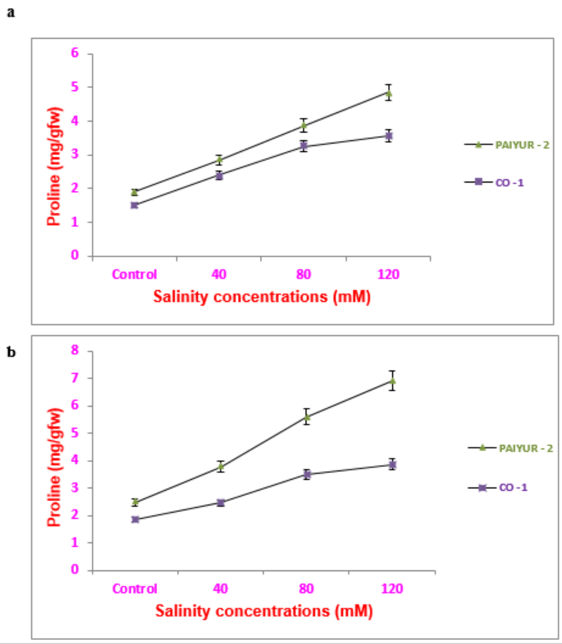

Fig. 6. Levels of proline content in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b) under varying levels of salinity. Each value represents mean ± SE of five independent determinations ($p<0.05$).

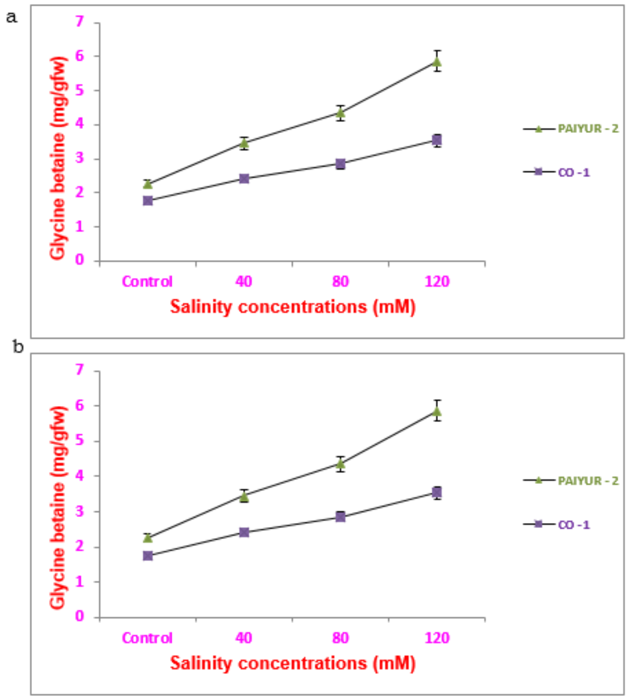

Fig. 7. Levels of Glycine betain content in leaves of horse gram varieties on 15th DAT (a), 30th DAT (b) under varying levels of salinity. Each value represents mean ± SE of five independent determinations ($p<0.05$).

Acknowledgements

The authors are highly thankful to Dr. P. Ravikumar, Professor and Head, for providing necessary facilities.

Author contributions

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

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