



EFFECT OF SALINITY STRESS ON PHOTOSYNTHETIC ENZYMES OF TWO SESAME (*SESAMUM INDICUM* L.) VARIETIES

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Abstract

The effect of increasing NaCl concentrations (0, 40, 80 and 120mM) was studied of two different varieties (VIR-2 and PAIYUR-1) of sesame (*Sesamum indicum* L.) plants grown in pots. Sampling was done on 15th Days After Treatment (DAT) and 30th DAT from control and salinity treated plants. The response of the sesame plants to salinity stress was analysed by estimating the levels of Photosynthetic enzymes activity. Photosynthetic rate, activities of RuBP carboxylase and sucrose phosphate synthase (SPS) decreased with increasing salinity. In addition, the activities of Dichlorophenol Indophenol (DCPIP) Reduction were decreased. The results indicated that plants of variety VIR-2 exhibited higher adaptive potential under salinity stress as judged by increased photosynthetic rate and activities of photosynthetic when compared to variety PAIYUR-1. From the results of this investigation, it may be concluded that plants of variety VIR-2 have high adaptive potential under salinity when compared to variety PAIYUR-1.

Key words : NaCl, Photosynthesis, RuBP carboxylase, SPS, DCPIP.

Introduction

Saline water or soil salinity is one of the most harmful environment problems in agriculture. It affects plant growth and crop production directly. About 37% of the world cultivated land is sodic and 23% is saline (Khan and Duke., 2001). Salinity effects over 70 million hectares of agricultural land which is about 20% irrigated land and 2% dry land (FAO., 2005). Salt Stress induced changes in Photosynthetic pigments, proteins, growth, and yield potential (Kanagaraj and Sathish., 2017). Global network on integrated soil management for sustainable use of salt-affected soils. Currently, salinity affects nearly 33% of all irrigated land in the world (Munns., 2005). Influence of NaCl stress on photosynthetic enzymes parameters such as RuBP carboxylase, sucrose phosphate synthase (SPS) and DCPIP reduction of decreased in plants (Kanagaraj and Desingh., 2019). Salt stress affects plant growth in three different ways: reduction of soil water potential called osmotic stress, causing an ionic imbalance in cells and lastly ion toxicity. Reduction in plant growth is directly related to the soluble salt concentration and duration of stress (Tavakkoli *et al.*, 2011 Salinity can influence several physiological processes including

photosynthesis, transpiration, stomatal conductance and water potential, growth and yield production (Wang *et al.*, 2012, Desingh and kanagaraj., 2019). During salt stress large amount of salt present in the leaf decreased water potential. Leaf stoma was closed due to presence of large amount of salts in the leaf that decreases transpiration and closed stomata reduced CO₂, due to this photosynthesis is decreased (Parida *et al.*, 2004). Salinity reduces photosynthesis by inducing stomatal closure preventing CO₂ diffusion (Brugnoli and Lauteri., 1991). Salinity may also affect non stomatal properties such as chlorophyll synthesis, photosystem structure, electron transport (Lee., 2004). Efficiency of the ribulose-1, 5-bisphosphate carboxylase/oxygenase for carbon fixation (Megdiche., 2008) and photophosphorylation (Stoeva and Kaymakanova., 2008). A major control point for the partitioning of photosynthetic between sucrose and starch in the leaves is SPS (Huber, 1983). Found that not only Rubisco is affected, but also the enzymes involved in regeneration of the Rubisco substrate, ribulose-1, 5-bisphosphate (RuBP) are regulated by salt stress at calvin cycle (Ghosh, 2001). Sesame (*Sesamum indicum* L.) is one of the oldest cultivated plants in the world. It is a

member of the Pedaliaceae family. Gingelly is one of the ancient oil seed crop cultivated for its superior quality oil seed, hence it is regarded as “Queen of the oil seed”. It was a highly prized oil crop of Babylon and Assyria at least 4,000 years ago. Today, India and China are the world’s largest producers of sesame, followed by Burma, Sudan, Mexico, Nigeria, Venezuela, Turkey, Uganda and Ethiopia. Tamilnadu is one of the major sesame growing state in India. Sesame is used in traditional system of medicine for curing many diseases. It has a good composition of amino acids, protein, niacin, folic acid, vitamin E, calcium and phosphorus. In India, sesame has a wide geographic distribution extending over a range of environmental conditions. However, as other crops in India, sesame is also subjected to environmental stresses, particularly salinity. Although much information is available on the agronomic aspects of sesame, very little is known about the effects of salinity on physiological and biochemical aspects of sesame. The present study was undertaken to evaluate the salinity responses of two sesame varieties (*Sesamum indicum* L.), usually used for cultivation.

Materials and Methods

The certified sesame (*Sesamum indicum* L.) seeds (Variety: PAIYUR–1, VIR-2) were procured from Tamilnadu Agriculture University Coimbatore and Paiyur. Seeds with uniform 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 mmol m⁻²s⁻¹ on a clear day. Daily maximum and minimum temperatures were 29-33°C and 20-22°C, respectively. Plants were watered for the first 20 days after germination. 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. 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. 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. Photosynthesis was

measured by following the method of (Sundar and Reddy., 2000). RuBP carboxylase activity was assayed by the method of (Lorimer *et al.*, 1977). Values are expressed as mYYmoles/mgprotein/min. The enzyme sucrose phosphate synthase (SPS) was assayed by the method of (Sinha *et al.*, 1997). Isolation of chloroplast and photochemical activities were done by following the method of (Lilley *et al.*, 1975). The photoreduction of DCPIP by isolated chloroplasts was measured following the decrease in absorbance at 620 nm using the spectrophotometer. The reaction was terminated by turning off the light. The rate of dye reduction was calculated with the help of a standard curve prepared for DCPIP under identical conditions. The values are expressed as mmol/mgpro/min. 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≤0.05) limits.

Results and Discussion

Data in table 1 and table 2 clearly indicates the effects of salinity on photosynthetic enzymes parameters such as Photosynthetic rate, activities of RuBP carboxylase, sucrose phosphate synthase (SPS) and activities of Dichlorophenol Indophenol (DCPIP) Reduction in leaves of two sesame varieties. The rate of photosynthesis in various sesame varieties under salinity stress on different DAT. Under high (120mM) salinity, variety VIR-2 showed highest photosynthetic rate on 30th DAT (2.60 nmol/gfw/s), and relative to control plants (3.72 nmol/gfw/s, respectively), while lowest photosynthetic rate was recorded in PAIYUR-1 on 30th DAT (1.68 nmol/gfw/s) over the control plants (3.03 nmol/gfw/s respectively). 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. In the present study, with the increase of salinity, there was a progressive and evident decrease in photosynthesis in leaves of sesame varieties on all the sampling days. During the whole experiment, photosynthesis in the leaves of plants treated with 0mM NaCl (control) kept relatively constant. However, maximum photosynthetic rate was noticed in the variety VIR-2 on two sampling days under salinity stress, while

PAIYUR-1 recorded lowest. The osmotic adjustment in leaves contributes to the maintenance of water uptake and cell turgor, allowing for physiological processes like stomata conductance, photosynthesis and cell expansion (Serraj and Sinclair., 2002). Higher photosynthetic rate of VIR-2 were positively correlated with RuBP carboxylase activity. Our data clearly demonstrate that VIR-2 is much superior in terms of photosynthetic rates compared to other sesame varieties. The activity of RuBP carboxylase in leaves of two sesame varieties under salinity stress on different sampling days. Highest RuBP carboxylase activity was recorded in VIR-2 on 30th DAT under 120mM salinity and it was 189.2 $\mu\text{molCO}_2/\text{mgpro}/\text{min}$ respectively, relative to control plants (269.12 $\mu\text{molCO}_2/\text{mgpro}/\text{min}$ respectively), whereas lowest RuBP carboxylase activity was noted in the variety

PAIYUR-1 it was 132.93 $\mu\text{molCO}_2/\text{mgpro}/\text{min}$ respectively, over the control plants (251.35 $\mu\text{molCO}_2/\text{mgpro}/\text{min}$ respectively). The decline in the rate of Rubisco-catalyzed reaction was reported to be due to alteration in CO_2 availability at the chloroplast level or the availability of the substrate, RUBP, or the deactivation of Rubisco. Oxidative stress may lead to small subunit degradation, subsequently leading to translational arrest of the large subunit. Alternatively, oxidative stress could initially arrest large subunit translation, resulting in rapid degradation of the unassembled small subunit. Increased activity of RuBP carboxylase in maize and rice under salt stress has also been demonstrated (Kim *et al.*, 2005). In the present investigation, the calvin cycle enzyme RuBP carboxylase was measured in control and salinity treated plants of all sesame varieties. Our results showed the

Table 1: Salinity stress effects on photosynthetic enzymes parameters in the leaves of two sesame varieties on 15th DAT (a), 30th DAT (b). Each value represents mean \pm SE of five independent determinations ($p < 0.05$).

A

Plants and parameters	Salinity concentrations (mM)			
	Control	40mM	80mM	120mM
Photosynthesis				
Vir-2	3.52 \pm 0.42	2.97 \pm 0.28	2.76 \pm 0.15	2.66 \pm 0.13
Paiyur-1	2.98 \pm 0.28	2.11 \pm 0.08	1.94 \pm 0.06	1.89 \pm 0.03
Rulp carboxylase				
Vir-2	262.23 \pm 7.05	237.25 \pm 7.07	222.64 \pm 6.89	194.25 \pm 6.59
Paiyur-1	241.85 \pm 6.32	196.48 \pm 6.24	168.68 \pm 6.13	143.17 \pm 6.05
Sucrose phosphate synthase				
Vir-2	31.74 \pm 1.86	28.67 \pm 1.39	25.82 \pm 1.37	22.74 \pm 1.28
Paiyur-1	29.22 \pm 1.32	22.58 \pm 1.08	19.98 \pm 1.03	17.49 \pm 0.97
Dcpip reduction				
Vir-2	151.90 \pm 6.37	134.42 \pm 6.29	118.34 \pm 6.03	113.45 \pm 5.66
Paiyur-1	140.27 \pm 6.05	115.38 \pm 5.94	91.83 \pm 5.31	84.28 \pm 4.93

B

Plants and parameters	Salinity concentrations (mM)			
	Control	40mM	80mM	120mM
Photosynthesis				
Vir-2	3.72 \pm 0.43	2.92 \pm 0.34	2.69 \pm 0.18	2.60 \pm 0.15
Paiyur-1	3.03 \pm 0.32	1.99 \pm 0.10	1.84 \pm 0.08	1.68 \pm 0.05
Rulp carboxylase				
Vir-2	269.12 \pm 7.08	236.4 \pm 7.10	219.2 \pm 6.92	189.2 \pm 6.64
Paiyur-1	251.35 \pm 6.35	194.9 \pm 6.28	163.6 \pm 6.16	132.93 \pm 6.09
Sucrose phosphate synthase				
Vir-2	32.97 \pm 1.89	25.52 \pm 1.41	24.98 \pm 1.39	21.88 \pm 1.37
Paiyur-1	32.03 \pm 1.82	21.86 \pm 1.26	19.08 \pm 1.20	16.01 \pm 1.05
Dcpip reduction				
Vir-2	155.43 \pm 7.25	132.44 \pm 6.21	116.12 \pm 6.01	109.53 \pm 5.45
Paiyur-1	147.42 \pm 6.16	111.35 \pm 5.90	89.17 \pm 5.25	78.26 \pm 4.85

highest activities of RuBP carboxylase in the leaves of variety VIR-2 under high salinity stress on all the sampling days (15th DAT, 30th DAT), while lowest activity was observed in the variety PAIYUR-1 under salinity stress. In C_3 plants, this enzyme is responsible for the primary step in CO_2 fixation and its carboxylating capacity can be the limiting factor in photosynthesis (Woodrow and Berry 1988). In plants, the salt stress conditions hinder the basic metabolism like photosynthesis, photorespiration, amino acid and carbohydrate synthesis (Sengupta and Majumdar., 2009). Sucrose phosphate synthase (SPS) activity decreased with increasing salinity levels on all the sampling days in leaves of sesame varieties. Significantly higher decrease of SPS activity was observed in variety PAIYUR-1 (16.01 $\mu\text{mol UDP}/\text{mg}$ on 30th DAT, under 120mM salinity stress, compared to control plants

(32.03 $\mu\text{mol UDP/mgpro/min}$ respectively), while lower decrease of SPS activity was recorded in the variety VIR-2 (21.88 $\mu\text{mol UDP/mgpro/min}$) relative to control plants (32.97 $\mu\text{mol UDP/mgpro/min}$ and 29.02 $\mu\text{mol UDP/mgpro/min}$, respectively). Recently, attention has focused on the potential regulatory role of sucrose phosphate synthase (SPS). Sucrose synthesis is catalyzed by SPS and sucrose-6-phosphate and its degradation is catalyzed by sucrose synthase or invertase (Burleigh and Harrison., 1999). In our study, significant decrease of SPS activity was observed in leaves of all sesame varieties under salinity stress on all the sampling days (15th DAT, 30th DAT). However, highest SPS activity was measured in the variety VIR-2 under salinity stress, while lowest SPS activity was observed in the varieties under salinity stress. SPS is a key enzyme in the synthesis of sucrose (Baxter *et al.*, 2003) and is the major transport carbohydrate in a range of plant species (Farrar, 1996) DCPIP reduction was studied under salinity stress on different sampling days in two sesame varieties. Higher 'DCPIP reduction' was recorded in the variety PAIYUR-1 on 30th DAT under 120mM salinity stress and it was 78.26 $\mu\text{mol/mgpro/min}$ respectively, over the control plants (147.42 $\mu\text{mol/mgpro/min}$ respectively), while lower 'DCPIP reduction' was measured in the variety VIR-2 (109.53 $\mu\text{mol/mgpro/min}$) relative to control plants (155.43 $\mu\text{mol/mgpro/min}$). In the same time many plant species are capable of adapting to extreme salinity. Photosynthetic membranes are stress sensitive sites but little information is available regarding the structural changes associated with salt stress (Carter and Chessman., 1993) and the exact mechanisms of membrane damage and protection are still unknown. In this respect the analysis of chloroplast structure and function is an important part of the study of NaCl salinity effect on the photosynthetic apparatus. In the present study, all the sesame varieties shows inhibition of Hill reaction activity (with DCPIP as electron acceptor) in isolated chloroplasts under salinity stress on all the sampling days. However, lower inhibition of DCPIP reduction was observed in the variety VIR-2 even under high salinity on two sampling days, while higher inhibition of Hill reaction activity was noticed in PAIYUR-1 under varying salinity levels as compared relative controls. Photosynthetic machinery is highly sensitive to abiotic stresses such as low temperature, drought, salinity and light intensity (Prasad and Parthasarathi, 2004, Lu *et al.*, 2009). In conclusion, based on the relative tolerance results provided, it seems that VIR-2 is highly salt tolerant in comparison with the other sesame varieties. In fact, for most parameters recorded, better performance was observed in VIR-2 under salt stress on two sampling days at all salinity levels. This variety probably maintains the

photosynthetic enzymes activities and osmotic adjustment with prevents oxidative and other stresses induced by Na^+ with well regulation of biochemical constituents and these traits would be useful as selection criteria during breeding for salt tolerance in sesame.

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