DIFFERENTIAL RESPONSES TO SALT STRESS ON ANTIOXIDANT ENZYMATIC ACTIVITY OF TWO HORSE GRAM [*MACROTYLOMA UNIFLORUM* (LAM.) VERDC] VARIETIES.

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ABSTRACT: *: The present study was carried out to examine salt-induced to effects of different concentrations (0, 40, 80 and 120mM) of salinity on horsegram [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 horsegram plants to salinity stress was analyzed by estimating the levels of antioxidant enzyme activity. In addition, the activities of key antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) also significantly increased. The results indicated that plants of variety PAIYUR-2 exhibited higher adaptive potential under salinity stress as judged by increased antioxidative enzymes activities when compared to variety CO-1. 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: *Salt Stress, Antioxidant System, Horse gram, Osmotic stress*

INTRODUCTION

Plants throughout their life cycle experience various types of environmental stresses (such as drought, salinity, high temperature, cold, heavy metal and other similar stresses) due to their sessile nature. Among these stresses, salinity stress has become the limiting factor for the productivity of agricultural crops by affecting germination, plant vigor and finally crop yield[1]. Salinity effects are more conspicuous in arid and semi-arid areas where 25% of irrigated land is salt affected. Salinity hampers growth and yield of plants [2]. There are various effects of salinity stress on plants such as ion toxicity, water stress, oxidative stress, nutritional imbalances, alterations in metabolic processes, disorganization of membranes, reduction in division and expansion of cells, and genotoxicity[3]. Oxidative mechanisms in plants include the production of reactive oxygen species (ROS) (superoxide radicals (O2.-), hydrogen peroxide (H2O2), and hydroxyl radicals (OH)[3]. Under physiological steady-state conditions, there is a balance between the production and scavenging of ROS[4]. However, this homeostasis can be disturbed by a number of adverse environmental factors. Enzymatic ROS-scavenging mechanisms in plants include production of superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), and glutathione reductase (GR). The extent of oxidative stress experienced in a cell is determined by the levels of superoxide, H2O2, and hydroxyl radicals. Additionally, a balance among SOD, APX, and CAT activities is crucial for suppressing toxic ROS levels within cells[5]. GR activity regulates the redox potential of cells and is important for the physiological needs of cells under oxidative stress. The role of GR is to protect the cell against oxidative stress effects by maintaining a high reduced glutathione-to-oxidized glutathione (GSH/GSSG) ratio[1]. These adverse effects collectively lead to the reduction in plant growth, development and finally biological yield. The decrease in photosynthesis under saline conditions is considered as one of the most important factors restricting plant growth and productivity[6]. Photosynthesis is severely affected during salinity stress which is mediated through a decrease in stomatal conductance[7], internal CO_2 partial pressure and gaseous exchange through stomata[8]. Different studies in plants with salinity stress are increasing; sucrose phosphate synthesis enzymes activity increasing and inverts enzymes activity was reduced[9]. A major control point for the partitioning of photosynthetic between sucrose and starch in the leaves is SPS [12]. Found that not only Rubisco is affected, but also the enzymes involved in regeneration of the Rubisco substrate, ribulose-1,5-bisphophate (RuBP) are regulated by salt stress at calvin cycle.[13] 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 of the highly nutritious vegetable pulse crops with ethno-medicinal values in India, which is commonly known as *Kollu* (Tamil). In India, horsegram has a wide geographic distribution extending over a range of environmental conditions. However, as other crops in India, horsegram is also subjected to environmental stresses, particularly salinity. Although much information is available on the agronomics aspects of horsegram, very little is known about the effects of salinity on physiological and biochemical aspects of horsegram. The present study was undertaken to evaluate the salinity responses of two horsegram 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 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. 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 .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.

ANTIOXIDANT ENZYMES

The supernatants were collected and used for the assays of protein content by the method of[11].

SUPEROXIDE DISMUTASE

The activity of SOD was assayed by the method of[11].

CATALASE

The activity of CAT was estimate by the method of[12].

GLUTATHIONE REDUCTASE

GR activity was measured by using the method of[13].

ASCORBATE PEROXIDASE

APX activity was estimated by monitoring the decline in absorbance at 240nm following [14].

PEROXIDASE

POD activity was determined the method of [15].

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

SUPEROXIDE DISMUTASE ACTIVITY (SOD)

SOD activity increased in the leaves of horsegram varieties with increasing salinity concentrations and it was shown in **Figure 1.** In the variety PAIYUR-2 maximum SOD activity was monitored (140.50 µmol/mgpro/min) under high salinity (120mM) on 30th DAT, relative to control plants (112.08 µmol/mgpro/min respectively), while in CO-1 minimum SOD activity was recorded (136.60 µmol/mgpro/min) over the control plants (109.75 µmol/mgpro/min respectively).

CATALASE ACTIVITY (CAT)

Salinity stress also caused increase of catalase activity in leaf extracts of two horsegram varieties (Figure 2). The increased activity of catalase was observed in PAIYUR-2 and CO-1 at 150 mM salinity to the tune of 51% (31.93 mmol/mg protein/min) and 50% (28.09 mmol/mg protein/min) respectively when compared to the respective control plants (16.79 mmol/mg protein/min, 14.16 mmol/mg protein/min, respectively).

GLUTATHIONE REDUCTASE ACTIVITY (GR)

GR activity increased in the leaves of two horsegrame varieties with increasing salinity concentrations and it was shown in **Figure 3.** In the variety PAIYUR-2 maximum GR activity was monitored (57.49 µmol/mgpro/min) under high salinity (120mM) on 30th DAT, relative to control plants (32.82 µmol/mgpro/min respectively), while in CO-1 minimum GR activity was recorded (48.75 µmol/mgpro/min) over the control plants (30.67 µmol/mgpro/min respectively).

ASCORBATE PEROXIDASE ACTIVITY (APX)

With increasing salinity level, APX activity increased on all the sampling days in the leaves of two varieties **(Figure 4).** On 30th DAT, under 120mM salinity, significantly horsegram higher enhancement of APX activity was recorded in PAIYUR-2 (31.81 mmol/mgpro/min) followed by over the control plants (23.43 mmol/mgpro/min respectively), whereas lower level of APX activity was observed in the variety CO-1 (28.34 mmol/mgpro/min) relative to control plants (20.03 mmol/mgpro/min respectively).

PEROXIDASE ACTIVITY (POD)

Peroxidase (POD) activity was increased with increase in salt stress level on all the sampling days in leaves of horsegram varieties **(Figure 5).** Significantly higher increase of POD activity was observed in the leaves of PAIYUR-2 by 66% (35.10 µmol/mgpro/min) over the control plants (12.09 µmol/mgpro/min respectively) with 120mM salinity on 30th DAT, while lower increase of POD activity was noticed in the variety CO-1 by 35%(30.28 µmol/mgpro/min) relative to control plants (10.89 µmol/mgpro/min and respectively).

DISCUSSION

SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutase, since discovered by[16] attracted the attention of many researchers because they are essential component in an organism's defense mechanism[17]. In the present study, SOD activity significantly increased under salinity stress (40, 80, 120mM) and the pattern of increase was differed among the varieties with sampling days (15th DAT, 30th DAT) (**figure 1)**. However, higher SOD activity was observed in the leaves of PAIYUR-2 with increased salinity levels on all the sampling days relative to respective control plants, while lower level of SOD activity was noticed in PAIYUR-1. Enhanced activity of SOD to NaCl stress seems to provide PAIYUR-2 variety with better protection against salinity including oxidative damage of cell membranes, as judged by the lower rates of lipid peroxidation.

CATALASE ACTIVITY (CAT)

Catalase (CAT) activity is used as a marker involved in the primary defense against oxidative damage[18]In the current study, PAIYUR-2 showed higher increase of CAT activity in the leaves, on all the sampling days, lowest increment of CAT was monitored in CO-1 under all salinity levels **(Figure 2).** In citrus, high CAT activity has been associated with resistance of the fruit to chilling stress[19].

GLUTATHIONE REDUCTASE ACTIVITY (GR)

Reduced GSH is the major nonprotein sulfhydryl compound in all living organisms, including plants and it has been implicated by numerous metabolic processes[20].In the current study, GR activity was significantly higher in the leaves of horsegram variety PAIYUR-2 on three sampling days (15th DAT, 30th), whereas lower increase of GR activity was observed in CO-1 on all the sampling days under varying level of salinity stress in comparison to the control plants **(Figure 3).** GSH is also an important cofactor, both for enzyme activities and for enzyme synthesis, as well as being control to the metabolism of reduced sulfer[21].

ASCORBATE PEROXIDASE ACTIVITY (APX)

APX is the key enzyme in the scavenging of H_2O_2 in chloroplasts with ascorbate as the electron donar [22]. In the present observation, APX activity significantly higher in the leaves of horsegrame variety PAIYUR-2 at high salinity on all the sampling days than that of control plants, while lower APX activity was observed in CO-1 under salinity stress **(Figure 4).** High levels of intercellular H_2O_2 induced cytosolic APX activity under salt stress [23].

PEROXIDASE ACTIVITY (POD)

Plant peroxidases are among the enzymes where reaction intermediates were first identified and their molecular and enzymatic properties have been characterized [24] and many isozymes of guaicol peroxidase have been found in plant tissues. It is noteworthy that POD activity is more stimulated by NaCl in the tolerant varieties. In our study, under salinity stress, the peroxidase activity highly increased with increasing NaCl concentration in the leaves of PAIYUR-2 on 30th DAT. while less increment was observed in the variety CO-1 under salinity stress **(Figure 5).** Thus, the enhanced POX activity suggests the involvement

of this antioxidant enzyme in the redox homeostasis during salt acclimation in the leaves of horsegram plants.

CONCLUSIONS

In conclusion, based on the relative tolerance results provided, it seems that PAIYUR-2 is highly salt tolerant in comparison with the other horsegram varieties. In fact, for most parameters recorded, better performance was observed in PAIYUR-2 under salt stress on all the sampling days at all salinity levels. This variety probably maintains the antioxidative enzymatic activity and osmotic adjustment and 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 horsegram.

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Figure 1. The effect of salinity on the activity of glutathione reductase in the leaf extracts of two cotton varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± s.e. of five independent determinations.

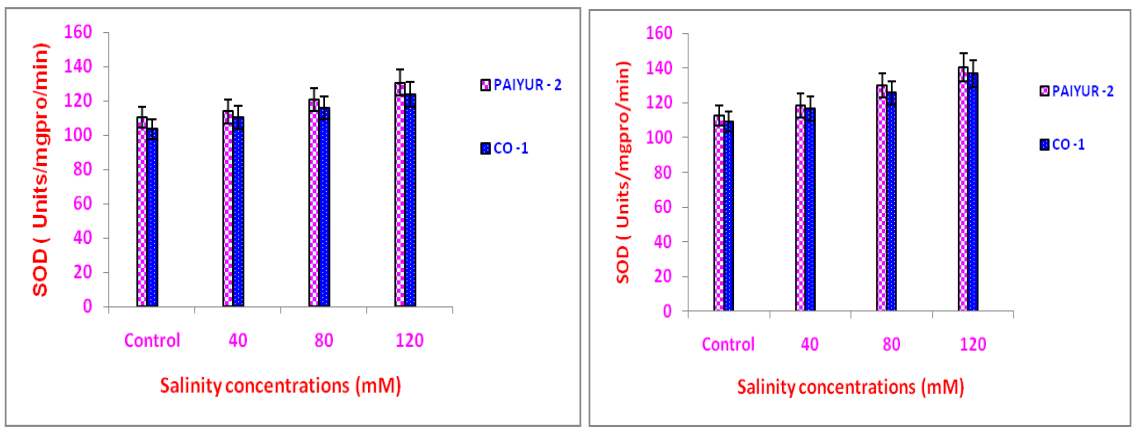

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Figure 2. The effect of salinity on the activity of catalase in the leaf extracts of two cotton varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± s.e. of five independent determinations.

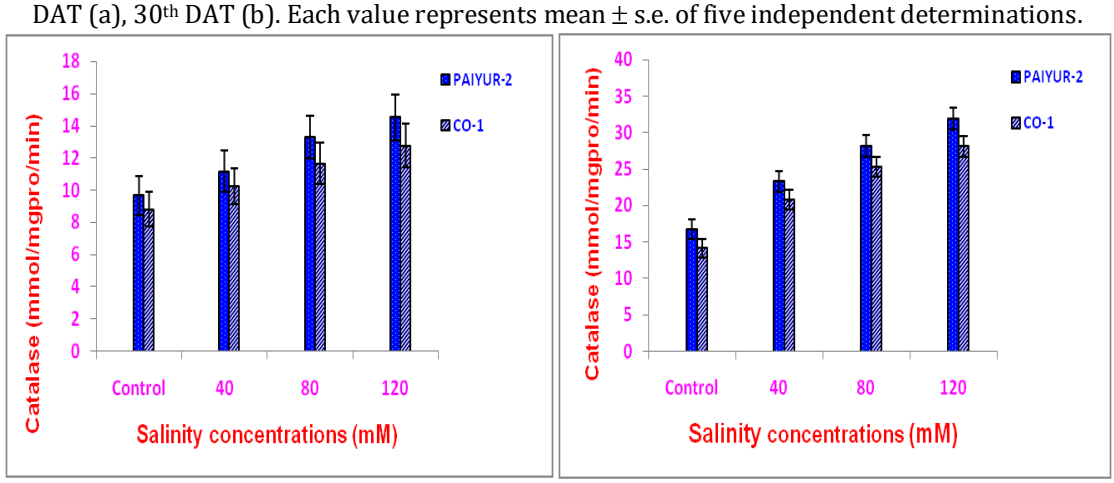

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Figure 3. The effect of salinity on the activity of glutathione reductase in the leaf extracts of two cotton varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± s.e. of five independent determinations.

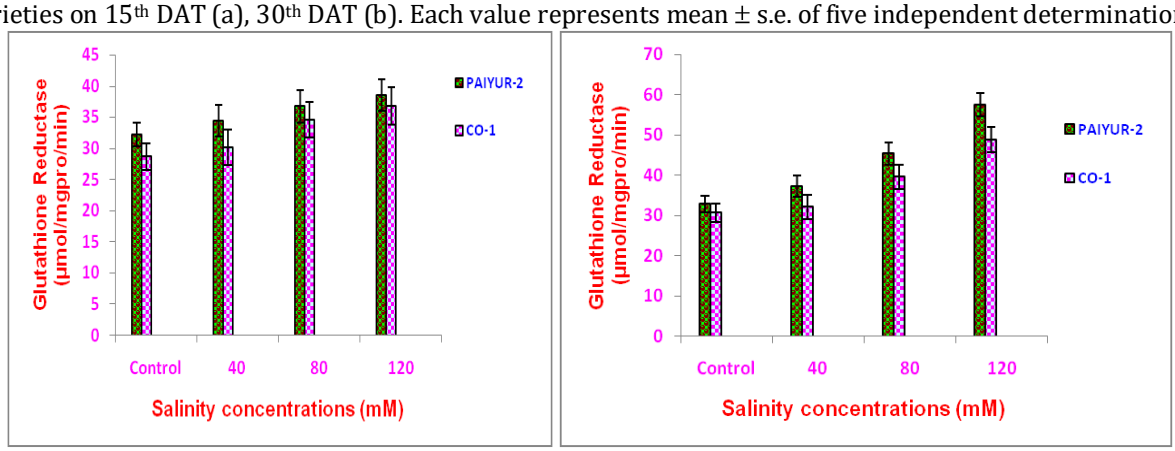

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Figure 4. The effect of salinity on the activity ascorbate peroxidase in the leaf extracts of two cotton varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± s.e. of five independent determinations.

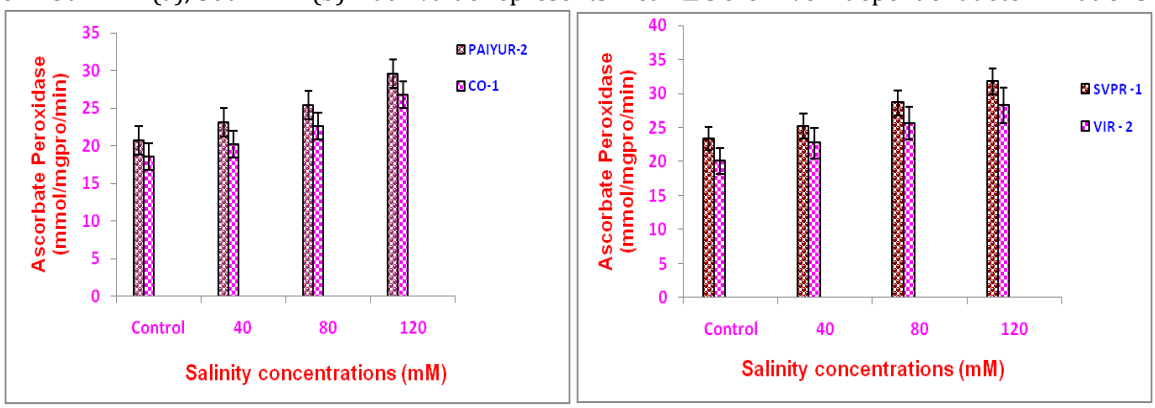

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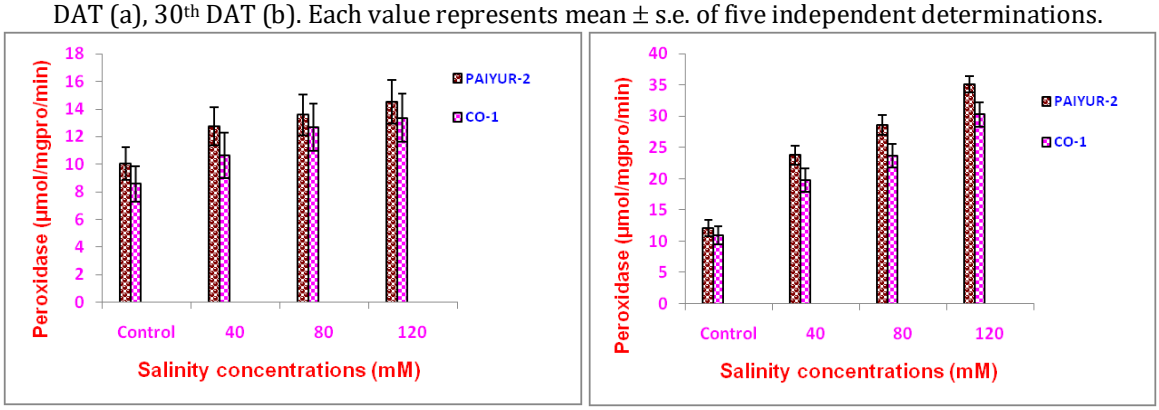
Figure 5. The effect of salinity on the activity of peroxidase in the leaf extracts of two cotton varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± s.e. of five independent determinations.



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