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Effect of exogenous application of salicylic acid on antioxidative enzymes in green gram (*Vigna radiate* (L.) wilczek) irrigated with saline water

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Abstract
Green gram (*Vigna radiata* (L.) Wilczek) is one of the major pulse crops of India cultivated from humid tropic to arid and semi-arid regions. Salt stress is one of the most serious limiting factors for growth and production in most of the crops including green gram and salicylic acid an important phenolics known to alleviates its adverse effect. Thus, the green house experiment was conducted to investigate the effect of exogenous application of salicylic acid (SA) under salt stress. The experimental design was factorial completely randomized design with four salicylic acid concentrations (0.0, 0.5, 1.0 and 1.5 mM), four level of irrigation to induce salinity stress by appropriate dilution of sea water (< 2 EC-tap water, 4.0 EC, 6.0 EC and 8.0 EC). The pots were irrigated with different concentration of saline water and plants were sprayed with salicylic acid after 20 and 40 DAS (Days after Sowing), samples were withdrawn after 21, 41 and 61 DAS for the analysis. The observations were recorded for the antioxidant enzymes *viz*, polyphenol oxidase, peroxidase and catalase. The results suggest that the antioxidant enzymes activity was affected due to salinity stress in mung bean. The peroxidase and polyphenol oxidase activity were increased with increasing level of salt stress, however, the catalase activity decreased. The plant treated with salicylic acid showed similar trends except at higher dose. This investigation has suggested salicylic acid as a potential biomolecule affecting the ROS scavenging enzyme under abiotic stress like salinity.

Keywords: Antioxidative enzymes, green gram, growth, salicylic acid, salinity stress, *Vigna radiate* (L.) wilczek

Introduction
Pulse crops, such as green gram (*Vigna radiata* L.), play an important role in protein production and economics of arid and semi-arid regions of the world. It grows on a wide range of soils but prefers well-drained loams or sandy loams, with a pH ranging from 5 to 8. Worldwide, about one-third of irrigated arable land is already affected and that level is still rising (Lazof and Bernstein, 1999) [7]. Soil salinity adversely affects plant growth and development. It is the one of the major factors responsible for losses in crop production and quality. Increase in salt tolerance is the highly attractive approach to overcome the salinity threat for a crop. Under high salinity stress, reactive oxygen species (ROS) formed and accumulated in plant cells cause severe damage to plants. However, plants equipped with a variety of defense mechanism scavenging ROS formed due to biotic as well as abiotic stresses. These mechanism includes, accumulation of phenolics, induction of antioxidant and its related enzymatic system etc., (Kandoliya and Vakharia, 2013; Patel *et al.* 2015; Kandoliya and Vakharia, 2015; Joshi *et al* 2018) [6, 10, 5, 4]. Salicylic acid belongs to an extraordinary diverse group of plant phenolic defined as substance that possesses an aromatic ring bearing hydroxyl group (Raskin, 1992) [12]. In most of the crop, it was well documented the role of salicylic acid for inducing the defense mechanism against abiotic stress. Thus, the green house experiment was conducted to investigate the effect of exogenous application of salicylic acid (SA) under salt stress on antioxidative enzymatic activities in green gram.

Materials and Methods
The green house experiment was conducted during *Rabi* 2016-17 at Food testing Laboratory, Department of Biotechnology, Junagadh Agricultural University, Junagadh. Green gram (*Vigna radiate* (L.) Wilczek) seeds of varieties Green gram Gujarat-4 were obtained from Main Pulse Research Station, Junagadh Agricultural University, Junagadh for the experiment.

Treatments

a) Salinity Level (4): Plant irrigated with saline water prepared by appropriate dilution of sea water [S_1-≤2 EC (Control, Tap Water), S_2-4 EC, S_3-6 EC and S_4-8 EC]

b) Salicylic acid (4): T_1-0.0 mM, T_2-0.5 mM, T_3-1.0 mM and T_4-1.5 mM

c) Growth stage (3): D_1-21 DAS, D_2-41 DAS and D_3-61 DAS

Salicylic acid spray of appropriate concentration was done at 20 and 40 days after the sowing (DAS). The sample was collected at 21, 41 and 61 DAS respectively. Green gram leaf were collected for enzymatic activity at different stages (D_1 to D_3) after the spray of salicylic acid (T_1 to T_4) from the pot irrigated with saline water having a different concentration (S_1 to S_4) and packed in plastic bag and brought to the laboratory under ice cold condition.

Enzyme Assay

Polyphenol oxidase (PPO) activity (EC 1.14.18.1): Leaf tissue weighed 0.1 gm and grind in 5 ml of 100 mM sodium phosphate buffer, pH 6.5. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used for enzyme assay. The reaction mixture contained 2.9 ml of catechol (10 mM catechol in 10 mM phosphate buffer, pH 6.5) and reaction was initiated by the addition of 100 µl of enzyme extract. The changes in the colour due to the oxidized catechol were read at 490 nm for one minute at an interval of 15 second. Blank was carried out without substrate. The enzyme activity was expressed as Δ OD.min.$^{-1}$g.$^{-1}$fr.wt. tissues (Esterbaner *et al*. 1977) [2].

Peroxidase (POX) activity (EC 1.11.1.7): Leaf tissue (100 mg) was homogenized in a pre-chilled mortar and pestle with 2 ml of extraction buffer, containing 50 mM sodium phosphate buffer pH 7.0. The homogenates were centrifuged at 10,000 rpm for 15 minutes and the supernatant was used for the assay of antioxidant enzymes *viz*. peroxidase and catalase. The reaction mixture contained 2.99 ml of 0.03% H_2O_2 in 0.1M phosphate buffer (pH 6.0) containing 0.01 % orthodianisidine dye (freshly prepared, dissolved in methanol). The reaction was initiated by the addition of 10 µl of enzyme extract. The change in color of oxidized dye was read at 460 nm up to 1 minute at the interval of 15 seconds. Blank was run without the addition of enzyme (Malik and Singh (1980) [8]. The enzyme activity was expressed as Δ OD.min.$^{-1}$g.$^{-1}$fr.wt.

Catalase activity (EC 1.11.1.6): Catalase activity was measured immediately in fresh extract and was assayed as described by Aebi (1984) [1]. Three ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 18 mM H_2O_2 and 50 µl enzyme extract. The hydrogen peroxide dependent oxidation was estimated by measuring the decrease in the absorbance at 240 nm. The enzyme activity was expressed as Δ OD.min.$^{-1}$ g.$^{-1}$fr. Wt.

Results and Discussion

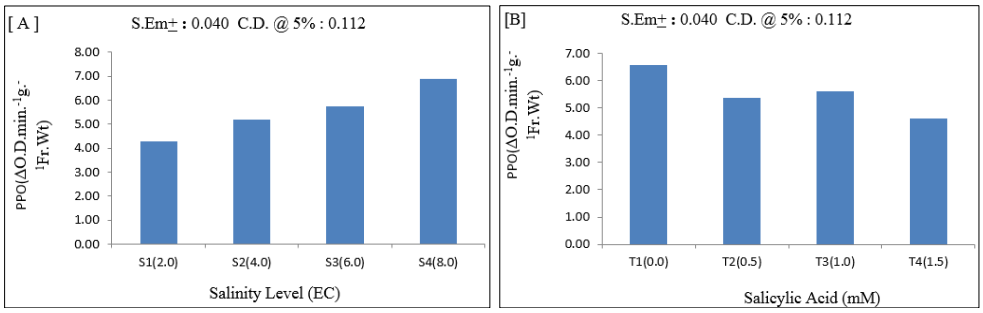
Polyphenol Oxidase (EC 1.14.18.1)

The data on enzyme activity of polyphenol oxidase activity (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) analyzed from leaf tissue of green gram collected from plants treated with different concentrations of salicylic acid (T_1 to T_4) grown in a pot irrigated with different concentration of saline water concentrations (S_1 to S_4) at different stages (D_1 to D_3)are depicted in Fig.1 and 2.

Mean effect of salinity level irrespective of salicylic acid and growth stages were found statistically significant for polyphenol oxidase activity (Fig. 1 A). Among the salinity level, treatment the S_1 (pot irrigated with tap water) showed lowest amount of polyphenol oxidase activity (4.286 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt).WhileS_4 (irrigated with saline water 8EC) showed highest amount of polyphenol oxidase activity (6.88 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) In general, activity increased with the increase in salinity in pot. Imposition of different treatments of salicylic acid resulted significant difference for the polyphenol oxidase activity (Fig. 1 B). The mean lowest activity was noted for the tissues received from T_4- pots treated with salicylic acid 1.5 mM (T_4) (4.598 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt). The tissues obtain from green gram pots treated with salicylic acid 0.0 mM (T_1) revealed higher amount of mean polyphenol oxidase activity (6.582 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) which was followed by T_3- 1.0 mM salicylic acid (5.59 mg.g^{-1}Fr.Wt.) and T_2-0.5 mM salicylic acid (5.352 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) irrespective of salinity level and growth stage. In general the exogenous application of salicylic acid decreased the enzyme activity. Among the different stages, mean value of polyphenol oxidase activity significantly varied between 6.320 and 4.672 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt. (Fig. 1 C). The activity was decreased from 21 DAS (6.320 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) to 61 DAS (4.672 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt).

Interaction effects of S × T, S × D and T × D for polyphenol oxidase activity were showed significant differences (Fig. 2 A, B and C). The lowest value (3.280Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) of polyphenol oxidase activity was observed in plant irrigated with tap water combine with salicylic acid 1.5 mM (S_1T_4). The highest value of polyphenol oxidase activity was observed for the S_4T_1 i.e. in plant irrigated with saline water 8 EC combine with salicylic acid 0.0 mM (7.813 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt). Interaction effects of T × D for polyphenol oxidase activity were revealed that the highest value of polyphenol oxidase activity observed for T_1D_1 i.e. in plant treated with 0.0 mM salicylic acid at 21 DAS (7.472 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt). The lowest value of polyphenol oxidase activity was observed for T_4D_3.

Polyphenol Oxidase Activity



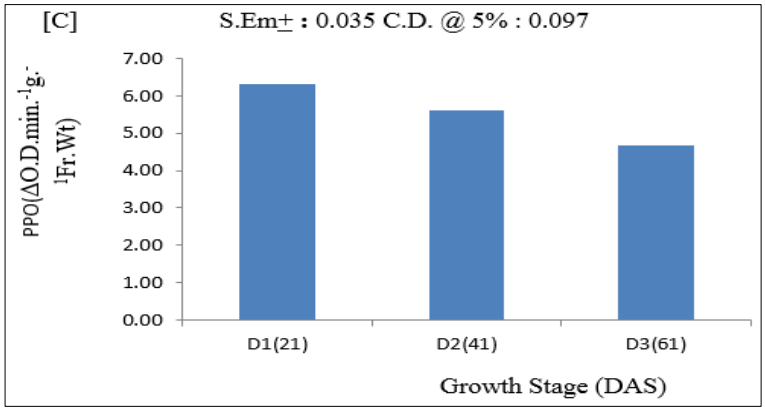


Fig 1: Mean effect of (S) salinity, (T) salicylic acid and (D) growth Stage on polyphenol oxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in seedling of green gram.

Polyphenol oxidase activity

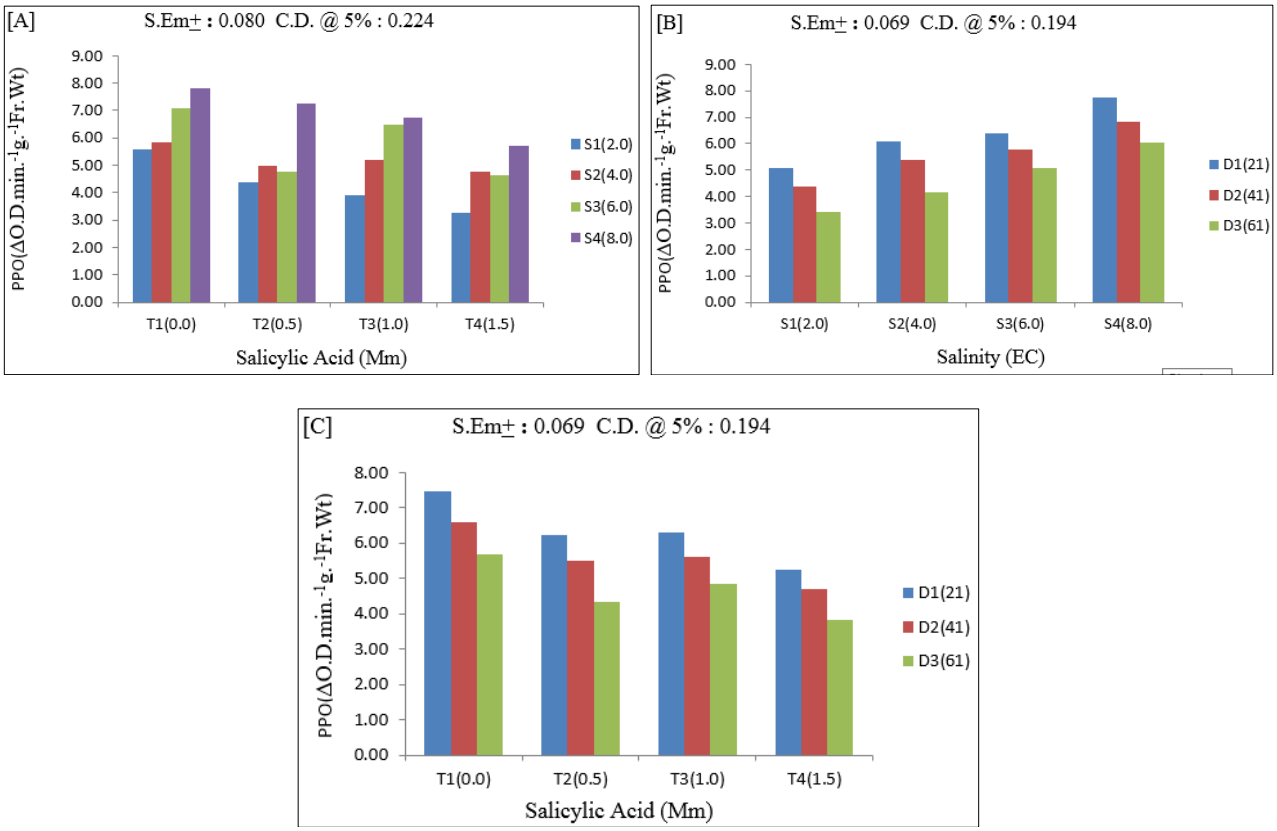
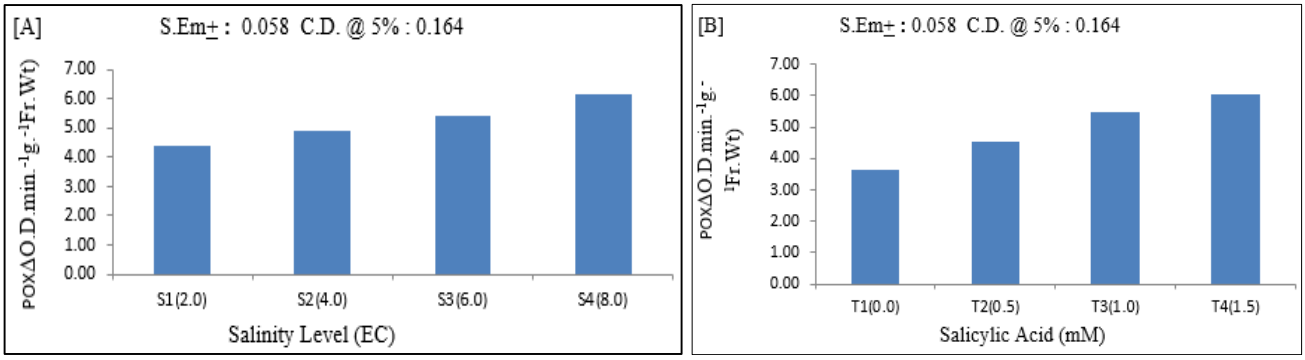


Fig 2: Interaction effect of salinity and salicylic acid, salinity and growth stages, salicylic acid and growth stages on polyphenol oxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaf tissue of green gram.

Peroxidase activity



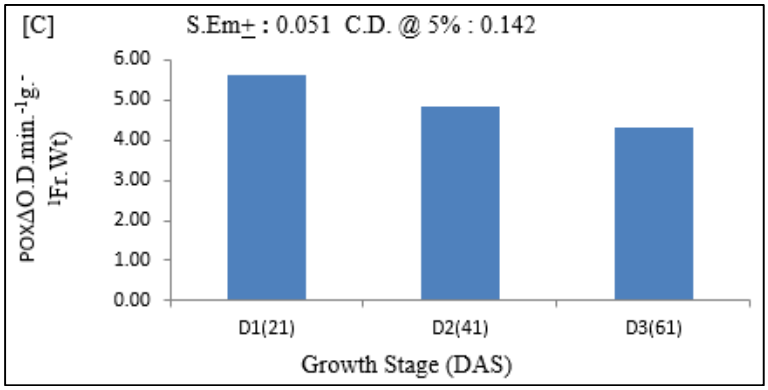
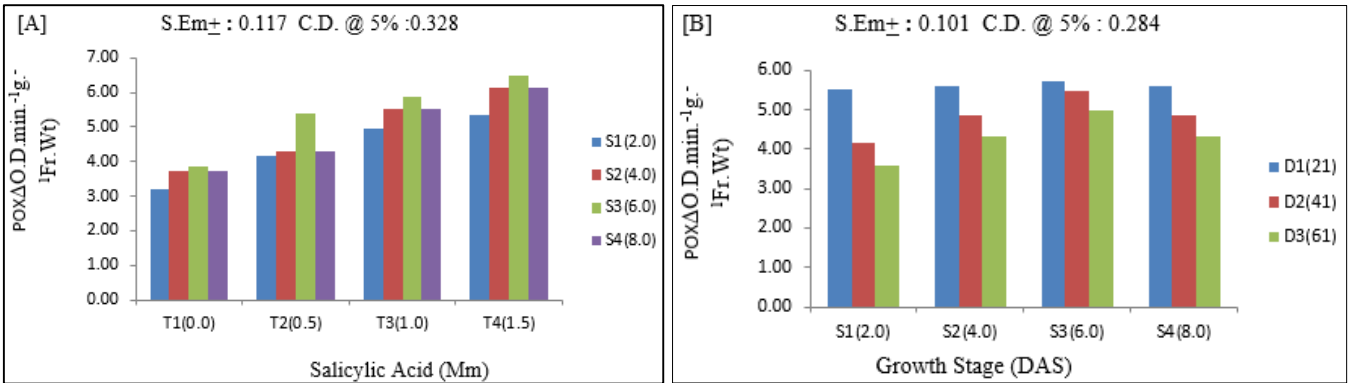


Fig 3: Mean effect of (S) salinity, (T) salicylic acid and (D) growth Stage on peroxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in seedling of green gram.

Peroxidase Activity



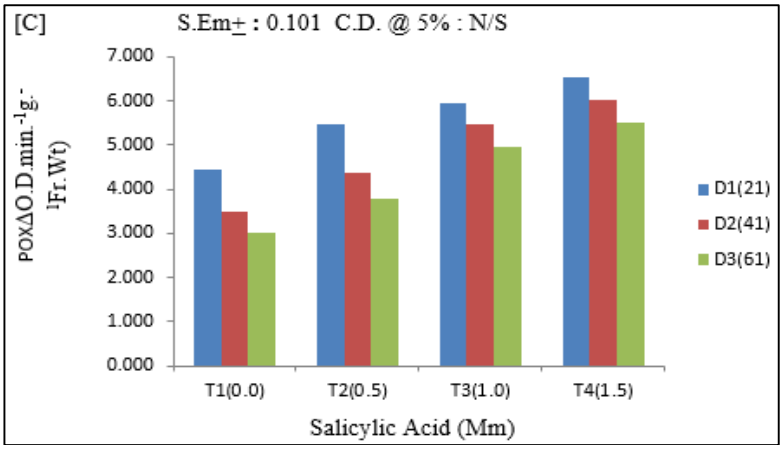
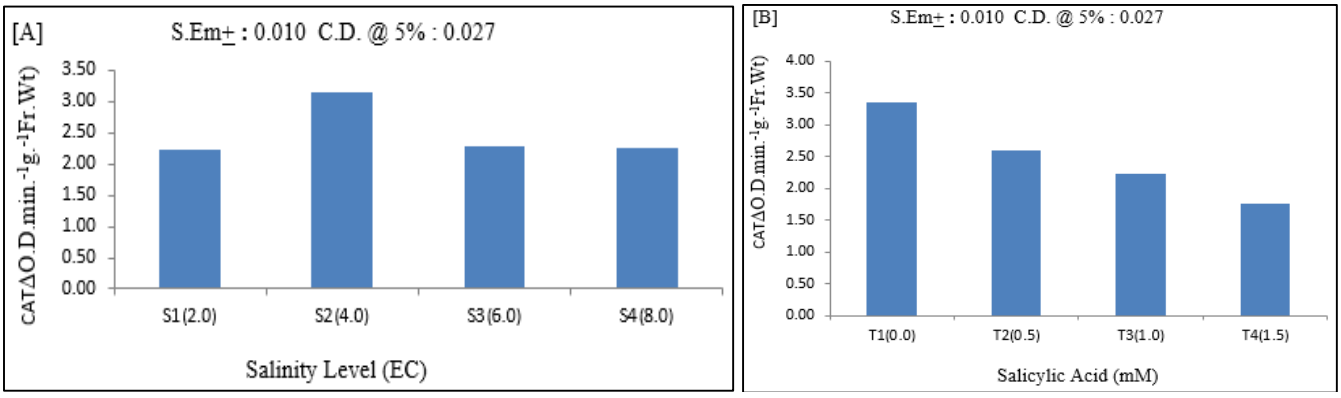


Fig 4: Interaction effect of salinity and salicylic acid, Salinity and growth stages, salicylic acid and growth stages on peroxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaf tissue of green gram.

Catalase Activity



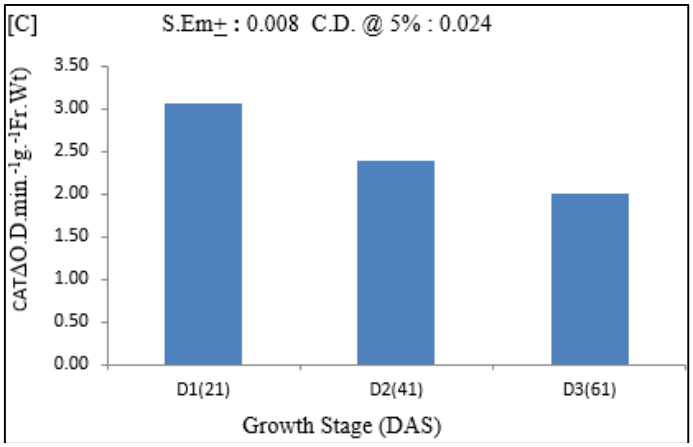


Fig 5: Mean effect of (S) salinity, (T) salicylic acid and (D) growth Stage on catalase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in seedling of green gram.

Catalase Activity

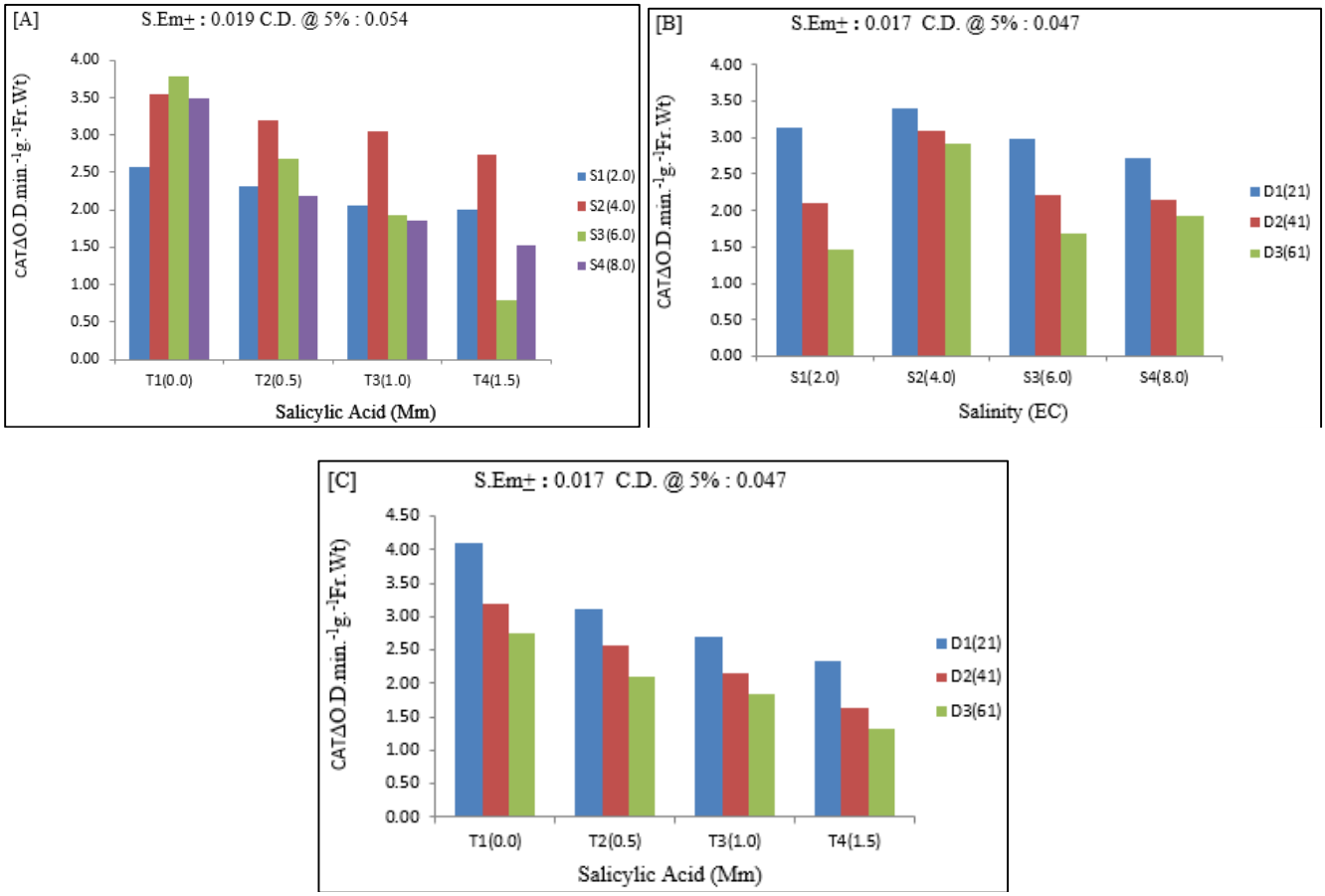


Fig 6: Interaction effect of salinity and salicylic acid, salinity and growth stages, salicylic acid and growth stages on catalase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaf tissue of green gram.

Peroxidase (EC 1. 11.1.7)

POX (EC 1.11.1.7) is the most important among the H_2O_2 scavenging enzymes. The enzyme activity (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) analyzed from leaf tissue of green gram collected from plants treated with different concentrations of salicylic acid (T_1 to T_4) grown in a pot irrigated with different concentration of saline water concentrations (S_1 to S_4) at different stages (D_1 to D_3) are depicted in Fig. 3 and 4.

Mean effect of salinity level irrespective of salicylic acid and growth stages were found to be significant for peroxidase activity (Fig. 3 A). Among the salinity level, treatment S_1 (irrigated with tap water) showed lowest amount of peroxidase activity (4.416 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) while the S_4 (irrigated with saline water 8 EC) showed increased in peroxidase activity (6.149 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) Imposition of different treatments of salicylic acid resulted significant difference for the peroxidase activity (Fig. 3 B). The tissues obtain fromgreen gram pots treated with salicylic acid 1.5 mM (T_4) revealed highest activity of peroxidase (6.030 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) whilegreen gram pots treated with salicylic acid 0.0 mM (T_1) revealed lowest activity of peroxidase (3.641 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). It suggests the inducing effect of of salicylic acid on paroxidase activity. Among the different stages, mean value of peroxidase activity significantly varied between 4.314and 5.597 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt. (Fig. 3 C). Interaction effects of S × T for peroxidase activity were revealed significant differences in leaf tissue of green gram (Fig. 4 A). The lowest value (3.216 Δ O.D. min.$^{-}$

$^{1}g.^{-1}Fr.Wt.$) of peroxidase activity was observed in plant irrigated with tap water combine with salicylic acid 0.0 mM (S_1T_1). The highest value of peroxidase activity was observed for the S_3T_4 i.e. in plant irrigated with saline water 6 EC combine with salicylic acid 1.0 mM (6.478 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). However, at higher salinity level (S_4) the activity was remained lower compared to S_3.

Interaction effects of S × D for peroxidase activity were revealed significant differences in green gram leaves (Fig. 4 B). The lowest value of peroxidase activity was observed for S_1D_2 in plant irrigated with tap water after 41 DAS (4.149 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). The highest value of peroxidase activity was observed for the S_2D_1 i.e. in plant irrigated with saline water 4 EC after at 21 DAS (5.591 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). Interaction effects of T × D for peroxidase activity were revealed non-significant differences in green gram (Fig. 4 C). The lowest value of peroxidase activity was observed for T_1D_3i.e in plant for control condition 0.0 mM salicylic acid after 61 DAS (3.1015 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). The highest value of peroxidase activity was observed for T_4D_1 i.e. in plant treated with 1.5 mM salicylic acid after at 21 DAS (6.539 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$).

Catalase (E.C 1.11.1.6)

CAT (E.C 1.11.1.6) is the most important among the H_2O_2 scavenging enzymes. The enzyme activity (Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$) analyzed from leaf tissue of green gram collected from plants treated with different concentrations of salicylic acid (T_1 to T_4) grown in a pot irrigated with different concentration of saline water concentrations (S_1 to S_4) at different stages (D_1 to D_3)are depicted in Fig. 5 and 6.

Mean effect of salinity level were found significant for catalase activity (Fig. 5 A). Among the salinity level, treatment S_2 (irrigated with saline water 4 EC) showed highest amount of catalase activity (3.138 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$) while the S_1 (pot irrigated with tap water) showed lowest amount of catalase activity (2.232 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). Qados (2015) observed that activities of CAT enzymes increased insignificantly with increasing salt stress until 4000 ppm, above which the activities of all enzymes were decreased as compared with control plants. SA treatment showed a linear increase in enzyme activities in salt stressed and unstressed plants until 4000 ppm. In present investigation, imposition of different treatments of salicylic acid showed significant difference for the catalase activity (Fig. 5 B). The tissues obtain from green gram pots treated with salicylic acid 0.0 mM (T_1) revealed higher amount of mean catalase activity (3.343 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$) and which was followed by T_2- 0.5 mM salicylic acid (2.594 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$) and T_3-1.0 mM salicylic acid (2.226 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$) irrespective of salinity level and growth stage. The mean lowest activity was noted for the tissues received from pots treated with salicylic acid 1.5 mM (T_4) (1.765 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). Among the different stages, mean value of catalase activity significantly varied between 2.000 and 3.057Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$ (Fig. 5 C). The activity was decreased from 21 DAS (3.057Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$) to 61 DAS (2.0 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$).

Interaction effects of S × T for catalase activity were revealed significant differences in green gram (Fig. 6 A). The highest value of catalase activity was observed for the S_3T_1 i.e. in plant irrigated with saline water 6 EC combine with salicylic acid 0.0 mM (3.775Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). The lowest value (1.518 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$) of catalase activity was observed in plant irrigated with saline water 8 EC combine with salicylic acid 1.5 mM (S_4T_4). Interaction effects of S × D for catalase activity were revealed significant differences in green gram (Fig. 6 B). The highest value of catalase activity was observed for the S_2D_1 i.e. in plant irrigated with saline water 4 EC after at 21 DAS (3.395 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). The lowest value of catalase activity was observed for S_1D_3 in plant irrigated with tap water after 61 DAS (1.454 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). Interaction effects of T × D for catalase activity were revealed significant differences in green gram (Fig. 6 C). The highest value of catalase activity was observed for T_1D_1 i.e. in plant treated with 0.0 mM salicylic acid after at 21 DAS (4.086 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$). The lowest value of catalase activity was observed for T_4D_3i.e in plant for control condition 1.5 mM salicylic acid after 61 DAS (1.322 Δ O.D. $min.^{-1}g.^{-1}Fr.Wt.$).

This results were in agreement with Qados (2015) also observed that the activities of peroxidase and polyphenol oxidase enzymes increased insignificantly with increasing salt stress until 4000 ppm, above which the activities of all enzymes were decreased as compared with control plants. Salicylic acid treatment showed a linear increase in enzyme activities in salt stressed and unstressed plants until 4000 ppm. The increase in enzyme activity at 6000 ppm stressed plants, in the presence of salicylic acid, was insignificant for polyphenol oxidase. Tuna *et al.* (2007) [14] noted that the application of salicylic acid in 2 mM dose considerably increased polyphenol oxidase activity and peroxidase activity. Ghasemzadeh and Jaafar (2013) [3] also studied the effect of foliar salicylic acid (SA) applications on activities of peroxidase (POX) and concluded that the exogenous application of SA increased antioxidant enzyme activities. Sedghi *et al.* (2013) [13] studied effects of salicylic acid on the antioxidant enzymes activity in sunflower and reported that all three enzymes activity increased by SA spraying compared to control. In general, salicylic acid treatment showed a linear increase in enzyme activities in salt stressed.

Conclusion

The results suggest that the antioxidant enzymes activities were affected due to salinity stress in mung bean. The peroxidase and polyphenol oxidase activity increased with increasing level of salt stress however, catalase activity decreased. The plant treated with salicylic acid showed similar trends but higher dose of salicylic acid reverts catalase and polyphenol oxidase enzymatic activity. This investigation has proved salicylic acid as a potential biomolecules affecting the ROS scavenging enzyme under abiotic stress like salinity.

References

1. Aebi H. Catalase *in vitro*. Methods Enzymology. 1984; 105:121-126.
2. Esterbaner H, Schwarz E, Hayn M. A rapid assay for catechol oxidase and laccase using 2-nitro-5-thio benzoic acid. Anal. Biochem. 1977; 77:486.
3. Ghasemzadeh A, Jaafar HZE. Interactive effect of salicylic acid on some physiological features and antioxidant enzymes activity in ginger (*Zingiber officinale* Roscoe). Molecules. 2013; 18(5):5965-5979.
4. Joshi AK, Kandoliya UK, Bodar NP, Golakiya BA. Diversity of coconut (*Cocos nucifera* L.) Genotypes and hybrids for root total phenol content and different enzymatic activity. J Agri Sci Res. 2018; 2(1):13-16.
5. Kandoliya UK, Vakharia DN. Ascorbic acid and ascorbate peroxidase based defence system induced by

Pseudomonas fluorescens against wilt pathogen in chickpea. Internat. J Plant Protec. 2015; 8(1):86-92.
6. Kandoliya UK, Vakharia DN. Induced resistance and phenolic acid accumulation in biological control of chickpea wilt by *Pseudomonas fluorescens*. Asian J Bio Sci. 2013; 8(2): 184-188.
7. Lazof DB, Bernstein N. The NaCl inhibition of shoot growth: The case for disturbed nutrition with special consideration of calcium. Adv. Bot. Res. 1999; 29(2):113-189.
8. Malik CP, Singh MB. Plant enzymology and histoenzymology. Kalyani Publishers. 1980; 53:45-55.
9. Neto L, Jose P, Joaqim E, Eneas F. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Envi. and Exp. Bot. 2005; 56(1):87-94.
10. Patel NJ, Kandoliya UK, Talati JG. Induction of Phenol and Defence-Related Enzymes During Wilt (Fusarium udum Butler) Infestation in Pigeon Pea. Int J Curr Microbiol App Sci. 2015; 4(2):291-299.
11. Qudos A. Examine effects of salicylic acid on growth, yield and chemical contents of pepper (*capsicum annuum* L.) plants grown under salt stress conditions. Int. J of Agri. and Crop Sci. 2015; 8(2):107-113.
12. Raskin I. Role of salicylic acid in plants. Ann. Rev. Plant physiol. Plant Mol. Boil. 1992; 4:439-463.
13. Sedghi M, Basiri HK, Sharifi RS. Effects of salicylic acid on the antioxidant ezymes activity in sunflower. Annals of West Uni. of Timisoara, ser. Biology. 2013; 16(2): 67-72.
14. Tuna L, Kaya C, Dikilitas M, Yokas I, Burun B, Altunlu H. Comparative effects of various salicylic acid derivatives on key growth parameters and some enzyme activities in salinity stressed maize (*Zea mays* L.) Plants. Pak. J Bot. 2007; 39(3):787-798.