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Effect of gibberellic acid, potassium nitrate and silicic acid on enzymes activity in cowpea (*Vigna unguiculata* L. Walp) irrigated with saline water

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Abstract
In Indian context, cowpea (*Vigna unguiculata* L. Walp) is a minor pulse cultivated mainly in 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 cowpea. While silicic, gibberellic acid and potassium nitrate is known to alleviate its adverse effect. Thus, Green house experiment was conducted to investigate the effect of exogenous application of gibberellic acid, potassium nitrate and silicic acid under salt stress on the enzymatic activities in cowpea. The experimental design was factorial completely randomized design with different eight treatment combinations, two level of irrigation to induce salinity stress i.e. tap water (S_1) and 4 EC (S_2). The plants were sprayed with GA_3, KNO_3 and silicic acid after 10 and 30 DAS (Days after sowing) and samples withdrawn after 20 and 40 DAS for the analysis. The activities of enzymes were recorded for polyphenol oxidase, peroxidase, nitrate reductase and phenylalanine ammonialyase. The enzymes activities *viz.,* polyphenol oxidase, phenylalanine ammonialyase and peroxidase that increased with increase in concentration of salt however, the nitrate reductase activity decreased. On application of GA_3, KNO_3 and silicic acid increased polyphenol oxidase, peroxidase, nitrate reductase and phenylalanine ammonialyase enzymatic activity. This investigation has suggested silicic acid, gibberellic acid, potassium nitrate as a potential biomolecules affecting the nitrate reductase as well as ROS scavenging enzyme under abiotic stress like salinity.

Keywords: Enzymes, cowpea (*Vigna unguiculata* L. Walp), growth, GA_3, Silcic acid, KNO_3, salinity stress

Introduction
Cowpea (*Vigna unguiculata* L. Walp) is an important leguminous vegetable crop mainly grown both in *Kharif* and spring summer season crop in most parts of India. It is early, multi-seasonal and multipurpose crop. It has multifarious uses like as fodder, cover crop and green manure and provi des high quality protein in the form of vegetable and pulse to human diet. Its young leaves, pods and grains contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding (Nielson *et al*., 1997) [16]. Cowpea seeds provide a rich source of proteins and calories, as well as minerals and vitamins. The seeds contain small amounts of β-carotene (precursor of vitamin A), thiamin, riboflavin, niacin, folic acid and ascorbic acid (Kay 1979; Tindall 1983) [10, 22]. 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) [8, 17, 9, 7]. Induced salt tolerance by exogenious application of various chemicals and hormones is a highly attractive approach to overcome the salinity threat (Trivedi *et al.*, 2018; Solanki *et al.*, 2018) [20, 23]. Gibberellic acid, growth hormone which enhances the flowering, increases fruit set as well as fruit size. Potassium enhanced resistance toward the bacterial, viral, nematodes and fungal pathogens (Perrenoud, 1990) [18]. Silicon deposited on the plant surfaces and serves as a protective layer against the biotic and abiotic stress as well as enhances the rate of photosynthesis and yield of the crop (Miyake and Takahashi, 1983) [14]. Thus, Green house experiment was conducted to investigate the effect of exogenous application of gibberellic acid, potassium nitrate and silicic acid under salt stress on the enzymatic activities in cowpea.

Material and Methods
The green house experiment was conducted during kharif 2018-19 at Food testing Laboratory, Department of Biotechnology, Junagadh Agricultural University, Junagadh using Pusa phalguni variety of cowpea (*Vigna unguiculata* L. Walp).

Treatments

a) Salinity level: Plant irrigated with saline water prepared by appropriate dilution of sea water. [S_1- Tap water, S_2- Saline water (4 EC)].

b) Gibberellic acid, Potassium nitrate, Silicic acid: Eight treatment combinations viz., T_1 - Control (without spray), T_2 – Sprayed with GA_3 @ 100 ppm, T_3 – Sprayed with KNO_3 @ 500 ppm, T_4 – Sprayed with Silicic acid @ 50 ppm, T_5 – Sprayed with GA_3 @ 100 ppm + KNO_3 @ 500 ppm, T_6 – Sprayed with KNO_3 @ 500 ppm + Silicic acid @ 50 ppm, T_7 – Sprayed with GA_3 @ 100 ppm + Silicic acid @ 50 ppm andT_8 - Sprayed with GA_3 @ 100 ppm + KNO_3 @ 500 ppm + Silicic acid @ 50 ppm.

Cowpea leaf samples were collected at two different stages (D_1 and D_2) after the spray of gibberellic acid, potassium nitrate and silicic acid from the pot irrigated with tap water as well as saline water having a 4 EC concentration. Leaf tissues were taken for first two stages (D_1 and D_2) at one day after the gibberellic acid, potassium nitrate and silicic acid spray.

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) [4].

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 peroxidase. The reaction mixture contained 2.99 ml of 0.03% H_2O_2 in 0.1M phosphate buffer (pH 6.0) containing 0.01 % ortho dianisidine 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) [13]. The enzyme activity was expressed as Δ OD.min.$^{-1}$g.$^{-1}$fr.wt.

Nitrate reductase (EC 1.6.6.1)

200 mg leaf tissue was cut into small slices. The leaf tissue was then suspended having a reaction mixture of 1 ml 5% isopropanol, 1 ml 1 M potassium nitrate and 3 ml phosphate buffer. Incubation was carried out at 30 °C for 2 hours. After incubation 1 ml aliquot was withdrawn and to this following reagent were added

i) 0.2 ml sulphanilamide solution

ii) 0.2 ml 0.2% NEDH (N- Napthyline diamine hydrochloride) solution

After 20 minutes 4 ml water was added and intensity of the colour developed was recorded at 570 nm in a spectrophotometer. The enzyme activity was expressed as Δ O.D.h.$^{-1}$g.$^{-1}$fr. wt. tissues.

Phenylalanine ammonialyase (PAL, EC 4.3.1.5)

Five hundred milligram of leaf tissues homogenized with a pre-chilled mortar and pestle in 5 ml of extraction buffer containing 50 mM borate-HCl buffer, pH 8.8 and 0.04 % ß-mercaptoethanol. The homogenate was centrifuged at 10,000 rpm for 15 min. The clear supernatant after appropriate dilution was used as the enzyme source for the assay of PAL (Mahadevan and Sridhar, 1986) [11]. The reaction mixture contained 3.0 ml of 0.1 M sodium borate buffer, pH 8.8, 0.5 ml of 0.1 M phenylalanine (dissolved in 0.1 M sodium borate buffer, pH 8.8). The reaction was initiated by the addition of 100 µl enzyme extract after appropriate dilution. The tubes were incubated at 37°C for 2 hr. The O.D. was read at 290 nm after 2hr. The enzyme activity was expressed as Δ O.D.h.$^{-1}$g.$^{-1}$fr. wt. tissues.

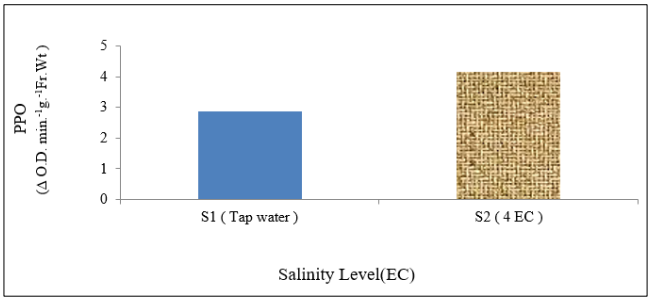
Result and Discussion

Polyphenol oxidase (PPO) activity (EC 1.14.18.1)

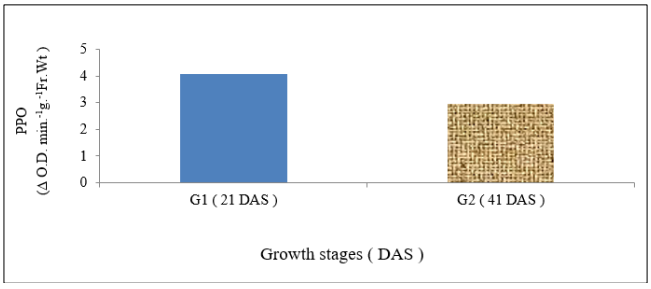
The data on enzyme activity of polyphenol oxidase activity (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) analyzed from leaf tissue of cowpea collected from plants treated at 10 and 30 DAS with concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T_1 to T_8) grown in a pot irrigated with tape water (S_1) and saline water (S_2) 4 EC at stages G_1 and G_2 are depicted in Fig. 1, 2.

Polyphenol oxidase

[A] S.Em±: 0.015 C.D. @ 5%: 0.042



[B] S.Em±: 0.015 C.D. @ 5%: 0.042



[C] S.Em±: 0.030 C.D. @ 5%: 0.084

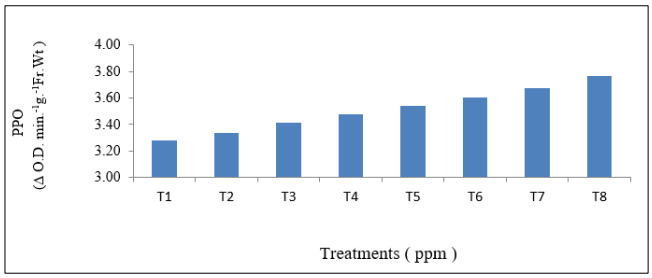
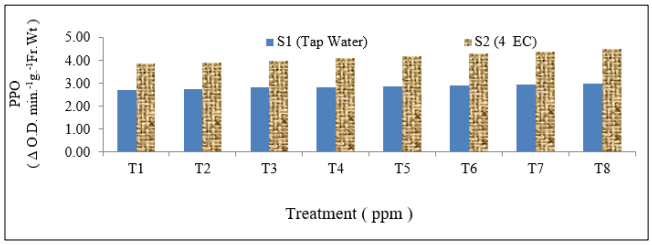
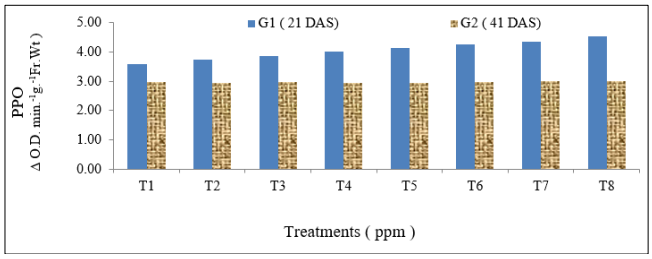


Fig 1: Mean effect of [A] salinity(S), [B]growth stage(G) and [C]treatments(T) on polyphenol oxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaves of cowpea

[A] S.Em±: 0.042 C.D. @ 5%: 0.118



[B] S.Em±: 0.042 C.D. @ 5%: 0.118



[C] S.Em±: 0.021 C.D. @ 5%: 0.059

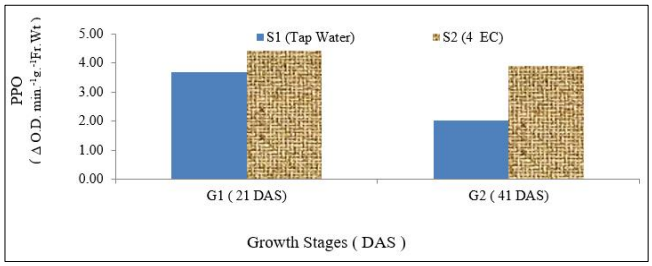


Fig 2: Interaction effect of [A]salinity(S) X treatments(T), [B] growth stages (G) X treatment(T) and [C] salinity(S) X growth stages(G) on polyphenol oxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) in leaves of cowpea.

Mean effect of salinity level were found to be significant for polyphenol oxidase activity (Fig.1 A). Among the salinity level, treatment S_2 irrigated with saline water showed highest value for polyphenol oxidase activity (4.16 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) while the S_1 pot irrigated with tap water showed declined value for polyphenol oxidase activity (2.86 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Among the different stages, mean value of cowpea significantly varied between 2.96 and 4.06 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt (Fig.1 B). The content was increased from 41 DAS (2.965 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) to 41 DAS (4.06 Δ O.D. min.$^{-1}$g.$^{-1}$ Fr. Wt.). This may be the effect of saline irrigation water as well the property of crop species. Application of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination result to be found significant difference for (Fig.1 C). However, Treatment (T_8) showed highest value for polyphenol oxidase activity in cowpea. The tissues obtain from cowpea pots with treatment (T_8) GA_3@ 100 ppm + KNO_3@ 500 ppm + Silicic acid@ 50 ppm showed higher amount of mean polyphenol oxidase activity (3.77 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The mean lowest content was noted for the tissues received from T_1 (3.28 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.).

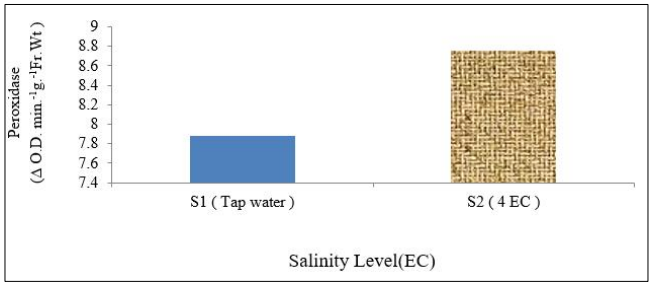
S X T interaction effects for polyphenol oxidase activity were revealed significant differences in cowpea (Fig.2 A). The highest value of polyphenol oxidase activity was observed for the S_2T_8 i.e. in plant irrigated with saline water and plant treated with GA3@ 100 ppm + KNO3@ 500 ppm + Silicic acid@ 50 ppm (4.52 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value (2.70 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) of polyphenol oxidase activity was obsereved in plant irrigated with tap water without treatment (S_1T_1). Interaction effect of G X T for polyphenol oxidase activity were revealed significant differences in cowpea (Fig.2 B). The highest value of polyphenol oxidase activity was observed for G_1T_8 i.e in plant treated with GA_3@ 100 ppm + KNO_3@ 500 ppm + Silicic acid@ 50 ppm after at 21 DAS (4.55 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value of polyphenol oxidase was observed for G_2T_4 i.e plant was in control conditon after at 41 DAS (2.93 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The observation from interaction effect of S X G for polyphenol oxidase activity were found significant differences in leaf tissue of cowpea (Fig.2 C). The highest value of polyphenol oxidase activity was observed for S_2G_1 i.e. in plant irrigated with saline water after 21 DAS (4.43 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value of cowpea was observed for S_1G_2 in plant irrigated with tape water after 21 DAS (2.04 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). This results were in agreement with Qados (2015) also observed that the activities of 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. Patel *et al.* (2015) [17] also noted that the polyphenol oxidase generated H_2O_2 could also be a component of significantly process for defense against stress condition. Mori *et al.* (2017) [15] also reported the increased in antioxidant enzymes in response to biotic stress. Noted that silicon alleviates salt stress by modulating antioxidant enzyme activities.

Peroxidase (EC 1.11.1.7)

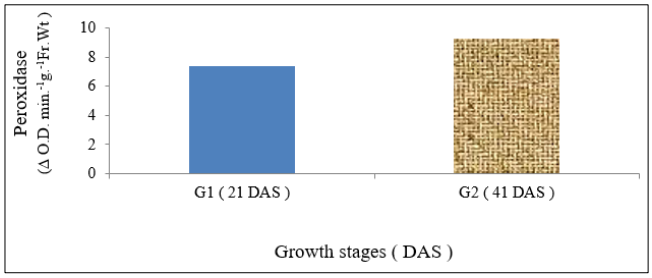
Salinity stress is one of the major factors amongst abiotic stress limiting cowpea production. Antioxidant enzymes like peroxidase plays a vital role in plants tolerant to the stress condition by efficiently scavenging ROS product due to stress. The data on enzyme activity of peroxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) analyzed from leaf tissue of cowpea collected from plants treated at 10 and 30 DAS with concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T_1 to T_8) grown in a pot irrigated with tape water (S_1) and saline water (S_2) 4 EC at stages G_1 and G_2 are depicted in Fig. 3.4.

Peroxidase

[A] S.Em+: 0.014 C.D. @ 5%: 0.039



[B] S.Em±: 0.014 C.D. @ 5%: 0.039



[C] S.Em±: 0.027 C.D. @ 5%: 0.077

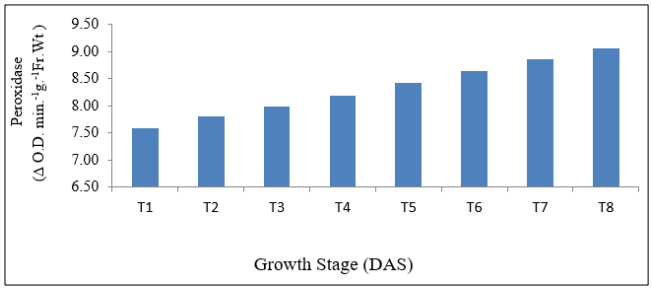
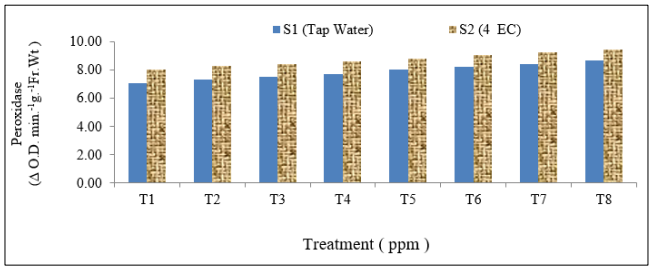
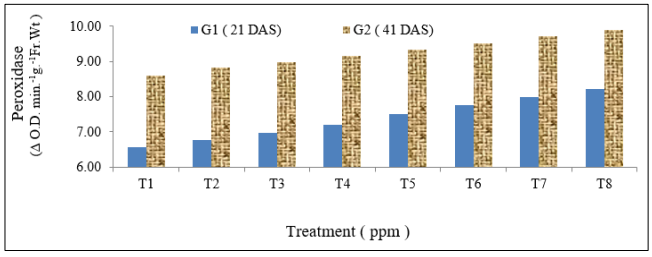


Fig 3: Mean effect of [A] salinity(S), [B]growth stage(G) and [C]treatments(T) on peroxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaves of cowpea

[A] S.Em±: 0.039 C.D. @ 5%: NS



[B] S.Em±: 0.039 C.D. @ 5%: 0.10



[C] S.Em+: 0.019 C.D. @ 5%: 0.055

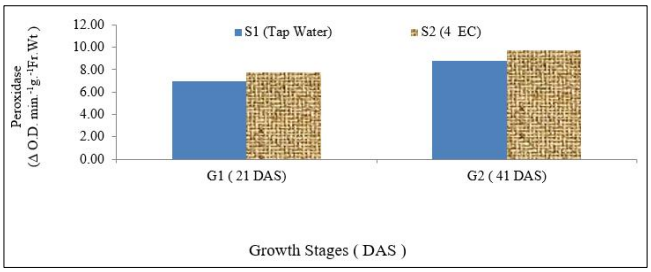


Fig 4: Interaction effect of [A]salinity(S) X treatments(T), [B] growth stages (G) X treatment(T) and [C] salinity(S) X growth stages(G) on peroxidase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaves of cowpea

Mean effect of salinity level should significant difference for peroxidase activity (Fig.3 A). Among the salinity level, treatment S_2 irrigated with saline water showed highest value for peroxidase activity (8.75 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) while the S_1 pot irrigated with tap water showed declined value for peroxidase activity (7.88 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Among the different stages, mean value of peroxidase activity significantly varied between 7.37 and 9.26 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt (Fig.3 B). The content was increased from 21 DAS (7.37 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) to 41 DAS (9.26 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Statistical significant differences were found on imposition of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination (Fig.3 C). However, Treatment (T_8) showed highest value for peroxidase activity in cowpea. The tissues obtain from cowpea pots with Treatment (T_8) GA_3@ 100 ppm + KNO_3@ 500 ppm + Silicic acid@ 50 ppm showed higher amount of mean peroxidase activity (9.06 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The mean lowest content was noted for the tissues received from Treatment (T_1) plant was control condition (7.58. Δ O.D. min.$^{-1}$ g.$^{-1}$ Fr.Wt.).

S X T interaction effects for peroxidase activity were showed non-significant differences (Fig.4 A). However, the highest value of peroxidase activity was observed for the S2T8 i.e. in plant irrigated with saline water and plant treated with GA_3@ 100 ppm + KNO_3@ 500 ppm (9.46 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value (7.09 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) of polyphenol oxidase activity was observed in plant irrigated with tap water without treatment (S_1T_1). Interaction effect of G X T for peroxidase activity were revealed significant differences for peroxidase (Fig.4 B). The highest value of peroxidase activity was observed for G_2T_8 i.e plant treated with GA_3@ 100 ppm + KNO_3@ 500 ppm + Silicic acid@ 50 ppm after at 41 DAS (9.90 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest peroxidase activity was observed for G_1T_1 i.e plant was under control condition after at 21 DAS (6.56 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Interaction effects of S X G for peroxidase activity were revealed significant differences in leaf tissue of cowpea (Fig. 4 C). The highest value of peroxidase activity was observed for S2G2 i.e. in plant irrigated with saline water after 41 DAS (9.72 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value of cowpea was observed for S1G1 in plant irrigated with tape water after 21 DAS (6.69 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Ahmad and Haddad (2011) [6] reported that, the application of silicon was increased the level of peroxidase enzyme activity.

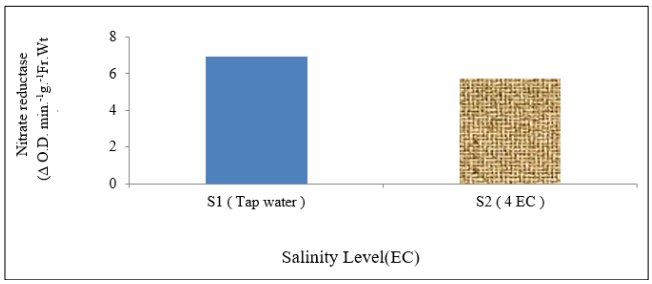
Nitrate reductase

The data on enzyme activity of nitrate reductase (Δ O.D. min.$^{-}$

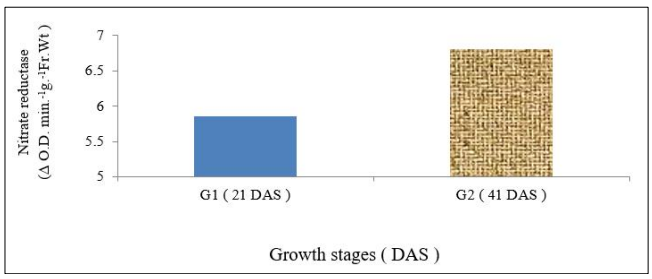
^{1}g.$^{-1}$Fr.Wt.) analyzed from leaf tissue of cowpea collected from plants treated at 10 and 30 DAS with concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T_1 to T_8) grown in a pot irrigated with tape water (S_1) and saline water (S_2) 4 EC at two stages G_1 and G_2 are depicted in Fig. 5, 6.

Nitrate reductase

[A] S.Em±: 0.026 C.D. @ 5%: 0.075



[B] S.Em+: 0.026 C.D. @ 5%: 0.075



[C] S.Em+: 0.053 C.D. @ 5%: 0.149

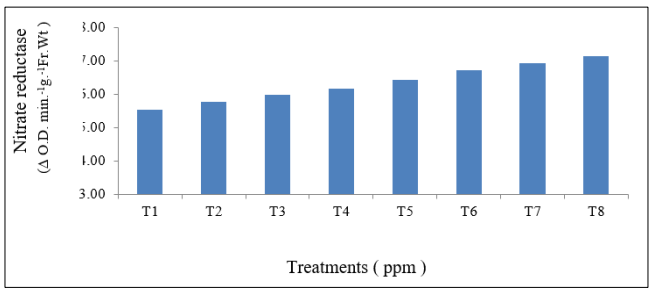
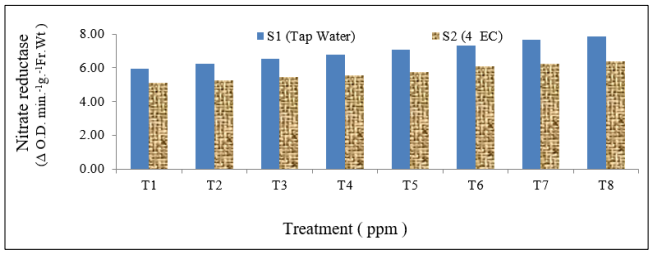
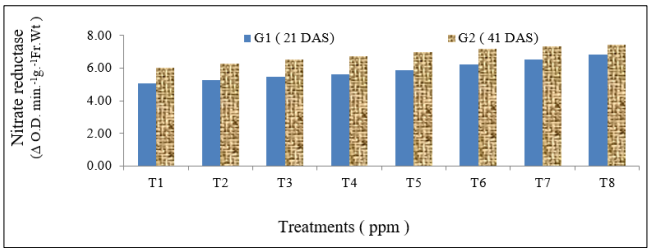


Fig 5: Mean effect of [A] salinity(S), [B]growth stage(G) and [C]treatments(T) on nitrate reductase activity (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) in leaves of cowpea

[A] S.Em+: 0.075 C.D. @ 5%: 0.2



[B] S.Em+: 0.075 C.D. @ 5%: 0.211



[C] S.Em+: 0.037 C.D. @ 5%: 0.105

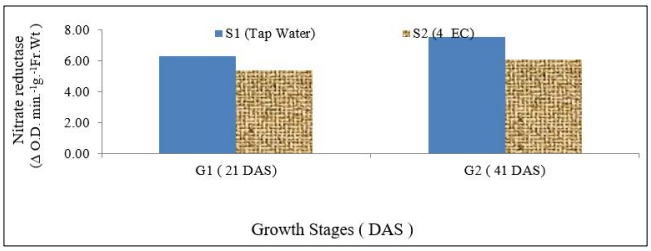


Fig 6: Interaction effect of [A]salinity(S) X treatments(T), [B] growth stages (G) X treatment(T) and [C] salinity(S) X growth stages (G) on nitrate reductase (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaves of cowpea

Mean effect of salinity level were found statistically significant for nitrate reductase activity (Fig. 5 A). Among the salinity level, treatment S_1 irrigated with tap water showed highest value for nitrate reductase activity (6.93 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). While the S_2 pot irrigated with saline water showed declined value for nitrate reductase activity (5.73 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) Among the different stages, mean value of nitrate reductase activity significantly varied between 6.81 and 5.86 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt. (Fig. 5 B). The content was increased from 21 DAS (5.86 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) to 41 DAS (6.801 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). This may be the effect of saline irrigation water as well the property of crop species. Imposition of gibberellic acid, potassium nitrate, silicic acid and their combination showed significant difference (Fig. 5 C). However, Treatment (T_8) revealed highest value for nitrate reductase activity in cowpea. The tissues obtain from cowpea pots with treatment T_8 [GA3 @ 100 ppm + KNO3 @ 500 ppm + Silicic acid @ 50 ppm] showed higher amount of mean nitrate reductase activity (7.14 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The mean lowest content was noted for the tissues received from treatment (T_1) i.e. plant grown in control condition (5.54 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.).

Interaction effects of S X T for nitrate reductase activity were revealed significant differences in cowpea (Fig. 6 A). The highest value of nitrate reductase activity was observed for the S_1T_8 i.e. in plant irrigated with saline water and plant treated with GA_3@ 100 ppm + KNO_3@ 500 ppm (7.87 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value (5.11 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) of nitrate reductase activity was observed in plant irrigated with saline water without treatment (S_2T_1). Interaction effects of G X T for nitrate reductase activity were revealed significant differences in cowpea (Fig. 6 B). The

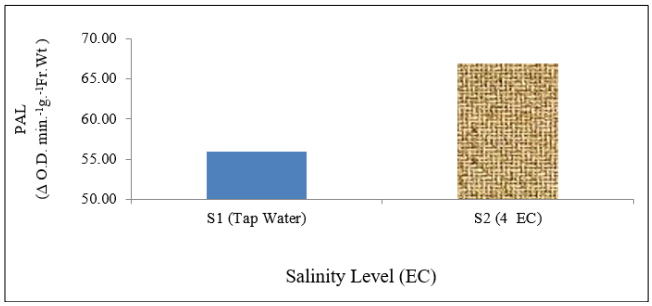
highest value of nitrate reductase activity was observed for G_2T_8 i.e. plant treated with GA_3@ 100 ppm + Silicic acid@ 50 ppm after at 21 DAS (7.44 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value of nitrate reductase activity was observed for G_1T_1 i.e plant treated with GA_3@ 100 ppm + Silicic acid@ 50 ppm after at 21 DAS (5.06 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Interaction effects of S X G for nitrate reductase activity were revealed significant differences in leaf tissue of cowpea (Fig. 6 C). The highest value of nitrate reductase activity was observed for S_1G_2 i.e. in plant irrigated with saline water after 21 DAS (7.55 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value of cowpea was observed for S_2G_1 in plant irrigated with tape water after 41 DAS (5.40 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Jabeen and Ahmad (2011) [6] reported that foliar application of KNO3 under saline water was increased the level of nitrate reductase enzyme. Bybordi (2010) [3] studied that nitrate reductase activity (NRA) was significantly decreased by salinity stress treatment. Under conditions of salt stress, nitrate reductase activity could be lowered initially due to enzyme degradation/inactivation and the reduction in gene expression and nitrate reductase.

Phenylalanine ammonialyase (EC 4.3.1.5)

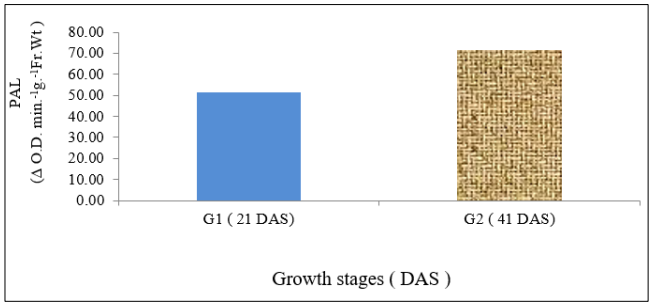
The data on enzyme activity of phenylalanine ammonialyase activity (Δ O.D. min.$^{-1}$g.$^{-1}$ Fr. Wt.) analyzed from leaf tissue of cowpea collected from plants treated at 10 and 30 DAS with concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T_1 to T_8) grown in a pot irrigated with tape water (S_1) and saline water (S_2) 4 EC at stages G_1 and G_2 are depicted in Fig. 7, 8.

Phenylalanine ammonialyase

[A] S.Em+: 0.026 C.D. @ 5%: 0.075



[B] S.Em+: 0.026 C.D. @ 5%: 0.075



[C] S.Em+: 0.053 C.D. @ 5%: 0.149

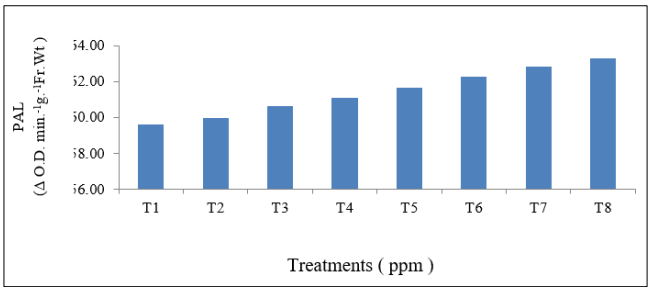
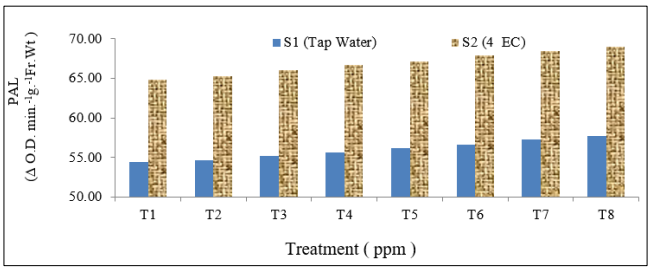
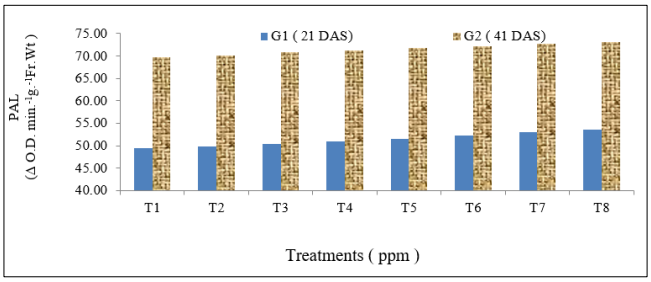


Fig 7: Mean effect of [A]Salinity(S), [B]Growth Stage(G) and [C]Treatment(T) on Phenylalanine ammonialyase activity (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt)in leaves of cowpea

[A] S.Em+: 0.075 C.D. @ 5%: 0.211



[B] S.Em+: 0.075 C.D. @ 5%: 0.211



[C] S.Em+: 0.037 C.D. @ 5%: 0.105

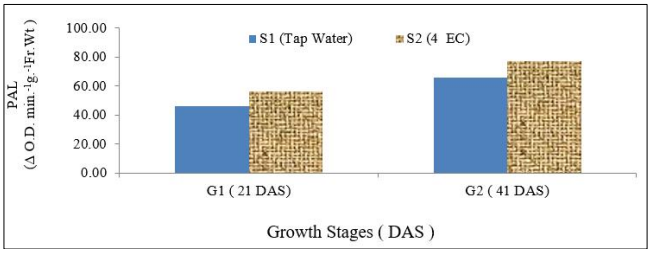


Fig 8: Interaction effect of [A]salinity(S) X treatments(T), [B] growth stages (G) X treatment (T) and [C] salinity(S) X growth stages(G) on phenylalanine ammonialyase activity (Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt) in leaves of cowpea

Mean effect of salinity level were found to be significant for phenylalanine ammonialyase activity (Fig. 7 A). Among the salinity level, treatment S_2 irrigated with saline water showed

highest value for phenylalanine ammonialyase activity (66.89 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) while the S_1 pot irrigated with tap water showed declined value for phenylalanine ammonialyase activity (55.97 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) Among the different stages, phenylalanine ammonialyase activity mean value of cowpea significantly varied between 71.44 and 51.41 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt. (Fig. 7 B). The content was increased from 21 DAS (51.41) to 41 DAS (71.44 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). This may be the effect of saline irrigation water as well the property of crop species. Imposition of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination result to be found significant difference for (Fig. 7 C). Treatment (T_8) showed highest value for phenylalanine ammonialyase activity in cowpea. The tissues obtain from cowpea pots with Treatment (T_8) GA_3@ 100 ppm + KNO_3@ 500 ppm + Silicic acid@ 50 ppm showed higher amount of mean phenylalanine ammonialyase activity (63.31 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The mean lowest content was noted for the tissues received from Treatment (T_1) plant was control condition (59.63).

Interaction effects of S X T for phenylalanine ammonialyase activity were revealed significant differences in cowpea (Fig.8 A). The highest value of phenylalanine ammonialyase activity was observed for the S_2T_8 i.e. in plant irrigated with saline water and plant treated with GA_3@ 100 ppm + KNO_3@ 500 ppm + Silicic acid@ 50 ppm (68.93 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value (54.41 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.) of phenylalanine ammonialyase activity was observed in plant irrigated with tap water and plant was control condition (S_1T_1). Interaction effects of G X T for phenylalanine ammonialyase activity were revealed significant differences in cowpea (Fig. 8 B). The highest value of phenylalanine ammonialyase activity was observed for G_2T_8 i.e. plant treated with GA_3@ 100 ppm + KNO_3@ 500 ppm + Silicic acid@ 50 ppm after at 41 DAS (73.05 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value of phenylalanine ammonialyase activity was observed for G_1T_1 i.e. plant was under control condition after at 21 DAS (49.52 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Interaction effect of S x G for phenylalanine ammonialyase activity were revealed significant differences in leaf tissue of cowpea (Fig. 8 C). The highest value of phenylalanine ammonialyase activity was observed for S_2G_2 i.e. in plant irrigated with saline water after 41 DAS (77.27 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). The lowest value of cowpea was observed for S_1G_1 in plant irrigated with tape water after 21 DAS (46.33 Δ O.D. min.$^{-1}$g.$^{-1}$Fr.Wt.). Gao *et al.* (2008) [5] reported that the level of phenylalanine ammonialyase was increased under saline water. PAL activity was increased under salt stress and this enzyme Modified is involved in the biosynthesis of phenolic and flavonoids method for the determination of pyruvic acid compounds.

Conclusion

The results suggest that the antioxidant enzymes activities were affected due to salinity stress in cowpea. The polyphenol-oxidase, phenylalanine ammonialyase and peroxidase that increase with higher concentration of salt stress but nitrate reductase activity that decrease. On application of GA_3, KNO_3 and silicic acid increased polyphenol oxidase, peroxidase, nitrate reductase, phenylalanine ammonialyase enzymatic activity. This investigation has proved gibberellic acid, potassium nitrate and silicic acid as a potential biomolecules affecting the ROS scavenging enzyme under abiotic stress like salinity.

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