Effect of gibberellic acid, potassium nitrate and silicic acid on antioxidative enzymes in groundnut (Arachis hypogaea L.) seedling irrigated with saline water

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Abstract
Salinity is one of the most serious limiting factors for growth and production in groundnut and silicic an important phenolics, gibberellic acid an important growth hormone, potassium nitrate known to alleviates its adverse effect. Thus, Green house experiment was conducted to investigate the effect of gibberellic acid, potassium nitrate and silicic acid under salt stress on physiological, biochemical parameters and enzymatic activities of groundnut. The observations were recorded for enzymes viz., polyphenol oxidase, peroxidase and catalase. The result suggested that the antioxidant enzymes activity was affected due to salinity stress in groundnut. The antioxidant enzymes activities viz., polyphenol oxidase, peroxidase and catalase that increased with higher concentration of salt stress. On application of GA3, KNO3 and silicic acid decreased polyphenol oxidase, peroxidase and catalase enzymatic activity. This investigation has suggested gibberellic acid, potassium nitrate and silicic acid are potential biomolecules affecting the ROS scavenging enzyme under abiotic stress like salinity.

Keywords: Groundnut (Arachis hypogaea L.), gibberellic acid, silicic acid, potassium nitrate and salinity stress

Introduction
Groundnut (Arachis hypogaea L.) is an annual prostrate herbaceous leguminous oilseed crop. Groundnut is native of South America (Hammons, 1982) [1]. After cereals, oilseeds are the second largest agricultural commodity sharing 14 per cent of country’s gross cropped area and account for nearly 5 per cent of gross national products and 10 per cent of the value of all agricultural products. Soil salinity adversely affects plant growth and development. Worldwide, about one-third of irrigated arable land is already affected and that level is still rising (Lazof and Bernstein, 1999) [8]. 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 various abiotic stresses. These mechanism includes, accumulation of different osmolites and phenolics compounds, induction of antioxidant and its related enzymatic system etc., (Vakharia et al., 1997; Kandoliya and Vakharia, 2013; Kandoliya and Vakharia, 2015 and Joshi et al., 2018) [20, 6, 7, 5]. Induced salt tolerance by exogenous application of various chemicals and hormones is a highly attractive approach to overcome the salinity threat (Trivedi et al., 2018; Solanki et al., 2018 a, b and Patel et al., 2019) [19, 17, 18, 14]. Gibberellic acid, growth hormone which enhances the flowering, increases fruit set as well as fruit size. It shows positive responses to increase the shelf life as well as quality parameters of the fruits and vegetables. Potassium enhanced resistance toward the bacterial, viral, nematodes and fungal pathogens (Perrenoud, 1990) [15]. 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) [10].

Materials and Methods
The green house experiment was conducted during kharif 2018-19 at Food testing Laboratory, Department of Biochemistry, Junagadh Agricultural University, Junagadh. Groundnut (Arachis hypogaea L.) seeds of varieties GG-20 were obtained from Main Oilseeds Research Station, J.A.U., Junagadh for the experiment.
Results and Discussion

Polyphenol oxidase (PPO) activity (EC 1.14.18.1)

Polyphenol oxidase (PPO, O-diphenol: O2-oxidoreductase) is also known as phenolase, phenol oxidase, catechol oxidase and tyrosinase. The enzyme is widely distributed in nature. It is known to be present in soluble forms in cytoplasm or bound to mitochondria, chloroplasts and certain other sub-cellular organelles. The data on enzyme activity of polyphenol oxidase activity (ΔOD.min.-1 g-1 Fr.Wt.) analyzed from leaf tissues of groundnut collected from plants treated at 20 DAS and 40 DAS with different concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T1 to T3) grown in a pot irrigated with tap water (S1) and saline water (S2) at two different stages G1 (30 DAS) and G2 (50 DAS) are depicted in Fig. 1.

Mean effect of salinity level irrespective of gibberellic acid, potassium nitrate, silicic acid treatment and their combination and growth stages i.e. before and after spraying of gibberellic acid, potassium nitrate, silicic acid and their treatment combination were found statistical significant for polyphenol oxidase activity (Fig. 1 A). Among the salinity level, treatment S2 irrigated with tap water showed lowest amount of polyphenol oxidase activity (5.86 ΔOD.min.-1 g-1 Fr.Wt.) while the pot irrigated with saline water 4 EC (S2) showed highest value for polyphenol oxidase activity (6.45 ΔOD.min.-1 g-1 Fr.Wt.). Among the different stages, mean value of polyphenol oxidase activity significantly varied between 6.48 ΔOD.min.-1 g-1 Fr.Wt. and 5.84 ΔOD.min.-1 g-1 Fr.Wt. (Fig. 1 B). The content was decreased from 30 DAS (6.48 ΔOD.min.-1 g-1 Fr.Wt.) to 50 DAS (5.84 ΔOD.min.-1 g-1 Fr.Wt.). Application of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination found statistical significant (Fig. 1 C). Treatment T3 [GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acid @ 50 ppm] caused marked decrease in polyphenol oxidase activity in groundnut leaf tissues. The tissues obtain from groundnut pots treated with T3 [GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acid @ 50 ppm] revealed lower amount of mean polyphenol oxidase activity (4.57 ΔOD.min.-1 g-1 Fr.Wt.) and which was followed by T1 [GA3 @ 100 ppm + silicic acid @ 50 ppm (5.70 ΔOD.min.-1 g-1 Fr.Wt.)] and T3 [KNO3 @ 500 ppm + silicic acid @ 50 ppm (5.79 ΔOD.min.-1 g-1 Fr.Wt.)] irrespective of salinity level and growth stages. The mean highest content was noted for the tissues received from T1 (7.11 ΔOD.min.-1 g-1 Fr.Wt.).

S X T interaction effect for polyphenol oxidase activity was revealed significant differences in leaf tissues of groundnut (Fig. 2 A). The lowest value of polyphenol oxidase activity content was observed for the S1T1 i.e. in plant irrigated with tap water combine with GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acids @ 50 ppm treatment (4.38 ΔOD.min.-1 g-1 Fr.Wt.). The highest value (7.42 ΔOD.min.-1 g-1 Fr.Wt.) of polyphenol oxidase activity was observed in plant irrigated with saline water (4 EC) under control condition (S1T1).

Interaction effect of G X T for polyphenol oxidase activity was revealed significant differences in leaf tissues of groundnut (Fig. 2 B). The lowest value of polyphenol oxidase activity was observed in G3T3 i.e. in plant treated with GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acid @ 50 ppm after at 50 DAS (4.02 ΔOD.min.-1 g-1 Fr.Wt.). The highest value of polyphenol oxidase activity was observed for G1T2 i.e. plant was control condition after 30 DAS (7.50 ΔOD.min.-1 g-1 Fr.Wt.). The observation from interaction effect of S X G for polyphenol oxidase activity was found significant.
differences in leaf tissues of groundnut (Fig. 2 C). The lowest value of polyphenol oxidase activity was observed in S\textsubscript{i}, G\textsubscript{j}; i.e. in plant irrigated with tap water after 50 DAS (5.46 ΔO.D.min. g\textsuperscript{-1} Fr.Wt.). The highest value of polyphenol oxidase activity was observed for S\textsubscript{i}, G\textsubscript{j} i.e. in plant irrigated with tap water after 50 DAS (6.69 ΔO.D.min. g\textsuperscript{-1} Fr.Wt.). These results were in agreement with Mohan and Shashidharan (2018) \cite{11} who reported that salinity increase polyphenol oxidase activity in groundnut seedling. Patel et al. (2010) \cite{13} also noted that the polyphenol oxidase generated \textit{H}\textsubscript{2}O\textsubscript{2} could also be a component of significantly process for defense against stress condition.

**Polyphenol oxidase**

![Polyphenol oxidase graph](image1)

![Salinity Level (EC)](image2)

![Peroxidase activity graph](image3)

![Peroxidase activity graph](image4)

![Peroxidase activity graph](image5)

**Fig 1:** Mean effect of [A] salinity (S), [B] growth stages (G) and [C] treatments (T) on polyphenol oxidase activity (ΔO.D.min. g\textsuperscript{-1} Fr.Wt.) in leaf tissues of groundnut

**Fig 2:** Interaction effect of [A] salinity (S) X treatments (T), [B] growth stages (G) X treatments (T), [C] salinity (S) X growth stages (G) on polyphenol oxidase activity (ΔO.D.min. g\textsuperscript{-1} Fr.Wt.) in leaf tissues of groundnut

**Peroxidase (EC 1.11.1.7 )**

Peroxidase, a group of heme-containing glycosylated protein, was found to play significant role in defense mechanisms in plant against infection with pathogen. The data on enzyme activity of peroxidase activity (ΔO.D.min. g\textsuperscript{-1} Fr.Wt.) analyzed from leaf tissues of groundnut collected from plants treated at 20 DAS and 40 DAS with different concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T\textsubscript{i} to T\textsubscript{8}) grown in a pot irrigated with tap water (S\textsubscript{1}) and saline water (S\textsubscript{2}) at two different stages (G\textsubscript{i}, G\textsubscript{j}) of growth are depicted in Fig. 3.
Mean effect of salinity level irrespective of gibberellic acid, potassium nitrate, silicic acid treatment and their combination and growth stages i.e. before and after spraying of gibberellic acid, potassium nitrate, silicic acid and their treatment combination were found statistical significant for peroxidase activity (Fig. 3 A).

Among the salinity level, treatment S₁ irrigated with tap water showed lowest amount of peroxidase activity (6.30 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.) while the pot irrigated with saline water 4 EC (S₂) showed highest value for peroxidase activity (7.23 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.). Trivedi et al. (2018) also reported an increased in peroxidase activity under salinity stress in green gram.

Among the different stages, mean value of peroxidase activity significantly varied between 7.14 and 6.38 (ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.) (Fig. 3 B). The content was decreased from 30 DAS (7.14) to 50 DAS (6.38) (ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.). Differences were found on imposition of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination statistical significant (Fig. 3 C). Treatment T₃ [GA₃ @ 100 ppm + KNO₃ @ 500 ppm + silicic acid @ 50 ppm] caused marked increase in peroxidase activity in groundnut leaf tissues. The tissues obtain from groundnut pots treated with T₃ [GA₃ @ 100 ppm + KNO₃ @ 500 ppm + silicic acid @ 50 ppm] revealed higher amount of mean peroxidase activity (7.98 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.) and which was followed by T₅ [GA₃ @ 100 ppm + silicic acid @ 50 ppm (7.31 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.)] and T₆ [KNO₃ @ 500 ppm + silicic acid @ 50 ppm (7.37 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T₁ (5.22 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.).

S X T interaction effect for peroxidase activity was revealed significant differences in leaf tissue of groundnut (Fig. 4 A). The highest value of peroxidase activity was observed for S₁T₅ i.e. in plant irrigated with saline water combine with GA₃ @ 100 ppm + KNO₃ @ 500 ppm + silicic acids @ 50 ppm treatment after 50 DAS (8.35 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.). The lowest value (4.78 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.) of peroxidase activity was observed in plant irrigated with tap water under control condition (S₁T₁). Interaction effect of G X T for peroxidase activity was revealed significant differences in leaf tissue of groundnut (Fig. 4 B). The highest value of peroxidase activity was observed in G₁T₃ i.e. in plant treated with GA₃ @ 100 ppm + KNO₃ @ 500 ppm + silicic acid @ 50 ppm after at 30 DAS (8.33 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.). The lowest value of peroxidase activity was observed for G₅T₁ i.e. plant was control condition after 50 DAS (4.87 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.).

Interaction effect of S X G for peroxidase activity was revealed significant differences in leaf tissue of groundnut (Fig. 4 C). The highest value of peroxidase activity was observed in S₂G₁ i.e. in plant irrigated with saline water (4 EC) after 30 DAS (7.69 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.). The lowest value of peroxidase activity was observed for S₁G₂ (5.99 ΔO.D.min.⁻¹ g⁻¹ Fr.Wt.). These results were in agreement with Shinde et al. (2017) who reported that salinity increase peroxidase activity in groundnut seedling.

Imposition of abiotic stress significantly reduced relative water content, membrane stability and total carotenoid content in all the cultivars.

Relationship among different physiological parameters showed that the level of oxidative stress, in terms of production of reactive oxygen species, was negatively correlated with activities of different antioxidant enzymes such as catalase and peroxidase (Chakraborty et al., 2015) [3].
One of the main enzymes playing a role in catabolism of hydrogen peroxide is catalase. The catalase is tetrameric heme protein, occurring in almost all aerobic organisms including plants. The data on enzyme activity of catalase activity (ΔO.D.min. -1.g.-1 Fr.Wt.) analyzed from leaf tissues of groundnut collected from plants treated at 20 DAS and 40 DAS with different concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T1 to T8) grown in a pot irrigated with tape water (S1) and saline water (S2) 4 EC at two different stages G1 (30 DAS) and G2 (50 DAS) are depicted in Fig. 5. Mean effect of salinity level irrespective of gibberellic acid, potassium nitrate, silicic acid treatment and their combination and growth stages i.e. before and after spraying of gibberellic acid, potassium nitrate, silicic acid and their treatment combination were found statistical significant for catalase activity (Fig. 5 A). Among the salinity level, treatment S1 irrigated with tap water showed lowest amount of catalase activity (3.45 ΔO.D.min. -1.g.-1 Fr.Wt.) while the pot irrigated with saline water 4 EC (S2) showed highest value for catalase activity (4.06 ΔO.D.min. -1.g.-1 Fr.Wt.). Earlier it was reported that the catalase increase with the increase in the concentration of NaCl.

Among the different stages, mean value of catalase activity significantly varied between 4.08 ΔO.D.min. -1.g.-1 Fr.Wt. and 3.43 ΔO.D.min. -1.g.-1 Fr.Wt. (Fig. 5 B). The content was decreased from 30 DAS (4.08 ΔO.D.min. -1.g.-1 Fr.Wt.) to 50 DAS (3.43 ΔO.D.min. -1.g.-1 Fr.Wt.).

Mean data on imposition of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combinations were found statistical significant (Fig. 5 C). Treatment T8 [GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acid @ 50 ppm] caused marked decrease in catalase activity in groundnut leaf tissues. The tissues obtain from groundnut pots treated with T8 [GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acid @ 50 ppm] revealed lower amount of mean catalase activity (2.67 ΔO.D.min. -1.g.-1 Fr.Wt.) and which was followed by T7 [GA3 @ 100 ppm + silicic acid @ 50 ppm (3.29 ΔO.D.min. -1.g.-1 Fr.Wt.)] and T6 [KNO3 @ 500 ppm + silicic acid @ 50 ppm (3.32 ΔO.D.min. -1.g.-1 Fr.Wt.)] irrespective of salinity level and growth stages. The mean highest content was noted for the tissues received from T1 (4.97 ΔO.D.min. -1.g.-1 Fr.Wt.).

Interaction effect of S X T for catalase activity was revealed significant differences in leaf tissues of groundnut (Fig. 6 A). The lowest value of catalase activity content was observed for the S1T8 i.e. in plant irrigated with tap water combine with GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acids @ 50 ppm treatment (2.45 ΔO.D.min. -1.g.-1 Fr.Wt.). The highest value (5.39 ΔO.D.min. -1.g.-1 Fr.Wt.) of catalase activity was observed in plant irrigated with saline water (4 EC) under control condition (S1T1).

G X T interaction effect for catalase activity was observed statistically significant in leaf tissues of groundnut (Fig. 6 B). The lowest value of catalase activity was observed in G2T8 i.e. in plant treated with GA3 @ 100 ppm + KNO3 @ 500 ppm + silicic acid @ 50 ppm after at 50 DAS (2.33 ΔO.D.min. -1.g.-1 Fr.Wt.). The highest value of catalase activity was observed for G1T1 i.e. plant was control condition after 30 DAS (5.38 ΔO.D.min. -1.g.-1 Fr.Wt.).

The data recorded from S X G interaction effect for catalase activity was revealed significant differences in leaf tissue of groundnut (Fig. 6 C). The lowest value of catalase activity was observed in S1G2 i.e. in plant irrigated with tap water after 50 DAS (3.18 ΔO.D.min. -1.g.-1 Fr.Wt.). The highest value of catalase activity was observed for S1G1 (4.44 ΔO.D.min. -1.g.-1 Fr.Wt.). These results were in agreement with Mohan and Shashidharan (2018) [11] who reported that salinity increase catalase activity in groundnut seedling.
**Fig 5:** Mean effect of [A] salinity (S), [B] growth stages (G) and [C] treatments (T) on catalase activity ($\Delta$O.D.min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaf tissues of groundnut.

**Fig 6:** Interaction effect of [A] salinity (S) X treatments (T), [B] growth stages (G) X treatments (T), [C] salinity (S) X growth stages (G) on catalase activity ($\Delta$O.D.min.$^{-1}$g.$^{-1}$Fr.Wt.) in leaf tissues of groundnut.

**Conclusion**

The results suggest that the antioxidant enzymes activities were affected due to salinity stress in groundnut. The polyphenol oxidase, peroxidase and catalase were increased with higher concentration of salt stress. On application of GA$_3$, KNO$_3$ and silicic acid decreased polyphenol oxidase, peroxidase and catalase 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.

**References**


