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Effect of gibberellic acid, potassium nitrate and silicic acid on biochemical constituents of groundnut (*Arachis hypogaea* L.) seedling exposed to saline water

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Abstract
Salinity reduces the ability of plant utilize water and causes a reducing in growth rates, as well as changes in plant metabolic processes in most of the crops including groundnut. The molecules like silicic, gibberellic acid and potassium nitrate known to alleviates 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 biochemical parameters of groundnut. The observations were recorded for the biochemical parameters total soluble sugars, reducing sugars, total phenol, proline and glycine betaine. The result was suggested that the biochemical constituents were adversely affected due to salinity stress in groundnut. On application of GA_3, KNO_3 and silicic acid increased total soluble sugars, reducing sugars, total phenol, proline and glycine betaine. This investigation has suggested gibberellic acid, potassium nitrate and silicic acid, as a potential biomolecules affecting certain biomolecules related to osmotic adjustment as against the abiotic stress like salinity.

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

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop cultivated mainly in arid and semiarid regions. Salinity reduces the ability of plant to utilize water and causes a reducing in growth rates, as well as changes in plant metabolic processes in most of the crops including groundnut. Soil salinity adversely affects plant growth and development. However, plants equipped with a variety of defense mechanism against biotic as well as various abiotic stresses. This mechanism include, accumulation of different osmolites and phenolics compounds, induction of antioxidant and its related enzymatic system etc., (Vakharia *et al.*, 1997; Patel *et al.*, 2010; Kandoliya and Vakharia, 2013; Kandoliya and Vakharia, 2015 and Joshi *et al.*, 2018) [28, 16, 9, 10, 8]. Induced salt tolerance by exogenous application of various chemicals and hormones is a highly attractive approach to overcome the salinity threats (Trivedi *et al.*, 2018; Solanki *et al.*, 2018; Patel *et al.*, 2019a, Patel *et al.*, 2019b and Purohit *et al.*, 2020) [27, 25, 17, 18, 20]. Gibberellic acid, growth hormone which enhances the flowering, fruit set, fruit size, 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) [19]. 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) [13]. Thus, present experiment was conducted to investigate the effect of exogenous application of gibberellic acid, potassium nitrate and silicic acid under salt stress on various biochemical parameters in groundnut crop.

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 variety GG-20 were obtained from Main Oilseeds Research Station, J.A.U., Junagadh for the experiment.

Treatments

(a) Salinity level (2): 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 and Silicic acid (8): 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, T_8- Sprayed with GA_3 @ 100 ppm + KNO_3 **@** 500 ppm + Silicic acid @ 50 ppm.

(c) Growth stage (2): G_1- 30 DAS, G_2- 50 DAS

Groundnut leaf were collected at different stages (G_1 and G_2) after the spray of gibberellic acid, potassium nitrate and silicic acid the pot irrigated with saline water having a concentration 4 dSm^{-1} and packed in plastic bag and brought to the laboratory under ice cold condition. Leaf tissues were taken for first two stages (G_1 and G_2) at ten days after the gibberellic acid, potassium nitrate and silicic acid spray. The experimental materials were cleaned, weighed and then transferred immediately to the respective medium for various biochemical and physiological analysis.

(d) Biochemical parameter

Seedlings (100 mg) were extracted with 5 ml of 80% ethanol and total soluble sugar was estimated as per Dubois *et al.* (1956) [5]. Reducing sugar was estimated from the same extract as described by Somogyi (1952) [26]. Suitable aliquot (0.1 ml) of was taken from methanol extract prepared for total phenol analysis and evaporated to dryness in water bath and total phenol estimated as per Bray and Thorpe (1954) [4].

The free proline was estimated as stated by Bates *et al*. (1973) [3]. Glycine betaine was done from fresh leaves as per the method of Hendawey (2015) [7].

Results and Discussion

The data on various biochemical parameters analyzed from leaf tissue 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_1 to T_8) grown in a pot irrigated with tape water (S_1) and saline water (S_2) 4 EC at two different stages G_1 (30 DAS) and G_2 (50 DAS) are depicted in respective figures.

Total soluble sugar

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 total soluble sugar (Fig. 1 A).

Among the salinity level, treatment S_1 irrigated with tap water showed lowest amount of total soluble sugar (3.94%) while the pot irrigated with saline water 4 EC (S_2) showed highest value for total soluble sugar (4.76%). As compared to S_1 (Tap water) 20.81% total soluble sugar content was increased in S_2 (4 EC).

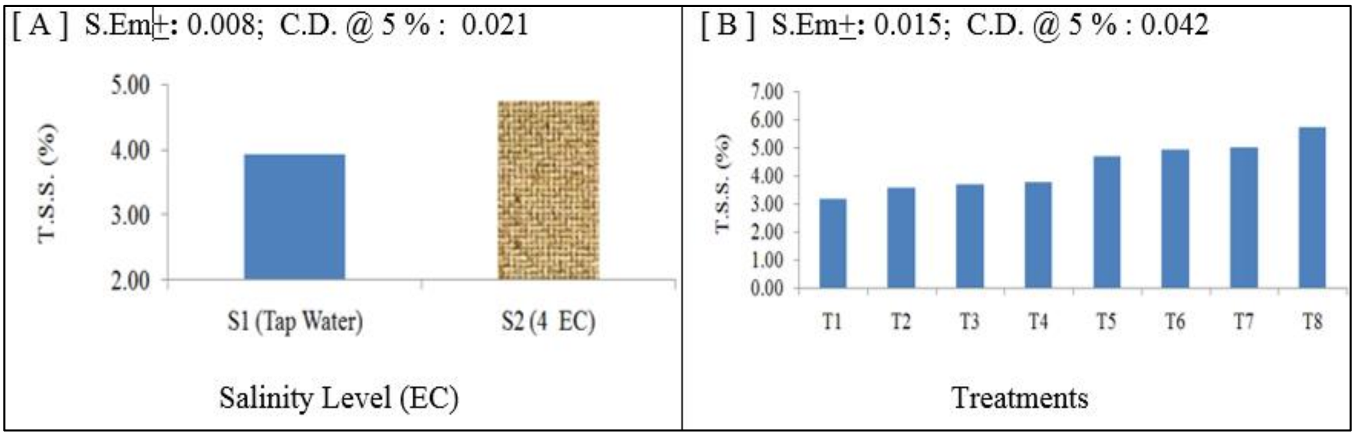

Fig 1: Mean effect of [A] salinity (S) and [B] treatments (T) on total soluble sugar content (%) in leaf tissues of groundnut.

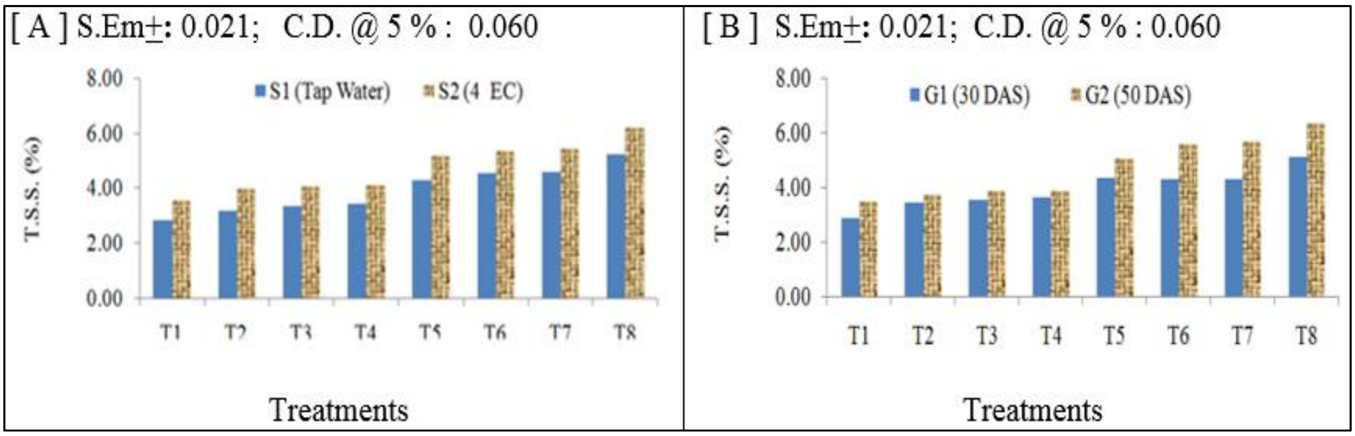

Fig 2: Interaction effect of [A] salinity (S) X treatments (T) and [B] growth stages (G) X treatments (T) on total soluble sugar content (%) in leaf tissues of groundnut.

Imposition of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination found statistical significant (Fig. 1 B). Treatment T_8 [GA_3@ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm] caused marked increase in total soluble sugar in groundnut leaf tissue. The tissues obtain from groundnut pots treated with T_8 [GA_3 @ 100 ppm + KNO3 @ 500 ppm + silicic acid @ 50 ppm] revealed higher amount of mean total soluble sugar (5.76%) and which was followed by T_7 [GA_3 @ 100 ppm + silicic acid @ 50 ppm (5.04%)] and T_6 [KNO_3@ 500 ppm + silicic acid @ 50 ppm (4.96%)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T_1 (3.20%).

Interaction effect of S X T for total soluble sugar was revealed significant differences in leaf tissue of groundnut (Fig. 2 A). The highest value of total soluble sugar content was observed for the S_2T_8 i.e. in plant irrigated with saline water combine with GA_3 @ 100 ppm + KNO_3 @ 500 ppm + silicic acids @ 50 ppm treatment after 50 DAS (6.26%). The lowest value (2.82%) of total soluble sugar content was observed in plant irrigated with tap water under control condition (S_1T_1).

Interaction effect of G X T for total soluble sugar content was revealed significant differences in leaf tissue of groundnut (Fig. 2 B). The highest value of total soluble sugar content was observed in G_2T_8 i.e. in plant treated with GA_3 @ 100 ppm + KNO_3 @ 500 ppm + silicic acid @ 50 ppm after at 50 DAS (6.37%). The lowest value of total soluble sugar content was observed for G_1T_1 i.e. plant was control condition after 30 DAS (2.91%).

These results were in agreement with Kazemi (2013) [12] who reported that salinity increase total soluble sugar in groundnut seedling. Almost all-metabolic process is affected by water deficits. Severe water deficits cause decreases in enzymatic activity. Complex carbohydrates and proteins are broken down by enzymes into simpler sugars and amino acids, respectively (Reddy *et al*., 2003) [21].

Reducing sugar

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 reducing sugar (Fig. 3 A). Among the salinity level, treatment S_1 irrigated with tap water showed lowest amount of reducing sugar (2.81%) while the pot irrigated with saline water 4 EC (S_2) showed highest value for reducing sugar (3.30%).

Application of spray treatment including gibberellic acid, potassium nitrate, silicic acid and their combination found statistical significant (Fig. 3B). Treatment T_8 [GA_3@ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm] caused marked increase in reducing sugar in groundnut leaf tissue. The tissues obtain from groundnut pots treated with T_8 [GA_3 @ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm] revealed higher amount of mean reducing sugar (4.34%) and which was followed by T_7 [GA_3 @ 100 ppm + silicic acid @ 50 ppm (3.70%)] and T_6 [KNO_3@ 500 ppm + silicic acid @ 50 ppm (3.65%)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T_1 (1.77%).

Interaction effect of S X T for reducing sugar was revealed significant differences in leaf tissue of groundnut (Fig. 4 A). The highest value of reducing sugar content was observed for the S_2T_8 i.e. in plant irrigated with saline water combine with GA_3@ 100 ppm + KNO_3 @ 500 ppm + silicic acids @ 50 ppm treatment after 50 DAS (4.67%). The lowest value (1.56%) of reducing sugar content was observed in plant irrigated with tap water under control condition (S_1T_1).

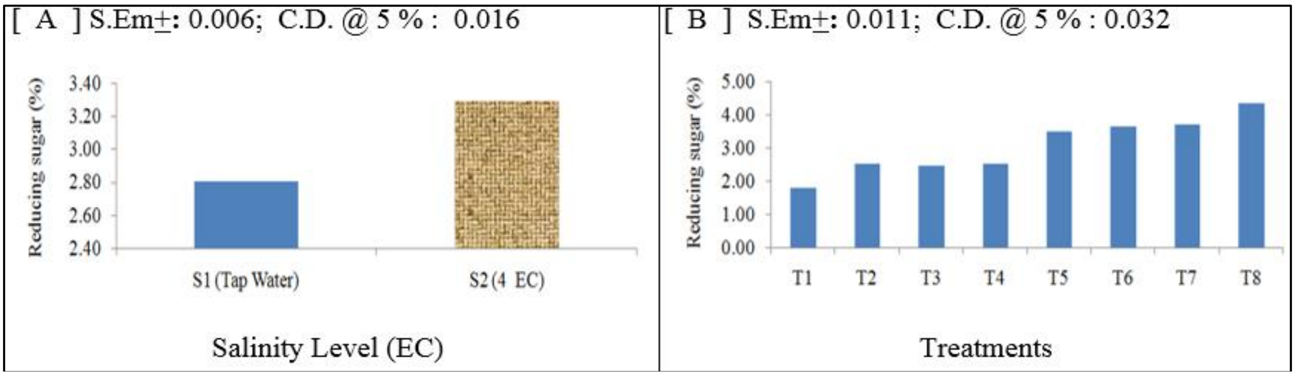

Fig 3: Mean effect of [A] salinity (S) and [B] treatments (T) on reducing sugar content (%) in leaf tissues of groundnut.

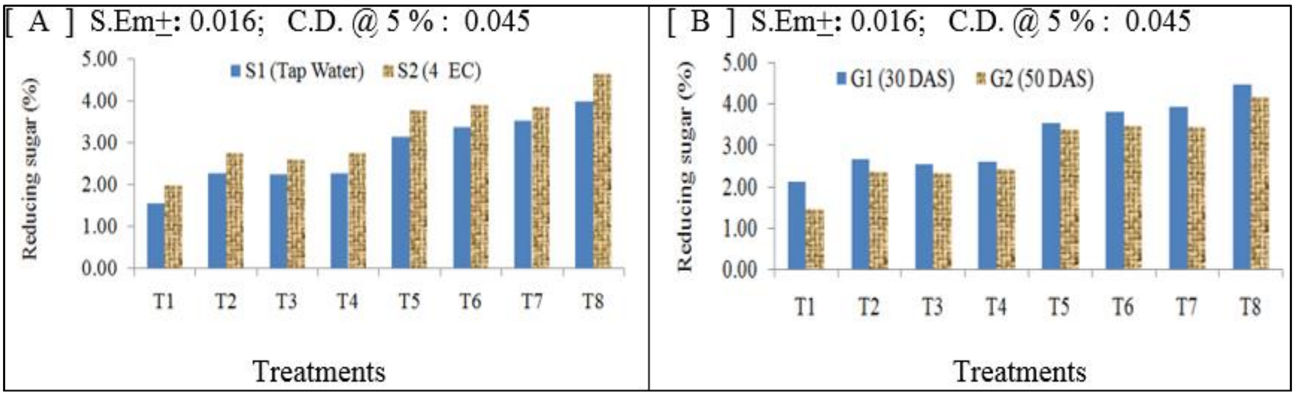

Fig 4: Interaction effect of [A] salinity (S) X treatments (T) and [B] growth stages (G) X treatments (T) on reducing sugar content (%) in leaf tissues of groundnut.

Interaction effect of G X T for reducing sugar content was revealed significant differences in leaf tissue of groundnut (Fig. 4 B). The highest value of reducing sugar content was observed in G_1T_8 i.e. plant treated with GA_3 @ 100 ppm + KNO_3 @ 500 ppm + silicic acid @ 50 ppm after at 30 DAS (4.48%). The lowest value of reducing sugar content was observed for G_2T_1 i.e. plant was control condition after 50 DAS (1.44%).
These results were in agreement with Sangeetha and Subramani (2014) [22] who reported that salinity increase reducing sugar content in groundnut seedling.

Total phenol
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 total phenol (Fig. 5 A). Among the salinity level, treatment S_1 irrigated with tap water showed lowest amount of total phenol (13.07 $mg.g^{-1}$) while the pot irrigated with saline water 4 EC (S_2) showed highest value for total phenol (13.90 $mg.g^{-1}$).
Spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination found statistical significant (Fig. 5 B). Treatment T_8 [GA_3@ 100 ppm + KNO_3 @ 500 ppm + silicic acid @ 50 ppm caused marked increase in total phenol in groundnut leaf tissue. The tissues obtain from groundnut pots treated with T_8 [GA_3 @ 100 ppm + KNO_3 @ 500 ppm + silicic acid @ 50 ppm] revealed higher amount of mean total phenol (16.29 $mg.g^{-1}$) and which was followed by T_7 [GA_3 @ 100 ppm + silicic acid @ 50 ppm (14.08 $mg.g^{-1}$)] and T_6 [KNO_3@ 500 ppm + silicic acid @ 50 ppm (13.98 $mg.g^{-1}$)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T_1 (11.90 $mg.g^{-1}$).

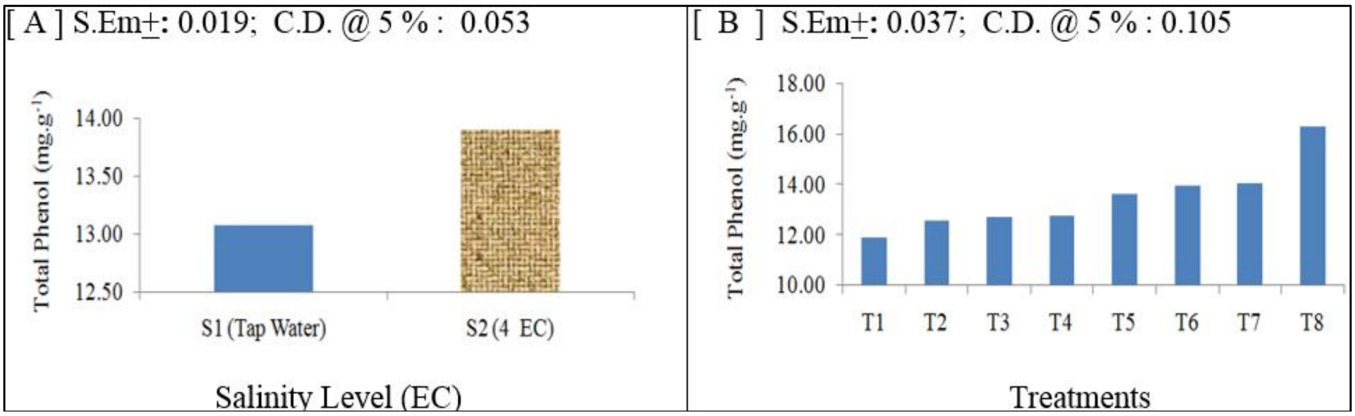

Fig 5: Mean effect of [A] salinity (S) and [B] treatments (T) on total phenol content ($mg.g^{-1}$) in leaf tissues of groundnut.

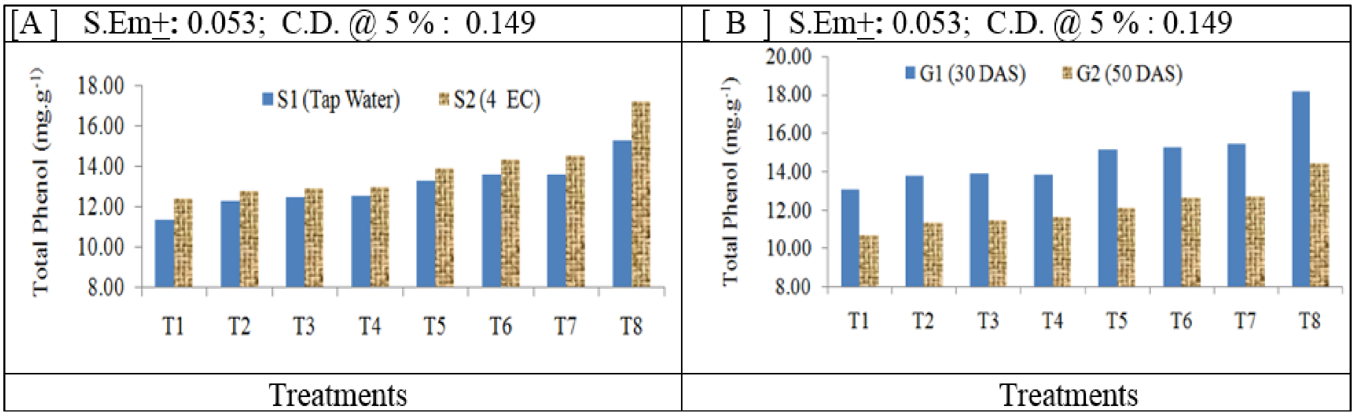

Fig 6: Interaction effect of [A] salinity (S) X treatments (T) and [B] growth stages (G) X treatments (T) on total phenol content ($mg.g^{-1}$) in leaf tissues of groundnut.

Interaction effect of S X T for total phenol showed significant differences in leaf tissue of groundnut (Fig. 6 A). The highest value of total phenol content was observed for the S_2T_8 i.e. in plant irrigated with saline water combine with GA_3@ 100 ppm + KNO_3 @ 500 ppm + silicic acid @ 50 ppm treatment after 50 DAS (17.26 $mg.g^{-1}$). The lowest value (11.38 $mg.g^{-1}$) of total phenol content was observed in plant irrigated with tap water under control condition (S_1T_1).
Interaction effect of G X T for total phenol content was found statistically significant differences for total phenol content in groundnut (Fig. 6 B). The highest value of total phenol content was observed in G_1T_8 i.e. in plant treated with GA_3 @ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm after at 30 DAS (18.15 $mg.g^{-1}$). The lowest value of total phenol content was observed for G_2T_1 i.e. plant was control condition after 50 DAS (10.73 $mg.g^{-1}$). Zohra *et al.* (2016) [29] also suggest that the polyphenol compounds involved in the defense against salt stress. Kandoliya *et al*. (2016) [11] noted that the phenolic compound may contribute directly to the antioxidant action.
These results were in agreement with Mohan *et al.* (2018) [14] who reported that salinity increase phenolic compound in groundnut seedling. The phenolic contents increased under prevalence of saline conditions in mungbean. This increase might be due to cellular adaptive mechanism for scavenging the reactive oxygen species (Aslam *et al.*, 2016) [1]. In general, the higher phenol content was associated with higher antioxidant capacity (Santas *et al*., 2008) [23]. Several studies

have also reported a good correlation between total phenol of plant extract and antioxidant activity (Bahorun *et al*., 2004) [2].

Proline

Mean data 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 proline (Fig. 7 A). Among the salinity level, treatment S_1 irrigated with tap water showed lowest amount of proline (2.99 $mg.g^{-1}$) while the pot irrigated with saline water 4 EC (S_2) showed highest value for proline (3.98 $mg.g^{-1}$). As compared to S_1 (Tap water) 33.11% proline content was increased in S_2 (4 EC).

Spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination found statistical significant (Fig. 7 B). Treatment T_8 [GA_3@ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm] caused marked increase in proline in groundnut leaf tissue. The tissues obtain from groundnut pots treated with T_8 [GA_3 @ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm] revealed higher amount of mean proline (4.73 $mg.g^{-1}$) and which was followed by T_7 [GA_3 @ 100 ppm + silicic acid @ 50 ppm (4.02 $mg.g^{-1}$)] and T_6 [KNO_3@ 500 ppm + silicic acid @ 50 ppm (3.94 $mg.g^{-1}$)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T_1 (2.22 $mg.g^{-1}$).

Interaction effect of S X T for proline was revealed significant differences in leaf tissue of groundnut (Fig. 8 A). The highest value of proline content was observed for the S_2T_8 i.e. in plant irrigated with saline water combine with GA_3@ 100 ppm + KNO_3 @ 500 ppm + silicic acids @ 50 ppm treatment after 50 DAS (5.33 $mg.g^{-1}$). The lowest value (1.73 $mg.g^{-1}$) of proline content was observed in plant irrigated with tap water under control condition (S_1T_1).

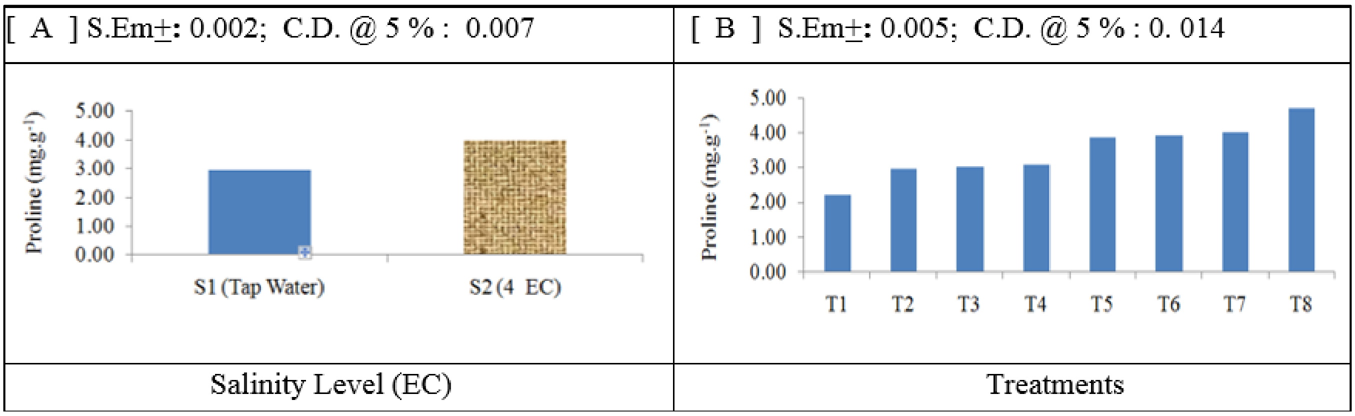

Fig 7: Mean effect of [A] salinity (S) and [B] treatments (T) on proline content ($mg.g^{-1}$) in leaf tissues of groundnut.

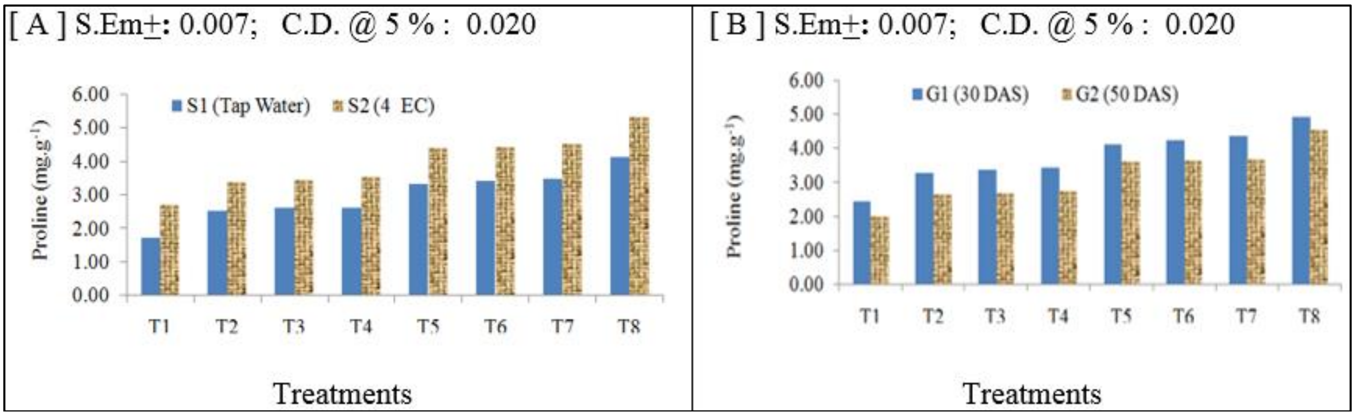

Fig 8: Interaction effect of [A] salinity (S) X treatments (T) and [B] growth stages (G) X treatments (T) on proline content ($mg.g^{-1}$) in leaf tissues of groundnut.

G X T interaction effect for proline content was revealed significant differences in leaf tissue of groundnut (Fig. 8 B). The highest value of proline content was observed in G_1T_8 i.e. in plant treated with GA_3 @ 100 ppm + KNO_3 @ 500 ppm + silicic acid @ 50 ppm after at 30 DAS (4.91 $mg.g^{-1}$). The lowest value of proline content was observed for G_2T_1 i.e. plant was control condition after 50 DAS (2.01 $mg.g^{-1}$).

These results were in agreement with Nithila *et al*. (2013) [15] who reported that salinity increase proline content in groundnut seedling. Girija *et al*. (2001) [6] also revealed same trend.

Glycine betaine

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 glycine betaine (Fig. 9 A). Among the salinity level, treatment S_1 irrigated with tap water showed lowest amount of glycine betaine (3.37 $mg.g^{-1}$) while the pot irrigated with saline water 4 EC (S_2) showed highest value for glycine betaine (4.10 $mg.g^{-1}$).

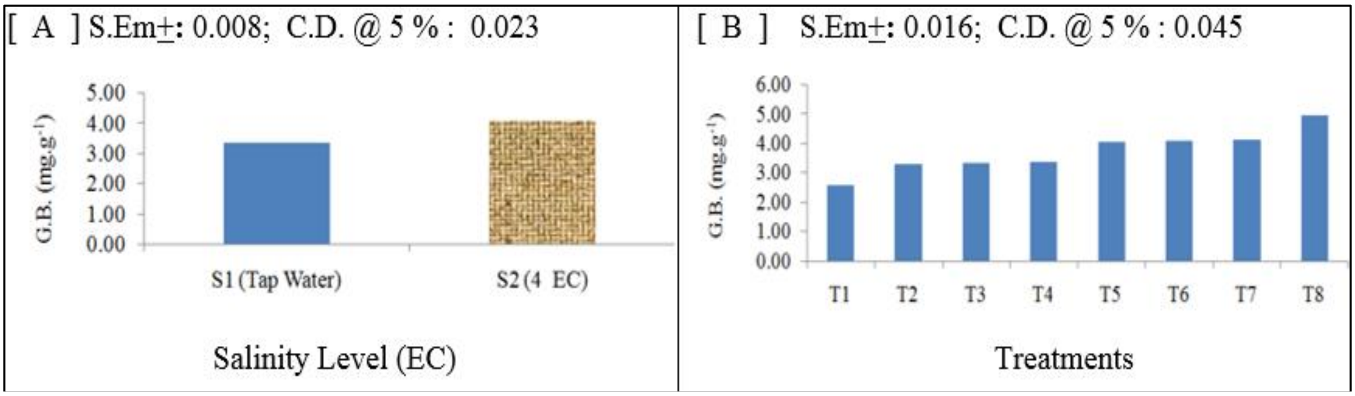

Fig 9: Mean effect of [A] salinity (S) and [B] treatments (T) on glycine betaine content ($mg.g^{-1}$) in leaf tissues of groundnut.

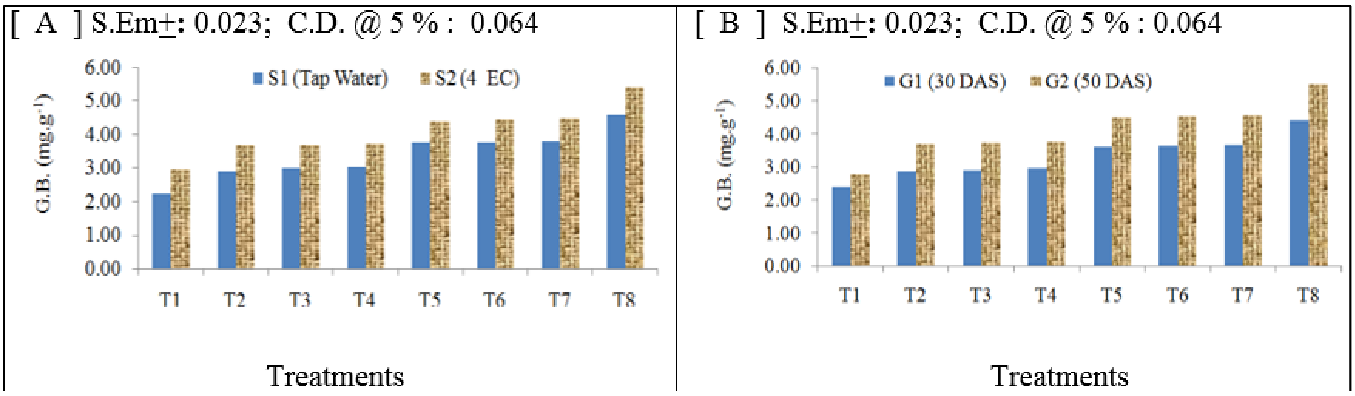

Fig 10: Interaction effect of [A] salinity (S) X treatments (T) and [B] growth stages (G) X treatments (T) on glycine betaine content ($mg.g^{-1}$) in leaf tissues of groundnut.

Treatment of gibberellic acid, potassium nitrate, silicic acid and their combination found statistical significant (Fig. 9 B). Treatment T_8 [GA_3@ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm] caused marked increase in glycine betaine in groundnut leaf tissue. The tissues obtain from groundnut pots treated with T_8 [GA_3 @ 100 ppm + KNO_3@ 500 ppm + silicic acid @ 50 ppm] revealed higher amount of mean glycine betaine (4.97 $mg.g^{-1}$) and which was followed by T_7 [GA_3 @ 100 ppm + silicic acid @ 50 ppm (4.12 $mg.g^{-1}$)] and T_6 [KNO_3@ 500 ppm + silicic acid @ 50 ppm (4.11 $mg.g^{-1}$)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T_1(2.59 $mg.g^{-1}$).

Interaction effect of S X T for glycine betaine was revealed significant differences in leaf tissue of groundnut (Fig. 10 A). The highest value of glycine betaine content was observed for the S_2T_8 i.e. in plant irrigated with saline water combine with GA_3@ 100 ppm + KNO_3 @ 500 ppm + silicic acids @ 50 ppm treatment after 50 DAS (5.39 $mg.g^{-1}$).

The lowest value (2.21 $mg.g^{-1}$) of glycine betaine content was observed in plant irrigated with tap water under control condition (S_1T_1).

Interaction effect of G X T for glycine betaine content was revealed significant differences in leaf tissue of groundnut (Fig. 10 B).

The highest value of glycine betaine content was observed in G_2T_8 i.e. in plant treated with GA_3 @ 100 ppm + KNO_3 @ 500 ppm + silicic acid @ 50 ppm after at 50 DAS (5.52 $mg.g^{-1}$). The lowest value of glycine betaine content was observed for G_1T_1 i.e. plant was control condition after 30 DAS (2.40 $mg.g^{-1}$).

These results were in agreement with Shaddad *et al.* (2013) [24] studied effect of salinity stress, and found that the regulation of osmotic pressure, protection of membrane that increased due to accumulation of glycine betaine.

Conclusion

The result was suggested that the biochemical constituents were affected due to salinity stress in groundnut. The biochemical constituents *viz.*, total soluble sugars, reducing sugars, total phenol, proline and glycine betaine that increase with higher concentration of salt stress. Biochemical constituents showed further increasing trend on application of GA_3, KNO_3 and silicic acid increased total soluble sugars, reducing sugars, total phenol, proline and glycine betaine. This investigation has suggested gibberellic acid, potassium nitrate and silicic acid as a potential biomolecules affecting biochemical parameter under abiotic stress like salinity.

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