



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(6): 2162-2172

© 2019 IJCS

Received: 09-09-2019

Accepted: 13-10-2019

Patel Riddhi

Department of Biochemistry,
College of Agriculture, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Purohit HB

Department of Biochemistry,
College of Agriculture, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Kandoliya UK

Department of Biochemistry,
College of Agriculture, Junagadh
Agricultural University,
Junagadh, Gujarat, India

BA Golakiya

Department of Biotechnology,
College of Agriculture, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Effect of gibberellic acid, potassium nitrate and silicic acid on biochemical constituents and physiological parameter in cowpea (*Vigna unguiculata* L. Walp) seedling irrigated with saline water

Patel Riddhi, Purohit HB, Kandoliya UK and BA Golakiya

Abstract

Salinity is a major environmental constraint influencing plants productivity and plant distribution worldwide. Salinity reduces the ability of plant utilize water and causes a reducing in growth rates, as well as changes in plant metabolites processes. 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 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 exogenous application of gibberellic acid, potassium nitrate and silicic acid under salt stress on physiological, biochemical parameters in seedlings of cowpea. The result suggest that biochemical constituents *viz.*, total soluble sugars, reducing sugars and proline that increase under salt stress conditions while, the physiological parameter like relative water content that decreased. On an application of GA₃, KNO₃ and silicic acid under salt stress increased total soluble sugars, reducing sugars, proline. This investigation has suggested a silicic acid, gibberellic acid, potassium nitrate as a potential biomolecules affected the various biochemical and physiological parameter under abiotic stress like salinity.

Keywords: Biochemical parameter, physiological parameter, cowpea (*Vigna unguiculata* L. walp)

Introduction

Cowpea [*Vigna unguiculata* (L.)Walp.] is an ancient crop probably domesticated during the Neolithic period. In India, it is commonly known as lubia, black eye, roungi, kaffir pea, china pea and southern bean. It is grown throughout the tropics and subtropics as a grain legume. It is one of the most important leguminous vegetable crops, native of West Africa but, Steele (1976) [17] suggested Ethiopia as the primary and Africa as the secondary centre of diversity. Cowpea (*Vigna unguiculata* L. Walp) is a diploid species with $2n=2x=22$ chromosomes. It is a self-pollinated crop, with natural cross-pollination of up to one percent. It has multifarious uses like as fodder, cover crop and green manure and provides 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) [11]. 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) [9]. Salinity can induce abiotic stress tolerance by altering ROS scavenging enzyme system (Solanki *et al.*, 2018; Trivedi *et al.*, 2018; Patel *et al.*, 2019) [16, 18, 13]. 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, 12, 4]. 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) [19, 16]. Increase crop salt tolerance is a highly attractive approach to overcome the salinity threat.

Corresponding Author:**Patel Riddhi**

Department of Biochemistry,
College of Agriculture, Junagadh
Agricultural University,
Junagadh, Gujarat, India

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) [14]. 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].

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 biochemical constituents and physiological parameters 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. Cowpea (*Vigna unguiculata* L. Walp) seeds of varieties Pusa phalguni were obtained from Agro center, Junagadh for the experiment.

Treatments

a) Salinity level (2): Plant irrigated with saline water prepared by appropriate dilution of sea water. [S₁- Tap water, S₂- Saline water (4 EC)].

b) Gibberellic acid, Potassium nitrate, Silicic acid (8) : T₁ - Control (without spray), T₂ - Sprayed with GA₃ @ 100 ppm, T₃ - Sprayed with KNO₃ @ 500 ppm, T₄ - Sprayed with Silicic acid @ 50 ppm, T₅ - Sprayed with GA₃ @ 100 ppm + KNO₃ @ 500 ppm, T₆ - Sprayed with KNO₃ @ 500 ppm + Silicic acid @ 50 ppm, T₇ - Sprayed with GA₃ @ 100 ppm + Silicic acid @ 50 ppm, T₈ - Sprayed with GA₃ @ 100 ppm + KNO₃ @ 500 ppm + Silicic acid @ 50 ppm.

c) Growth stage (2): D₁- 21 DAS, D₂- 41 DAS

Cow pea leaf were collected at different stages (D₁ and D₂) after the spray of gibberellic acid, potassium nitrate and silicic acid the pot irrigated with saline water having a concentration 4 ds m⁻¹ and packed in plastic bag and brought to the laboratory under ice cold condition. Leaf tissues were taken for first two stages (D₁ and D₂) at one day 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 bio chemical and physiological analysis.

Biochemical parameters and physiological parameter assay

Total soluble sugar

Seedlings (100 mg) were extracted with 5 ml of 80% ethanol and centrifuged at 3000 rpm for 10 minutes. Extraction was repeated 4 times with 80% ethanol and supernatants were collected into 25 ml volumetric flasks. Final volume of the extract was made to 25 ml with 80 % methanol. The extract (0.3 ml) was pipetted into separate test tubes and the tubes were placed in a boiling water bath to evaporate the methanol. One ml of millipore water and 1ml of 5% phenol was added in each test tube. Then 5 ml of sulphuric acid was added. The tubes were allowed to cool in ice-bath for 10-15 minutes. The intensity of colour was read at 490 nm on spectrophotometer. A standard curve was prepared using 10 mg glucose per 100

ml distilled water. Dubois *et al.* (1956) [3]. The amount of total soluble sugar present in the sample was calculated by appropriate formula.

Reducing sugar

The dinitrosalicylic acid (DNSA) method was used to estimate the glucose and galacturonic acid released by cellulase, polygalacturonase and β-1, 3 glucanase enzymes (Somogyi, (1952) [15]). A known volume of aliquot was taken in test tube and final volume of 1.0 ml adjusted with distilled water. To this 0.5 ml DNSA reagent (1g DNSA + 200mg crystalline phenol + 50mg sodium sulphite in 100ml of 1% sodium hydroxide) was added and mixed properly. The content was heated in a boiling water bath for 5 min. When the contents of the tubes were still warm, 1.0 ml of 40% sodium potassium tartrate (Rochelle salt) solution was added. Cool it and final volume was made 5.0 ml with distilled water. After that the tubes were read at 540nm using spectrophotometer. Reagent blank was also performed by addition of 1.0 ml of distilled water in place of enzyme aliquot. A known concentration of standard (0.5-2.5 μM) of glucose or galacturonic acid was carried out and was calibrated and expresses as appropriate.

Proline

The sample (0.3 ml) and standard proline (0.1-0.6 ml from 0.05 mg/ml proline stock) were taken in a series of test tubes and the volume was made up to 1.0 ml with distilled water. Then 2 ml glacial acetic acid and 2 ml acid ninhydrin reagent were added. Then tubes were kept in boiling water bath for 1 hrs. The tubes were cooled in running water at room temperature. After that 4 ml toluene was added. The absorbance was recorded from toluene phase at 520 nm in spectrophotometer. The free proline was calculated as stated below and expressed as mg.g⁻¹.

Relative water content (RWC)

Known green gram leaf sample weight (gm) of green gram leaf sample was transferred in a petri dish, and to this 25 ml distilled water was added and kept for four hour. Then the leaves were taken out, dried by blotting paper and weighed (Turgid weight). The leaf was kept in oven at 84°C for 5 hr and weighted until constant weight was obtained. After this RWC were estimated as per formula and expressed as per cent relative water content (Weatherley, 1962) [22].

$$\text{Relative Water Content (\%)} = \frac{\text{Fresh weight (g.)} - \text{Dry weight (g.)}}{\text{Turgid Weight (g.)} - \text{Dry Weight (g.)}} \times 100$$

Result and Discussion

Total soluble sugar

The data on total soluble sugar (%) 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 (T₁ to T₈) grown in a pot irrigated with tap water (S₁) and saline water (S₂) 4 EC at stages G₁ and G₂ are depicted in Fig. 1, 2.

Mean effect of salinity level irrespective of gibberellic acid, potassium nitrate, silicic acid and their treatment 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 S₂ irrigated with saline water showed highest value for total soluble sugar (4.59 %) while the S₁ pot irrigated with tap

water showed declined value for total soluble sugar (4.17 %). Among the different stages, mean value of total soluble sugar significantly varied between 5.26 and 3.50 %. (Fig.1 B). The content was increased from 21 DAS (3.50 %) to 41 DAS (5.26 %). 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 stastical significant (Fig.1 C). Treatment (T8) [GA3@ 100 ppm + KNO3@ 500 ppm + Silicic acid@ 50 ppm] caused marked increased in total soluble sugar in cowpea. The tissues obtain from cowpea pots treated with T8 [GA3@ 100 ppm + KNO3@ 500 ppm + Silicic acid@ 50 ppm] revealed higher amount of mean total soluble sugar (4.74 %). The mean lowest content was noted for the tissues received from T1 when plant was in control condition (4.01 %).

Interaction effect of S X T for total soluble sugar were revealed significant differences in cowpea (Fig.2 A). The highest value of total soluble sugar was observed for the S2T8 i.e. in plant irrigated with saline water combine with GA3@ 100 ppm + KNO3@ 500 ppm (4.96 %). The lowest value (3.85 %) of total soluble sugar was observed in plant irrigated with saline water without treatment (S1T1). Khodary (2004)^[8] reported that the maize plants submitted to salinity treatment, showed a progressive increase in their soluble sugar content with increasing the salinity level. G X T interaction effect for total soluble sugar were revealed significant differences in cowpea (Fig.2 B). The highest value of total soluble sugar was observed for G2T8 i.e in plant treated with GA3@ 100 ppm + KNO3@ 500 ppm + Silicic acid @ 50 ppm after at 41 DAS (5.61 %). The lowest value of total soluble sugar was observed for G1T1 i.e in plant treated with GA3@ 100 ppm + Silicic acid@ 50 ppm after at 21 DAS (3.12 %). Interaction effect of S X G for total soluble sugar were revealed significant differences in leaf tissue of cowpea (Fig.2 C). The highest value of total soluble sugar was observed for S2G2 i.e. in plant irrigated with saline water after 41 DAS (5.43 %). The lowest value of total soluble sugar was observed for S1G1 in plant irrigated with tape water after 21 DAS (3.26 %).

Reducing sugar

The data on total soluble sugar (%) 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 (T₁ to T₈) grown in a pot irrigated with tape water (S₁) and saline water (S₂) 4 EC at stages G₁ and G₂ are depicted in Fig. 3, 4.

Mean effect of salinity level irrespective of gibberellic acid, potassium nitrate, silicic acid and their treatment 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 S2 irrigated with saline water showed highest value for reducing sugar (3.80%) while the S1 pot irrigated with tap water showed declined value for reducing sugar (3.66 %). Kandoliya *et al.* (2015)^[7] and Vakharia *et al.* (1997)^[21] showed that 3 to 16 % high reducing sugar under abiotic stress condition. Among the different stages, mean value of reducing sugar significantly varied between 3.81 and 3.65 %. (Fig.3 B). The content was increased from 21 DAS (3.65 %) to 41 DAS (3.81 %). This may be the effect of saline irrigation water as well the property of crop species. Application of spray treatment including gibberellic acid,

potassium nitrate, silicic acid and their combination result stastical significant (Fig.3 C). Treatment (T8) [GA3@ 100 ppm + KNO3@ 500 ppm + silicic acid@ 50 ppm] caused marked increased in reducing sugar in cowpea. The tissues obtain from cowpea pots treated with T8 [GA3@ 100 ppm + KNO3@ 500 ppm + Silicic acid@ 50 ppm] revealed higher amount of mean reducing sugar (3.85%). The mean lowest content was noted for the tissues received from T1 when plant was in control condition (3.63%).

Interaction effect of S X T for reducing sugar were revealed non significant differences in cowpea (Fig.4 A). However, the highest value of reducing sugar was observed for the S2T8 i.e. in plant irrigated with saline water combine with GA3@ 100 ppm + KNO3@ 500 ppm (3.95 %). The lowest value (3.58 %) of reducing sugar was observed in plant irrigated with tape water without treatment (S1T1). Interaction effect of G X T for reducing sugar were revealed non significant differences in cowpea (Fig.4 B). However, the highest value of reducing sugar was observed for G2T8 i.e in plant treated with GA3@ 100 ppm + KNO3@ 500 ppm + Silicic acid@ 50 ppm after at 41 DAS (3.88 %). The lowest value of reducing sugar was observed for G1T1 i.e in plant was under control condition after at 21 DAS (3.51 %). Interaction effect of S X G for reducing sugar were revealed significant differences in leaf tissue of cowpea (Fig.4 C). The highest value of reducing sugar was observed for S2G2 i.e. in plant irrigated with saline water after 41 DAS (3.94 %). The lowest value of reducing sugar was observed for S1G1 in plant irrigated with tape water after 21 DAS (3.63 %).

Proline

The data on proline content (mg.g⁻¹Fr.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 (T₁ to T₈) grown in a pot irrigated with tape water (S₁) and saline water (S₂) 4 EC at stages G₁ and G₂ are depicted in Fig. 5, 6.

Mean data of salinity level were found significant for proline contents (Fig.5 A). Among the salinity level, treatment S2 irrigated with saline water showed highest value for proline (2.59 mg.g⁻¹ Fr.Wt.) while the S1 pot irrigated with tap water showed declined value for proline (2.17 mg.g⁻¹ Fr.Wt.). Among the different stages, mean value of cowpea significantly varied between 1.50 and 3.26 mg.g⁻¹ Fr.Wt. (Fig.5 B). The content was increased from 21 DAS (1.50 mg.g⁻¹ Fr.Wt.) to 41 DAS (3.26 mg.g⁻¹ Fr.Wt.). This may be the effect of saline irrigation water as well the property of crop species. Spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination result to be found significant difference for (Fig.5 C). Treatment (T8) showed highest value of proline in cowpea. The tissues obtain from cowpea pots with Treatment (T8) treated with GA₃@ 100 ppm + KNO₃@ 500 ppm + Silicic acid@ 50 ppm showed higher amount of mean proline (2.74 mg.g⁻¹ Fr.Wt.). The mean lowest content was noted for the tissues received from T₁ i.e plant was under control condition (2.00 mg.g⁻¹ Fr.Wt.). Interaction effect of S X T for proline were revealed significant differences in cowpea (Fig.6 A). The highest value of proline was observed for the S2T8 i.e. in plant irrigated with saline water and plant treated with GA₃@ 100 ppm + KNO₃@ 500 ppm + Silicic acid@ 50 ppm (2.96 mg.g⁻¹ Fr.Wt.). The lowest value (1.84 mg.g⁻¹ Fr.Wt.) of proline was observed in plant irrigated with tap water and plant was under control condition (S₁T₁). G X T interaction effect for proline were revealed significant differences in cowpea (Fig.6 B).

The highest value of proline was observed for G2T8 i.e plant was under control condition after 41 DAS (3.61 mg.g⁻¹ Fr.Wt.). The lowest value of proline was observed for G1T1 i.e plant was under control condition after 21 DAS (1.10 mg.g⁻¹ Fr.Wt.). S X G interaction effect for proline was concerned, it showed significant differences in leaf tissue of cowpea (Fig.6 C). The highest value of proline was observed for S2G2 i.e. in plant irrigated with saline water after 41 DAS (3.43 mg.g⁻¹ Fr.Wt.). The lowest value of cowpea was observed for S1G1 in plant irrigated with tap water after 21 DAS (1.25 mg.g⁻¹ Fr.Wt.).

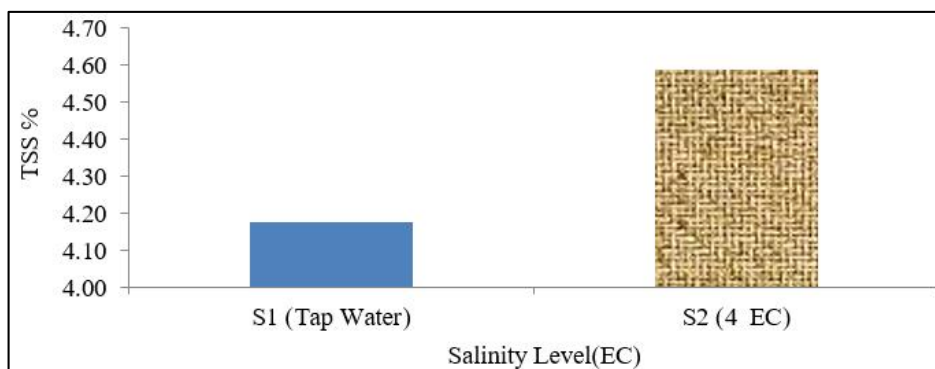
Relative water content (RWC)

The relative water content of a leaf is a measurement of its hydration status relative to its maximum hydration capacity of full turgidity. It provides a measurement of degree of stress expressed. In present experiment, the data on Relative Water Content (RWC) (%) analyzed from leaf tissue of cow pea collected from plants treated at 10 DAS and 30 DAS with different concentration of gibberellic acid, potassium nitrate, silicic acid and their combination (T1 to T8) grown in a pot irrigated with tap water (S1) and saline water (S2) 4 EC at two different stages G1 (21 DAS) and G2 (41 DAS) are depicted in Fig. 7, 8.

Mean observation for 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 relative water content (Fig.7 A). Among the salinity level, treatment S₁ irrigated with tap water showed highest amount of relative water content (87.31%) while the pot irrigated with saline water 4 EC (S₂) showed lowest value for relative water content (78.29%). Aranda *et al.* (2001) [1] also reported that the salinity condition decrease water uptake was the possible reason for decrease in percent relative water content. The decrease in relative water contents in present experiment was low may be the effect of accumulation of osmolytes like proline, glycine betaine and total soluble sugar. Trivedi (2018) [18, 19] also reported the same trend for salinity of relative water content in moong bean crops. Vakharia *et al.* (1997) [21] also reported that the decreased in leaf RWC from 81.72% to 75.92% in groundnut imposed to abiotic stress. Among the different stages, mean value of relative water content significantly varied between 84.29 % and 81.31 %

Total soluble sugars

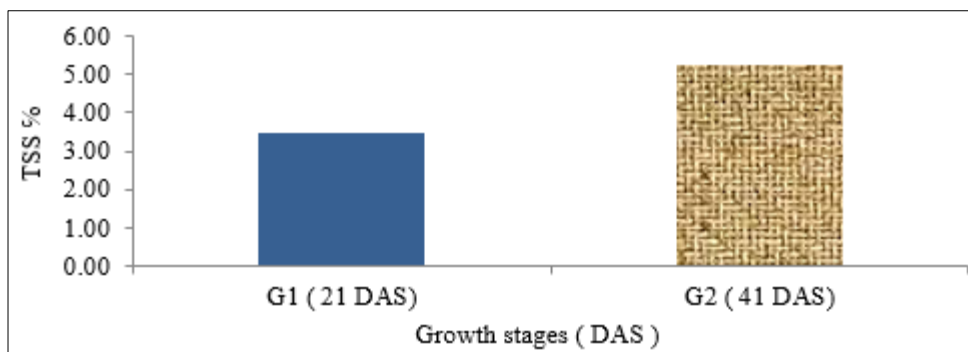
[A] S.Em±: 0.015 C.D. @ 5%: 0.043



(Fig.7 B). The content was decreased from 21 DAS (84.29 %) to 41 DAS (81.31 %). Imposition of spray treatment of gibberellic acid, potassium nitrate, silicic acid and their combination found statistical significant (Fig.7 C). Treatment (T8) [GA₃@ 100 ppm + KNO₃ @ 500 ppm + Silicic acid@ 50 ppm] caused marked increased in relative water content in cowpea leaves. The leaves obtain from cowpea pots treated with T8 [GA₃ @ 100 ppm + KNO₃@ 500 ppm + silicic acid@ 50] revealed higher amount of mean relative water contents (87.06%) and which was followed by T7 [GA₃@100 ppm + silicic acid@ 50 ppm (85.92%)] and T6 [KNO₃@ 500 ppm + Silicic acid @ 50 ppm (84.82%)] irrespective of salinity level and growth stages. The mean lowest content was noted for the tissues received from T1 (78.49 %).

Interaction effect of salinity and treatment S X T for relative water content revealed significant differences in leaves of cowpea (Fig.8 A). The highest value of relative water content was observed for the S1T8 i.e in plant irrigated with tap water and treated with [GA₃@ 100 ppm + KNO₃ @ 500 ppm + Silicic acids@ 50 ppm] treatment (88.71%). The lowest value (71.02 %) of relative water content was observed in plant irrigated with saline water 4 EC under control condition (S2T1). In general, salt stress decreased the relative water content which was maintained by different treatments of GA₃, KNO₃ and silicic acid. Tuna *et al.* (2007) [20] also noted that the relative water content (RWC) decreases with the application of salt compared to control treatment, but GA₃ application increased it either moderately or remained stable. Interaction effect of G X T for relative water content was revealed non significant differences for relative water content in cowpea (Fig.8 B). The highest value of relative water content was observed in G1T8 i.e in plant treated with [GA₃ @ 100 ppm + KNO₃ @ 500 ppm + silicic acid @ 50 ppm] after at 21 DAS (87.79%).The lowest value of relative water content was observed for G2T1 i.e plant was control condition after 41 DAS (77.55 %). However in general gibberellic acid and potassium nitrate treatment improved the relative water content (%) in leaves of cowpea. Interaction effect of S X G for relative water content were revealed significant differences in leaf tissue of cowpea (Fig.8 C). The highest value of relative water content was observed in S₁G₁ i.e in plant irrigated with tap water (control condition) after 21 DAS (88.05 %). The lowest value of relative water content was observed for G₂S₂ (76.04 %).

[B] S.Em±: 0.015 C.D. @ 5%:0.043



[C] S.Em±: 0.031 C.D. @ 5%:0.087

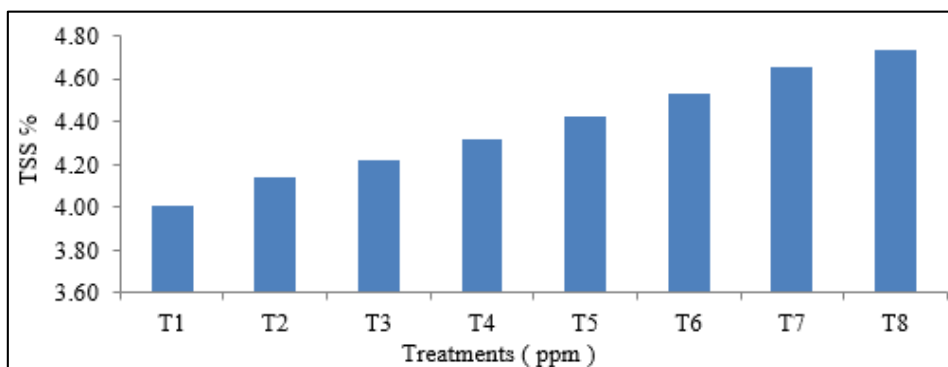
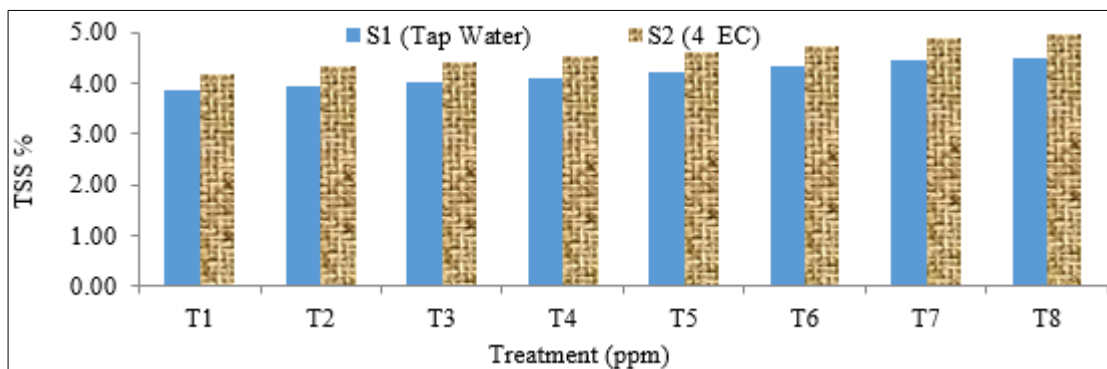
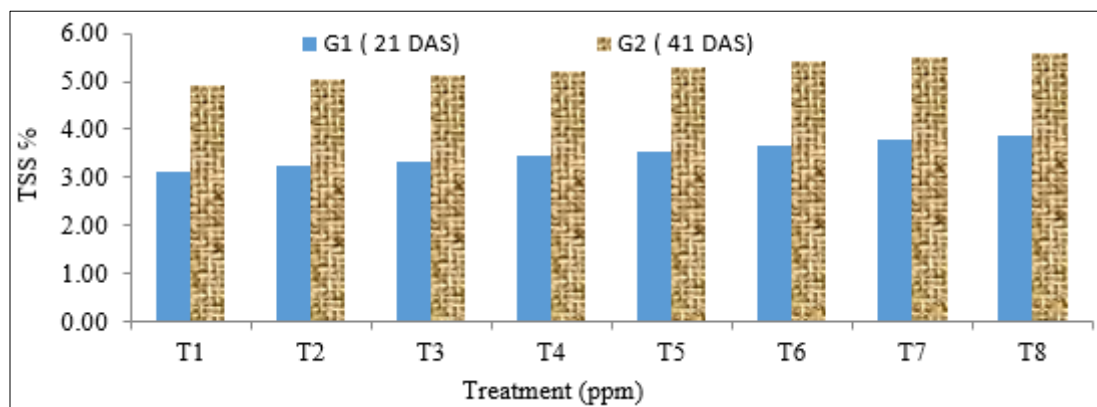


Fig 1: Mean effect of [A] Salinity(S), [B] Growth Stage (G) and [C] Treatments (T) on Total soluble sugars (%) in leaves of Cowpea

[A] S.Em±: 0.043 C.D. @ 5%: NS



[B] S.Em±: 0.043 C.D. @ 5%: NS



[C] S.Em±: 0.022 C.D. @ 5%:0.061

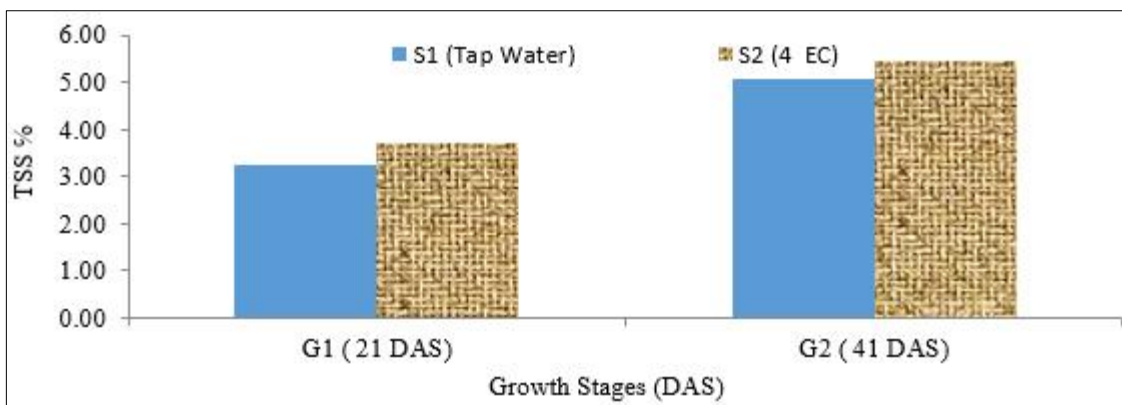
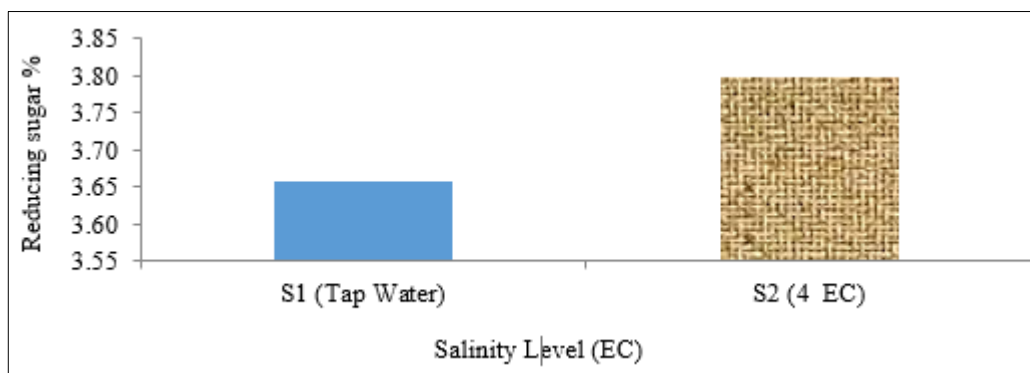


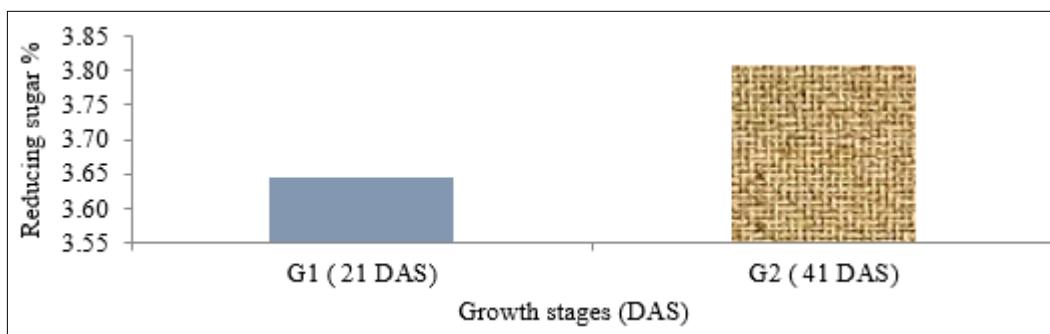
Fig 2: Interaction effect of [A] Salinity (S) X Treatment (T), [B] Growth Stages (G) X Treatment (T), [C] Salinity (S) X Growth Stages (G) on Total soluble sugar (%) in leaves of Cowpea.

Reducing Sugars

[A] S.Em±: 0.014 C.D. @ 5%: 0.040



[B] S.Em±: 0.014 C.D. @ 5%: 0.040



[C] S.Em±: 0.029 C.D. @ 5%: 0.081

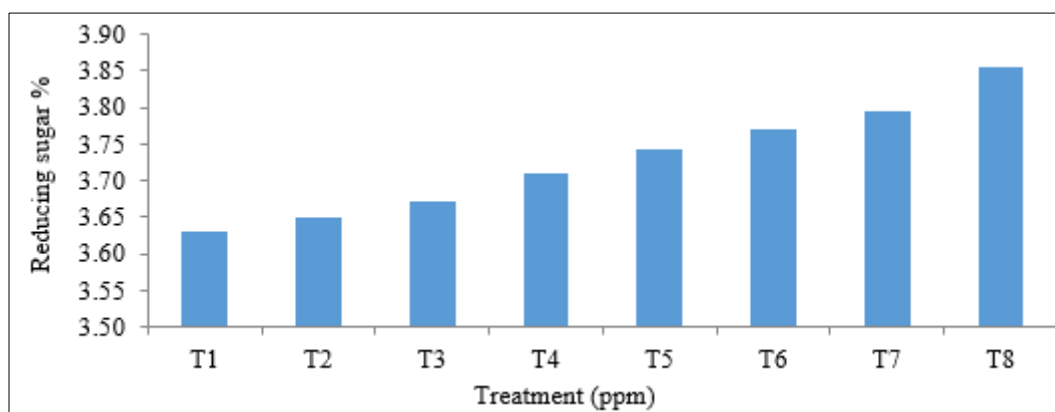
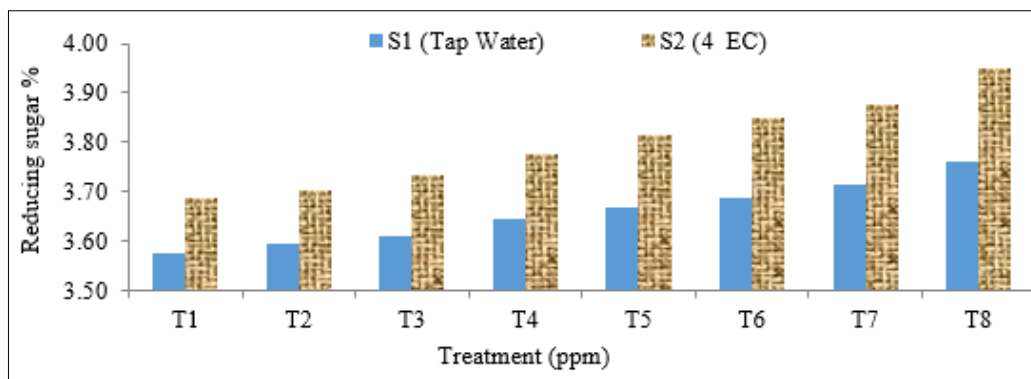
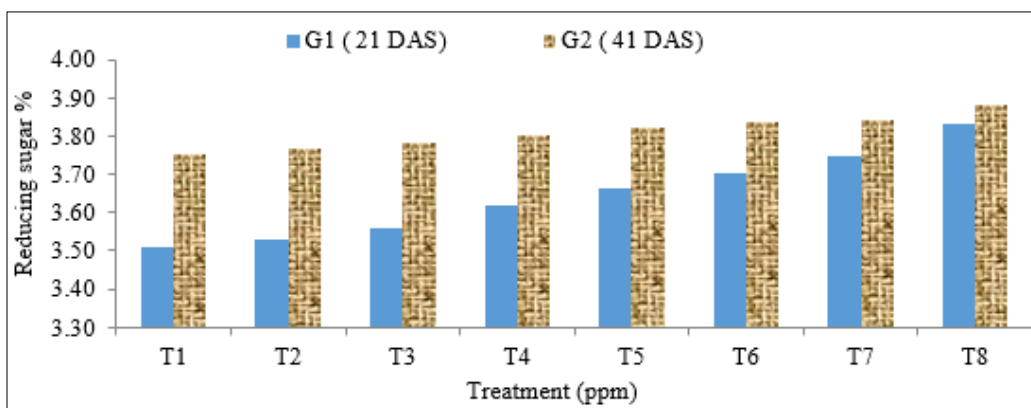


Fig 3: Mean effect of [A] Salinity (S), [B] Growth Stage and [T] Treatments on reducing sugars (%) in leaves of Cowpea.

[A] S.Em±: 0.041 C.D. @ 5% : NS



[B] S.Em±: 0.041 C.D. @ 5%: NS



[C] S.Em±: 0.020 C.D. @ 5%: 0.057

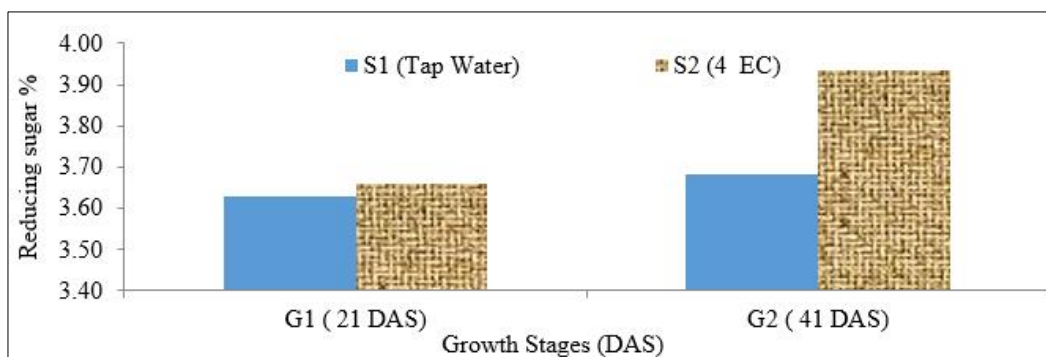
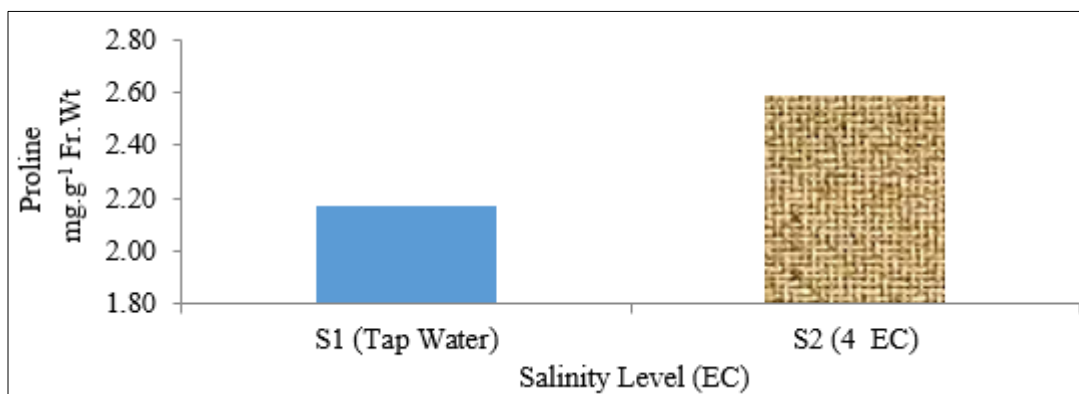


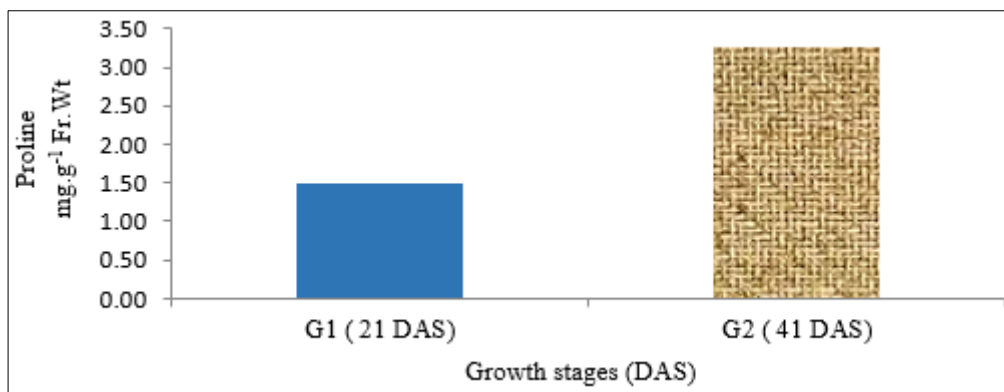
Fig 4: Interaction effect of [A] Salinity (S) X Treatments (T), [B] Growth Stages (G) X Treatment (T), [C] Salinity (S) X Growth Stages (G) on Reducing sugars (%) in leaves of Cowpea.

Proline

[A] S.Em±: 0.002 C.D. @ 5%: 0.006



[B] S.Em±: 0.002 C.D. @ 5%: 0.006



[C] S.Em±: 0.004 C.D. @ 5%: 0.012

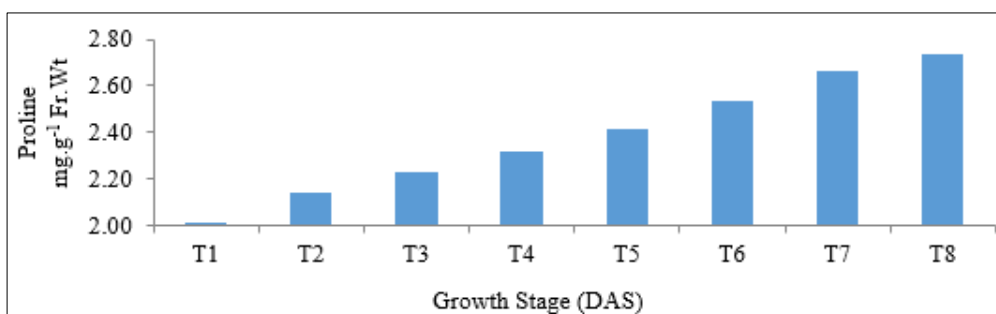
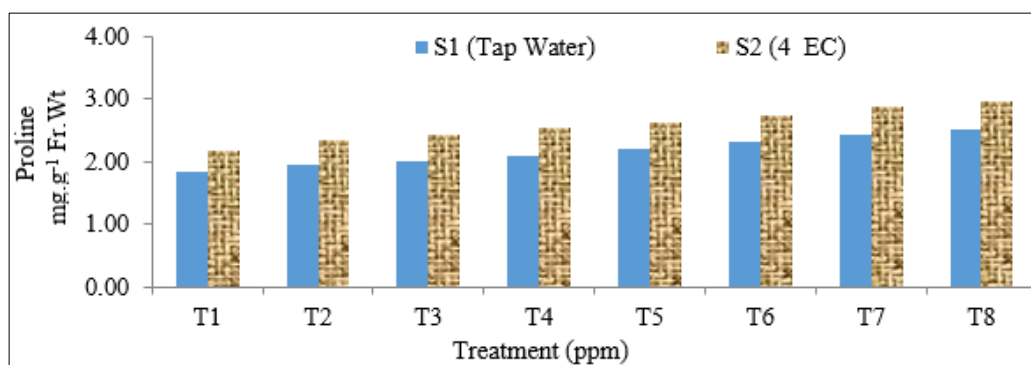
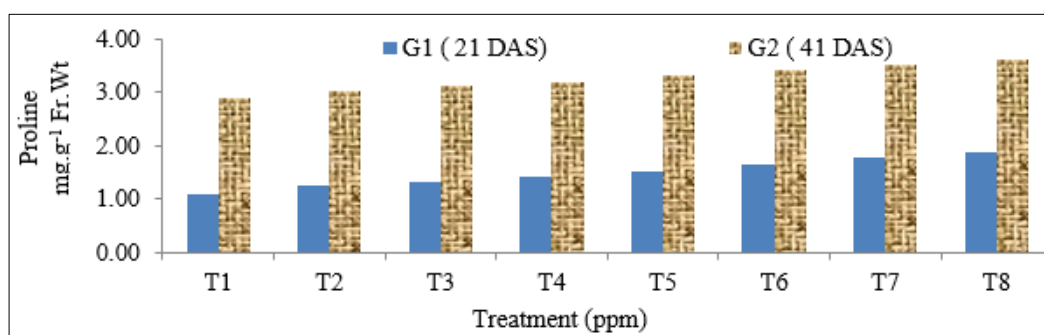


Fig 5: Mean effect of [A] Salinity (S), [B] Growth Stage (G) and [C] treatments (T) on Proline (mg.g⁻¹ Fr.Wt.) in leaves of Cowpea.

[A] S.Em±: 0.006 C.D. @ 5%:0.017



[B] S.Em±: 0.006 C.D. @ 5%:0.017



[C] S.Em±: 0.003 C.D. @ 5%:0.009

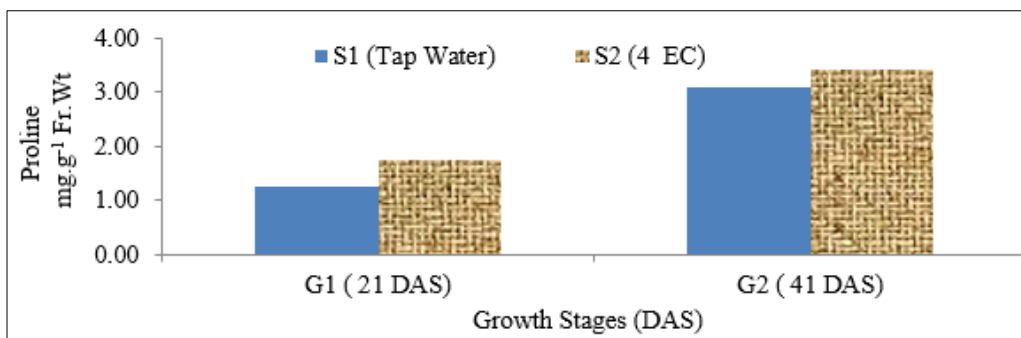
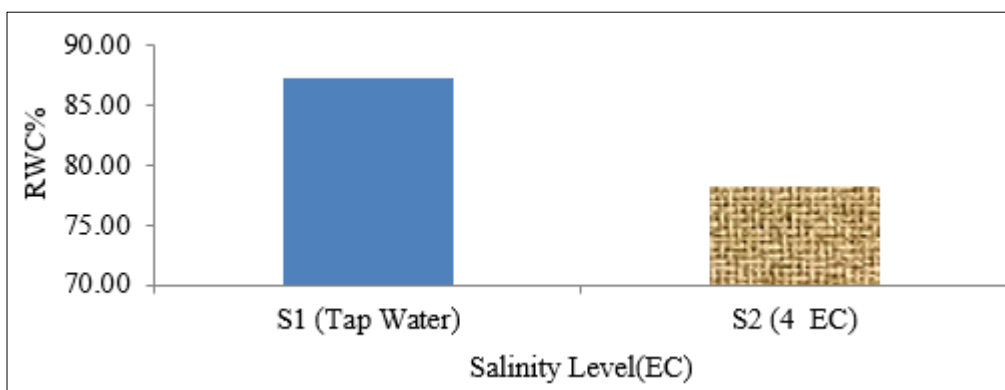


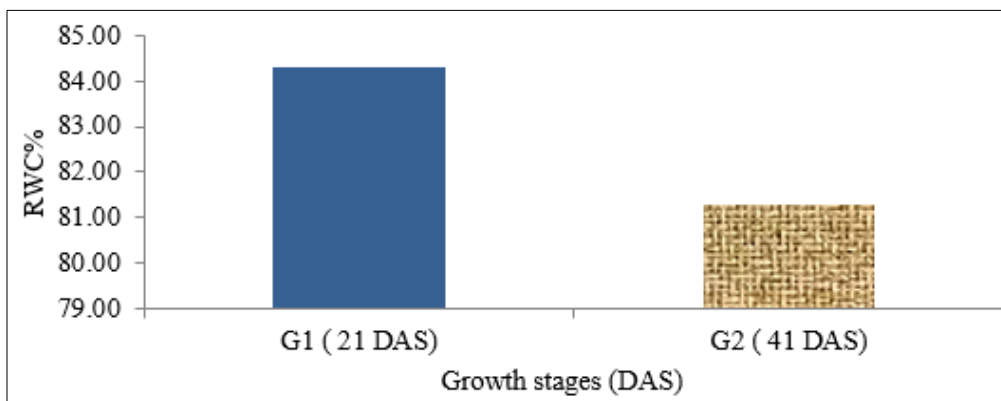
Fig 6: Interaction effect of [A]Salinity(S) X Treatments(T), [B]Growth Stages (G) X Treatment(T), [C]Salinity(S) X Growth Stages(G) on Proline (mg.g⁻¹ Fr. Wt.) in leaves of Cowpea

Relative water content

[A] S.Em±: 0.351 C.D. @ 5%: 0.992



[B] S.Em±: 0.351 C.D. @ 5%: 0.992



[C] S.Em±: 0.702 C.D. @ 5%: 1.985

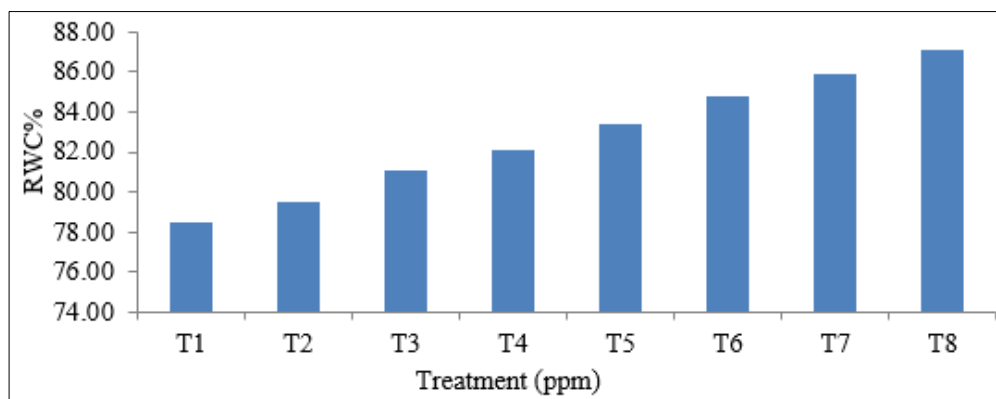
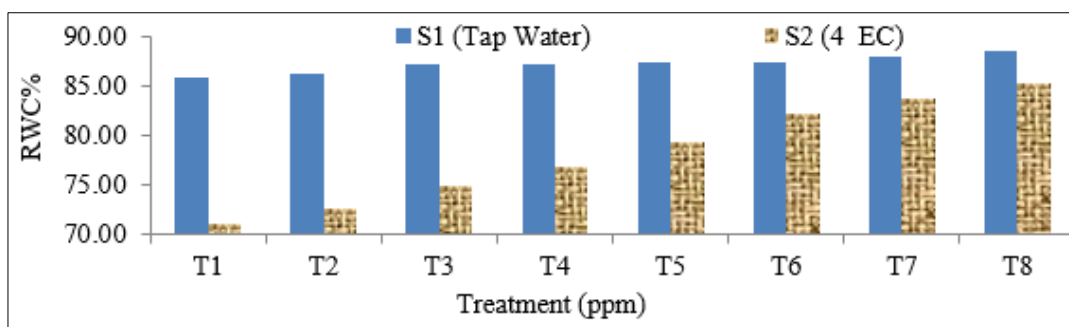
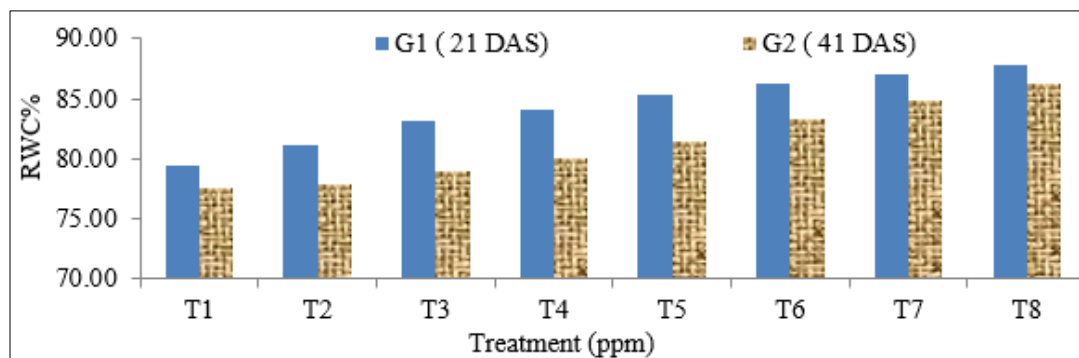


Fig 7: Mean effect of [A] Salinity(S), [B] Growth stages(G) and [C] Treatments (T) on Relative Water Content (%) in leaves of Cowpea.

[A] S.Em ±: 0.993 C.D. @ 5%: 2.807



[B] S.Em ±: 0.993 C.D. @ 5%: NS



[C] S.Em ±: 0.497 C.D. @ 5%:1.404

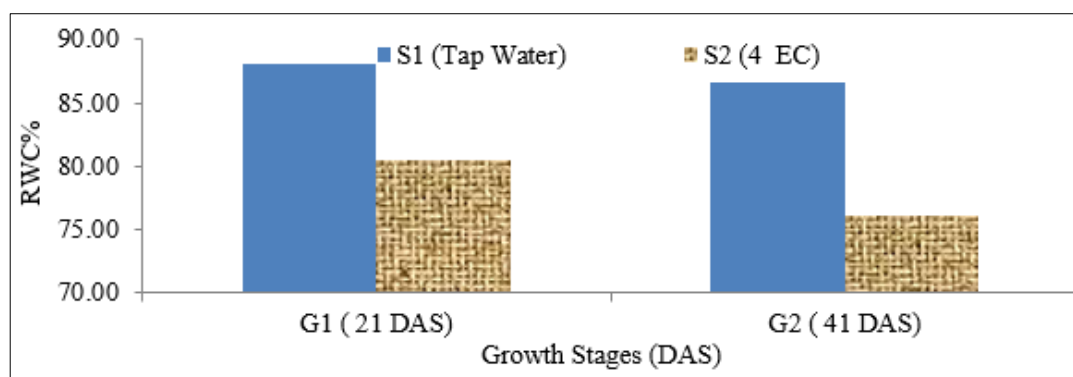


Fig 8: Interaction effect of [A] Salinity (S) X Treatments (T), [B] Growth stages (G) X Treatments (T) and [C] Salinity (S) X Growth stages (G) on Relative Water Content (%) in leaves of Cowpea.

Conclusion

The result suggests that the biochemical constituent and physiological parameter were affected due to salinity stress in cowpea. The biochemical constituents *viz.*, total soluble sugars, reducing sugars and proline that increase with higher concentration of salt stress but physiological parameter like relative water content that decrease. Biochemical constituents showed trend on application of GA₃, KNO₃ and silicic acid increased total soluble sugars, reducing sugars, proline and physiological parameter relative water content also increased. This investigation has suggested silicic acid, gibberellic acid, potassium nitrate as a potential biomolecules affecting the ROS scavenging biochemical and physiological parameter under abiotic stress like salinity.

References

- Aranda KV, Jail PK, Kumar VK. Effect of salt stress on physiological analysis: growth parameters in green gram. *Plant physiology*. 2001; 13(4):15-26.
- Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant and soil*. 1973; 39(1):205-207.
- Dubois M, Gillies KA, Hamilton JK, Reber FA, Smith F. Colorimetric methods of determination of sugar and related substance. *Analytical Chem*. 1956; 28(30):350-352.
- 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.
- Kandoliya UK, Marvia GV, Vakharia DN, Golakiya BA. Effect of drought growth stage on carbohydrates and lipids composition of groundnut. *International Journal of Current Research and Academic Review*. 2015; 3(10):281-287.
- 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. 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.
 8. Khodary S. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int. J Agri. Biol. 2004; 6(1):1560-8530.
 9. Lazof DB, Bernstein N. The NaCl inhibition of shoot growth: The case for disturbed nutrition with special consideration of calcium. Advances in Botanical Research. 1999; 29(2):113-189.
 10. Miyake Y, Takahashi E. Effect of silicone on the growth of cucumber plant in soil culture. Soil Science and Plant Nutrition. 1983; 29(3):463-471.
 11. Nielson S, Ohler T, Mitchell C. Cowpea leaves for human consumption: production, utilization and nutrient composition. In: Singh, S. and Rachie, K.(eds.). Cowpea researches, production and utilization. Wiley, New York, 1997, 145-162.
 12. Patel NJ, Kandoliya UK, Talati JG. Induction of phenol and defence related enzymes during wilt (*Fusarium udum* butler) infestation in pigeon pea. International Journal of Current Microbiology and applied science. 2015; 4(2):291-299.
 13. Patel RS, Kadam DD, Kandoliya UK, Golakiya BA. Effect of gibberellic acid, potassium nitrate and silicic acid on enzymes activity in cowpea (*Vigna unguiculata* L. Walp) irrigated with saline water. Journal of Pharmacognosy and Phytochemistry. 2019; 8(5):1022-1029.
 14. Perrenoud S. Potassium and plant health. IPI Research Topics No. 3, 2nd rev. edition. Basel/Switzerland, 1990.
 15. Somogyi M. Determination of Reducing Sugars by Nelson-Somogyi Method. Journal of Biochemistry. 1952, 200-245.
 16. Solanki MV, Trivedi SK, Kandoliya UK, Golakiya BA. Effect of exogenous application of salicylic acid on biochemical constituent in black gram (*Vigna mungo* L.) Hepper irrigated with saline water. European Journal of Biotechnology and Bioscience. 2018; 6(5):28-34.
 17. Steele WM. Cowpea (*Vigna unguiculata* (L.) Walp.). In: Evolution of crop plants, (Eds. Summerfield, R. J. and Bunting, A. H.), HMSO, London, pp.183-185.
 18. Trivedi, S. K. 2018. Effect of exogenous application of salicylic acid on antioxidative enzymes in green gram (*Vigna radiate* L.) irrigated with saline water. M.Sc. (Agri.) Thesis submitted at Junagadh Agricultural University, Junagadh, 1976.
 19. Trivedi SK, Solanki MV, Kandoliya UK, Golakiya BA. Effect of exogenous application of salicylic acid on antioxidative enzymes in green gram (*Vigna radiate* L.) irrigated with saline water. International Journal of Chemical Studies. 2018; 6(4):2668-2674.
 20. Tuna AL, Kaya C, Dikilitas M, Higgs D. The combined effect of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environmental and Experimental Botany. 2007; 62:1-9.
 21. Vakharia D, Kandoliya U, Patel N, Parameswaran M. Effect of drought on leaf metabolites: relationship with pod yield in groundnut. Plant physiology & Biochemistry. 1997; 24(2):102-105.
 22. Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences. 1962; 15(3):413.