ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Gibberellic Acid on Black Gram (*Vigna mungo*) Irrigated with Different Levels of Saline Water.

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ABSTRACT

Salinity is one of the major environmental factors limiting plant growth and productivity. Excess salt in the soil may adversely affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. *Vigna mungo* was selected for the study. Different concentration of NaCl (10, 20, 30, 40 and 50mM) were used in test control groups and 100 mg of gibberellic acid (GA) was used in each bag of test group by foliar spray (on 14th day) and plants were analyzed 0n 30th day. The results of this study indicate that salinity impairs seed germination, retards plant development and reduces crop yield. Increase in salinity affect the growth of the *Vigna mungo* by decreasing germination percentage, no of leaves, shoot length, root length, dry weight, fresh weight, pigments, protein content, free amino acid, and increase in carbohydrate, proline, phenol content, catalase and super oxide dismutase activity. Among different concentration of salinity (10, 20, 30, 40 and 50mM) in the present investigation the maximum inhibition is found at 50mM. The application of GA treatment showed to increase in all the parameters decreased by salt stress and restores the increased parameters to normal value. Thus we may conclude from the present study that the application of GA alleviates the adverse effect of salinity on black gram seedlings and can be attempted in the field trials in future.

Keywords: *Vigna mungo*, NaCl, Gibberellic acid, foliar spray

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ISSN: 0975-8585

INTRODUCTION

Salinity is an environmental stress that inhibits the growth and development of plants. Excess NaCl is a built complex, changes in their morphology, physiology and metabolism of plants [1, 2]. The solution of saline soil composed of dissolved salts such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl and Na_2CO_3. Each salt contribute to salinity stress, NaCl is the most prevalent salt [3]. It occurs through natural or human induced processes that result in the accumulation of salts in the soil. Water enhances the plant growth and consequences of osmotic stress and it releases the excess of Na^+ and Cl^- ions on critical biochemical process [2, 4]. Salinity causes significant reduction in the growth medium for plant growth parameters like leaf area, leaf length, root and shoot dry weight [5]. Salinity decreases caroteniod and induce reduction in chlorophyll, photosynthetic activity due to the lack of activity of ribulose 1, 5-biphosphate carboxylase, which results reduction in the formation of carbohydrates [6]. Plant adapt many strategies in response to different abiotic stresses such as high salt, dehydration, which ultimately affect the plant growth and productivity, different mechanisms including change in morphological and developmental pattern as well as physiological and biochemical responses [7, 8]. Adverse effects of salinity on plant growth may be due to ion cytotoxicity (mainly due to Na^+,Cl^-,So_4^-) and osmotic stress metabolic imbalances caused by ion toxicity, osmotic stress and nutritional deficiency under saline conditions may also lead to oxidative stress [9]. According to these reports, suggested that salt tolerance could be induced by enhancing antioxidant capacity of plants. Plant hormones are active members of the signal compounds involved in the induction of plant stress responses abiotic stresses result in both altered levels of phytohormones and decreased plant growth. The GA involves decreased cytokinin and increased abscisic acid content reported in salt stress plants [10].

GA is a very potent hormone which occurs naturally in plants, controls their development. It is able to reduce NaCl of rice seedling growth, GA has been reported to promote the growth of cotton and some halophytes in saline condition [11]. Addition of exogenous GA causes an increase in germination and seedling growth by enhancing the availability of endogenous GA [12]. GA has been the main focus of some plant scientists [13, 14]. Black gram (*Vigna mungo*) is a member of the Asian crop group. It is a summer pulse crop with short duration (90-120 days) and high nutritive value especially protein and it is valued for its high digestibility and flatulence effect [15].

Comparatively, few studies have been carried out on problems with salinity stress and methods to overcome salinity injuries in black gram. Therefore, this study investigated the effect on morphological changes, biochemical changes, salt stress on the photosynthetic pigments and enzyme activities on black gram under salt stress and hormone. This study investigates the growth enhancing ability of GA in salinity stressed black gram plants, with an examination on several growth parameters like pigments, carbohydrates, proteins, amino acids, proline and enzyme activity.

MATERIAL AND METHODS

Collection of Seed, Soil and Hormone

The certified seeds of *Vigna mungo* (ADT5) were collected from the paddy research institute, Aduthurai, Tamilnadu, India. Uniform sized seeds were surface sterilized with 0.5% mercuric chloride for 2 to 3 min to avoid fungal infection and taken out immediately, then washed with distilled water several times. Soil was collected from the agricultural fields near by Aduthurai. GA was collected from Insecticide India Limited, Jammu & Kashmir, India.

Experimental Protocol

Culture experiments were conducted under uniform conditions of light, soil and water. Sterilized seeds were divided into five test control and five test groups. Control plants were also maintained. The plants were grouped for experimental work as given in Table 1.

ISSN: 0975-8585

Table 1: experimental protocol

Test Group	NaCl (mM)	Gibberellic acid (mg)
Control	0	-
Test control 1	10	-
Test 1	10	100
Test control 2	20	-
Test 2	20	100
Test control 3	30	-
Test 3	30	100
Test control 4	40	-
Test 4	40	100
Test control 5	50	-
Test 5	50	100

Polyethylene bag experiment

Polyethylene bag culture experiments were conducted to study the effect of GA on black gram under different levels of saline water. The growth medium in the polyethylene bags consist of 5 Kg soil and polluted by different concentration of NaCl that is 10, 20, 30, 40 and 50 mM NaCl. By making 2 cm deep holes, sowed eight sterilized seeds in each bag. Afterwards, each seed was covered with a small amount of soil. Moisture content was adjusted regularly by water holding capacity with tap water. The experiments comprised of three replicates and were laid out in a completely randomized design. Test control groups received NaCl alone and test groups on 14th day after sowing 20 ml (100mg) per bag of GA was sprayed on leaves.

Growth parameters

Growth parameters like germination percentage, root and shoot length (cm), fresh and dry weight (gm), number of leaves were analysed on 30th day.

Vigour Index

Vigour index data were recorded on germination basis. Using the mean value of root length and shot length, Vigour index was calculated by the formula (1).

$$\text{Vigour index} = (\text{Mean shoot length} + \text{mean root length}) \times \text{Germination \%} \quad (1)$$

Biochemical analysis

After 30 days the plants were harvested and biochemical parameters like chlorophyll [16], carotenoids [17], total carbohydrates [18], total proteins [19], amino acids [20], total phenols [21] and proline [22] were analyzed by using standard methods.

Enzyme analysis

The antioxidant enzymes like Catalase [23], Superoxide Dismutase (SOD) [24] were also analysed.

Statistical Methods

The statistical analysis were done using SPSS package.

RESULTS AND DISCUSSION

Growth Parameters

The growth parameters were measured in salinity stressed plants such as root length, shoot length and number of leaves of black gram (Table 2). The observations were recorded at 30th day after sowing. In the

present study, we found that germination of seeds of test plants was significantly inhibited by increasing concentration of NaCl and that the salt stress-induced suppression of seed germination was alleviated by GA. At 50mM NaCl 75% of germination was recorded. **Chenshuo Chang et al**., demonstrated that the salt stress-induced inhibition of seed germination was related to its suppression of ethylene production during imbibition [25]. Ethylene has been reported to stimulate germination in nondormant seeds and break dormancy in seeds that display embryo dormancy A positive correlation exists between seed germination and ethylene production, suggesting that ethylene plays a role in modulation of seed germination. **Petruzzelli et al. [26]** reported that the onset of seed germination in pea is accompanied by ethylene evolution from the embryonic axis. It is observed from results (Table 2) that the increased salinity concentration (10, 20, 30, 40 and 50 mM) progressively decreased the germination percentage and number of leaves of black gram. Iterbeormaetxe *et al*., 1998 showed that high salinity adversely affected germination, growth physiology and productivity by causing ionic and osmotic stresses as well as oxidative damage and our results also well agree with their findings [27].

Table 2: Effect of GA on the growth parameters of *Vigna mungo* under different concentration of salinity stress.

Groups	Germination (%)	Shoot length (cm)	Root length (cm)	No of leaves
Control	100	14.08±0.18	9.5±0.2	5.15±0.35
Test control 1	100	12.25±0.16*	9.15±0.12	5.123±0.11
Test 1	100	12.66±0.152*	9.7±0.15	5.142±0.03
Test control 2	100	11.925±0.17*	8.5±0.2*	5.11±0.02
Test 2	100	12.22±0.21*	8.9±0.23*	5.2±0.24
Test control 3	87.5	11.47±0.14**	8.16±0.14*	4.91±0.11
Test 3	87.5	11.9±0.11**	8.88±0.2*	5.10±0.25
Test control 4	87.5*	11.11±0.11**	7.13±0.16**	4.52±0.37*
Test 4	87.5*	11.63±0.12**	7.95±0.21**	4.90±0.02
Test control 5	75**	10.23±0.13***	6.15±0.12***	3.96±0.01**
Test 5	75**	10.7±0.15***	6.94±0.2***	4.43±0.25*

Values are expressed as mean ±SD. N=8, Stastistical significant test for comparison was done by ANOVA, followed by T test (n=6) ***p< 0.05 vs control, **p< 0.01 vs control. *p< 0.001 vs control

The same results were reported by Lin and Kao., 1986 who showed that salinity reduced the germination of plants [3,28]. It is observed that the root and shoot length were decreased at maximum salinity conditions 6.15 and 10.24 cm (50 mM) respectively. The GA treatment restores the salinity stress and increases the root and shoots length values near to control (Table 2). The shoot and root length of control plants were 14.08 cm and 9.5 cm respectively. The shoot length (cm) of plant under salinity at different concentration were noted as 12.25 (10 mM), 11.92 (20 mM), 11.47(30 mM), 11.11 (40 mM) and 10.23 (50 mM) and the root length (in cm) as 9.15 (10 mM), 8.5 (20 mM), 8.16 (30 mM), 7.13 (40 mM) and 6.15 (50 mM). The increasing concentration of salt progressively decreases root length, shoot length and number of leaves. Percentage of germination, root length, shoot length and number of leaves affected by salt and its values highly decrease in higher salt concentration than low salt concentration and control [4, 29]. The observation supported by the work of Raptan *et al*., 2001 who reported that salinity decreases root and shoot length [30]. Plant growth maximized with applying an organo-mineral fertilizer under salinity [31]. The seeds treated with GA showed a significant increase in root and shoot length. The shoot length (cm) of the plants received NaCl and GA were recorded as 12.66 (10 mM), 12.22 (20mM), 11.9 (30mM), 11.63 (40mM) and 10.7 (50mM). Results confirm the alleviation effect of GA against NaCl. In the same way root length of the GA supplied plants increased when compared to the test control plants which received NaCl alone. However it is interesting to note from table 2 that the GA treatment is able to overcome the effect of salinity stress and improves the growth parameters. Similar results were reported by Tejera et al. [32], who indicated that growth of common bean plants considerably decreased by salt stress. Salinity can inhibit plant growth by altering the water potential, increasing the ion toxicity, inhibiting the cell division and cell expansion, or causing an ion imbalance [33]**.** Younis et al. [34] reported that the growth reduction caused by salinity stress is due to inhibited apical growth in plants as well as endogenous hormonal imbalance. In both cases, reduction could have been caused by the toxic effects of ions (Na+ and Cl-) on metabolism or from adverse water relations. In addition, a secondary aspect of salinity stress in plants is the stress-induced production of ROS [35]. The enhanced production of ROS during salinity stress lead to the progressive oxidative damage and ultimately cell death and growth suppression [36].

ISSN: 0975-8585

Fresh weight, Dry weight and Vigor index

The effect of GA treatment on fresh and dry weight, vigour index of *Vigna mungo* under different concentration of salt stress (10, 20, 30, 40 & 50 mM NaCl) is given in Table 3. The observations were recorded at 30th day after sowing black gram seeds by polythene bag experiment. The marked reduction in germination percentage, root length and shoot length influence decreased vigour index, fresh and dry mass. The highest seedling vigour is noted in control plants as 2300 and lowest vigour is noted in plants under maximum salinity concentration ie 50mM (1700). The similar results have been observed that excess salinity decreases the vigour of seedling [37, 38].

Table 3: Effect of Gibberellic acid on the vigour index, fresh and dry weight of *V.mungo* under different concentration of salinity stress.

Groups	Vigour index	Fresh weight	Dry weight
Control	2300±0.12	2.35±0.04	0.45±0.01
Test control 1	2130±0.21	2.01±0.11	0.38±0.11*
Test 1	2220±0.32	2.32±0.32	0.42±0.25
Test control 2	2030±0.11	1.89±0.13*	0.30±0.5*
Test 2	2110±0.35	2.05±0.5	0.36±0.05*
Test control 3	1950±0.02*	1.72±0.23**	0.21±0.07**
Test 3	2020±0.13	1.90±0.12*	0.28±0.11**
Test control 4	1810±0.53**	1.58±0.35***	0.13±0.02***
Test 4	1900±0.12*	1.71±0.05**	0.20±0.05**
Test control 5	1700±0.32**	1.40±0.25***	0.08±0.07***
Test 5	1790±0.24**	1.55±0.11***	0.15±0.01***

Values are expressed as mean ±SD. N=8, Stastistical significant test for comparison was done by ANOVA, followed by T test (n=6) ***p< 0.05 Vs control, *p< 0.01 Vs control *p< 0.001 Vs control

It is observed from the table 3 that the fresh weight and dry weight of control plant were 2.35g and 0.45 g respectively. The fresh weight of plant (g) under salinity at different concentration were noted for 10 mM (2.01), 20 mM (1.89), 30 mM (1.72), 40 mM (1.58) and 50 mM (1.40) and dry weight as 10 mM (0.38), 20mM (0.30), 30 mM (0.21), 40 mM (0.13) and 50 mM (0.08). The same results were reported by Dhingra and Sharma., 1992 who showed that salinity reduced the fresh and dry weight of plants [38].

It has been further supported by Ashraf *et al*., 1996 that saline conditions reduced the growth parameters such as fresh and dry weight of plants. Several studies suggested that reduction of dry weight may be due to a turgor limitation or cell wall hardening by limited extension growth [39]. It is quite interesting to note from table that the application of GA is able to overcome the effect of different concentration of salinity stress on vigour index, fresh weight and dry weight and restores the value. These values were nearer to control.

The inhibitory effect of high levels of salinity were mitigated partially or completely alleviated by GA. The maximum fresh and dry weight (g) of test plants (NaCl + GA) 2.32 and 0.42 at 10mM NaCl treatment. This is probably by increasing the efficiency of water uptake and utilization as well as protecting the photosynthetic pigments and the photosynthetic apparatus. The observation supported by Radi *et al*., 1989 who reported that salinity decreases growth parameters such as fresh weight and dry weight of plant [4].

Biochemical analysis

Carbohydrate and protein content

Carbohydrate content of the experimental plants have been presented in Table 4. It is clear from the results that total carbohydrate level in the control plants of black gram leaves is 26.4 mg/g and the higher concentration of NaCl induced significant increase in total carbohydrate content of black gram. An increase in carbohydrate content (mg/g) was noted in plants under different salt concentration of 10, 20, 30, 40 and 50mM were 28.1, 31.5, 35.2, 38.6 and 41.2 respectively. The similar results were observed and reported that

the salinity can affect the carbohydrate metabolism and overall production of carbohydrate [28, 40]. The significant increase in carbohydrate fraction in Z*ea mays* was observed [41].

Table 4: Effect of GA on the carbohydrate, protein and amino acid of Vigna mungo under different concentration of salinity stress.

Groups	Carbohydrate (mg/g)	Protein (mg/g)	Amino acid (mg/g)
Control	26.4± 0.36	23.5 ±0.5	41.28± 0.03
Test control 1	28.1± 0.12*	23.1± 0.12	39.56 ±0.12*
Test 1	26.8± 0.5	26.0± 0.32**	40.13± 0.5
Test control 2	31.5± 0.42**	22.9 ±0.56	37.82± 0.16**
Test 2	28.7± 0.16*	25.6 ±0.12**	39.20 ±0.4*
Test control 3	35.2± 0.25**	22.5 ±0.4	34.96 ±0.1**
Test 3	32.1± 0.5**	24.8± 0.36*	36.58± 0.25**
Test control 4	38.6± 0.18***	22.2± 0.11*	31.60± 0.52***
Test4	34.9± 0.6**	23.9± 0.16	33.87 ±0.13**
Test control 5	41.2 ± 0.13***	21.5± 0.5*	28.96 ±0.6***
Test 5	38.6± 0.56***	22.6± 0.4	31.17± 0.05***

Values are expressed as mean ±SD. N=8, Stastistical significant test for comparison was done by ANOVA, followed by T test (n=6) ***p< 0.05 vs control, **p< 0.01 vs control. *p< 0.001 vs control

Salt and water deficit stresses caused a significant increase in soluble carbohydrate content of barley leaves. With increasing salt doses, the rate of increase in soluble carbohydrate content was increased, indicating a role of soluble carbohydrate in the osmotic adjustment. The accumulation of sugars in plants under stress conditions might be involved in the osmotic adjustment was reported [42]. Supply of GA decreased the carbohydrate content of plants significantly. The carbohydrate content (mg/g) of plants of Test 1 to Test 5 are 26.8, 28.7, 32.1, 34.9 and 38.6 respectively.

The total protein content of *Vigna mungo* given in Table 3. The protein content of control plant was recorded as 23.5 mg/g. The salt stress not only impairs the carbohydrate metabolism but also interferes with the protein metabolism. From the table 4 it was found that marked decrease in protein content (mg/g) of test control plants in decreasing order of 10mM (23.1), 20mM (22.9), 30mM (22.5), 40mM (22.2) and 50 mM (21.5). There was a significant difference between the test control groups when compared to control.These similar results have been observed by Chakrabarti and Mukherji, 2003 showed that NaCl concentration caused greatest reduction in growth, nitrogen fixation, total nitrogen contents and protein contents [43, 44]. Metabolic toxicity of Na+ is largely a result of its ability to compete with K+ for binding sites of multiple enzymes in the cytoplasm that are required for cellular function. Protein synthesis also requires high concentrations of K+, owing to the K+ requirement for the binding of tRNA to ribosomes and probably other aspects of ribosome function [45]. Thus, disruption of protein synthesis by elevated concentrations of Na+ appears to be an important cause of damage by NaCl. Salt stress can also increase the production of ROS and cause damage to proteins. One possibility is that autophagy might be responsible for degrading oxidized proteins under salt stress [46].

The plants under salinity stress after receiving GA the protein content was increased. The protein content of Test 1 to 5 plants are 26, 25.6, 24.8, 23.9 and 22.6 mg/g which are are significantly increased when compared to test control groups. In the presence of Ascorbic acid, the soluble protein content increased, indicating that it may have an important role in plant adaptation to a high NaCl content [47].

Amino Acid Content

Total free amino acid content of *V. mungo* is expressed in Table 4. The amino acid content of control plants were recorded as 41.28 mg/g. Total amino acids were measured on 30 days old leaves. The drastic reduction of amino acid content in the salt concentration 10, 20, 30, 40 and 50 mM of NaCl were 39.56, 37.82, 34.96, 31.60 and 28.96mg/g respectively. It has been further supported by many researchers, that salinity conditions reduced the amino acid content and nitrogen content [4, 44]. The similar results were found by Nemoto and Sasakuma., 2002 who showed that salinity can affect the amino acid metabolism [8]. It is interesting to note that application of GA increases the amino acid content. Total amino acid content of test control groups 1 to 5 were recorded as 40, 39.2, 36.5, 33.8 and 31.1 mg/g respectively. The application of GA

treatment is able to overcome the adverse effect of salt stress and increase the value of carbohydrate, protein and amino acid in *Vigna mungo* under salinity. These values were nearer to control [8]. Signaling of GA was required for adjustment to adverse environmental conditions and helped maintain source–sink relationship [48] as salinity caused a reduction in sink enzyme activities.

Proline and total phenol content

The values represented in table 5 shows the effect of GA treatment on proline and phenol content of *V.mungo* under different concentration of salt stress. The observations were noted 30th day after sowing and compared with control. Results showed that salt stress caused marked increase in proline content (mg/g) as compared to control plant. The proline content of control black gram is 6.50 mg/g. It is found to be increased in the order of salt concentration 10, 20, 30, 40 and 50 mM as 6.73, 6.95, 7.13, 7.31 and 7.55 respectively. Similar observation has been reported in salt stressed Rice [49], Soybean [50] and Maize [51] seedlings. Proline is an important parameter to measure stress tolerance capacity of plants [52] moreover proline is a marker of stress tolerance and has been reported by many scientists. This highly water soluble amino acid protects membranes against the harmful effects of high concentrations of inorganic ions, and it can also higher electrical conductivity of the treatment solutions on growth parameters, especially on leaf water potential [53]. Application of GA to the salt stressed plants reduces the level of proline content which indicates that this phyto hormone reduces the stress caused by salinity (Fig 2).

Table 5: Effect of Gibberellic acid treatment on the pigments of V.mungo under different concentration of salinity stress.

	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Carotenoid (mg/g)
Control	0.25±0.007	0.14±0.005	0.27±0.005	0.054±0.003
Test control 1	0.23±0.012	0.13±0.007	0.24±0.16	0.048±0.006
Test 1	0.24±0.003	0.14±0.002	0.26±0.007	0.052±0.001
Test control 2	0.21±0.017*	0.12±0.004*	0.23±0.008*	0.043±0.007*
Test 2	0.22±0.006*	0.13±0.002	0.24±0.002	0.047±0.002
Test control 3	0.18±0.11**	0.11±0.007**	0.21±0.013**	0.039±0.008**
Test 3	0.20±0.003*	0.12±0.003*	0.23±0.001*	0.042±0.007*
Test control 4	0.17±0.01***	0.10±0.008**	0.20±0.018**	0.036±0.11**
Test4	0.18±0.07**	0.11±0.002**	0.21±0.002**	0.038±0.008**
Test control 5	0.16±0.13***	0.09±0.008***	0.18±0.008***	0.033±0.007***
Test 5	0.17±0.11***	0.10±0.003***	0.19±0.009***	0.036±0.002***

Values are expressed as mean ±SD. N=8, Stastistical significant test for comparison was done by ANOVA, followed by T test (n=6) ***p< 0.05 Vs control, **p< 0.01 vs control. *p< 0.001 Vs control

Fig.1 Effect of Gibberlic acid on *Vigna mungo* irrigated with different levels of saline water.

ISSN: 0975-8585

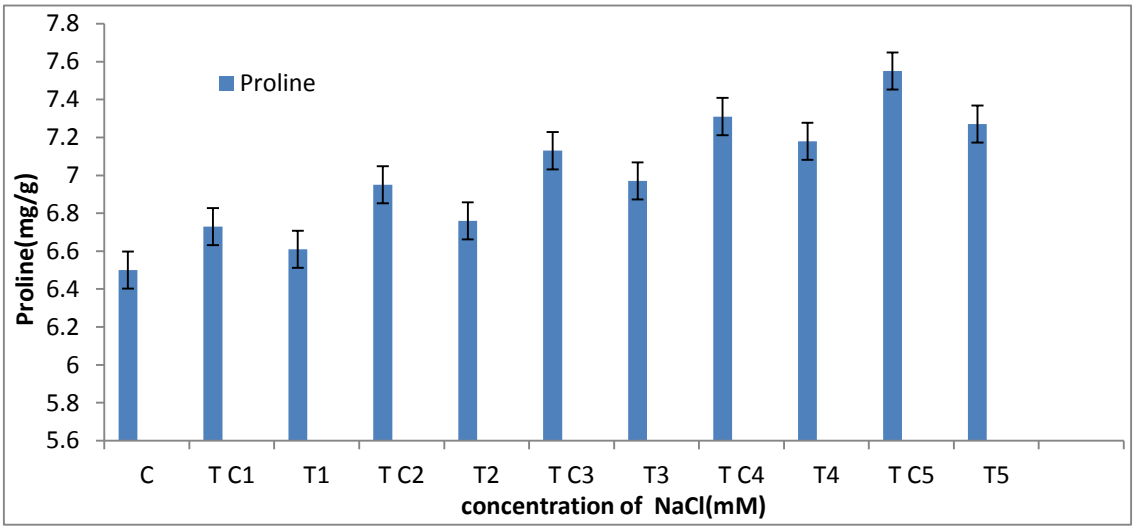

Fig. 2 Effect of Gibberellic acid on Proline of *Vigna mungo* under different concentration of salinity stress.

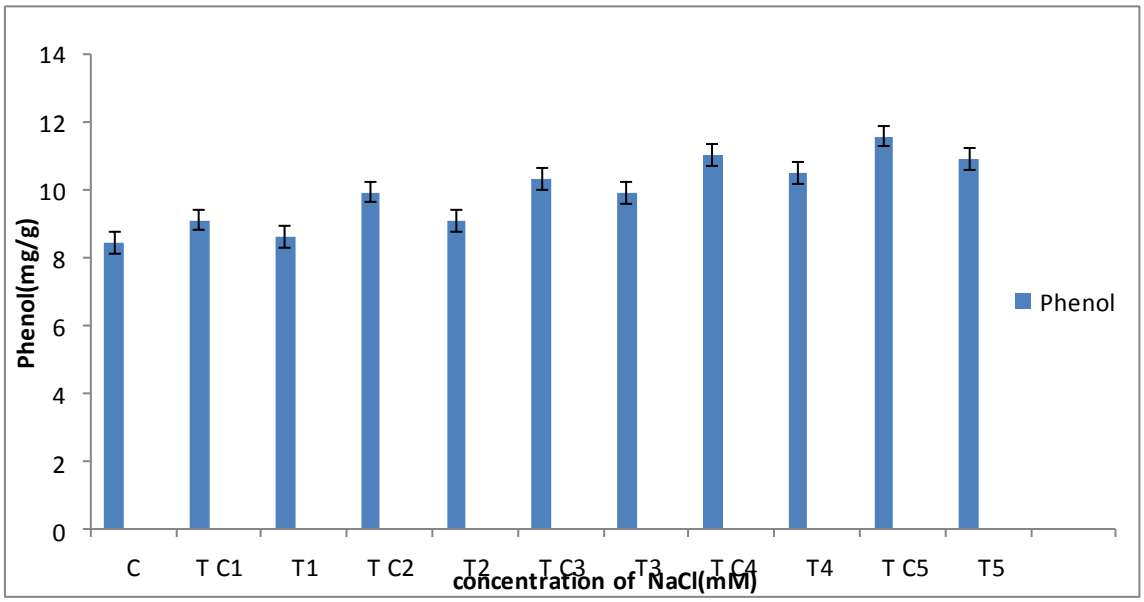

Fig. 3 Effect of Gibberellic acid on Phenol of *Vigna mungo* under different concentration of salinity stress.

Fig 3 represents the total phenol level in the control plants and it is found to be 8.45mg/g. The amount of phenol under various concentration of NaCl 10,20,30,40 and 50 mM were 9.12, 9.95, 10.32, 11.06 and 11.6 respectively. Phenols are the defense system would be triggered in order to protect the plant from further pathological injury, while the plant is suffering out of salinity. An increase in phenol level is noted with increasing concentration of salinity and application of Gibberellic acid in saline condition shows to restore the proline and phenol content nearer to control. Phenol content of Test groups 1 to 5 are 8.63, 9.1, 9.9, 10.5 and 10.93 mg/g respectively which shows significant difference when compare to control.

Chlorophyll and Carotenoid

The effect of GA on the chlorophyll 'a' and 'b', total chlorophyll and carotenoid content of black gram under different concentration of salt stress (Table 5). Increased salinity levels showed a sharp decline in total pigment content of black gram leaves such reduction was attributed to the decline in the chlorophyll a and b as well as the carotenoid content and also quite interesting to mention here that the GA treatment prevent the toxic effect of salt stress and also restores the pigments value nearer to control. The chlorophyll a and b level of control plants has been recorded as 0.25 mg/g and 0.14 mg/g and drastic reduction in plant pigments

were noticed under higher salinity. Increasing salinity decreases pigment content. These results are in harmony with those observed by Rabie *et al*., 2005 and Zhao *et al*., 2002 that salinity caused decrease in plant pigment and chlorophyll contents in leaves of green gram. The high salinity caused a disturbed chloroplast structure, number and size, which affected chlorophyll content and/or caused disruption of chloroplasts by oxidative stress that causes a decrease in chlorophyll content [54, 55]. Supplementation of caffeic acid to soybean plants grown under salinity conditions prevents salinity-induced chlorophyll loss [56]. Gul., 1998 further found that a decrease in chlorophyll concentration in salinized plants could be attributed to increase the activity of chlorophyll degrading enzyme chlorophyllase [57]. Further, chlorophyllase activity increases during stress conditions [58], suggesting that the observed low chlorophyll content could be a result of both decreased synthesis and increased degradation under salt stress.

Application of GA to the saline stressed plants increased the pigment level. GA3 also plays a vital role in tolerance to salt stress by improving plant growth and chlorophyll synthesis. In addition, the inhibitory effect of GA3 on chlorophyll catabolism might be partly due to the down regulation of the activities of enzymes involved in chlorophyll catabolism and the alleviation of oxidative chlorophyll bleaching [25].

The total carotenoid content of control black gram has been recorded as 0.054 mg/g and drastic decrease in carotenoid level was observed in higher concentration of NaCl ie 50mM (0.033mg/g). The decrease in total chlorophyll content under salinity stress is commonly reported phenomenon, because of its adverse effects on membrane stability [59]. Bassia diffusa would not survive prolonged submergence (> 7 days) but could survive submergence of short duration (< 7 days) through continuous underwater photosynthesis, accumulation of osmolytes such as oxalic acid and carotenoid, and maintenance of relative water content and succulence within control levels [60]. Hamada and Al-Hakimi., 2002 shows the similar result of salinity decrease carotenoid and induce reduction in chlorophyll and photosynthetic activity [5]. The application of GA to salinised plants alleviate the effect of salt and increases the pigment content.

Catalase and Superoxide Dismutase

Effect of Gibberellic acid treatment on the catalase (CAT) and superoxide dismutase (SOD) content of *Vigna mungo* under different concentration of salt stress were analysed (Table 6). It is clear from table that increased salinity (10, 20, 30, 40 and 50 mM) progressively increased the CAT and SOD activity. The CAT and SOD activity of control plants has been noted as 115.3 U/g and 7.9 U/g respectively and drastic increase was observed at higher NaCl concentration like at 10, 20, 30, 40 and 50 mM of NaCl CAT activity (U/g) were recorded as 122.4, 129.4, 135.3, 141.1 and 152.8 respectively and the SOD activity (U/g) as 8.1, 8.7, 9.49, 9.52 and 10.30 respectively. SOD catalyses the conversions of superoxide anion to hydrogen peroxide and water. Several reports have been shown that over expression of SOD leads to increased tolerance to abiotic stress such a temperature and water [9]. Zhao *et al.,* 2004 had similar findings and expressed that increased SOD activity was because increased in oxidative damage were closely related [55]. Under stressful conditions, plants have evolved complex mechanisms to struggle against these oxidative stresses by the synchronous action of various enzymatic and non enzymatic antioxidants. Of these, SOD and CAT form the antioxidant enzymatic component [61]. These antioxidants play a significant role in detoxifying ROS [62]. SOD dismutates superoxide radicals to H2O2, which is the initial reaction of ROS detoxification and a key component of the ROS_scavenging system [61], whereas CAT and peroxidase are involved in converting H2O2 into water and oxygen. Thus, antioxidants may provide for a strategy to enhance plant salinity tolerance. There is enough evidence that alleviation of oxidative damage and increased salinity tolerance are often correlated with an efficient antioxidant defense system in plants . Similarly, increased SOD, CAT and Peroxidase may be correlated to salinity tolerance [63].

Application of GA reduces the oxidative stress caused by salinity and reduces the CAT and SOD activity (Table 6). The results obtained in the present study indicated the positive effect of GA in salt stress. It is interesting to note that the application of GA is able to overcome the effect of different concentration of salinity stress on catalase, peroxidase and super oxide dismutase restores the value which is nearer to the control [53, 64-66].

ISSN: 0975-8585

CONCLUSION

From the results obtained from the present study we can conclude that the salt concentration of the soil severely affects plant growth by interfering with metabolism of carbohydrate, protein, amino acid. More over higher salt concentration reduces the activity of antioxidant enzymes so that plants face oxidative stress. But the supply of GA alleviates the activity of salt on *Vigna mungo* when it is applied as foliar spray. GA increases plant growth and helps to nullify the effects caused by salt.

ACKNOWLEDGEMENT

The authors are highly thankful to the Vice Chancellor, SASTRA University for providing the required amenities for carrying out this work.

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