International Journal of Plant & Soil Science

18(6): 1-10, 2017; Article no.IJPSS.36420
ISSN: 2320-7035

Effect of PGRs and Nutrients on Growth, Physiological Parameters and Yield of *Vigna mungo* L. under Saline Stress

R. Sivakumar[1*] and S. Jaya Priya[1]

[1]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, India.

Authors' contributions

This work was carried out in collaboration between two authors. Author RS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SJP carried out the collection of literatures, carried out the work and measurement of growth parameters and physiological traits. Both the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/36420
Editor(s):
(1) Peter A. Roussos, Agricultural University of Athens, Lab. Pomology, Greece.
Reviewers:
(1) Kürşat Çavuşoğlu, Süleyman Demirel University, Turkey.
(2) E. G. Oboho, University of Benin, Nigeria.
(3) Badran E. Ayman, Desert Research Centre, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/21130

Original Research Article

Received 28th August 2017
Accepted 21st September 2017
Published 25th September 2017

ABSTRACT

Aim: Pots culture experiment was conducted in order to alleviate the salinity effect in *Vigna mungo* L. using plant growth regulators and nutrients by assessing growth, physiological and yield parameters.
Study Design: The experiment was carried out with seven treatments of different PGRs and nutrients with three replications organized in a complete randomized design.
Place and Duration of Study: The experiment was conducted in glass house, Department of Crop Physiology, TNAU, Coimbatore, Tamil Nadu, India during 2016.
Methodology: Red sandy soil mixture was used for pots culture experiment using medium size pots with 12 kg capacity. The salinity was imposed by 125 mM NaCl concentration. Plant growth regulators and nutrients like jasmonic acid (50 µM), gibberellic acid (10 ppm), brassinolide (0.5 ppm), salicylic acid (100 ppm), ascorbic acid (100 ppm), benzyl amino purine (5 ppm), K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%) and TNAU Pulse Wonder (1%) were used as foliar spray at 20 and 40 days after sowing except jasmonic acid, which used as seed soaking. The plant height, root length, chlorophyll

*Corresponding author: E-mail: sivatnau5@gmail.com;

content, soluble protein content, proline content and NR activity were estimated at 30 and 50 days after sowing. Finally the yield was recorded during harvest.
Results: Salinity significantly reduces the plant height, root length, chlorophyll and soluble protein contents, NR activity and yield. However, the proline content was increased under saline condition over absolute control. Among the PGRs and nutrients, 0.5 ppm brassinolide showed its supremacy in terms of increased plant height, soluble protein, NR activity and yield compared to other treatments. However, benzyl amino purine recorded higher chlorophyll content (2.25 mg g^{-1}) followed by TNAU Pulse Wonder (2.12 mg g^{-1}) and brassinolide (2.04 mg g^{-1}). Brassinolide registered the highest proline content of followed by ascorbic acid and TNAU Pulse Wonder. Brassinolide increased nitrate reductase activity up to 36.78 per cent followed by salicylic acid (32.18%) over control. Brassinolide recorded the maximum grain yield of 8.85 g $plant^{-1}$ followed the salicylic acid (8.72) which is on par with TNAU Pulse Wonder (8.60 g).
Conclusion: Salinity causes 40 per cent yield reduction in black gram. All the PGRs and nutrients improved the growth and yield of black gram under salinity. Brassinolide showed its supremacy of 21.07 per cent yield increment under salinity followed by salicylic acid (16.98%) and TNAU Pulse Wonder (13.21%).

Keywords: Growth regulators; NaCl; NR activity; Vigna mungo L.

1. INTRODUCTION

The human population is rapidly increasing and needs a substantial increase in agricultural productivity worldwide. However, various abiotic stresses are major factors limiting crop productivity. Among that, salinity is one of the major abiotic stresses that adversely affect crop productivity and quality with increasing impact on the socioeconomic fabric and health, especially of the farming communities. Salinity is a major abiotic stress that affects approximately 7% of the world's total land area and more than 800 million hectares of land around the world are affected by salinity [1], which results in billions of dollars in crop production losses. In India about 7 million hectare area produces less yield due to salinity. Soil salinity problems are encountered in almost all the districts in Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Gujarat and Rajasthan. The area extends to about 0.176 million ha in Andhra Pradesh, 0.2 million ha in Karnataka, 0.0427 million ha in Tamil Nadu and about 0.03 million ha in Kerala. Salinity inhibits seed germination and plant growth, affects the leaf anatomy and physiology of plants and, thereby, influences their photosynthesis, water relations, protein synthesis, energy production and lipid metabolism [2]. It is recognized as major constraint in the production of crops where it can cause yield losses more than 70% [3].

Black gram assumes considerable importance from the opinion of food and nutritional security in Asian countries. Black gram is favorable short duration pulse crop and well suited in all seasons either as sole or as intercrop or fallow crop. India is the world's largest producer as well as consumer of black gram [4]. It is very sensitive to salinity, in early stage of growth and affects the ability to utilize water and cause a reduction in growth rate as well as plant metabolic processes ultimately yield [5].

Plant growth regulators (PGRs) and nutrients have been used to promote plant growth and development of plants under various stress conditions. Brassinosteroids (BRs) are a class of polyhydroxy steroidal lactones that play essential roles in plant development [6]. They play a significant role in amelioration of the salt-stress in different plant species [7]. BRs promote seed germination in wheat, tobacco, tomato and groundnut under salt stress condition [8]. Foliar application of salicylic acid increased the photosynthetic rate in corn and soybean under salt stress [9]. Ascorbic acid application alleviate the destructive effects of salinity on osmotic potential, shoot and root dry mass, K^+/Na^+ ratio and contents of photosynthetic pigments in wheat seedlings under salinity stress [10]. Application of GA increases nitrogen and magnesium in leaves and roots under salinity [11]. GA_3 stimulated the accumulation of K in shoots of wheat under saline condition [12]. Cytokinins could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and ABA [13]. Plant growth and salt tolerance were sharply reduced when exposed to a combination of salt stress and potassium deficiency stress. Potassium deficiency significantly increased the negative effects that were induced by salt in the photosynthesis of barley and was accompanied by an increase in salt sensitivity [14]. Hence, the experiment was conducted to study the performances of different plant growth regulators and nutrients on chlorophyll content, proline, soluble protein, NR enzyme activity and yield of blackgram under saline conditions.

2. MATERIALS AND METHODS

2.1 Experimental Location and Materials

The pots culture experiment was conducted at glass house condition at Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2016. Red sandy soil was used for pot culture experiment at glass house. Soil mixture was prepared by using red soil, sand and vermicompost in the ratio of 2:1:1. Medium size pots with the capacity of 12 kg of soil. The seeds of black gram variety were collected from the Department of Pulses, TNAU.

2.2 Methodology and Treatments

Black gram is a direct seed sown crop hence, seeds were sown in a pot then the salt solution was poured on the soil. Salinity was imposed from sowing onwards till the end of the harvest. Crop was applied with recommended dose of fertilizers (25 kg N + 50 kg P_2O_5 + 25 kg K_2O ha^{-1}) and other cultivation operations including plant protection measures were carried out as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore. The experiment was laid out in completely randomized block design with three replications organized in a complete randomized design. The salinity was imposed by 125 mM NaCl concentration.

Plant growth regulators and nutrients like jasmonic acid (50 µM), gibberellic acid (10 ppm), brassinolide (0.5 ppm), salicylic acid (100 ppm), ascorbic acid (100 ppm), benzyl amino purine (5 ppm), K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%) and TNAU Pulse Wonder (1%) were used as foliar spray at 20 and 40 DAS by adopting completely randomized design with three replications. Jasmonic acid (50 µM) used in this study as seed soaking method. The observations like morphological, physiological and biochemical parameters were recorded at 30 and 50 DAS. Finally the yield and quality parameters were estimated after harvest.

2.3 Measurement of Growth Parameters

Plant height was measured from the ground level to the tip of the growing point and expressed as cm. The plant was uprooted from the pot and the root was taken with minimum damage and the length from the cotyledonary node to the root tip was measured and expressed as cm.

2.4 Estimation of Physiological Parameters

The total chlorophyll content was estimated by adopting the acetone method [15] and the content was expressed as mg g^{-1} of fresh weight. Soluble protein content in the leaf was estimated by using Folin Ciocalteau reagent [16].

The proline content was estimated by acid ninhydrin protocol [17]. The leaf sample was homogenized with 10 ml of 3 per cent sulphosalicylic acid and centrifuged at 3000 rpm for 10 minutes. Two ml of the supernatant was taken and 2 ml of glacial acetic acid, 2 ml of ortho phosphoric acid and 2 ml of acid ninhydrin mixture were added. The contents were allowed to react at 100℃ for 1 hour and then it is incubated on ice for 10 minutes to terminate the reaction. The reaction mixture was mixed vigorously with 4 ml toluene for 15 to 20 seconds in separating funnel.

The chromophore containing toluene was aspired from the aqueous phase, warmed to room temperature and optical density was read at 520 nm and the amount of proline was quantified by using pure proline as standard.

Nitrate reductase activity was estimated in fully expanded functional leaves by using assay medium. 10 ml of the assay medium (phosphate buffer + KNO_3 + Iso propanol) was added to 500 mg of leaf sample pieces and kept in desiccator for 5 minutes and one hour for reaction. After one hour, 2 ml of the aliquot was taken and 1 ml of sulphanilamide (1%) and 1 ml of NEDD (0.02%) were added. The OD was measured at 540 nm in the spectrophotometer and the enzyme activity was expressed as µg NO_2 g^{-1} h^{-1} using KNO_2 as a standard [18].

2.5 Estimation of Yield and Statistical Analysis

The total weight of grains harvested from each plant of all picking was added and average yield per plant was worked out and expressed in gram per plant. The data on various parameters were analyzed statistically [19]. Wherever the treatment differences are found significant, critical differences were worked out at five per cent probability level and the values were furnished.

3. RESULTS

3.1 Impact of Salinity and Effect of PGRs and Nutrients on Growth Parameters

The growth parameters like plant height and root length were reduced by salinity shown in Table 1. The highest value of plant height (30.3 cm) was recorded in absolute control while the lowest (21.50 cm) was recorded in salinity at 50 DAS. Among the PGRs and nutrients used, highest plant height was observed in brassinolide treatment (25.1 cm) which is on par with TNAU Pulse Wonder (25.00 cm) followed by gibberellic acid (24.50 cm).

Significant differences were observed in all the treatments in the case of root length. Absolute control recorded 16.7 cm root length and 12.00 cm was recorded under salinity at 50 DAS. The application of plant growth regulators and nutrients showed an intense effect on root length. Among the treatments, TNAU Pulse Wonder recorded the highest root length (13.9 cm) which is on par with brassinolide (13.8 cm) and salicylic acid (13.6 cm) followed by gibberellic acid (13.4 cm) at 30 DAS. At 50 DAS, the highest root length was observed by TNAU Pulse Wonder and brassinolide (14.0 cm), followed by gibberellic acid (13.8 cm) and salicylic acid (13.7 cm) (Table 1).

3.2 Impact of Salinity and Effect of PGRs and Nutrients on Chlorophyll and Soluble Protein

Significant differences were noticed in all treatments with respect to chlorophyll content under saline conditions was shown in Table 2. Due to salinity, the total chlorophyll content drastically reduced from 2.53 (Absolute control) to 1.65 mg g^{-1} (Salinity). Among the plant growth regulators and nutrients used, benzyl amino purine recorded highest chlorophyll content (2.25 mg g^{-1}) followed by TNAU Pulse Wonder (2.12 mg g^{-1}) and brassinolide (2.04 mg g^{-1}) at 50 days after sowing.

Table 2 shows that the soluble protein content was recorded 15.42 mg g^{-1} under non-saline condition and comparatively lower soluble protein content was registered in control (10.57 mg) at 30 DAS. Among the PGRs, brassinolide recorded higher soluble protein content (12.30 mg g^{-1}) which is on par with TNAU Pulse Wonder (12.25 mg g^{-1}) followed by ascorbic acid (11.70 mg g^{-1}) and salicylic acid (11.41 mg g^{-1}) at 50 DAS.

3.3 Impact of Salinity and the Effect of PGRs and Nutrients on NR Activity and Proline

Fig. 1 show that nitrate reductase activity was drastically reduced from 145 µg NO_2 g^{-1} h^{-1} to 78 under salinity condition at 50 days after sowing. The data exhibited the significant difference with in treatments according to salinity. Among the ameliorants used in this study, nitrate reductase activity was higher in salicylic acid treatment (109 µg NO_2 g^{-1} h^{-1}) followed by brassinolide (106 µg NO_2 g^{-1} h^{-1}) and TNAU Pulse Wonder (95 µg NO_2 g^{-1} h^{-1}).

The proline content was showed higher value in stressful environment compared to non-stress condition was shown in Fig. 2. Absolute control recorded the lowest proline content of 201.08 µg g^{-1} while the control recorded higher proline content (347.58 µg g^{-1}). Among the PGRs and nutrients used, brassinolide registered the highest proline content of 384.24 µg g^{-1} followed by ascorbic acid (382.35 µg g^{-1}) and TNAU Pulse Wonder (380.25 µg g^{-1}) at 50 days after sowing.

3.4 Impact of Salinity and Effect of PGRs and Nutrients on Yield

Significant differences were recorded with the treatments in the case of grain yield under salinity condition was shown in Fig. 3. Grain yield was reduced from 10.30 g $plant^{-1}$ (Absolute control) to 7.18 g $plant^{-1}$ (Salinity). Among the PGRs and nutrients, brassinolide recorded the maximum grain yield (8.85 g) followed the salicylic acid (8.72 g) which is on par with TNAU Pulse Wonder (8.60 g).

4. DISCUSSION

Plant height is an important parameter that determines the growth and development of a plant. Generally, the plants with vigorous growth usually produce taller plants until maturity and are expected to give higher yield. But in this present investigation, the plant height was declined due to salinity. As a result of salinity, there was a significant reduction in plant height (28.33%) was noticed compared to absolute control. The salinity caused the reduction in plant height which might be due to the reduced water uptake and building up of salts in the apoplast of growing tissues leading to cell and tissue dehydration. Salt stress at growth stage caused a reduction in growth and development due to reduced turgor. The reduced plant height is due to impaired mitosis, cell elongation and expansion [20].

Under saline condition, both brassinolide and TNAU Pulse Wonder showed increment of plant height up to 16.27 per cent compared to control. Application of BRs improved tolerance against salt in rice and wheat [21]. Foliar application of BRs on wheat plants increased plant height and significantly overcome the depressive effect of salinity [22].

Root length is the most important indicator of salt stress because roots are in direct contact with the soil and absorbs water. Reduced root length under salinity is commonly occurring phenomenon [23] which leads to reduction in shoot and root dry weight. Under high saline condition water uptake by plant was reduced due to increment of osmotic pressure which creates the physiological drought [24].

In this present study, root length was reduced up to 28.14 per cent due to salinity. This result is in harmony with previous finding reported that soil salinity suppresses the shoot and root growth [25]. Such decline in shoot and root length in response to salinity stress might be due to either decrease in cell elongation resulting from the inhibiting effect of water shortage in turn, led to a decrease in each of cell turgor, cell volume and eventually cell growth due to blocking up of xylem and phloem vessels [26].

Table 1. Effect of PGRs and nutrients on plant height and Root Length in black gram under salinity

Treatments	Plant height (cm)		Root length (cm)	
	30 DAS	50 DAS	30 DAS	50 DAS
T_1 : Absolute control (Without salinity)	25.4^A	30.3^A	15.5^A	16.7^A
T_2 : Control (Water spray)	15.2^d	21.5^c	11.4^d	12.0^c
T_3 : Seed soaking with jasmonic acid (50 µM)	18.5^c	22.0^c	12.6^c	12.9^b
T_4 : Gibberellic acid (10 ppm)	20.5^a	24.5^a	13.4^b	13.8^a
T_5 : Brassinolide (0.5 ppm)	21.0^a	25.1^a	13.8^a	14.0^a
T_6 : Salicylic acid (100 ppm)	19.6^b	23.4^b	13.6^a	13.7^a
T_7 : Ascorbic acid (100 ppm)	18.6^c	21.2^c	12.2^c	13.5^a
T_8 : Benzyl amino purine (5 ppm)	19.5^b	24.3^a	12.0^d	13.0^b
T_9 : K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%)	18.0^c	23.0^b	11.8^d	12.5^b
T_{10} : TNAU Pulse Wonder (1%)	20.8^a	25.0^a	13.9^a	14.0^a
Mean	19.7	24.0	13.02	13.60
SEd	0.31	0.53	0.21	0.32
CD (P=0.05)	0.66	1.12	0.44	0.66

SEd – Standard Deviation; CD – Critical Difference

Table 2. Effect of PGRs and nutrients on total chlorophyll content and soluble protein content of blackgram under salinity

Treatments	Total chlorophyll (mg g^{-1})		Soluble protein (mg g^{-1})	
	30 DAS	50 DAS	30 DAS	50 DAS
T_1 : Absolute control (Without salinity)	3.03^A	2.53^A	15.42^A	12.50^a
T_2 : Control (Water spray)	2.26^c	1.65^d	10.57^c	9.52^d
T_3 : Seed soaking with jasmonic acid (50 µM)	2.28^c	1.68^d	10.65^c	9.54^d
T_4 : Gibberellic acid (10 ppm)	2.37^c	1.72^d	10.72^c	10.20
T_5 : Brassinolide (0.5 ppm)	2.54^a	2.04^b	12.65^a	12.30^a
T_6 : Salicylic acid (100 ppm)	2.61^a	1.96^c	12.00^a	11.41^b
T_7 : Ascorbic acid (100 ppm)	2.40^b	1.88^c	11.88^b	11.70^b
T_8 : Benzyl amino purine (5 ppm)	2.67^a	2.25^a	11.75^b	10.65^c
T_9 : K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%)	2.38^b	2.00^b	10.90^c	10.45^c
T_{10} : TNAU Pulse Wonder (1%)	2.48^b	2.12^a	12.42^a	12.25^a
Mean	2.50	1.98	11.89	11.05
SEd	0.04	0.04	0.32	0.21
CD (P=0.05)	0.10	0.08	0.67	0.45

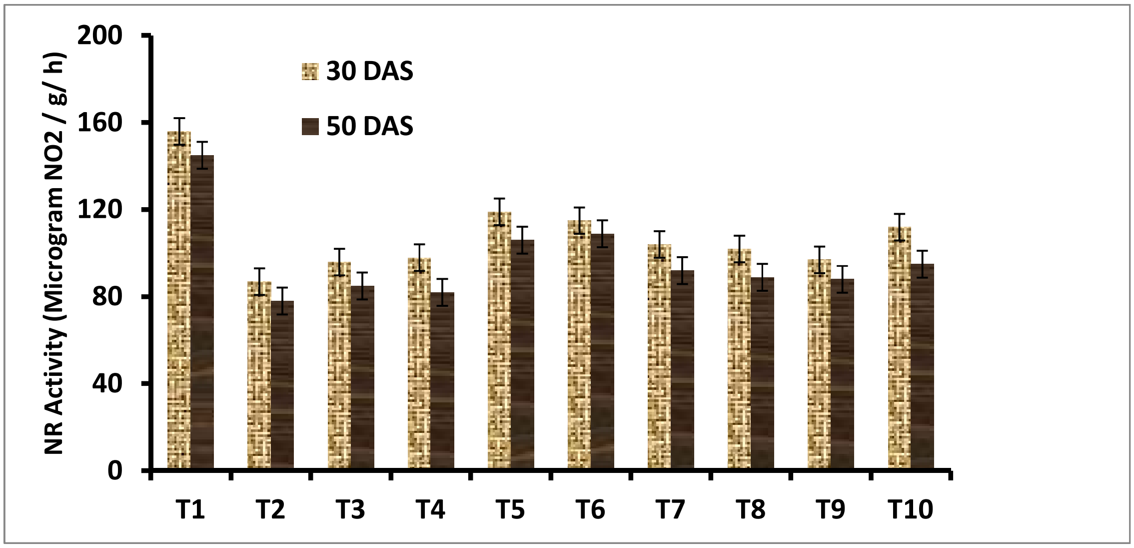

Fig. 1. Effect of PGRs and nutrients on NR activity (µg NO_2 g^{-1} h^{-1}) of blackgram under salinity
T_1 - Absolute control (Without salinity), T_2 - Control (Salinity), T_3 - Jasmonic acid (50 µM), T_4 - Gibberellic acid (10 mM), T_5 – Brassinolide (0.5 ppm), T_6 - Salicylic acid (100 ppm), T_7 - Ascorbic acid (100 ppm), T_8 - Benzyl amino purine (50 ppm), T_9 - K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%), T_{10} - TNAU Pulse Wonder (1%)

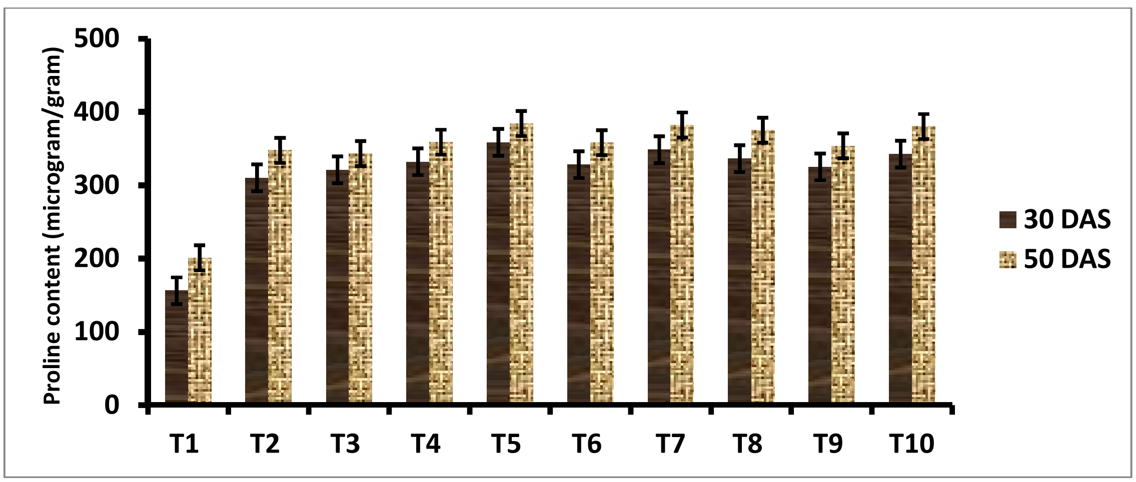

Fig. 2. Effect of PGRs and nutrients on Proline content (µg g^{-1}) of blackgram under salinity
T_1 - Absolute control (Without salinity), T_2 - Control (Salinity), T_3 - Jasmonic acid (50 µM), T_4 - Gibberellic acid (10 mM), T_5 – Brassinolide (0.5 ppm), T_6 - Salicylic acid (100 ppm), T_7 - Ascorbic acid (100 ppm). T_8 - Benzyl amino purine (50 ppm), T_9 - K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%), T_{10} - TNAU Pulse Wonder (1%)

In this present investigation, brassinolide spray was increased the root length up to 16.27 per cent which is on par with TNAU Pulse Wonder compared to control. Brassinolide causes a significant increase in root and shoot length of wheat, showed that brassinolide counteracted the inhibitory effects of salinity [27].

Chlorophyll content can reflect the photosynthetic rate of the plants and it is strongly influenced by environmental factors [28]. In this present study, the chlorophyll content was reduced up to 28.76 per cent due to salinity. Decreased chlorophyll content may attributed to a salt-induced weakening of protein-pigment-lipid complex and due to the suppression of the specific enzyme which is responsible for synthesis of green pigments or increases chlorophyllase enzyme activity which degrades chlorophyll.

In this present investigation, benzyl amino purine recorded increased chlorophyll content up to 31.97 per cent followed by brassinolide (31.70%) compared to control.

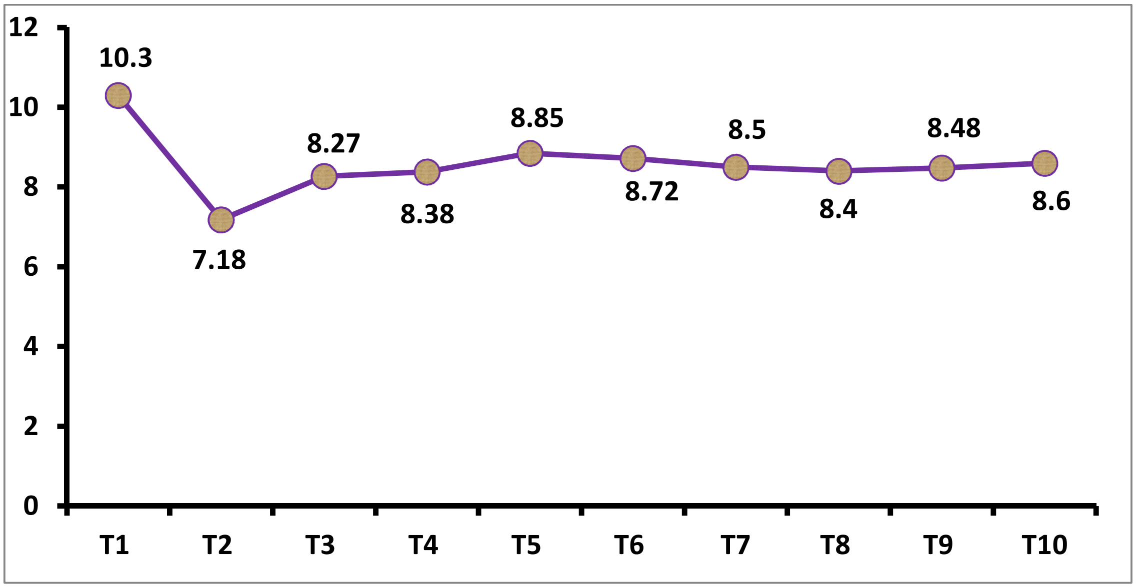

Fig. 3. Effect of PGRs and nutrients on grain yield (g plant^{-1}) of blackgram under salinity

T_1 - Absolute control (Without salinity), T_2 - Control (Salinity), T_3 - Jasmonic acid (50 µM), T_4 - Gibberellic acid (10 mM), T_5- Benzyl amino purine (50 ppm), T_6 - Ascorbic acid (100 ppm), T_7 - Salicylic acid (100 ppm), T_8 - KCl (1%), T_9 - $CaCl_2$ (0.5%)

The enhancement of chlorophyll content by benzyl amino purine might be due to act as anti-senescence hormone which protect the chlorophyll degradation under salinity. Benzyl amino purine has proved to increases the total chlorophyll content in wheat under salt stress condition [29]. The positive effect of brassinolide on chlorophyll protection under salinity might be due to involvement in membrane integrity.

Soluble protein content was reduced up to 31.45 per cent due to salinity. This might be due to breakdown of proteins by proteolytic process and diversion of energy for growth and metabolism to overcome the stress situations. Brassinolide treatment found to most effective to increase the soluble protein content by 19.67 percent followed by TNAU Pulse Wonder (17.50%) compared with control. Rice plants treated with brassinolide showed increased amount of soluble protein content over control [30]. Increase in nitrogen fixation and enhanced soluble protein content in groundnut by exogenous application of brassinosteroids have also been reported [31]. The increment of soluble protein by TNAU Pulse Wonder over control under salinity might be due to increased synthesis of soluble protein through catalytic role as it contain magnesium, which act as cofactor nutrient for rubisco assembly.

Proline is multifunctional amino acid and also a signaling molecule protects the membrane against the harmful effects of high concentration of inorganic ions and high electrical conductivity. The plant growth regulators and nutrients increased the proline content over control which indicates that these phytohormone and nutrients reduces the stress caused by salinity. In this present experiment, brassinolide treatment increased the proline content by 10.54 per cent followed by ascorbic acid (10%) compared to control. The results were supported and the seedlings exposed to NaCl exhibited a significant decline in growth parameters and changes in the levels of antioxidant enzymes, however, treatment with brassinolide showed an improvement in growth, levels of protein and proline content and antioxidant enzyme activity [32]. In addition, the increased content of chlorophylls could result from protection by proline of thylakoid membranes against the attack of ROS [33]. The results are also coincides with that the application of brassinosteroids increases the accumulation of proline and enhances activities of antioxidant enzymes in salt stressed *Cicer arietinum* and *Vigna radiata* [34].

Nitrogen assimilation in plants is regulated by nitrate reductase and its activity plays a constructive role in the nitrogen utilization by the plants through nitrogen metabolism. In this present study, the nitrate reductase activity was reduced (44.23%) significantly by salinity stress in control. In this present experiment, brassinolide treatment increased the NR activity up to 36.78 percent followed by salicylic acid (32.18%) over control. The increase in NR activity due to plant growth regulators could be attributed to increased availability of assimilates and the substrates required for its activity.

Amelioration of salinity was due to enhanced N status and nitrate reductase activity through pre-sowing wheat seeds with plant growth regulators like brassinolide and cytokinin [35]. Foliar spray of salicylic acid protects NR activity and maintains protein and nitrogen content under abiotic stress in wheat seedlings [36].

Reduction in grain yield under salinity might be due to the high salt concentrations in the soil which caused shrinkage of cell contents, damage of membrane, disturbed avoidance mechanism, unbalanced nutrition, reductions in the development and differentiation of the tissues. These entire factors contribute towards the reduction in plant yield.

Grain yield was recorded higher in brassinolide which is on par with TNAU Pulse Wonder (18.48) over control. The possible reason for improvement in yield by TNAU Pulse Wonder might be due to nature of this crop booster with combination of nutrients and growth regulators for pulse resulted in decreased flower shedding and improvement in crop tolerance to abiotic and biotic stress. Foliar spray of brassinolide enhanced the growth, nuuleic acid content and seed yield under salt stress condition in *brassica juncea* [37]. One ppm brassinolide increased significantly the plant height, number of pods per plant, biological yield, seed yield per plant, number of seeds per pod under stress conditions in *Cicer arietinum* L. cultivars [38].

5. CONCLUSION

Salinity is worldwide problem in current situation and it is increasing day by day. Pulse crop like blackgram is highly sensitive to salinity and mostly growing under rainfed condition where salinity is a major crisis. Hence, protecting the crop under salinity is a major task and foliar spray of PGRs and nutrients alleviated the salinity stress effect. In the present study, foliar spray of 0.5 ppm brassinolide at 20 and 40 days after sowing showed its supremacy for alleviation of salinity stress effect followed by 100 ppm salicylic acid and 1 per cent TNAU Pulse Wonder.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Munns R. Genes and salt tolerance: Bringing them together. New Phytology. 2005;167:645-663.
2. Parida AK, Das AB. Salt tolerance and salinity effects on plants: A review. Ecotoxicology and Environmental Safety. 2005;60:324-349.
3. Hasanuzzaman M, Nahar K, Fujita M. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. Springer, New York, NY. 2013;25-87.
4. MoA. Agricultural statistics at a glance. Ministry of Agriculture, Government of India; 2012.
5. Munns R. Comparative physiology of salt and water stress. Plant Cell and Environment. 2002;25: 239-250.
6. Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment. Environmental and Experimental Botany. 2010;68 (1):14-25.
7. Ali B, Hayat S, Ahmad A. 28-homobrassinolide ameliorates the saline stress in *Cicer arietinum* L. Environmental and Experimental Botany. 2007;59:217-223.
8. Vardhini BV, Rao SSR. Effect of brassinosteroids on nodulation and nitrogenase activity in groundnut (*Arachis hypogea* L.). Plant Growth Regulation. 1999;28:165-167.
9. Khan NB, Prithviraj, Smith DL. Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology. 2003;160:485-492.
10. Kaydan D, Yagmur M, Okut N. Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). Tarim Bilimleri Dergisi. 2007;13:114-119.
11. Tuna AL, Kaya C, Dikilitas M, Higgs D. The combined effects 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.

12. Ashraf M, Karim F, Rasul E. Interactive effects of gibberellic acid (GA3) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. Plant Growth Regulation. 2002;36:49 -59.
13. Iqbal N, Ashraf MY, Javed F, Martinez V, Ahmad K. Nitrogen reduction and nutrient accumulation in wheat grown in soil salinization with four different salts. Journal of Plant Nutrition. 2006;29:409-421.
14. Degl'Innocenti E, Hafsi C, Guidi L, Navari-Izzo F. The effect of salinity on photosynthetic activity in potassium-deficient barley species. Journal of Plant Physiology. 2009;166:1968-1981.
15. Arnon DI. Copper enzymes in isolated chloroplasts- polyphenol oxidase in Beta vulgaris. Plant Physiology. 1949;24(1):1-15.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 1950;193(1):265-275.
17. Bates LS, Waldren RP, Teare ID. Rapid determination of proline for water stress studies. Plant Soil. 1973;39:205-207.
18. Nicholas JC, Harper JE, Hageman RH. Nitrate reductase activity in soybeans. Effect of light and temperature. Plant Physiology. 1976;58:731-735.
19. Gomez KA, Gomez AA. Statistical procedures for agricultural research. (2nd Ed.) John Wiley and sons, New York, USA; 1984.
20. Hussain TM, Chandrasekhar T, Hazara M, Sultan Z, Saleh BZ, Gopal GR. Recent advances in salt stress biology – a review. Biotechnology and Molecular Biology. 2008;3:8-13.
21. Sairam RK, Srivastava GC, Agarwal S, Meena RC. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biologia Plantarum. 2005;49:85–89.
22. Eleiwa ME, Bafeel SO, Ibrahim SA. Influence of brassinosteroids on wheat plant (*Triticum aestivum* L.) production under salinity stress conditions. I. Growth parameters and photosynthetic pigments. Australian Journal of Basic and Applied Sciences. 2011;5:58-651.
23. Raaza SH, Athar HR, Ashraf M. Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. Pakistan Journal of Botany. 2006;38:341–351.
24. Jamil M, Rehman S, Lee KJ, Kim JM, Kim HS, Rha, ES. Salinity reduced growth PSII photochemistry and chlorophyll content in radish. Scientia Agriculturae. 2007;64:111-118.
25. Mehrafarin A, Badi HN, Noormohammadi G, Zand E, Rezazadeh S, Qaderi A. Effects of environmental factors and methanol on germination and emergence of Persian Fenugreek (*Trigonella foenum-graecum* L.). African Journal of Agricultural Research. 2011;6:4631-4641.
26. Banon SJ, Ochoa J, Franco JA, Alarcon JJ, Sanchez-Blanco MJ. Hardening of oleander seedlings by deficit irrigation and low air humidity. Environmental and Experimental Botany. 2006;56:36-43.
27. Soad S, AboHamad A. Effect of exogenous application of brassinolide on growth and metabolic activity of wheat seedlings under normal and salt stress conditions. Annual Research and Review in Biology. 2014;4(24):3687-3698.
28. Qiu D, Lin P, Guo SJ. Effects of salinity on leaf characteristics and CO2/H2O exchange of *Kandelia candel* (L.) Druce seedlings. Journal of Forestry Science. 2007;53:13-19.
29. Bagdi DL, Afria BS. Alleviation of salinity effects using plant growth regulators in wheat. Indian Journal of Plant Physiology. 2008;13(3):272-277.
30. Tania D, Shukla YM. Effect of brassinolide on biochemical constituents in rice (*Oryza sativa* L.) under salinity stress. Asian Journal of Experimental Chemistry. 2011; 6(1):22-25.
31. Vardhini BV, Rao SSR. Effect of brassinosteroids on nodulation and nitrogenase activity in groundnut (*Arachis hypogea* L.). Plant Growth Regulation. 1999;28:165-167.
32. Sharma P, Sardana V, Singh SK. Dry matter partitioning and source–sink relationship as influenced by foliar sprays in groundnut. Bioscan. 2013;8:1171–1176.
33. Kavi-Kishor PB, Gandonou CB, Srikant S. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science. 2005;88:424-438.
34. Hayat S, Hasan SA,Yusuf S, Hayat M, Ahmad A. Effect of 28-homobrassinolide

on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiata*. Environmental and Experimental Botany. 2010;69:105-112.
35. Angrish R, Kumar B, Datta KS. Effect of gibberellic acid and kinetin on nitrogen content and nitrate reductase activity in wheat under saline conditions. Indian Journal of Plant Physiology. 2001;6:172-177.
36. Bhupinder S, Usha K. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regulation. 2003;39: 137-141.
37. Hayat S, Ali B, Ahamad A. Response of *Brassica juncea*, to 28-homobrassinolide, grown from the seeds exposed to salt stress. Journal of Plant Biology. 2006;33: 169-174.
38. Jyoti Verma K, Kakralya BL, Jakhar ML. Effect of brassinolide on physiological aspects of chick pea under drought conditions. Journal of Plant Science. 2012; 28:151-155.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21130