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PLANT PHYSIOLOGY

Impact of Plant Growth Regulators and Nutrient Consortium on Growth, Superoxide Dismutase, Na^+/K^+ ratio and Yield of Blackgram under Salinity Stress

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ABSTRACT

An experiment was conducted to study the effect of plant growth regulators like jasmonic acid (50 μM), brassinolide (0.5 ppm), salicylic acid (100 ppm), ascorbic acid (100 ppm), gibberellic acid (10 ppm), benzyl amino purine (5 ppm) and nutrient consortium (K_2SO_4 - 0.5% + $FeSO_4$ - 0.5%) + Borax - 0.3%) and 1% TNAU pulse wonder on blackgram (*Vigna mungo* L.) variety TNAU Blackgram CO 6 under salinity condition. The salinity was imposed by 125 mM NaCl concentration in pot culture. Plant growth regulators and nutrient consortium were used as foliar spray at 20 and 40 days after sowing except jasmonic acid, which used as seed soaking. Under salinity stress, root volume, leaf area, specific leaf weight, net assimilation rate and yield were significantly reduced compared to normal condition. Among the treatments, brassinolide showed its supremacy on higher leaf area (149.13 cm^2 $plant^{-1}$), specific leaf weight (0.41 mg cm^{-2}) and super oxide dismutase activity (325 Units mg^{-1} protein) followed by salicylic acid and TNAU pulse wonder. However highest root volume of 3.5 cc was recorded by TNAU pulse wonder. Salicylic acid registered highest net assimilation rate of 0.398 mg cm^{-2} d^{-1} followed by brassinolide (0.396 mg cm^{-2} d^{-1}). The minimum Na^+/K^+ ratio (0.325) was observed in brassinolide followed by salicylic acid (0.355) which is on par with nutrient mixture (0.357) and TNAU pulse wonder (0.362). Among the plant growth regulators and nutrients, brassinolide recorded the maximum grain yield of 8.85 g $plant^{-1}$ followed the salicylic acid (8.72 g) which is on par with TNAU Pulse Wonder (8.60 g).

Highlights

- Root volume and leaf area were reduced up to 46.8% and 14.07% respectively by salinity in blackgram
- Foliar application of 0.5 ppm brassinolide significantly increased the SOD activity by 13.6% compared to control.
- Exogenous application of plant growth regulators and nutrients reduces the sodium potassium ratio in blackgram under salt stress condition.
- Higher grain yield was recorded by foliar spray of 0.5 ppm brassinolide treatment which is on par with salicylic acid and TNAU pulse wonder over control

Keywords: Brassinolide, TNAU pulse wonder, blackgram, SOD, leaf area, SLW, Na^+/K^+, Yield

Diverse environmental factors affect the plant growth, development and biological yield of the crop. These factors include salinity, drought and high temperature which limit the crop productivity worldwide. Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation may be insufficient for leaching of salts (Zhao *et al*. 2007).

Sairam and Tyagi (2004) reported that the salinity stress has a major impact on plant growth and development. It affects seed germination, seedling vigour and vegetative growth that ultimately cause poor plant establishment. The harmful effects of salinity on plant growth are associated with low osmotic potential of soil solution, specific ion effect, nutritional and hormonal imbalance, induction of oxidative stress and combination of these factors (Rahnama *et al*. 2010). Ashraf and Harris (2004) suggested that the plant biomass production depends on the accumulation of photo-assimilates through photosynthesis, which adversely affected by salinity.

Blackgram is one of the important pulse crops which is short duration and well suited in all seasons either as sole or as intercrop or fallow crop. It has high nutritive value and consist high protein, vitamins and minerals. Munns (2002) found that the blackgram is very sensitive to salinity in early stage of growth which causes reduction in growth rate as well as plant metabolic processes and yield.

Plant growth regulators (PGRs) and nutrients have been used to promote plant growth and development of plants under various abiotic stress conditions. Brassinosteroids are a class of polyhydroxy steroidal lactones which are amelioration for the salt stress in different plant species (Ali *et al*. 2007). Kamlesh *et al.* (2017) registered that foliar spray treatment with 1.0 ppm brassinolide significantly increased grain yield and biological yield under saline condition.

The activities of antioxidant enzymes *viz.*, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were up-regulated by NaCl stress and these enzymes activities were further enhanced by foliar spray of 1.5 mM salicylic acid in *Nitraria tangutorum* which indicate that salicylic acid effectively alleviated the adverse effects of NaCl stress (Liu *et al.* 2016). Zeinab *et al.* (2014) registered that the application of ascorbic acid not only mitigated the inhibitory effect of salt stress in kidney bean, but also in some cases induced a stimulatory effect greater than that estimated in the control plants on growth parameters. The greatest value of plant dry weight was obtained by the foliar spray of cytokinin at pollination and grain filling stages in wheat. The results showed that cytokinin foliar spray had the greatest positive effect on seed germination, plant growth and development under saline conditions (Esmail 2014).

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 of salt on photosynthesis in barley and was accompanied by an increase in salt sensitivity (Degl'Innocenti *et al*. 2009). TNAU Pulse Wonder is a nutrient and PGR consortium developed by Tamil Nadu Agricultural University, Coimbatore which contains essential nutrients and PGRs required for pulse crops like greengram and blackgram. Hence, the experiment was conducted to study the performance of different plant growth regulators and nutrient consortium on root volume, leaf area, SLW, NAR, SOD enzyme activity, Na^+/K^+ ratio and yield of blackgram under salinity condition.

MATERIALS AND METHODS

A pot culture experiment was conducted in glass house at Department of Crop Physiology, TNAU, Coimbatore in blackgram. Red sandy soil mixture (red soil, sand and vermicompost in the ratio of 2:1:1 respectively) was used for pot culture experiment. Blackgram is a direct seed sown crop, hence seeds were sown in a pot then the salt solution was poured on the soil. 125 mM NaCl saline water was used in this experiment and it 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 per hectare) 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 ten treatments and three replications.

Plant growth regulators like gibberellic acid (10 ppm), brassinolide (0.5 ppm), salicylic acid (100 ppm), ascorbic acid (100 ppm), benzyl amino purine (5 ppm), nutrient consortium (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. In this study, 50 μM jasmonic acid was used as seed soaking PGR which was already standardized under *in-vitro* condition by assessing seed germination and seedling characters. The seeds were soaked in jasmonic acid solution for four hours and then shade dried. The observations like root volume, leaf area, SLW, NAR, SOD activity and Na^+/K^+ were recorded at 30 and 50 DAS. The yield parameters were estimated after harvest.

Measurement of growth and growth analytical parameters

Root volume

The root volume was measured by water displacement method. Individual plant roots were immersed in known volume of water and the amount of water displaced was measured and expressed in cubic centimeter.

Leaf area

Leaf area per plant was measured using a Leaf Area Meter (LICOR, Model LI 3000) and expressed as cm^2 $plant^{-1}$.

Specific Leaf Weight (SLW)

Specific leaf weight was calculated by using the formula of Pearce *et al.* (1968) and expressed in mg cm^{-2}.

$$SLW = \frac{\text{Leaf dry weight per plant}}{\text{Leaf area per plant}}$$

Net Assimilation Rate (NAR)

It is the rate of increase of total dry weight per unit leaf area per unit time. It was calculated by using following formula given by Williams (1946) and expressed as mg cm^{-2} day^{-1}.

$$NAR = \frac{Log_e L_2 - Log_e L_1}{L_2 - L_1} \times \frac{W_2 - W_1}{t_2 - t_1}$$

Where, $Log_e L_2$ - Natural log of leaf area at time t_2; $Log_e L_1$ - Natural log of leaf area at time t_1;

L_2 & L_1 - Leaf area at t_2 and t_1 respectively; W_2 & W_1 - Plant dry weight at t_2 and t_1 respectively.

Estimation of superoxide dismutase (SOD) activity

SOD activity was determined by nitro blue tetrazolium (NBT) salt as described by Beau-Champ and Fridovich (1971) and expressed in enzyme Units mg^{-1} protein. 500 mg of leaf sample was weighed and macerated with 10 ml HEPES-KOH buffer containing 0.1 mM EDTA. The content was centrifuged at 15000 ppm for 15 minutes and collected supernatant was made up to 50 ml. 1 ml of enzyme extract was taken and mixed with 3 ml of reaction mixture. One unit SOD activity was defined as the amount of enzyme required to result a 50 per cent inhibition of the rate of NBT reduction at 560 nm.

Estimation of sodium potassium ratio

Leaf sodium and potassium content of digested solution was estimated by using atomic absorption spectrometer and the ratio was worked out.

Estimation of yield

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.

Statistical analysis

The statistical analysis was done as per design of the experiment as suggested by Panse and Sukhatme (1987) and Ranganna (1995). The critical difference (CD) was worked out at five per cent probability level for significant treatments.

RESULTS AND DISCUSSION

Root volume and root length can be considered as an important trait for determining tolerant capacity of crops under saline condition. Root volume was reduced up to 46.8 per cent under salt stressed condition over absolute control. This result was supported by Azooz (2009), who stated that salinity can affects the growth parameters like root and shoot length. Decreased root system under salinity condition can be attributed to the decreased absorption of water from the soil. Root volume revealed that there was a significant difference in all the treatments. Higher root volume was observed in absolute control (4.5 cc and 4.7 cc) whereas in control recorded 2.4 cc and 2.5 cc at 30 DAS and 50 DAS respectively (Table 1).

Among the plant growth regulators and nutrients used, TNAU Pulse Wonder recorded the uppermost root volume of 3.5 cc followed by gibberellic acid (3.4 cc) and brassinolide (3.3 cc) which is on par with ascorbic acid (3.2 cc) and nutrient mixture (3.1 cc). Plant growth regulators and nutrient treatments in many vegetable crops have improved the physiological activities and root proliferation especially under stress condition (Halmer 2004).

Table 1: Effect of PGRs and nutrient consortium on root volume (cc) and leaf area (cm^2) in blackgram under salinity

Treatments	Root volume (cc)		Leaf area (cm^2)	
	30 DAS	50 DAS	30 DAS	50 DAS
Absolute control (Without salinity)	4.5	4.7	168.49	152.00
Control (Water spray)	2.4	2.5	121.98	130.61
Seed soaking with jasmonic acid (50 μM)	2.7	2.8	132.72	135.13
Gibberellic acid (10 ppm)	2.9	3.4	138.15	136.61
Brassinolide (0.5 ppm)	3.2	3.3	141.41	149.13
Salicylic acid (100 ppm)	2.6	3.2	138.66	142.98
Ascorbic acid (100 ppm)	3.1	3.2	123.33	132.85
Benzyl amino purine (5 ppm)	2.8	3.0	137.02	140.51
K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%)	3.0	3.1	127.37	128.70
TNAU Pulse Wonder (1%)	3.4	3.5	139.52	137.66
Mean	3.07	3.27	136.86	138.61
SEd	0.08	0.07	3.29	3.11
CD (P=0.05)	0.17	0.16	6.86	6.50

Among the treatments, TNAU Pulse Wonder increases the root volume up to 40 per cent followed by gibberellic acid (36%) over control. Positive effect of GA_3 on root volume under salinity might be due to the involvement in cell elongation. Significant effect of GA_3 on increased the root length and dry weight under salinity was observed by Patel and Saxena (1994). This statement was supported by Naeem and Muhammud (2006), who reported that seed priming with low concentrations of GA_3 could increase root length, root volume, dry weight, fresh weight and tissue water content.

The index of photosynthesizing surface is represented by leaf area which is always subjected to boundaries by the salinity stress. The leaf area is an important component that is closely related to physiological processes controlling the dry matter production and yield and it's significantly reduced under saline conditions. Leaf area was reduced up to 27.60 per cent by salinity compared to absolute control. This reduction in leaf area was attributed by negative influence of salinity on cell division, cell enlargement and accelerated leaf senescence. Also the reduction in leaf area due to salt stress may be resulted from nutritional imbalance due to interference of salt ions, such as Na^+ and Cl^- with K^+ involved in both uptake and translocation processes (Errabi *et al.* 2007). Highest leaf area of 168.49 cm^2 $plant^{-1}$ was recorded in absolute control and lowest leaf area (121.98 cm^2 $plant^{-1}$) in control at 30 DAS. Among the ameliorants, brassinolide registered higher leaf area (149.13 cm^2 $plant^{-1}$) which is on par with salicylic acid (142.98 cm^2 $plant^{-1}$) followed by benzyl amino purine (140.51 cm^2 $plant^{-1}$) and TNAU Pulse Wonder (137.66 cm^2 $plant^{-1}$) compared to control (130.61 cm^2 $plant^{-1}$) at 50 DAS.

Mostafa and Abou Al-Hamd (2011) reported that application of plant growth regulators enhanced leaf area, leaf weight, plant growth and yield by improving the plants tolerance to salinity injury. In this study, brassinolide treatment increased the leaf area up to 15.92 per cent over control. This result was supported by Fatima *et al.* (2000) who reported that, application of brassinolide increases the leaf area, leaf weight and number of leaf in *Vigna mungo* under salt stress condition.

Specific leaf weight is an excellent parameter to assess the translocation efficiency of crop plants. In the present study specific leaf weight was reduced up to 21.42 per cent due to salinity. The reduction in specific leaf weight under salinity might be due to less production of photo assimilates by reduced photosynthesis due to lower leaf area. However, the proportion of reduction of leaf area under salinity is lower than leaf weight. The maximum specific leaf weight was recorded in absolute control (0.42 mg cm^{-2}) and the minimum in control (0.33 mg cm^{-2}) at 50 DAS. Among the PGRs and nutrients used, maximum specific leaf weight was observed in brassinolide (0.41 mg cm^{-2}) which is on par with ascorbic acid (0.40 mg cm^{-2}), followed by TNAU Pulse Wonder (0.39 mg cm^{-2}). In this present investigation, foliar application of brassinolide treatment increased the specific leaf weight up to 24.24 per cent followed by ascorbic acid (21.21). This is might be due to enhancement of photosynthesis through increases the activity of rubisco leads to increased photo assimilates. This result was supported by Gograj Jat *et al*. (2012) who reported that use of brassinolide up to 1.0 ppm was observed to increase significantly leaf area, specific leaf weight, and photosynthetic rate.

Net assimilation rate indicates the assimilatory capacity of the leaves for biomass production per unit time. Salinity stress causes the reduction in NAR. This reduction might be due to occurrence of salinity stress reduces the photosynthesis of the leaves and thus reduces net assimilation rate. In this experiment, salt stress caused 17.23 per cent reduction in net assimilation rate compared to absolute control. The most prominent net assimilation rate was recorded in absolute control (0.412 mg cm^{-2} d^{-1}) and the least net assimilation rate was recorded in control (0.341 mg cm^{-2} d^{-1}).

Among the plant growth regulators and nutrients used, the maximum net assimilation rate was recorded in the salicylic acid (0.398 mg cm^{-2} d^{-1}) which is on par with brassinolide (0.396 mg cm^{-2} d^{-1}) followed by TNAU Pulse Wonder (0.388 mg cm^{-2} d^{-1}). Among the treatments, salicylic acid increased the net assimilation rate up to 16.72 per cent followed by brassinolide (16.13%) over control. Meena (2011) observed that brassinolide significantly increased photosynthetic rate, transpiration rate, stomatal conductance and net assimilation rate whereas significant decrease in osmotic potential was recorded in the wheat under saline conditions.

Among the PGRs and nutrients used, maximum SOD activity was registered in brassinolide (325) which is on par with ascorbic acid (323) followed by salicylic acid (318) and TNAU Pulse Wonder (310). The statistical analysis also exhibited significant differences among all the treatments with respect to control. Salt stress resulted in a considerable increase in the activity of SOD in blackgram plants. The increase in SOD activity in leaves of salt-stressed blackgram might be due to the activation of pre-existing SOD or due to synthesis of new SOD under salt stress conditions. These results are in accordance with Sairam *et al.* (2002) who showed that total SOD activity is increased in salt-stressed wheat plants.In this experiment, brassinolide caused high significant increase in the activity of SOD (13.64%) of stressed plants as compared with their respective control (Fig. 1). These results are in agreement with Arora *et al.* (2008) who found that the level of SOD is increased by the application of brassinolide to overcome the stress generated by NaCl and to boost the tolerance capacity of plants. The higher level of these enzymes suggests a possible role of brassinolide in amelioration of oxidative stress

Table 2: Effect of PGRs and nutrient consortium on SLW (mg cm^{-2}) and NAR (mg cm^{-2} d^{-1}) in blackgram under salinity

Treatments	SLW (mg cm^{-2})		NAR (mg cm^{-2} d^{-1})	Grain yield (g plant^{-1})
	30 DAS	50 DAS		
Absolute control (Without salinity)	0.39	0.42	0.412	10.30
Control (Water spray)	0.28	0.33	0.341	7.18
Seed soaking with jasmonic acid (50 μM)	0.30	0.35	0.367	8.27
Gibberellic acid (10 ppm)	0.34	0.38	0.366	8.38
Brassinolide (0.5 ppm)	0.38	0.41	0.396	8.85
Salicylic acid (100 ppm)	0.31	0.36	0.398	8.72
Ascorbic acid (100 ppm)	0.36	0.40	0.354	8.50
Benzyl amino purine (5 ppm)	0.32	0.37	0.369	8.40
K_2SO_4 (0.5%) + $FeSO_4$ (0.5%) + Borax (0.3%)	0.29	0.34	0.362	8.48
TNAU Pulse Wonder (1%)	0.37	0.39	0.388	8.60
Mean	0.33	0.37	0.37	8.57
SEd	0.01	0.01	0.01	0.17
CD (P=0.05)	0.03	0.02	0.02	0.26

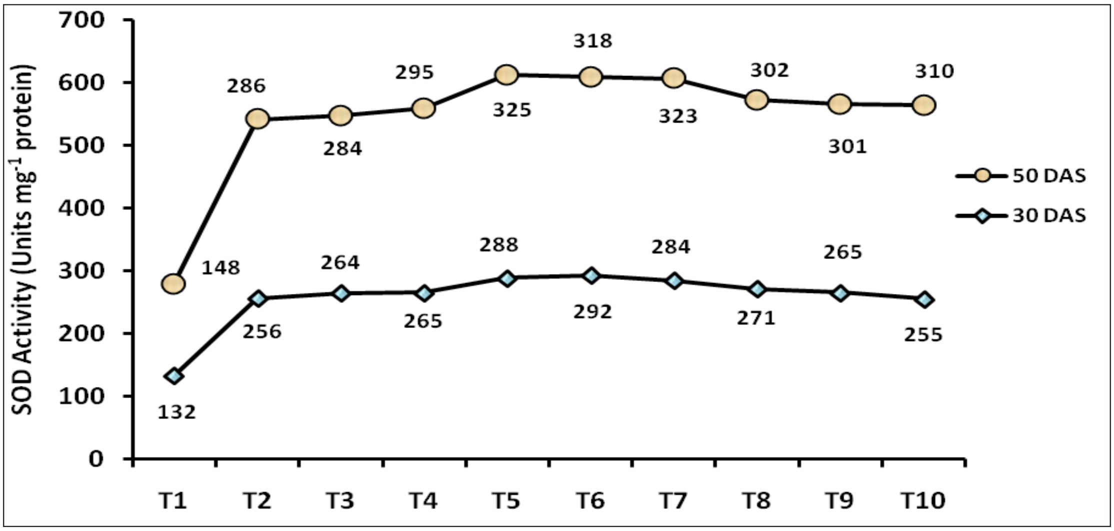

Fig. 1: Effect of PGRs and nutrient consortium on SOD activity in blackgram under salinity

Treatment details

T_1 - Absolute control, T_2 - Control, T_3 - Seed soaking with Jasmonic acid, T_4 - Gibberellic acid, T_5 - Brassinolide, T_6 - Salicylic acid, T_7 - Ascorbic acid, T_8 - Benzyl amino purine, T_9 - K_2SO_4 + $FeSO_4$ + Borax, T_{10} - TNAU Pulse Wonder.

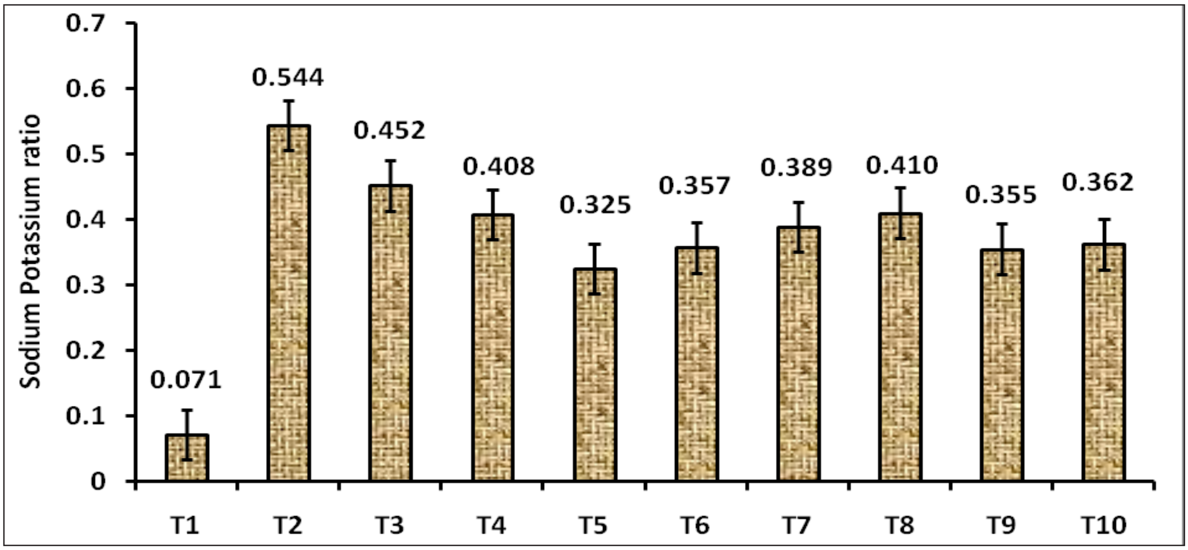

Fig. 2: Effect of PGRs and nutrient consortium on Na^+/K^+ ratio in blackgram under salinity

Treatment details

T_1 - Absolute control, T_2 - Control, T_3 - Seed soaking with Jasmonic acid, T_4 - Gibberellic acid, T_5 - Brassinolide, T_6 - Salicylic acid, T_7 - Ascorbic acid, T_8 - Benzyl amino purine, T_9 - K_2SO_4 + $FeSO_4$ + Borax, T_{10} - TNAU Pulse Wonder.

generated by salt stress. Brassinolide may confer tolerance to salt stress by increasing the activities of antioxidative enzymes. Similar conclusion was offered by Ozdemir *et al.* (2004) in rice and Arora *et al.* (2008) in maize. Sodium/potassium ratio was high in plants when subjected to salt stress. Sivritepe *et al.* (2003) found that NaCl salinity increased Na content in plant tissue of crops. Essa (2002) reported that NaCl salinity may produce extreme ratios of Na/Ca and Na/K in the plants, causing them to be susceptible to osmotic and specific-ion injury, as well as to nutritional disorders.

But application of plant growth regulators and nutrients reduces the sodium potassium ratio in salt stress condition (Fig. 2). The maximum reduction percentage was observed in brassinolide (40.25%) followed by nutrient mixture (34.74%) and salicylic acid (34.75%). The treatments with brassinolide and salicylic acid also showed their efficacy in decreasing Na/K ratio. El-Tayeb (2005) found that exogenous salicylic acid applications inhibited Na accumulation, but stimulated N, P, K, Mg, Fe, Mn and Cu uptake in barley plants. Comparing the treatments, absolute control recorded the highest grain yield of 10.30 g and control recorded the least value of 7.18 gram (Table 2). Significant difference was recorded with treatments with respects to grain yield under saline condition.

Among the PGRs and nutrients, brassinolide (8.85 g) recorded the maximum grain yield followed the salicylic acid (8.72 g) which is on par with TNAU Pulse Wonder (8.60 g). Foliar spraying of plant growth regulators and nutrients might have exploited favorably for indeterminate crop for continuous translocation of photosynthates to economic part and ultimately yield increment. The production of higher seed yield due to PGRs may be attributed to the fact that plants treated with growth regulators remained physiologically more active to build up sufficient food reserves for developing flowers and seeds.

Bera *et al*. (2008) found that the foliar application of brassinolide and salicylic acid could be beneficial for improving yield and nutritional quality of green gram. Jeyakumar *et al*. (2008) reported that application of 125 ppm salicylic acid to blackgram plants increased seed yield. One ppm brassinolide significantly increased the plant height, number of pods per plant, biological yield, seed yield per plant, number of seeds per pod stress conditions in *Cicer arietinum* L. cultivars namely RSG888 and RSG895 (Jyoti Verma *et al*. 2012). With respect to TNAU Pulse Wonder, the possible reason for improvement in yield 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.

CONCLUSION

Foliar application of 1% TNAU Pulse Wonder at 20 and 40 days after sowing increased the root volume up to 40% in black gram under salinity condition followed by 0.5 ppm brassinolide (32%). The leaf area increased up to 14.2% by the application of brassinolide followed by salicylic acid (9.5%). Foliar application of brassinolide increased the SLW up to 24% and salicylic acid increased the NAR up to 16.7% under salinity condition. The lowest Na/K ratio of 0.325 and highest SOD activity (13.6% increased over control) were registered by brassinolide followed by 100 ppm salicylic acid (0.357 & 11% respectively). Foliar application of 0.5 ppm brassinolide increased the grain yield up to 23% in black gram under salinity condition followed by 100 ppm salicylic acid (21.4%) over control.

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