Effect of Plant Growth Regulators on Chlorophyll Content and Relative Water Content of Green Gram under Salinity

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Received:- 08 October 2022/ Revised:- 15 October 2022/ Accepted:- 21 October 2022/ Published: 31-10-2022
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Abstract— *An investigation was carried out in green gram CO8. The objective of the experiment was standardization of NaCl to study the effects of salinity during seed germination of green gram and to study the response of green gram treated with plant growth regulators to salinity. In laboratory study, the standardization of NaCl was done first by using 75mM, 100mM, 150mM and 200mM NaCl. Among the four concentrations, 50% germination was observed in 150mM NaCl and it was standardized for further experiment. The plant growth regulators used were T3: NAA 100ppm, T4: NAA 200ppm, T5: kinetin 50ppm, T6: kinetin 100ppm, T7: GA₃ 100ppm, T8: GA₃ 200ppm along with T1: Absolute control and T2: Control (150mM NaCl). The experimental results showed a decrease in seedling growth due to salinity but with the seed treatment with the above mentioned PGRs showed an increased stress tolerance index, chlorophyll content and relative water content. The seeds treated with GA₃ 200ppm recorded the maximum stress tolerance index (STI) (83.09) as well as higher relative water content (88.44%). While the seeds treated with kinetin 100ppm recorded the maximum chlorophyll content (1.22 gm/g). The whole study revealed that, in laboratory condition, with the imposition of salinity stress by 150mM NaCl, the seed treatment with GA₃ 200ppm responded better compared to other treatments.*

Keywords— Greengram, PGR, NaCl, Stress tolerance index, Chlorophyll content, Relative water content.

I. INTRODUCTION

Green gram (*Vigna radiata*), also called Mungbean is a pulse crop from botanical family of Fabaceae. It is a warm season, frost-intolerant plant and suitable for being planted in temperate, sub-tropical and tropical regions. The most suitable temperature for mung bean's germination and growth is 15-18 °C. It has high adaptability to various soil types, while the best pH of the soil is between 6.2 and 7.2. Since, it is a short-day plant, long day condition will delay its flowering and podding.

Salinity is one of the most important abiotic stress factors limiting plant growth and productivity (Flowers, 2004). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, induce water deficit, cause ionic imbalance of the cellular ions resulting in ion toxicity and osmotic stress (Khan and Panda, 2009). Specific effects of salt stress on plant metabolism have been related to the accumulation of toxic Na+ and Cl- ions or to K+ and Ca2+ ions depletion (Sreenivasulu *et al.*, 2000). As a consequence of ion imbalance and hyperosmotic stress, which are primary effects of salt stress, secondary stress such as oxidative damage may occur (Rahman *et al.*, 2018). Plant hormones, also known as phytohormones, are small chemical messenger that are produced within the plant at extremely low concentrations and plays a crucial role in plant growth and development by co-ordinating their cellular activities. It control all aspects of plant growth and development, from embryogenesis, regulation of organ size, pathogen defence, stress tolerance and up-to reproductive development. Aziz Khan *et al*., (2009) concluded that phytohormones are known to play vital roles in the ability of plants to acclimatize to varying environments, by mediating growth, development, source/sink transitions and nutrient allocation.

Auxin plays an important role in cell elongation in the shoot, apical dominance, root initiation, prevention of abscission, induction of parthenocarpy, stimulation of respiration, activate cell division and induce callus formation, induce vascular differentiation in plants. NAA is a synthetic plant hormone in the auxin family and is an integral component in many commercial plant rooting horticultural products. It is a rooting agent and used for the vegetative propagation of plants from

stem and leaf cuttings. It is also used for plant tissue culture. Kinetin is a cytokinin derivative which promotes cell division and plant growth. It has been shown to naturally exist in DNA of organisms including humans and various plants. While kinetin is used in tissue cultures to produce new plants, it is also found in cosmetic products as an anti-aging agent. Gibberllic acid, a plant hormone stimulating plant growth and development is a tetracyclic di-terpenoid compound. GAs stimulate seed germination, trigger transitions from meristem to shoot growth, juvenile to adult leaf stage, vegetative to flowering, determines sex expression and grain development along with an interaction of different environmental factors viz., light, temperature and water (Sivakumar R *et al.*, 2018).

II. MATERIALS AND METHODS:

The present investigation was carried out to evaluate the effect of plant growth regulating chemicals in alleviating the effect of salinity in Green gram CO8. The effect of salinity was evaluated by seed soaking method of plant growth regulators. The research trail was carried out as a laboratory study at Crop Physiology Lab, Department of Crop Management, Thanthai Roever Institute of Agriculture and Rural Development, Perambalur. The experiment was laid out under completely randomized block design with eight treatments and three replications. The seeds of Greengram CO8 were placed in petriplates. The petridishes for the experiment were sterilized using 0.01 per cent HgCl2 and 70 per cent ethanol and finally repeated washing with distilled water. The salinity was imposed through using NaCl at different concentrations viz., 75mM, 100mM, 150mM and 200mM. Among these concentrations of NaCl used, there was no seed germination in 200mM. In 75mM and 100mM, almost all the seeds were germinated along with control. However, only 50 per cent of the seeds were germinated in 150mM concentration of NaCl compared to control. Hence, NaCl 150mM was standardized to carry out the experiment to evaluate the effect of plant growth regulators in mitigation of salinity stress effect in greengram. Seeds were soaked in T3: NAA 100ppm, T4: NAA 200ppm, T5: kinetin 50ppm, T6: kinetin 100ppm, T7: GA3 100ppm and T8: GA3 200ppm. After that, seeds were dried up under shade for four hours. These treated seeds were later placed on germination sheet in each petriplates separately, untreated seeds in control and absolute control. The germination paper was moistened at regular intervals with NaCl 150mM solution for salinity and water for absolute control. The petriplates were kept in laboratory under room temperature. The seeds were allowed to germinate by pouring the NaCl 150mM solution of approximately ten ml each once in three days. Distilled water was used for maintaining the absolute control. Stress tolerance index of the seeds was calculated using the following formula proposed by Dhopte and Livera-Muñoz (1989). The contents of chlorophyll 'a', 'b' and total chlorophyll were estimated by adopting the procedure of Arnon (1949) and the content was expressed as mgg-1 of fresh weight. The relative water content (RWC) was estimated according to Barrs and Weatherley (1962) and calculated by using following formula and expressed as per cent.

III. RESULT AND DISCUSSION

The results showed the significant differences among the treatments. The highest stress tolerance index was noticed in GA3 200 ppm (T8) treatment (83.09 %) followed by GA3 100 ppm (T7) (79.54, the lowest stress tolerance index was recorded by NAA 200 ppm (T4) (24.31%). Stress tolerance index (STI) indicates the tolerant potential of the plants during stress. In this present investigation GA3 200ppm (T8) noticed up to (70%). High stress tolerance index compared to control followed by GA3 100ppm (T7) (6.8%) **(Table 1)**. This increment may be due to GA3 200ppm (T8) induced germination, vigour index, shoot and root length under saline environment.

TABLE 1
EFFECT OF PGRS ON STRESS TOLERANCE INDEX (%) OF GREEN GRAM UNDER SALINITY

S.No	Treatments	Stress tolerance index (%)
1	T2: Control (Salinity)	15.22
2	T3:NAA 100ppm	31.31
3	T4:NAA 200ppm	24.31
4	T5:Kinetin 50 ppm	28.95
5	T6:Kinetin 100ppm	38.54
6	T7:GA3 100 ppm	79.54
7	T8:GA3 200ppm	83.09
Mean		42.99
SEd		2.70
CD (P=0.05)		5.78

The chlorophyll content was reduced by the salinity compared to control. Significant difference was noticed in all the treatments with respect with respect to chlorophyll extent. GAʒ 200ppm (T8) was obtained the highest chlorophyll content (1.090 mg\g). Seed treatment with PGRs showed a significant increment in chlorophyll content. Among the PGRs, highest chlorophyll content was obtained in seed treatments with GAʒ 200ppm (T8) (1.09 mg\g) followed by kinetin 100ppm (T6) (1.22mg\g) and GAʒ 100ppm (T7) (0.76 mg\g). However the lowest chlorophyll content was observed in the treatment NAA 100 ppm (T3) (0.43mg\g) followed by NAA 200 ppm (T4) (0.69 mg\g) **(Figure 1)**. Chlorophyll content of the absolute control (T1) is (0.90mg\g). A Decrease in photosynthesis pigment content of green gram plants under salt stress was observed. There was a decrease of 90% of Chlorophyll a in response to the 150 mM NaCl treatments. When respectively compared to the control. In the case Chlorophyll b decrease was 93% in response to 150mM NaCl treatments respectivety, compared to control total Chlorophyll was reduces by 92% under high salinity. The reduction in leaf Chlorophyll content under Nacl stress has been attributed to the destruction of Chlorophyll pigments. In the present investigation kinetin 100ppm (T6) is high Chlorophyll content 92% compared to control. Kinetin improves the Chlorophyll content similar response Cengiz Kaya et al. (2010) start that foliar application kinetin improved the Chlorophyll level in salinity stressed.

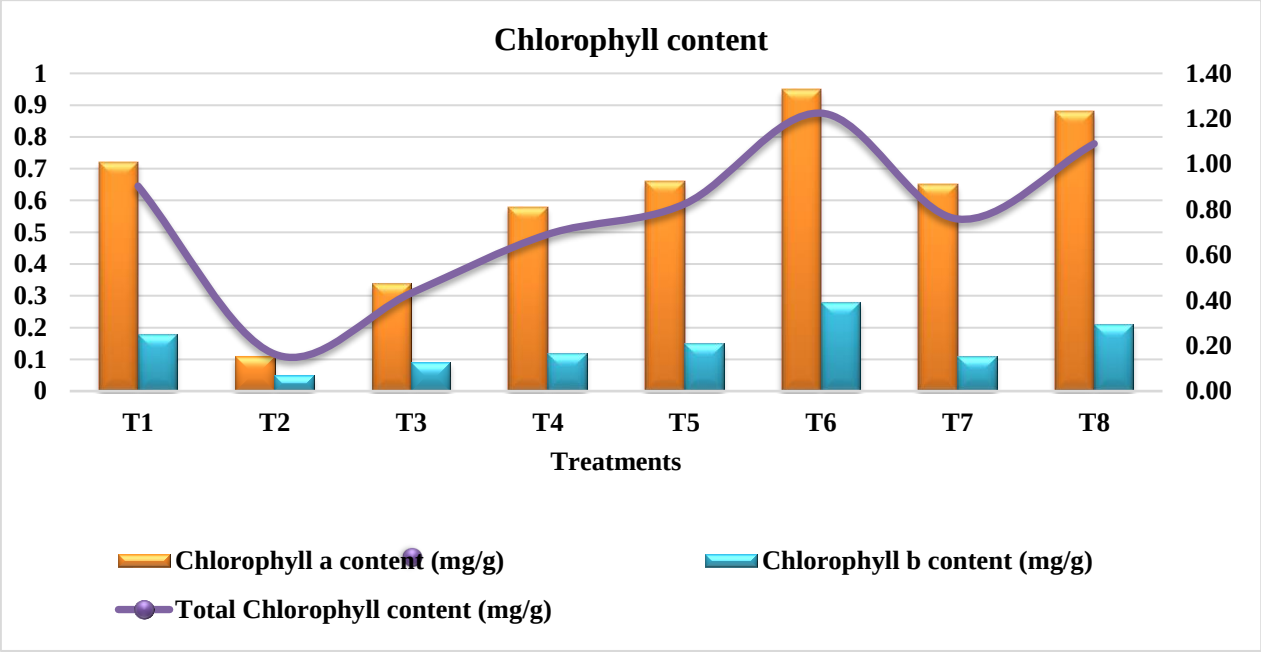


FIGURE 1: Effect of PGRs on chlorophyll a and chlorophyll b of green gram under salinity

Significant differences were noticed in all the treatments with respect to relative water content. The GAʒ 200ppm (T8) treatment recorded the higher RWC (88.44%). The control (T2) showed the lowest RWC (61.85%). Among the PGRs, there was a significant increment in RWC. Highest RWC was observed in seed treatment with GAʒ 200ppm (T8) followed by kinetin 100ppm (T6) (82.33%) and GAʒ 100ppm (T7) (80.70%). The lowest RWC in treatments is NAA 100ppm (T3) (68.43%) followed by NAA 200ppm (T4) (72.12%) **(Figure 2)**. The absolute control (T2) RWC is (82.10%). Relative water content was decreased up to 26.59% by salinity stress. This might be due to high level of sodium chloride changed the water potential and decrease the water absorption of the plant, hence Relative water content is decreased. Reduction in Relative water content is a common effect of salinity stress. Mohammed arifsadik polash et al. (2018) stated, When the plants are subjected to salinity they faced an osmatic challenge that reduces water uptake by roots. In the present investigation GAʒ 200ppm (T8) increase the Relative water content up to 26.59% compared to control. Relative water content declined with water deficit similar reports have been made for many species during water stress condition. Such a decrease in Relative water content is due to unavailability of water in the soil (Shalhevet 1993), both 25& 50 Mg\L. GAʒ treatments elevated water status in water stressed plant.

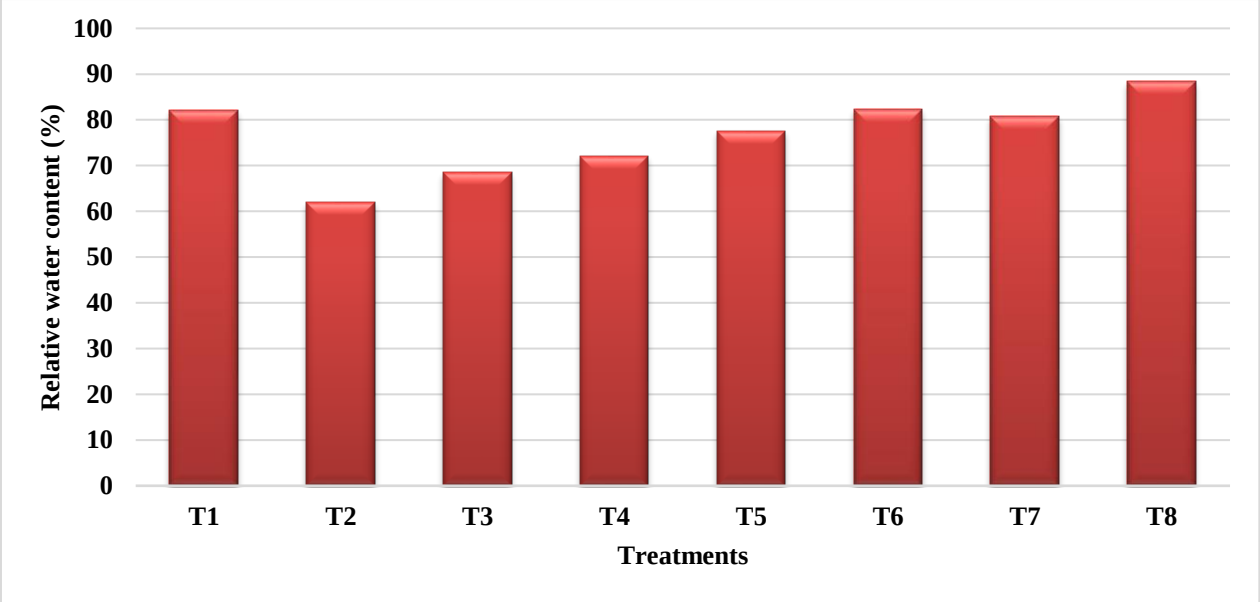
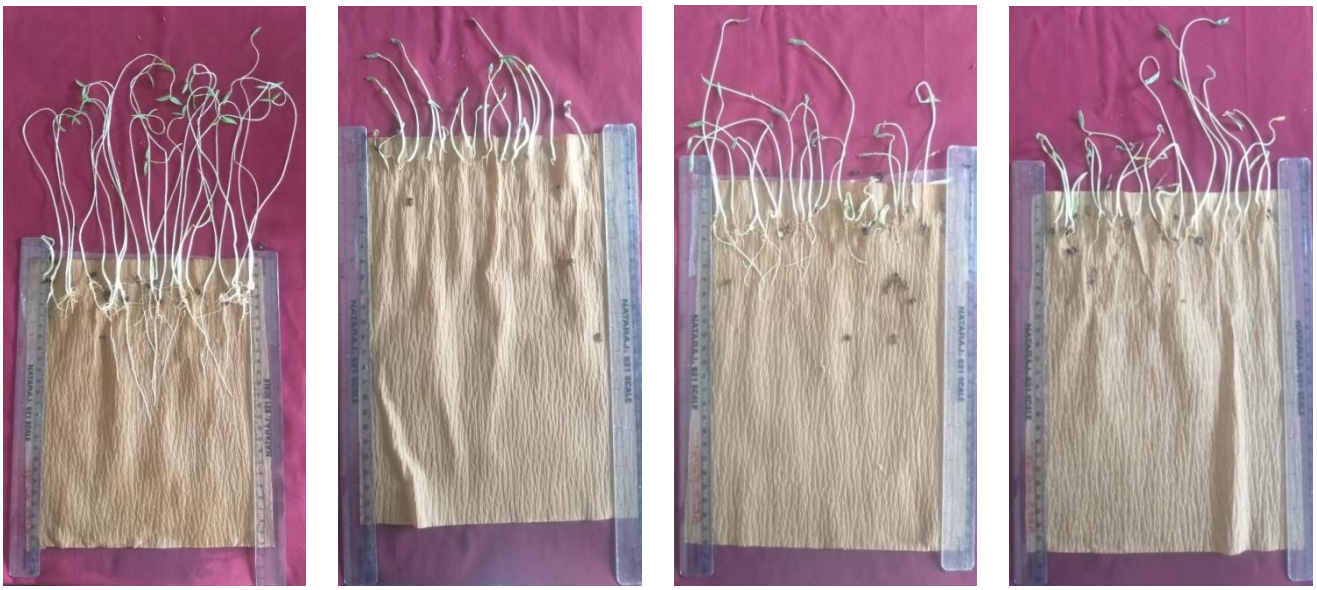


FIGURE 2: Effect of PGRs on RWC (%) of green gram under salinity

IV. SUMMARY AND CONCLUSION

The objectives of the study were to find the effects of salinity during seed germination of Greengram and to study the effect of the plant growth regulators in response to salinity stress during the seed germination in Greengram **(Figure 3)**. The NaCl solution was first standardized so that the resulting germination would be in the range of 50%. This is done with the trial of germinating the seeds with different concentrations NaCl solutions, such as 75 mM, 100 mM, 150 mM and 200 mM solutions. In these the 150 mM NaCl solution yielded the desired result and this solution was used for the study going forward. The seeds are treated by soaking the seeds with the 100 ppm (T3) and 200 ppm (T4) of NAA, 50 ppm (T5) and 100 ppm (T6) of Kinetin and 100 ppm (T7) and 200 ppm (T8) of GA3, for 8 hours. Then the treated seeds are places in the germination papers and are regularly saturated with the 150 mM NaCl solution except for the Absolute control which is saturated with normal irrigation water.



T1:Absolute control (Without salinity) **T5:Kinetin 50 ppm** **T7:GA3 100 ppm** **T8:GA3 200 ppm**

FIGURE 3: Effect of plant growth regulators on seedling growth of Greengran CO8 under salinity

Stress tolerance index (STI) indicates the tolerant potential of the plants during stress. In this present investigation the STI of GA3 200ppm (T8) treatment noticed up to (70%) with high stress tolerance index compared to control followed by GA3 100ppm (T7) (6.8%). This increment may be due to GA3 200ppm (T8) induced germination, vigour index, shoot and root length under saline environment.

There was a decrease in the total chlorophyll content upto 92%, chlorophyll a upto 90% and chlorophyll b upto 93% compared to the control in response to the 150 mM NaCl treatment. The seeds treated with the Kinetin 100 ppm (T6) had a high chlorophyll content of 92%. Relative water content was decreased up to 26.59% by salinity stress. This might be due to high level of sodium chloride changed the water potential and decrease the water absorption of the plant, hence Relative water content is decreased. Reduction in relative water content was a common effect of salinity stress. GA3 200 ppm (T8) had increased the relative water content upto 26.59%.

ACKNOWLEDGEMENT:

The results presented in this paper are a part of project work done for the completion of undergraduate degree programme supported by Thanthai Roever Institute of Agriculture and Rural Development, Perambalur.

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