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Enhancing Chickpea (*Cicer arietinum* L.) Tolerance to Salinity through Plant Growth Regulators

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

During the period of 2020-21, a research study was carried out at the Plant Protective Culture Unit within the semi-controlled environmental settings of the Bangladesh Agricultural Research Institute. The aim of the study was to mitigate the negative impacts of salinity stress on chickpea by applying various concentrations of salicylic acid (SA) and gibberellic acid (GA_3). Plants were subjected to four different levels of salinity stress, including a control group, mild stress, moderate stress, and severe stress. This was achieved by irrigating them with four varying concentrations of saline water (5, 7.5, 10 and 12.5 dSm^{-1}) starting from the 14th day after sowing (DAS) and continuing until maturity at 100 DAS. On the other hand, the control plants received regular irrigation with tap water. Two concentrations of salicylic acid (SA) specifically 200 ppm and 400 ppm, along with gibberellic acid (GA_3) at 10 ppm and 20 ppm, were administered as a foliar spray once a week,

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commencing at 20 days after sowing (DAS) and continuing until the flowering stage. Data related to chlorophyll levels in the leaves and water-related traits, including relative water content (RWC) and water retention capacity (WRC) were collected seven days after the foliar spray of these plant growth regulators at the flowering stage. Additionally, measurements of total dry weight, yield, and various yield-contributing factors were taken at the point of maturity. The results indicated that chickpea suffered adverse consequences due to salinity stress which included a reduction in chlorophyll content, a decrease in relative water content, a decline in water retention capacity, a decrease in total dry weight and a lower overall yield. However, the foliar application of different doses of salicylic acid (SA) and gibberellic acid (GA_3) under varying levels of salinity stress had beneficial effects in alleviating the impact of salinity stress. Interestingly, it was observed that lower concentrations of SA at 200 ppm and GA_3 at 10 ppm were more effective in mitigating the adverse effects of salinity stress and improving salinity tolerance in chickpea, especially under mild salinity stress conditions (5 dSm^{-1}) as evidenced by the positive influence on the parameters mentioned above.

Keywords: Salinity tolerance; Chickpea; gibberellic acid; salicylic acid; tolerance.

1. INTRODUCTION

Salinity poses a significant challenge to agriculture on a global scale, and Bangladesh is no exception, with substantial crop yield losses being a consequence. In Bangladesh, approximately 30% of the country's total crop land, covering around 2.86 million hectares, is located in coastal areas. Within this expanse, nearly 1.056 million hectares are affected by varying degrees of salinity [1]. The severity of salinity in this region intensifies as the soil becomes more desiccated. This salinity impacts crops differently depending on the degree of salinity during critical growth stages, leading to reduced yields and, in severe cases, complete crop loss. Therefore, it has become imperative to explore ways to harness the potential of these saline lands to increase crop production, as noted by Alam and Islam in [2]. Among the cultivable saline areas along the coast, approximately 31% (328 thousand hectares), 26% (274 thousand hectares), and 18% (190 thousand hectares) of the land are affected by very slight salinity (2.0-4.0 dSm^{-1}), slight salinity (4.1-8.0 dSm^{-1}), and moderate salinity (8.1-12.0 dSm^{-1}), respectively, and hold promise for successful crop production [3].

Various approaches are being implemented to address the decline in plant growth caused by salinity. One of these strategies involves the introduction of salt-resistant varieties of various crops. However, efforts to enhance the salt tolerance of these varieties through traditional plant breeding methods are both time-consuming and labor-intensive, primarily due to limitations in the existing genetic diversity [4]. Hence, an alternative method to address salinity stress might involve the external application of growth regulators to mitigate its adverse effects on plants, bringing these effects to a tolerable level. Plant growth regulators such as salicylic acid (SA) and gibberellic acid (GA_3) are acknowledged as internal regulators of plant metabolism, primarily engaged in responding to both biotic and abiotic stressors [5]. Salicylic acid plays a role in regulating the water balance within plant cells when subjected to abiotic stress conditions, helping to alleviate the negative effects caused by water scarcity in plants [6]. Gibberellic acid controls the growth and developmental processes in plants, while also decreasing stomatal resistance and improving plant water utilization under conditions of low salinity [7].

Chickpea (*Cicer arietinum* L.) serves as the main source of dietary protein for approximately 20% of the global population and ranks as the second most significant pulse crop [8]. Chickpea seeds comprise 21% protein, 5.6% fat, 60% carbohydrates, and boast a wealth of minerals and vitamins. However, chickpeas are susceptible to salt stress, leading to significant reductions in crop yields [9]. The existing data on the role of salicylic acid and gibberellic acid in improving salt tolerance and promoting the growth of chickpea are insufficient to provide specific recommendations to end-users. There is a lack of comprehensive research on the impact of plant growth regulators such as SA and GA_3 on various chickpea varieties in terms of their salinity tolerance. Consequently, the objective of this experiment was to investigate the effects of SA and GA_3 in enhancing the salt tolerance of chickpea.

2. MATERIALS AND METHODS

During the period of 2021-22, a pot experiment was carried out in the plant protective Culture unit, within a semi-controlled environmental setting at the regional horticulture research station of the Bangladesh Agricultural Research Institute (BARI) in Patuakhali. Chickpea variety BARI Chola-5 was used as plant material in the experiment. The soil for the experiment was gathered prior to seed sowing. The soil exhibited a clay loam texture and had neutral pH with a medium fertility status in terms of its physical and chemical properties. To ensure uniformity, each pot soil was enriched with compost, comprising 25% of the soil volume, and received 0.27-0.28-0.20 grams of urea, triple superphosphate, and muriate of potash, respectively, per pot to provide the necessary nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O). The compost was made mostly from cow dung which contained 0.8% N, 0.6% P_2O_5 and 1.0% K_2O on dry weight basis. Ten seeds were sown in each plastic pots filled with soil inside plastic house under natural light. After seedling establishment, five uniform and healthy plants were allowed to grow in each pot. Weeding and pest control measures were taken for proper growth of plants.

Salt solution was prepared by adding tap water in sea water to make 5, 7.5, 10 and 12.5 dSm^{-1} salinity levels. Plants were irrigated with these 5, 7.5, 10 and 12.5 dSm^{-1} concentrates saline water from 14^{th} days after sowing (DAS) to maturity (100 DAS) and control plants were irrigated with tap water. Different concentration of SA and GA_3 were applied as foliar spray once in a week from 20 DAS to flowering stage. The experiment was laid out in Randomized Complete Block Design (RCBD) having three replications. Four level of salinity stress treatments were used in this experiment. A treatment detail is given below-

a) Withoutstress (Control)
b) Mild stress

5 dSm^{-1} salinity
5 dSm^{-1} salinity + 200 ppm SA
5 dSm^{-1} salinity + 400 ppm SA
5 dSm^{-1} salinity + 10 ppm GA_3
5 dSm^{-1} salinity + 20 ppm GA_3
c) Moderate stress

7.5 dSm^{-1} salinity
7.5 dSm^{-1} salinity + 200 ppm SA
7.5 dSm^{-1} salinity + 400 ppm SA
7.5 dSm^{-1} salinity + 10 ppm GA_3
7.5 dSm^{-1} salinity + 20 ppm GA_3
10 dSm^{-1} salinity
10 dSm^{-1} salinity + 200 ppm SA
10 dSm^{-1} salinity + 400 ppm SA
10 dSm^{-1} salinity + 10 ppm GA_3
10 dSm^{-1} salinity + 20 ppm GA_3

d) Severe stress

12.5 dSm^{-1} salinity
12.5 dSm^{-1} salinity + 200 ppm SA
12.5 dSm^{-1} salinity + 400 ppm SA
12.5 dSm^{-1} salinity + 10 ppm GA_3
12.5 dSm^{-1} salinity + 20 ppm GA_3

The data for chlorophyll content and water relation traits in leaf such as relative water content (RWC) and water retention capacity (WRC) were recorded 7 days after foliar spray of SA and GA_3 at flowering stage.

Chlorophyll content was estimated from the fully expanded uppermost leaf samples using the method described by Witham et al*.* [10].

To measure the relative water content (RWC), fresh weight of the collected leaves sample was measured immediately. Thereafter, the leaves sample were then incubated in deionized water for 4 hours as described by Sairam et al*.* [11] and turgid weight of the leaf sample was recorded. The leaf sample was then filled into a brown paper bag and oven dried at 70^0C temperature for 48 hours and the dry weight of the sample was taken. The relative water content (RWC) was estimated as follows:

$$RWC = (FW - DW) / (TW - DW) \times 100$$

Where, FW = Fresh weight of the leaf,
DW = Dry weight of the leaf,
TW = Turgid weight of the leaf.

Water retention capacity is the ratio of the turgid weight and dry weight of a tissue. After the harvest both treated and controlled plants were segmented intodifferent components (root, stem, leaf). The segmented parts were then oven dried for 72 hours and the dry weights were recorded. Total dry weight of both treated and control plant were estimated by summing up the dry weight of root, stem and leaf. Yield and yield contributing characters like number of pod $plant^{-1}$, number of seed pod^{-1}, 100 seed weight and seed yield $plant^{-1}$etc. data were recorded on in case of both treated as well as control plants in each pot. The data recorded on different parameters were statistically analyzed with the help of R program. Means were compared to adjust the difference in plant performance under growth regulators treatments experiencing both salinity and control conditions by Least Significant Different Test [12].

3. RESULTS AND DISCUSSION

3.1 Yield and Yield Contributing Characters

3.1.1 Pods number per plant

The findings indicated a notable impact of salinity on the number of pods per plant, as shown in Table 1. The highest number of pods per plant (35.33) was observed under non-saline conditions (control). As the salinity level increased, the pod count per plant decreased. The lowest number of pods (16.08) was recorded under severe stress conditions, where the plants were irrigated with a salinity level of 12.5 dSm^{-1}. This represented a decrease of 7.16 pods per plant accounting for only 20.27% of the pod count in the control plants. However, when the plants were subjected to saline conditions and treated with SA and GA_3 through foliar spraying, there was an increase in the number of pods. Specifically, when SA was applied at a concentration of 200 ppm to plants irrigated with saline water levels of 5, 7.5, 10 and 12.5 dSm^{-1}, the average pod counts were 33.23, 23.07, 18.00 and 8.72 respectively. These figures represented 94%, 65%, 51% and 25% of the pod count compared to the control group. On the other hand, when SA was applied at a concentration of 400 ppm, the pod counts were 28.81, 24.48, 19.52 and 8.28 for the same salinity levels, corresponding to 82%, 69%, 55% and 23% of the pod count compared to the control group. Conversely, when GA_3 was sprayed at a rate of 10 ppm to plants subjected to saline water levels of 5, 7.5, 10 and 12.5 dSm^{-1}, chickpea plants produced an average of 31.60, 24.06, 21.37 and 9.01 pods per plant respectively. These figures represented 89%, 68%, 60% and 26% of the pod count compared to the control group. However, when GA_3 was applied at a concentration of 20 ppm, the pod counts were 82%, 66%, 52%, and 21%, respectively, compared to the control. It is evident that the higher concentration of SA and GA_3 did not demonstrate a significant mitigating effect on salinity stress in chickpea, particularly concerning the number of pods produced.

3.1.2 100-seed weight

The impact of salinity on the 100-seed weight was statistically significant. The greatest seed weight per plant was observed under non-saline conditions (control), with a weight of 25.18 grams. As the salinity level of the irrigation water increased, there was a decline in seed weight, and this reduction rate became more pronounced with higher salinity levels. The lowest seed weight recorded was 3.56 grams, representing only 14.13% of the control's seed weight, when the plants were irrigated with saline water at a level of 12.5 dSm^{-1} (as shown in Table 1). However, there was an increase in seed weight when SA and GA_3 were applied through foliar spraying under saline conditions. For instance, when SA was sprayed at 200 ppm on chickpea plants irrigated with saline water levels of 5, 7.5, 10 and 12.5 dSm^{-1}, the average 100-seed weights were 19.06 g, 15.01 g, 12.82 g and 4.55 g respectively. These values represented 76%, 60%, 51% and 18% respectively compared to the control group. When SA was applied at 400 ppm, the 100-seed weights were 70%, 63%, 55% and 17% of the control for the same salinity levels.

Similarly, when GA_3 was sprayed at 10 ppm on chickpea plants irrigated with saline water levels of 5, 7.5, 10 and 12.5 dSm^{-1}, the 100-seed weights were 17.39 g, 15.60 g, 12.72 g and 5.08 g respectively, which represented 73%, 63%, 55% and 21% of the control. However, when GA_3 was applied at 20 ppm, the 100-seed weights were 69%, 61%, 52% and 20% of the control for the same salinity levels. It is evident that the higher concentrations of SA and GA_3 did not demonstrate a significant mitigating effect on salinity stress in chickpea concerning seed weight.

3.2 Seed Yield

The analysis of variance revealed a significant impact of salinity stress on plant biomass. The greatest seed yield per plant was achieved in the control group, amounting to 6.77 grams. However, in plants subjected to saline conditions, the average seed yield decreased and this reduction rate became more pronounced with higher salinity levels. The lowest seed yield recorded was 1.88 grams, which represented only 28% of the seed yield in the control group, specifically when the plants were irrigated with saline water at a level of 12.5 dSm^{-1} (as indicated in Table 1). However, there was a significant increase in seed yield when SA and GA_3 were applied through foliar spraying under saline conditions. For example, when SA was sprayed at 200 ppm on chickpea plants irrigated with saline water levels of 5, 7.5, 10, and 12.5 dSm^{-1}, the seed yields per plant were 6.25 g, 4.66 g, 3.70 g and 2.62 g respectively. These figures represented 92%, 69%, 55% and 39% of the seed yield compared to the control group. When SA was applied at 400 ppm, the seed yields were 83%, 56%, 48% and 35% of the control for the same salinity levels.

Similarly, when GA_3 was sprayed at 10 ppm on chickpea plants irrigated with saline water levels of 5, 7.5, 10 and 12.5 dSm^{-1}, the seed yields per plant were 6.08 g, 4.44 g, 2.71 g and 2.12 g respectively, which represented 90%, 66%, 40% and 31% of the seed yield compared to the control. However, when GA_3 was applied at 20 ppm, the seed yields were 81%, 60%, 39% and 29% of the control for the same salinity levels. It can be suggested that the positive impact of SA on enhancing yield may be attributed to the increased translocation of photosynthates to the seeds. These results may be linked to the role of salicylic acid in enhancing certain physiological and biochemical aspects under stressful conditions, as suggested by Maity and Bera in [13]. The reduction in chickpea seed yield due to terminal salinity stress has been previously reported by Hossain et al. in [14].

Table 1. Influence of plant growth regulators on seed yield and yield-related traits of chickpea under varied salinity stress levels

Sl. No.		Treatment	Number of pod/plant	100-seed weight (g)	Seed yield/ plant (g)
1	(Control) without stress	Control	35.33	25.18	6.77
2	Mild stress	5 dSm^{-1} salinity	28.59	17.23	5.18
3		5 dSm^{-1} salinity + (200 ppm) SA	33.23	19.06	6.25
4		5 dSm^{-1} salinity + (400 ppm)SA	28.81	17.60	5.60
5		5 dSm^{-1} salinity + (10 ppm)GA_3	31.60	18.43	6.08
6		5 dSm^{-1} salinity + (20 ppm) GA_3	29.06	17.39	5.46
7	Moderate stress	7.5 dSm^{-1} salinity	23.10	11.55	3.77
8		7.5 dSm^{-1} salinity + (200 ppm) SA	23.07	15.01	4.66
9		7.5 dSm^{-1} salinity +(400 ppm) SA	24.48	15.91	3.81
10		7.5 dSm^{-1} salinity + (10 ppm) GA_3	24.06	15.92	4.44
11		7.5 dSm^{-1} salinity + (20 ppm) GA_3	23.38	15.60	4.05
12		10 dSm^{-1} salinity	17.68	9.77	2.80
13		10 dSm^{-1} salinity + (200 ppm) SA	18.00	12.82	3.70
14		10 dSm^{-1} salinity + (400 ppm) SA	19.52	13.93	3.27
15		10 dSm^{-1} salinity + (10 ppm) GA_3	21.37	13.90	2.71
16		10 dSm^{-1} salinity + (20 ppm) GA_3	18.50	12.72	2.63
17	Severe stress	12.5 dSm^{-1} salinity	7.16	3.56	1.88
18		12.5 dSm^{-1} salinity + (200 ppm) SA	8.72	4.55	2.62
19		12.5 dSm^{-1} salinity + (400 ppm) SA	8.28	4.42	2.38
20		12.5 dSm^{-1} salinity + (10 ppm) GA_3	9.01	5.28	2.12
21		12.5 dSm^{-1} salinity +(20 ppm) GA_3	7.56	5.08	1.99
STDEV			**8.79**	**6.03**	**1.52**
CV (%)			**17.05**	**14.26**	**13.72**

3.3 Dry Matter Production

There was a significant decrease in dry matter accumulation under saline conditions during the vegetative stage, with the most substantial reduction occurring at 12.5 dSm^{-1} saline conditions, where it reached 5.21 g per plant, representing only 28% of the control (refer to Fig. 1). The control plants produced the highest total dry matter at 18.67 g per plant and even under 5 dSm^{-1} saline conditions, it was relatively high at 16.23 g per plant.

The application of plant growth regulators led to an increase in total dry weight under salinity stress. Among the plants treated with plant growth regulators, the highest dry matter production was recorded at 17.36 g per plant when SA was sprayed at a concentration of 200 ppm. This was followed by 17.12 g per plant when GA_3 was applied at 10 ppm under 5 dSm^{-1} salinity conditions. When plants were sprayed with SA at 400 ppm and GA_3 at 20 ppm under the same 5 dSm^{-1} salinity condition, the total dry matter per plant was 16.65 g and 16.35 g respectively. At 12.5 dSm^{-1} saline conditions, when plants were sprayed with SA at 200 ppm and GA_3 at 10 ppm, dry matter production reached 8.54 g and 8.51 g per plant respectively. On the other hand, when plants were sprayed with SA at 400 ppm and GA_3 at 20 ppm under the same 12.5 dSm^{-1} salinity stress, dry matter production amounted to 7.45 g and 7.67 g per plant, respectively. Lower doses of SA and GA_3 demonstrated a remarkable effect on dry matter production under saline conditions. Similar findings were reported by Hossain et al. in [14] and Yildirim et al. in [15], who observed that the exogenous application of SA and GA_3 mitigated the negative impact of salt stress on plant dry weight.

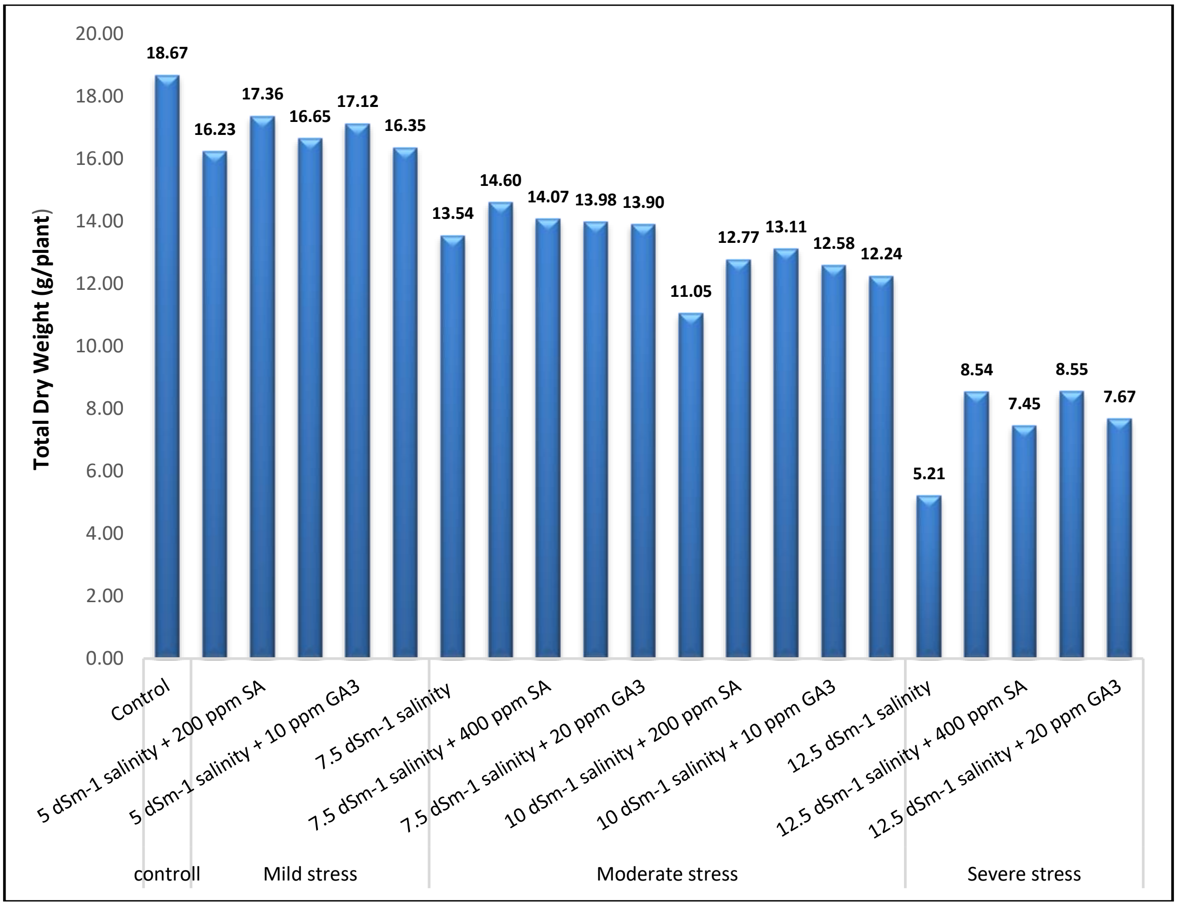

Fig. 1. Influence of plant growth regulators on total dry weight of chickpea under varied salinity stress levels

3.4 Relative Water Content (RWC)

The relative water content (RWC) exhibited a significant decrease under saline conditions when compared to the control, as illustrated in Fig. 2. Under salinity stress, the RWC percentage decreased and this decrease became more pronounced with increasing salinity levels. Specifically, at salinity levels of 5, 7.5, 10 and 12.5 dSm^{-1}, the RWC values were 81%, 74%, 65% and 43% respectively, whereas the control plants maintained the highest RWC at 87%.

However, the foliar application of SA and GA_3 led to an increase in RWC under various salinity conditions. For example, when plants were sprayed with SA at 200 ppm and GA_3 at 10 ppm, the RWC values reached 84% and 82% respectively, under 5 dSm^{-1} salinity conditions. Under 12.5 dSm^{-1} saline conditions, the RWC was 51% and 49% when SA was sprayed at 200 ppm and GA_3 at 10 ppm respectively. Conversely, when plants were sprayed with SA at 400 ppm and GA_3 at 20 ppm under the same 5 dSm^{-1} salinity stress, the RWC values were 83% and 82% respectively. It is noteworthy that the rate of RWC increase was lower when higher doses of SA and GA_3 were applied. These results are consistent with the fact that SA has the potential to induce a wide range of metabolic and physiological responses, including its impact on plant water relations, as discussed by Hayat et al. in [16].

3.5 Water Retention Capacity (WRC)

Water retention capacity (WRC) was influenced by the application of plant growth regulators. Control plants exhibited the highest WRC, approximately 7.30 surpassing that of the saline-treated plants, which showed a gradual decrease with increasing salinity levels. Under severe salinity conditions (12.5 dSm^{-1}), WRC dropped to 3.10. However, SA and GA_3 were found to enhance salinity tolerance and increase water retention capacity, as depicted in Fig. 3. The rate of increase was more pronounced when plants were sprayed with 200 ppm SA and 10 ppm GA_3 under 5 dSm^{-1} saline conditions. In the presence of 5 dSm^{-1} salinity conditions, the application of 200 ppm of SA and 10 ppm of GA_3 resulted in respective relative water contents (RWC) of 7.15 and 7.10. However, when the salinity level increased to 12.5 dSm^{-1}, the RWC values decreased to 4.10 and 4.08 for the same SA and GA_3 treatments. Conversely, under the same 5 dSm^{-1} salinity stress, using higher concentrations of SA (400 ppm) and GA3 (20 ppm) led to RWC values of 7.00 and 6.90, respectively. It is noteworthy that the rate of increase in RWC was lower when higher doses of SA and GA3 were applied. Ahmad et al. [17] observed that the application of GA3 mitigated the detrimental effects of NaCl salinity on relative water content.

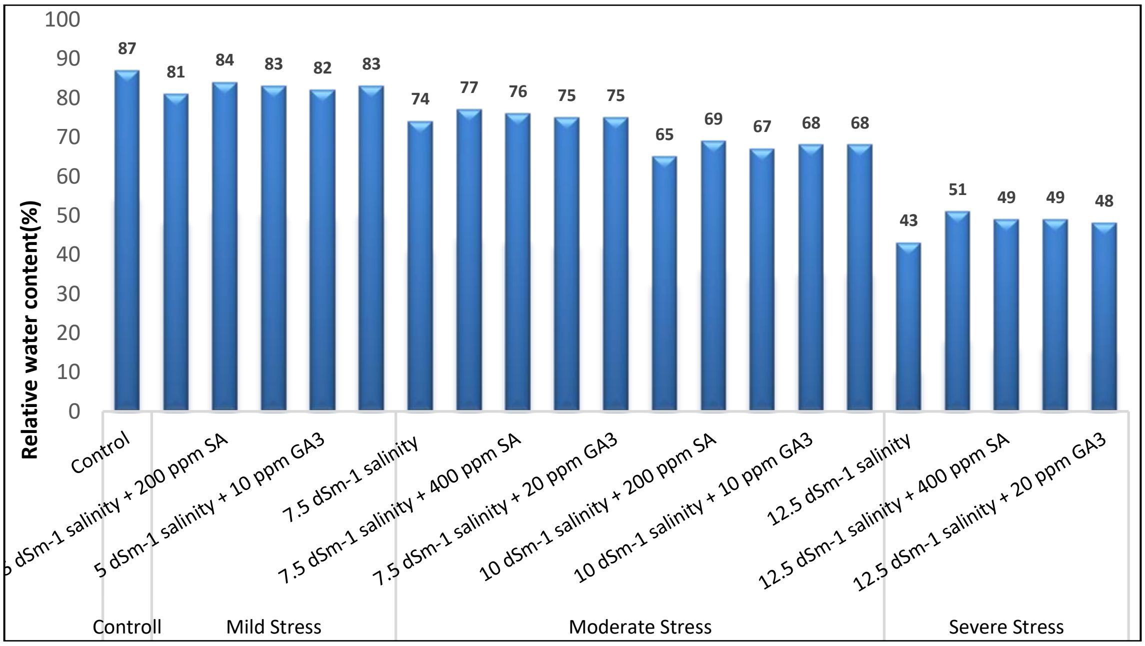

Fig. 2. Influence of plant growth regulators on relative water content of chickpea under varied salinity stress levels

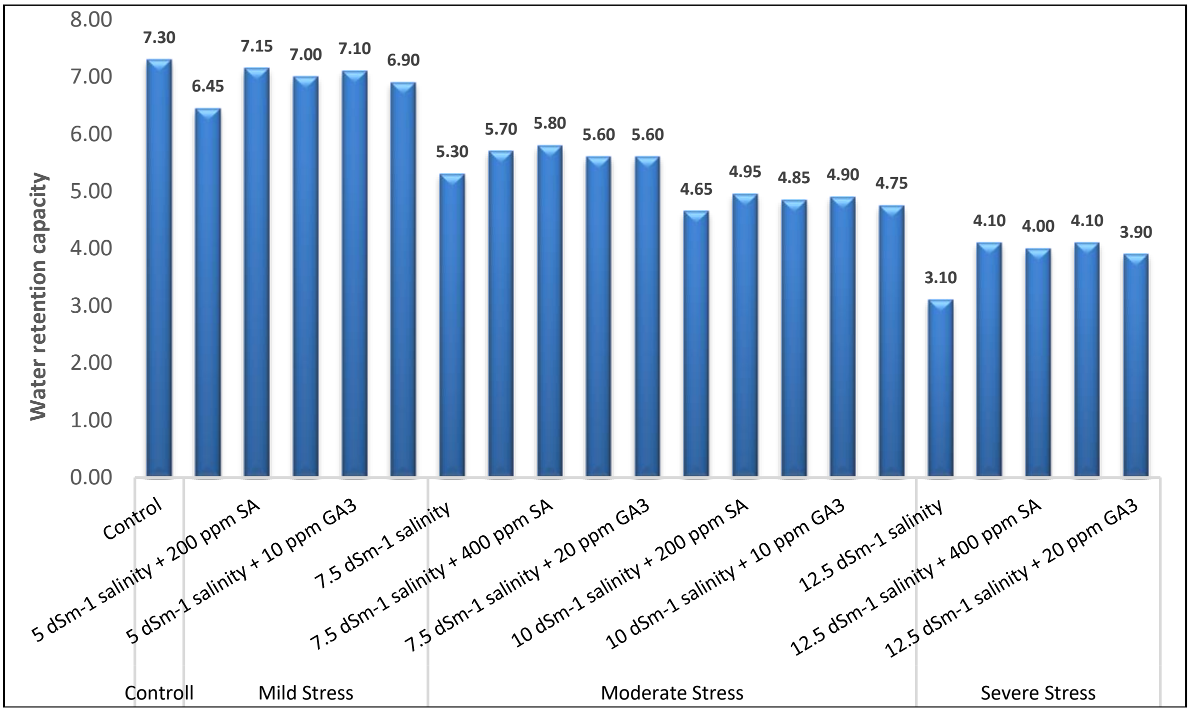

Fig. 3. Influence of plant growth regulators on water retention capacity of chickpea under varied salinity stress levels

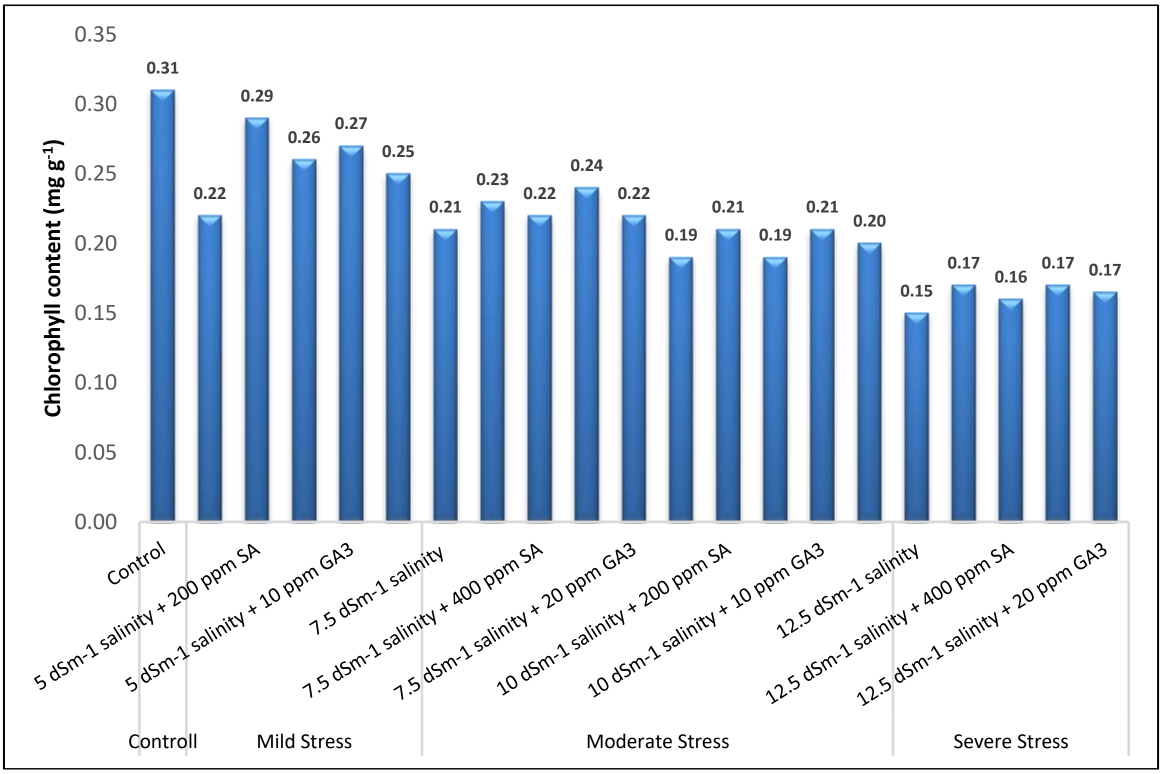

Fig. 4. Influence of plant growth regulators on chlorophyll content of chickpea under varied salinity stress levels

3.6 Chlorophyll Content

The chlorophyll content in chickpea leaves exhibited a significant reduction under salinity stress conditions. The highest total chlorophyll content, measured at 0.31 mg g^{-1} of leaf tissue, was observed under non-saline conditions. As the salinity level increased, the chlorophyll content in chickpea leaves decreased, with the most substantial reduction occurring at the highest salinity level of 12.5 dSm^{-1} (as depicted in Fig. 4). However, when plant growth regulators were applied under saline conditions, the total chlorophyll content increased. Foliar application of SA and GA_3 led to an enhancement in chlorophyll levels in salt-treated plants, although the rate of increase was more pronounced when lower doses of SA and GA_3 were used. Notably, the most prominent effects on chlorophyll content were recorded when SA and GA_3 were applied at concentrations of 200 ppm and 10 ppm, respectively, under lower salinity conditions (5 dSm^{-1}). When plants are subjected to saline conditions, photosynthetic activity tends to decrease, resulting in reduced plant growth, leaf area, chlorophyll content and chlorophyll fluorescence as reported by Muhammad et al. in [18]. Szepsi et al*.* in [19] suggested that the positive effect of SA could be attributed to increased CO_2 assimilation, photosynthetic rate, and enhanced mineral uptake by stressed plants under SA treatment. Zeid in [21] reported the alleviation of adverse effects of salinity on chlorophyll content in barley through GA_3 treatment. Likewise, Ali et al*.* in [21] documented the restoration of altered pigments through the application of GA_3 under saline conditions in *Hibiscus sabdariffa*.

4. CONCLUSION

In summary, this study highlights the negative impact of salinity on various aspects of chickpea including yield, dry matter production, water-related traits, and chlorophyll content. However, the application of low concentrations of plant growth regulators, specifically salicylic acid at 200 ppm and gibberellic acid at 10 ppm, demonstrated positive effects on several parameters such as the number of pods per plant, 100-seed weight (g), seed yield per plant (g), total dry weight (g), chlorophyll content (mg g^{-1}) and water-related traits in chickpea. Furthermore, these plant growth regulators were particularly effective in alleviating the adverse effects of salinity under mild and moderate salinity stress conditions (5 and 7.5 dSm^{-1}) showing the greatest potential for improving salinity tolerance in chickpea. It's important to note that higher concentrations of salicylic acid and gibberellic acid did not exhibit significant performance in mitigating the adverse effects of salinity under moderate and severe salinity stress conditions. Therefore, it can be concluded that the reduction in chickpea growth and yield caused by salinity stress can be ameliorated through the exogenous foliar application of plant growth regulators at low concentrations, especially under mild to moderate salinity stress conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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