

Response of sweet basil plants (*Ocimum basilicum* L.) to Gibberellin acid (GA) under salinity

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Abstract:

The research was conducted during the year 2024 according to a randomized complete design (R.C.D) in three replicates in the village of Al-Hennadi- Lattakia-Syria. The treatments consisted of three levels of Gibberellin (100, 200 and 300 μM) In addition to the control. And to induce salinity stress, sodium chloride salt (NaCl) was used at concentrations (6, 12, 18 dmol/cm) in addition to the control. Traits such as plant height (cm/plant), number of branches (branch/plant), plant leaf area area (cm^2), leaf specific gravity (g/cm^2), leaf total chlorophylls content ($\mu\text{g/g}$) and proline ($\mu\text{mol/g}$) were measured. High salinity concentration led to negative effects on all indicators studied. Foliar treatment with Gibberellin acid led to an increase in plant height, total leaf surface area, specific gravity, and leaf chlorophyll content. On the other hand, treatment with Gibberellin acid under salinity stress conditions at a low concentration (100 μM) outperformed all treatments and the control. Therefore, we recommend foliar spraying with Gibberellin acid at a concentration of (100 μM), due to its role in improving the morphological, physiological, and biochemical characteristics of the sweet basil plant.

Keywords: Sweet basil, Gibberellin , salt stress, salinity.

استجابة نباتات الريحان الحلو (*Ocimum basilicum* L.) لحمض الجبرلين (GA) تحت

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الملخص:

أجري البحث خلال عام 2024 في تجربة عاملية وفقاً للتصميم العشوائي الكامل (R.C.D) في قرية الهنادي – اللاذقية – سورية. بثلاثة مكررات لكل معاملة. حيث عوملت النباتات بثلاثة تراكيز من الجبرلين (100، 200 و 300 ميكرومول) إضافة إلى معاملة الشاهد، ولتحفيز إجهاد الملوحة، تم استخدام ملح كلوريد الصوديوم (NaCl) بتراكيز (6، 12، 18 ديسي مول/سم) إضافة إلى معاملة الشاهد. تم قياس العديد من الصفات، منها: ارتفاع النبات (سم/نبات)، عدد التفرعات (فرع/نبات)، مساحة المسطح الورق الكلي (سم²)، الوزن النوعي للأوراق (غ/سم²)، محتوى الأوراق من الكلوروفيل (ميكروغرام/غ) والبرولين (ميكرومول/غ)، أدى ارتفاع تركيز الملوحة إلى تأثيرات سلبية في جميع المؤشرات المدروسة. أدت المعاملة الورقية بحمض الجبرلين إلى زيادة في ارتفاع النبات ومساحة مسطح الورق الكلي والوزن النوعي ومحتوى الأوراق من الكلوروفيل، وعلى الجانب الآخر تفوقت المعاملة بحمض الجبرلين تحت ظروف الإجهاد الملوحة بتركيز منخفض (100 ميكرومول) على جميع المعاملات والشاهد. لذلك، ننصح بالرش الورقي بحمض الجبرلين بتركيز (100 ميكرومول)، نظراً لدوره في تحسين الخصائص الشكلية والمورفولوجية والبيوكيميائية لنبات الريحان الحلو.

الكلمات المفتاحية: تبغ، بصما، الأسكوربيك، الإجهاد الملحي.

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Introduction:

The sweet basil (*Ocimum basilicum* L.) plant is a member of the family Lamiaceae, commonly known as mint, and a healing plant family because it contains about 150 aromatic species that differ in leaf size, flower color, outward characteristics, and flavor. Africa and Asia are the original home of basil (Al-Ibrahemi *et al.*, 2023). Sweet basil's aromatic leaf directly used as flavoring agent in different foods and beverages industries. Oil and oleoresin of sweet basil indirectly widely used for flavor and fragrance in the food, pharmaceutical, cosmetic, and aromatherapy industries. It is also used in the pharmaceutical and cosmetic industries. The other use of sweet basil is a pasture for bees to produce honey and can be used as ornamental plant. Generally, Sweet basil plant can be considered as; easily available, easy to grow, have multipurpose advantages that should be produced as high value crop (Egata, 2021).

Extensive agricultural lands in coastal zones have experienced elevated soil salinity levels, attributed to marine intrusion and climate change, resulting in decreased crop production rates (Haddaji *et al.*, 2021), environments have an adverse effect on plant nutrition and increase soil osmotic pressure, which both hinder plant growth (Huang *et al.*, 2019).

Salinity is a common issue in arid and semi-arid climates because of the limited water availability and high evaporation rates, which can lead to the accumulation of salts in soil and water (Pitman and Läuchli, 2002). In arid and semi-arid regions, water is scarce and often comes from underground sources, such as wells or aquifers (Ahmed *et al.*, 2020). These sources are typically rich in dissolved salts, which can accumulate in the soil as the water evaporates (Zafar-ul-Hye *et al.*, 2019). The salts can also be brought to the surface through capillary action, where they can accumulate and create salt pans or salt flats (Ahmadvand *et al.*, 2019). High salinity levels in the soil can be detrimental to plant growth and can limit agricultural productivity (Zhao *et al.*, 2021).

On the contrary, Gibberellin (GA), a plant hormone, plays a crucial role in regulating various aspects of plant growth and development, spanning from seed germination to flower formation. Indeed, faster germination allows plants to develop a robust root system that captures more soil moisture (Hedden, 2016). Moreover, there is increasing evidence in the literature highlighting the dynamic involvement of plant growth regulators and phytohormones like Gibberellins in responding to and tolerating abiotic stresses (Ullah *et al.*, 2022).

Gibberellin acid plays a pivotal role in regulating gene expression levels that govern plant physiological responses to stressors such as drought and salinity (Ritika Bhatt *et al.*, 2015). Under drought and salt stress conditions, the biosynthesis, accumulation, and metabolism of various phytohormones, including Gibberellins, can be affected (Eyidogan *et al.*, 2012), the application of exogenous Gibberellin significantly increased parameters such as plant height, root length, stem diameter, and leaf area (Matos *et al.*, 2020). Results showed that the Gibberellin acid was significantly higher in all studied characteristics when it gave the highest average of plant height, number of leaves, number of side branches, leaves content of total chlorophyll (Shahmani and Al-Tufaili, 2020).

The aim of this work is to the response of basil plants treated with in graded concentrations Gibberellin acid under salinity conditions (Artificially stressed by many concentrations of sodium chloride (NaCl) to know the effect these treatments on some different growth and biochemical traits.

Material and Methods:

The experiment was carried out during the 2024 season. The field experiment was conducted in the Al-Hanadi village, within a Latakia greenhouse.

Experimental design:

Seeds of basil (*O. basilicum*) were sown in pots with a depth of 25 cm and a diameter of 20 cm filled with clay loam (pH of 7.5, 10% organic material, 1.30 dS/m, 50% field capacity), sown in a depth of 1 cm.

The field experiment was performed as a factorial in a randomized complete design (R.C.D) with three replications. Experimental treatments included Gibberellin at four levels (0, 100, 200 and 300 μ M). The foliar application Gibberellin (Sigma Aldrich, Germany) was done with the concentrations mentioned above on basil at the 3–4-leave stage (18-day-old plants). The plants were sprayed at 6:00 AM in cool weather with almost no wind. In the control treatment, the plants were sprayed with distilled water (without hormones). After applying treatments, the studied traits were measured at the flowering stage (55- to 60-day-old plants), and biochemical analyses were performed in leaves.

Irrigation with NaCl (S) solutions (0, 6, 12 and 18 dS/m) was carried out during active vegetative growth (before flowering), at a rate of three irrigations every week.

Treatment:

GA₀S₀: Con.

S₁: 6 dS/m NaCL.

S₂: 12 dS/m NaCL.

S₃: 18 dS/m NaCL.

GA₁: 100 μ M Gibberellin acid.

GA₂: 200 μ M Gibberellin acid.

GIBBERELLIN : 300 μ M Gibberellin acid.

GA₁S₁: 100 μ M Gibberellin acid, 6 dS/m NaCL.

GA₁S₂: 100 μ M Gibberellin acid, 12 dS/m NaCL.

GA₁S₃: 100 μ M Gibberellin acid, 18 dS/m NaCL.

GA₂S₁: 200 μ M Gibberellin acid, 6 dS/m NaCL.

GA₂S₂: 200 μ M Gibberellin acid, 12 dS/m NaCL.

GA₂S₃: 200 μ M Gibberellin acid, 18 dS/m NaCL.

GA₃S₁: 300 μ M Gibberellin acid, 6 dS/m NaCL.

GA₃S₂: 300 μ M Gibberellin acid, 12 dS/m NaCL.

GA₃S₃: 300 μ M Gibberellin acid, 18 dS/m NaCL.

Many different traits were measured during flowering:

Morphological Characteristics:

7. Plant Height (cm): was measured for each experimental treatment, starting from the soil surface level to the growing top (Soufi *et al.*, 2021).
8. Number of branches/ plant (branch/plant): the number of branches on the - plant branch/plant (Soufi *et al.*, 2021).

Physiological characteristics:

9. Plant leaf area (PLA) (cm²):

From the following equation: PLA (cm²/plant) = sum of the area of all leaves of a plant.

LAI = plant leaf area (cm²) / area occupied by the plant on the ground (cm²)

The plant leaf area was measured by the gravimetric method according to (Vivekanandan *et al.*, 1972).

10. Specific gravity of leaves (g/cm²):

The leaf-specific weight (SLW) was determined after measuring the dry weight of the leaves at the beginning of the technical maturity of the leaves according to the researcher (Pearce *et al.*, 1968):

SLW = dry leaf weight (g/plant)/leaf area (cm²/plant).

Chemical Characteristics:

11. Determination of Chlorophyll content in leaves (μ g/g):

As per the methodology outlined by reference (Lichtenthaler, 1987), leaf sections used for measuring chlorophyll and carotenoids were extracted using 5 mL of pure methanol for subsequent spectrophotometric determination.

12. Determination of proline content in leaves ($\mu\text{mol/g}$):

Plant tissue samples weighing 0.5 g were crushed in 5 mL of 95% (v/v) ethanol. The insoluble fraction of the extract was then washed twice with 5 mL of 70% ethanol. Subsequently, all soluble fractions were centrifuged at $3500 \times g$ for 10 min, and the resulting supernatants were collected and stored at 4°C for proline determinations (Irigoyen *et al.*, 1991). The free proline content was measured spectrophotometrically at 515 nm.

Statistical Analysis:

Statistical analyses were performed by the analysis of variance (ANOVA) with Tukey. All data are presented as means \pm standard deviation (SD) of three replicates. Differences at $P < 0.05$ were significant.

Results and Discussion:

7. Effect of treatment with Gibberellin and salt stress on plant height (cm):

The data in Figure (1) indicate that there are significant differences at the level ($P < 0.05$) between the studied treatments in terms of the height of sweet basil plants (cm). Salt stress led to a decrease in plant height. Results showed that the low concentrations of Gibberellin GA_1 and GA_2 had significant effect in plant height compare to control treatment. The interaction between 100 μM concentration of Gibberellin and 6 dS/m concentration of NaCL was reached the highest average in plant height trait compare to control and remaining treatment.

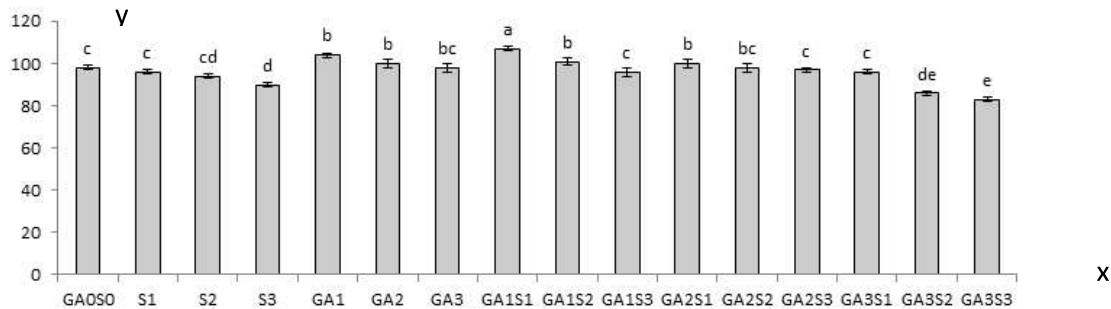


Figure (1): Effect of Gibberellin on plant height under salt stress.

All data refer to averages plus standard error (means \pm SE), $n=3$, and different letters (a, b, c... to show the significant differences between the averages for each indicator at each treatment ($P < 0.05$), ANOVA-Tukey test. The negative effect of salinity on plant growth and water content may be due to the occurring of defect metabolism in plant cells. Since high osmotic pressure resulted from high salinity restricted plant cells to uptake water and some mineral nutrients dissolved in the culture medium (Cicek and Cakirlar, 2002). Exogenous Gibberellin was observed to enhance the salt tolerance of basil plants, manifested through increased plant height, root length, and foliar area (Akoudad *et al.*, 2024).

8. Effect of treatment with Gibberellin and salt stress on Number of branches/ plant (branches/ plant):

The data in Figure (2) indicate that there are significant differences at the level ($P < 0.05$) between the studied treatments in terms of the number of branches. Salt stress led to a decrease in plant height. Results showed that the low concentration of Gibberellin 100 μM GA_1 had significant effect in number of branches compare to control treatment. The interaction between 100 μM concentration of Gibberellin and 6 dS/m concentration of NaCL was reached the number of branches trait compare to control and remaining treatment.

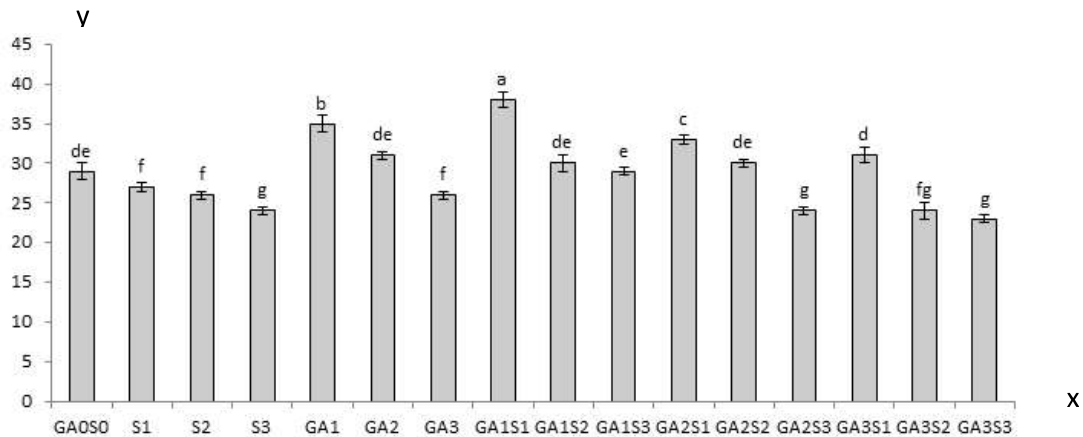


Figure (2): Effect of Gibberellin on Number of branches/ plant under salt stress.

Salinity stress creates osmotic stress and ion imbalance in the plant due to high salt and low water availability outside the sap; it also leads to ion toxicity due to the accumulation of salts in transpiring leaves (Acosta-Motos *et al.* 2017; Farooq *et al.* 2017)

9. Effect of treatment with Gibberellin acid and salt stress on plant leaf area (cm²/plant):

There are significant differences at the level ($P < 0.05$) between the studied treatments in terms of the plant leaf area. Salt stress led to a decrease the plant leaf area traits. Results showed that the low concentration of Gibberellin 100 μm had significant effect in plant leaf area GA₁ compare to control treatment. The interaction between 100 μm concentration of Gibberellin and 6 ds/m concentration of NaCL was reached the plant leaf area trait compare to control and remaining treatment. Figure (2).

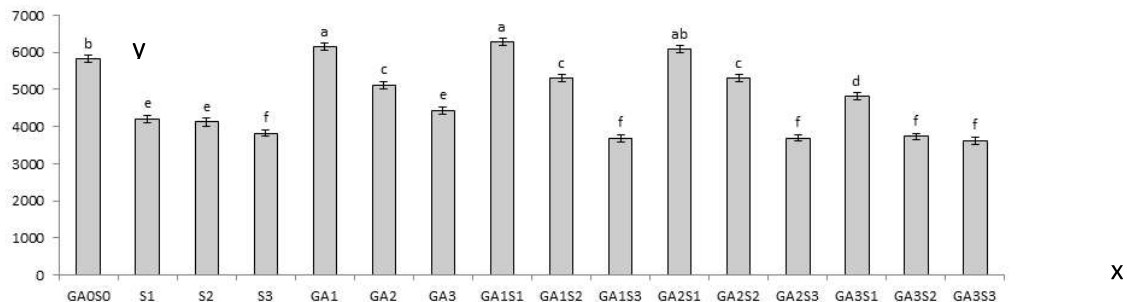


Figure (3): Effect of Gibberellin on total leaf surface area under salt stress.

growth inhibitory is the immediate response toward salinity stress because of the attenuation of plant biomass due to diminutions in the rate of cell division and differentiation, exosmosis occurs by disturbance in plant water uptake, aberrations in ion balance, and overproduction of toxic ROS, initiating oxidative damage to the antioxidant system (Husen *et al.*, 2018; Jamshidi Goharrizi *et al.*, 2020).

In the canopy, leaves have the largest portion and area for atmospheric exchange processes. LAI represents the amount of the leaf area per ground area that controls important processes such as the net production rate, photosynthesis, respiration, evapotranspiration, light absorption, and, to a large extent, other ecological processes (Hopkins, Huner, 2008). The application of Gibberellin increased the leaf area, which has been reported in the study of Shaddad *et al.* (2013). When Gibberellin was sprayed on wild mint plants, in addition to its effect on stem elongation, it could also increase the leaf area (Bose *et al.*, 2013). The larger leaf area may be due to stimulated cell division and elongation (Hopkins, Huner, 2008). The

foliar application of SA in basil and marjoram increased the number of branches, leaves, and leaf area (Gharib, 2006).

The results indicate that the application of Gibberellic acid alleviated the adverse effects of high salinity and resulted in enhanced biomass production. In comparison to the control treatment, foliar surface values for basil increased by 15% (Akoudad *et al.*, 2024).

10. Effect of treatment with Gibberellin acid and salt stress on specific gravity of leaves (g/cm²):

The data in Figure (4) indicate that there are significant differences at the level ($P < 0.05$) between the studied treatments in terms of the specific gravity of leaves of sweet basil plants. Salt stress led to a decrease in Specific gravity of leaves, and this decrease increased with increasing concentration of added salt. While treatment with Gibberellin acid at two concentrations (100 and 200 μM) increased specific gravity of leaves compared to the control. Treatment with Gibberellin acid at a concentration of 100 μM and salinity of 6 dS/m also outperformed all other parameters and the control.

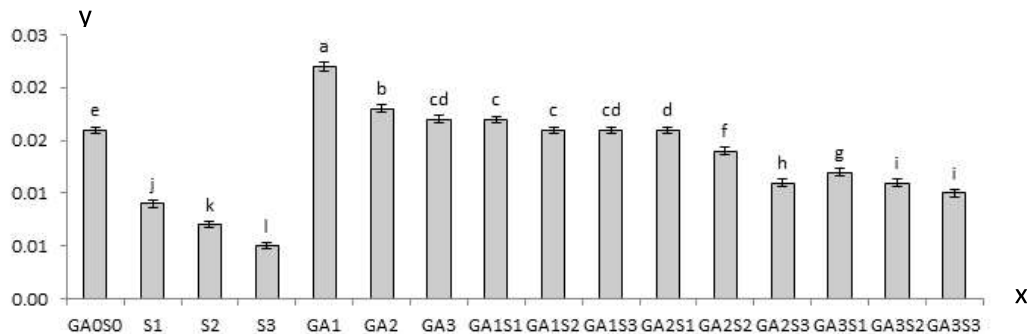


Figure (5): Effect of Gibberellin on Specific gravity of leaves under salt stress.

Decreasing the rate of cell division and elongation, and ultimately reducing root and shoot length (Bhatt *et al.*, 2015). The length, fresh weight, and dry weight of plants are the outcomes of proper cell division and photosynthesis, leading to proper assimilation and accumulation of storage material ultimately resulting in proper plant growth (Hafez *et al.*, 2021). Decreased water potential and oxidative stress by salinity could have adversely affected the enzymes of the carbon and nitrogen assimilation cycle, resulting in low root and shoot dry weight of affected plants (Ashraf, 2009). The positive role of GA in the regulation of shoot growth has been confirmed by many researchers (Bose *et al.*, 2013). Also, Gibberellin acid increases the photosynthetic rate and the elongation of leaves (Emamverdian *et al.*, 2020). Gibberellin can affect the expansion of increased biomass of the basil plants by exerting a positive influence on the growth and division of cells, plant water status, chlorophyll content, and enhanced photosynthetic capacity of the plant. The application of different concentrations of GA indicated that there was a greater increase in plant biomass in response to the GA. This may be due to rapid increment of cell division and elongation with GA treatment (Abbasi *et al.*, 2020).

5. Effect of treatment with Gibberellin acid and salt stress on chlorophyll content in leaves ($\mu\text{g/g}$):

The data in Figure (5) indicate that there are significant differences at the level ($P < 0.05$) between the studied treatments in terms of chlorophyll content in leaves of sweet basil plants. Salt stress led to a decrease in chlorophyll content in leaves, and this decrease increased with increasing concentration of added salt. Treatment with Gibberellin acid at two concentrations (100 and 200 μM) increased chlorophyll content in leaves compared to the control. Treatment

with Gibberellin acid at a concentration of 100 μM and salinity of 6 dS/m also outperformed all other parameters and the control.

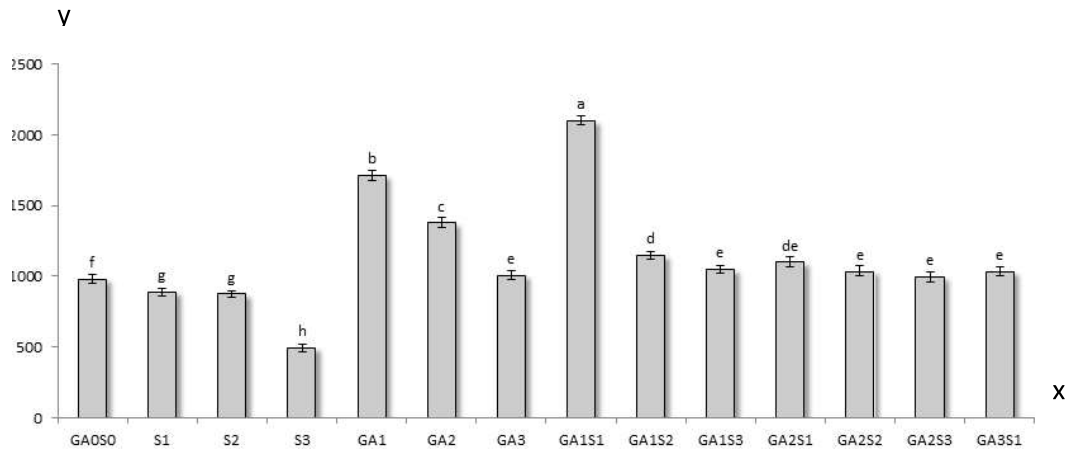


Figure (5): Effect of Gibberellin on chlorophyll content in leaves under salt stress

This noticeable decrease in the content of photosynthetic pigments (chlorophyll and carotenoids) in basil leaves is explained by the fact that high concentrations of sodium chloride salts lead to increased decomposition of chlorophyll molecules, the destruction of chloroplasts, and a decrease in their physiological activity in the plant (Taleisnik-Gertel *et al.*, 1983).

It has also been proven in other studies that the amount of chlorophyll a, b, and total was increased with Gibberellin treatment (Alharby *et al.*, 2021). This incremental effect in plants after the application of Gibberellin was attributed to the positive influence of Gibberellin on the genes encoding the chlorophyll biosynthesis pathway (Shaddad *et al.*, 2013). It has been reported that there is a clear interaction between Gibberellin and salinity, both under non-stress and stress conditions. The expression of Gibberellin-responsive genes increases the endogenous levels of salinity (Emamverdian *et al.*, 2020).

6. Effect of treatment with Gibberellin acid and salt stress on proline content in leaves ($\mu\text{mol/g}$):

The data in Figure (6) indicate that there are significant differences at the level ($P < 0.05$) between the studied treatments in terms of proline content in the leaves of sweet basil plants. Salt stress increased the proline content, and the proline content increased with increasing concentration of added salt. Treatment with Gibberellin acid at two concentrations 100 and 200 μM (GA_1 and GA_2) led to a decrease in the proline content in plants compared to the high concentration and the control. Treatment with Gibberellin acid at a concentration of 100 μM and a salinity of 6 dS/m also gave the lowest proline content compared to the rest of the treatments.

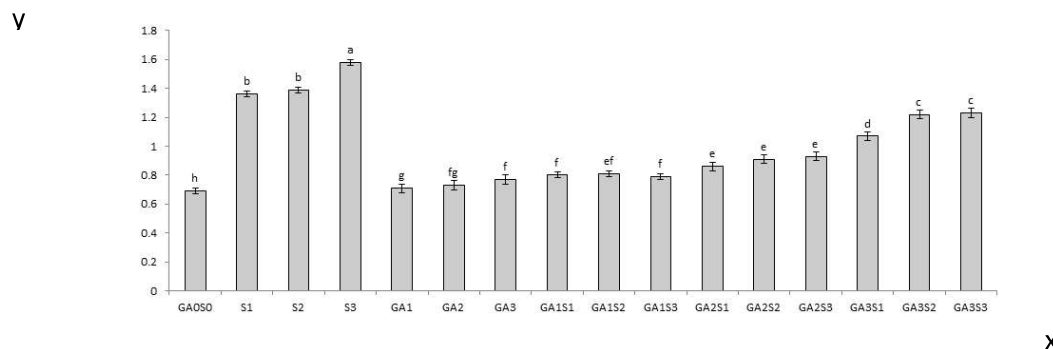


Figure (6): Effect of Gibberellin on proline content under salt stress.

Proline is preferentially accumulated in plant leaves to uphold chlorophyll levels and cell turgor, thereby safeguarding photosynthetic activity under saline stress conditions (Silva-Ortega *et al.*, 2008). The accumulation of proline in response to unfavorable conditions reflects a stress-resistant nature in many plant species. This accumulation of compatible solutes during salinity stress serves as a metabolic adaptation for osmotic balance between the vacuole and cytosol, with higher concentrations generally observed in plants exhibiting greater tolerance to salinity stress conditions (Madan *et al.*, 1995). Gibberellic acid plays a crucial role in enhancing plant tolerance to salinity by positively impacting various aspects, including growth (roots, stems, leaf area), physiological parameters (water content, and pigments), and biochemical parameters (proline) (Akoudad *et al.*, 2024).

Conclusions:

foliar spraying application of Gibberellic acid at low concentration 100 µM demonstrated positive effects in alleviating the negative impacts of high salinity levels by improving various growth and chemical characteristics.

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