

The Role of Salicylic Acid in Improving the Tolerance of Broad Bean to Salinity: A Study on Growth, Antioxidant Enzymes, And Oxidative Damage Index to Achieve Sustainable Agriculture

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Abstract

The experiment was conducted out in Anbar Governorate, Ramadi District during the 2024–2025 growing season, to study the impact of salinity levels and concentrations of salicylic acid, and their interaction on growth traits, yield and some biochemical & physiological indicators for Broad bean plant. Experimental design was based on a split-plot nested within a randomized complete block design with three replicates. The experiment included three levels of salinity (normal water, moderate salinity, and high salinity) and four doses of salicylic acid (SA) 0, 50, 100, and 150 mg l⁻¹. The results implies that increasing the salinity level led to a clear increase in the activity of the catalase enzyme and superoxide dismutase enzyme, and an increase in the level of hydrogen peroxide, with a significant decrease in all vegetative and productivity traits. High salinity (100 mM) significantly reduced plant yield from 23.69 g plant⁻¹ under normal water to 9.57 g plant⁻¹, a decrease of about 60%. The number of pods per plant decreased from 3.28 to 2.10, number of seeds per pod from 4.45 to 2.73, and pod weight from 14.00 g to 8.65 g. Seed soaking with salicylic acid at 150 mg l⁻¹ increased plant yield under high salinity to 12.21 g plant⁻¹ (an 80% increase), number of pods to 2.40 pod⁻¹, number of seeds per pod to 3.10, and pod weight to 10.30 g. The same treatment reduced hydrogen peroxide level by 43% (from 10.39 to 5.92 units) and enhanced catalase and superoxide dismutase activities to 18.50 and 15.03 units, respectively. The calculated indicators showed that the highest total activity of the two enzymes was recorded at the highest salinity level with the highest dose of SA. This was the same treatment that recorded the lowest level of hydrogen peroxide and the lowest oxidative damage index, while the highest oxidative damage was recorded under high salinity without the addition of SA. The salinity tolerance index also showed that plant yield was the most affected by salinity, while the vegetative traits were the most tolerant. We conclude from these results that SA at a concentration of 150 mg l⁻¹ is an effective technique for improving the tolerance of broad bean plants to salt stress by improving the efficiency of antioxidant systems, in a manner consistent with the sustainable development goals. It is recommended to use this concentration for soaking broad bean seeds before planting in areas that suffer from soil salinity or irrigation water salinity.

Keywords: broad bean, salinity, salicylic acid, antioxidant enzymes, oxidative damage index, sustainable development.

دور حامض الساليسليك في تحسين تحمل الباقلاء للملوحة: دراسة في النمو والإنزيمات المضادة للأكسدة ومؤشر الضرر التأكسدي لتحقيق الزراعة المستدامة

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المستخلص

نفذت هذه التجربة في محافظة الأنبار، قضاء الرامادي، خلال الموسم الزراعي 2024–2025، بهدف دراسة تأثير مستويات الملوحة وتركيز حامض الساليسليك والتفاعل بينهما في نمو وإنتاجية نبات الباقلاء، وبعض المؤشرات البيوكيميائية والفسيولوجية. استخدم تصميم الألواح المنشقة ضمن تصميم القطاعات الكاملة المعشاة بثلاثة مكررات. تضمنت التجربة ثلاثة مستويات من الملوحة (ماء عادي، ملوحة متوسطة، ملوحة عالية) وأربعة تراكيز من حامض الساليسليك (0، 50، 100، 150 ملغم لتر⁻¹). أظهرت النتائج أن زيادة مستوى الملوحة (100 ملغم لتر⁻¹) أدت إلى انخفاض ملحوظ في محصول النبات من 23.69 غم نبات⁻¹ في ظل ظروف المياه العادية إلى 9.57 غم نبات⁻¹، أي بانخفاض قدره 60% تقريباً. كما انخفض عدد القرون في النبات الواحد من 3.28 إلى 2.10، وعدد البذور في القرون الواحدة من 4.45 إلى 2.73، ووزن القرون من 14.00 غرام إلى 8.65 غرام. في المقابل، أدى نقع البذور في حمض الساليسليك بتركيز 150 ملغم لتر⁻¹ إلى زيادة محصول النبات في ظل الملوحة العالية إلى 12.21 غم نبات⁻¹ (زيادة قدرها 80%)، وزيادة عدد القرونات إلى 2.40 قرون نبات⁻¹، وعدد البذور في القرون الواحدة إلى 3.10، ووزن القرون إلى 10.30 غرام. أدى نفس العلاج إلى خفض مستوى بيروكسيد الهيدروجين بنسبة 43% (من 10.39 إلى 5.92 وحدة) وزيادة نشاط الكاتالاز وديسموتاز الفائق الأكسيد إلى 18.50 و 15.03 وحدة على التوالي. أظهرت المؤشرات المحسوبة أن أعلى مجموع لنشاط الإنزيمين سجل عند أعلى مستوى ملوحة مع التركيز الأعلى للساليسليك، وهي نفس المعاملة التي سجلت أقل مستوى لبيروكسيد الهيدروجين وأقل مؤشر ضرر تأكسدي، بينما سجل أعلى ضرر تأكسدي عند الملوحة العالية وبدون إضافة الساليسليك. كما أظهر مؤشر التحمل الملحي أن حاصل النبات كان الأكثر تأثراً بالملوحة، بينما كانت الصفات الخضريّة الأكثر تحملاً. نستنتج من هذه النتائج أن حامض الساليسليك بتركيز 150 ملغم لتر⁻¹ يعد تقنية فعالة لتحسين تحمل نبات الباقلاء للإجهاد الملحي من خلال تحسين كفاءة الانظمة المضادة للأكسدة بما ينسجم مع أهداف التنمية المستدامة، ويوصى باستخدام هذا التركيز لنقع بذور الباقلاء قبل الزراعة في المناطق التي تعاني من ملوحة التربة أو مياه الري.

الكلمات المفتاحية: الباقلاء، ملوحة، حامض الساليسليك، إنزيمات مضادة للأكسدة، مؤشر الضرر التأكسدي، أهداف التنمية المستدامة.

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Introduction

Broad beans (*Vicia faba* L.) are a globally important food and forage legume, valued for their high protein, fiber, and mineral content, as well as their role in improving soil fertility through biological nitrogen fixation (Yehmed et al., 2024). Global production reached approximately 1.81 million tons for green beans and 6.77 million tons for dry beans in 2022, with China and Ethiopia being the leading producers (Raman et al., 2024).

Salinity is a major abiotic stress limiting crop productivity in arid and semi-arid regions, affecting over 20% of irrigated lands worldwide—a proportion expected to rise due to climate change and unsustainable agricultural practices (Qadir and Oster, 2026; El-Sayed et al., 2025). Salinity induces osmotic stress, ionic toxicity, and oxidative imbalance through excessive production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide radicals (Challabathula et al., 2022). While low H_2O_2 levels act as signaling molecules, their accumulation damages membranes, DNA, and proteins, ultimately reducing growth and yield (Iqbal and Chen, 2025). Plants counteract ROS via antioxidant enzymes including catalase (CAT) and superoxide dismutase (SOD) (Kesawat et al., 2023).

Among various strategies to mitigate salt stress, salicylic acid (SA) a key signaling molecule—has gained considerable attention for enhancing salt tolerance by boosting antioxidant enzyme activity, improving chlorophyll content, and reducing ion toxicity (Hayat et al., 2025; El-Beltagi et al., 2025; Ogunsij et al., 2023).

Despite the importance of broad bean, research on the combined effects of salt stress and SA seed soaking on this crop remains limited, particularly regarding the relationships among enzyme activity, H_2O_2 levels, and yield. This study aimed to evaluate the effects of different salinity levels and salicylic acid concentrations on the growth and productivity of broad bean as a crop affected by soil degradation associated with desertification. It also assessed oxidative stress indicators, analyzed physiological indices related to salt tolerance, and examined the relationship between H_2O_2 levels and yield to clarify tolerance mechanisms under salinity conditions in degraded and desertification-prone environments.

Materials and Methods

This experiment was conducted in Anbar Governorate, Ramadi District, on a farmer's field during the 2024-2025 growing season. The Ramadi region is characterized by an arid and semi-arid climate, where agricultural lands are exposed to increasing salinity problems as a result of climatic conditions and the irrigation methods followed.

The broad bean crop was used in this experiment (the local cultivar available in the local markets). Three levels of saline water were prepared: normal water, medium salinity (50 mM), and high salinity (100 mM). This was achieved by adding 2.922 g of table salt per liter of water to the first level and 5.844 g of table salt per liter of water to the second level. SA was also used at four concentrations: 0, 50, 100, and 150 mg of SA per liter of water.

The experiment was implemented using a split-plot arrangement RCBD Design, where salinity levels (three levels) represented the main plots, while SA concentrations (four concentrations) represented the subplots. The experiment was carried out with three replicates. The total number of treatments was 36 experimental units, and the dimensions of the experimental unit were 2×2.5 m. Samples were taken and chemical and physical analysis was performed on the field soil (Table 1).

Broad bean seeds were soaked before planting in SA solutions at its four concentrations for a full 24 hours before planting (Al-Siriti et al., 2025). After the soaking period ended, the seeds were slightly dried and then planted in their assigned experimental units on 1 November 2024. Planting was done in hills, where three seeds were placed in each hill. The space between one hill and the next was 25 cm, while the distance between the lines measured 75 cm. After the saplings reached the stage of 3–4 leaves, the extra plants were thinned.

All agricultural service operations, including plowing, leveling, hoeing, and pest control, were carried out according to the scientific recommendations recommended for the broad bean crop in the region. Irrigation was carried out with the three salinity levels throughout the growth season by preparing the saline solution in 2000-L tanks, and then the experimental units were irrigated using water pipes, while taking into account the arrival of an equal amount of water to each experimental unit. Fertilization was applied at a rate of 40 kg nitrogen and 20 kg superphosphate fertilizer per donum (Mam-Rasul, 2018).

Studied traits:

The following traits were estimated for ten guarded plants from each experimental unit.

First: Biochemical traits

They were studied during the stage of complete vegetative growth and before flowering by taking samples from plant leaves, which were transferred directly to the laboratory. The analyses included the following:

1. Catalase enzyme activity: It was estimated according to the method of Aebi (1984), and was estimated in the unit of $\mu\text{mol g}^{-1}$ fresh weight (FW).
2. Superoxide dismutase enzyme activity: It was estimated according to the method of Beyer and Fridovich (1987), and was estimated in the unit of unit $\mu\text{mol g}^{-1}$ FW.

3. Hydrogen peroxide level: It was estimated according to the method of Jana and Choudhuri (1982), and was estimated in the unit of unit $\mu\text{mol g}^{-1}$ FW.

Second: Vegetative traits

Measurements of the vegetative traits were taken at the completion of the flowering process and included:

1. Plant height (cm per plant): The height of the plant was measured from the soil surface to the apex of the main stem.
2. Total chlorophyll content: It was measured using a SPAD device.
3. Number of branches per plant: The lateral branches of the plants were counted.

Third: Yield traits and its components

1. Number of pods per plant: All mature pods per plant were counted.
2. Number of seeds per pod: Ten random pods were taken from each guarded plant in each experimental unit. The seeds were removed and counted, and the average number of seeds per pod was calculated.
3. Seed weight in one pod (g pod^{-1}): The seeds of each pod were weighed using a sensitive balance.
4. Weight of one pod (g pod^{-1}): The pods were weighed after drying.
5. Yield of one plant (g plant^{-1}): The total seed weight for each plant was calculated.

Fourth: Calculated physiological indices

1. Ratio of catalase to superoxide dismutase: It was estimated by dividing the value of catalase activity by the value of superoxide dismutase activity for each treatment (Ritter et al., 2006).
2. Salinity tolerance index: It was calculated according to the equation adopted by IUPAC (2009):
3. Salinity tolerance index = (trait value under the high salinity level $\div$ trait value under the normal water level) $\times 100$.
4. Oxidative damage index: It was calculated according to the equation adopted by Aiyaz et al. (2021):
5. Oxidative damage index = (hydrogen peroxide level $\div$ total activity of catalase and superoxide dismutase) $\times 100$.

Statistical Analysis

Data were statistically analyzed using the GenStat software package, and the Least Significant Difference test at a probability level of 0.05 was employed for comparison among arithmetic means (Suza and Lamkey, 2023). Furthermore, simple correlation coefficients were calculated using Pearson's correlation coefficient among treatments and studied traits, and the relationship between hydrogen peroxide level and yield per plant was plotted. These analyses were performed using the statistical software package SPSS, version 27 (George and Mallery, 2022).

Table 1. Some physical and chemical properties of the experimental field soil before the experiment

Value	Unit	Trait
7.45	-	pH (Soil reaction)
1.89	dS m^{-1} (decisiemens per meter)	Electrical Conductivity (EC)
75	mg kg^{-1}	(N) content
23.3	Ppm	(P) content
25.78	mg kg^{-1}	(K) content
985	ppm	TDS
300.4	mg l^{-1}	Ca
50.1	mg l^{-1}	Mg
46.79	meq L^{-1}	Na
15.76	meq L^{-1}	So ₂
Soil Texture		
24	%	Clay
41.2	%	Silt
34.8	%	Sand
Sandy Loam	Soil Texture	

Results and Discussion

First: Biochemical traits: The results in Table 2 showed significant effects of salinity levels, SA concentrations, and their interaction on catalase (CAT) and superoxide dismutase (SOD) activities, as well as hydrogen peroxide (H_2O_2) levels in broad bean leaves.

Increasing salinity significantly enhanced the activity of both antioxidant enzymes. CAT activity increased from 11.14 units under normal water to 12.99 units at moderate salinity (50 mM) and to 15.01 units under high salinity

(100 mM), representing a 35% increase over the control. Similarly, SOD activity rose from 7.08 units under normal water to 9.84 units at moderate salinity and to 11.45 units under high salinity, a 62% increase. In contrast, H_2O_2 levels were lowest under moderate salinity (6.66 units) but increased markedly to 7.95 units under high salinity, indicating that ROS production surpassed the capacity of the antioxidant defense system.

SA seed soaking at increasing concentrations progressively enhanced antioxidant enzyme activities while decreasing H_2O_2 levels (Table 2). In the control treatment (without SA), CAT activity was 10.24 units, which increased to 12.30, 13.94, and 15.71 units at 50, 100, and 150 mg l⁻¹ SA, respectively. Likewise, SOD activity increased from 6.12 units in the control to 9.01, 10.94, and 11.76 units at the three SA concentrations. Concurrently, H_2O_2 levels decreased from 8.50 units in the control to 7.67, 6.80, and 6.13 units at 50, 100, and 150 mg l⁻¹ SA, respectively, representing a 28% reduction at the highest concentration.

The interaction between salinity and SA concentrations revealed a complex but clear response pattern (Table 2). Under normal water, SA addition gradually increased CAT activity from 9.55 units (control) to 13.49 units at 150 mg l⁻¹ SA, and SOD from 5.44 to 8.15 units. Under moderate salinity, CAT increased from 10.14 to 15.14 units, SOD from 6.07 to 12.11 units, while H_2O_2 decreased substantially from 8.40 to 5.18 units (a 38% reduction). Under high salinity, the most pronounced responses were observed: CAT activity increased from 11.02 units (without SA) to 18.50 units at 150 mg l⁻¹ SA, SOD from 6.84 to 15.03 units, while H_2O_2 dropped sharply from 10.39 to 5.92 units, a 43% reduction.

Notably, CAT and SOD activities increased in parallel across all treatments, indicating coordinated regulation of these enzymes under salt stress, with SA further enhancing this protective synergy. The treatment combining high salinity with 150 mg l⁻¹ SA consistently recorded the highest enzyme activities and the lowest H_2O_2 levels, confirming that SA seed soaking at 150 mg l⁻¹ effectively enhances ROS scavenging, reduces oxidative damage, and maintains cellular integrity, which positively influenced subsequent growth and yield. These findings are consistent with Souana et al. (2025), Layachi (2024), and Hayat et al. (2025), who reported that SA enhances antioxidant enzyme activity and reduces H_2O_2 accumulation under salt stress.

Table 2. Effect of salinity levels and salicylic acid concentrations on catalase (CAT) activity, superoxide dismutase (SOD) activity, and hydrogen peroxide (H_2O_2) content in faba bean leaves

Treatment	CAT	L.S.D	SOD	L.S.D	H2O2	L.S.D
S0	11.135	0.465	7.077	0.412	7.212	0.514
S1	12.990		9.842		6.660	
S2	15.012		11.454		7.949	
SA0	10.236	0.537	6.116	0.475	8.500	0.594
SA1	12.296		9.012		7.667	
SA2	13.940		10.940		6.796	
SA3	15.709	0.930	11.763	0.823	6.133	1.028
S0 SA0	9.549		5.440		6.710	
S0 SA1	10.469		7.130		7.800	
S0 SA2	11.030		7.590		7.040	
S0 SA3	13.490		8.150		7.300	
S1 SA0	10.140		6.070		8.400	
S1 SA1	12.810		9.920		7.053	
S1 SA2	13.870		11.270		6.010	
S1 SA3	15.139		12.110		5.180	
S2 SA0	11.020		6.840		10.389	
S2 SA1	13.610		9.986		8.150	
S2 SA2	16.920		13.959		7.340	
S2 SA3	18.500		15.030		5.919	

Second: Vegetative traits: plant height, chlorophyll content, and number of branches.

Salinity significantly reduced all vegetative traits (Table 3). Under high salinity (100 mM), plant height decreased by 29% (from 46.30 to 32.73 cm), chlorophyll content by 36% (from 67.50 to 43.00 SPAD units), and branch number by 19% (from 3.50 to 2.85 branches plant⁻¹) compared to normal water.

SA seed soaking at 150 mg l⁻¹ improved plant height by 20%, chlorophyll by 13%, and branch number by 19% compared to the control without SA.

The best vegetative performance was recorded under normal water with 150 mg l⁻¹ SA (plant height: 50.60 cm; chlorophyll: 73.00 SPAD; branches: 3.80 plant⁻¹), while the lowest values were under high salinity without SA (29.40 cm, 41.00 SPAD, 2.60 branches plant⁻¹). Under high salinity, the same SA treatment (150 mg l⁻¹) increased

plant height to 35.20 cm (20% increase), chlorophyll to 44.00 SPAD 7% increase, and branch number to 3.20 plant⁻¹ 23% increase (Table 3).

The salinity-induced reductions in vegetative traits are consistent with Anaya et al. (2025) and Dawood et al. (2022), who attributed similar declines to H₂O₂-mediated oxidative damage affecting membranes, chloroplasts, and cell division. In the present study, H₂O₂ levels reached 10.39 units under high salinity without SA, supporting this mechanism. Osmotic stress and hormonal disruption further contributed to reduced growth.

The magnitude of reduction in plant height 29% was less severe than that reported by El-Sayed et al. (2025) 42% but greater than Souana et al. (2025) 18%, likely due to differences in cultivar tolerance, growth duration, or environmental conditions.

SA seed soaking at 150 mg l⁻¹ improved vegetative traits by reducing H₂O₂ (from 10.39 to 5.92 units) and enhancing CAT and SOD activities (from 11.02 to 18.50 and from 6.84 to 15.03 units, respectively). This aligns with Hayat et al. (2025), who reported SA-mediated antioxidant activation. Similarly, Uçar et al. (2024) found that foliar SA at 150 mg l⁻¹ improved faba bean growth, supporting our findings with seed soaking. The preservation of photosynthetic efficiency and cell division confirms SA as an effective strategy for mitigating salinity stress (El-Beltagi et al., 2025; Ogunsiji et al., 2023).

Table 3. Effect of salinity levels and salicylic acid concentrations on plant height, chlorophyll content, number of branches, and number of pods of faba bean (*Vicia faba* L.)

Treatment	Plant Height (cm)	L.S.D	Total chlorophyll (SPAD units)	L.S.D	Number of branches per plant	L.S.D	Number of pods per plant	L.S.D
S0	46.300	1.198	67.500	2.735	3.500	0.219	3.275	0.224
S1	40.775		59.250		3.125		2.775	
S2	32.725		43.000		2.850		2.100	
SA0	36.166	1.384	52.666	3.158	2.866	0.253	2.166	0.259
SA1	38.966		56.000		2.933		2.666	
SA2	41.099		58.000		3.433		2.833	
SA3	43.500		59.666		3.400		3.200	
S0 SA0	42.900	2.397	63.000	5.469	3.000	0.439	2.600	0.448
S0 SA1	44.400		66.000		3.199		3.199	
S0 SA2	47.300		68.000		3.999		3.200	
S0 SA3	50.600		73.000		3.800		4.100	
S1 SA0	36.199		54.000		3.000		2.300	
S1 SA1	39.800		59.000		3.000		2.700	
S1 SA2	42.400		62.000		3.300		3.000	
S1 SA3	44.699		62.000		3.199		3.100	
S2 SA0	29.400		41.000		2.600		1.599	
S2 SA1	32.699		43.000		2.600		2.100	
S2 SA2	33.600		44.000		3.000		2.300	
S2 SA3	35.199		44.000		3.199		2.400	

Third: Yield traits and its components: number of pods, number of seeds per pod, seed weight per pod, pod weight, and yield of one plant.

Salinity significantly reduced all yield traits (Table 4). Under high salinity 100 mM, plant yield showed the greatest reduction 60%, from 23.69 to 9.57 g plant⁻¹, followed by number of seeds per pod 39%, from 4.45 to 2.73 seeds pod⁻¹, pod weight 38%, from 14.00 to 8.65 g, number of pods per plant 36%, from 3.28 to 2.10 pods plant⁻¹, and seed weight per pod 40%, from 7.08 to 4.25 g.

The percentage decrease in plant yield 60% was greater than that observed for vegetative traits plant height: 29%; branch number: 19%, indicating that the reproductive stages (flowering, pod setting, and seed formation) are more sensitive to salinity than vegetative growth.

SA seed soaking improved all yield traits in a dose-dependent manner (Table 4). The highest SA concentration 150 mg l⁻¹ increased plant yield by 107% under normal water from 14.73 to 34.91 g plant⁻¹ and by 80% under high salinity from 6.76 to 12.21 g plant⁻¹ compared to untreated controls. Similar improvements were observed for number of pods (from 1.60 to 2.40 pods plant⁻¹, number of seeds per pod from 2.30 to 3.10 seeds pod⁻¹,

seed weight per pod from 3.10 to 5.10 g, and pod weight from 6.40 to 10.30 g under high salinity with SA seed soaking at 150 mg l⁻¹.

The interaction results revealed that the highest yield was achieved under normal water with 150 mg l⁻¹ SA 34.91 g plant⁻¹, while the lowest was under high salinity without SA 6.76 g plant⁻¹ (Table 4).

High salinity 100 mM reduced plant yield by 60%, number of seeds per pod by 39%, pod weight by 38%, and number of pods per plant by 36%. These results are consistent with Uçar et al. (2024), who reported a 55% yield reduction in faba bean under similar salinity levels, and with El-Sayed et al. (2025), who found that yield components were more sensitive to salinity than vegetative traits.

However, the magnitude of yield reduction in the present study 60% was greater than that reported by Anaya et al. (2025), who observed a 45% reduction under comparable salinity conditions. This discrepancy may be attributed to differences in cultivar tolerance, duration of stress exposure, or the growth stage at which salinity was applied. Souana et al. (2025) also reported a less severe reduction 48%, possibly due to the use of a more salt-tolerant faba bean variety or different growing conditions.

The greater sensitivity of yield traits compared to vegetative traits (as indicated by the Salt Tolerance Index, Table 5) is due to the high energy demands of flowering, fertilization, and seed filling, which are easily disrupted by oxidative stress. The biochemical results of the present study support this mechanism, as H₂O₂ levels increased to 10.39 units under high salinity without SA (Table 2), causing oxidative damage to floral and fruit tissues. Additionally, the 36% reduction in chlorophyll content (Table 3) under high salinity inhibited photosynthesis, reducing carbohydrate availability for seed filling. Salinity also disrupts water and nutrient balance and impairs assimilate translocation from leaves to pods and seeds, leading to flower drop and incomplete pod setting.

The improvement in yield traits with SA seed soaking (especially at 150 mg l⁻¹) can be explained by the observed reduction in H₂O₂ levels (from 10.39 to 5.92 units, Table 2) and enhanced CAT and SOD activities (from 11.02 to 18.50 and from 6.84 to 15.03 units, respectively). This finding agrees with Hayat et al. (2025), who reported that SA activates antioxidant defense systems and protects floral tissues from oxidative damage. Similarly, El-Beltagi et al. (2025) found that foliar SA improved yield components of faba bean under salinity by enhancing photosynthetic efficiency and reducing ion toxicity.

The 80% increase in plant yield under high salinity with SA seed soaking (from 6.76 to 12.21 g plant⁻¹) is consistent with Ogunsiji et al. (2023), who reported that SA treatment improved pod and seed formation under salt stress by reducing flower drop and increasing assimilate translocation. Layachi (2024) also confirmed that SA seed soaking at 150 mg l⁻¹ was the most effective concentration for improving faba bean yield under saline conditions, which aligns perfectly with the present study.

The greater improvement in yield under normal water with SA (107% increase) compared to under high salinity (80% increase) suggests that SA has a synergistic effect with optimal growing conditions, while still providing substantial protection under stress. This observation is supported by Dawood et al. (2022), who noted that SA not only mitigates stress damage but also promotes growth under non-stress conditions.

Fourth: Calculated physiological indices:

Ratio of catalase to superoxide dismutase:

This ratio expresses the balance between the two-antioxidant enzymes, where its increase indicates the dominance of catalase activity, and its decrease indicates the dominance of superoxide dismutase activity or joint regulation between them. Through Table 5, we notice that the highest value of this ratio was recorded in the treatment that combined irrigation with normal water and without the addition of salicylic acid, where it reached 1.76, while the lowest value was recorded in the treatment that combined the highest salinity level and the second concentration of salicylic acid, where it reached 1.21, as well as in the treatment that combined the highest salinity level and the third concentration, where it reached 1.23. Under irrigation conditions with normal water, the ratio was relatively high, where it ranged between 1.45 and 1.76. When salicylic acid was added, a slight decrease in the ratio was observed at the first and second concentrations, then a slight increase at the third concentration, which indicates balanced regulation of the activity of the two enzymes.

Likewise, under moderate salinity conditions, the ratio was high in the control treatment (1.67), then it decreased clearly when salicylic acid was added to 1.29, 1.23, and 1.25, respectively. This decrease indicates that the addition of salicylic acid led to an increase in superoxide dismutase activity greater than the increase in catalase activity. Under high salinity conditions, the ratio decreased from 1.61 without salicylic acid to 1.36, 1.21, and 1.23 when salicylic acid was added, and the lowest ratio was at the second concentration (1.21).

It can be said that the decrease in the ratio of catalase to superoxide dismutase under high salinity conditions when salicylic acid was added reflects the ability of salicylic acid to stimulate both enzymes in a balanced

manner. Which contributed to reducing the level of hydrogen peroxide from 10.39 to 5.92 units, and this decrease was associated with a marked improvement in vegetative growth and yield

Table 4. Effect of salinity levels and salicylic acid concentrations on number of seeds per pod, seed weight per pod, pod weight, and yield per plant of faba bean

Treatment	Number of seeds per pod	L.S.D	Seed weight per pod	L.S.D	Pod weight (g)	L.S.D	Yield per plant (g)	L.S.D
S0	4.450	0.203	7.075	0.271	14.000	0.351	23.693	1.440
S1	3.574		5.175		10.150		14.575	
S2	2.733		4.249		8.650		9.574	
SA0	3.099	0.235	4.399	0.313	8.833	0.406	10.515	1.663
SA1	3.400		5.400		10.600		14.896	
SA2	3.777		5.733		11.533		16.583	
SA3	4.066		6.466		12.766		21.795	
S0 SA0	3.800	0.406	5.700	0.543	11.400	0.703	14.733	2.881
S0 SA1	4.200		6.900		13.600		22.106	
S0 SA2	4.700		7.200		14.400		23.023	
S0 SA3	5.100		8.499		16.599		34.910	
S1 SA0	3.199		4.399		8.700		10.050	
S1 SA1	3.400		5.100		9.899		13.803	
S1 SA2	3.700		5.399		10.600		16.183	
S1 SA3	4.000		5.800		11.400		18.266	
S2 SA0	2.300		3.100		6.399		6.763	
S2 SA1	2.600		4.200		8.299		8.780	
S2 SA2	2.933		4.600		9.600		10.543	
S2 SA3	3.100		5.100		10.299		12.210	

Sum of the activity of the two enzymes:

The combined activity of the two enzymes represents the overall capacity of the plant's antioxidant defense system. The higher this total is, the greater the plant ability to face oxidative stress. From the results of Table 5, we notice that the highest value was recorded at the high salinity level with the highest concentration of salicylic acid, reaching 33.53, while the lowest value was recorded in the normal irrigation water treatment and the salicylic acid control treatment, reaching 14.99. The remaining values ranged between 14.99 and 21.64, with a gradual increase when the concentration of salicylic acid increased. Under moderate salinity conditions, the total ranged between 16.21 and 27.25. Under high salinity conditions, the total ranged between 17.86 and 33.53, with a very large increase when salicylic acid was added.

It is important to note that the highest total activity of the two enzymes was recorded in the high salinity treatment with the highest concentration of salicylic acid. It is the same treatment that recorded the lowest level of hydrogen peroxide of 5.92 units, which indicates that the plant greatly activated its defense system under the effect of salicylic acid, which enabled it to remove the large amounts of reactive oxygen species.

Oxidative damage index:

This index expresses the ratio of hydrogen peroxide to the total activity of the two enzymes. The lower this ratio is, the greater the capacity of the defense system to remove hydrogen peroxide, and therefore the lower the oxidative damage.

From the results of Table 5, we notice that the lowest oxidative damage of 17.7 was recorded in the treatment S2 SA3, while the highest oxidative damage of 58.2 was recorded in the treatment of the high salinity level (100 mmol l⁻¹). Under irrigation conditions with normal water, the index ranged between 33.7 and 44.8, with a gradual decrease as salicylic acid concentration increased. Under moderate salinity conditions, the index decreased from 51.8 without salicylic acid to 19.0 at the third concentration, with a decrease of 63%. Under high salinity conditions, the index decreased from 58.2 to 17.7, with a decrease of 70%. It is very important to note that the decrease in the oxidative damage index was inversely associated with the improvement in vegetative growth and yield. The treatment S2 SA3, which recorded the lowest damage index 17.7, is the same treatment that recorded the highest improvement in vegetative traits under high salinity, as well as the highest plant yield 12.21 g under the same salinity level.

Table 5. Oxidative Stress Index (OSI) of faba bean as affected by salinity and salicylic acid treatments

Treatment	CAT/SOD	SOD + CAT	H ₂ O ₂	OSI
S0 SA0	1.76	14.989	6.71	44.8
S0 SA1	1.47	17.599	7.8	44.3
S0 SA2	1.45	18.62	7.04	37.8
S0 SA3	1.66	21.64	7.3	33.7
S1 SA0	1.67	16.21	8.4	51.8
S1 SA1	1.29	22.73	7.05	31
S1 SA2	1.23	25.14	6.01	23.9
S1 SA3	1.25	27.249	5.18	19
S2 SA0	1.61	17.86	10.39	58.2
S2 SA1	1.36	23.596	8.15	34.5
S2 SA2	1.21	30.879	7.34	23.8
S2 SA3	1.23	33.53	5.92	17.7

By reviewing all the calculated indices together, it can be noticed that whenever the total activity of the two enzymes increased, the level of hydrogen peroxide decreased and the oxidative damage index decreased. Also, the decrease in the oxidative damage index was associated with a clear improvement in all vegetative and productivity traits. The third concentration of salicylic acid was the most effective in reducing the oxidative damage index under all salinity levels.

The general conclusion of the calculated indices confirms the effective role of salicylic acid in enhancing the antioxidant defense system in broad bean plants. The addition of salicylic acid, especially at the third concentration, led to a large increase in the total activity of the two enzymes, which led to reducing the level of hydrogen peroxide and consequently decreasing the oxidative damage index by up to 70% under high salinity conditions. This decrease in oxidative damage created suitable internal conditions for growth and production, which was reflected positively on all vegetative and productivity traits.

Salinity tolerance index

After the effect of salinity on the different traits was presented, and to evaluate the extent of broad bean plant tolerance to salt stress quantitatively, the salinity tolerance index was calculated for each of the main traits. This index expresses the percentage of the trait value under high salinity conditions compared with its value under normal water conditions. The higher this percentage is, the greater the tolerance to salinity.

The values of the salinity tolerance index ranged between 40.4% and 70.7%, which indicates that high salinity negatively affected all studied traits, but at different degrees (Table 6). The results showed that the lowest value of the salinity tolerance index was recorded for the trait of plant yield, where it reached 40.4%. This means that the plant lost about 60% of its production under high salinity conditions. In contrast, the vegetative traits, plant height and number of branches, were the most tolerant, as they recorded the highest index value, which reached 70.7%. The intermediate values were recorded for the trait of number of pods 64.1%, chlorophyll content 63.7%, pod weight 61.8%, and seed weight per pod 60.1%. This order reveals that the vegetative traits were the most tolerant to salinity, followed by yield component traits, while total plant yield was the most affected.

The lower effect on the vegetative traits is due to the fact that the plant tries to maintain its vegetative growth as an attempt to survive. The biochemical results showed that the plant activated its defense system, where the total activity of the two enzymes increased from 14.99 to 17.86 under high salinity. The greater effect on yield traits is due to the high sensitivity of yield traits, because the processes of flowering, setting, and seed formation require high energy. The results showed that salinity led to an increase in hydrogen peroxide to 10.39 units, which caused oxidative damage to floral and fruit tissues. As for plant yield, it was the most affected because yield represents the outcome of the interaction of all other traits. Any decrease in any of these traits is reflected cumulatively on the final yield.

It is important to note that the addition of salicylic acid led to a large improvement in the salinity tolerance index. When comparing the high-salinity treatment without salicylic acid yield of 6.76 g with the high-salinity treatment with the third concentration of salicylic acid yield of 12.21 g, the improved salinity tolerance index reached 82.9%, which is much better than the percentage of 40.4% recorded without the addition of salicylic acid.

The results of the salinity tolerance index showed that high salinity negatively affected all traits at different degrees. The vegetative traits were the most tolerant, and plant yield was the most affected. The results also indicate that the use of salicylic acid can greatly improve the index.

Table 6. Salt Tolerance Index (STI) for different traits of faba bean under saline conditions

Trait	Value at S0	Value at S2	STI (%)
Plant yield (g)	23.693	9.574	40.4
Number of pods	3.275	2.100	64.1
Pod weight (g)	14.000	8.650	61.8
Seed weight per pod (g)	7.075	4.249	60.1
Plant height (cm)	46.300	32.725	70.7
Chlorophyll (SPAD)	67.500	43.000	63.7

Correlation analysis among the studied traits

After completing the presentation of the detailed results, correlation analysis was conducted to determine the nature and strength of the relationships among the different traits. From the results of the correlation analysis table (Table 7), we notice that there were strong positive correlation values among the productivity traits. Plant yield recorded the same correlation value with the number of pods, number of seeds, seed weight per pod, and pod weight, reaching 0.98. Meanwhile, the number of seeds was associated with seed weight per pod and pod weight by the highest positive correlation, reaching 0.99. These correlations confirm that improving any of these traits is reflected positively on the total yield.

As for the correlation relationship among the vegetative traits, it was positive. Correlation values of 0.97 were recorded between plant height and chlorophyll, and 0.88 between plant height and number of branches. The correlation between chlorophyll and number of branches reached 0.83. These correlations confirm that the improvement of vegetative growth in general is associated with the improvement of all its components. As for the correlations between vegetative and productivity traits, positive correlation values were recorded. The correlation between plant height and yield reached 0.94, between chlorophyll and plant yield 0.92, and between number of branches and plant yield 0.83. These correlations confirm that the improvement of vegetative growth is reflected positively on final productivity. We also notice that the correlations between the antioxidant enzymes were positive, as catalase with superoxide dismutase recorded a very strong correlation of 0.99, indicating joint regulation. Catalase with grain yield recorded a moderate correlation of 0.66. Likewise, superoxide dismutase with plant yield recorded a moderate correlation of 0.61. These moderate correlations indicate that the increase in enzyme activity was a response to stress, but it was not sufficient to completely eliminate it.

As for the negative correlations, hydrogen peroxide was negatively correlated with yield, reaching -0.79. The number of pods was correlated with the number of seeds, seed weight per pod, plant yield, hydrogen peroxide, chlorophyll, and plant height by negative correlation relationships that reached -0.73. These strong negative correlations confirm the toxic role of hydrogen peroxide when it accumulates in large quantities inside the cells, -0.78.

Correlation analysis reveals a complex network of relationships among the studied traits. There were strong positive correlations among all vegetative and productivity traits, which confirms that improving vegetative growth is reflected positively on yield. There was also a very strong positive correlation between the two antioxidant enzymes, indicating their joint regulation. In contrast, there were strong negative correlations between hydrogen peroxide and most vegetative and productivity traits, which confirms the negative role of the accumulation of this compound under salt stress conditions. These results strongly support that salicylic acid improves growth and yield by reducing hydrogen peroxide level and enhancing the activity of antioxidant enzymes.

Table 7. Pearson correlation matrix among all studied traits of faba bean under salinity and salicylic acid.

	Plant Height (cm)	Total chlorophyll (SPAD units)	Number of branches per plant	Number of pods per plant	Number of seeds per pod	Seed weight per pod	Pod weight (g)	Yield per plant (g)	CAT	SOD	H ₂ O ₂
Plant Height (cm)	1										
Total chlorophyll (SPAD units)	0.97	1									
Number of branches per plant	0.88	0.83	1								
Number of pods per plant	0.96	0.94	0.84	1							
Number of seeds per pod	0.96	0.95	0.85	0.99	1						
Seed weight per pod	0.95	0.94	0.84	0.99	0.99	1					
Pod weight (g)	0.96	0.94	0.84	0.99	0.99	0.99	1				
Yield per plant (g)	0.94	0.92	0.83	0.98	0.98	0.98	0.98	1			
CAT	0.58	0.48	0.68	0.61	0.63	0.61	0.63	0.66	1		
SOD	0.53	0.43	0.65	0.56	0.58	0.56	0.58	0.61	0.99	1	
H ₂ O ₂	-0.81	-0.74	-0.65	-0.78	-0.79	-0.79	-0.78	-0.78	-0.2	-0.17	1

Conclusion

Irrigation with saline water (100 mM) caused a 60% reduction in faba bean yield, accompanied by significant increases in catalase (CAT) and superoxide dismutase (SOD) activities, as well as hydrogen peroxide (H₂O₂) levels. Seed soaking with salicylic acid at 150 mg l⁻¹ effectively mitigated salt damage, reducing H₂O₂ by 43% and increasing plant yield under high salinity by 80%. The treatment combining high salinity with the highest SA concentration 150 mg l⁻¹ recorded the maximum total activity of CAT and SOD and the lowest oxidative damage index, confirming the pivotal role of SA in activating the antioxidant defense system. Correlation analysis revealed strong positive correlations among vegetative and yield traits, while H₂O₂ exhibited strong negative correlations with most traits, underscoring its toxic role under salt stress.

Sustainability Statement

This study demonstrates the role of salicylic acid in improving the tolerance of broad bean (*Vicia faba* L.) to salinity stress through enhancing growth performance, antioxidant enzyme activity, and reducing oxidative damage. The findings support sustainable agricultural strategies aimed at improving crop resilience in saline environments using eco-friendly plant growth regulators, thereby contributing to food security and sustainable production systems.

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Data Availability Statement

The data supporting the findings of this study are available from the author upon reasonable request. All data generated or analyzed during this study are included in the published article and its supplementary materials, where applicable.

Author Contributions (CRediT)

Single author: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Conflict of Interest

The author declares no conflict of interest.

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