Effects of wounding and salinity stress on faba bean (*Vicia faba*):

role of salicylic acid in plant defense responses

By:

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Abstract

Plants are often exposed to multiple stress factors at the same time, and one stress can influence how a plant responds to another. While salinity and mechanical wounding are both known to affect plant growth and physiology, their combined effects, and the role of salicylic acid (SA), are not well understood in Faba bean (*Vicia faba)*. In this study, Faba bean (*Vicia faba* cv. Broad Windsor) plants were exposed to salinity (150 mM NaCl), mechanical wounding, and foliar application of SA (0.5 mM) in different combinations. Several physiological, biochemical, and growth parameters were measured, including chlorophyll fluorescence (Fv/Fm), stomatal conductance, transpiration, biomass, water content, protein content, chlorophyll levels, and leaf elemental composition. Salinity had the strongest effect on the plants as it significantly reduced stomatal conductance, transpiration, and shoot biomass, while increasing the accumulation of Na, Cl, and N in the leaves. Fv/Fm values remained relatively stable, suggesting that photosystem II was not severely affected. An unexpected infection by *Colletotrichum* sp. caused high levels of necrosis in control plants, while salt-treated plants showed less injury. Chlorophyll and protein levels were higher in salt-treated plants, likely because these plants had less fungal damage. Wounding had only minor effects overall, with small changes in water content and shoot biomass, and slightly lower levels of necrosis. Salicylic acid did not have a significant effect on most measured parameters. Overall, these results show that salinity was the main factor affecting plant responses and may also provide indirect protection against pathogen infection. This study highlights the importance of considering multiple stresses and unexpected factors, such as pathogen presence, when studying plant stress responses.

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1 - Introduction

Salinity stress is an important issue in the world as it impacts plant growth and crop yield; around 25% of land is affected by high salinity in soil (Shokri et al, 2024). This stress is a detrimental abiotic factor encountered by plants and can be defined as the concentration of salt cations (Na^+, Ca^{2+}, Mg^{2+}, and K^+) and anions (Cl^-, SO_4^{2-}, HCO_3^-, and NO_3^-) present in the soil (Tanji, 2002). The threshold for saline soil is an electrical conductivity greater than 4 dS/m (Hanin et al., 2016). Excess salt in soil can occur through salt deposits, marsh areas, mining, agricultural practices such as overirrigation and de-icing of roads (Bai et al, 2016). Salinity stress imposes physiological strain on plants, disrupting osmotic balance and photosynthesis, which can result in stunted growth, chlorosis, and increased oxidative stress (Zhang et al, 2025). The osmotic stress is caused by an increase in salts which lowers the soil water potential and reduces the plant water uptake (Zhang et al, 2025). The increase in salt ions specifically Na^+ and Cl^- leads to ion toxicity disrupting plant membranes and reducing nutrient uptake through ion competition (Jan et al, 2024). In addition, ROS (Reactive Oxygen Species) accumulation in plants leads to oxidative stress, which damages DNA, proteins, and membrane lipids. This disrupts cellular function, compromises membrane integrity and reduces photosynthesis efficiency due to damage of photosystems II and I in chloroplasts (Stefanov et al, 2023). Tolerant plants have developed several survival mechanisms such as sequestering salt ions in salt bladders or vacuoles to reduce the effects of ion toxicity (Yamada et al, 2025). Some plants can also increase the number of membrane transporters and perform selective ion uptake of K^+ instead of Na^+ to maintain nutrient balance (Othman et al, 2023). To deal with the osmotic stress, plants can produce osmolytes, increase the aquaporins, and/or produce longer roots for water uptake (Lui et al, 2014). Plants

can manage oxidative stress by producing antioxidants (Rao et al, 2025), dissipating energy through reactions such as the xanthophyll cycle; they can also convert excess energy through photorespiration (González-Moro et al, 2003).

Mechanical wounding is a common biotic stress that can be caused by animals or human activities; herbivory attacks and agricultural practices (Myers et al, 2022). This stress impacts plants by damaging tissues, decreasing water content, and increasing susceptibility to pathogenic attacks (Fuchs et al, 2024). Plants can survive this stress through the production of phytohormones such as ethylene (Kolanchi et al, 2025). Ethylene helps in mediating plant response by expressing genes that protect the plant. These genes activate signaling pathways (via EIN2/EIN3) that turn on protective genes involved in antioxidant production, pathogen defense, and stress-responsive proteins (Huang et al, 2023). It also coordinates crosstalk with other hormones like jasmonic acid and abscisic acid, which enables the plant to survive stress more efficiently (Liu et al., 2024). Ethylene also elongates stem and stimulates bud development (Fan et al, 2024). In response to the damage of plant tissues, the production of lignin increases to strengthen cell walls (Garcia et al, 2015).

In the natural occurring environment of a plant, stressors are often found together; one stress could in fact induce another stress such as high temperature induces drought (Rhizhsky, 2002). These combined stresses can be either beneficial or further detrimental to plants depending on the interaction and their intensity and duration (Alhaithloul, 2019). A beneficial combination of stresses could be synergistic as the interaction of stresses has been shown to enhance survival and regulate stress response genes (Dombrowski, 2003; Capiati et al, 2006). A common survival mechanism under salinity stress is the production of metabolites such as

osmolytes like proline to upkeep osmoregulation, this in turn could help protect plant tissues damaged from wounding, because proline stabilizes proteins and membranes (Khan et al, 2014). Plants can also produce hormones to protect themselves, a common survival mechanism under wounding is the production of ethylene, which mediates plant growth through root and stem elongation (Bailey et al, 2005). Under salinity stress the production of ethylene could mediate plant growth and reduce oxidative stress as well (Wang et al, 2020).

Phytohormones play a key role in plant defense under various environmental stressors by mitigating defense responses and increasing tolerance (Liao et al, 2025). Salicylic acid (SA) is one of theses phytohormones that is essential in the survival mechanism of plants during both salinity and wounding stress (Abo-Shanab et al, 2025). This phytohormone has been shown to decrease oxidative stress (by increasing antioxidant enzyme activity) within plants and regulate photosynthesis (Bakhshandeh et al, 2025; González-Villagra et al, 2022). Both endogenous and exogenous application of SA improves osmotic balance through the production of osmolytes such as proline and glycine betaine (Vijayalakshmi et al., 2016). Salicylic acid also stabilizes plant membranes and leads to the production of stronger cell walls through lignin and callose deposition which results in thicken plant cell walls (González-Villagra et al, 2022). Overall, it has been shown that the exogenous application of SA can increase stress tolerance (Liu et al, 2008).

Faba beans (*Vicia faba*) are common legumes originated in the Mediterranean region and are now found around the world and grow in typically cool season climates. This legume is particularly efficient at nitrogen fixation which is essential for protein synthesis and plant growth (De Notaris et al, 2023). This is a unique biological process in which atmospheric nitrogen gas is converted to a usable form of nitrogen such as ammonia. This is made possible through a

symbiotic relationship between plant and *Rhizobium* bacteria that work in the rhizosphere of the legume (Haddad et al, 2022).

Faba bean can be sensitive to environmental stressors, especially salinity and wounding. These stressors impact plant survival by reducing protein synthesis, metabolic efficiency and overall plant growth (Walters et al, 2006; Abdelhamid et al, 2014). Faba bean is usually not tolerant to high salinity in soil and can be severely damaged from high concentrations such as 150 mM NaCl (Abo-Shanab et al, 2025). However, different cultivars of the faba bean have expressed varying degrees of tolerance to different saline concentrations. Faba bean cultivar Giza 3, when inoculated with *Rhizobium leguminosarum*, showed significant reduction in nodule number and nitrogenase activity even at moderate salinity (50–125 mM NaCl) (Abd-Alla, M.H., 1992). There was also a reduction in dry weight of stems and roots. When salinity reaches 150 mM NaCl, the plant experienced much stronger effects, including drops in chlorophyll content, photosynthetic rate, stomatal conductance, and chlorophyll fluorescence (Fv/Fm), along with higher oxidative and cellular damage (Elshobary et al., 2025; Ma et al., 2025). At even higher levels (150–200 mM), four different faba bean cultivars show reduced germination and early seedling growth, demonstrating that the crop becomes increasingly sensitive as salinity rises (El-Bastawisy, 2018). In term of mechanical wounding, faba bean plants can handle partial leaf removal with minimal impact on their overall growth. Even though leaf area dropped, the plants can adjust their resource allocation to keep biomass production relatively stable (Casierra-Posada et al, 2017).

When plants are treated with SA, they can activate SA-dependent signalling which triggers ROS species, in response jasmonic acid, which is found naturally in plants, suppresses these ROS species (Tian et al., 2024). This demonstrates how there is an antagonistic relationship

between these two stress-induced pathways (Myers et al., 2023; Pieterse et al., 2012). When faba beans are exposed to SA before or during salt stress, they are "primed," meaning they respond faster and stronger to later stress, including wounding (M'sehli et al, 2020). SA helps stabilize the plant water status and photosynthesis in the presence of salt, so when wounding happens, the plant is not already weakened (Khan et al, 2016). This combined effect leads to less leaf damage, better protection from ROS, and a more controlled defense response. When faba beans grow in salty soil (around 100–150 mM NaCl), they normally suffer from leaf damage, low chlorophyll, and reduced water content. However, studies show that applying SA as a foliar spray can reduce these negative effects. For example, SA improves photosynthesis, increases chlorophyll levels, and boosts antioxidant enzymes like superoxide dismutase, catalase, and peroxidase, which help remove harmful reactive oxygen species (ROS) caused by saline soils (Elkelish et al., 2020). Another recent study found that SA helps faba bean to maintain a better Na^+/K^+ balance, protecting cell membranes and improving growth even under high salt concentration (Taha et al., 2022).

Although there have been few published studies on the effect of salt and mechanical wounding on faba bean (*Vicia faba*), these interactions have been studies in other plant species. Based on these studies, it has been suggested that the interaction between salinity and wounding could potentially benefit and enhance the plant's resilience (Capiati et al., 2006; Li & Gong, 2011; Liu et al, 2024). This study aimed to determine the individual and combined effects of salinity and mechanical wounding on growth, physiology and biochemistry of faba bean. It also aimed to determine if salicylic acid can alleviate the effects of the combined stresses upon the faba bean. I hypothesized that the interaction between high salinity and wounding will result in an increased

tolerance to stress within faba bean. I also hypothesized that SA will help in alleviating and modulating both salinity and wounding stress in faba bean

The significance of this study was to determine the interaction between salinity and wounding stress and how it will enhance the understanding of stress physiology in legumes. This study will also help us understand the effects of salicylic acid in modulating stress in legumes/crops.

2 - Material and Methods

2.1 - Plant material and growth conditions

Faba bean (*Vicia faba* cv. Broad Windsor) seeds were purchased from T&T Seeds, Ltd. (Headingley, MB). The seeds were soaked in water for 24 hours before potting to allow imbibition. One seed was planted per 2L pot for a total of 32 pots (4 replicates with 8 treatments) in Sungro Sunshine Professional Growing Mix No. 4 Aggregate Plus. All plants were grown for 2 weeks in the greenhouse under 16-hour light photoperiod and 23°C day/18°C night temperatures. The plants were irrigated with reverse osmosis (RO) water every two to three days depending on moisture levels. Moisture levels in the soil were checked using a ML3 ThetaProbe (Delta -T Devices, Cambridge, UK) and aim for a moisture level of around 48 to 50%. Plants were fertilized with 250 mL of half-strength Hoagland nutrient solution every two weeks.

2.2 - Treatments

After two weeks of growth, half of the plants (16 plants) were sprayed with salicylic acid (0.5 mM) (Table 1). This concentration was selected based on previous studies performed on faba

bean (Azooz et al, 2011; Souana et al, 2020). This concentration has been shown to reduce oxidative stress and overall improve photosynthetic capacity, therefore alleviating stress on faba bean. This treatment was intended to illicit a response to SA before salinity and wounding stresses were applied (Khan et al, 2014).

Two days later, half of the control plants (8) and half of the SA treated plants (8) were exposed to wounding (Table 1). Mechanical wounding was performed by cutting the third mature leaves from the base with sterilized scissors (Plavčak et al, 2021).

After two days, to allow time for induction of a response to wounding, saline solution (NaCl) was applied (250 ml per pot) to half of the plants, 4 in each treatment (treated or non-treated with SA and wounded or non-wounded, see Table 1). To avoid osmotic shock, plants were irrigated with increasing concentrations of NaCl, 50 mM for 2 days, then 100 mM for 2 days and finally 150 mM (our desired concentration to test). This concentration was selected based on a previous study conducted in our lab that showed that applying 50 mM and 100 mM saline solution over 3 weeks did not affect the gas exchange, protein or phenolic compounds of faba bean (Lavallée-Shrupka, 2025). Salinity solution (150 mM) was applied to salt-treated plants every few days for a total of 3 weeks as needed to maintain the target moisture levels. Control (no salt) plants were irrigated with reverse osmosis water in the greenhouse to maintain moisture levels relatively constant. There were 4 replicates (4 pots) for each of the 8 treatments.

Table 1. Experimental design for faba bean (*Vicia faba*) plants with 8 treatments and 4 replicates. Treatments include Salicylic Acid (SA), Wounding (W) and NaCl (Salt).

<table>
<tr><th colspan="8">Treatment Groups</th><th>Treatment Application</th><th>Dates</th></tr>
<tr><td colspan="4">No SA
(16 plants)</td><td colspan="4">SA
(16 plants)</td><td>0.5 mM
Foliar Spray</td><td>2 weeks after planting</td></tr>
<tr><td colspan="2">No W
(8)</td><td colspan="2">W
(8)</td><td colspan="2">No W
(8)</td><td colspan="2">W
(8)</td><td>Cut mature leaf in half
3rd mature leaves from the base</td><td>2 days after SA</td></tr>
<tr><td>No Salt
(4)</td><td>Salt
(4)</td><td>No Salt
(4)</td><td>Salt
(4)</td><td>No Salt
(4)</td><td>Salt
(4)</td><td>No Salt
(4)</td><td>Salt
(4)</td><td>150 mM
NaCl
Irrigation</td><td>2 days after W
For 3 weeks</td></tr>
</table>

2.3 – Physiological measurements

2.3.1 Chlorophyll Fluorescence (Fv/Fm)

The maximum quantum efficiency of photosystem II (Fv/Fm) was determined using an OPTI-SCIENCES OS30P fluorometer, two days after the beginning of each treatment, SA, wounding and salt irrigation and also 3 weeks after salinity exposure. Fv is the variable fluorescence and Fm is the maximum fluorescence. The third mature leaf from the base of the plant was selected for the measurements. I kept the leaves in a dark environment for 30 minutes. Measurements were taken during the day, between 10am- 1pm with a light pulse of 3000 μmoles m^{-2} s^{-1}.

2.3.2 – Transpiration and Stomatal conductance

Leaf transpiration and stomatal conductance were measured using a steady state porometer (LI-1600, LI-COR Inc.) on the 3rd mature leaf from the bottom. Measurements were taken the same days as for chlorophyll fluorescence, between 11am and 1 pm.

2.4 – Growth measurements

Growth measurements such as plant height, number of lateral shoots were taken every week throughout the experiment. Height of the main and lateral shoots was measured after 3 weeks of exposure of 150 mM NaCl. Three weeks after exposure to 150 mM NaCl, after harvesting, all plant tissues were washed thoroughly with distilled water. The fresh weight of roots, stems, damaged leaves, and non-damaged leaves were recorded and then all plant tissues were frozen (-20°C), then freeze-dried using a LABCONCO freeze dryer for a minimum of 3 days to determine dry weights. Injury levels (percentages of chlorosis and necrosis) were recorded using a scale from 1 to 5 (table 2) at time of harvest. Non-damaged dry leaves were grinded using a Cuisinart grind central grinder for 3 minutes to a fine texture for further analysis. Water content (WC) was measured using fresh and dry weights according to the formula: WC = 100 x [(fresh weight – dry weight)/fresh weight].

Table 2. Injury scale and percent of injury:

Injury Scale	Percent of Injury (necrosis and chlorosis)
0	No Injury
1	1-24%
2	25-49%
3	50-74%
4	75-99%
5	Dead plant

2.5 – Proteins and chlorophylls

2.5.1 Protein analysis

Total soluble protein content in *Vicia faba* leaf tissues was determined using a phosphate buffer with bovine serum albumin (BSA) as the standard (Kumar et al, 2022). Freeze-dried leaf samples (0.05g) were homogenized in a mortar and pestle with 50 mM phosphate buffer (pH 7.25) containing 1 mM EDTA, 1 mM Ascorbic Acid and 1% polyvinylpyrrolidone. The samples were then placed on a shaker (130 RPM) for 20 min on ice and then centrifuged at 15,000g for 20 min at 4 °C. The supernatant (200 µl) was collected, and samples were mixed with 5ml of Bradford reagent (Biorad). Absorbance was measured spectrophotometrically at 595 nm, and protein concentration was calculated using the standard calibration curve with bovine serum albumin. All samples were triplicated to ensure accuracy and reproducibility.

2.5.2 - Chlorophyll a and b analysis

Chlorophyll a and b concentrations were measured using the methanol extraction method. Twenty mg of freeze-dried leaf samples were mixed with 6 ml of methanol and incubated in the dark for 24 hours, to prevent pigment degradation (MacKinney, 1941; Saadaoui et al, 2022). Extracts were collected in vials, and the absorbance was read at 650 nm and 665 nm. Calculations were done using the following equations:

Chlorophyll a= (16.5*A665)- (8.3*A650)

Chlorophyll b= (33.8*A650)- (12.5*A665).

2.6 - Soil and leaf elemental analysis

Soil electrical conductivity was measured at the end of the experiment using an Orion 3 Star conductivity meter (Kumarthunga, 2022). Soil samples were collected from each pot and dry in an oven (60°C) for one week. Five grams of oven dry soil were mixed with 25 ml of deionized water to create a saturated paste. The paste was then transferred to a Buchner funnel, containing a filter paper, attached to a flask and with the help of a hand pump, the leachate was extracted into the beaker. Soil conductivity and pH were measured from the leachate using a Thermo Scientific Orion 3 Star pH meter and conductivity meter.

For elemental analysis of the following ions, Sodium (Na), Chloride (Cl), Calcium (Ca), Magnesium (Mg), Nitrogen (N), and Phosphorus (K), dried faba bean leaf samples were ground into a fine powder. These samples were sent to Stratford Agri Analysis (Stratford, ON) to quantify elemental concentrations (Shao et al., 2020). For chloride analysis, 50 mg of dried faba bean leaf samples were used and were mixed with 0.5 M HNO_3, the mixture was stirred for 30 min on the shaker at 150 rpm. 200 µl Ionic Strength Adjuster (ISA) was added and then the reading was recorded using a Fisher Scientific dual channel pH/Ion meter. Calculations were performed based on a chloride standard curve.

2.7 - Data analysis

Data were analyzed through a 1, 2 or 3-way ANOVA (after SA treatment, wounding and NaCl treatment respectively) using JMP with Tukey's Honest Significant Difference test to compare treatment means (Yi et al, 2025).

3 - Results

Two weeks after the beginning of the salt treatment, we observed a *Colletotrichum sp.* Infection (identified by the Crop Diagnostic Centre, Winnipeg, MB) that impacted the experiment by causing leaf necrosis on some of the plants (mainly no-salt plants). Consequently, the results have been reported to indicate treatment effects before and after the fungal infection.

3.1-Photosynthetic Efficiency

Photosystem II efficiency (determined by Fv/Fm) was consistent throughout the experiment and around 0.8 for the first 2 weeks (2 days after SA spraying, 2 days after wounding and 2 days after exposure to 125 mM NaCl) for all treatments prior to fungal infection (Table 3). After 3 weeks of salinity exposure (post infection with *Colletotrichum sp*), ANOVA analysis indicated a significant decrease for the wounding treatment ($P=0.045$), while none of the other treatments or interactions were statistically significant. Further analyses with Tukey's test showed that none of the means were significantly different from each other.

3.2-Stomatal conductance and transpiration

Stomatal conductance (Table 3) was constant after SA treatment and 2 days after wounding, but it was reduced 2 days after 125 mM NaCl (prior to infection) ($P<0.0001$). After 3 weeks of exposure to 150 mM NaCl, post fungal infection, results from the ANOVA showed that stomatal conductance was significantly reduced by salinity ($P=0.01336$). Salt treated plants had lower stomatal conductance prior to infection as compared to control (no salt). Salt treatment reduced stomatal conductance across all treatment combinations. Prior to infection, under 125 mM NaCl exposure, stomatal conductance decreased by 52.6% in salt treated plants compared

to control, 55.0% in salt + SA compared to control + SA, 50.0% in salt + W compared to control + W, and 57.1% in salt + SA + W compared to control + SA + W. After three weeks of 150 mM NaCl exposure following fungal infection, stomatal conductance declined by 71.4% in salt-treated plants relative to control (no salt), 57.1% in salt + SA compared to control + SA, 66.7% in salt + W compared to control + W, and 20.0% in salt + SA + W compared to control + SA + W. Further Tukey's test confirmed that stomatal conductance was affected by salinity after 125 mM NaCl and after 3 weeks of exposure to salt.

Transpiration (Table 3) was not significantly different for any treatments 2 days after SA application and wounding. Two days after 125 mM NaCl exposure, the means decreased for all treatments containing salt ($P<0.0001$). After 3 weeks of salt exposure and post fungal infection, salinity significantly reduced ($P=0.00873$) the transpiration rates for all salt treated plants.

3.3-Injury levels

Injury level (chlorosis and necrosis) after 3 weeks salt exposure was higher ($P <0.0001$) in control plants, in comparison to salt treated plants in all treatments (Fig 1), there was no effect of SA and wounding and no interaction between the 3 factors. The injury in plants non-exposed to salt (Fig 2) was likely due to a fungus, *Colletotrichum sp* as necrosis was mainly reported in control plants. Leaves of control + W appeared to have less necrosis as compared to other control groups.

Post infection, after 3 weeks of treatments, salinity reduced root water content (Fig 3) ($P=0.02121$). However, Tukey test shows no differences between any treatment groups. Leaf water content (Fig 4) was increased for salt ($P<0.00001$), for wounding ($P=0.03343$) and there

was a significant interaction SA+ W+ Salt (P=0.04337). Further analysis with Tukey's test revealed significant difference between control and salt group and also between control + SA+ W and salt+ SA+ W groups.

3.4-Plant growth

Root dry weight (Table 4) was significantly reduced by salinity (P=0.00001). Tukey test show differences for only wounded plants (control + W and salt +W), no other groups were statistically different. ANOVA analysis showed that dry weight of damaged leaves was significantly decreased by salt (P=0.00042) and there was a significant interaction of SA+ wounding (P=0.04132). It was not significant for any other treatments. However, Tukey's test showed no significant differences among any treatment groups. Dry weight for non-damaged leaves was significantly decreased for salt treatment only (P=0.00068). Tukey tests show no differences among any treatment groups. Shoot dry weight was significantly affected by salt (P<0.00001), wounding (P=0.01063), and SA (P=0.02420). There was also a significant interaction between salt and wounding (P=0.01794). Tukey's test for shoots confirmed these differences for the above treatment groups.

Plant height before infection and post infection (Table 5) was not significantly affected for any treatment groups and this was confirmed by Tukey's Tests. The number of mature leaves before and post infection (Table 5) were not significantly different for any treatment groups and this was confirmed by Tukey's Tests.

3.5-Protein and chlorophylls

Leaf protein content of non-damaged leaves post infection (Fig 5) was higher in salt-treated plants as compared to control ($P=0.0001$). No effect of wounding, SA or any interactions were significant. Following further analysis with Tukey's tests, the only treatments with statistical difference were control + SA + W and salt + SA + W.

Chlorophyll a and b content post infection (Fig 6 and 7) were higher in salt-stressed plants as compared to control plants ($P<0.0001$). Tukey's test results confirmed that the only groups that were significantly different were control and salt groups.

After 3 weeks of salt treatment, at harvesting time, salinity significantly increased soil pH ($P=0.003$) but after further Tukey test there was no differences found between any groups (Fig 8). Soil conductivity (Fig 9) was also significantly increased for salt treated groups only ($P<0.0001$), and Tukey's test confirmed this.

3.6-Elements

Post fungal infection, leaf Na, Cl and N content were significantly increased for salt treatment ($P<0.0001$) with no effects of SA and wounding and this was confirmed by Tukey's Test (Tables 6 and 7). Leaf Ca level increased for salt treatment only ($P<0.0001$) and Tukey's test confirmed the difference between control and salt groups with the exception of plants exposed to wounding (Table 7). Mg was significantly higher in the leaves for salt treatment only ($P<0.0001$). Further analysis with Tukey's test showed differences only in salt-treated plants in absence of SA and wounding. ANOVA test showed that K level was significantly increased for salt treatment only ($P<0.0001$), however there were no differences between any treatment groups post Tukey's Test.

Table 3. Fv/Fm ratio, transpiration, and stomatal conductance (mean and SE) of faba bean (*Vicia faba*) after plants were exposed to SA, W and Salt. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

	Fv/Fm	Transpiration ($mol/m^2/s$)	Stomatal Conductance ($mol/m^2/s$)
2 days after SA			
SA	0.80 (0.00)[a]	2.26 (0.27)[a]	0.18 (0.02)[a]
No SA	0.80(0.00)[a]	2.32 (0.11)[a]	0.19(0.02)[a]
2 days after W			
Control	0.81 (0.00)[a]	2.76 (0.15)[a]	0.18(0.012)[a]
Control + W	0.81 (0.00)[a]	2.77 (0.14)[a]	0.17(0.011)[a]
SA	0.81 (0.00)[a]	2.95(0.22)[a]	0.19 (0.016)[a]
SA + W	0.81 (0.00)[a]	2.98(0.13)[a]	0.19 (0.010)[a]
Pre infection: After 125 mM NaCl			
Control	0.80 (0.01)[a]	2.74 (0.24)[a]	0.19 (0.02)[a]
Salt	0.80 (0.01)[a]	1.37 (0.15)[b]	0.09 (0.01)[b]
Control + SA	0.77 (0.01)[a]	2.79(0.36)[a]	0.20 (0.03)[a]
Salt + SA	0.80 (0.00)[a]	1.43(0.12)[b]	0.09 (0.01)[b]
Control + W	0.77 (0.02)[a]	3.08 (0.16)[a]	0.22 (0.01)[a]
Salt + W	0.80 (0.01)[a]	1.62(0.20)[b]	0.11 (0.01)[b]
Control + SA + W	0.78 (0.01)[a]	2.92 (0.25)[a]	0.21 (0.02)[a]
Salt + SA + W	0.79 (0.01)[a]	1.41 (0.06)[b]	0.09 (0.00)[b]
Post Infection: After 3 weeks of 150 mM NaCl			
Control	0.67 (0.06)[a]	0.92 (0.12)[ab]	0.07 (0.01)[abc]
Salt	0.72 (0.02)[a]	0.30 (0.06)[c]	0.02 (0.00)[d]
Control + SA	0.71(0.02)[a]	0.92 (0.18)[ab]	0.07 (0.01)[ab]
Salt + SA	0.74 (0.03)[a]	0.33 (0.08)[c]	0.03 (0.01)[cd]
Control + W	0.70 (0.02)[a]	1.11 (0.16)[a]	0.09 (0.01)[a]
Salt + W	0.57 (0.12)[a]	0.33 (0.08)[c]	0.03 (0.01)[d]
Control + SA + W	0.50 (0.09)[a]	0.66 (0.16)[abc]	0.05 (0.01)[abcd]
Salt + SA+ W	0.61 (0.14)[a]	0.49 (0.12)[bc]	0.04 (0.01)[bcd]

Table 4. Height (cm) and number of mature leaves (mean + SE) for faba bean (*Vicia faba*) before and after fungal infection. Means followed by different letters are significantly different according to Tukey's test (P ≤ 0.05). Control refers to no salt.

Treatments	Height (cm)	# of mature leaves
Pre infection: After 125 mM NaCl		
Control	24.7 + 1.9 [a]	4.4 + 0.2 [a]
Salt	25.7 + 1.7 [a]	4.8 + 0.2 [a]
Control +SA	23.7 + 0.9 [a]	4.4 + 0.2 [a]
Salt + SA	24.6 + 2.1 [a]	4.8 + 0.2 [a]
Control + W	26.4 + 1.4 [a]	4.8 + 0.2 [a]
Salt + W	23.5 + 0.6 [a]	4.8 + 0.2 [a]
Control + SA + W	25.3 + 1.9 [a]	4.8 + 0.5 [a]
Salt + SA +W	26.3 + 3.0 [a]	4.8 + 0.2 [a]
Post Infection: After 2 weeks of 150 mM NaCl		
Control	42.0 + 3.2[a]	5.8 + 0.2[a]
Salt	40.7 + 2.8[a]	5.0 + 0.3[a]
Control +SA	44.5 + 3.1[a]	5.8 + 0.4[a]
Salt + SA	34.9 + 2.3[a]	6.0 + 0.3[a]
Control + W	44.0 + 3.3[a]	6.0 + 0.6[a]
Salt + W	37.7 + 0.8[a]	5.8 + 0.2[a]
Control + SA + W	36.3 + 5.2[a]	5.4 + 0.4[a]
Salt + SA +W	39.9 + 4.6[a]	5.6 + 0.2[a]

Table 5. Dry weight (g) (mean and SE) for damaged leaves, non-damaged leaves, shoot and roots of faba bean (*Vicia faba*) after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

Treatments:	Damaged leaves (g)	Non-damaged leaves (g)	Shoots (g)	Roots (g)
Control	0.93 (0.28)^{ab}	4.08 (0.66)^{ab}	12.08 (1.78)^{ab}	4.67 (0.25)^{abc}
Salt	0.16 (0.09)^{b}	2.62 (0.25)^{b}	7.37 (0.91)^{c}	2.63 (0.44)^{bc}
Control + SA	0.73 (0.26)^{a}	4.76 (0.77)^{a}	13.04 (1.72)^{a}	4.47 (0.48)^{abc}
Salt + SA	0.24 (0.06)^{ab}	2.98 (0.28)^{ab}	8.30 (1.23)^{c}	2.62 (0.28)^{bc}
Control + W	0.21 (0.12)^{b}	3.36 (0.28)^{ab}	9.62 (1.47)^{ab}	5.68 (0.56)^{a}
Salt + W	0.20 (0.12)^{b}	2.71 (0.35)^{ab}	7.49 (0.80)^{c}	2.19 (0.21)^{a}
Control + SA + W	1.02 (0.16)^{a}	3.58 (0.45)^{ab}	11.03 (1.47)^{ab}	4.82 (0.89)^{ab}
Salt + SA + W	0.31 (0.12)^{ab}	2.53 (0.20)^{b}	7.99 (0.63)^{c}	3.49 (0.74)^{abc}

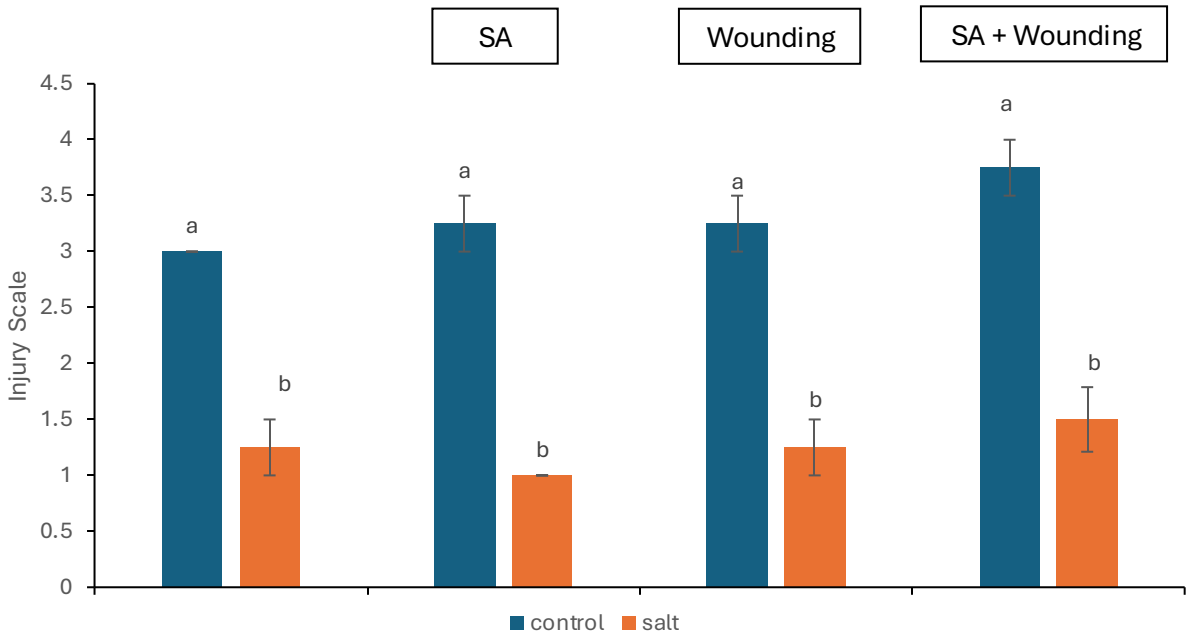

Fig 1. Injury levels (mean + SE) of faba bean (*Vicia faba*) after 3 weeks exposure to 0or 150 mM NaCl and *Colletotrichum sp.*. Injury scale of 1-5: 0=No Injury, 1=1-24% injury, 2=24-49% injury, 3=50-74% Injury, 4=75-99% Injury, 5= Dead plant. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

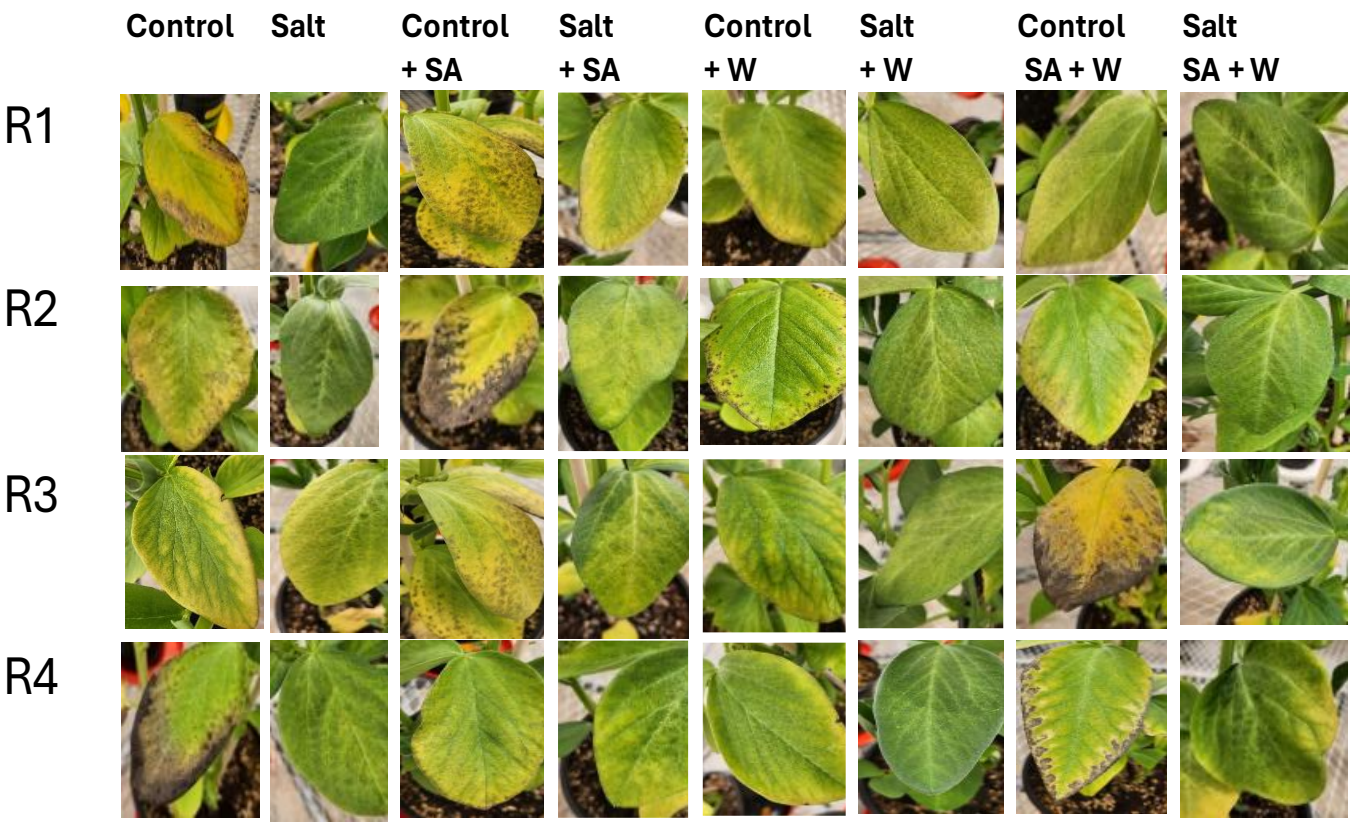

Fig 2. Faba bean (*Vicia faba*) leaves after 3 weeks exposure to 0 or 150 mM NaCl with fungal infection (necrosis) *Colletotrichum sp.* Control refers to no salt.

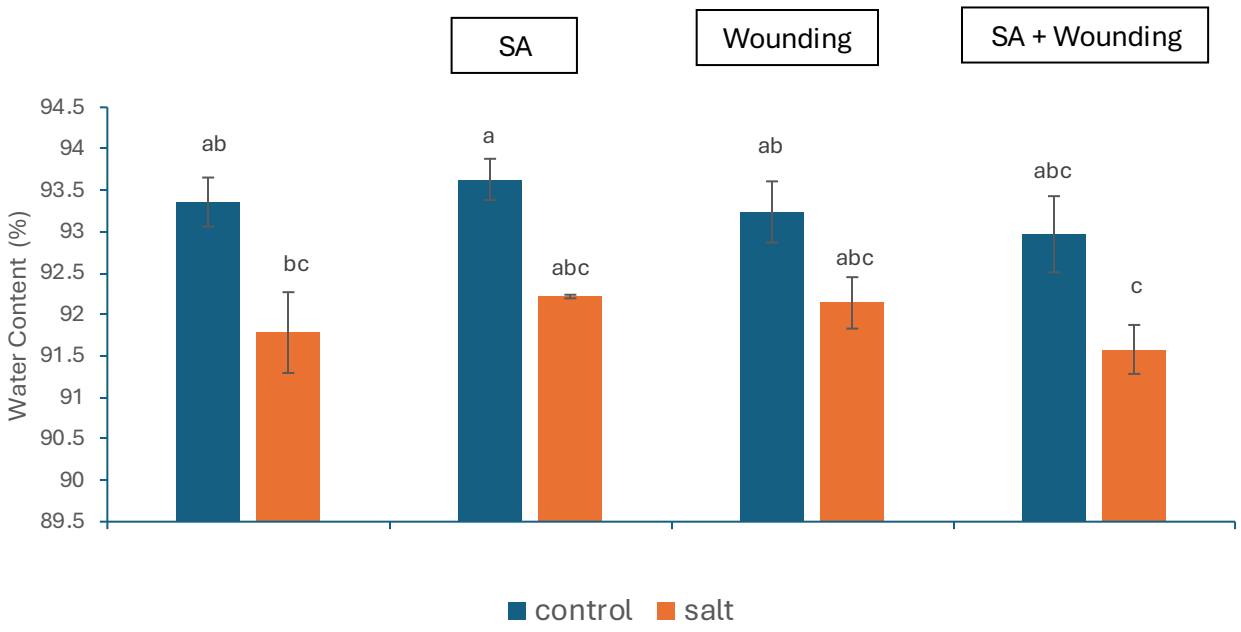

Fig 3. Root water content (%) (mean+ SE) of faba bean (*Vicia faba*) after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test (P ≤ 0.05). Control refers to no salt.

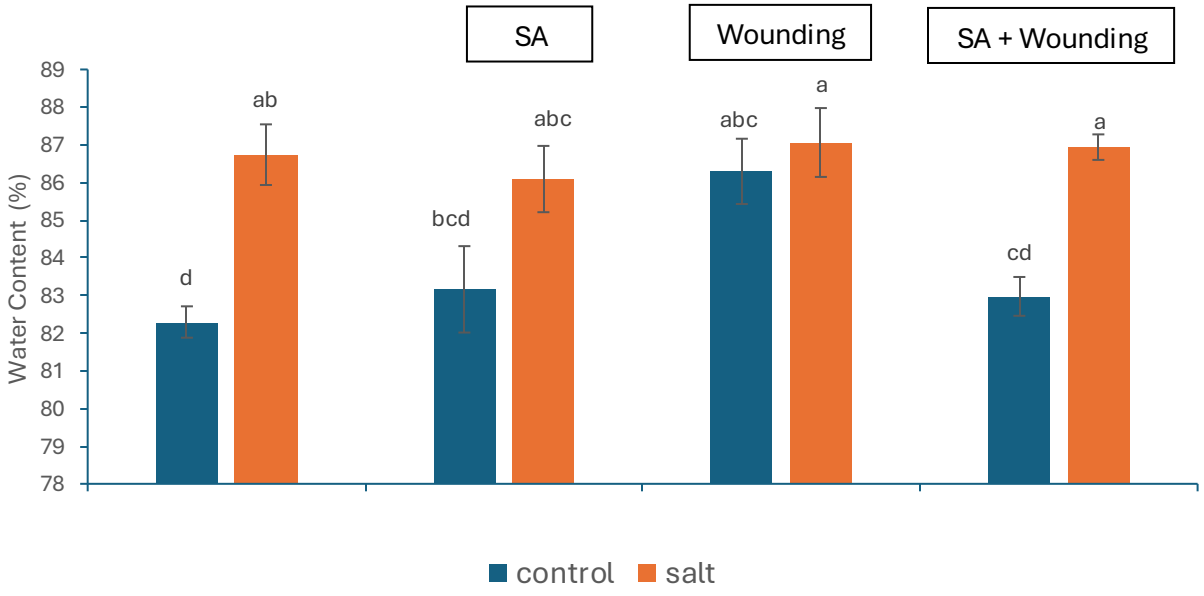

Fig 4. Leaves water content (mean+ SE) of faba bean (*Vicia faba*) after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test (P ≤ 0.05). Control refers to no salt.

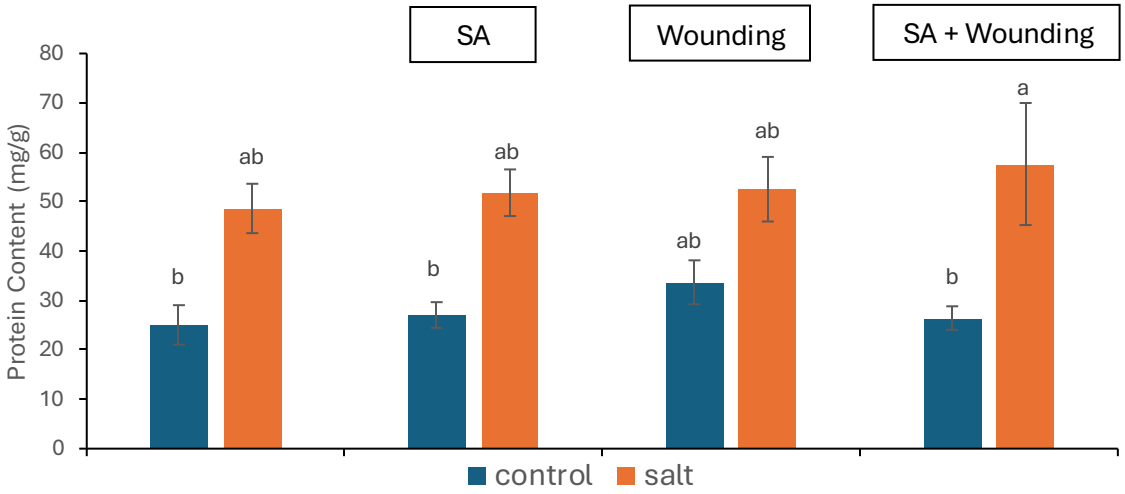

Fig 5. Protein content (mean + SE) of faba bean (*Vicia faba*) leaves after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

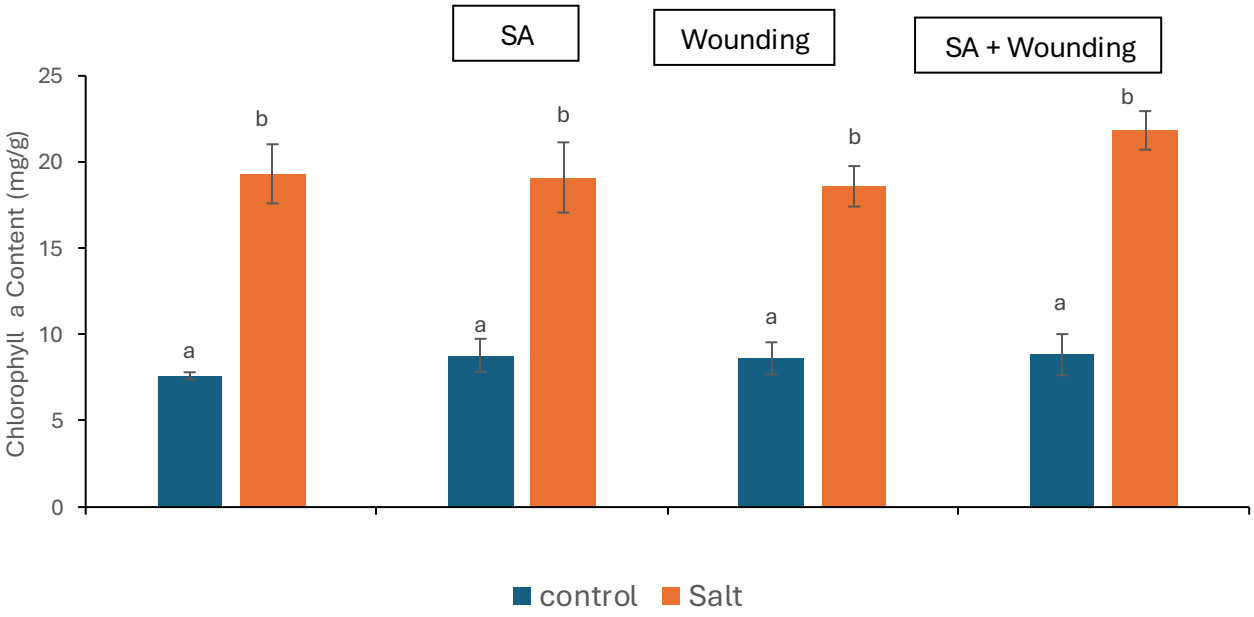

Fig 6. Chlorophyll a content (mean + SE) of faba bean (*Vicia faba*) after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

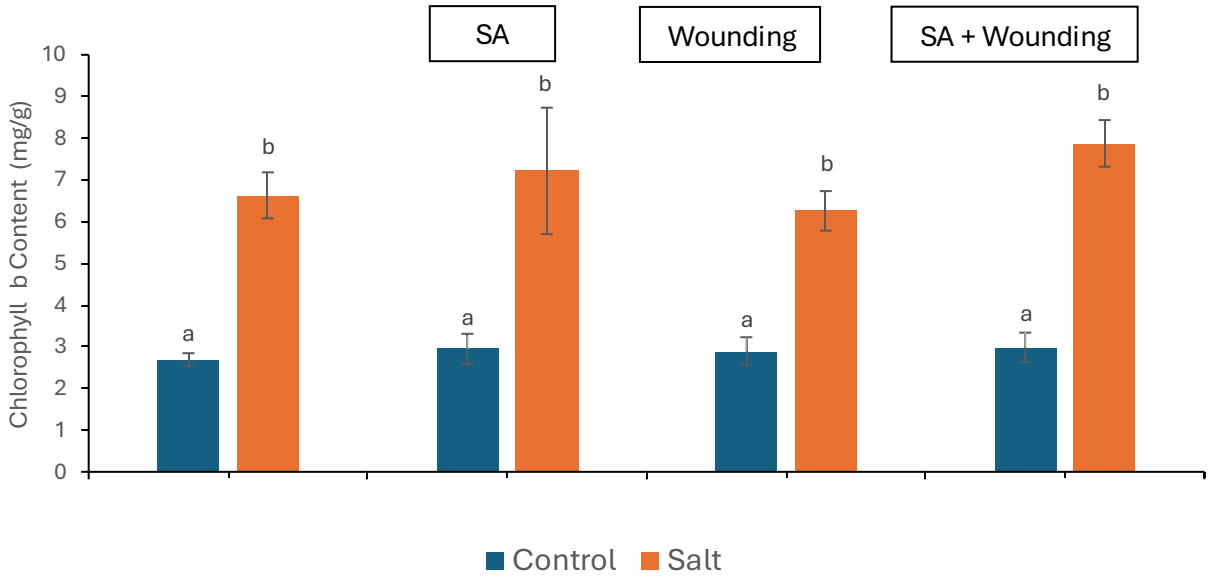

Fig 7. Chlorophyll b content (mean + SE) of faba bean (*Vicia faba*) after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

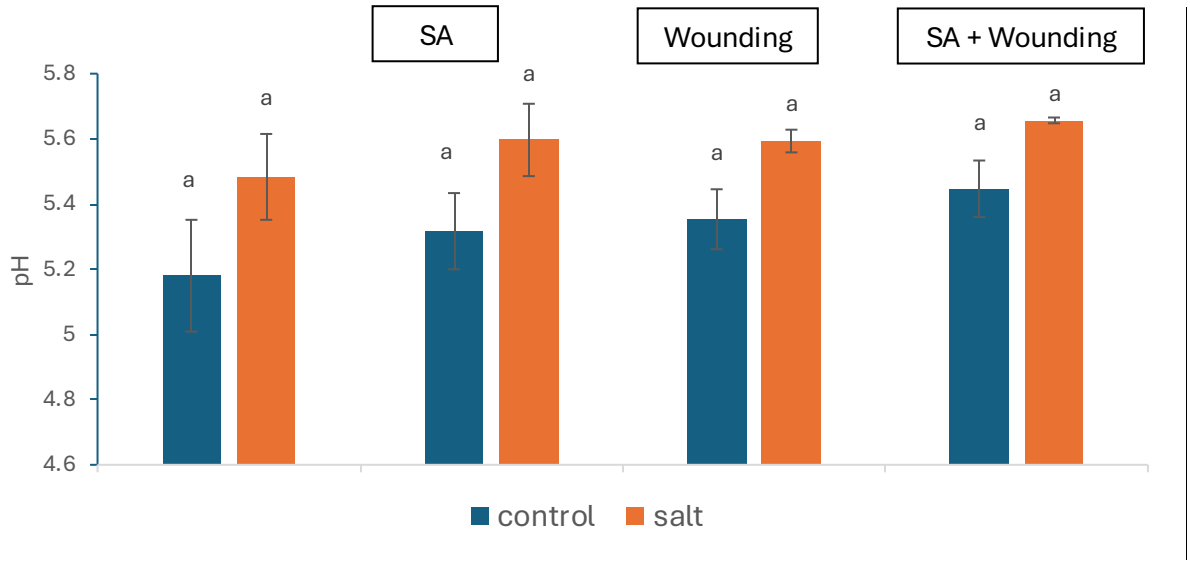

Fig 8. Soil pH (mean + SE) of faba bean (*Vicia faba*) after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test (P ≤ 0.05). Control refers to no salt.

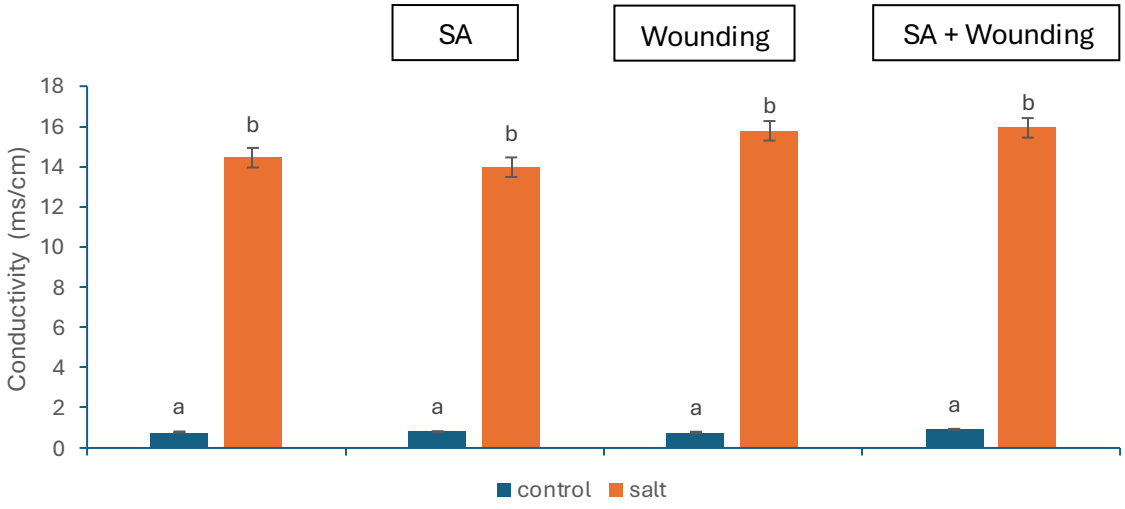

Fig 9. Soil Conductivity mS/cm (mean + SE) of faba bean (*Vicia faba*) after 3 weeks exposure to 0 or 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test (P ≤ 0.05). Control refers to no salt.

Table 6. Leaf Na and Cl content (mg/g) of faba bean (*Vicia faba*) (Mean + SE) for all 8 treatment groups after 3 weeks exposure to 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

Treatment	Na	Cl
Control	$0.16 + 0.02^{a}$	$0.27 + 0.03^{a}$
Salt	$2.41 + 0.18^{b}$	$44.85 + 3.64^{b}$
Control + SA	$0.17 + 0.04^{a}$	$0.28 + 0.05^{a}$
Salt + SA	$2.22 + 0.51^{b}$	$37.70 + 9.47^{b}$
Control + W	$0.28 + 0.03^{a}$	$0.30 + 0.04^{a}$
Salt + W	$3.21 + 0.71^{b}$	$59.90 + 15.45^{b}$
Control + SA + W	$0.12 + 0.01^{a}$	$0.35 + 0.08^{a}$
Salt + SA + W	$3.18 + 0.23^{b}$	$48.00 + 5.57^{b}$

Table 7: Leaf tissue elemental composition (mg/g) of faba bean (*Vicia faba*) (Mean + SE) for all 8 treatment groups after 3 weeks exposure to 150 mM NaCl. Means followed by different letters are significantly different according to Tukey's test ($P \leq 0.05$). Control refers to no salt.

Treatment	Ca	Mg	K	N
Control	$1.21 + 0.14^{d}$	$0.31 + 0.03^{b}$	$0.86 + 0.11^{a}$	$1.54 + 0.12^{a}$
Salt	$2.77 + 0.21^{a}$	$0.62 + 0.06^{a}$	$1.11 + 0.19^{a}$	$2.76 + 0.09^{b}$
Control + SA	$1.48 + 0.28^{cd}$	$0.41 + 0.09^{ab}$	$0.69 + 0.08^{a}$	$1.52 + 0.1^{a}$
Salt + SA	$2.4 + 0.20^{ab}$	$0.5 + 0.04^{ab}$	$1.23 + 0.14^{a}$	$2.67 + 0.15^{b}$
Control + W	$1.76 + 0.08^{bcd}$	$0.45 + 0.02^{ab}$	$0.81 + 0.08^{a}$	$1.74 + 0.15^{a}$
Salt + W	$2.52 + 0.19^{ab}$	$0.5 + 0.05^{ab}$	$1.34 + 0.26^{a}$	$2.81 + 0.12^{b}$
Control + SA + W	$1.31 + 0.14^{d}$	$0.36 + 0.05^{b}$	$0.78 + 0.12^{a}$	$1.39 + 0.05^{a}$
Salt + SA + W	$2.33 + 0.17^{abc}$	$0.54 + 0.04^{ab}$	$0.98 + 0.21^{a}$	$2.89 + 0.12^{b}$

4. Discussion

4. 1-Pathogen infection and Injury

An unexpected fungal infection by *Colletotrichum* sp. occurred during the experiment and resulted in necrosis on the leaves of several plants. Necrotrophic fungal pathogens such as *Colletotrichum* infect plant tissues by inducing host cell death and forming necrotic lesions that reduce photosynthetic ability and damage plant tissues (Dean et al., 2012). In this study, injury levels were higher in control plants than in salt-treated plants. One possible explanation is that exposure to salinity activated stress defense pathways that enhanced resistance to fungal infection, control plants might not have activated any defense pathways (Danial et al, 2014). Plants experiencing abiotic stress often activate antioxidant systems and defense signaling pathways that can also provide protection against pathogens. This is known as cross-tolerance, it occurs when exposure to one stress increases resistance to another stress (Belkhodja et al, 1999).

Anthracnose is a common fungal disease of plants caused mainly by species of the genus *Colletotrichum*. These fungi infect leaves, stems of all types of plants including fruits. The mode of infection is typically entering the plant through natural openings or sites of injury, such as wounds from mechanical damage, insects, or environmental stress (Liu et al, 2024). Once inside, *Colletotrichum* species penetrate plant tissue and colonize cells, leading to symptoms like dark, sunken lesions, necrosis, and tissue collapse (Wang et al, 2020; Gouse et al, 2026). Wounding of leaves can increase susceptibility to anthracnose because it damages the plant physical barriers, allowing easier fungal entry. This results in wounded plants showing more severe anthracnose

symptoms compared to non wounded plants. However, in my experiment plants not exposed to salt but previously wounded had lower observed injury levels as compared to other control plants. Salinity could also have affected the fungus; a study on bell pepper (*Capsicum annuum)* anthracnose demonstrated that sodium and potassium salts significantly reduced the growth and spore germination of *Colletotrichum capsici*, resulting in smaller lesion development (Ajith et al, 2011). This inhibitory effect is likely due to salt-induced osmotic stress and ionic imbalances, which can disrupt fungal cell membrane. As a result, increased salinity may decrease fungal growth and infection, which could help explain the reduced disease severity observed under salt-treated conditions in this study.

The necrotic lesions observed in this study are also similar to those caused by chocolate spot disease in faba bean, which is caused by the fungal pathogen *Botrytis fabae*. This disease produces necrotic lesions on leaves and stems and is one of the most common fungal diseases affecting faba bean crops and causes severe yield loss (Tivoli & Banniza, 2007; Sahile et al, 2008). Although the pathogen identified in this experiment was *Colletotrichum*, both pathogens are necrotrophic fungi that induce tissue death. Several studies have shown that salinity can modify plant–pathogen interactions by activating defense responses. For example, salinity stress has been reported to induce antioxidant enzymes and defense-related proteins in faba bean, which may enhance resistance to fungal pathogens and reduce injury levels compared to control plants (Mahmoudi et al., 2014; Bouhassan et al, 2004). This could help explain why plants exposed to salt stress in our study exhibited lower levels of necrotic injury than control plants.

4.2-Physiological responses to salinity

Salinity had the largest physiological impact on faba bean *Vicia faba* in this study, particularly on stomatal conductance and transpiration. Both parameters decreased significantly prior to fungal infection, indicating that plants responded to salinity by reducing stomatal opening. This response is commonly observed in plants experiencing osmotic stress, as increased salt concentrations lower soil water potential and limit water uptake by roots. As a result, plants close their stomata to conserve water and reduce transpiration losses (Abdul Qados, 2011; Rajhi et al, 2023). This reduction in stomatal conductance could have reduced a possible entry point for infection for the pathogen. With increasing salt concentration and exposure, the stomates closed further. This further closing of stomates could have contributed to the lower level of injury in salt treated plants. Whereas, the control plants that did show higher stomatal conductance allowed for more entry points for pathogens, resulting in increased injury levels.

Reduced stomatal conductance under saline conditions is often accompanied by limitations in gas exchange and photosynthetic efficiency. Salt stress can also impair photosystem activity by affecting electron transport and inducing oxidative stress in chloroplasts (Zhu, 2016). Although Fv/Fm values remained relatively stable during the early stages of treatment, the overall reduction observed later in the experiment suggests that prolonged salt exposure and additional stress factors, such as pathogen infection, may have affected photosystem II efficiency (ElDakak et al, 2022).

4.3-Biochemical responses to salinity

Salinity significantly increased leaf protein content in plants that were treated with SA and then wounded. This increase in protein content may be a response stress-related proteins that help plants like the faba bean cope under stressed conditions, such as aquaporins and heat shock proteins (Abid et al, 2025). The application of SA under salinity stress on faba bean often produce higher levels of protein which may be responsible for plant protection as many proteins are involved in antioxidant enzymes and stress-responsive proteins, which help reduce reactive oxygen species and stabilize cellular structures (Anaya et al, 2017; Ma et al, 2024). There was a lower level of protein content in control plants which could be, in part attributed to the increase in fungal infection in control plants (Embaby & Abdel-Galil, 2006). Salt treated plants had higher protein content potentially due to the lower infection in those plants.

Salinity also increased chlorophyll a and chlorophyll b concentrations in salt-treated plants which was unexpected, as salinity often leads to chlorophyll degradation (Dawood et al, 2015; ElDakak et al, 2020). Some studies have found that plants primed with SA had maintained or even increased levels of chlorophyll content in salt treated plants (Souana et al, 2020). A possible explanation for the higher chlorophyll levels in salt-treated plants in this study relates to the fungal infection that occurred during the experiment. Control plants exhibited greater levels of necrosis caused by *Colletotrichum* which could have resulted in increased degradation of chlorophyll pigments in infected tissues. Although studies have shown that under salt stress, usually the rate of pathogen growth increases, there are some pathogens such as *Fusarium acuminatum* and *Clonostachys rosea* that showed reduced growth, such as *Colletotrichum* in this

study (Haddoudi et al, 2014). Some studies have found the growth of *Colletotrichum* species to be reduced with increasing salt concentration, specifically at 100mM NaCl (Davy et al, 2025)

4.4-Growth responses to stress

Salinity significantly reduced shoot and root dry weight. These reductions in biomass are consistent with previous studies showing that salt stress negatively affects plant growth by limiting water uptake, disrupting ion balance, and through oxidative damage (Afza et al, 2022). Despite these reductions in biomass, plant height and number of mature leaves were not significantly affected by any of the treatments. This suggests that while salinity reduced overall plant productivity, some growth parameters were maintained during the duration of the experiment. Similar patterns have been reported in other crop species where salt stress primarily affects biomass accumulation rather than plant height (El-Beltagi et al, 2025). There was a lower biomass recorded for damaged leaves of wounded plants, this could be due to wounded plants having previously activated defense pathways that resulted in lower injury levels. Wounded plants exhibited lower level of necrosis as compared to other treatment groups.

Salinity caused a small increase in soil pH; however, differences between individual treatment groups were not statistically significant. In contrast, soil electrical conductivity increased significantly in salt-treated soils compared with the control, reflecting the accumulation of soluble salts in the soil solution. Increased electrical conductivity is a well-known indicator of soil salinity and is commonly observed in experiments involving NaCl application to soil. Similar increases in soil conductivity have been reported in studies examining salinity effects on faba bean growth, where salt treatments elevated soil electrical conductivity

(Abdel-Latef et al, 2020; Maggio et al, 2007). These findings confirm that the salt treatment successfully increased soil salinity in our study.

Salt stress often leads to the accumulation of sodium and chloride ions in plant tissues, which can interfere with cellular metabolism and enzyme activity. As a result, plants regulate energy towards maintaining ion homeostasis and osmotic balance which results in balance of growth and biomass production, explaining why there was no significant differences between plant height (Shoukry et al, 2014). Na, Cl, and N concentrations increased significantly in salt-treated plants. The increase levels of Na and Cl are expected under saline conditions and represents the uptake and translocation of these ions from the soil solution into plant tissues. Under salinity stress, Na and Cl can accumulate in the leaves, roots and older tissues of faba bean (Tavakkoli et al, 2012). Excess chloride can impair photosystem II and lead to chlorophyll degradation, while Na disrupts K and Ca balance, causing further damage (Tavakkoli et al., 2010; Geilfus, 2018). Increased nitrogen levels may reflect stress-induced metabolic adjustments or altered nutrient uptake associated in presence of salinity. Nitrogen accumulation under salt stress may also be associated with an increased synthesis of amino acids and stress-related proteins. These compounds contribute to osmotic adjustment and cellular protection under saline conditions (Iqbal et al., 2014). Legumes such as faba bean partake in nitrogen fixation which can increase nitrogen concentrations in plant tissues (Cordovilla et al., 1999). Nitrogen concentration plays a significant role in plant–pathogen interactions, with elevated nitrogen levels often associated with increased susceptibility to fungal infection (Dordas, 2008; Mur et al., 2017). In addition, pathogens can exploit plant tissues for nitrogen to increase their own growth and reproduction, resulting in increased infection severity (Mur et al., 2017). Although studies on faba

bean are limited, similar responses have been widely reported in legume systems, suggesting that increased nitrogen concentrations may contribute to enhanced fungal infection (Dordas, 2008). In this study, nitrogen concentrations were elevated in salt-treated plants, yet fungal infection was reduced, suggesting that salinity stress may override the benefits of pathogens to attack and feed on plant nutrients. Plants in this study were not inoculated, so nodulated plants may have had higher nitrogen level than seen in this study.

A notable increase was also observed for calcium (Ca), which showed a significant increase in salt-treated plants with the exception of the wounded plants. Calcium plays an essential role in plant stress signaling and membrane stabilization, and its accumulation under salinity may represent a protective response to ionic and osmotic stress (Ali et al, 2014). Calcium is known to function as a secondary messenger in salt stress signaling pathways and can reduce Na toxicity by stabilizing plasma membranes and regulating ion transport systems, thereby helping maintain cellular homeostasis during high salinity (Danial et al, 2024; Wu et al, 2012). Increased Ca availability has also been shown to improve plant tolerance to salinity by enhancing selective ion uptake and maintaining membrane integrity (Abo Shanab et al, 2025; Tavakkoli et al, 2010). The absence of a significant difference in wounded plants may suggest that mechanical damage alters Ca redistribution or interferes with Ca-mediated signaling pathways involved in stress responses.

Magnesium (Mg) concentration was increased in salt treated plants but there were no significant differences among any treatment groups. Magnesium is a central component of chlorophyll and is involved in numerous enzymatic reactions related to photosynthesis and energy metabolism. It is essential for carbon assimilation, which drives nitrogen metabolism and

overall nutrient use (Cakmak & Yazici, 2010; Parisa et al, 2025). Mg also interacts with other elements such as K, Ca, and Na, and under salinity stress, high Na levels can increase Mg uptake, leading to nutrient imbalance (Lavallée-Shrupka, 2025). Salt treated plants did not have reduced Mg levels, this shows that the salt treated plants were efficient at balancing elemental levels. Control plants had lower levels of Mg possibly due to the fungal infection (Dash et al, 2025). Magnesium plays an indirect but important role in fungal interactions in faba bean, its availability can influence plant nutrients through ion selectivity and impact physiological responses, which in turn may affect susceptibility to pathogens (Dash et al, 2025). Another study has looked at soil Mg and has shown that Mg is a key factor in shaping fungal community in faba bean cropping systems, highlighting its role in regulating plant–microbe interactions (Siczek et al., 2024). Having similar Mg levels in both salt and control plants may reflect physiological adjustments aimed at maintaining photosynthetic processes and microbial growth under salt stress and pathogenic attack (AbdelHamid et al, 2010). Salt treated plants were also able to maintain K levels like control plants. Potassium plays a critical role in osmotic regulation, enzyme activation, and stomatal function, the lack of clear difference among treatments may indicate that K homeostasis remains tightly regulated even under saline stress conditions (Parisa et al, 2026). Overall, these findings suggest that salinity was the primary factor influencing leaf mineral composition following infection.

4.5-Effects of Wounding

Mechanical wounding had relatively limited effects on physiological and biochemical parameters measured in this study (an increase in the water content of leaves and a decrease in the dry weight of shoots). Post fungal infection, there was a lower level of necrosis observed in

wounded plants as compared to control plants that has not been wounded. This suggests that faba bean (*Vicia faba)* plants can tolerate moderate levels of tissue damage without significant damage to physiological or biochemical processes. Many plant species have shown increased peroxidase activity following mechanical damage, showing detoxifying capabilities (Walters et al, 2006; Deshpande et al, 2025). Wounding can also activate phytohormone signaling pathways, particularly those involving jasmonic acid, which play important roles in plant defense responses. Activation of these pathways can lead to the production of defense compounds and proteins that protect plants against pathogens (Metoui et al, 2002). In our study, the limited impact of wounding may indicate that this level of tissue damage was not sufficient to produce strong responses.

4.6. Interaction of Stressors

The interaction between salinity and wounding was significant only for a few parameters in this study: dry weight of damaged leaf and shoots. Salinity and wounding could possibly have been a detrimental interaction for shoot dry weights, as salinity treated plants had lower shoot dry weight. Water content for non damaged leaves was significant for the interaction of salinity and wounding. Although it was hypothesized that salinity and wounding would act synergistically to enhance stress responses, the results did not support this interaction. Instead, the combined treatment did not produce effects greater than those observed under salinity or wounding alone. This could be due to several factors such as timing and intensity of wounding and fungal infection near the end of the experiment. Several studies have found the interaction of stressors such as salinity and wounding to be significant for multiple different parameters such as physiological and biochemical parameters, this is probably due to the lack of fungal infection in these studies, also the duration of salinity or intensity and mechanism of wounding (Ma et al, 2025; Xu et al, 2024).

4.7-Effect of Salicylic Acid

Salicylic acid treatment did not improve the plant response to salinity and wounding in this study. Although SA is widely known to enhance plant tolerance to environmental stress through regulation of antioxidant systems and stress-responsive genes, the effects of SA can vary depending on plant species, treatment concentration, and environmental conditions (Khan et al., 2015; Ucar et al, 2024). It is also possible that the fungal infection overridden any possible effects that could have been seen in this study. Furthermore, the concentration of SA applied in this experiment may not have been sufficient to produce strong physiological responses. Choosing a higher concentration or repeated treatments could provide significant results. Other studies have used 1mM SA and have seen more significant results (Anaya et al, 2017; Anaya et al, 2025). However, studies using even lower SA concentrations (0.14-0.28mM) have shown a decrease in phytoplasma pathogens in faba bean (Fandino et al, 2026). Therefore, we cannot conclude that this concentration was not sufficient. Salicylic acid had no effect on any of the physiological, biochemical and growth parameters of the faba bean, thus the results of the study did not confirm my hypothesis.

4.8-Limitations and implications for future research

Salinity has shown to have a greater effect on faba bean in this study as compared to salicylic acid and wounding. A major limitation of this study was the fungal infection that occurred during the experiment, as the infection affected control plants more strongly than salt-treated plants, it introduced an additional stress factor that complicated interpretation of the treatment effects. There were no significant differences between any of the treatment groups for the

interaction of salinity and wounding. It was difficult to determine the effect and combined effect of stressors on the faba bean especially the effect of salicylic acid.

Future research should aim to repeat this experiment under controlled conditions that minimize pathogen infection in order to better isolate the effects of salinity, wounding, and salicylic acid treatments. Additional studies could also examine different concentrations of salicylic acid and different wounding mechanisms to further explore how interactions between abiotic stressors influence plant defense responses in legumes. Understanding how plants respond to combined environmental stresses is increasingly important in agricultural systems, where crops are frequently exposed to multiple stress factors simultaneously.

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