Herbivory resistance to generalist feeder *Trichoplusia ni* of faba bean (*Vicia faba)* exposed to salinity stress

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Abstract

Plants are often exposed to multiple stress factors simultaneously. Exposure to one stress can exacerbate or mitigate the response to a subsequent stress. While salinity and herbivory can impair growth and physiological function individually, their combined effects remain poorly understood. To investigate this interaction and determine if salt tolerance influences resistance to herbivory, *Vicia faba* (cv. Broad Windsor) plants were grown under salinity (0, 50, 75, 100 mM NaCl) and exposed to cabbage looper (*Trichoplusia ni*) herbivory. Gas exchange parameters (photosynthesis, stomatal conductance, transpiration) were not significantly affected by salinity after 11 days of treatment. After 20 days, salinity did not affect plant height, stem biomass or water content of most tissues but significantly reduced root and lateral leaf biomass. Total phenolic compounds and total soluble proteins in the leaves were also unchanged. Elemental analysis revealed that salinity significantly increased Na and Cl concentrations in all three tissue types (leaves, stems, and roots), with the highest accumulation in roots. Salinity significantly increased the concentrations of macronutrients (N, P, K, Ca, and Mg) and micronutrients (Fe, Mn, Cu, Zn, and Mo) in the leaves, suggesting efficient nutrient retention in photosynthetically active tissues. Constitutive resistance to herbivory was not significantly affected by salinity. However, induced resistance was significantly impaired in salt-treated plants. Together, these findings indicate that *V. faba* exhibits moderate tolerance to salinity, maintaining gas exchange and nutrient homeostasis under salt stress while minimizing growth impairments. The compromised induced resistance highlights the vulnerability of inducible defenses and suggests that salinity may force a trade-off between preserving physiological function and sustaining inducible defences against herbivory. These results emphasize the importance of considering stress interactions when evaluating plant defence.

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1. Introduction

Plants are regularly exposed to numerous environmental factors originating from both abiotic (non-living) or biotic (living) sources and become stress factors when they negatively impact plant function and performance (Redondo-Gómez, 2013; Suzuki et al., 2014; Parihar et al., 2015). In this context, stress refers to the adverse effects of any factor on a plant, impacting its growth and physiological function (Cornara et al., 2023). Understanding these stress factors as well as the responses they induce in plants has become a major area of study. One of the most prevalent abiotic stresses to global crop production and plant productivity is soil salinity (Parihar et al., 2015). It is estimated that over 20% of the irrigated land used for crop production globally is affected by salinity (Sairam & Tyagi, 2004; Negrão et al., 2017). In addition to salinity, plants often experience insect herbivory (Renault et al., 2016). While the broad impacts of salinity and herbivory are known, their interactive effects remain less studied.

Salinity stress evokes a plant response which can be separated into two distinct phases (Munns & Tester, 2008; Negrão et al., 2017). During the first phase, the shoot ion-independent response which occurs on a timescale of minutes to days, the plant experiences osmotic stress caused by a reduced capacity to take up water from the roots due to the reduction of the external water potential (Zhao et al., 2020). This can bring about reductions in growth rate and major alterations to the plant physiology, such as a decreased turgor pressure of cells (Zhao et al., 2020), closure of stomates (James et al., 2008), inhibition and reductions in enzymatic activity (Tuteja, 2007), modifications to membranes and cell walls (Parihar et al., 2015), reduced photosynthetic efficiency (Manchanda & Garg, 2008), and the induction of oxidative stress as a result of the formation of reactive oxygen species (ROS) within plant cells (Upadhyaya et al., 2013). The second phase, the ion-dependent response (ionic stress) develops over days to weeks (Munns & Tester, 2008; Negrão et al., 2017; Zhao et al., 2020). This is associated with the accumulation of toxic levels of Na^+ and Cl^- ions within the cytosol of the plant that cause an imbalance in the K^+/Na^+ ratio, reducing the activity of many enzymes involved in primary metabolism, and leading to nutrient deficiencies as Na^+ and Cl^- ions outcompete and prevent the uptake of nutrients such as K^+ and NO_3^- (Hu & Schmidhalter, 2005; Tuteja, 2007; Parihar et al., 2015; Zhao et al., 2020). These changes can result in the premature senescence of leaves and in some cases the death of the plant (Munns & Tester, 2008; Negrão et al., 2017).

The severity of salinity stress in plants depends on various physiological/biochemical mechanisms that can be activated within plant cells of different tissues. Three categories of salinity tolerance mechanisms are recognized in the literature: 1) Ion exclusion – the limitation and exclusion of ion entry into shoot tissues 2) Tissue tolerance – the compartmentation of toxic ions at the cellular and subcellular level to prevent toxic levels within the cytosol 3) Osmotic tolerance – the maintenance of water uptake and growth (Munns & Tester, 2008; Costa et al., 2018). These categories encompass mechanisms such as the synthesis of solutes compatible with cellular metabolism (proline, glycine betaine, etc.), the induction of antioxidants, membrane uptake and transporter modifications, and changes in gene expression leading to the production of plant hormones like abscisic acid (ABA) (Sairam & Tyagi, 2004; Tuteja, 2007; Munns & Tester, 2008; Manchanda & Garg, 2008).

In addition to abiotic stress factors like salinity, plant growth is often hindered by biotic stress factors such as insect herbivory (Renault et al., 2016). The evolutionary arms race between insects and plants over the past 350 million years has led to the evolution of various plant attributes that deter their consumption by insects (War et al., 2012; War et al., 2018). Such attributes include trichomes, thorns, and secondary metabolites such as terpenoids, alkaloids, phenolics, and proteinase inhibitors (Mithöfer & Boland, 2012; War et al., 2018; Kessler & Baldwin, 2002). These act as direct defences against herbivory by preventing mechanical damage, acting as toxins, and anti-digestives (Kessler & Baldwin, 2002). Plants may also deter herbivory through the production of volatile organic compounds (VOCs) which act as indirect defences by attracting predators of the herbivores (War et al., 2012). Some of these traits are expressed constitutively, that is, they are present in the plant in the absence of herbivory. Traits that are expressed in response to herbivore attack are said to be induced (Fürstenberg-Hägg et al., 2013, Kessler & Baldwin, 2002). Furthermore, some of these traits, like trichome density and secondary metabolite concentrations, exhibit quantitative variation and can be expressed both constitutively and in an induced manner (Kessler & Baldwin, 2002). Herbivore attacks induce defence signalling pathways involving secondary metabolites and regulatory elements like jasmonic acid (JA), Ca^{2+}, and kinases (Fürstenberg-Hägg et al., 2013). Because these anti-herbivore responses are resource-intensive, many resistance traits are inducible rather than constitutively expressed, allowing the plant to avoid unnecessary fitness costs in the absence of herbivore attack. Upon activation, these responses may lead to decreased growth but can improve photosynthesis and nutrient uptake (War et al., 2018, Kessler & Baldwin, 2002). In addition, different defence signalling pathways are activated based on the herbivore species present and its feeding behaviour (War et al., 2012; Moreira et al., 2015).

In the wild, plants are typically exposed to numerous stress factors simultaneously. The response to one stress can alter the response to a subsequent stress; the interaction between various stress factors can have positive or negative effects on plants (Rejeb et al., 2014). The crosstalk among hormonal signalling pathways has been shown to play a major role in the interactions between abiotic and biotic stresses (Ku et al., 2018). Understanding these interactions is crucial for addressing how plants respond to simultaneous abiotic and biotic stress in natural and agricultural systems. The effects of salinity on the resistance to herbivory seem to vary based on the plant and insect species involved. Notably, much of the research on these interactions has focused on halophytic plants, which are naturally adapted to saline environments. As a result, far less is known about how non-halophytic (glycophytic) plants, which are more sensitive to salinity, respond to herbivory under salt stress—representing an important gap in our understanding of plant stress interactions.

Certain studies report reduced resistance (Marroquin et al., 2023; Moon & Stiling, 2000; Quijano-Medina et al., 2021; Welti & Kaspari, 2021; Avila-Sakar et al., 2018), while other reported increased resistance under salinity (Quais et al., 2019; Renault et al., 2016). Findings on the interaction between salinity and herbivory resistance are reported in Tables 1 & 2.

Table 1. Summary table of the interaction between salinity and herbivory resistance in halophytic plants.

Species	Herbivore	Salinity level	Resistance	Reference
Atriplex suspicata	*Spilosoma virginica*	1.32 ppt salinity	Decreased	Nabity et al., 2006
Borrichia frutescens	*Pissonotus quadripustulatus*	Not specified	Decreased	Moon & Stiling, 2000
Spartina alterniflora	Simulated (leaf clipping)	0-14 ppt salinity	Increased	Wittyngham, 2021
Spartina foliosa	*Haliaspis spartinae*	27-59 ppt salinity	No change	Long & Porturas, 2014

Table 2. Summary table of the interaction between salinity and herbivory resistance in glycophytic plants.

Species	Herbivore	Salinity level	Resistance	Reference
Gossypium hirsutum	Simulated (leaf clipping)/*Spodoptera frugiperda*	215-430 mM NaCl	Decreased	Quijano-Medina et al., 2021
Glycine max L.	*Trichoplusia ni*	100 mM NaCl	Decreased	Avila-Sakar et al., 2018
Solanum lycopersicum	*Spodoptera exigua*	0-150 mM NaCl	Increased	Marsack & Connolly, 2022
S. *lycopersicum*	*Helicoverpa zea*	0-200 mM NaCl	Increased	Pawar et al., 2024
S. lycopersicum	*T. ni*	200 mM NaCl, 20 mM $CaCl_2$	No change	Thaler & Bostock, 2004
Oryza sativa L.	*Nilaparvata lugens*	0-18 dS/m NaCl	Increased	Quais et al., 2019
Brassica juncea	*T. ni*	0-100 mM NaCl	Increased	Renault et al., 2016

A field-based study conducted on the halophyte *Atriplex suspicata* has shown that long-term exposure to moderate salinity (approximately 1.32 ppt) reduced the resistance to the generalist feeder *Spilosoma virginica* due to a reduced ability of the plant to allocate energy resources when grown under salinity (Nabity et al., 2006). Studies conducted on the halophyte *Spartina alterniflora* (syn. *Sporobolus alterniflorus*) found that *S. alterniflora* from mesohaline marshes (6-14 ppt salinity) had higher resistance than plants from oligohaline marshes (0-3 ppt) (Wittyngham, 2021) after approximately three months of exposure. Greater resistance in mesohaline marshes may reflect the combined influence of a longer evolutionary history of herbivory and salinity-driven shifts in resource allocation. In *Spartina foliosa*, increasing salt stress (27-59 ppt) reduced foliar nitrogen concentration as well as the plant's ability to compensate for herbivory by the scale insect *Haliaspis spartinae,* resulting in a neutral response under high salinity (Long & Porturas 2014) after six months of exposure.

Studies conducted on glycophytic plants have reported mixed results. Salinity was found to increase herbivory resistance in tomato (*Solanum lycopersicum*) when exposed to generalist herbivore *Spodoptera exigua* after 12 days of salt exposure, and the corn earworm caterpillar (*Helicoverpa zea*) after three days of exposure, at moderate to high NaCl concentrations (50 – 200 mM). This may be due to an accumulation of phenolics (Marsack & Connolly, 2022), and reductions in both total leaf protein and relative water content (Pawar et al., 2024). Furthermore, Dombrowski (2003) demonstrated that a JA-dependent accumulation of proteinase inhibitors and other wound-related genes occurred in tomatoes exposed to high salinity stress (100 – 300 mM NaCl) for up to five days. This suggests that salinity stress can trigger physiological changes which reduce nutritional quality and induce chemical defences, thereby contributing to increased resistance to herbivores. On the other hand, when tomatoes were subjected to short-term exposure (24h) at concentrations of 200 mM NaCl and 20 mM $CaCl_2$, no change in resistance to the generalist herbivore *Trichoplusia ni* were observed (Thaler & Bostock, 2004), likely due to a lack of enhanced JA-dependent defences. In the rather salt-sensitive soybean (*Glycine max* (L.) Merr. variety Pekko R2), resistance to *T. ni* was decreased with salinity stress, likely due to increased total leaf N content and impaired photosynthetic function (Avila-Sakar et al., 2018). Interestingly, constitutive resistance to the same herbivore was increased in the more salt-tolerant *Brassica juncea*, while no effect was found for induced resistance (Renault et al., 2016), possibly due to a decreased foliar N content.

Faba bean (*Vicia faba,* Fabaceae) is a species with great genetic diversity (Duc et al., 2015). Generally, faba bean is considered mildly tolerant to salinity but a wide range of cultivars exist with respect to their tolerances to various abiotic stresses (Matijević et al., 2014). It is also cold-hardy and can thrive on nearly any soil type (Singh et al., 2013). Faba bean is a worthy candidate for further investigations as it is a staple protein source worldwide (Abdelhamid et al., 2010; Etemadi et al., 2018), is one of the oldest crops in the world, and is the fourth most widely produced cool season legume in the world (Singh et al., 2013; Maalouf et al., 2018) that is commonly grown as a rainfed crop in saline-sodic soils (Tavakkoli et al., 2012).

Considering the inconsistent trends observed in existing studies and our limited understanding of how salinity influences plant resistance to herbivores, particularly in glycophytic crop species, further investigation is warranted. Studying this interaction in faba bean can help determine if salinity compromises or enhances herbivore resistance in this widely cultivated legume, informing future stress-resilience breeding and long-term crop management strategies. To investigate the effects of salt treatments on faba bean resistance to herbivory, the cabbage looper (*T. ni*), was selected for its generalist feeding behaviour. This species is widely distributed, with populations found all over North America. They typically overwinter in the southern United States, before migrating north in the spring (Lingren et al., 1993). Furthermore, they have been shown to feed on over 160 different plant species, making them an excellent model organism to study insect herbivory. The insect herbivore feeding preference has been linked to increased levels of nitrogenous compounds such as amino acids (proline) and proteins (Renault et al., 2016; Avila-Sakar et al., 2018). Increased levels of nitrogenous compounds have been demonstrated in faba bean exposed to salinity (Khalafallah et al., 2008; Dawood & El-Awadi, 2014). This current study aims to determine how salinity affects the composition of faba bean leaves, which in turn influences the resistance to *T. ni* herbivory. I hypothesize that salinity increases the nutritional value of the leaves, making them more appealing to *T. ni* and resulting in higher leaf tissue consumption, thereby reducing the plant herbivory resistance. To determine how salinity impacts faba bean's performance, we assessed various growth and gas exchange (photosynthesis, stomatal conductance, transpiration) parameters. We also analyzed the nutrient composition of roots, stems, and leaves to understand how salinity alters whole-plant resource allocation. To evaluate how salinity-induced changes in leaf traits influence resistance to herbivory, we measured leaf phenolic compound levels as an indicator of chemical defence. Additionally, we quantified total soluble leaf protein and nutrient concentrations, as these traits are known to affect herbivore feeding preference and overall host tissue quality.

2. Materials & Methods

2.1 Plant and insect material

Faba bean (*Vicia faba* cv. Broad Windsor) seeds were purchased from T&T Seeds, Ltd. (Headingley, MB). Faba bean was chosen for this study due to its mild salinity tolerance, fast-growing nature and widespread global production (Matijević et al., 2014; Maalouf et al., 2018). The salinity tolerance of cultivar Broad Windsor is not known; however, it was selected for this study because the cultivar is commonly grown cultivar locally, making the findings directly relevant for local agriculture. Cabbage looper (*Trichoplusia ni*) eggs were obtained from Natural Resources Canada Insect Production and Quarantine Laboratories (Great Lakes Forestry Centre, Ontario).

2.2 Plant growth conditions

Faba bean seeds were soaked overnight to allow imbibition. Following imbibition, seeds were randomly selected and planted in 2 L pots (1 per pot) filled with Sungro Sunshine Professional Growing Mix No.4 Aggregate Plus. Plants were grown in a climate-controlled greenhouse (18–22 °C day / 17–21 °C night) under a 16:8 hour photoperiod maintained using automated greenhouse shades, and supplemented with Verjure^{TM} Pro Series^{TM} LED lights, maintained throughout the experiment.

2.3 Salinity treatments

After 10 days of growth, 10 groups were formed, each consisting of 4 plants (40 plants total). Within each group plants were randomly assigned, one each, to the following salinity treatments: 0, 50, 75, 100 mM NaCl. These concentrations were selected to represent a gradient of salt concentrations commonly observed in soils ranging from low to moderate salt stress conditions. For each salinity treatment, plants were irrigated with 250 mL of a solution of the corresponding of NaCl (0, 50, 75, 100 mM) every 2-3 days. Soil moisture was monitored with an ML3 ThetaProbe (Delta -T Devices, Cambridge, UK) throughout the experiment to maintain adequate moisture levels (40-50%). Plants were fertilized with 250 mL of half-strength Hoagland solution (Al-Tahir et al., 1997) every 2 weeks.

2.4 Insect rearing

Trichoplusia ni larvae were reared under SunBlaster T5HO fluorescent lights (6400 K, 110-130V, relative intensity peaks at 435, 545, and 615 nm) (SunBlaster, Surrey, B.C) at a temperature of 21.5°C with a 14:10 hour light:dark photoperiod and raised on an artificial

McMorran diet (http://insect.glfc.cfs.nrcan.gc.ca/cart-panier/diets-dietes.cfm?lang=eng, Natural Resources Canada Insect Production) until they reached the 4th/5th instar (1-2cm) approximately 14 days after hatching. As insects began to hatch, they were transferred to separate cups containing the artificial diet to avoid overcrowding. As they continued to grow, they were transferred into larger cups with artificial diet to allow sufficient nutrition for all individuals.

2.5 Resistance to herbivory

After 18 days of salinity treatments, constitutive resistance was assessed through both *in vitro* and *in vivo* experimentation. First *T. ni* larvae were starved for 24 hours prior to conducting experimentation. To evaluate the resistance to herbivory *in-vitro*, a ‘choice’ style assay was performed. One mature leaflet (5th mature leaf from the base of the stem) was excised from each control and salt-treated plants. Two disks (2.16 cm^2) were cut from each leaf using a 12mm copper cork borer. One disc from each treatment (including control) was placed adaxial side up in a petri plate with two *T. ni* larvae for 1 hour. Trials were conducted between 10:00 am and 3:00 pm at constant ambient temperature and petri plates were covered to simulate the late day/dusk peak feeding activity of *T. ni* (Goodspeed et al., 2012). As T. ni feeding behavior is circadian-regulated and persists under constant conditions, maintaining consistent assay time minimized variation in feeding activity across trials. The second disc from each treatment was placed on a control plate in the absence of larvae to later determine the leaf disc area changes due to desiccation alone. Following the end of the assay, the larvae were removed, and the area of the remaining leaf disc tissue was measured using ImageJ software (Version 1.53t). The area of these leaf discs was compared to the initial leaf area, obtained from their counterpart in the control plate. Resistance was calculated according to Avila-Sakar et al. (2018) with the following modifications: Here we consider the remaining leaf area instead of the leaf area consumed according to the formula R = (Ac/Ai) where Ac = the remaining leaf area (corrected for dessication), Ai = the initial leaf disc area, and R = resistance. *In-vivo* constitutive resistance was conducted by first placing larvae on the leaves (4th mature leaf from the base of the stem with 2 leaflets) of each treatment (1 larva per leaflet) for 24 hours in mesh bags to prevent their escape. Prior to this, the initial leaf area was assessed by tracing leaves on transparent paper and measuring them using a leaf area meter (Li-3100 Area Meter, LICOR USA). After 24 hours, larvae were removed, leaves were excised, and the leaf area determined. Resistance was calculated as above. To measure induced resistance, leaves were collected 24 hours after the removal of the larvae from the *in-vivo* experiment to allow sufficient time for biochemical responses to herbivory to occur (Reglinski et al., 2023). Induced resistance was measured following the ‘choice’ assay protocol mentioned above, using a new set of larvae.

2.6 Growth

The growth measurements recorded after 20 days of salinity treatments were stem (main and lateral), root, leaf biomass, stem height, and number of leaves. Pre-salt leaves (formed prior to salinity treatments) were considered as the 1-3rd leaves from the base of the plant, while post-salt leaves (formed during salinity treatments) were considered as the 6th leaves from the base of the plant and up. Following harvest, roots, stems and leaves were rinsed thoroughly with distilled water, fresh weights were recorded, and tissues were freeze-dried to obtain the final dry weights. Water content (WC) of the plants was obtained from fresh and dry final weights according to the formula WC = 100 x [(fresh weight – dry weight)/fresh weight].

2.7 Gas exchange

To assess the level of tolerance to salinity exhibited by the plants, gas exchange and water relations of the leaves were evaluated after 11 days of salinity treatments. A portable infrared gas analyser (IRGA) (Li-6400, LICOR USA) was used to measure stomatal conductance, transpiration and photosynthesis. Reference CO_2 was set to 400 µmol mol^{-1} with a flow rate of 400 µmol s^{-1} and a light level (photosynthetically active radiation) of 400 µmol m^{-2} s^{-1}, this approximates the ambient atmospheric CO_2 concentration, making it a standard baseline for measuring photosynthesis. Measurements were taken between the hours of 10 am - 2 pm on fully expanded leaves (3rd leaf from the base of the stem).

2.8 Soil chemistry

To determine how salinity affects soil chemistry, soil electrical conductivity and soil pH were measured. During the harvest of plants, soil was collected and dried in an oven at (60°C) for one week. Subsequently, 35 mL of de-ionized water was added to 5g of the dried soil to form a saturated paste and the mixture was filtered through a Buchner funnel fit with a Whatman #1 filter paper using a hand vacuum pump. The soil filtrate was collected in a filtering flask and transferred to a falcon tube. The conductivity and pH of the filtrate were measured using a conductivity meter (Thermo Scientific Orion 3-Star Portable Conductivity Meter) and a pH electrode (Fischer Scientific AR25 Dual Channel pH/Ion Meter).

2.9 Leaf biochemical composition

To determine if the leaves have undergone any biochemical changes, the remaining tissue from the mature leaves used to determine constitutive and induced resistance was harvested following the herbivory assays and freeze-dried for future measurements.

2.9.1 Phenolic compounds

The total amount of phenolic compounds was assessed following the protocol described by Mujic et al. (2009) using the Folin-Ciocalteu's reagent to evaluate changes in a commonly used biochemical defence compound. Freeze-dried leaf tissue (0.05g) was finely ground with liquid nitrogen and extracted with 10 mL of 40% ethanol (24 h on a shaker in the dark). After centrifugation at 4900g for 10 minutes, 2 mL of supernatant was diluted with 8 mL of 40% ethanol. Then, 1 mL of diluted extract was mixed with 0.5 mL of Folin-Ciocalteu reagent for 5 minutes, followed by the addition of 1 mL of 5% Na_2CO_3. The solution was incubated in the dark for 30 minutes and absorbance was measured at 750 nm. Total phenolic content was quantified using a gallic acid standard curve (0, 10, 20, 30, 40, and 50 µg/mL in 40% ethanol).

2.9.2 Protein content

Total soluble proteins were determined through colorimetric assays according to the Bradford assay (1976). Freeze-dried leaf tissue (0.05g) was finely ground with liquid nitrogen, extracted with 25 mL of cold 50 mM potassium phosphate buffer (pH 7) containing 1 mM EDTA and 1 mM ascorbic acid. Subsequently, 1% PVPP was added to the extraction buffer, and the mixture was vortexed for 1 minute and incubated on a shaker for 20 minutes on ice. After centrifugation at 15000g for 20 minutes at 4°C, 200 µL of supernatant was diluted with 5 mL of Bio-Rad reagent. After vortexing, the absorbance was measured at 595 nm following a 5-minute incubation. The total soluble protein concentration was determined using a bovine serum albumin standard curve (0, 5, 10, 20, 40, 60, 80 µg).

2.10 Elemental analysis

Freeze-dried leaf tissue collected during harvest was finely ground, 0.5g of each was sent to Stratford Agri Analysis (Stratford, ON) to determine their elemental composition (Na, N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Mo, B) (Shao et al., 2020). Total nitrogen was measured via Leco TruSpec N analyzer, while the other elements were analyzed by Direct Current Plasma technique. In addition, Cl content was determined with a chloride electrode (Accumet, USA). To prepare the samples, 0.05 g of dry leaf tissue was mixed with 10 mL of 0.5 M HNO_3 and placed on a shaker for 30 minutes. Afterward, 200 µL of Ionic Strength Adjuster (ISA) was added, and the chloride electrode was used to measure Cl content in the solution. Quantification of Cl content was based on a standard curve prepared with concentrations of 10, 50, 100, and 500 ppm Cl, covering the range of interest.

2.11 Data analysis

Simple linear regression was used to determine the effects of salinity in comparison to the controls. To ensure sufficient material for the different analyses, sample pooling was performed where necessary. Six groups of all four treatments (0, 50, 75, 100 mM NaCl) were harvested and evaluated for growth and physiological measurements, resulting in a total degrees of freedom (df) of 22; however, two plants lacked fully developed lateral stem leaves, reducing the df to 20 in that scenario. For elemental analysis, tissue from the six groups was pooled into three combined samples per treatment to ensure adequate materials for submission, resulting in df = 10. For protein and phenolic analyses, leaves were harvested from all 10 groups (40 plants total), but pooled into five combined groups per treatment (20 total samples), yielding df = 18. Resistance assays were performed on all individual plants (40 total), and no pooling was necessary, resulting in df = 38. Additionally, we conducted parallel simple linear regression on log-transformed data and ANOVA analyses, but these did not qualitatively improve results or provide further insight into the effects of salinity and herbivory.

3.0 Results

3.1 Growth

Salinity had no significant effect on the plant height, number of leaves, stem biomass stems, or on the biomass of pre- and post-salt leaves (Table 3). Similarly, there were no significant salinity effects on the water content of stems, roots, and both the pre-salt leaves of the main stem and the leaves of lateral stems (Table 4). In contrast, salinity significantly reduced the biomass of roots and lateral stem leaves with every 25 mM increase in salinity leading to a 6.75% and 13% decrease in biomass respectively (Table 3). Salinity also significantly affected the water content of main stem post-salt leaves, with every 25 mM increase in NaCl resulting in a 0.75% increase in leaf water content (Table 4).

3.2 Elemental analysis

Faba bean plants exposed to salinity showed significant changes in the elemental composition of the leaves, stems and roots. Salinity significantly increased Na and Cl in all three tissue types (leaves, stems, roots), with the highest mean concentrations in the roots

(Tables 7). In the leaves, most macronutrients (N, P, K, Ca, Mg) increased significantly, while S remained unchanged as salinity increased (Table 5). Significant increases were also observed for the following micronutrients (Na, Cl, Fe, Mn, Cu, Zn) with salinity, while no significant changes occurred for Mo and B (Table 5). In the stems, N and P increased significantly with salinity, while S decreased significantly (Table 6). No effects of salinity were found for K, Ca, and Mg (Table 6). Significant increases with salinity were observed for Fe, while no significant relationship was found for Mn, Cu, Zn, Na, Mo, B (Table 6). In the roots, a significant decrease was observed for the Mg (Table 7). Furthermore, salinity significantly increased the concentration of Zn, Na, Cl, B (Table 7).

3.3 Soil chemistry measurements

Soil pH ($F_{1,22} = 44.77$, $P < 0.0001$; Fig. 1A) and soil conductivity ($F_{1,22} = 300$, $P < 0.0001$; Fig. 1B) were significantly affected by salinity ($F_{1,22} = 44.77$, $P < 0.0001$), with every 25 mM increase in NaCl increasing pH by 0.12 and conductivity 2.24 mS/cm.

3.4 Physiological and biochemical measurements

After 11 days of salinity treatments, photosynthesis ($F_{1,22} = 2.65$, $P = 0.12$; Fig. 2A), stomatal conductance ($F_{1,22} = 2.90$, $P = 0.10$; Fig. 2B), and transpiration rates ($F_{1,22} = 3.90$, $P = 0.06$; Fig. 2C) were not significantly affected by salinity. Similarly, total constitutive ($F_{1,18} = 0.78$, $P = 0.39$; Fig. 3A) and induced ($F_{1,18} = 0.29$, $P = 0.60$; Fig. 3B) phenolics content of the leaves were not significantly affected by salinity. Finally, the total soluble constitutive ($F_{1,18} = 3.15$, $P = 0.09$; Fig. 4A) and induced ($F_{1,18} = 3.99$, $P = 0.06$; Fig. 4B) protein content were not significantly affected by salinity.

3.5 Resistance to herbivory

Constitutive resistance was not significantly affected by salinity whether *in vivo* ($F_{1,38}$ = 0.19, P = 0.67; Fig. 5A) or *in vitro* ($F_{1,38}$ = 1.15, P = 0.29; Fig. 5B). However, *in vitro* induced resistance was significantly affected by salinity ($F_{1,38}$ = 5.23, $P < 0.05$; Fig. 5C).

4.0 Discussion

Salinity did not significantly affect the height of the faba bean, the number of leaves, the biomass of both stems and leaves, or the water content of stems, roots, lateral stem leaves, or main stem pre-salt leaves. These results contrast with previous studies on faba bean that found significant reductions in multiple growth parameters when exposed to similar concentrations of NaCl (0 – 150 mM NaCl) (Abdelhamid et al., 2010; Tavakkoli et al., 2010; Hussein et al., 2017; Filipović et al., 2020). One interpretation of this discrepancy is that the cultivar used in our study could be more tolerant to salinity than those used in previous studies (Duc et al., 2015). Another possibility could be the shorter duration of the experiment, our plants experienced salinity for 20 days during the vegetative stage of the plant following 10 days of initial growth, whereas the aforementioned studies, subjected plants to salinity for periods of four to nine weeks which may better represent real-world field conditions. These extended treatment durations likely caused a more pronounced ionic stress, which may have limited plant growth and could explain the discrepancies between their findings and ours.

Salinity significantly reduced root biomass, which is consistent with the results of other studies (Cordovilla et al., 1994, 1995, 1999; Abdelhamid et al., 2010; Hussein et al., 2017). The effects of salinity stress seem to vary in faba bean, with some studies reporting a more pronounced effect on root growth (Cordovilla et al., 1999; Abdelhamid et al., 2010;

Hussein et al., 2017), and others on shoot growth (Cordovilla et al., 1994, 1995). Moreover, we found that roots accumulate more salt than other plant parts, as they serve as the primary entry point for salt (Tester & Davenport, 2003). Elevated soil salinity lowers the water potential, making it more difficult for roots to take up water and causing osmotic stress. Prolonged osmotic stress has been demonstrated to reduce root growth through decreased cell elongation and proliferation (Royer et al., 2016).

Salinity also significantly reduced the biomass of lateral shoot leaves, while stem and main stem leaf biomass remained unaffected, indicating a potential trade-off in resource allocation. This shift could reflect an energy investment in osmotic and ionic stress tolerance rather than the growth of lateral shoot tissues, conserving key resources to sustain primary architecture. Furthermore, the higher water content of the post-salt leaves of the main stem in response to salinity could be due to osmotic adjustment mechanisms enabling young tissues to remain hydrated despite the increased osmotic stress. Studies have shown glycine betaine accumulates preferentially in newly formed leaves, which can help maintain turgor pressure by aiding in water retention (Sharma et al., 2019).

Salinity stress is known to affect the nutrient composition of plant tissues. This study found that salinity significantly increased the concentration of Na and Cl ions in all tissues, with roots having the highest mean concentrations, conditions typically contributing to ionic stress. However, the presence of these ions in aboveground tissues suggests that faba bean may tolerate some degree of cytoplasmic ion accumulation through tissue tolerance mechanisms rather than avoiding stress entirely via exclusion. Specifically, the high root concentrations may reflect intracellular compartmentalization of Na^+ and Cl^- ions into vacuoles of root cells, preventing cytoplasmic toxicity and contributing to the significant reductions in root growth under salinity (Munns & Tester, 2008; Tavakkoli et al., 2010).

This aligns with previous findings that Na^+ and Cl^- ions are often sequestered in the vacuoles of roots and older leaves to protect metabolically active tissues (Munns et al., 2016).

While most studies on faba bean reported decreased total nitrogen (N) content with increasing salinity (Cordovilla et al., 1995; Abd-Alla et al., 2001; Abdelhamid et al., 2010), this study showed that salinity significantly increases the concentration of N in both leaves and stems. This increased N content could potentially be attributed to increased synthesis of osmolytes such as proline and other amino acids often seen in salt-stressed plants (Renault et al., 2016), which has been demonstrated in faba bean (Khalafallah et al., 2008; Dawood & El-Awadi, 2014). Additionally, other N-containing compounds, such as polyamines or glycine betaine, could also have contributed to the increased N content in the leaves and stems (Mansour, 2000).

This study found that salinity significantly increases the concentration of phosphorus (P) in the leaves and stems, with no significant effect on the roots. While other studies report decreased P content with salinity (Srinivasarao et al., 2004; Abdelhamid et al., 2010), our results suggest that faba bean may prioritize P allocation to aerial tissues under salt stress, maintaining essential metabolic functions like nucleic acids and ATP synthesis in photosynthetically active tissues (Lambers, 2022) and endowing the plant with some tolerance to salinity. Sulphur (S), an essential nutrient for stress signalling and glutathione synthesis (Noctor et al., 2012), showed no significant salinity-induced changes in the leaves or roots, consistent with what is reported in the literature (Matijević et al., 2014). However, salinity significantly reduced stem S, suggesting that sulphur may be reallocated to the leaves to support the antioxidant defence system through glutathione synthesis (Avashthi et al., 2018).

Although Na can compete with other cations like K, Ca, and Mg for plasma membrane transporters (Zhu, 2003), this study found that salinity significantly increased K, Ca, and Mg in the leaves, with no effects in the stems, and decreased Mg in the roots. These findings contrast with previous findings on faba bean, which found reduced levels of these nutrients (Cordovilla et al., 1995; Abdelhamid et al., 2010; Bulut & Akinci, 2010; Matijević et al., 2014), suggesting that this cultivar (Broad Windsor) may have mechanisms to maintain cation homeostasis, like high-affinity K^+ transporters (Wu et al., 2018). Furthermore, my study found that all leaves exposed to NaCl had Na^+/K^+ ratios below 1, a value generally accepted as a threshold for the toxic effects of sodium (Assaha et al., 2017; Ran et al., 2022), suggesting that critical K^+-dependent physiological processes remained functional. Elevated Ca^{2+} may result from stress-induced dysregulation of non-selective cation channels (Tester & Davenport, 2003), allowing for excessive entry of Ca^{2+} into the cytosol, and enabling continued Ca-dependent signalling. (Danial & Basset, 2024). Similarly, the trends observed with Mg suggest that it is actively redistributed to leaves to support normal physiological and photosynthetic function under stress (Cakmak & Yazici, 2010; Zirek & Uzal, 2020).

These patterns of nutrient retention and redistribution paired with the significant increases in micronutrients (Fe, Mn, Cu, Zn, Mo) may explain why salinity had no significant effect on photosynthesis, stomatal conductance or transpiration after 11 days. Fe, Mn, Cu, and Zn play important roles in the antioxidant response (Hasanuzzaman et al., 2012) and are essential for photosynthesis (Schmidt et al., 2016; Kroh & Pilon, 2020). Increased Mo in the leaves may reflect a physiological response to support nitrogen metabolism, as Mo is a nitrate reductase cofactor (Mendel & Hänsch, 2002; Minner-Meinen et al., 2022). Alternatively, the duration of exposure may have been too short to cause observable declines in gas exchange, as previous studies reporting such declines often assessed gas exchange after three or more weeks of exposure (Hussein et al., 2017; Neji et al., 2021). This suggests that faba bean may have maintained ion homeostasis early in the stress period, preventing immediate physiological impairment caused by the accumulation of Na^+ and Cl^- ions (Tavakkoli et al., 2010).

Phenolic compounds can be induced following exposure to salinity and are known for their role as antioxidants, aiding in the scavenging of ROS (Waśkiewicz et al., 2013). This study found no significant effect on the constitutive (baseline concentration) or induced (following herbivore exposure) phenolic content of faba bean leaves, suggesting that in this cultivar, phenolics may not be the primary antioxidant mechanism under salt or herbivory stress. The observed increase in leaf N may instead point to a greater reliance on nitrogen-containing osmoprotectants like proline, glycine betaine, and polyamines. These compounds play important roles in enhancing the antioxidant system, ROS scavenging, maintaining osmotic balance and membrane stabilization (Saha et al., 2015; El Moukhtari et al., 2020; Islam et al., 2021). The exogenous application of these compounds helped alleviate salinity-induced damage, increased growth, antioxidant enzyme activity and osmoprotectant levels in faba bean plants (Gadallah, 1999; Mahdi, 2016). However, they were not assessed in this study, highlighting the need for further investigations.

This study found that salinity had no significant effect on the constitutive or induced total soluble protein content of faba bean leaves. The increased leaf N observed may indicate the reallocation of N to non-proteinic nitrogenous compounds (proline, polyamines, etc.) rather than proteins. These results could also indicate an equilibrium between protein degradation and synthesis in this cultivar (Broad Windsor) or that it cannot upregulate defensive protein synthesis when salt-stressed. Studies involving faba bean report mixed results. Elsherif et al. (2024) found that salinity significantly decreases the total soluble protein content of faba bean leaves at higher concentrations (150 mM NaCl) over a longer period of time (~45 days), while Ahmed and Abd El Sattar (2024) found that the total soluble protein content increased with salinity at comparable concentrations and durations, suggesting that exposure to salt can upregulate or downregulate protein synthesis depending on the cultivar, the duration, and intensity of the stress. However, further investigations are required to determine which proteins are involved in faba bean defensive response and what role they play in plant resistance to herbivory.

Despite the physiological stress imposed by salinity, constitutive resistance to herbivory by *T. ni* was unaffected, indicating that faba bean maintained its baseline defensive traits. This aligns with observations that soluble protein (proteinase inhibitors, oxidative enzymes, etc.) and phenolic levels were not significantly affected by salinity, suggesting that baseline chemical defences remained constant. Additionally, the constant gas exchange parameters early in the treatment period suggest that faba bean was not yet experiencing the negative physiological effects associated with salinity stress, which could otherwise compromise constitutive resistance to herbivory (Züst & Agrawal, 2017). Cross-tolerance may explain the sustained constitutive defences, as overlapping stress responses can bolster defences against both abiotic and biotic stressors (Rejeb et al., 2014). In soybean, a relatively salt-sensitive species, salinity significantly reduced constitutive resistance to *T. ni* herbivory due to increased leaf nitrogen (Avila-Sakar et al., 2018). In contrast, a more salt-tolerant species, Indian mustard showed increased resistance stemming from decreased leaf nitrogen (Renault et al., 2016), highlighting the role of nitrogen in herbivore feeding preference. Since nutrient levels were only assessed postherbivory, further investigations are required to determine how constitutive leaf nitrogen affects herbivory resistance.

Strong induced resistance to herbivory requires substantial resource investment toward secondary metabolites and defensive compounds (Poelman et al., 2023). This study found that salinity significantly reduced induced resistance to *T. ni*, suggesting a compromised response to herbivory. One factor which could have contributed to the reduced induced resistance is crosstalk among hormonal signalling pathways between abscisic acid (ABA) and jasmonic acid (JA). Salinity-induced ABA may suppress JA-mediated defences (Anderson et al., 2004; Sah et al., 2016). As JA regulates induced defences against herbivory (Poelman et al., 2023), increased ABA levels during salinity stress may impair defence compound synthesis. However, hormone levels were not measured in this study, further investigations directly measuring ABA and JA levels are required to understand their roles in faba bean's defensive response.

Faba bean may also have allocated resources towards a defensive strategy that proves ineffective in a greenhouse setting. In the cultivar Broad Windsor (the one used in the present study), Mondor at al. (2013) found that leaf damage induced extrafloral nectary (EFN) production. In nature, EFNs attract predatory insects like ants or ladybugs; these insects form a mutualistic relationship with the plant, gaining carbohydrate-rich nectar in exchange for protecting the plant from herbivores (Marazzi et al., 2019). Without these mutualists in the greenhouse, increased EFN production would provide no defensive benefit. This suggests that the plant may have prioritized EFN production over direct chemical defences like phenolic compounds or defensive proteins, contributing to the reduced induced resistance. However, EFN's were not assessed in the present study.

While likely not the sole cause, the additional 48 hours of salinity may have compounded with other mechanisms to impair the induced response. Greater ionic stress could increase ROS accumulation, exacerbating oxidative stress and damaging cellular structures (Zhao et al., 2020).

Increased leaf nutritional quality under salinity stress may also have contributed to the weakened induced resistance to herbivory. However, the constitutive defences of the plant may have been sufficiently robust to counteract the effects of enhanced nutrient availability on herbivore feeding preference, which could explain why baseline constitutive resistance was unaffected by salinity. This study found significant increases in both leaf macronutrient (N, P, K, Ca, Mg) and micronutrient (Fe, Mn, Cu, Zn, Mo) content with increasing salinity, suggesting a greater nutrient availability for herbivores. Insect herbivores like *T. ni* often prefer nutrient-rich leaves (Molinari & Knight, 2010; Caldwell et al., 2016), particularly for nitrogen, which supports fast growth and larval development. Larvae feeding on high-quality leaves form larger pupae, with higher fecundity (Lill & Marquis, 2001). Furthermore, insect herbivores with slow growth rates are more susceptible to predators and pathogens (Shikano et al., 2018), suggesting that insects that feed on higher quality leaves will be less susceptible to predation. This relationship between leaf tissue quality and herbivory resistance has been demonstrated in prior studies. As previously mentioned, Avila-Sakar et al. (2018) attributed the significant reduction in resistance of soybean (*Glycine max*), in part, to increased leaf nitrogen content leading to a higher *T. ni* consumption. While Renault et al. (2016) found that significant decreases in leaf nitrogen content led to increased resistance as *T. ni* consumed less tissue of salt-stressed Indian mustard (*Brassica juncea*).

In summary, this study showed that salinity did not impact overall plant growth or constitutive resistance to *T. ni* herbivory, but it caused significant reductions in root and lateral leaf biomass, and increased ion accumulation in the leaves. These results suggest that the cultivar Broad Windsor exhibits a moderate degree of salt tolerance, likely due to nutrient redistribution and ion compartmentalization.

Salinity significantly increased leaf macro- and micronutrient concentrations, particularly nitrogen, which is known to enhance herbivore feeding preference. These results partially support our hypothesis; while salinity did increase the nutritional value of the leaves, the predicted decrease of resistance was only observed after exposure to *T. ni* herbivory, suggesting that this was not the sole factor involved in faba bean's defensive response. Despite the changes in leaf nutritional quality, constitutive resistance remained unchanged, possibly due to maintained ion homeostasis supporting sustained gas exchange (photosynthesis, stomatal conductance, transpiration), and enhanced antioxidant capacity. However, induced resistance was significantly impaired under salinity stress, suggesting that faba bean's defensive response was compromised following herbivore attack. We speculate that this compromised induced response may be due to hormonal crosstalk, where salinity-induced ABA interferes with JA-mediated defences, the allocation of resources towards a defensive strategy that proves ineffective under greenhouse conditions or increased oxidative stress resulting from the additional 48 hours of salinity exposure.

Collectively, these findings suggest that salinity alters the plant-herbivore interaction in faba bean. While baseline defences are maintained, the ability to enhance defences in response to attack is weakened, possibly increasing vulnerability to herbivory under saline conditions in greenhouse settings. Further studies assessing hormone dynamics, specifically ABA and JA, constitutive nutrient concentrations, specific antioxidant enzyme or nitrogenous defence compound concentrations (proteins, proline, glycine betaine), and EFN production are needed to better understand the mechanisms driving these changes and to inform crop management strategies in salt-affected areas.

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Table 3. Growth parameters (mean and SE.) of faba bean (*Vicia faba*) exposed to salinity stress for 20 days. *F* & *P* values obtained from linear regression.

Parameter		NaCl (mM) 0	50	75	100	$F_{1,22}$	*P*
Height (cm)	Total stem	102.22 (9.29)	106.53 (10.30)	97.70 (7.81)	87.42 (5.80)	1.51	0.23
Leaf Number (#)	Main stem	7.83 (0.17)	7.50 (0.34)	7.12 (0.48)	7.50 (0.34)	0.99	0.33
	Lateral stem	6.83 (1.40)	6.33 (0.67)	7.00 (1.73)	5.33 (1.09)	0.42	0.52
Dry Biomass (g)	Total stem	3.26 (0.21)	3.04 (0.26)	3.07 (0.33)	2.80 (0.15)	1.65	0.21
	Main stem pre-salt leaf	0.65 (0.06)	0.74 (0.07)	0.57 (0.13)	0.63 (0.05)	0.27	0.61
	Main stem post-salt leaf	0.95 (0.09)	0.75 (0.15)	0.72 (0.12)	0.69 (0.13)	2.62	0.12
	Lateral stem leaf	0.92 (0.08)	0.51 (0.11)	0.51 (0.11)	0.44 (0.14)	9.18	< 0.01
	Root	2.53 (0.29)	2.05 (0.17)	1.85 (0.14)	1.89 (0.08)	7.91	< 0.05

Table 4. Water content (mean and SE) of faba bean (*Vicia faba*) exposed to salinity stress for 20 days. *F* & *P* values obtained from linear regression.

Parameter		NaCl (mM) 0	50	75	100	$F_{1,22}$	P
Water content (%)	Total stem	87.55 (0.34)	87.85 (0.32)	86.80 (0.59)	87.08 (0.31)	1.44	0.24
	Main stem pre-salt leaf	87.81 (0.82)	88.83 (0.72)	88.75 (0.78)	88.52 (0.27)	0.73	0.40
	Main stem post-salt leaf	80.31 (0.32)	81.66 (0.48)	82.25 (1.02)	84.01 (0.45)	17.37	< 0.001
	Lateral stem leaf	83.93 (0.83)	85.43 (0.40)	85.50 (0.97)	82.09 (3.59)	0.17	0.68
	Root water content	93.24 (0.17)	93.07 (0.13)	92.77 (0.14)	93.21 (0.28)	0.39	0.54

Table 5. Leaf elemental composition (mean ± s.e.) of faba bean (*Vicia faba*) exposed to salinity stress (mg/g) for 20 days. *F* & *P* values obtained from linear regression. Mean and s.e. values obtained from raw data.

	NaCl (mM)					
Element	0	50	75	100	$F_{1, 10}$	*P*
Na	1.78 (0.33)	7.68 (0.24)	9.81 (2.17)	11.38 (0.06)	48.5	< 0.001
Cl	0.41 (0.03)	10.93 (0.56)	17.91 (3.35)	24.14 (3.32)	66.43	< 0.001
N	20.53 (0.15)	19.99 (1.03)	25.54 (3.10)	28.08 (0.93)	9.29	< 0.05
P	1.78 (0.09)	2.05 (0.18)	1.94 (0.10)	2.19 (0.02)	5.37	< 0.05
K	9.72 (0.53)	14.80 (0.40)	15.20 (0.47)	17.31 (0.76)	76.95	< 0.001
Ca	11.42 (0.76)	15.32 (0.53)	14.26 (0.92)	19.09 (0.74)	24.11	< 0.001
Mg	3.17 (0.21)	3.99 (0.16)	3.82 (0.30)	4.90 (0.40)	13.78	< 0.01
S	2.00 (0.21)	1.52 (0.11)	1.76 (0.16)	1.99 (0.16)	0.02	0.88
Fe*	55.09 (0.87)	56.95 (4.16)	59.28 (4.67)	70.69 (4.63)	6.14	< 0.05
Mn*	26.84 (4.05)	43.12 (1.93)	35.14 (2.06)	48.66 (4.36)	10.78	< 0.01
Cu*	2.38 (0.23)	3.55 (0.55)	3.75 (0.29)	3.85 (0.00)	12.05	< 0.01
Zn*	31.06 (3.36)	52.50 (3.12)	50.24 (4.55)	51.12 (2.25)	12.35	< 0.01
Mo*	0.59 (0.30)	0.90 (0.34)	1.27 (0.25)	3.32 (0.78)	9.66	< 0.05
B*	19.94 (1.50)	18.35 (0.93)	16.32 (2.42)	19.06 (1.89)	0.55	0.47

*For elements denoted with an asterisk, mean concentrations are shown in µg/g

Table 6. Stem elemental composition (mean ± s.e.) of faba bean (*Vicia faba*) exposed to salinity stress (mg/g) for 20 days. *F* & *P* values obtained from linear regression. Mean and s.e. values obtained from raw data.

	NaCl (mM)					
Element	0	50	75	100	$F_{1, 10}$	*P*
Na	3.92 (0.31)	14.78 (0.29)	14.75 (0.84)	18.91 (0.49)	100.4	< 0.001
Cl	1.34 (0.10)	23.38 (1.12)	25.42 (2.15)	30.34 (1.26)	86.67	< 0.001
N	15.99 (0.68)	16.40 (0.68)	20.08 (2.13)	21.01 (1.19)	9.02	< 0.05
P	3.08 (0.04)	3.54 (0.08)	3.47 (0.07)	3.62 (0.03)	23.97	< 0.001
K	21.12 (0.12)	25.92 (1.39)	24.38 (2.23)	22.47 (0.56)	0.57	0.47
Ca	4.91 (0.29)	5.16 (0.17)	4.87 (0.31)	5.57 (0.24)	1.80	0.21
Mg	2.04 (0.07)	2.11 (0.09)	2.08 (0.10)	2.11 (0.07)	0.37	0.56
S	2.07 (0.10)	1.60 (0.11)	1.42 (0.21)	1.42 (0.13)	13.3	< 0.01
Fe*	42.08 (3.25)	50.81 (3.43)	47.94 (3.23)	75.42 (15.1)	5.58	< 0.05
Mn*	11.14 (0.50)	13.86 (0.98)	12.97 (0.06)	13.70 (1.12)	4.68	0.06
Cu*	2.49 (0.34)	2.94 (0.39)	3.17 (0.13)	2.81 (0.40)	1.04	0.33
Zn*	27.49 (1.71)	38.59 (3.76)	31.49 (2.00)	37.08 (2.41)	3.32	0.09
Mo*	14.29 (1.63)	17.76 (1.38)	14.99 (3.32)	18.06 (1.53)	1.05	0.33
B*	18.51 (0.62)	21.34 (0.55)	21.30 (0.98)	20.46 (0.55)	3.99	0.07

*For elements denoted with an asterisk, mean concentrations are shown in µg/g

Table 7. Root elemental composition (mean ± s.e.) of faba bean (*Vicia faba*) exposed to salinity stress (mg/g) for 20 days. *F* & *P* values obtained from linear regression. Mean and s.e. values obtained from raw data.

	NaCl (mM)					
Element	0	50	75	100	$F_{1,\ 10}$	*P*
Na	10.90 (1.13)	23.42 (1.93)	26.49 (1.40)	30.79 (2.07)	79.82	< 0.001
Cl	0.59 (0.10)	28.60 (1.63)	29.85 (3.99)	38.67 (3.67)	62.66	< 0.001
N	22.03 (0.68)	21.07 (0.99)	23.38 (0.92)	24.85 (0.77)	4.62	0.06
P	2.65 (0.24)	2.35 (0.27)	3.21 (0.13)	3.19 (0.07)	3.93	0.08
K	12.89 (1.44)	10.84 (1.83)	14.60 (1.19)	13.91 (1.40)	0.60	0.46
Ca	7.04 (0.15)	6.80 (0.63)	6.46 (1.16)	6.64 (0.56)	0.32	0.58
Mg	6.27 (0.51)	3.47 (0.40)	2.61 (0.15)	1.73 (0.05)	19.69	<0.01
S	7.78 (0.41)	7.03 (0.15)	7.67 (0.77)	7.38 (0.20)	0.21	0.66
Fe	0.17 (0.02)	0.19 (0.03)	0.12 (0.01)	0.14 (0.01)	1.81	0.21
Mn*	18.93 (4.36)	19.98 (0.34)	17.66 (0.27)	19.32 (1.49)	0.01	0.93
Cu*	9.55 (0.44)	8.84 (0.50)	9.94 (1.95)	9.08 (0.87)	0.01	0.91
Zn*	19.61 (1.16)	21.65 (0.92)	25.63 (1.40)	24.98 (1.21)	13.84	< 0.01
Mo*	6.77 (087)	7.30 (0.43)	6.29 (0.90)	6.23 (0.80)	0.45	0.52
B*	12.38 (0.77)	13.93 (0.27)	13.88 (0.38)	13.98 (0.51)	5.75	< 0.05

*For elements denoted with an asterisk, mean concentrations are shown in µg/g

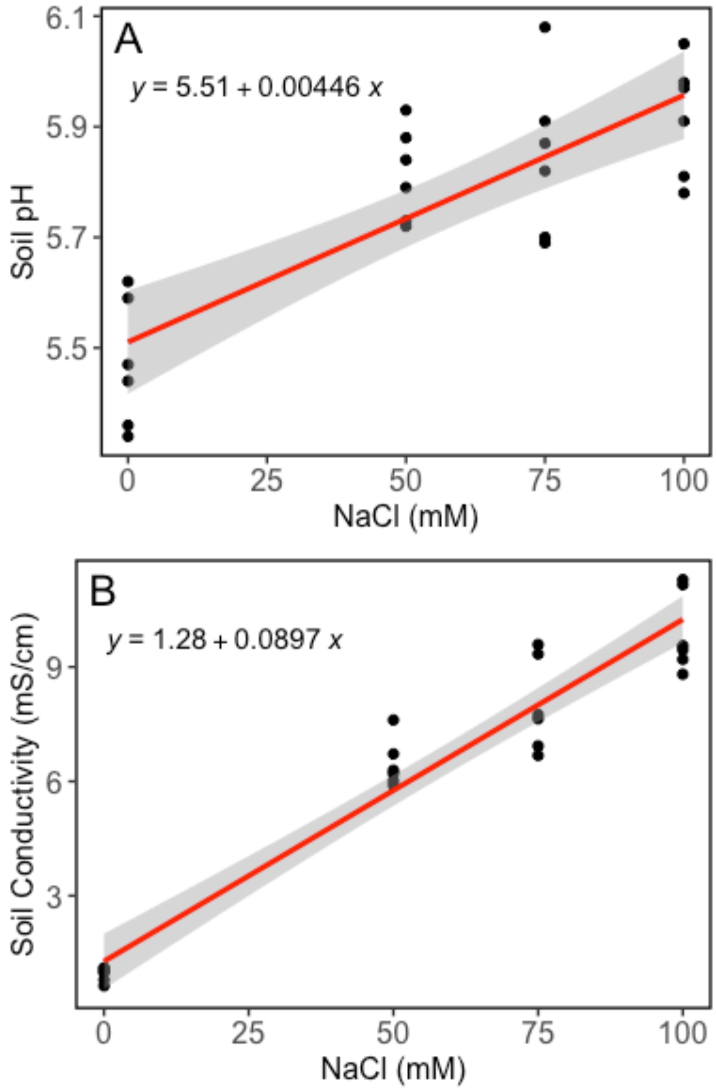

Figure 1. Soil pH (A) and soil conductivity (m*S*/cm) (B) of *V. faba* treated with 0, 50, 75, and 100 mM NaCl for 20 days. Linear regression was used for statistical analysis. Shaded area represents 95% CI.

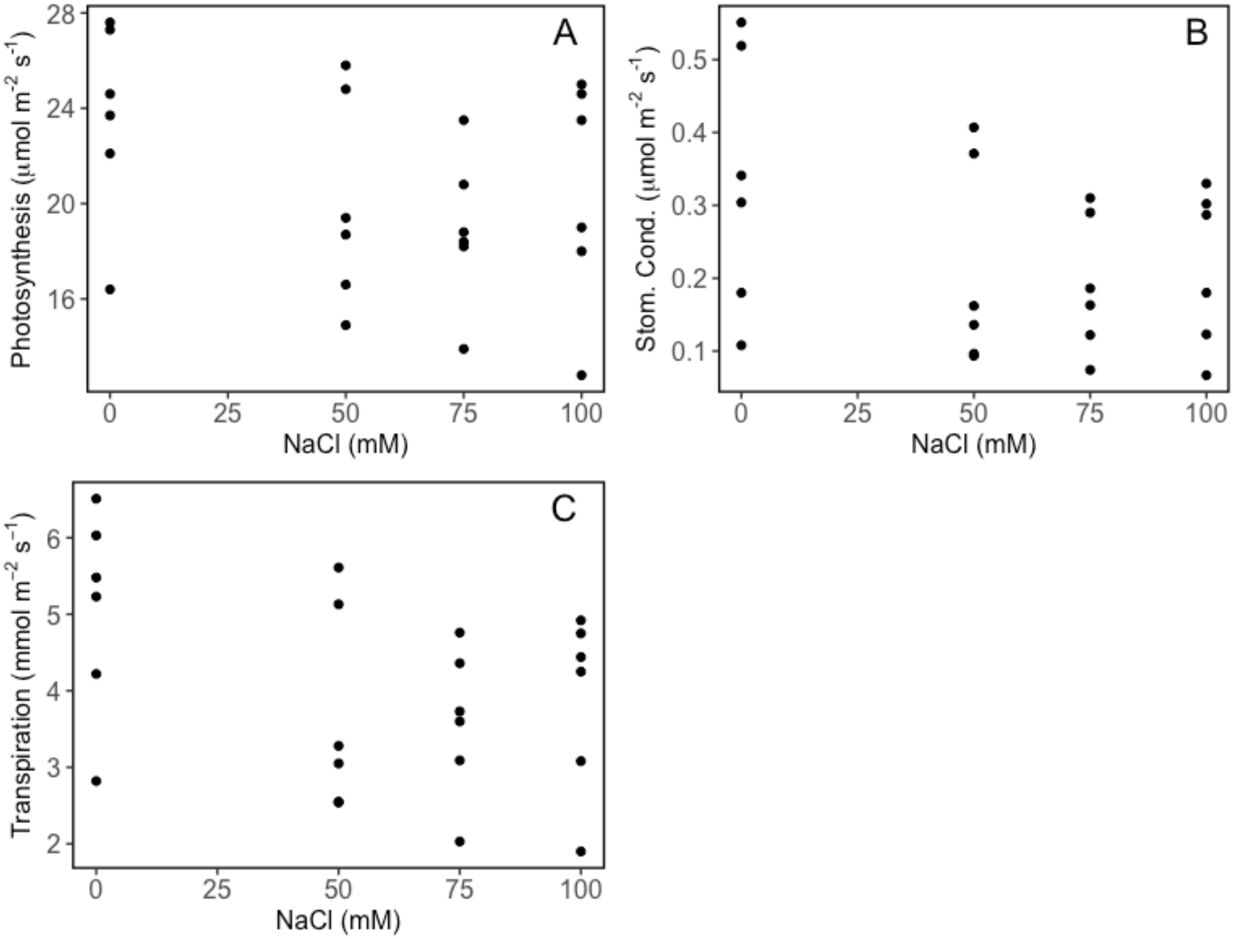

Figure 2. Photosynthetic rate (μmol m^{-2} s^{-1}) (A), stomatal conductance (μmol m^{-2} s^{-1}) (B), and transpiration rate (mmol m^{-2} s^{-1}) (C) of *V. faba* treated with 0, 50, 75, and 100 mM NaCl for 11 days. Linear regression was used for statistical analysis.

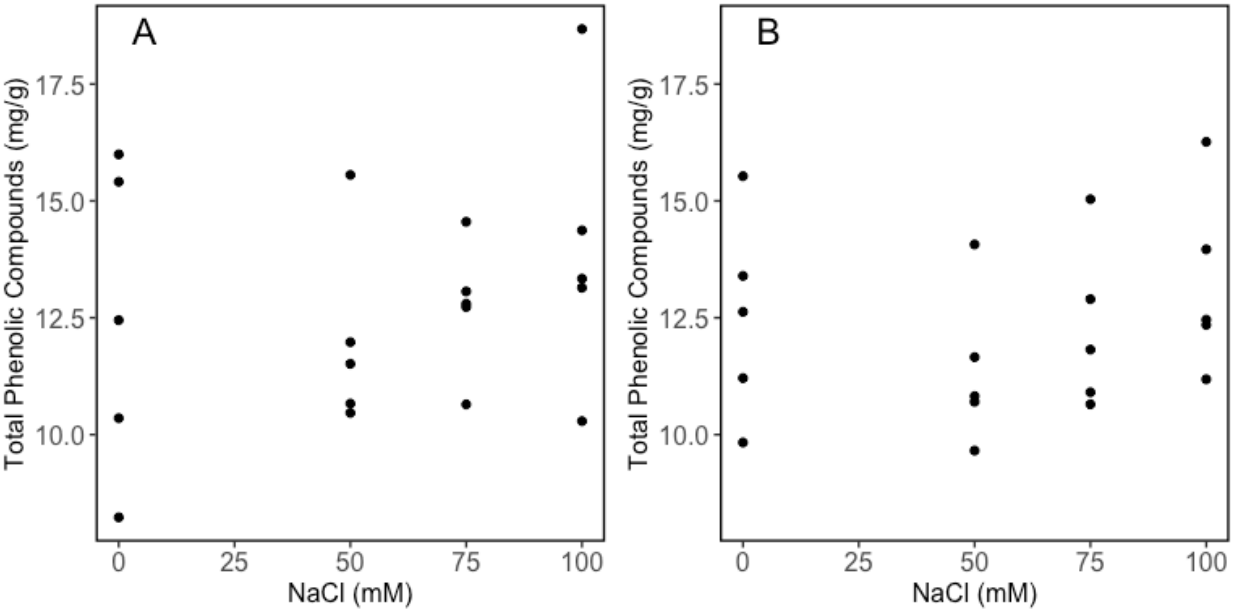

Figure 3. Total constitutive phenolic compounds (mg/g) (A) and total induced phenolic compounds (mg/g) (B) of *V. faba* treated with 0, 50, 75, and 100 mM NaCl for 20 days. Linear regression was used for statistical analysis.

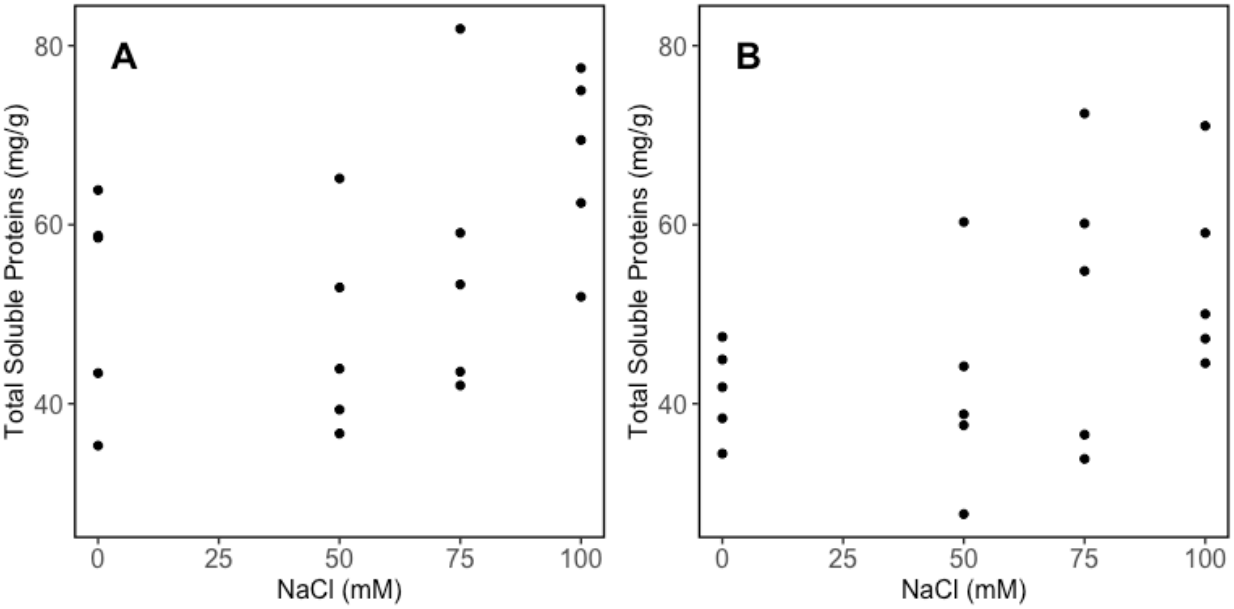

Figure 4. Total constitutive soluble proteins (mg/g) (A) and total induced soluble proteins (mg/g) (B) of *V. faba* treated with 0, 50, 75, and 100 mM NaCl for 20 days. Linear regression was used for statistical analysis.

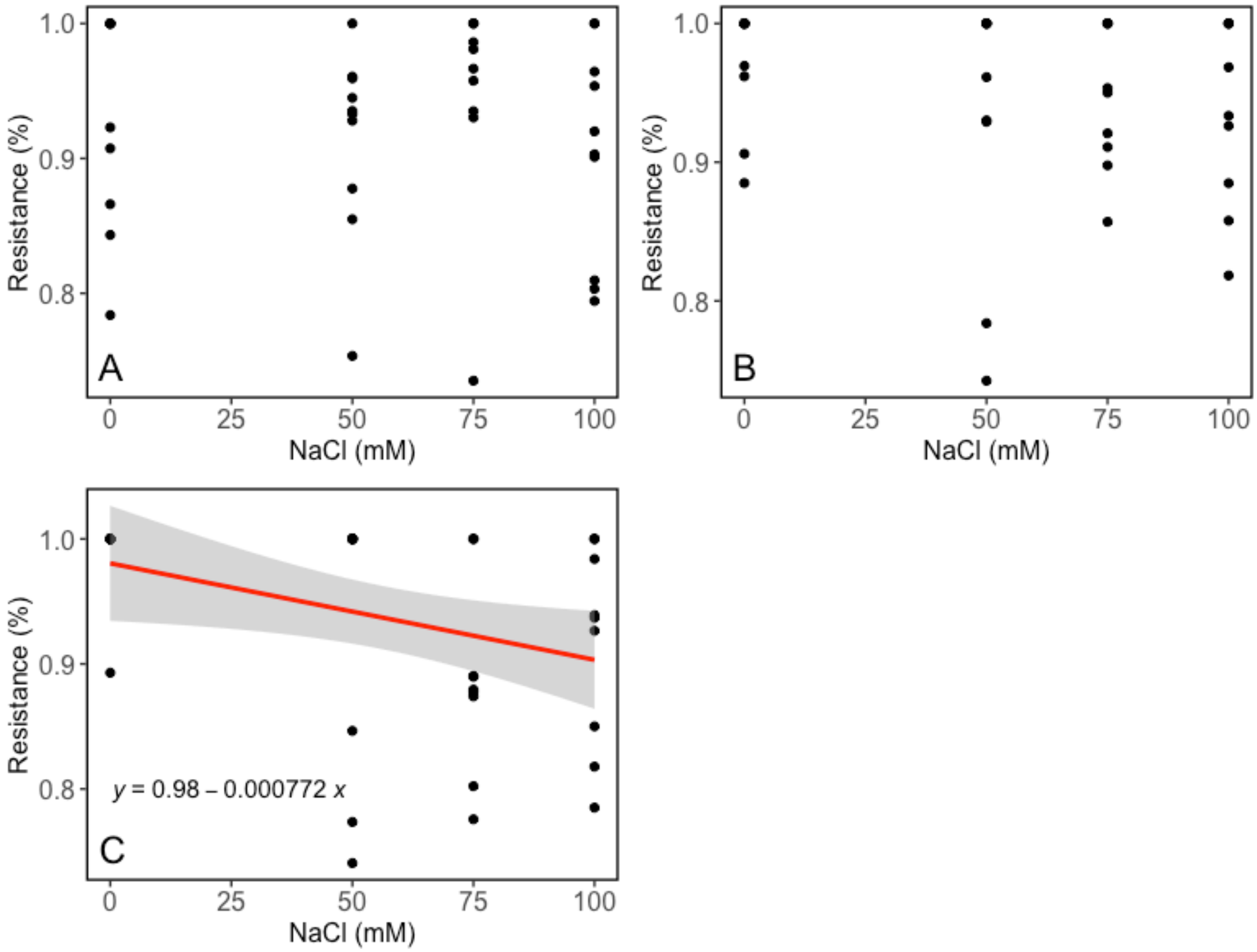

Figure 5. *In vivo* constitutive resistance (A), *in vitro* constitutive resistance (%) (B) and *in vitro* induced resistance (C) (%) of *V. faba* treated with 0, 50, 75, and 100 mM NaCl for 20 days. Linear regression was used for statistical analysis. Shaded area represents 95% CI