



Research paper

## Plant resistance to the whitefly *Bemisia tabaci* is compromised in salt-stressed *Capsicum*

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## ABSTRACT

Climate change has profound effects on crop production, for example through increasing temperatures, and more frequent extreme weather events. Climate change can also lead to increased pest pressure. How plants cope under double stress conditions is dependent on pest species, environment, and plant genotype, and for many plant-insect interactions, this knowledge is lacking. The whitefly *Bemisia tabaci* is an important pest worldwide and can be destructive for pepper (*Capsicum*) production. Breeding resistant varieties could aid in combatting whiteflies in a sustainable manner. In this study, we aimed to identify *Capsicum* accessions with resistance to *B. tabaci*, and study how this resistance was affected by salt stress. We grew 25 *Capsicum* accessions under salt treatment, and measured *B. tabaci* survival and oviposition. We identified four accessions with increased whitefly resistance, exhibited as higher adult mortality. Under salt stress, growth of most accessions was inhibited, and Na<sup>+</sup> accumulated in shoots. Importantly, in all plants that had experienced salt stress, whitefly survival and oviposition increased, essentially nullifying resistance in salt-stressed plants. When plants were treated with salt, the phytohormone jasmonic acid was reduced compared to whitefly-infested plants without salt, possibly resulting in reduced defense to whiteflies. The results of this study will contribute to a better understanding of pest resilient plants in a changing climate.

## 1. Introduction

Climate change leads to increasing temperatures, and extreme weather events are expected to happen more frequently, bringing about floods or long periods of drought. This will lead to increased (abiotic) stress conditions for plants and crop systems (Chaudhry and Sidhu, 2022). At the same time, climate change can lead to increased pest pressure, due to an increase in range or an increase in annual number of generations (IPCC Secretariat, 2021). Low-lying deltas are particularly vulnerable to sea-level rise and associated saltwater intrusion. Here, climate change is expected to exacerbate existing stresses and have an impact on food production, for example on pepper production, via

increasing salination, drought, and pests (Siegmond-Schultze et al., 2023). Various technical, behavioral and institutional changes in the broader food system are required to cope with the stress factors. Using crops with increased tolerance to biotic and abiotic stress factors is one component that could contribute to a solution.

*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is among the most devastating pests on crops. It is a generalist that can infest many plant species, including ornamental, field, and vegetable crops (De Barro et al., 2011). It causes direct damage by feeding, and indirectly by the viruses it transmits. It can vector over 400 plant viruses, among which geminiviruses are a major threat to agriculture (Navas-Castillo et al., 2011). *Bemisia tabaci* is a group of over 40 cryptic, morphologically

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indistinguishable species, of which MEAM1 is one of the most widespread. (Dinsdale et al., 2010; De Barro et al., 2011; Firdaus et al., 2013b; Legg et al., 2014; Vyskočilová et al., 2019; Mugerwa et al., 2021) Control of whiteflies is until now mostly achieved by use of pesticides. As an alternative to pesticide use, breeding for host plant resistance has been explored in several plant species, and identification of resistance in crop plants or wild relatives has been attempted. Resistance to *B. tabaci* in tomato is associated with glandular trichomes and presence of acyl sugars (Firdaus et al., 2013a; Vosman et al., 2019). In the genus *Capsicum*, exploration of germplasm revealed differences in survival or performance of *B. tabaci*. Several accessions were identified with lower oviposition (Firdaus et al., 2011; Pantoja et al., 2018; Hernández-Alvarado et al., 2019), higher nymphal mortality (Ballina-Gomez et al., 2013; Pantoja et al., 2018; Hernández-Alvarado et al., 2019) or adult mortality of whiteflies (Sandra et al., 2022), but the mechanism of resistance in these accessions is not known.

Increased salinity of the soil is characterized by a high concentration of soluble salts (often Na<sup>+</sup> and Cl<sup>-</sup>). Its effect on plants is two-fold: first, an immediate osmotic effect, as high concentrations of salt make it harder for roots to take up water. Secondly, ion toxicity, resulting from accumulation of ions in the shoot (Munns and Tester, 2008). In *Capsicum*, salt stress results in reduced growth, measured as a reduction in fresh and dry weight, plant height and leaf area (Günes et al., 1996; Chartzoulakis and Klapaki, 2000; Aktas et al., 2006). Yield can also be affected: total fruit yield and fruit size were decreased under higher salinity levels (Chartzoulakis and Klapaki, 2000; Navarro et al., 2002).

To cope with stress conditions, plants have evolved several response mechanisms. Multiple components of the regulatory network involved in stress responses, such as reactive oxygen species (ROS) signaling, redox status, ion fluxes, and accumulation of phytohormones: jasmonic acid, salicylic acid, abscisic acid, are shared between different stresses (Kissoudis et al., 2014; Nguyen et al., 2016b). These mechanisms may function antagonistically or some responses may be prioritized over others. Moreover, physical and chemical resistance traits may be affected by abiotic stress responses. Consequently, resistance to pests or pathogens may be affected, if they are accompanied by a (prior) abiotic stress (Kissoudis et al., 2014; Nguyen et al., 2016a; Bai et al., 2018; Quais et al., 2020). The outcome is likely to be plant, genotype, pathogen, and stress intensity-dependent, but for many plant-insect-abiotic stress, knowledge on the interaction is lacking.

In this study, we investigated the impact of salt stress on the pepper – whitefly interaction. While some whitefly resistance has been found in *Capsicum* and can be used to breed resistant varieties, it is not known how these plants would perform under an additional stress, in this case higher salt, and which mechanisms in the plant are affected. Here, we aimed to identify pepper (*Capsicum* sp) accessions with resistance to the whitefly *B. tabaci*, and we studied how this resistance is affected by salt stress. To do this, we screened 25 *Capsicum* accessions and identified accessions resistant to whitefly and accessions with tolerance to salt stress. Then, we assessed how resistance to whitefly is affected when the plants are exposed to salt stress as well, and how phytohormone accumulation changed in response to the dual stress. The results of the study will contribute to growing pest-resilient plants in a changing climate.

## 2. Material and methods

### 2.1. Plant material

Twenty-five *Capsicum* accessions from four different species were used in this study; Sixteen *C. annuum* (Linnaeus), five *C. chinense* (Jacquin), two *C. frutescens* (Linnaeus), and two *C. pubescens* (Ruiz & Pavón). Seeds were obtained from the Plant Breeding collection (Wageningen University and Research, the Netherlands). The selection of the accessions was based on previously performed unpublished evaluations.

### 2.2. Salt treatment experiment

Plants were sown out on vermiculite #3. Four weeks after sowing, eight seedlings per accession were potted in vermiculite #3, and arranged in a randomized complete block design consisting of eight blocks, four per salt concentration, with one replicate of each accession per block. The plants were grown at a temperature of 25°C /19°C for day/night with 60 % relative humidity and a day/night cycle of 16/8 hours. Plants were watered via a dripping system with a 0.5 x Hoagland solution. Three weeks after potting the plants, the salt treatment was started by adding NaCl to a concentration of 0 mM or 75 mM to the irrigation solution. The salt treatment continued for three weeks.

### 2.3. Plant measurements

Plant growth was monitored by measuring plant height using a one meter ruler from the topmost leaf to the base of the stem. Plant height was measured at 3 days, 13 days, and 20 days after the start of the salt treatment (DAT). To measure dry weight of the shoots at the end of the experiment (21 DAT), plants were cut at the stem and dried at 70°C for five days.

### 2.4. Ion chromatography

To measure the amount of sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), and calcium (Ca<sup>2+</sup>), all dried shoot samples were ground using an IKA-A11 grinder, or a mortar and pestle. After grinding, 25–50 mg of dry powder per sample was weighed and ashed in glass tubes at 550 degrees for 5 hours. Ash samples were dissolved by shaking for 30 minutes in 1 mL 3 M formic acid, and then diluted with 9 mL Milli-Q water. Of this dilution, 100 µL was added to 9.9 mL Milli-Q (final dilution sample is 1000x). The ion content of each shoot sample was assessed using the Ion Chromatography (IC) system 850 Professional (Metrohm Switzerland).

### 2.5. Whitefly rearing and assay

The population of *B. tabaci* MEAM1 was maintained on Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) plants in insect rearing cages with W60 x D60 x H60 and mesh size of 150 x 150 (160 µm aperture) (Bugdorm, Taiwan). The rearing was kept in a climatized greenhouse at Unifarm, Wageningen, the Netherlands at 25/24°C (day/night temperature), relative humidity of 60–70 %, and a day/night cycle of 16/8 hours. A no-choice assay was performed two weeks after start of the salt treatment as described by Lucatti et al. (2013). Two clip-on-cages containing 5 synchronized one-day-old female whiteflies were placed on the abaxial side of the first and second fully expanded leaf of each plant. After five days, the leaves with clip cages were detached by cutting clip cages along with the smallest portion of leaf possible without whitefly escape. Living and dead whiteflies and the number of eggs present on the leaf were counted using a stereomicroscope binocular and used to calculate Adult Survival (AS) and Oviposition (OR).

### 2.6. Whitefly assay for measuring hormone accumulation

Plants were grown in a separate assay to measure the amount of phytohormones in plant tissue with salt stress and whitefly infestation. Two accessions, *C. annuum* – 51 and *C. annuum* – 21, were included in this assay. The plants were grown and the whitefly assay was performed as described above. Two weeks after the salt treatment, two clip-on-cages containing 5 synchronized one-day-old female whiteflies were placed on the abaxial side of the first and second fully expanded leaf of each plant. After 24 hours, the whiteflies were removed and the leaf area under the clip-cage was punched out with a 1 cm leaf puncher, harvested in a 2 mL Eppendorf tube and flash-frozen in liquid nitrogen. Punches of leaves without whitefly infestation were harvested similarly for the none-whitefly treatment.

## 2.7. Hormone extraction, measurement, and quantification

Around 20 mg of flash-frozen plant material was used per sample and extracted as previously described (Schiessl et al., 2019; Gühl et al., 2021) with the addition of abscisic acid (ABA), OPDA, Jasmonic acid (JA), JA-Isoleucine (JA-Ile) and salicylic acid (SA) stable isotope-labeled Internal Standards (IS, Table S1). Sample residues were re-dissolved in 100  $\mu$ L of acetonitrile/water (20:80, v/v) for the acid fraction (ABA, IAA, OPDA, JA, JA-Ile, and SA) and filtered through a 0.45 mm Minisart SRP4 filter (Sartorius, Göttingen, Germany). Analyses were performed by comparing retention times and mass transitions with those of unlabeled standards (Table S1) using a Waters XevoTQs mass spectrometer equipped with an electrospray ionization source coupled to an Acquity UPLC system (Waters, Milford, USA), as previously described (Schiessl et al., 2019; Gühl et al., 2021). Multiple Reaction Monitoring (MRM) transitions, cone voltage and collision energy selected for compound identification and quantification are shown in Table S1. To determine sample concentrations, a 10-point calibration curve was constructed for each compound ranging from 190 pM to 1  $\mu$ M, and each dilution also contained a known amount of an appropriate deuterium-labelled internal standard.

## 2.8. Statistical analysis

For the experiment assessing the effect of salt on growth, and whitefly performance, four replicates per accession and salt treatment were included (8 plants total per accession). An ANOVA analysis was carried out on height and weight in salt-treated and non-salt-treated plants, and whether salt affected height and weight per accession was determined with T-tests. For survival and oviposition of whiteflies, adult survival was calculated as  $AS = \frac{\text{living whiteflies}}{\text{living} + \text{dead whiteflies}}$ . Oviposition rate was calculated as the number of eggs per average number of living females, calculated as  $OR = \frac{\text{eggs}}{\text{living whiteflies} + (\text{dead whiteflies}/2)}$ . The effect of salt and accession on survival of whiteflies was tested using ANOVA analysis. For ANOVA analysis, AS data was transformed using arcsine(sqrt(AS)) and the OR data was transformed as sqrt(OR). Significance of differences in

accession means was tested on salt-treated or without salt-treated separately, using an LSD test ( $P < 0.05$ ) on the transformed data. For the experiment measuring phytohormone accumulation in plants with and without whitefly infestation and growing with and without a salt treatment, four replicates per accession per treatment were used (16 plants total per accession). The effect of salt and whitefly on hormone accumulation was tested using ANOVA analysis.

## 3. Results

### 3.1. Salt treatment reduces bio-mass of pepper plants

To study the effect of salt stress on growth of *Capsicum* plants, we grew 25 accessions of *C. annuum*, *C. pubescens*, *C. frutescens*, and *C. chinense* for three weeks in vermiculite pots watered with 0.5 x Hoagland solution containing either 0 mM or 75 mM NaCl. Plant height was measured three, 13, and 20 days after the start of the salt treatment. Over all accessions, the addition of salt did not reduce plant height at 3 days or 13 days, but led to significantly reduced plant height 20 days after the start of the treatment (Fig. 1, p-value < 0.001). Similarly, shoot dry weight, measured at the end of the experiment, was significantly lower for salt-treated plants (Fig. 1, p-value < 0.001). Considering each accession separately, reduction in weight of salt-stressed plants was up to 70 % compared to non-stressed plants, while height was reduced by up to 40 % (Table 1). Ten accessions did not significantly decrease in weight and height at 20 DAT. From these, three accessions, all *Capsicum annuum*, height after 20 days and dry weight were not reduced in salt-treated plants, i.e. *C. annuum* - 14, *C. annuum* - 118 and *C. annuum* - 23 (Table 1).

### 3.2. Salt treatment increases $Na^+$ in leaf tissues

When plants were grown without the NaCl treatment, all genotypes had similar  $Na^+$  levels in the shoot and the  $K^+/Na^+$  ratio were similar between genotypes (Table 2). When plants grew under salt treatment, the concentration of sodium increased in the shoot of all accessions but one (*Capsicum annuum*-39). In addition, the content of  $K^+$  in shoots was

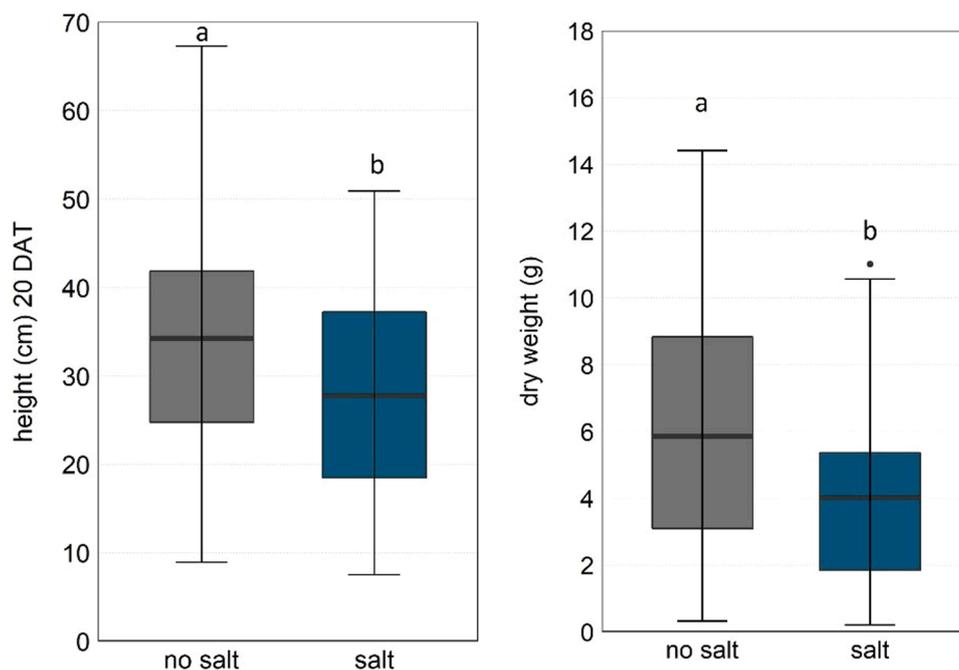


Fig. 1. Effect of salt on plant growth. Height and dry weight of all plants growing for three weeks in conditions of 0.5 x Hoagland solution supplemented with 0 mM NaCl (no salt) or 75 mM NaCl (salt). Height was measured 20 days after the start of the treatment (DAT), dry weight was measured at the end of the experiment by cutting shoot at the stem and drying. Different letters indicate statistically significant differences between no-salt and salt-treated plants (Anova, p-value < 0.001).

**Table 1**

Effect of salt on plant growth depends on accession. Plant height in cm 3 days (3DAT), 13 days (13DAT), and 20 days after the start of the treatment (20DAT), and shoot dry weight in g for each accession. The difference between height and 20 DAT and 3 DAT in cm is also shown. For dry weight, the difference in percentage between non-salt treated and salt-treated plants is shown. Accessions are ordered on increasing percentage of difference in height. An asterisk in the 75 mM NaCl column indicates a significant difference in height or dry weight between non-salt and salt treated plants (T-tests,  $p < 0.05$ ).

accession	Height 3DAT (cm)		Height 13DAT (cm)		Height 20DAT (cm)		Difference 20–3 DAT (cm)		Dry weight (g)		
	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	%
<i>C. annuum</i> - 14	10.8	11.4	15.7	21.0	30.8	33.9	20.0	22.5	2.7	2.7	0.0
<i>C. annuum</i> - 118	8.0	11.7	17.1	21.7	30.4	33.1	22.4	21.5	3.3	3.8	-15.2
<i>C. annuum</i> - 23	10.9	10.7	17.0	18.9	30.6	30.5	19.8	19.8	2.5	2.6	-4.0
<i>C. annuum</i> - 2	15.7	12.4*	28.7	26.0	43.3	40.4	27.6	27.9	11.0	6.6*	40.0
<i>C. annuum</i> - 30	8.6	10.1	16.2	16.2	26.4	24.4	17.8	14.3	2.7	2.2	18.5
<i>C. annuum</i> - 39	15.6	14.2	30.5	26.9	40.4	37.2	24.8	23.1	9.0	4.9*	45.6
<i>C. annuum</i> - 50	14.9	15.5	30.1	28.6	42.1	38.3	27.2	22.8	9.2	6.4	30.4
<i>C. annuum</i> - 119	13.3	14.4	23.4	22.4	35.4	32.2	22.1	17.8*	7.8	6.2	20.5
<i>C. frutescens</i> - 38	6.4	7.4	13.2	13.2	24.5	22.0	18.1	14.5*	3.1	2.7	12.9
<i>C. chinense</i> - 37	13.2	13.2	23.6	22.2	37.9	32.7	24.7	19.4	6.0	5.1	15.0
<i>C. annuum</i> - 53	20.9	19.7	42.0	38.0	57.6	49.3*	36.7	29.6*	13.5	9.7*	28.1
<i>C. annuum</i> - 51	20.5	19.0	38.3	33.0*	54.5	46.0	34.0	27.1	7.9	4.2*	46.8
<i>C. chinense</i> - 35	5.2	4.7	10.0	8.6	15.2	12.8	10.0	8.1	1.7	0.9	47.1
<i>C. annuum</i> - 52	18.4	13.7*	29.6	24.6	39.3	31.9*	20.9	18.2	13.2	6.7*	49.2
<i>C. annuum</i> - 21	8.0	6.9	16.6	13.2	29.0	22.1	21.0	15.2	5.2	3.2	38.5
<i>C. annuum</i> - 12	15.3	14.4	26.9	23.4	40.5	30.6*	25.2	16.2*	9.9	5.4*	45.5
<i>C. chinense</i> - 20	7.7	6.8	14.6	9.8	23.8	17.0	16.2	10.3	2.7	1.2*	55.6
<i>C. annuum</i> - 18	18.0	17.1	34.5	28.9	53.0	37.8	35.0	20.7	8.0	5.6	30.0
<i>C. chinense</i> - 31	8.9	6.5	19.4	13.9	34.1	24.2	25.1	17.8*	7.6	3.0*	60.5
<i>C. annuum</i> - 80	13.3	10.3	27.3	20.2	42.0	29.8	28.8	19.4	5.9	3.4	42.4
<i>C. frutescens</i> - 22	4.9	5.2	8.1	7.8	17.1	11.7	12.2	6.6	1.6	0.9	43.8
<i>C. pubescens</i> - 2	10.0	10.2	18.6	17.0	32.3	21.7*	22.3	11.5	7.6	5.1*	32.9
<i>C. chinense</i> - 33	9.7	6.3	15.2	11.4	22.4	14.1*	12.8	7.8	4.0	1.4*	65.0
<i>C. annuum</i> - 40	7.0	5.9	17.6	11.8*	31.9	18.6*	24.9	12.7*	4.8	1.6*	66.7
<i>C. pubescens</i> - 1	9.1	9.6	16.9	13.7	29.8	16.8*	20.7	7.2*	7.1	4.3	39.4

**Table 2**

Increase of  $\text{Na}^+$ , reduction of  $\text{K}^+$ , in leaf tissue of salt-treated plants. Shown are the means in  $\mu\text{g}/\text{mg}$  of four replicates per treatment (0 mM NaCl or 75 mM NaCl). The ratio  $\text{K}^+/\text{Na}^+$ , and  $\text{Ca}^{2+}/\text{Na}^+$  are also given. Accessions are ordered from higher to lower  $\text{K}^+/\text{Na}^+$  ratio under saline conditions.

Accession	$\text{Na}^+$ ( $\mu\text{g}/\text{mg}$ )		$\text{K}^+$ ( $\mu\text{g}/\text{mg}$ )		$\text{Ca}^{2+}$ ( $\mu\text{g}/\text{mg}$ )		$\text{K}^+/\text{Na}^+$ ( $\mu\text{g}/\text{mg}$ )	
	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl
<i>C. annuum</i> - 39	5.57	5.58	84.4	49.7	16.7	13.8	15.2	8.9
<i>C. chinense</i> - 37	5.22	10.00	90.4	74.5	19.6	16.0	17.3	7.4
<i>C. chinense</i> - 35	3.88	7.12	67.9	50.0	16.4	12.2	17.5	7.0
<i>C. annuum</i> - 12	3.55	7.62	76.8	51.0	14.4	15.5	21.6	6.7
<i>C. annuum</i> - 52	2.72	7.75	75.0	49.0	14.2	16.3	27.5	6.3
<i>C. annuum</i> - 53	3.72	8.60	66.8	53.5	14.8	16.0	17.9	6.2
<i>C. annuum</i> - 30	6.60	9.70	67.6	53.7	16.8	14.1	10.2	5.5
<i>C. annuum</i> - 50	3.17	9.80	75.0	52.8	14.3	17.1	23.6	5.4
<i>C. annuum</i> - 18	5.35	10.35	77.0	50.4	18.8	14.2	14.4	4.9
<i>C. annuum</i> - 2	4.92	9.92	73.5	47.0	14.1	14.6	14.9	4.7
<i>C. annuum</i> - 14	3.05	9.18	49.9	42.8	11.2	11.7	16.3	4.7
<i>C. annuum</i> - 51	4.45	10.47	69.3	47.8	18.7	17.6	15.6	4.6
<i>C. chinense</i> - 20	6.03	9.38	85.5	40.8	19.1	14.1	14.2	4.3
<i>C. pubescens</i> - 2	4.08	9.43	71.4	40.6	18.6	20.5	17.5	4.3
<i>C. annuum</i> - 21	4.22	9.43	55.9	37.6	8.1	7.1	13.2	4.0
<i>C. pubescens</i> - 1	4.30	10.62	63.5	40.8	23.5	20.4	14.8	3.8
<i>C. annuum</i> - 119	3.78	10.93	62.3	41.4	8.5	9.7	16.5	3.8
<i>C. annuum</i> - 80	4.03	14.80	69.7	43.7	14.6	13.2	17.3	3.0
<i>C. annuum</i> - 23	3.85	13.43	53.2	34.6	14.8	13.9	13.8	2.6
<i>C. frutescens</i> - 38	4.85	15.53	83.1	37.6	15.1	12.6	17.1	2.4
<i>C. annuum</i> - 118	5.50	16.38	82.6	38.5	18.2	13.3	15.0	2.3
<i>C. chinense</i> - 31	5.08	20.65	68.1	37.4	22.3	14.1	13.4	1.8
<i>C. frutescens</i> - 22	5.10	22.52	94.2	39.2	18.3	14.2	18.5	1.7
<i>C. chinense</i> - 33	5.15	30.12	57.1	37.3	10.0	6.1	11.1	1.2
<i>C. annuum</i> - 40	4.53	41.98	54.3	22.5	8.4	4.7	12.0	0.5

lower in salt-treated plants and the ratio  $\text{K}^+/\text{Na}^+$  was reduced (Table 2). There was no correlation between  $\text{Na}^+$  accumulation and reduction in weight or height, but there was a slight positive correlation between the ratio  $\text{K}^+/\text{Na}^+$  and reduction in height and weight.

### 3.3. Pepper genotypes vary in resistance to whiteflies

Overall, whitefly survival was high on all *Capsicum* accessions tested. Reproduction of the whiteflies varied between accessions and ranged from 13 and 30 eggs per living female (Table S2). Without salt treatment, whitefly survival was significantly reduced on four accessions, i.e.

*C. annuum*-119, *C. annuum*-118, *C. annuum*-21 and *C. annuum*-40 (Fig. 2, Table S2;  $df = 24$ ,  $F = 5.81$   $p$ -value  $< 0.001$ ). On these accessions, mean survival of adults was between 38 % and 45 %. Oviposition rate on these four accessions was also lower than on other accessions (Table S2). This indicates that these four *C. annuum* accessions contain resistance against *B. tabaci*, affecting survival and reproduction of adult whiteflies.

### 3.4. Salt treatment increases survival of whitefly in resistant accessions

When plants were growing under salt stress, survival of whiteflies increased. Overall, survival of whiteflies increased from 85 % to 95 % (Fig. 2). Oviposition rate also increased slightly on most accessions (Table S2). Both accession and salt had a significant effect on adult survival, and the interaction of the two was also significant. However, only on the four accessions that were identified as resistant in the treatment without salt, survival of the whiteflies significantly increased after salt treatment, to over 88 % (Fig. 2, Table S2).

### 3.5. Phytohormone accumulation in salt-treated and whitefly-infested plants

To understand why plant resistance to whiteflies was negatively affected under salt stress, we examined three phytohormones involved in abiotic and biotic stress in plants, i.e. abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA), in two *C. annuum* accessions. One that was susceptible, *C. annuum*-51, and one in which whitefly adults had reduced survival, *C. annuum*-21. When growing under salt stress, the accumulation of ABA increased in both accessions (Fig. 3). When plants were infested with whiteflies for 24 hours, the levels of ABA did not differ significantly from plants without whiteflies for both accessions. The accumulation of SA was not significantly affected by salt, but whitefly infestation led to a higher accumulation of SA in the resistant accession, while it did not change in the susceptible accession. JA levels were affected by both salt treatment and whitefly infestation. Without salt, whitefly infestation increased JA levels in both accessions. But when grown under salt stress, these levels were reduced, in the resistant accession *C. annuum* - 21 to levels measured without whitefly infestation (Fig. 3).

## 4. Discussion

We investigated the impact of salt stress on the pepper–whitefly interaction for 25 *Capsicum* accessions and found variation between accessions for plant growth and ion accumulation in response to salt and for resistance to *B. tabaci*. In general, plants grown under salt stress were inhibited in their shoot growth and accumulated  $\text{Na}^+$  in their leaves accompanied by a reduction in  $\text{K}^+$  content, similar to previous observations (Navarro et al., 2002; De Pascale et al., 2003; Aktas et al., 2006). Three accessions were identified for which weight and height were not affected when growing under salt stress. The identified accessions are interesting materials to be used in breeding salt-tolerant *Capsicum* varieties.

Most accessions tested were susceptible to the whitefly *B. tabaci*. Four accessions were identified on which survival of *B. tabaci* adults was significantly reduced compared to other accessions. In non-salt treated plants, the average survival of adults on these accessions was below 50 %. In addition, oviposition was also lower. Variation in the performance of *B. tabaci* on *Capsicum* accessions has been found before (Firdaus et al., 2011; Ballina-Gomez et al., 2013; Pantoja et al., 2018; Hernández-Alvarado et al., 2019; Sandra et al., 2022). Further studies of the identified accessions are needed to reveal the genetics and mechanism(s) of whitefly resistance in them. Also, they could be used as a starting point for breeding more resilient, whitefly resistant varieties.

On all resistant accessions, when plants had been growing under salt stress, survival of the adult whiteflies increased. This indicates that plant resistance to whiteflies is negatively affected by the response to abiotic stress. Previously, the opposite effect, i.e., an increase in resistance to pests after plants experienced an abiotic stress, was found. For example, drought stress negatively affected the aphid *Myzus persicae* and caterpillar *Mamestra brassicae* on *Arabidopsis thaliana* (Pineda et al., 2016). In tomato, drought stress led to a lower whitefly population growth, but only on two of four cultivars tested (González-Klenner et al., 2022). On salinity-stressed rice, the effect was also dependent on the cultivar used, either increasing or decreasing the population of brown planthopper (Quais et al., 2020). The outcome of the interaction between biotic and abiotic stress is thus expected to be plant, genotype, pathogen, and stress intensity dependent. Here, we see that in four plant genotypes there was a reduction in resistance to whiteflies. Reallocation of resources to deal with the salt stress could have led to less investment in biotic stress

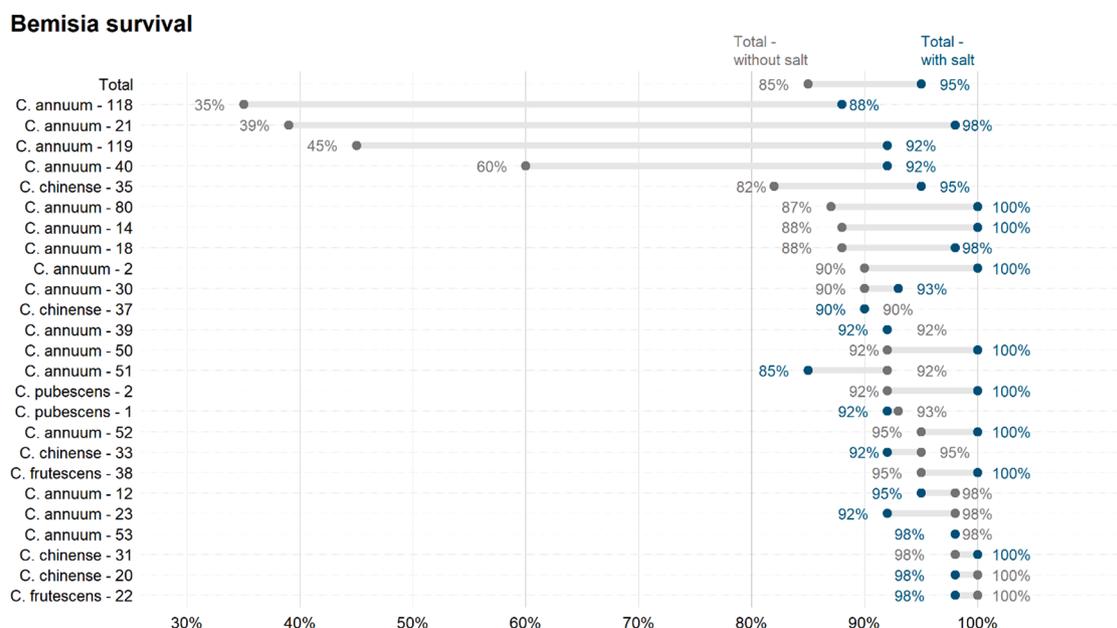
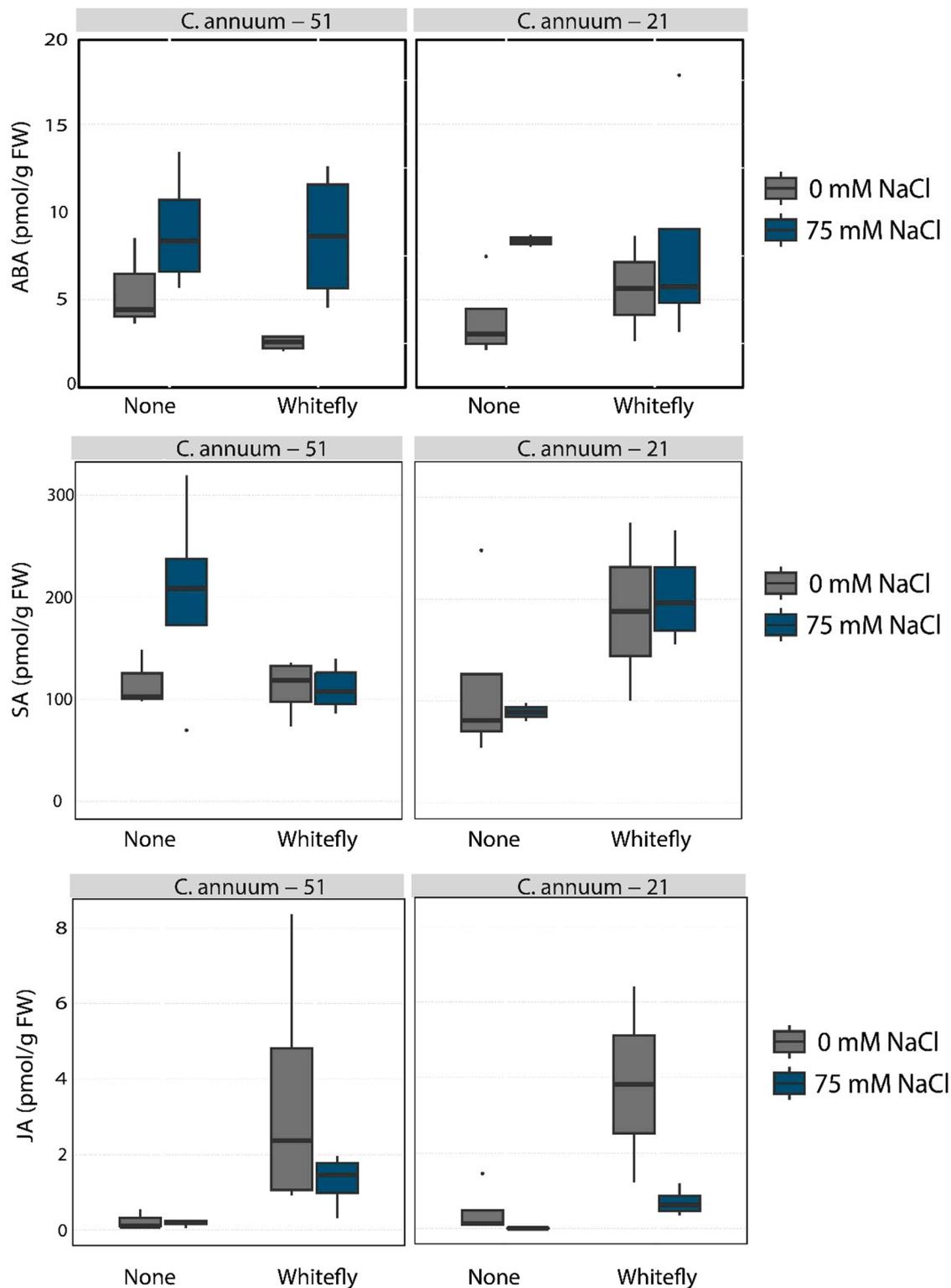


Fig. 2. Whitefly survival increases in salt-treated plants. Shown is the average adult survival (AS) with and without salt for 25 accessions. Grey dots indicate AS in plants without salt treatment, blue dots indicate AS in plants that were treated with salt.



**Fig. 3.** Accumulation of the phytohormones abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) under salt and whitefly in a susceptible accession (*C. annuum* - 51) and a whitefly resistant accession, *C. annuum* - 21.

resistance in these plants. Other processes that are affected by the salt treatment could also have led to a reduction in whitefly mortality. For example, abiotic stress, and ABA accumulation were found to reduce the expression of resistance genes (Yang et al., 2021). Further study into the resistance mechanisms that lead to lower whitefly survival in the here identified resistant *Capsicum* accessions, and how these mechanisms are affected by abiotic stress, can help answer these questions.

In response to salt stress, as expected, an increase in ABA accumulation was found in both accessions (Munns and Tester, 2008). We found an increase in the phytohormone SA in response to whiteflies only in the accession that had increased resistance to whiteflies. Induction of SA by whiteflies was previously observed and linked to whitefly-manipulated suppression of the JA defense (Zhang et al., 2013), but was also shown to be required for resistance-gene-mediated resistance to

whiteflies (Rodríguez-Álvarez et al., 2015). Possibly, in our resistant accession, SA is involved in a resistance-gene mediated resistance, but further study of the resistant accession is needed to provide evidence for this. In whitefly-infested plants, without salt-treatment, we found an increase of jasmonic acid (JA) in the two accessions tested. JA treatment has been shown to negatively affect whitefly nymph development, result in lower oviposition by adult whiteflies and lower survival rate from eggs to adults (Shi et al., 2017; Zhang et al., 2018; Li et al., 2023). In the combined stress, accumulation of JA was reduced, which was accompanied by a lower plant defense to whiteflies.

Our work shows that in a changing climate that forces us to grow plants under more saline conditions in river deltas, not only the direct effect of the salt need to be taken into account, but also the indirect effects, such as an increase in susceptibility towards pests and possibly diseases (Bai et al., 2018). In addition, when studying resistance to pests, using different circumstances and testing their stability in the field, it is important to understand how durable these resistance will be in a changing climate. More study into how resistance mechanisms are affected, and how these could be manipulated, may give us tools to grow plants with higher resilience to both abiotic and biotic stress under changing conditions.

### CRedit authorship contribution statement

**Lotte Caarls:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Faith Enigimi:** Investigation, Formal analysis. **Wouter Kohlen:** Writing – review & editing, Formal analysis. **Gerard van der Linden:** Writing – review & editing. **Wendy P. C. van 't Westende:** Investigation, Conceptualization. **Kas Swinkels:** Formal analysis. **Ben Vosman:** Writing – review & editing, Conceptualization.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2025.106101](https://doi.org/10.1016/j.envexpbot.2025.106101).

### Data Availability

The datasets generated and analyzed during the current study are available at a Zenodo repository (<https://doi.org/10.5281/zenodo.14134722>).

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