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RESEARCH

# Identifying resistance to aphid *Aphis gossypii* and whitefly *Bemisia tabaci* in *Cucurbita* and possibility of broad-spectrum resistance to both insects

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**Abstract** Aphids and whiteflies threaten the production of *Cucurbita* crops by causing damage through feeding and transfer of viruses. The cotton-melon aphid *Aphis gossypii* and the silverleaf whitefly *Bemisia tabaci* are two of the major species affecting *Cucurbita*. Current pest control heavily relies on chemical methods, but there is growing interest in exploring resistant crop varieties as an alternative strategy. To develop these resistant varieties, sources of resistance are needed. In search of potential resistant sources, we screened a total of 448 and 401 accessions in no-choice, clip-cage assays for resistance to *A. gossypii* and *B. tabaci*, respectively. The collection of accessions consisted of four of the cultivated species (*C. argyrosperma*, *C. maxima*, *C. moschata*, and *C. pepo*) and two wild relatives (*C. ecuadorensis* and *C. lundelliana*). By assessing survival and reproduction of the insects, we identified 44 accessions potentially resistant to *A. gossypii* and 16 accessions potentially resistant to *B. tabaci*, based on lower survival and reproduction. Accession PI 550694 showed resistance to both insects, which may indicate broad-spectrum resistance to phloem-feeding insects. These putative sources of resistance to aphids and whiteflies can be used for genetic mapping of resistance, contributing towards the development of resistant *Cucurbita* varieties.

**Keywords** *Cucurbita* · Pumpkin · Squash · Aphid · Whitefly · Host plant resistance · Resistance mechanism · Broad-spectrum resistance

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## Introduction

The *Cucurbitaceae* include economically important crops, such as melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), and pumpkin and squash (including zucchini) (*Cucurbita* spp.). The genus *Cucurbita* contains five domesticated species, *C. pepo* L., *C. moschata* Duchesne, *C. maxima* Duchesne, *C. argyrosperma* C. Huber, and *C. ficifolia* Bouché (Sanjur et al. 2002). The first three are the most widely cultivated, and the most important in terms of agricultural production and uses. The wild species of *Cucurbita*, such as *C. okeechobeensis* (Small) L.H. Bailey, *C. ecuadorensis* Cutler & Whitaker, and *C. lundelliana* Bailey could be useful sources of genetic materials for crop improvement

(Grumet et al. 2021; Khoury et al. 2020). Some of these wild relatives can be crossed with the cultivated species (Paris 2016). *Cucurbita* species originated from the Americas: the center of origin of the three main species has been traced to North America (*C. pepo*) and South America (*C. moschata* and *C. maxima*) (Sanjur et al. 2002). These species then spread worldwide with great diversification in Europe, Asia, and Africa (Montero-Pau et al. 2017; Paris 2016). All species of *Cucurbita* are diploid with 20 pairs of chromosomes ($2n = 2x = 40$) (Kates et al. 2017).

Pumpkins and squash are widely cultivated for food and ornamental purposes, which largely contribute to their economic value. In addition to their economic importance, they are rich in essential nutrients such as vitamins, minerals, and carotenoids, and have been associated with health-promoting properties including anti-inflammatory, anti-diabetic, anti-cancer, and anti-hypertension effects (Sharma et al. 2020). Globally, pumpkin and squash production is estimated at around 28 million tons across 3 million hectares. China and India contribute about 58% and 20% of the total production respectively (FAOSTAT 2016). However, like every other crop, pumpkins and squash productions are threatened by pests and pathogens.

Aphids and whiteflies are pests that seriously damage pumpkins and squash. The cotton-melon aphid *Aphis gossypii* (Glover) (Hemiptera: Aphididae) and the silverleaf whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) are two of the major species of concern. These pests are phloem feeders, they extract nutrients from the plant phloem which causes damage both directly and indirectly. Among the direct damage caused by *A. gossypii* are yellowing of leaves, foliar necrosis, deformation of tissues, wilting, and stunted growth (Baldin et al. 2009), while *B. tabaci* induces squash silverleaf disorder (SSD). The indirect damage that *A. gossypii* and *B. tabaci* cause through virus transmission is even more concerning. The aphid-vectored potyvirus, zucchini yellow mosaic virus (ZYMV) is one of the most widespread and destructive pathogens of pumpkins and squash (Ahsan et al. 2023; Tóbiás and Palkovics 2003). Furthermore, Squash-Leaf Curl, caused by a complex of begomoviruses which are vectored by *B. tabaci*, is becoming a major problem in the warmer production regions, particularly in Asia (Abudy et al. 2010; Brown et al. 2007; Verma et al. 2023). Virus infections often lead to fruit deformities, discoloration, and delayed ripening which may reduce the marketability of the produced fruit (Roberts et al. 2008). Also, both *A. gossypii* and *B. tabaci* excrete sticky honeydew which promotes black sooty mould growth on *Cucurbita* leaves and fruit surfaces (Roopa et al. 2014).

Chemical pesticides are still widely used to control aphids and whiteflies. However, the harmful effects of chemicals on the environment, especially on other beneficial arthropods such as pollinators, are of concern and makes them undesirable (Obregon et al. 2022; Sánchez-Bayo 2021). In addition, indiscriminate use of pesticides caused various pests to develop pesticide resistance (Georghiou 2012). Moreover, chemical pesticides are banned in organic agriculture, an agricultural system that is of growing interest among pumpkins and squash producers following governmental regulations and market demands (Toscano et al. 2021). These limitations of chemical control highlight the need for alternatives, such as the use of resistant varieties.

Resistant plants can interfere with insect–host plant selection leading to non-preference (antixenosis) or can adversely affect growth, development, survival, and reproduction of insects (antibiosis) (Broekgaarden et al. 2012; Daryanto et al. 2017). Resistance to major phloem feeding insects has been found in several plant species, for instance, resistance in *Brassica oleracea* against the whitefly *Aleyrodes proletella* (Broekgaarden et al. 2012), resistance in *Capsicum* species to *Myzus persicae* (Sun et al. 2018) and *Bemisia tabaci* (Rini et al. 2025), resistance to silver-leaf whitefly in cotton (Miyazaki et al. 2013), and resistance to soybean aphid in early maturing soybeans and some other soybean varieties (Bhusal et al. 2014; Enders et al. 2014; Hesler et al. 2017).

So far, only a few screenings have been done to identify sources of resistance to aphids or whiteflies in *Cucurbita*. Baldin et al. (2009) and Baldin and Beneduzzi (2010) reported two cultivars with resistance to aphids and one resistant to whiteflies among eight tested *C. pepo* cultivars. In a more extensive study, Luckew et al. (2022) screened over 200 accessions of four *Cucurbita* species for resistance to whiteflies and whitefly-transmitted viruses and identified six *C. moschata* accessions with lower adult whitefly counts and high tolerance to the silverleaf disorder caused by *B. tabaci*. Our objective was to conduct a large-scale screening of *Cucurbita* germplasm to identify additional and potentially stronger sources of resistance to aphids and whiteflies. An additional objective was to explore the potential for broad-spectrum resistance, defined here as the ability of an accession to exhibit resistance against both phloem-feeding insect species tested. To accomplish this, we screened 448 and 401 *Cucurbita* accessions for antibiosis type of resistance to aphids and whiteflies, respectively, and performed several validation assays. The identified putative resistant sources from this research have the potential to be used as source materials in breeding efforts towards the development of aphids and whiteflies resistant *Cucurbita* varieties.

## Materials and methods

### Plant materials

In total, 476 accessions were obtained from two genebanks, the Germplasm Resources Information Network of the U.S. Department of Agriculture (GRIN-USDA) and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK-Germany). Plant accessions were first selected based on availability across genebanks. From these, accessions were prioritized based on geographic origin, with emphasis on centers of origin and broad continental distribution. For each insect assay, four cultivated species (*C. argyrosperma*, *C. maxima*, *C. moschata* and *C. pepo*) and two wild relatives (*C. ecuadorensis* and *C. lundelliana*) were included (Table 1).

We screened 448 accessions (Supplementary Table 1) for resistance to *Aphis gossypii* and 401 accessions (Supplementary Table 2) for resistance to *Bemisia tabaci*, in multiple batches (Table 2).

All plants were grown at the UNIFARM greenhouse of Wageningen University and Research (WUR), the Netherlands at 23/21 °C (day/night) temperature, 60–70% relative humidity (RH), and 16/8 h (L/D) photoperiod. Seeds were sown into planting trays with potting compost. One week after sowing, germinated seedlings were transplanted into 17 cm diameter pots with potting compost. Plants were watered every other day and nutrients were supplied when needed. No pesticide treatment was done during growth and screenings.

**Table 1** Overview of the number of accessions per species used in each assay

| Species | Aphid assay | Whitefly assay |
|---|---|---|
| *C. maxima* | 172 | 154 |
| *C. moschata* | 85 | 79 |
| *C. pepo* | 182 | 159 |
| *C. argyrosperma* | 6 | 6 |
| *C. ecuadorensis* | 2 | 2 |
| *C. lundelliana* | 1 | 1 |
| Total | 448 | 401 |

### Insect rearing

The initial inoculum of *Bemisia tabaci* Mediterranean-Middle East-Asia Minor I (MEAM1) was obtained from a permanent rearing at WUR Plant Breeding on tomato 'Forticia F1', and that of *Aphis gossypii* from a cucumber rearing from the WUR Greenhouse Horticulture group, Bleiswijk,

**Table 2** Overview of the number of accessions screened in each batch for both aphid and whitefly as well as number of accessions selected per batch and the screening period

| Insect | Batch | N | n | Screening period |
|---|---|---|---|---|
| Aphid | Batch 1 | 120 | 17 | April 2023 |
| | Batch 2[a] | 89 | 9 | April 2023 |
| | Batch 3 | 107 | 10 | May 2023 |
| | Batch 4 | 95 | 8 | June 2023 |
| | Batch 5 | 37 | 0 | June 2023 |
| | Validation 1 | 36 | 4 | June 2023 |
| | Validation 2 | 4 | 3 | June 2024 |
| Whitefly | Batch 1 | 108 | 6 | August 2023 |
| | Batch 2 | 105 | 5 | September 2023 |
| | Batch 3 | 99 | 1 | September 2023 |
| | Batch 4 | 89 | 4 | September 2023 |
| | Validation 1 | 16 | 9 | May 2024 |
| | Validation 2 | 9 | 5 | July–August 2024 |

The table shows the number of accessions screened vs selected per batch and the screening period. All insect assays were done at temperature of 25/23 (°C), 65–70 (RH) and 16/8 h (L/D) photoperiod and evaluated at 9 days post infestation (dpi). N is the number of tested accessions excluding the 3 standard controls, and n is the number of accessions selected as potentially resistant from the assay

[a]This assay was evaluated at 8 dpi and different temperature of 21/19 (°C)

Netherlands. The rearing of both insects was continued on *Cucurbita maxima* 'Amoro-F1' at 25/23 °C (day/night), 65–70% RH, 16/8 h (L/D) photoperiod.

### No-choice clip-cage aphid assay

A three-phase aphid assay was conducted on 3-week-old *Cucurbita* plants to test for aphid survival and reproduction, using a no-choice clip-cage method (Supplementary Figure 1). First, a total of 448 accessions (Supplementary Table 1) were screened with a single replicate per accession in five batches (Table 1). 'Amoro-F1', 'Betternut', and 'Black Beauty' (Jansen Zaden, the Netherlands) were included as susceptible controls, in each batch. Based on insect response parameters, 44 accessions were identified as putatively resistant. Next, we performed a first validation assay on 36 out of the 44 selected accessions, due to no-germination of the other 8 accessions. Each accession and the three susceptible controls were tested with two replicates per genotype. In a second validation experiment, four accessions derived from the previous selected putative resistant accessions by self-pollination were evaluated in a final validation assay. In this phase, each accession and controls were tested with 12 replicates. A completely randomized design was used. The assays were performed in a climatized greenhouse with conditions 25/23 °C (day/night), except for batch 2 (Table 2), 65–70% RH, 16/8 (L/D) photoperiod. One clip-on-cage each containing five synchronized 1-day-old nymphs were placed on the abaxial side of the 2nd and 3rd fully expanded leaves of the plants. After 9 days, the number of living and dead aphids (from the initial 5 1-day-old nymphs), and the number of second-generation nymphs were counted. Survival rate and reproduction rate were further calculated using the formulas described by Sun et al. (2018) and Vosman et al. (2019). Survival rate (%) was calculated as: $\frac{living\ aphids}{living\ +\ deadaphids} \times 100$. Reproduction rate was calculated as: $\frac{number\ of\ nymphs}{\left(\frac{2 \times \text{living} + \text{dead adults}}{2}\right)}$ which assumes living females contribute twice as much as dead females to nymphal production.

### No-choice clip-cage whitefly assay

Similar to the aphid assays, a three-phase whitefly assay was conducted on 3-week-old *Cucurbita* plants to test for whitefly survival and reproduction, using a no-choice clip-cage method. First, a total of 401 accessions were screened with one replicate per accession. The three standard susceptible controls were included with two replicates each. Based on whitefly survival and reproduction, 16 accessions were selected for further testing. For two of the 16 accessions, seeds obtained by self-pollination were available prior to the experiment in the second phase (first validation assay). In this phase, the two selfed accessions and the three standard susceptible controls were evaluated with 10 replicates each. The remaining 14 accessions, for which selfed seeds were not available, were tested using genebank seed stock with two replicates per accession. In the second validation assay, nine accessions and two of the standard susceptible controls were assessed, using 2–4 replicates per genotype. The nine accessions included both self-pollinated and genebank-derived seed materials. For all experiments, a completely randomized design was used. The assays were performed at a climatized greenhouse with 25/23 °C (day/night), 65–70% RH, 16:8 L:D photoperiod.

For the first screening and the first validation experiment, two clip-cages containing five 0–2 days old adult females were placed on the abaxial side of the 2nd and 3rd fully expanded leaves of the plants. After 5 days, living and dead adults, and the number of eggs were counted. Survival rate (%) was calculated as: $Adult\ survival\ (AS) = \frac{living\ whiteflies}{living{+}dead\ whiteflies}$. The reproduction rate was calculated as $\frac{number\ of\ eggs}{\left(\frac{2\times\text{living} + \text{dead adults}}{2}\right)}$.

For the second validation experiment, two to three clip-cages were used per plant. Adult survival was calculated similarly as the first two experiments at 5 days post infestation. Then, adults were removed, and plants were enclosed in whitefly proof bags, to prevent escape of newly hatched adults later. At 27 dpi, the number of larvae and number of newly hatched adults were counted. The larvae rate (LR) was then calculated as $LR = \frac{number\ of\ larvae}{((2\times\text{living}+\text{dead adults})/2)}$ and hatched adult rate (AR) as $AR = \frac{number\ of\ hatched\ adults}{\left(\frac{2\times\text{living}+\text{dead adults}}{2}\right)}$.

### Statistical analysis

Statistical analysis was performed using R (version 4.3.1) (R Core Team 2023). Prior to statistical analysis, observations from the two clip-cages per plant were combined. Because the large-scale screenings were done without replication, these data were analyzed using descriptive statistics (histogram). Figures were created using the ggplot2 package (Wickham 2016). Summary tables (Supplementary Tables 3–6) including mean and standard deviation values per resistance parameter, and Pearson correlations between the two parameters (survival and reproduction) were generated.

For the validation experiments, both the parametric and non-parametric approaches were used to analyze the survival and reproduction data. For data that met the assumptions of normality (Shapiro–Wilk test $P > 0.05$) and homogeneity of variance (Levene's test $P > 0.05$), in this case the second whitefly validation experiment data, we applied a linear regression model (*lm*) to the transformed data. The survival data were transformed using the arcsine square root transformation [arcsin(sqrt(x)] to stabilize variance for proportion-based data, while the reproduction data were subjected to a square root transformation [sqrt(x)] to normalize its distribution. Following the regression analysis, we performed post-hoc comparisons using Tukey's honestly significant difference (HSD) test to identify and separate statistically significant differences between group means.

For data that violated the assumptions of the linear regression, in this case the aphid validation data and the first whitefly validation data, the Kruskal–Wallis test was used as a non-parametric alternative to analyze the untransformed data. The significance of differences in the means after the Kruskal–Wallis test was evaluated using dunn.test.

## Results

### Aphid performance on *Cucurbita* accessions in a large-scale screening

We evaluated 448 *Cucurbita* accessions and the three standard susceptible controls for resistance to *Aphis gossypii* by assessing aphid survival and reproduction. We observed that on two of the susceptible controls; 'Amoro-F1' (*C. maxima*) and 'Black Beauty' (*C. pepo*), survival and reproduction rates of aphids were high across batches, with little to no variation between the replicates. 'Betternut' (*C. moschata*) on the other hand had lower survival and/or reproduction compared to 'Amoro-F1' and 'Black Beauty' (Supplementary Table 3). In general, aphid survival and reproduction were high on almost all tested accessions (Fig. 1). The overall mean survival rate of aphids on all accessions was 93.2% (12.5–100.0%), and the mean reproduction rate was 15.6 nymphs per adult (0.7–38.4) (Supplementary Table 1; Fig. 1). A positive but weak correlation was found between survival and reproduction parameters for aphid resistance (Pearson correlation coefficient 0.34). On 29 accessions (6.5%), aphid survival was lower than 70%, and on 44 accessions (10%) aphid reproduction rate was < 10. These 44 accessions were selected

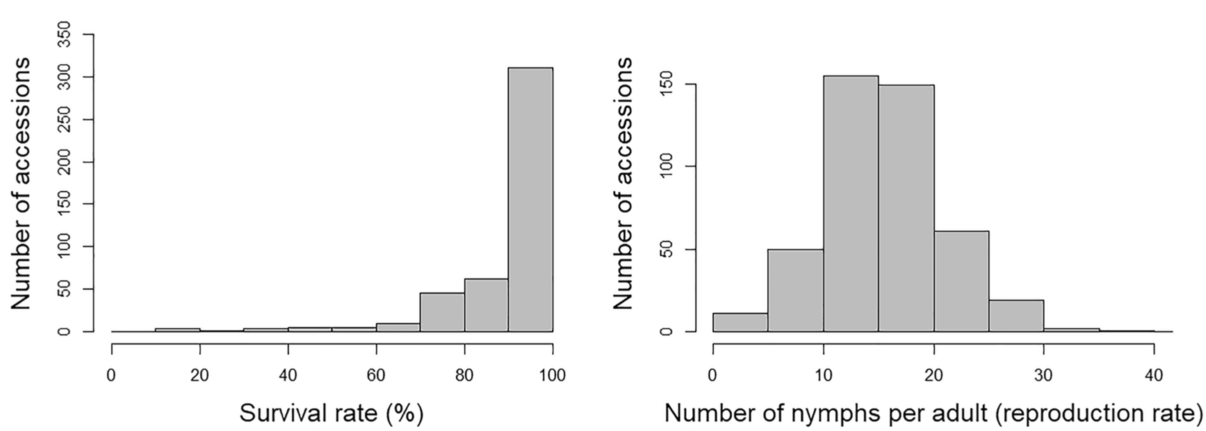


**Fig. 1** Performance of *A. gossypii* on *Cucurbita* accessions. In total, 448 *Cucurbita* accessions were evaluated for aphid resistance based on two parameters: survival rate (left) and reproduction rate (right). The *y*-axis represents the number of accessions, and the *x*-axis gives the value for survival or reproduction

as potentially aphid-resistant accessions. We also observed slower nymphal development on the majority of the accessions with lower reproduction rates compared to the susceptible ones (Fig. 2).

## Validation of aphid resistance on selected accessions

Out of the 44 selected accessions, we performed a first validation experiment with 36 accessions, due to no germination of the other 8 accessions, and we included the same 3 controls as before (Fig. 3). In general, survival and reproduction were higher in this experiment compared to the same accessions in the first screenings. Mean survival was 96.51% ± 7.38 (70–100%). For reproduction, the mean value was 16.62 ± 4.91 (7.1–30.5). The Kruskal–Wallis test showed that accessions were not significantly different for survival [$H(38) = 53.72$, $P = 0.05$]. Although the test showed a significant accession effect for reproduction [$H(38) = 57.75$, $P = 0.02$], the post-hoc analysis did not detect significant pairwise differences between accessions. Two *C. maxima* accessions (PI 302416 and PI 419080) and one *C. pepo* (PEP1658) were selected based on low reproduction rates observed in the first validation assay (Fig. 3). A second validation assay was done on plants grown from seeds that were obtained by self-pollinating plants selected from the large-scale screening. From this second assay, the *C. pepo* accession (PEP 1658) was confirmed resistant (Supplementary Figure 2). In addition, one *C. moschata* accession PI 550694 was selected from this assay as potentially resistant due to low reproduction rates. Furthermore, we observed that on PI 550694, nymphs were able to feed and develop into adults but died as adults (Supplementary Figure 3).

## Whitefly performance on *Cucurbita* accessions in a large-scale screening

We evaluated 401 *Cucurbita* accessions, and the 3 standard susceptible controls for survival and reproduction of whiteflies (Fig. 4). The controls showed high survival and reproduction of whiteflies, except for 'Betternut' in one batch. In general, survival and reproduction of whiteflies were high on almost all accessions. The mean survival rate (%) of whitefly on all accessions was 92.61% (40–100%), and the mean reproduction rate was 24.58 eggs per adult (5.78–57.56). The Pearson correlation coefficient between the two resistance parameters for whitefly resistance was 0.22. Out of 401 accessions tested on 26 accessions (6.5%) adult whitefly survival was lower than 70%, and on 20 accessions (5%) reproduction rate was lower than 10. Eventually, we selected 16 accessions from this screening. This selection included accessions with a low survival rate (≤ 70), and/or low reproduction rate (≤ 10) (Fig. 4).

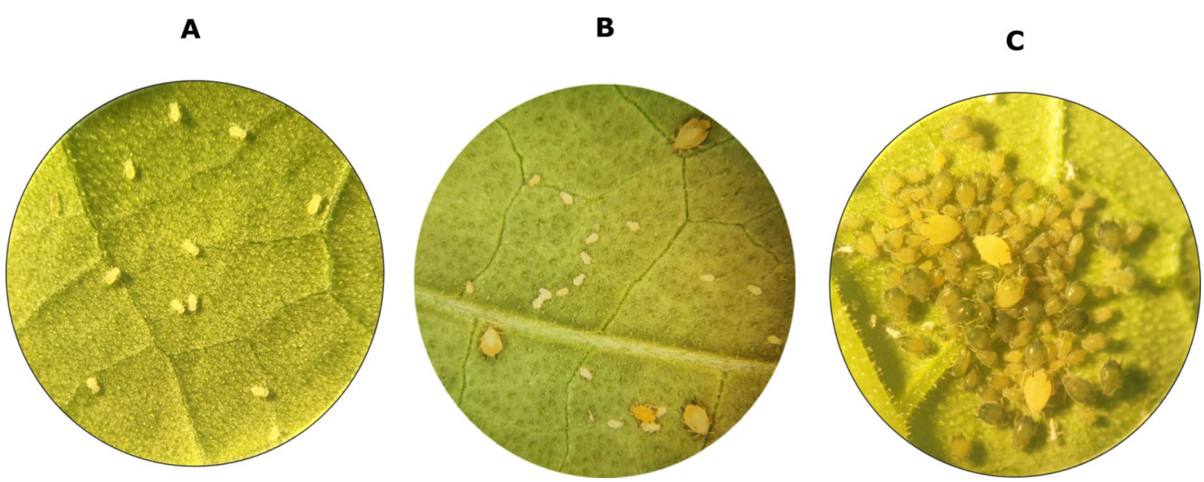


**Fig. 2** Delayed nymphal development on resistant accession compared to susceptible accession. **A** Some of the 1-day-old nymphs used for infestation, **B** nymphal development on a resistant plant at 9 dpi, and **C** nymphal development on a susceptible plant at 9 dpi. Scale: image captured under × 10 objective lens with × 1.0 zoom

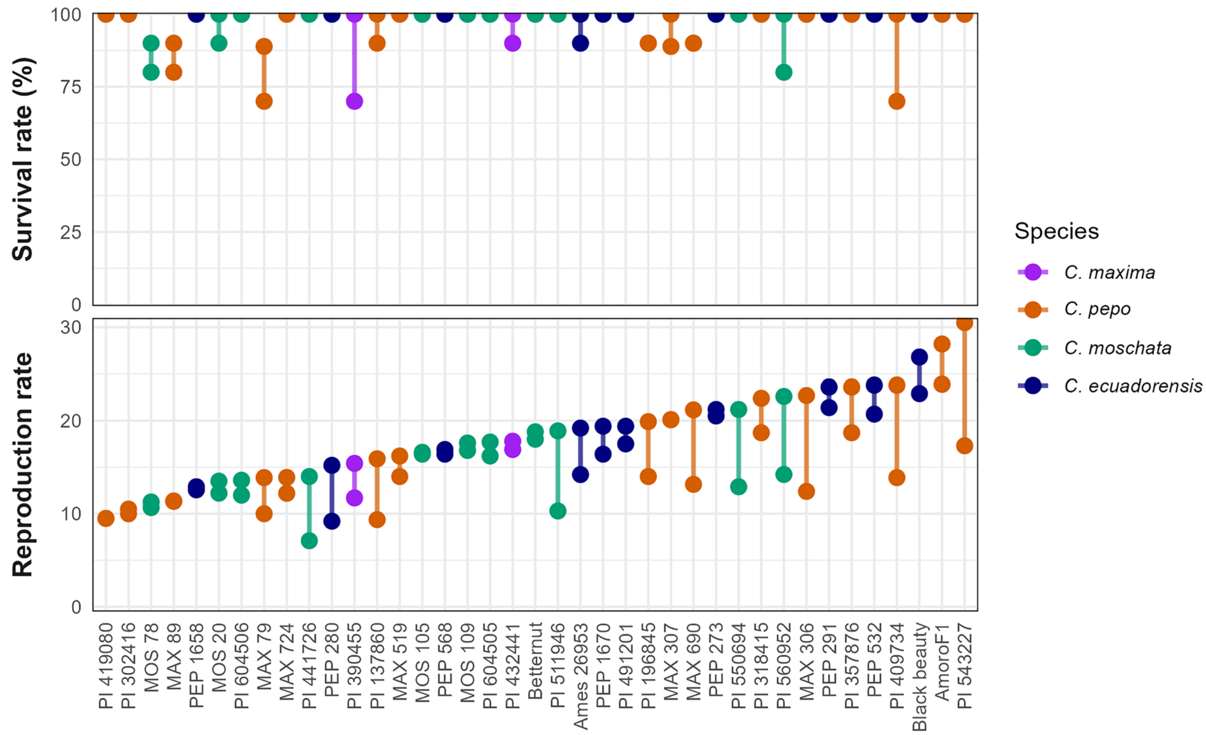


**Fig. 3** Dot plot showing aphid performance on selected accessions in a validation experiment. A total of 36 out of 44 accessions selected from the large-scale screening were re-tested for aphid resistance based on two parameters: survival rate (top) and reproduction rate (bottom). $n = 2$ per accession

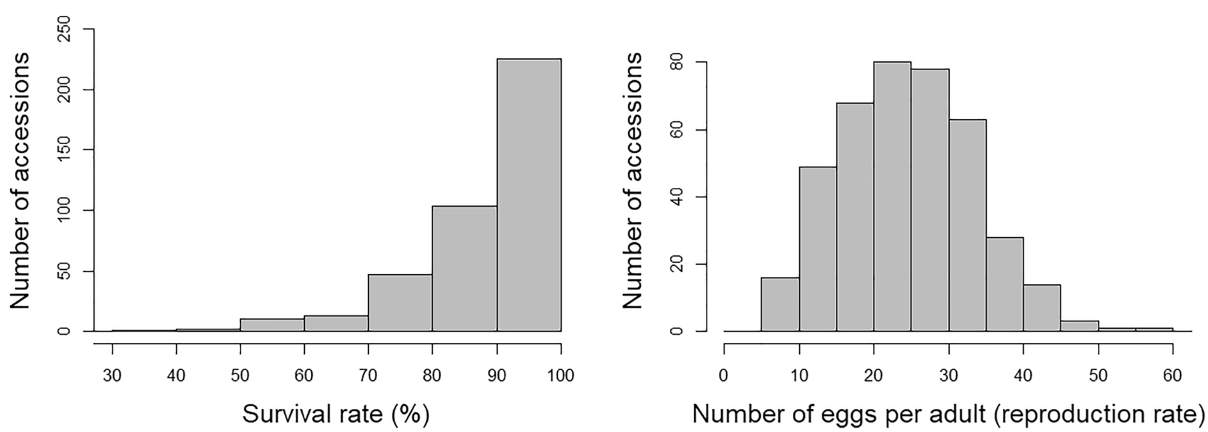


**Fig. 4** Performance of *B. tabaci* on *Cucurbita* accessions. In total, 401 *Cucurbita* accessions were evaluated for whitefly resistance based on two parameters: survival rate (left) and oviposition rate (right). The *y*-axis represents the number of accessions and the *x*-axis gives the value for survival or reproduction

## Validation of whitefly resistance on selected accessions

Resistance of *Cucurbita* accessions to whitefly was validated by retesting the 16 accessions previously selected as putatively resistant, and the three standard susceptible controls for the two whitefly resistance parameters; survival and reproduction (Fig. 5).

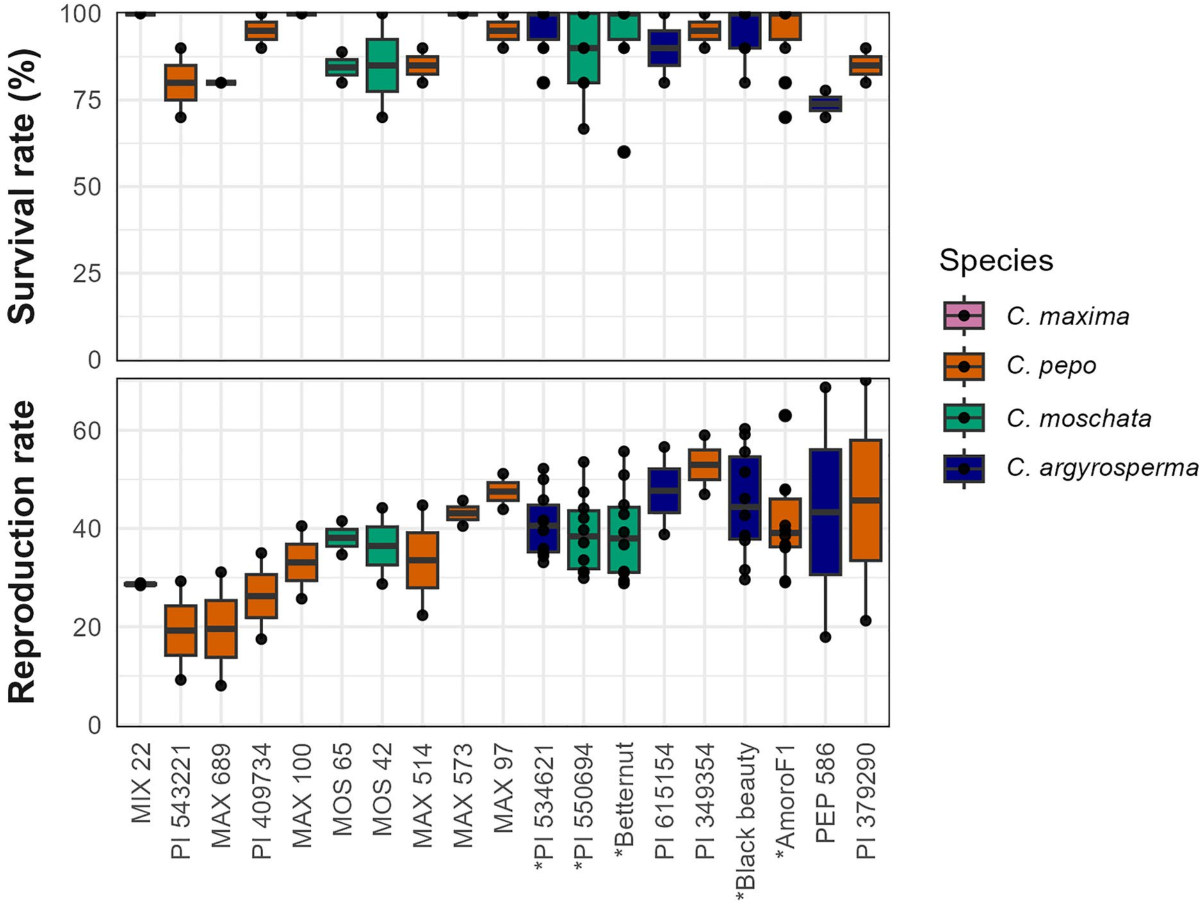


**Fig. 5** Boxplot showing whitefly performance on selected accessions from the large scale screenings. Accessions were re-tested for whitefly resistance based on two parameters: survival rate (top) and reproduction rate (bottom). Either 2 or 10 replicates per accession were used. Asterisk indicates accessions and controls tested with 10 replicates. MIX 22 was the only *C. argyrosperma* included

We tested two to ten replicates of these selected accessions. These accessions were not statistically different from each other, or from the standard susceptible controls survival [H(21) = 29.38, *P* = 0.105]; reproduction [H (21) = 28.54, *P* = 0.125]. The mean reproduction rate in the validation experiment was 38.85 ± 11.43, compared to 24.58 ± 8.94 in the large-scale screening.

## Resistance to whitefly further verified in a long-term infestation assay

Next, development from eggs into adults was studied on nine other accessions based on seed availability, and two of the control accessions (Table 3). The nine accessions included two of the previously tested 16 accessions (MOS 65 and PI 550694). Five days after infestation, adult survival was measured as $Adult\ survival\ (AS) = \frac{living\ whiteflies}{living + dead\ whiteflies}$. No statistically significant differences were found among accessions [F(10,21) = 1.54, *P* = 0.19] for survival, with survival rate ranging from 0.7 to 1.0. Twenty-seven days after infestation, total larvae and total hatched adults on each plant were counted. The LR was determined as the number of larvae relative to the average number of adults (living and dead), and ranged from 0.8 to 12.9 larvae/adult female (Table 3). All accessions of *C. moschata* and one accession of *C. pepo* PI 292014 showed a significantly lower LR when compared to Amoro-F1 and Black Beauty. The hatched AR was calculated as the ratio of hatched adults

**Table 3** Adult survival, developed larvae, and hatched adult whiteflies on different accessions of *C. maxima*, *C. moschata* and *C. pepo*

| Accession | n | i | Adult survival | | Larvae | | | Hatched adults | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rate | | Total | Rate | | Total | Rate | |
| | | | Mean | | Mean | | | Mean | | |
| *C. maxima* | | | 0.9 | | 155.7 | 11.0 | | 31.3 | 2.2 | |
| Amoro-F1 | 3 | 15 | 0.9 | *a* | 155.7 | 11.0 | *a* | 31.3 | 2.2 | *abcd* |
| *C. moschata* | | 12.1 | 0.9 | | 12.9 | 1.2 | | 1.9 | 0.2 | |
| MOS 58[a] | 3 | 13.3 | 0.9 | *a* | 14.7 | 1.1 | *b* | 1.7 | 0.1 | *acd* |
| MOS 65 | 3 | 15.0 | 0.9 | *a* | 12.7 | 0.9 | *b* | 0.3 | 0.0 | *cd* |
| PI 550694[a] | 4 | 10.0 | 1.0 | *a* | 7.3 | 0.8 | *b* | 0.0 | 0.0 | *d* |
| PI 634692 | 2 | 10.0 | 0.8 | *a* | 17.0 | 2.0 | *b* | 5.5 | 0.6 | *abcd* |
| *C. pepo* | | 13.8 | 0.8 | | 121.1 | 9.7 | | 54.8 | 4.5 | |
| PEP 212 | 3 | 15.0 | 0.8 | *a* | 153.0 | 11.3 | *a* | 41.3 | 3.2 | *abc* |
| PI 229688 | 3 | 13.3 | 0.7 | *a* | 119.3 | 10.3 | *a* | 62.0 | 4.8 | *ab* |
| PI 292014[a] | 3 | 15.0 | 0.8 | *a* | 20.0 | 1.5 | *b* | 46.3 | 3.7 | *abc* |
| PI 615103 | 3 | 13.3 | 0.9 | *a* | 159.7 | 12.9 | *a* | 77.3 | 6.6 | *b* |
| PI 615115 | 3 | 13.3 | 0.8 | *a* | 120.3 | 9.8 | *a* | 50.7 | 4.2 | *ab* |
| Black beauty | 2 | 12.5 | 0.9 | *a* | 154.0 | 12.7 | *a* | 51.0 | 4.4 | *ab* |

Number of replicates is indicated by "n", while "i" indicates number of initial adult for infestation per accession. "Total" is the absolute count per accession, mean is the average value per n, while Rate were adjusted for (i). Different letters indicate statistical differences according to the Tukey's HSD ($P < 0.05$). *C. pepo* 'Black Beauty', and *C. maxima* 'Amoro-F1' were included as reference

[a]Accessions from self-pollinated seeds

relative to the average number of initial adults (living and dead) at 27 days post infestation, and ranged from 0.0 to 6.6.

The LR for *C. moschata* accessions varied between 0.8 and 2.0, while the AR ranged from 0.0 to 0.6 (Table 3). These values were consistently lower than those observed in accessions from other species and the control accessions, indicating that whitelies were not able to complete their lifecycle on these tested *C. moschata* accessions.

Following the different whitefly experiments, we selected MAX 689 and PI 543221 (*C. maxima*), PI 550694 (*C. moschata*), and PI 615154 (*C. pepo*) as potential material for whitefly resistance.

## Discussion

### From large-scale to in-depth screening for resistance

The first step in every resistance breeding program is the identification of resistant sources. Our research aimed to identify sources of resistance in *Cucurbita* germplasm to *Aphis gossypii* and *Bemisia tabaci*. We screened a total of 448 accessions for aphid survival and reproduction, and 401 accessions for survival and reproduction of adult whiteflies, using the clip-cage method. Previously, Hernandez et al. (2023) characterized the genetic diversity of the USDA *Cucurbita* collection and identified 10 ancestral groups in *C. pepo* and 6 each in *C. moschata* and *C. maxima*. The accessions we screened for aphid and whitefly resistance represented all genetic groups except *C. maxima* group 4 (mainly from Argentina), which was likely part of the collection that was unavailable for ordering from the genebank at the time of screening.

From the resistance screenings, plants were selected as resistant based on lower survival and/or lower reproduction of aphids and whiteflies (quantified), delayed survival of aphid nymphs (visually observed), and hindered development of whitefly eggs into adults (quantified). Our screening effort identified 44 and 16 accessions with lower survival and/or lower reproduction of aphids and whiteflies, respectively, in the first large-scale screenings. Accessions from the first selection round were mainly distributed across the three main species (*C. maxima*, *C. moschata*, and *C. pepo*) (Supplementary Figure 4). The *Cucurbita* accessions selected as potentially resistant showed a broad geographical distribution (Fig. 6). The 44 accessions selected as putative resistant to aphids were distributed across continents. However, no *C. pepo* accession was selected from Asia or South America, and no *C. moschata* accession was selected from Asia (Fig. 6A). Among the 16 accessions selected for whitefly resistance, *C. maxima* accessions originated from all continents except Australia. In contrast, the selected *C. moschata* accessions were limited to Europe and North America, while those of *C. pepo* were from Asia and Europe (Fig. 6B).

Only one accession of the wild relative *C. ecuadorensis* was selected during the first round of aphid resistance screening, likely reflecting the limited representation of wild relatives in the initial germplasm set (Table 1). While wild relatives are often recognized as valuable sources of resistance due to their genetic diversity and adaptation to stress (Hajjar and Hodgkin 2007), the underrepresentation of these materials in our study may have reduced the likelihood of their inclusion in the selected group.

Finally, four accessions: PI 302416 and PI 419080 (*C. maxima*) from North America and Asia respectively, PI 550694 (*C. moschata*) from North America, and PEP1658 (*C. pepo*) from Australia were selected for further evaluation of aphid resistance. The two *C. maxima* accessions were selected following the large-scale screening and validated in a second experiment with two plants per accession, *C. pepo* PEP1658 was selected from the large screening, validated in a second experiment with two more plants, and confirmed in plants derived from self-pollinated plants. *C. moschata* PI 550694 was found in the first screening and confirmed in the second validation experiment with self-pollinated plants. For whitefly, four accessions: MAX 689 and PI 543221 (*C. maxima*), PI 550694 (*C. moschata*), and PI 615154 (*C. pepo*), were selected as putative resistant. PI 543221 originating from South America was selected from the large scale screening and found resistant again in the first validation experiment. PI 615154 from Asia was selected based on the large-scale screening only, while MAX 689 from Africa was selected after the first validation experiment and PI 550694 was selected from the long-term infestation assay with selfed plants.

Our initial screening efforts done without replication of the accessions resulted in the identification of potential sources of resistance. We expected between-accession variability to be larger than within-accession variability, and due to limitation of space and resources, we chose to test one plant for each of the accessions. This enabled us to test a wider variety of plants than testing 2 or more individuals of 100–200 accessions. Our approach was to use this initial screening to reduce the number of accessions, to enable a more focused and rigorous

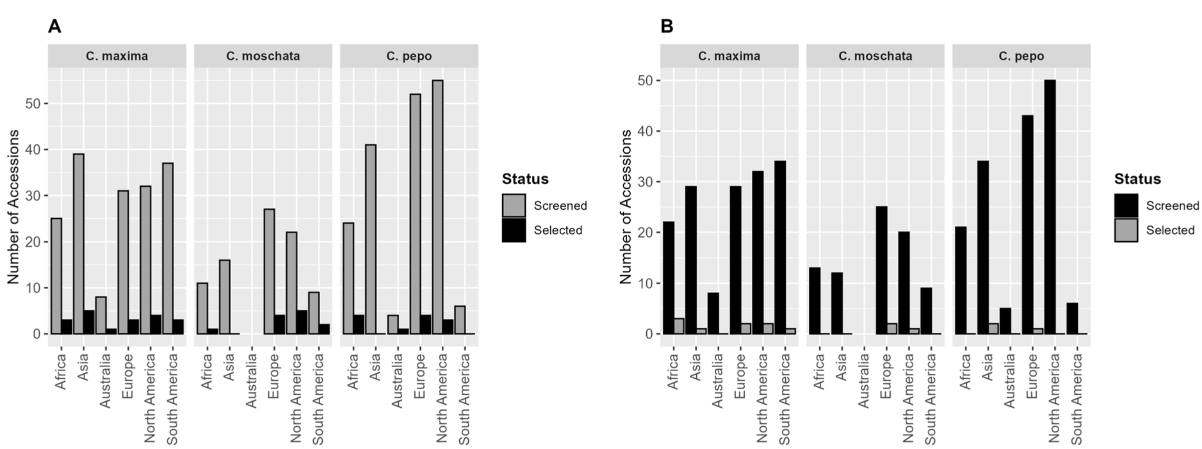


**Fig. 6** Geographic origin of screened vs selected *Cucurbita* accessions for aphid resistance (**A**) and whitefly resistance (**B**). The graphs showed the distribution of the 448 screened accessions compared with the 44 selected accessions for aphid resistance (**A**), and the 401 screened compared with the 16 selected accessions for whitefly resistance (**B**)

evaluation of selected accessions by testing progenies derived from self-pollination. Unfortunately, only a few of the selected accessions could be successfully selfed, due to biological limitations such as asynchronous male–female flower development, pollen sterility and self-incompatibility in some accessions. For those accessions that could not be selfed, validation was carried out using additional seeds from the original seed lot, with limited replication ($n=2$), acknowledging that these may represent different genotypes within the same accession, as these genebank accessions are heterogenous, due to the sib-mating propagation method reported by the genebanks. Therefore, testing three plants per accession does not equate to testing three replicates of a single genotype. A more robust approach would involve validating resistance in genetically uniform selfed lines, but this was only feasible for a subset of accessions. Therefore, further research is needed on the eight selected accessions to confirm and genetically map the resistance found. To do this, the selected resistant individuals were directly crossed to generate mapping populations for further genetic analysis.

So far, limited work has been done to evaluate pumpkin and squash for resistance to these two insect pests. In one case, the authors reported delayed nymphal development, feeding non-preference and mortality of *A. gossypii* on 2 of 8 tested squash (*Cucurbita pepo*) cultivars, in a no-choice leaf disc assay (Baldin et al. 2009). In another case, low whitefly attractiveness, oviposition non-preference and adult mortality of whiteflies were reported in eight squash varieties tested under choice and no-choice conditions, with one accession showing a high level of resistance (Baldin and Beneduzzi 2010). Finally, in a field screening of over 200 accessions of different *Cucurbita* species, 6 *C. moschata* accessions were reported to have lower adult whitefly count and reduced silverleaf disorder (Luckew et al. 2022). Accession PI 550694, was one of the 6 *C. moschata* identified by Luckew et al. (2022), and was also found in our screening to exhibit resistance against both aphids and whiteflies.

## Critical role of timing in resistance screening for accurate phenotyping

During our screenings, particularly in the aphid assays, we observed differences in reproduction between seasons, early or late spring. An implication of this may be that our initial selection from the first two batches of aphid assays done in April for low reproduction might be influenced by the season. However, the selected accessions still showed lower reproduction than non-selected ones within the same period, supporting the validity of our selection based on relative differences. The tested parameters, especially reproduction, showed clear differences between accessions when experiments were conducted during periods of optimal reproduction, such as May and June. The clear differences involved an observed increase in number of nymphs per adult and faster development of both first and second generation nymphs. At these time points, plants with low reproduction could be confidently interpreted as resistant. Despite maintaining controlled greenhouse conditions, aphid reproduction may be influenced by seasonal cues such as photoperiod, intrinsic biological rhythms, and plant phenology (Matsuda et al. 2020; Simon et al. 2010; Wellings et al. 1980). Plants may exhibit seasonal variations in physiology, nutrient composition, or secondary metabolites that may indirectly impact aphid reproduction and growth (Cruz et al. 2019; Soni et al. 2015). This highlights the importance of performing phenotyping during periods of optimal insect reproduction to clearly differentiate resistant from susceptible accessions. Such timing is essential for accurately observing segregation in phenotyping studies, which is crucial for resistance mapping, such as quantitative trait loci (QTL) studies.

## Challenges in using egg count as the determinant of whitefly reproduction rate

Resistance in crops is a complex and multifaceted concept usually assessed at the crop level. Therefore, using the correct parameters is pivotal to evaluate and identify resistance successfully. In our study we utilized measurable traits which included survival and reproduction as the determinant of resistance. These traits could be used to evaluate insect multiplication or population build-up, to give an indication of plant resistance. For whitefly resistance, initially, the reproduction rate was calculated based on the number of eggs (oviposition rate), determined at 5 days post infestation. In a validation experiment, previously classified resistant accessions showed higher egg counts (mean rate increased from 24.58 to 38.85), possibly due to seasonal effects. A subsequent study with a longer infestation period (27 days vs 5 days) allowed us to study the development of eggs to adults by evaluating LR and hatched AR. This way plants could be confidently classified as resistant or susceptible based on the ability of whiteflies to complete their developmental cycle from egg to adult, and to multiply.

However, counting larvae and hatched adults was labor-intensive, and the fast growth of *Cucurbita* accessions over the 27 days demanded much space. A practical approach to overcome these limitations for large-scale screenings is to use LR and AR in a complementary manner with oviposition rate. For large germplasm collections, oviposition rate, being faster and less resource-intensive to evaluate, can be used as a preliminary screening tool. Subsequently, the longer infestation assay can be conducted on cloned or selfed individuals of the selected accessions to evaluate egg development based on LR and AR, as demonstrated in this study.

## Differential impact of plant traits on insect survival and reproduction

In this study, we assessed *Cucurbita* accessions for aphid and whitefly resistance based on survival and reproduction rate. In the 44 selected accessions identified as potentially resistant to aphids, we observed that the initial nymphs were mostly able to survive and develop into adults. However, the development of these initial nymphs was slower, and reproduction was significantly lower when compared to the standard susceptible controls and other susceptible accessions (Fig. 2). Previously, Sun et al. (2018) also reported that resistance in pepper to *Myzus persicae* affected reproduction more than the aphid's survival. These findings show that survival and reproduction do not always go hand in hand, suggesting that accessions may possess different mechanisms affecting survival or reproduction. For example, resistance traits such as toxic secondary metabolites may not immediately kill the insects but can weaken them over time, affecting their reproductive capacity (Yousaf et al. 2018). Therefore, it is well possible that the present resistance mechanism only affects one of the two parameters, or that the same mechanism affects both parameters but one more strongly than the other (Sun et al. 2018).

Aphids and whiteflies are pests characterized by high reproduction efficiency, making it difficult to control them efficiently using natural enemies because they can quickly outgrow them. Studies have shown that moderately to highly resistant plants can help improve the efficiency of biological control (Messina and Sorenson 2001; Peterson et al. 2016). Hence, resistance that reduces aphid and whitefly reproduction can be combined with other control methods to improve efficiency.

## Possibility of broad-spectrum resistance in one *Cucurbita moschata* accession

In practice, plants, particularly field grown crops, are exposed to a wide array of pests that may vary in terms of virulence. This is a challenge for resistance breeding programs. In this study, we identified one *Cucurbita moschata* accession (PI 550694) that is potentially resistant to both *Aphis gossypii* and *Bemisia tabaci.* This accession showed low survival and low reproduction of aphids, and little to no development of whitefly eggs to subsequent developmental stages (Supplementary Figures 3, 5). Earlier studies have identified resistance to some viral diseases mainly in *C. moschata* and wild relatives (McCreight 1984; Provvidenti 1990; Luckew et al. 2022). Previously, Luckew et al. (2022) reported accession PI 550694 as resistant to whiteflies. These findings with PI 550694 raised the question whether a common mechanism affects both insects in this accession, or whether there are two different mechanisms, governed by different genes. The former would be a case of broad-spectrum resistance, whereas the latter would be a stacking of separate resistance mechanisms.

Broad-spectrum resistance is an intriguing focus in resistance breeding due to its potential to confer comprehensive protection against various insect pests. Therefore, our study does not only contribute to identifying resistance sources in the *Cucurbita* germplasm to aphids and whiteflies but also underlines the potential for developing broad-spectrum resistance strategies in *Cucurbita* breeding programs, in case of a common resistance mechanism in this specific accession. Further study of these accessions could be done to identify specific genetic regions or QTL associated with resistance traits in these accessions, which can be used by breeders to develop aphid- and whitefly-resistant *Cucurbita* varieties.

## Conclusion

We identified several accessions that could serve as potential sources of resistance to aphids and whiteflies. One *Cucurbita moschata* accession (PI 550694) may have a form of broad-spectrum resistance as it was resistant to the two insects evaluated. The resistant materials identified herein can be explored further for aphid and whitefly resistance, contributing towards the development of resistant *Cucurbita* varieties.

**Author contributions** LC, REV, OS, and MA conceived and designed the experiments. MA, LB, and MFS performed the experiments. MA carried out data analysis and drafted the manuscript. RGFV, OS, REV, and LC were involved in revising the manuscript.

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**Data availability** The datasets generated and analyzed in this study are deposited in Zenodo repository at https://doi.org/10.5281/zenodo.14965957.

### Declarations

**Conflict of interest** Richard Visser is a member of the editorial board of Euphytica. The authors declare that they have no conflict of interest.



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