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Evaluation of lettuce germplasm for resistance to impatiens necrotic spot virus

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Abstract Recently, impatiens necrotic spot virus (INSV) has emerged as a major pathogen of lettuce in the Salinas Valley of California. The virus is a member of the genus *Orthotospovirus*, and under natural conditions, is transmitted by the tiny insect, western flower thrips. Due to the high efficiency of virus transmission, chemical management of thrips vectors is often ineffective in preventing the spread of the disease. Utilization of genetic resistance will be a critical component for managing the disease; however, little is known about genetic sources of resistance in lettuce. Here, we evaluated 189 lettuce lines under natural disease pressure in four field experiments to identify INSV resistant germplasm. The stability of resistance was also evaluated using different inoculation methods under controlled conditions. Our results showed a significant variation ($p < 0.0001$) among lettuce cultivars for both INSV severity and incidence. We identified INSV resistant germplasm that belongs to different horticultural types and leaf color, including a red-leaf cultivar 'Eruption' with a high degree of genetic stability. Moreover, we characterized several factors influencing thrips and host plant relationships, including horticultural type, leaf color, and polyphenol concentration. Overall, INSV resistant germplasm identified in this study provide valuable resources for breeding of INSV resistant lettuce cultivars.

Introduction

Lettuce is the seventh most valuable agricultural commodity in California with a cash value over $2.03 billion in 2021 (https://www.cdfa.ca.gov/statistics/). In the United States, California ranks number one for fresh market lettuce production with ~75% of national production (USDA-NASS 2022). Within California, Monterey County (the Central Coast region) leads the total volume of lettuce production with ~58% of the state total (CDFA 2022). In the Central Coast region, lettuce is grown in the field year-round (except for a 2-week lettuce-free period from December 7 to 21 as enforced by the Monterey County Agricultural Commissioner's office) to supply domestic and international markets. To fulfil a broad range of consumer demands, cultivars grown in this

region belong to several commercial types, including crisphead (iceberg), romaine (cos), leaf (green and red), butterhead, and other specialty types. Cultivation practices, including planting densities and crop duration, vary from one field to another because of different post-harvest uses such as baby-leaf, spring mix, hearts, whole-head, or ready-to-eat bagged salad consumption (Smith et al. 2011; Turini et al. 2011). However, cultivars with novel characteristics, improved postharvest quality, and resistance to biotic and abiotic stresses are always in demand because of changes in production practices, consumer preferences, marketing innovations, and to overcome different emerging challenges.

Impatiens necrotic spot virus (INSV; family *Tospoviridae*, genus *Orthotospovirus*) has emerged as a major limiting factor for commercial lettuce production in the Salinas Valley and other parts of the Central Coast region of California ever since it was first reported in 2006 in Monterey County (Koike et al. 2008; Hasegawa and Del Pozo-Valdivia 2023). INSV has also been recently documented in winter lettuce production areas of Southern California and Arizona (Hasegawa et al. 2022). Until 2017, INSV incidence occurred as isolated outbreaks in limited numbers of commercial fields (Kuo et al. 2014; Simko et al. 2023). However, in recent years, disease occurrence is widespread, causing devastating losses to the lettuce industry. While reasons for the recent emergence of INSV in coastal California are not fully understood, there is a need to develop effective management strategies.

Typical symptoms of INSV infection include stunted plant growth and twisting of leaves, and tan to dark brown necrotic spots on the leaves and midribs (Koike et al. 2008; Kuo et al. 2014; Hasegawa and Del Pozo-Valdivia 2023). INSV can infect lettuce plants at any stage of plant growth, though infection at early stages of development often leads to more pronounced symptoms that can lead to a non-marketable product, and pre-mature death of plants.

INSV is transmitted by the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). The virus is acquired during the larval growth stages (first or second instar larvae), while transmission is performed by adult thrips (Moritz et al. 2004; Pappu et al. 2009). Previous studies revealed the existence of moderate to large populations of thrips in lettuce fields of the Salinas Valley with a significant positive correlation between thrips populations and INSV incidence (Kuo et al. 2014; Hasegawa and Del Pozo-Valdivia 2023). This suggests that disease incidence (DI) is likely to be higher when thrips populations are high.

Management of thrips is challenging due to their small size (1–2 mm) and western flower thrips specifically have evolved resistance to a wide range of classes of insecticides (Gao et al. 2012). Furthermore, INSV has a broad host range and can infect hundreds of vegetable and fruit crops, including lettuce, basil, bell pepper, blackberry, celery, faba bean, peanut, pepino, potato, radicchio, spinach, sweet pepper, tobacco, tomatillo, and tomato, as well as ornamental crops and weed plant species that are found in the Salinas Valley (Pappu et al. 2009; Kuo et al. 2014). Weedy plant species may also act as sources of primary inoculum for lettuce planted in the early spring, as well as during the peak lettuce production season in the summer and fall.

One of the most effective strategies to control orthotospoviruses is to identify and introgress resistance genes into cultivars that are adapted to current production systems. This approach was successfully employed in the past by exploiting the *Sw*-5 gene to control *Tomato spotted wilt virus* (TSWV) in tomato and pepper (Boiteux and de B. Giordano 1993; Boiteux and de Ávila 1994). Before initiating resistance breeding, it is important to evaluate germplasm to identify sources of the genetic resistance and such evaluations require efficient and reliable screening methods. Simko et al. (2018) initiated screening of lettuce germplasm for resistance to INSV under controlled conditions and natural disease incidence in the field and indicated that field evaluation may not be reliable under low disease incidence. However, in recent years, relatively uniform and higher disease incidence in the Salinas Valley have been frequently observed (average disease incidence as high as 83% in the field with 100% incidence in susceptible cultivars) (Hasegawa and Del Pozo-Valdivia 2023; Simko et al. 2023). Such high disease incidence provides a unique opportunity to conduct field evaluations to identify genetic sources of resistance to INSV.

Currently, the genetics underlying INSV resistance are not fully understood. There are many types of cultivated lettuce that comprise extensive variation for leaf color, texture, shape, growth habit, and other traits (Ryder 1999), however little is known

about genetic variation for INSV resistance and germplasm resources. The genetic basis of partial resistance to INSV was explored in recent studies (Simko et al. [2018], [2023]). While this work provided an important foundation for developing lettuce cultivars with increased resistance to INSV, it did not elucidate underlying mechanisms of resistance against the virus, thrips, or both in germplasm with partial resistance. No cultivar was identified to be completely immune to INSV, signifying the importance for continuing germplasm evaluations. Therefore, we designed this study to evaluate large numbers of lettuce cultivars belonging to different horticultural types and leaf colors under a uniform and severe natural INSV infection in field experiments. Our objects were to: (i) assess genetic variation for INSV resistance, (ii) identify highly stable INSV resistant lettuce germplasm, and (iii) examine factors affecting virus, vector (thrips), and host plant relationships.

Materials and methods

Plant material

Plant material used in this study was comprised of commercial lettuce cultivars, breeding lines and plant introductions from the USDA collection in Salinas, and materials received from the seed company Enza Zaden, USA through a material transfer agreement. Germplasm under evaluation belong to various market classes of head type and leaf color. Number of lines under evaluation varied in different field experiments (Table 1), however 71 entries were overlapped across all field experiments.

Evaluation of genotypes under natural infection conditions in field

The genotypes were evaluated for INSV resistance under natural infection conditions in field experiments. The experimental plots were located at the USDA Spence Research Farm (GPS location: 36.629046, -121.539598) in Salinas, California. A total of four field experiments were conducted during two consecutive years in 2021 and 2022. In each year, two experiments were planted with the first and second planting seeded in June and August, respectively. Two planting dates were

Table 1 Number of lettuce cultivars tested for impatiens necrotic spot virus (INSV) resistance in different field experiments

Field experiment	Number of lines tested[z]			
	USDA collection materials	Enza Zaden materials	Total	Included in analysis
2021 June[§]	39	54	93	93
2021 August[§†]	70	103	173	170
2022 June[§†]	61	57	118	110
2022 August[§†]	65	49	114	110

[z]71 entries are overlapped across all four experiments.

[§] Some entries are tested in single replication only (i. For USDA materials: 2021 June −1 entry, 2021 August −13 entries, and 2022 August −2 entries; ii. For Enza Zaden materials: 2021 August −46 entries, 2022 June −2 entries, and 2022 August −5 entries).

[†] Some entries have either no germination or poor stand count in the field, therefore, removed from data analysis (i. For USDA materials: 2022 June −1 entry; ii. For Enza Zaden materials: 2021 August −3 entries, 2022 June −7 entries, and 2022 August −4 entries).

chosen to capture variability of seasonal disease and thrips pressure that could influence genotypic variation. In 2021, the first trial was planted on June 23rd and the second trial was planted on August 18th. Similarly, in 2022, the first trial was planted on June 15th and the second trial was planted on August 24th. Each trial was randomized independently and planted in a randomized complete block design with three replications. Raised beds were 1.02 m wide with two seed-lines per bed. Each bed was partitioned into 6.1 m plots with 0.61 m alleys between plots. Conventional lettuce production practices were followed throughout the experiment. Briefly, seed planting was done using a precision Stanhay planter (Stanhay, UK), modified by Sutton AG Enterprises (Salinas, CA) to accommodate hand dropping of raw seed for planting four seed-lines (two beds) at a time, attached to a tractor. After planting, plots were sprayed with an anti-crustant fertilizer to prevent crusting of the surface layer. Irrigation was by overhead sprinklers three times per week until thinning of seedlings (between weeks 3 and 4) and then twice weekly for the length of the experiment. Plots were thinned to 20–25 cm spacing between plants once plants were established (3–4 weeks after planting). Fertilizer was applied

pre-planting (6-20-20 at 336.3 Kg ha^{-1}) and then at 4 and 6 weeks after planting (ammonium sulfate at 336.3 Kg ha^{-1}). Two herbicides, Kerb (3.5 L ha^{-1}) and Prefar (9.4 L ha^{-1}), were applied pre-emergence. During the experiment, weed control was performed with a tractor mounted cultivator and by hand weeding. Fungicides were applied as needed later in the growing season to control downy mildew using Reason 500 (0.6 L ha^{-1}), Aliete (2.2 Kg ha^{-1}), Revus (0.6 L ha^{-1}), or Tanos DF (0.6 L ha^{-1}). Insecticides were applied as needed for aphid and worm control using Exirel (0.4 L ha^{-1}), Asana XL (0.6 L ha^{-1}), Movento (0.4 L ha^{-1}), Assail (0.1 L ha^{-1}), Beleaf 50 SG (0.2 L ha^{-1}), or Actara (0.1 L ha^{-1}). None of the insecticides used are labelled for thrips control.

For data collection, 10 plants were marked within each plot and monitored for INSV infection. Disease severity was recorded for each plant weekly beginning six weeks after planting and until the tenth week. Week 6 was chosen as starting point to allow plants to establish in the field and avoid any chance of losing experimental plants during thinning. INSV severity data was recorded using a 0–5 rating scale as described by Hasegawa and Del Pozo-Valdivia (2023). To assure that visual symptoms were due to INSV, symptomatic plants were randomly sampled and tested for INSV and TSWV using rapid lateral flow serological tests (ImmunoStrips; Agdia, Elkhart, IN). Presence of INSV was confirmed, but no TSWV was detected in any symptomatic plant samples across all experiments. We calculated disease incidence data as the proportion of plants with INSV severity of 2 or more. Area under the disease progress stairs (AUDPS) for both INSV severity and incidence data was calculated to combine the five weekly measurements into a single value (Simko and Piepho 2012). For statistical analysis, plot mean of 10 plants were computed for each individual week data.

We recorded leaf color data as 'Green' (if leaves were totally green), 'Green and purple' (if both green and purplish colors were present on leaves), 'Green and red' (if both green and red colors were present on leaves), or 'Red' (if leaves were red). Moreover, horticultural head type was recorded as 'Butterhead', 'Crisphead', 'Head' (if head forming, but not a typical crisphead type), 'Leaf', 'Romaine', or 'Segregating' (if individual plants within a plot varied significantly for plant type).

Evaluations of genotypes under different inoculation methods in controlled conditions

A subset of 13 cultivars, that showed consistent reaction to INSV (either resistant, intermediate, or susceptible) in field tests under natural infection, was picked for further evaluation under controlled conditions to examine genetic stability for INSV resistance. Name, horticultural type, and leaf color of these 13 cultivars can be tracked in Table 2.

Mechanical INSV inoculation: To characterize the responses of cultivars against the virus alone, plants were evaluated under mechanical inoculation only. Mechanical inoculation was performed by gently rubbing the freshly prepared inoculum on leaves of five-week-old seedlings (~ four- to five-true leaf stage). Inoculum was prepared by grinding 0.5 g of INSV infected leaf tissue (~ 2.5 square cm) per 5 ml of buffer (1:10 wt/vol) with freshly prepared ice-cold 0.1 M Sodium Phosphate buffer (pH 7.0) containing 0.2% Sodium Sulphite and 1% Celite. Virus presence in INSV infected leaf tissue, originated from plants infected by thrips, was confirmed using ImmunoStrips (Agdia, Elkhart, IN). The experiment was conducted in a walk-in growth room (Conviron - model MTPS144; Conviron, Winnipeg, Canada) using a randomized complete block design with four replications. Each replication was comprised of three individuals per genotype. Growth room temperature was maintained at 25 °C and a photoperiod of 16 h (light intensity ≈ 350 μmol.m^{-2} s^{-1}).

Thrips inoculations: Five-week-old plants (~ four- to five-true leaf stage; previously grown in the growth chamber in the absence of INSV and thrips) were placed inside a greenhouse containing INSV-infected plants and a viruliferous population of western flower thrips. Experimental plants were placed along with spreader plants (susceptible plants on which the thrips population was maintained) in a randomized complete block design with four replications. Each replication was comprised of two individual plants per genotype. Climatic conditions of the greenhouse were set to 25 °C and a photoperiod of 16 h. Depending on cloudy or bright sunny days, and supplemental LED lights, the intensity of light fluctuated between ≈ 300 to 700 μmol m^{-2} s^{-1}).

Table 2 Comparison of impatiens necrotic spot virus (INSV) severity and incidence mean of lettuce cultivars at 10 weeks post-planting under different inoculation methods in growth room and greenhouse conditions[z]

Cultivar	Type	Leaf color	Disease Severity (Week 10)					Disease Incidence (Week 10)				
			Mechanical Inoculation	Thrips Inoculation		Mechanical + Thrips Inoculation		Mechanical Inoculation	Thrips Inoculation		Mechanical + Thrips Inoculation	
			GR 2022	GH 2022 A	GH 2022B	GH 2022 A	GH 2022B	GR 2022	GH 2022 A	GH 2022B	GH 2022 A	GH 2022B
Rubens Red	Romaine	Red	1.3 (0.3) [a]	3.8 (0.4) [bc]	3.6 (0.2) [bcd]	3.2 (0.3) [a]	4.1 (0.2) [cdef]	41.7 (8.3) [a]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Cavalry	Leaf	Red	3.7 (0.5) [ab]	2.3 (0.1) [a]	2.2 (0.1) [a]	3.0 (0.2) [a]	2.4 (0.2) [a]	75.0 (8.3) [abc]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Eruption	Leaf	Red	2.8 (0.9) [ab]	3.1 (0.3) [ab]	2.8 (0.2) [ab]	3.1 (0.1) [a]	2.8 (0.2) [ab]	58.3 (16.0) [ab]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Red Hot	Leaf	Red	4.8 (0.1) [b]	3.8 (0.2) [bc]	3.6 (0.3) [bcd]	3.5 (0.4) [ab]	3.7 (0.1) [bcde]	100.0 (0.0) [c]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Flashy Trout Back	Leaf	Green with red patches	3.5 (0.4) [ab]	2.1 (0.1) [a]	–	3.5 (0.6) [ab]	–	83.3 (9.6) [bc]	100.0 (0.0) [a]	–	100.0 (0.0) [a]	–
BL288	Segregating	Green	2.3 (1.0) [ab]	3.6 (0.1) [b]	3.2 (0.2) [abc]	3.4 (0.2) [a]	3.3 (0.3) [abc]	58.3 (21.0) [ab]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
BL280	Segregating	Green	2.3 (0.2) [ab]	4.1 (0.1) [bc]	3.2 (0.1) [abc]	4.0 (0.2) [ab]	3.4 (0.3) [abcd]	58.3 (8.3) [ab]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Beacon	Crisphead	Green	3.8 (0.4) [ab]	3.9 (0.4) [bc]	4.0 (0.3) [cde]	4.2 (0.3) [ab]	4.3 (0.2) [cdef]	83.3 (9.6) [bc]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Salinas	Crisphead	Green	2.3 (0.5) [ab]	4.4 (0.2) [bc]	3.5 (0.3) [bcd]	4.3 (0.3) [ab]	4.3 (0.2) [def]	58.3 (16.0) [ab]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Pacific	Crisphead	Green	2.5 (0.6) [ab]	4.2 (0.3) [bc]	4.0 (0.1) [bcd]	4.5 (0.3) [ab]	3.8 (0.3) [bcde]	58.3 (16.0) [ab]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Conquistador	Romaine	Green	2.5 (0.8) [ab]	4.0 (0.5) [bc]	4.3 (0.4) [cd]	4.0 (0.2) [ab]	3.8 (0.1) [bcde]	58.3 (16.0) [ab]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Defender	Romaine	Green	4.6 (0.3) [b]	4.1 (0.2) [bc]	4.7 (0.2) [d]	3.8 (0.1) [ab]	4.7 (0.2) [ef]	100.0 (0.0) [c]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]
Paris White	Romaine	Green	4.9 (0.1) [b]	5.0 (0.0) [c]	4.5 (0.3) [d]	5.0 (0.0) [b]	5.0 (0.0) [f]	100.0 (0.0) [c]	100.0 (0.0) [a]	100 (0.0) [a]	100.0 (0.0) [a]	100 (0.0) [a]

[z]GR and GH indicate growth room and greenhouse, respectively and year followed by different letter (A, or B) indicate that experiment was conducted at different time points.

Different letter grouping within a column indicates that mean value significantly differed at p value 0.05 (Disease severity data were analyzed using parametric method, whereas disease incidence data were analyzed using non-parametric method).

Value in parentheses represents standard error of mean.

Combined inoculation (mechanical + thrips): Five-week-old plants (~ four- to five-true leaf stage) were mechanically inoculated and also placed in the greenhouse containing viruliferous thrips as described above. Plants were placed in a randomized complete block design with four replications and each replication was comprised of two individual plants per genotype.

Choice and No-choice tests to assess thrips feeding preference and reproduction

To assess thrips preference of host plant for feeding and reproduction, we conducted choice and no-choice tests on the subset of 13 cultivars (described above) in a greenhouse experiment in the absence of INSV infection. For choice tests, one plant of each cultivar at the 3–4 true leaf stage was placed inside of a thrips-proof cage inside of a greenhouse maintained at 24 °C under natural lighting. The plants were oriented in a circle within the cage and 200 adult western flower thrips were released in the middle of the arena for 2 weeks. At the end of the trial, adult and immature thrips were recovered from each plant and counted. To assess thrips feeding damage, three leaf discs (2.54 cm diameter) were randomly collected from an outer, middle, and inner leaf of each plant. The total number of feeding scars were counted from those three leaf discs and assigned a score of 0–5 (0 = no scars, 1 = 1 to 5 scars, 2 = 6 to 10 scars, 3 = 11 to 15 scars, 4 = 15 to 20 scars, 5 = more than 20 scars). The experiments were repeated seven times.

For no-choice tests, a single leaf from each cultivar at the 3–4 true leaf stage was removed and placed inside of a clear plastic feeding chamber, in which thrips could feed on a 2.54 cm diameter circular area of the leaf. Ten adult thrips were placed inside of each chamber and incubated at 23 °C under a 16 h photoperiod for 3 days. At the end of the experiment, the number of feeding scars were counted for each leaf and assigned a score of 0–5 using the same scale mentioned earlier. The experiments were repeated three times.

Polyphenol assay

We performed polyphenol assay on the subset of 13 cultivars (described above). Three plants of each cultivar were grown in 10.2 cm pots in a growth room

chamber. Plants were direct seeded on moist potting soil by maintaining growth chamber temperature at 20 °C and photoperiod 16 h (light intensity ≈ 300 µmol.m^{-2}.s^{-1}). Plants were supplemented with nutrients (Miracle Gro) on a weekly basis and watered daily to maintain volumetric water content of soil to 0.2–0.3 m^{3}m^{-3}. Two trials were performed and from each trial, plant samples were harvested at 8-weeks after seed-sowing for polyphenol analysis. Entire plants were cut at the base, old chlorotic leaves were removed, and the sample was immediately flash frozen in liquid nitrogen and transferred to – 80 °C until further processing. For the total polyphenol extraction and quantification, we followed the protocol described by Medina ([2011]). Briefly, a core (1 cm diameter) of plant tissue was collected by capturing all stages of plant leaves (core passing through entire head up to the center but avoided the mid-ribs). The tissue was ground using liquid nitrogen and 0.25 g ground samples was transferred to1.5 mL centrifuge tube. The samples were then soaked in 1 ml of 70% methanol and centrifuged at 6,800 g for 10 min. Next, 100 µl of supernatant was distributed in triplicate to a 96 well polystyrene plate (Falcon 3072), followed by adding 40 µl of 0.1% aqueous Fast Blue BB (FBBB) to each well. Then, the plate was agitated for 3 min and 60 µl of 1.67% sodium hydroxide was added to each well. Finally, the plate was incubated in the dark at room temperature for 90 min, and absorbance was read at 450 nm on a Biotek HTX Multi-Mode plate reader (Agilent Technologies Inc., Santa Clara, CA). Total polyphenol content was reported based on a standard curve of gallic acid equivalents (GAE µM / 0.25 g tissue).

Statistical analysis

Statistical analyses of data were performed in R v. 4.1.1 (R Core Team [2021]), unless otherwise mentioned. Disease severity and incidence data were visualized as box plots using the package "ggplot2". These box plots show the distribution of data, including the median, mean, the 25th and 75th percentile, and outliers. We tested homogeneity of variance among data collected in four different field tests using the function 'leveneTest' in "car" package to check if the pooled estimates could be used in analysis. However, the test indicated a significant difference in the variances of the four field tests

data (Levene's test p value < 0.05), therefore we analyzed each field test dataset separately.

Field tests data were subjected to an analysis of variance (ANOVA) to test any significant difference among the cultivars for INSV severity and disease incidence. This analysis was performed using JMP Pro 15 (2020, SAS Institute, Cary, NC). In the ANOVA model, cultivar was treated as fixed effect and replication was considered random effect. Followed by ANOVA, we performed analysis of means (ANOM) to compare means of each cultivar with the overall mean. ANOM helps to classify cultivars into three categories: (i) Relatively resistant, if the mean value of any entry falls below the lower decision limit boundary of the 95% confidence interval, (ii) Intermediate, if the mean value of any entry falls within the boundary of the 95% confidence interval, and (iii) Relatively susceptible, if the mean value of any entry exceeds the upper decision limit boundary of the 95% confidence interval.

We examined the impact of different horticultural head types and leaf color on INSV severity and incidence. Because sample size and variance for each group were not equal, a nonparametric one-way ANOVA (Kruskal-Wallis test) was performed to determine group differences followed by Dunn's multiple rank comparison test with p-value adjustment using 'Holm' method and the analysis was performed using the function 'dunnTest' in the R packages "FSA".

We computed broad-sense heritability (H^2) to examine the contribution of the genetic factors on disease severity. We applied variance component method to estimate H^2 using the mathematical equation below (Hallauer et al. 1988),

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{n} + \frac{\sigma_e^2}{nr}}$$

where,

σ_G^2 = genotypic variance,

σ_{GE}^2 = genotypic × environment interaction variance.

σ_e^2 = error variance.

n = number of environments.

r = number of replications.

The variance components were estimated using the function 'remlVCA' in the R package "VCA".

Variability of INSV severity and incidence were decomposed into genetic, environmental, their interaction, and error components considering all variables to be random. For this analysis, we included INSV severity and incidence data of the 71 lines that were overlapped across all of the four field tests.

To compare the responses of cultivars under different inoculation methods (mechanical, thrips only, or both), we performed one way ANOVA, using either function 'aov' (if assumptions of parametric analysis were met), or 'kruskal.test (if assumptions of parametric analysis were not met). Following ANOVA, post-hoc analysis was performed using "Tukey" method in parametric analysis and "Holm" method in non-parametric analysis. Preference of host plants was also assessed by performing ANOM using the package "ANOM" for mature and immature thrips count and feeding score data. In ANOM method, a linear model was fit for feeding score data using function 'lm', whereas poisson generalized linear model with a logarithmic link function was fitted for count data using function 'glm'. Total polyphenol concentration (TPC) data for 13 cultivars were analyzed using the Kruskal-Wallis test followed by Dunn's multiple comparison test to determine differences between cultivars. TPC data of the two trials were significantly different for variance homogeneity (Levene's test p value < 0.05), therefore analyzed separately.

Genotype main effect plus genotype × environment interaction (GGE) biplot analysis was performed to explore genotype stability and the interrelationship among test environments. The graphical presentation of GGE biplot was based on the first two principal components (PCs) resulting from singular value decomposition (SVD). The GGE model was constructed by using the following equation (Yan 2002):

$$Y_{ij} - \mu - \beta_j = \sum_{l=1}^{k} \lambda_l \, \xi_{il} \, \eta_{lj} + \varepsilon_n$$

where,

Y_{ij} = the measured mean disease severity of genotype i (1, 2,…n) in environment j ($= 1, 2,…m$).

μ = the grand mean.

β_j = the main effect of environment j, ($\mu + \beta_j$) being the mean disease severity in environment j.

λ_l = the SV of lth principal component (PC), the square of which is the sum of squares explained by PCl (l = 1, 2,…k, with $k \leq \min(m, n)$ and $k = 2$ for a two- dimensional biplot).

ξ_{il} = the eigenvector of genotype i for PC l.

η_{lj} = the eigenvector of environment j for PC l.

ε_{ij} = the residual associated with genotype i in environment j.

Results

Genotypic response against INSV under natural infection conditions

Distribution of INSV severity and incidence data are visualized in Fig. 1 and summary statistics are presented in Online Resource 1. Both severity and incidence of INSV disease increased as the weeks advanced. In general, a strong correlation was observed between INSV severity and incidence data for each week of data collection in all environments (Online Resource 2). The average disease incidence was the highest at Week 10 in all environments. By this point, most cultivars tested were past the harvest maturity stage. However, we present ANOM results of both disease severity and incidence data at week 10 only because it appears to be the most appropriate to compare genotype responses to infection by INSV. We did not use AUDPS value in our analysis either because it could be misleading to discriminate between genotypes infected at early stage with slow progress of disease versus genotypes that were infected later stage with quick progress of disease. Differences in both mean and variance among different field experiments are evident from the boxplots (Fig. 1). Overall, the lowest disease incidence was recorded in the June 2021 experiment (40.9% ± 29.2) and the highest in the June 2022 experiment (83.4% ± 27.3) (Online Resource 1). A significant genetic variation ($p < 0.0001$) is observed for both INSV severity and incidence within each field experiment (Online Resource 8), yet the genotype performance was skewed toward the susceptible side (Fig. 1). In the June 2021 experiment, 3 cultivars fall within the relatively resistant category whereas the majority were categorized as intermediate as compared to the August 2021 experiment in which 21 cultivars showed a relatively resistant reaction based on
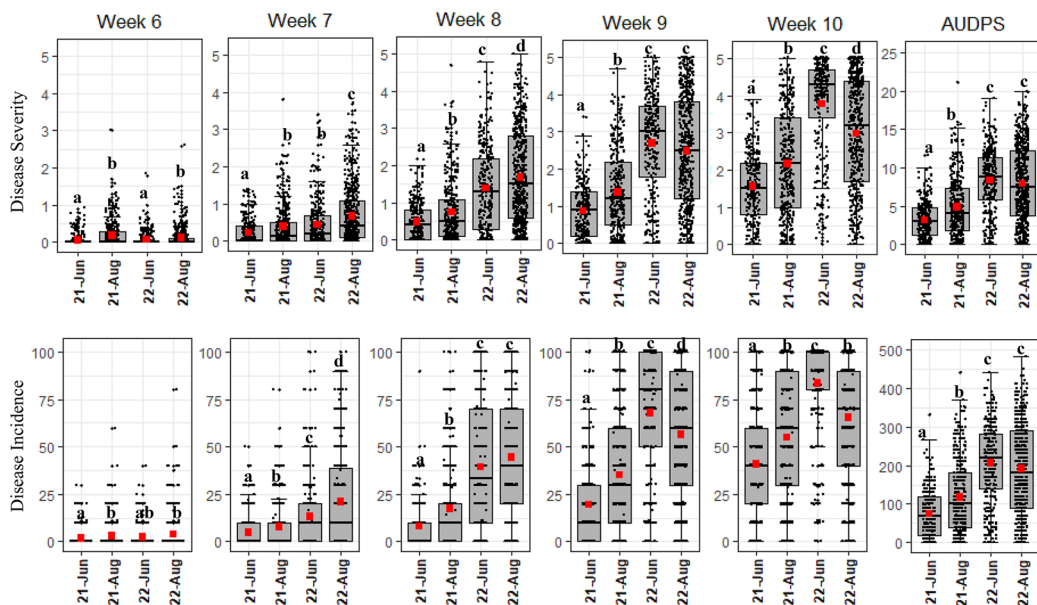
Fig. 1 Distribution of INSV severity and incidence from week 6 to 10 after planting and Area under disease progress stairs (AUDPS) data in different field experiments (21-Jun = June 2021, 21-Aug = August 2021, 22-Jun = June 2022, and 22-Aug = August 2022). Different letter grouping within a group indicates a significant difference at p-value 0.05. Red square represents mean value

ANOM classification. In the June 2022 and August 2022 experiments, a total of 23 and 31 cultivars were identified to be relatively resistant, respectively. After comparing ANOM results of all four field experiments, no genotype showed complete immunity to INSV. However, we identified that the cultivars 'Amazona', 'Anais', 'Ancora', 'Cavalry', 'Eruption', 'Ostinata', 'Oscar', 'Parade', 'Rubens Red', 'INSV-21-59', 'INSV-21-63', 'INSV-21-72', and 'INSV-21-89' showed consistently low INSV severity and incidence across all four field experiments (Online Resource 8). On the other hand, the cultivars 'Defender', 'Heart's Delight', 'King Henry', 'Paris White', 'PI 251246', 'Triple Threat', 'Xena', 'INSV-21-51', and 'INSV-21-54' were the most susceptible cultivars. Some cultivars showed a significant Genotype × Environment interaction such as 'INSV-21-77', which was classified as susceptible in August 2021 and June 2022, but relatively resistant in August 2022.

Impact of horticultural traits on INSV severity and incidence

Comparison of INSV severity among lettuce cultivars, belonging to different horticultural types and leaf colors, was performed to identify any significant differences. Results are presented in Fig. 2, Online Resource 3, and Online Resource 4. A range of variation was observed for both INSV severity and incidence within different horticultural types and leaf colors of lettuce. In general, romaine type lettuce cultivars had higher INSV severity and incidence compared to head forming lettuce cultivars, including crisphead and butterhead types. Furthermore, INSV incidence and severity were generally low in red leaf lettuce cultivars as compared to green leaf cultivars.

Variance component and heritability

Estimates of variance components are presented in Table 3. Combined ANOVA revealed substantial
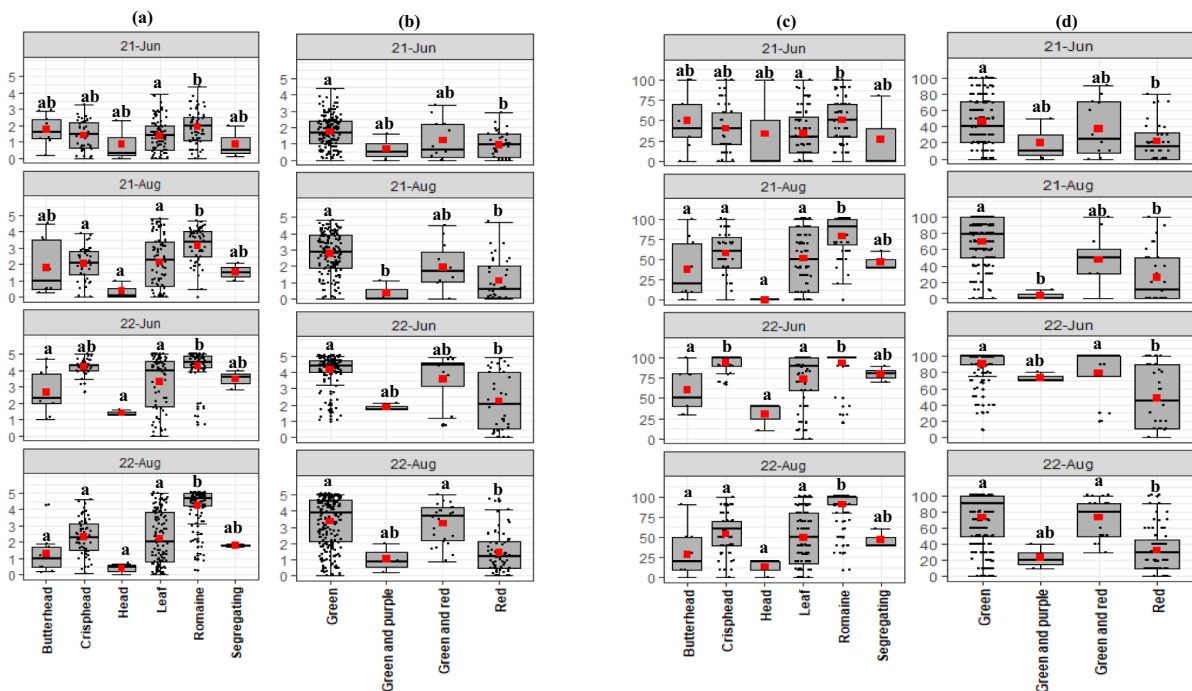
Fig. 2 Comparison among lettuce cultivars with horticultural types and leaf color for INSV disease severity and incidence at week 10 post-planting; **a** horticultural types vs. disease severity, **b** leaf color vs. disease severity, **c** horticultural types vs. disease incidence, and **d** leaf color vs. disease incidence in different field experiments (21-Jun=June 2021, 21-Aug=August 2021, 22-Jun=June 2022, and 22-Aug=August 2022). Different letter grouping within a group indicates a significant difference at p-value 0.05. Red square represents mean value

Table 3 Estimates of variance components and heritability using the 71 cultivars of lettuce that belongs to different horticultural types and leaf color and evaluated for INSV resistance across four field experiments

Source of variation	DF	Variance	%Total	H²
Disease Severity (Week 10)				
Cultivar	70	0.98***	36.0	0.88
Environment	3	0.86***	31.6	
Cultivar × Environment	210	0.37***	13.6	
Error		0.51	18.8	
Disease Incidence (Week 10)				
Cultivar	70	468.0***	36.7	0.87
Environment	3	290.6***	22.8	
Cultivar × Environment	210	158.2***	12.4	
Error		358.1	28.1	

DF; Degree of freedom

%Total; Percent of total (percentage of variation explained)

H^2; Broad-sense heritability

*** indicates *p* value < 0.0001

genetic variation for INSV severity and incidence along with significant environment and Genotype × Environment interaction. Most of the variation for disease severity was contributed by Genotype (36%), followed by Environment (32%), and Genotype × Environment interaction (14%). For disease incidence, variation contributed by Genotype and Genotype × Environment interaction were nearly the same as for disease severity, however variation contributed by environment reduced to 23% while error variance increased to 28%. Estimates of broad sense heritability for both INSV severity and incidence were very high ($H^2 = 0.88$ and 0.87, respectively).

Genotype performance against INSV under different inoculation methods in controlled conditions

The results of 13 cultivars evaluated for INSV resistance under various inoculation methods are shown in Table 2.

Responses of cultivars under mechanical inoculation: A significant variation for disease severity was observed among the 13 cultivars under mechanical inoculation. 'Rubens Red' had the lowest disease severity (1.3 ± 0.3) and 'Defender', 'Red Hot', and 'Paris White' had the highest (4.6 ± 0.3, 4.8 ± 0.1, and 4.9 ± 0.1, respectively). All other cultivars showed intermediate severity. For disease incidence, 'Rubens

Red' had the lowest percent of infected plants (41.7% ± 8.3) followed by 'Eruption' (58.3% ± 16.0). 'Defender', 'Red Hot', and 'Paris White' had the highest percent of infected plants (all 100%). Interestingly, 'Cavalry' (a relatively resistant cultivar in field experiment) had a disease incidence (75% ± 8.3) not significantly different from the most susceptible cultivars.

Responses of cultivars under inoculation by thrips: In two greenhouse tests conducted at different times (2022A and 2022B), 'Cavalry' showed the lowest mean disease severity (2.3 ± 0.1 and 2.2 ± 0.1) followed by 'Eruption' (3.1 ± 0.3 and 2.8 ± 0.2). 'Flashy Trout Back' was tested in a single test where it showed a low severity (2.1 ± 0.1) not significantly different from 'Cavalry'. 'Paris White' (5.0 ± 0.0 and 4.5 ± 0.3) and 'Defender' (4.1 ± 0.2 and 4.7 ± 0.2) showed the most susceptible reactions in both greenhouse tests. Interestingly, 'Rubens Red' (a relatively resistant cultivar in field experiments) had INSV severity in the two tests (3.8 ± 0.4 and 3.6 ± 0.2) not significantly different from the most susceptible cultivars. Not a single plant of any cultivar had an INSV severity score below 2, giving all cultivars a mean incidence of 100%.

Responses of cultivars under combination of mechanical and thrips inoculation: In two greenhouse tests (2022A and 2022B), where cultivars were tested under the combination of mechanical inoculation and natural thrips inoculations, 'Cavalry' (3.0 ± 0.2 and 2.4 ± 0.2) and 'Eruption' (3.1 ± 0.1 and 2.8 ± 0.2) had lower disease severity than any other cultivars in both tests. 'Paris White' had the highest severity in both tests. Interestingly, 'Rubens Red' (a relatively resistant cultivar in field experiments) had INSV severity in test 2022B (4.1 ± 0.2) not significantly different from the most susceptible cultivars. Similar to thrips inoculations, not a single plant of any cultivar had an INSV severity score below 2, giving all lines a mean incidence of 100%.

**Genotype plus genotype × environment interaction (GGE) biplot analysis**

We performed GGE biplot analysis to visualize performance of 13 genotypes and the interrelationship of 9 test environments (each field and greenhouse experiment represents a separate environment). The first two principal components (PC) of the GGE

biplot explained a total of 83.3% (PC1 = 71.04% and PC2 = 12.24%) of the variation for disease severity over nine test environments.

To examine discriminating power (how well a test environment can separate resistant and susceptible genotypes) and representativeness (how well a genotype's performance in a test environment is representative of its overall performance) of the test environments, a discriminativeness vs. representativeness plot was created (Fig. 3a). In the figure, the "Average-environment axis" (AEA) is represented by a line passing through the average environment co-ordinate (a point at the end of arrow) and biplot origin. Test environment vectors that have a smaller angle (more acute) with AEA are more representative. Concentric circles aid in comparing the length of the test environment vectors, the longer the vector from the biplot origin, the test environment had relatively more discriminating power (more informative). Most of the test environments have acute angles (< 90°) with each other (except between 'GR22 mechanical only' and 'GH22A thrips only'), indicating strong positive correlations between the test environments. Ideally, the
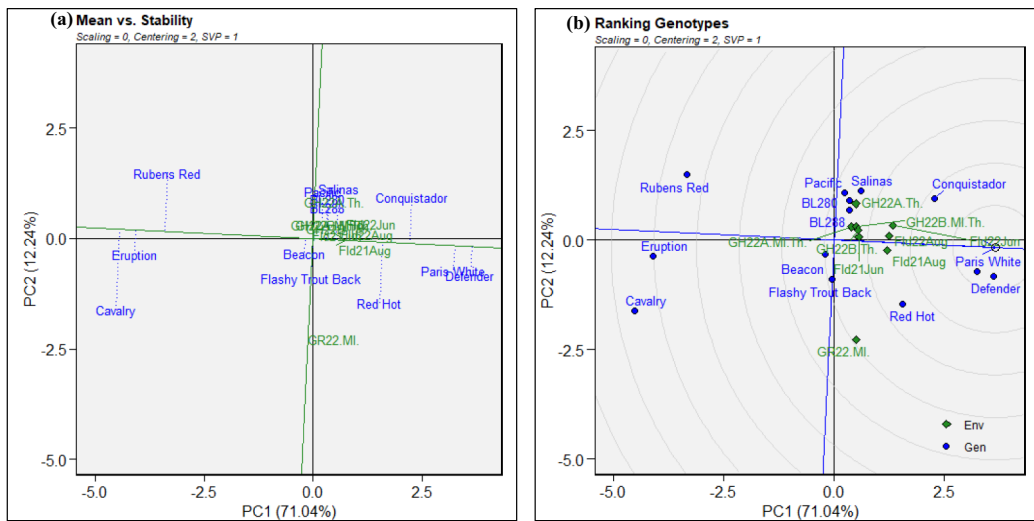
best environment would have a longer vector and the angle with AEA close to zero. Similarly, Ranking environment plot (Fig. 3b) shows the ideal test environment (the most discriminating and representative) as a center of the concentric circles. Looking at plot, it is evident that 'GR22 Mechanical only' is the least desirable test environment because it is located at the farthest distance from the center of the concentric circles.

'Mean vs. stability' (Fig. 4a) plot is visualized to assess mean performance and stability of all 13 genotypes evaluated across the nine test environments. The single-arrowed line is the AEA and points towards higher disease severity. Therefore, genotypes positioned the farthest to the left of the plot ('Cavalry', 'Eruption', and 'Rubens Red') are the most resistant. The length of vertical dotted line connecting the genotype that is perpendicular to AEA shows the genotype stability (shorter the line in either direction of AEA, the genotype is more stable). This indicates that 'Eruption' is the most stable resistant cultivar followed by 'Cavalry'. 'Defender' and 'Paris White' are the most stable susceptible cultivars. Likewise,
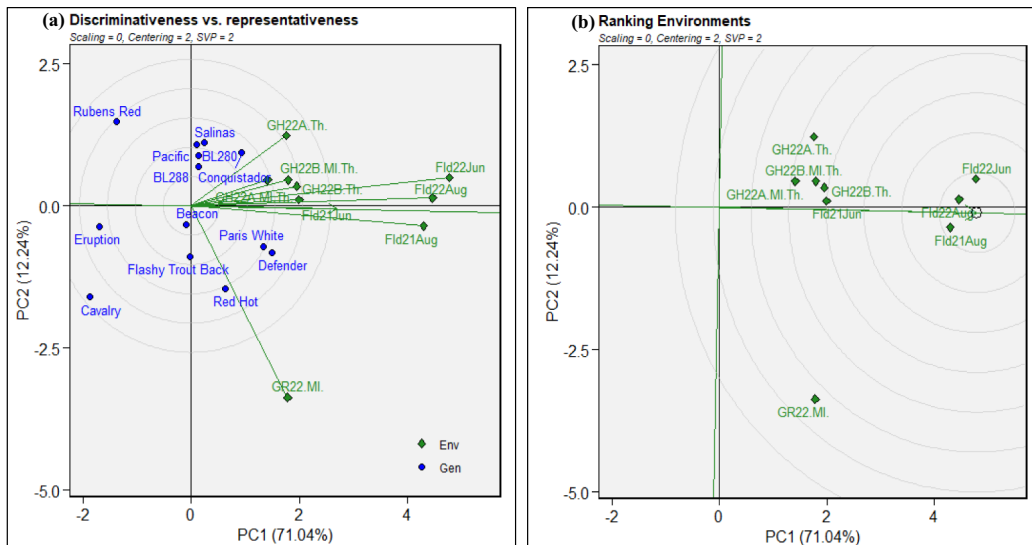
Fig. 3 GGE biplot **a** Discriminativeness vs. representativeness of nine test environments and **b** ranking environments based on relative performance of 13 cultivars. "Scaling = 0" means data were not scaled. "Centering = 2" means the data were centered by the means of environment. "SVP = 2" means the singular value were partitioned into the environment eigenvectors for visualizing the correlation among environments. Text in "Blue" font represents the name of cultivars. Text in

"Green" font represents the name of test conditions (Abbreviations: GR22.MI – Growth room 2022 Mechanical Inoculation, GR22A.MI.Th – Greenhouse 2022A Mechanical Inoculation + Thrips, GR22B.MI.Th – Greenhouse 2022B Mechanical Inoculation + Thrips, GR22A.Th – Greenhouse 2022A Thrips, GR22B.Th – Greenhouse 2022B Thrips, Fld21Jun – Field June 2021, Fld21Aug – Field August 2021, Fld22Jun – Field June 2022, Fld22Aug – Field August 2022)

Fig. 4 GGE biplot **a** Mean vs. stability of 13 cultivars and **b** ranking of genotypes performance across nine test environments. "Scaling = 0" means data were not scaled. "Centering = 2" means the data were centered by the means of environment. "SVP = 1" means the singular value were partitioned into the genotype eigenvectors for visualizing the correlation among genotypes. Text in "Blue" font represents the name of cultivars. Text in "Green" font represents the name of test conditions (Abbreviations: GR22.MI – Growth room 2022 Mechanical Inoculation, GR22A.MI.Th – Greenhouse 2022A Mechanical Inoculation + Thrips, GR22B.MI.Th – Greenhouse 2022B Mechanical Inoculation + Thrips, GR22A.Th – Greenhouse 2022A Thrips, GR22B.Th – Greenhouse 2022B Thrips, Fld21Jun – Field June 2021, Fld21Aug – Field August 2021, Fld22Jun – Field June 2022, Fld22Aug – Field August 2022)

'Ranking genotypes' is visualized in Fig. 4b. The center of concentric circles in this plot indicates the highest disease severity mean. Therefore, genotypes distantly located from this origin are more resistant ('Cavalry', 'Eruption', and 'Rubens Red').

'Which-won-where' plot (Fig. 5) is constructed by connecting vertex genotypes (the genotypes positioned at the farthest point from the biplot origin), thus forming a polygon such that all other genotypes are contained inside the polygon. The polygon resulted in seven vertex genotypes with both positive (a higher disease severity means) and negative (a lower disease severity means) PC1 scores. In this plot, 'Defender' is the vertex genotype that falls in the sector in which most of test environment colocalized. Based on disease rating scale used and high PC1 score, it is evident that 'Defender' is the most susceptible cultivar to INSV in all of test environments. 'Cavalry', 'Eruption', and 'Rubens Red' are the most resistant genotypes to INSV as indicated by the high negative PC1 scores. Because nearly all of test environments (except 2022 Mechanical only and 2022 GHA thrips only) fall in one sector, it can be considered one mega-environment and any of these

test environments would be sufficient to select lettuce germplasm for INSV resistance.

Choice and no-choice test to assess thrips feeding preference and reproduction

The means of thrips feeding score on different cultivars of lettuce (evaluated under choice and no-choice test in the absence of INSV infection) is summarized in Online Resource 5 and results of analysis of mean is presented in Fig. 6. Our results indicated no statistical differences among lettuce cultivar for feeding score, except that 'Pacific' had significantly more feeding scars (higher feeding scores) than any other cultivars in choice tests. The number of thrips on each cultivar of lettuce from the choice tests are presented in Online Resource 5 and Fig. 7. Our results showed significant differences in the number of mature and immature thrips among different lettuce cultivars. 'Cavalry' (4.4 ± 0.8) and 'Eruption' (3.3 ± 0.9) had significantly fewer numbers of mature thrips than the overall mean in the choice tests. When comparing the number of immatures thrips, significantly fewer immatures were observed on 'BL 280' (21.3 ± 6.2),
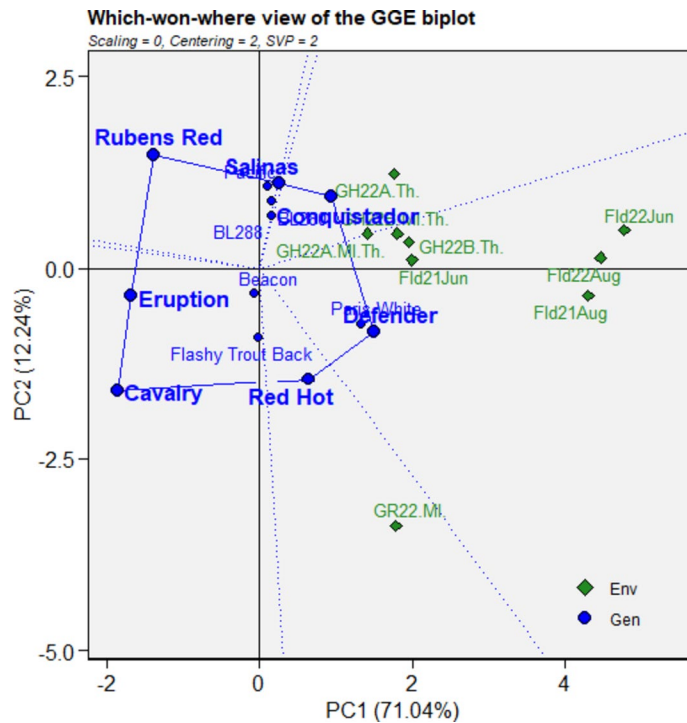
Fig. 5 'Which-won-where' view of the GGE biplot to visualize performance of 13 genotypes performance across nine test environments. "Scaling = 0" means data were not scaled. "Centering = 2" means the data were centered by the means of environment. "SVP = 2" means the singular value were partitioned into the environment eigenvectors for visualizing the correlation among environments. Text in "Blue" font represents the name of cultivars. Text in "Green" font represents the name of test conditions (Abbreviations: GR22.MI – Growth room 2022 Mechanical Inoculation, GR22A.MI.Th – Greenhouse 2022A Mechanical Inoculation + Thrips, GR22B.MI.Th – Greenhouse 2022B Mechanical Inoculation + Thrips, GR22A.Th – Greenhouse 2022A Thrips, GR22B.Th – Greenhouse 2022B Thrips, Fld21Jun – Field June 2021, Fld21Aug – Field August 2021, Fld22Jun – Field June 2022, Fld22Aug – Field August 2022)

'Cavalry' (26.7 ± 6.6), 'BL 288' (33.6 ± 10.1), and 'Eruption' (44.3 ± 12.1). A significantly greater number of mature thrips was observed on 'Beacon' (13.3 ± 2.2). We observed relatively higher numbers of immature thrips on 'Beacon' (103.7 ± 21.7), 'Conquistador' (91.4 ± 23.2), 'Pacific' (87.3 ± 13.7), 'Paris White' (78.1 ± 18.3), and 'Red Hot' (67.1 ± 16.0).

Genotypic variation for total polyphenol concentration

We also sought to understand the relationships between polyphenols and thrips preference for feeding and reproduction by performing a polyphenol assay using the same subset of 13 cultivars that showed either resistant, intermediate, or susceptible reactions to INSV. Results of the polyphenol assay are visualized in Fig. 8. A significant variation ($p < 0.05$) for total polyphenol concentration (TPC) was observed among the 13 lettuce cultivars. In trial 1, 'Eruption' (1180.5 GAE μM / 0.25 g ± 246.7) had significantly higher concentration of TPC than any other cultivars. In Trial 2, 'Flashy Trout Back' (3229.5 GAE μM / 0.25 g ± 1314.6) had the higher concentration of TPC, followed by 'Eruption' (2255.8 GAE μM / 0.25 g ± 1363.3) compared to other cultivars. Generally, red-leaf cultivars had a higher concentration of total polyphenol than green-leaf cultivars in both trials. Additionally, in Trial 2, all cultivars showed relatively higher concentration of TPC along with high variability as compared to Trial 1.

Discussion

The surge in INSV has significant economic repercussions for lettuce growers in the Salinas Valley and
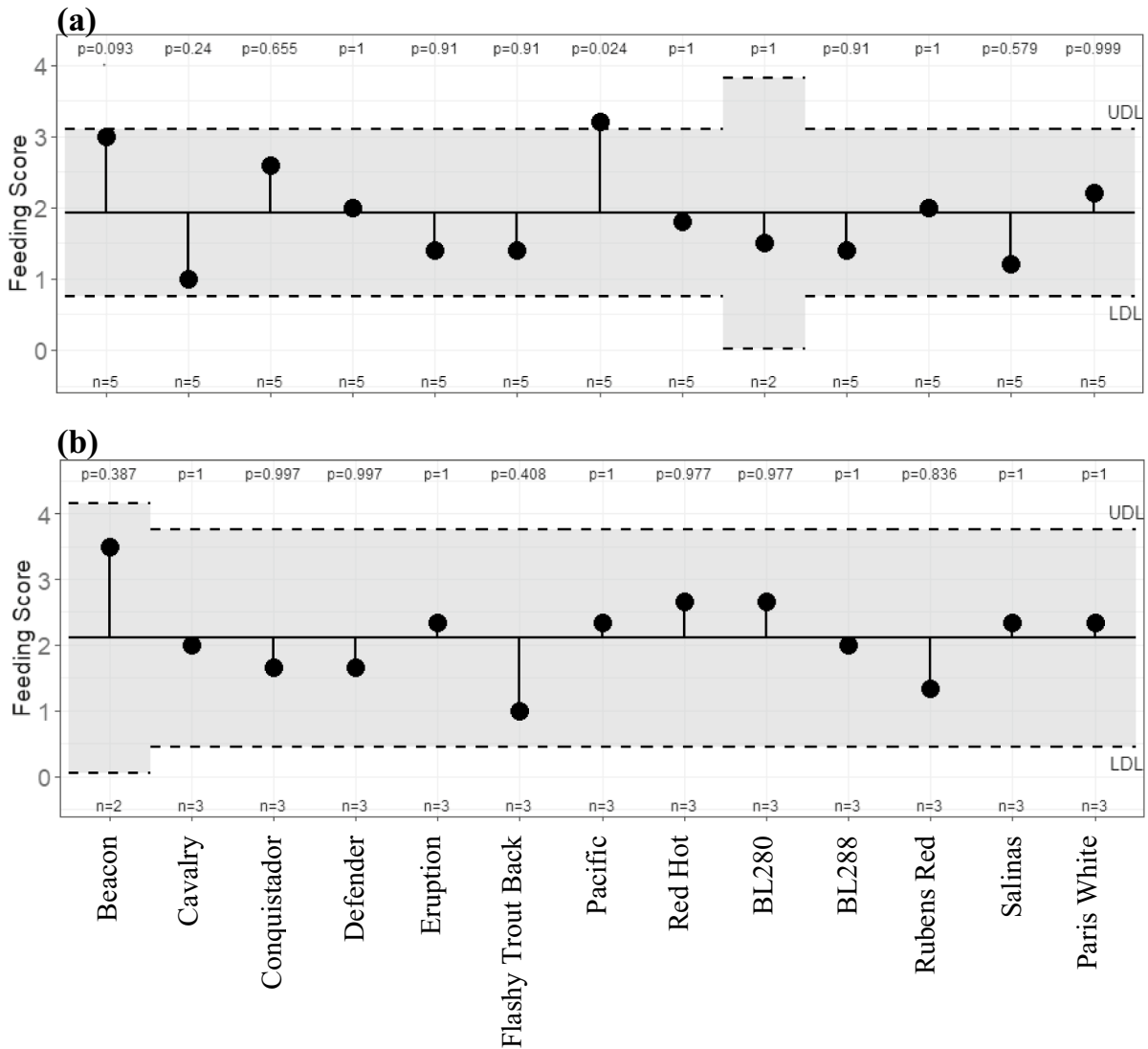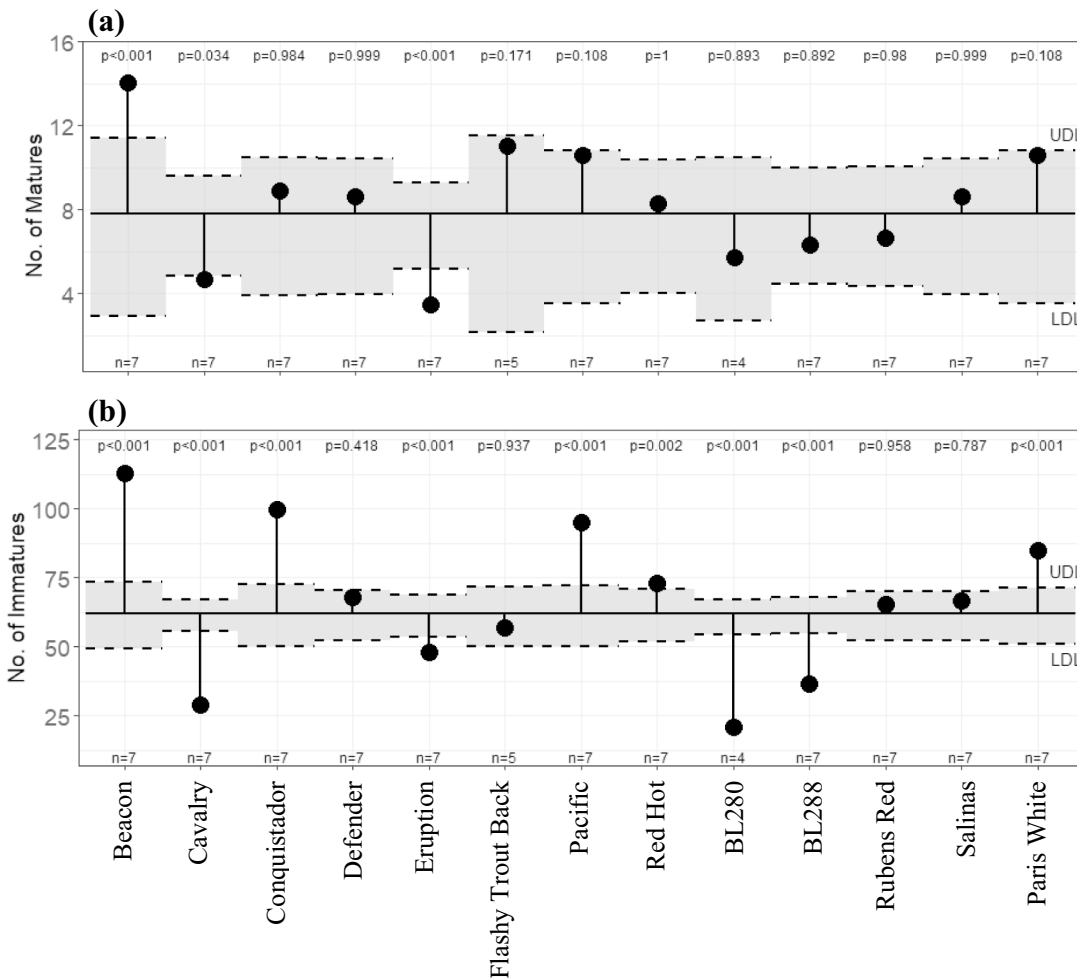
Fig. 6 Analysis of mean of thrips feeding score on different cultivars of lettuce under **a** choice test and **b** no-choice test. Horizontal black solid line indicates overall mean, gray-shaded area indicates 95% confidence interval (CI) of overall mean, UDL = upper decision limit, and LDL = lower decision limit. Cultivar mean outside the boundary of 95% CI are significantly ($p < 0.05$) different than overall mean (genotypes exceeding UDL are preferred hosts, whereas those below LDL are less preferred hosts for feeding). Number of trials (n) and p-Value for analysis of mean of each cultivar are indicated at bottom and top, respectively

other parts of the Central Coast region of California (Hasegawa and Del Pozo-Valdivia 2023). Management of INSV remains an ongoing challenge due to lack of holistic approaches that ensure high efficacy in managing the virus. No germplasm with complete immunity to INSV is known, while chemical control methods are not effective and may pose risks to human health and the environment. The broad host ranges of the virus and vector further complicate area-wide disease management strategies. Therefore, breeding for INSV resistance is imperative. Here, we conducted four field experiments over two years (2021 and 2022) to identify sources of resistance to INSV in lettuce germplasm. The average disease

Fig. 7 Analysis of mean for the number of thrips on different cultivars in lettuce choice tests, **a** mature and **b** immature. Horizontal black solid line indicates overall mean, gray-shaded area indicates 95% confidence interval (CI) of overall mean, UDL = upper decision limit, and LDL = lower decision limit. Cultivar mean outside the boundary of 95% CI are significantly ($p < 0.05$) different than overall mean (genotypes exceeding UDL are preferred hosts, whereas below LDL are less preferred hosts for reproduction). Number of trials (n) and *p*-Value for analysis of mean of each cultivar are indicated at bottom and top, respectively

incidence was substantially high in all four experiments and ranged from 41% in the June 2021 experiment to 83% in the June 2022 experiment, with several cultivars suffering 100% disease incidence by the end of the experiments, which is similar to the high disease incidence previously observed (Hasegawa and Del Pozo-Valdivia 2023; Simko et al. 2023). Such a high level of disease pressure in the field provided a unique opportunity to evaluate a large number of cultivars for INSV resistance under natural infection conditions. Research to investigate the epidemiological

factors responsible for the recent surge of INSV in the Salinas valley has been in progress (Hasegawa and Del Pozo-Valdivia 2023). Climate data from the USDA Spence Research Farm during the four field experiments show variation in average air temperature and precipitation levels throughout a growing season and among years (Online Resource 6). This could impact the thrips active season and transmission of the virus, which may explain the variation for number of thrips recorded during the field experiments (Online Resource 7). Our field experiments evaluated
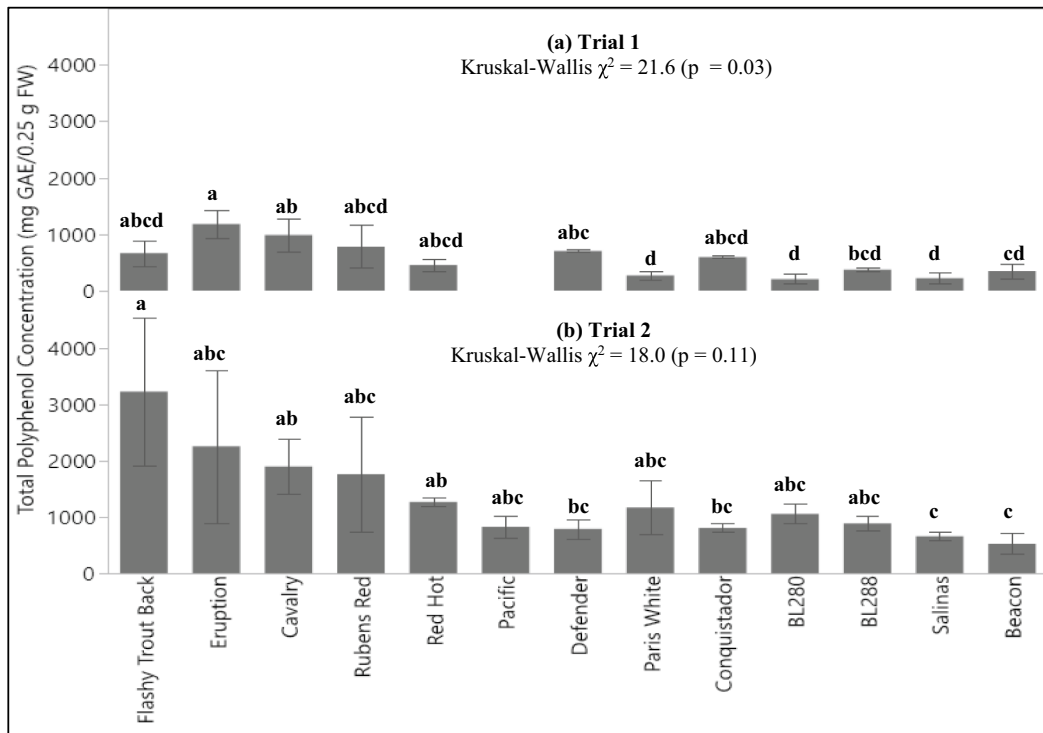
Fig. 8 Bar plot showing mean total polyphenol concentration for 13 cultivars as measured in mg of gallic acid equivalent (GAE) per 0.25 g of fresh weight of tissue collected from **a** Trial 1, and **b** Trial 2. Error bars represent the standard error of the mean. Different letter grouping of bars within a Trial indicates the significant difference at *p*-value 0.05 as detected by Dunn's test

189 lettuce cultivars and breeding lines. No single germplasm was completely immune to INSV, but we identified genotypes with a high level of partial resistance. In general, we observed a lower disease severity and incidence in the head forming lettuce cultivars, including crisphead and butterhead types and in red leaf lettuce cultivars. Effect of phytomorphological differences on INSV incidence, including negative correlation between disease incidence and anthocyanin (polyphenol compound that contributes to red color of leaves) was observed in a previous report (Simko et al. 2023). Some cultivars that showed a high level of partial resistance are 'Amazona' (leaf type with green and purple leaves), 'Anais' (head forming type with green leaves), 'Ancora' (butterhead type with green leaves), 'Cavalry' (leaf type with red leaves), 'Eruption' (leaf type with red leaves), 'Ostinata' (butterhead type with green leaves), 'Oscar' (butterhead type with green leaves), 'Parade' (butterhead type with green leaves), 'Rubens Red' (romaine type with green and red leaves), 'INSV-21-59' (leaf

type with red leaves), 'INSV-21-63' (leaf type with red leaves), 'INSV-21-72' (leaf type with red leaves), and 'INSV-21-89' (romaine type with green leaves). The green romaine cultivars such as 'Defender', 'Heart's Delight', 'King Henry', 'Paris White', and 'Triple Threat' were the most susceptible. In previous studies, 'Amazona', 'Ancora', 'Eruption', and 'Rubens Red' were also identified to be the most resistant cultivars, and 'Triple Threat' was also highly susceptible (Simko et al. 2018, 2023), indicating that genotype × environment interaction has little influence on the genetic determinants of these cultivars. Cultivars consistently showing resistant reactions can be directly utilized as genetic sources for cultivar breeding. Further, crosses between any combination of these resistant and susceptible cultivars can be used for creating mapping populations to identify significant genomic regions and underlying genes contributing to INSV resistance.

In this study, we partitioned variance components due to genotype, environment, and their interaction

contributing to INSV resistance in lettuce. To the best of our knowledge, no previous studies attempted to partition the variance components. Estimates of variance component and heritable genetic variation are helpful to breeders when designing breeding strategies. Our results showed that the genotype effect is the most powerful component that accounts for 36% of total variability which is true for both disease severity and incidence data. For disease severity, magnitude of genotypic effect is slightly higher than environment effect, with lower interaction component. These results indicate that both genetic traits and environmental conditions are contributing to variability of INSV severity, but their interaction is not as influential as each factor acting alone. Therefore, it can be inferred that the genetic and environmental factors are somewhat independent in their effects and each factor plays a substantial role in determining INSV severity, but their combined effect doesn't show a strong synergistic or antagonistic relationship. When we partitioned variance of disease incidence data, the scenario slightly changes i.e. environmental effect was reduced but residual (error) increased. The reduction in environmental effects suggests that the incidence of disease is less influenced by external factors. This might imply that certain environmental factors primarily affect the severity of disease rather than its occurrence. The residual component indicates that there are other important, measurement error or unaccounted factors influencing the trait. Based on variance components, we computed the broad sense heritability and estimates were high for both disease severity (0.88) and incidence (0.87), indicating opportunities for positive response to selection with a high precision. However, our estimates for broad sense heritability might seem somewhat discordant at first glance while relating to genotypic variance of 36%. Such discrepancy could be associated with the biological complexity associated with INSV resistance. In general, large effect of genetic factors and high heritability indicates ample opportunities to make genetic progress with a high precision for INSV resistance in lettuce.

When breeding for INSV resistance, the objective is to develop resistant cultivars with a high degree of genetic stability. However, complex dynamics between the virus, vector, and environment interaction with host plant may increase the degree of difficulty towards the selection of durable resistance sources. Therefore, it's crucial to understand underlying mechanisms of INSV resistance in lettuce by assessing responses of cultivars under different inoculation methods. In our studies, some genotypes performed better when inoculated mechanically, while some performed better when plants were naturally inoculated by thrips (with or without mechanical inoculation). For example, 'Rubens Red' had a relatively resistant response when mechanically inoculated, whereas 'Cavalry' was relatively more resistant when inoculated by thrips, and 'Eruption' showed an intermediate response under both. Variation in genetic stability of 'Cavalry', 'Eruption', and 'Rubens Red' is also noticeable in the GGE biplot. This indicates possible genotypic differences for resistance mechanism in these three genotypes. Such genotypic differences in lettuce are likely attributed to plant resistance to the virus, thrips feeding and reproductive preference, plant tolerance to thrips feeding damage, or a combination of these factors. Virus resistance may be associated with the ability to restrain systemic movement of the virus within the plant system. Tissue-specific differences in INSV titers were reported in lettuce (Simko et al. 2018). Such restriction of virus movement was also reported to confer field resistance against *Tomato spotted wilt virus* (another species of *Orthotospovirus*) in peanut (Murakami et al. 2006). It is difficult to confirm such possibility from our data, however, future studies focused on elucidating any genetic variation and potential effects on virus localization and movement would be beneficial for cultivar breeding.

In addition to developing virus resistant cultivars, there would be benefits to developing lettuce cultivars that are resistant to thrips or are less preferred hosts. Three different modes of host-plant resistance to insects have been defined by Painter (1951): (i) antixenosis (host plant ability to keep herbivore away i.e. less preferred), (ii) antibiosis (deleterious effect on herbivore biology such as reduction in reproduction), and (iii) tolerance (ability of host plant to withstands herbivore feeding). Among the three, antibiosis and antixenosis involve both plant response and herbivore response, whereas in tolerance only plant response is involved. We performed a series of choice and no-choice tests to investigate variation of the selected lettuce cultivars for host-plant resistance characteristics to thrips. We did not find any significant differences among lettuce genotypes for feeding damage,

indicating all cultivars were physiologically suitable hosts. However, under choice tests, we observed significant genotypic variation for the number of mature thrips and immature thrips (as an indicator of thrips preference egg laying). Of the genotypes we evaluated, 'Cavalry' and 'Eruption' had a significantly fewer number of both mature and immature thrips than the overall mean. USDA breeding lines 'BL280' and 'BL288' had the fewest number of immature thrips. 'Cavalry' and 'Eruption' are red leaf cultivars, whereas 'BL280' and 'BL288' are green leaf cultivars. More studies are needed to fully understand the underlying mechanisms of thrips preference to different lettuce germplasm.

Further, we measured the total polyphenol content to examine its influence on thrips preference of host plant. Polyphenols are a large group of plant secondary metabolites that are derived from the amino acid phenylalanine via the phenylpropanoid biosynthesis pathway. Lettuce plants contain many different phenolic compounds, including caffeic acid, chlorogenic acid, feruloyl quinic acid, rutin, kaempferol, anthocyanins, luteolin, and p-coumaric acids and its concentration drastically varies based on cultivar and growing environment (Bennett and Wallsgrove 1994; López et al. 2014; Shi et al. 2022). Among these compounds, chlorogenic acid and feruloyl quinic acid were reported to provide resistance against western flower thrips in chrysanthemums (Leiss et al. 2009). Chlorogenic acid and feruloyl quinic acid have unique combination of negative effect on thrips with positive effects on human health (Leiss et al. 2009). In this study we did not identify specific polyphenols, but our results showed that total polyphenol concentration was significantly high in 'Eruption' on which the number of mature and immature thrips were also low. Future studies focused on the role of different secondary metabolites linked with INSV resistance and thrips preference would be beneficial.

Overall, this study expanded the list of lettuce germplasm with a high level of partial resistance belonging to different horticultural types. The GGE biplot analysis has shown that 'Eruption' exhibits a high stability for INSV resistance, and this resistance is likely contributed by a combination of multiple genetic factors, including both virus resistance and vector resistance. 'Eruption' and other partially resistant cultivars identified in our study could be utilized as genetic sources for introgression of resistance genes in adapted cultivars. Selection for INSV resistance would be relatively simple because of minimal effect of cultivar × environment interaction effects and high heritability of this trait. Our results highlight the need for deeper exploration into the genetic and environmental factors, including virus-vector-host plant interactions and the role of secondary metabolites that may influence INSV severity and incidence.

Online Resource 1. Table of summary statistics of disease severity and incidence.

Online Resource 2. Table of Spearman rank correlation between disease severity and incidence at different week of data collection under different environment.

Online Resource 3. Table of summary statistics of INSV disease severity and incidence of different horticultural types of lettuce at 10-week post planting in different environment.

Online Resource 4. Table of summary statistics of INSV disease severity and incidence of lettuce having different leaf color at 10-week post planting in different environment.

Online Resource 5. Table of means of thrips feeding scores, and number of mature and immature thrips on different lettuce cultivars evaluated under choice and no-choice tests in greenhouse conditions.

Online Resource 6. Table of Climate data of test site during experiment period (2021 and 2022).

Online Resource 7. Figure showing the number of thrips captured on yellow sticky cards at different dates during the period of each field experiment.

Online Resource 8. Tables and Figures of Analysis of mean (ANOM) of lettuce cultivars evaluated in four different field experiments.

Data availability The data are contained within supplementary materials. Any additional information regarding data can be obtained from the corresponding author upon reasonable request. Online Resource 1. Table of summary statistics of disease severity and incidence. Online Resource 2. Table of Spearman rank correlation between disease severity and incidence at different week of data collection under different environment. Online Resource 3. Table of summary statistics of INSV disease severity and incidence of different horticultural types of lettuce at 10-week post planting in different environment.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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