



Determining relevant traits for selecting landrace accessions of *Phaseolus lunatus* L. for insect resistance

Roberto Rafael Ruiz-Santiago¹, Horacio Salómon Ballina-Gómez¹, Esau Ruiz-Sánchez¹, Jaime Martínez-Castillo², René Garruña-Hernández³ and Rubén Humberto Andueza-Noh³

¹Division de Estudios de Posgrado e Investigacion, Tecnológico Nacional de México/Campus Conkal, Conkal, Yucatan, Mexico

²Centro de Investigacion Cientifica de Yucatan, Merida, Yucatan, Mexico

³Division de Estudios de Posgrado e Investigacion, Conacyt-Tecnológico Nacional de México/Campus Conkal, Conkal, Yucatan, Mexico

ABSTRACT

Plant-insect interactions are a determining factor for sustainable crop production. Although plants can resist or tolerate herbivorous insects to varying degrees, even with the use of pesticides, insects can reduce plant net productivity by as much as 20%, so sustainable strategies for pest control with less dependence on chemicals are needed. Selecting plants with optimal resistance and photosynthetic traits can help minimize damage and maintain productivity. Here, 27 landrace accessions of lima beans, *Phaseolus lunatus* L., from the Yucatan Peninsula were evaluated in the field for morphological resistance traits, photosynthetic characteristics, insect damage and seed yield. Variation was found in physical leaf traits (number, area, and dry mass of leaves; trichome density, specific leaf thickness and hardness) and in physiological traits (photosynthetic rate, stomatal conductance, intercellular carbon, water-use efficiency, and transpiration). Five accessions (JMC1325, JMC1288, JMC1339, JMC1208 and JMC1264) had the lowest index for cumulative damage with the highest seed yield, although RDA analysis uncovered two accessions (JMC1339, JMC1288) with strong positive association of seed yield and the cumulative damage index with leaf production, specific leaf area (SLA) and total leaf area. Leaf traits, including SLA and total leaf area are important drivers for optimizing seed yield. This study identified 12 important morphological and physiological leaf traits for selecting landrace accessions of *P. lunatus* for high yields (regardless of damage level) to achieve sustainable, environmentally safe crop production.

Submitted 25 February 2021
Accepted 8 August 2021
Published 16 September 2021

Corresponding author
Horacio Salómon Ballina-Gómez,
horacio.bg@conkal.tecnm.mx

Academic editor
Hazem Kalaji

Additional Information and
Declarations can be found on
page 14

DOI 10.7717/peerj.12088

© Copyright
2021 Ruiz-Santiago et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

Subjects Agricultural Science, Ecology, Entomology, Plant Science

Keywords Lima bean, Plant defense, Leaf damage, Seed yield, Plant physiology

INTRODUCTION

In recent years, global crop productivity has been improved through the artificial selection of traits that increase yield (Lynch, 2007), but this approach has resulted in low levels of diversity, lack of expression of defense genes, and production systems that depend on high inputs of pesticides (Panda & Khush, 1995; Lynch, 2007; Chen et al., 2016). One of the

main constraints on crops is insect damage to leaves; up to 20% of the net productivity in important crops can be lost despite increased pesticide use (Oerke & Dehne, 2004; Agrawal, 2011). Stout (2013) proposed an effective sustainable alternative: selecting plants with resistance and tolerance traits that reduce the impact of insect damage. The efficacy of such resistance traits in protecting a crop against herbivorous insects depends on studies that clarify their influence, their impact on herbivorous insects and their expression under different environmental conditions (Strauss & Agrawal, 1999; Stinchcombe, 2002). Some accessions with larger leaves are likely to have greater vigor and tolerance to damage by herbivorous insects (Sekandi et al., 2016). Likewise, the thickness and hardness of leaves also have an important impact on resistance. In Fabaceae species, trichomes can also contribute to resistance against defoliating insects (Oghiakhe, Jackai & Makanjuola, 1992; Veeranna & Hussain, 1997) by hindering their movement on the plants (Tian et al., 2012; Figueiredo et al., 2013). In contrast, some plants with thinner, more fragile leaves are less preferred by insects, as in the case of *Vigna radiata* (L.) (Lakshminarayan, Singh & Mishra, 2008), *V. mungo* (Taggar & Gill, 2012), *Gossypium hirsutum* (Butter & Vir, 1989) and *Cucumis sativus* (Shibuya et al., 2009).

Legumes are the second most important group in past and current agricultural systems and for human nutrition (Blair et al., 2016). According to the FAO in 2018, beans were third in importance by planted area in Mexico, with 7.9% of the total. The Yucatan Peninsula has the greatest richness of cultivated domesticated beans in all of Mexico, and varieties have high levels of genetic diversity including those of *P. lunatus* (Martínez-Castillo et al., 2012), which are excellent germplasm sources for improving cultivated beans. We hypothesized that landrace accessions of broad beans (*Phaseolus lunatus*) have physical and physiological characteristics that confer defensive traits that may reduce the activity of herbivorous insects and optimize yields. In this context, the objective of this study was to identify accessions that were most resistant to damage caused by defoliating insects in 27 landrace accessions of lima beans (*P. lunatus*) by determining morphological, physiological and yield traits known to contribute to insect resistance such as dry mass of leaves, leaf area, specific leaf area (SLA), leaf thickness and hardness, and trichome density, improvement in photosynthetic capacity, and its impact on yield (Gong & Zhang, 2014).

MATERIALS AND METHODS

Seed sources

Seeds of 27 landraces accessions of lima beans (*P. lunatus*) were collected in the states of Quintana Roo, Campeche and Yucatán in the Yucatan peninsula from home gardens and rural markets. Information on the origin and genetic characterization of landraces is available in previous studies carried out by the Centro de Investigacion Cientifica de Yucatan (CICY), in Martínez-Castillo, Colunga-García & Zizumbo-Villarreal (2008) and Camacho-Pérez, Martínez-Castillo & Mijangos-Cortés (2008) (Table 1). Seeds were tested for germination, and only those lots with germination above 85% were sown.

Table 1 Origin of landrace accessions of lima bean (*P. lunatus*) evaluated in this study.

Accession Code	Accession	Species	Collector	State	Municipality	Coordinate	Local name
1	RRS0001	<i>Phaseolus lunatus</i>	Roberto Ruiz	Yucatán	Izamal	–	–
2	JMC1271	<i>Phaseolus lunatus</i>	Jaime Martínez	Quintana Roo	Tulum	87°46'16.55"	Putsicasutsuy
3	JMC1280	<i>Phaseolus lunatus</i>	Jaime Martínez	Quintana Roo	Felipe C. Puerto	88°02'43"	Mulición
4	RRS0002	<i>Phaseolus lunatus</i>	Roberto Ruiz	Yucatán	Izamal	–	–
5	JMC1255	<i>Phaseolus lunatus</i>	Jaime Martínez	Campeche	Calkiní	90°03'03"	Mulición
6	JMC1304	<i>Phaseolus lunatus</i>	Felix Dzúl Tejero	Campeche	Calkiní	90°03'03"	Mulición
7	JMC1240	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Peto	89°24'00"	Putsicasutsuy
8	JMC1350	<i>Phaseolus lunatus</i>	Jaime Martínez	Campeche	Hopelchén	89°44'51.98"	X-Nuk ib
9	JMC1254	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tahdziú	89°30'	Putsicasutsuy
10	JMC1327	<i>Phaseolus lunatus</i>	Jaime Martínez	Campeche	Hecelchakán	89°58'14.48"	Mulición
11	JMC1273	<i>Phaseolus lunatus</i>	Jaime Martínez	Campeche	Hopelchén	89°35.57'	Putsicasutsuy
12	JMC1357	<i>Phaseolus lunatus</i>	Jaime Martínez	Campeche	Calkiní	89°53'90"	Mejen ib
13	JMC1345	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tixmehuac	89°06'31.43"	Mulición
14	JMC1270	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tixmehuac	89°6'56.16"	Chak ib
15	JMC1337	<i>Phaseolus lunatus</i>	Jaime Martínez	Campeche	Hopelchén	89°11'30"	Mulición
16	JMC1245	<i>Phaseolus lunatus</i>	Felix Dzúl Tejero	Campeche	Calkiní	89°53'90"	Putsicasutsuy
17	JMC1208	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Chankom	88°30'48.00"	Sac ib
18	JMC1348	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Peto	89°24'00"	Sak X-nuk ib
19	JMC1339	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Yaxcabá	88°49'39.69"	Sac ib
20	JMC1288	<i>Phaseolus lunatus</i>	Felix Dzúl Tejero	Yucatán	Tekax	89°29'18"	Box ib
21	JMC1306	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tzucacab	89°57'35"	Mulición
22	JMC1264	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Yaxcabá	88°49'39.69"	Chak ib
23	JMC1325	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tekax	89°17'16"	Mejen ib
24	JMC1297	<i>Phaseolus lunatus</i>	Felix Dzúl Tejero	Yucatán	Tixmehuac	89°6'56.16"	Sac ib
25	JMC1313	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tekax	89°12'00"	Sacmejen
26	JMC1336	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tekax	89°17'16"	Sacmejen
27	JMC1364	<i>Phaseolus lunatus</i>	Jaime Martínez	Yucatán	Tekom	88°27'17"	Sac ib

Site conditions and crop establishment

The field experiment was carried out in September, October, November and December of 2019 in the horticultural production area of the National Technological Institute of Mexico, Campus Conkal when the monthly mean temperature was 26.4 °C, the maximum was 34.7 °C, and minimum was 17.3 °C, and the monthly mean precipitation was 100.66 mm. The soil is a Leptosol, with 0.93% N, and the total contents of P, K, Ca and Mg is 2.45, 3.5, 49.38 and 2.63 g kg⁻¹, respectively. Seeds were sown directly every 60 cm in a 50-m row with a distance of 120 cm between each row. Approximately 100 plants per accession were obtained. The field was irrigated each day (7:00 to 9:00) with a drip irrigation strip system, and traditional agronomic management was applied for weed control; no pesticides or chemical fertilizers were applied. Plots were established using a split-plot experimental design with a completely random arrangement of three subplots.

Data collection

At 60 days after plant emergence (DAE), morphological and physiological leaf traits of each landrace accession were evaluated. We randomly selected five plants for each of three experimental subplots for each accession for a total of 15 plants. All leaves on each plant were counted, and area of each leaf was measured with an area meter (LI-3000C portable meter, LI-COR, Lincoln NE, USA). The dry mass of leaves was obtained by placing fresh leaves in a drying oven at 60 °C until mass was constant. The SLA ($\text{cm}^2 \text{g}^{-1}$) was calculated by dividing the leaf area (cm^2) by the dry mass (g). Leaf thickness was measured with a digital micrometer (Mitutoyo model H-2780 JPN). Blade hardness (g cm^{-2}) was measured with a portable penetrometer (AMS 59032 OSHA, USA). Trichomes were counted on several parts of the adaxial surface of the fully expanded youngest leaf using a stereoscope (OPTIKA ST-30FX IT) at 40 \times to calculate density (no. cm^{-2}) (*Widstrom, McMillian & Wiseman, 1979*).

An infrared gas analyzer (LI-6400 IRGA; LI-COR, Lincoln NE, USA) was used to measure gas exchange in three fully extended young leaves for each of 15 new randomly selected plants of each accession. Each leaf was measured five times for photosynthetic assimilation rate (P_N), stomatal conductance (g_s), intercellular carbon (C_i), and transpiration (E). Water-use efficiency (WUE) was then calculated as photosynthetic assimilation rate (PN) divided by transpiration rate (E). Measurements were done between 7:00 and 10:00 when flowering had started (between 45 and 60 DAE).

Leaf damage and yield

To evaluate foliar damage by chewing insects on 15 plants randomly selected per plot at 30, 45 and 60 DAE, we used the percentage damage scale of *Dirzo & Domínguez (1995)*: (0) leaves without herbivory, (1) 1 to 5% damage, (2) 6 to 12% damage, (3) 13 to 25% damage; (4) 26 to 50% damage and (5) above 50% damage). At each sampling date, we also calculated a cumulative damage index for each accession by dividing the lowest damage value by the highest value for the accession (*Sohrabi, Nooryazdan & Gharati, 2017*).

For yield determinations, 15 plants were selected per accession, pods were harvested and beans removed and oven-dried at 60 °C until mass was constant. Beans were then weighted to obtain seed yield per plant (g plant^{-1}) for each accession.

Statistical analyses

We ran a one-way analysis of variance (ANOVA) to compare morphological and physiological traits, damage and seed yield among the accessions; thus, we used accession as the only factor (independent variable). When the data did not meet normality assumptions, data were transformed as follows: continuous data with the natural logarithm, discrete numbers with square root, and proportions with the arcsine of the square root. Hierarchical grouping of means tests of the Scott-Knott statistic were then applied.

These analyses were performed with the InfoStat software (*Di Rienzo et al., 2016*). The most important resistance and physiological tolerance traits for the accessions were identified using a principal component analysis (PCA) and the arithmetic means for each accession for a variable. The PCA was performed using correlation matrices and

normalization of the model by the varimax rotation method (Dien, Beal & Berg, 2005) using SPSS version 25 (IBM, Armonk, NY, USA).

To determine possible associations between variables, we subjected all data for physical and physiological traits, seed yield and cumulative damage index for each of the 27 accessions using a redundancy analysis (RDA) (Legendre & Legendre, 1998). The RDA was chosen over a canonical correspondence analysis due to the length of the gradient for the variables (Ter Braak & Smilauer, 2002). Gradient length was calculated using a detrended correspondence analysis (DCA) (Hill, 1979). The significance of the damage and yield index on the ordering of morphological and physiological variables, was analyzed using a Monte Carlo random permutation test (499 permutations, $p < 0.05$) using Canoco 4.5 (Ter Braak & Smilauer, 2002).

RESULTS

Physical traits of resistance

Significant differences in the physical traits were found among the accessions (Scott-Knott $p < 0.005$). The accessions with the most leaves were JMC1339, JMC1306, JMC1364, JMC1208, JMC1264, JMC1313, JMC1336, JMC1288 and JMC1348 had the fewest (Fig. 1A). JMC1339 had the largest leaf area, JMC1255 and JMC1348 the smallest (Fig. 1B). JMC1255, RRS0002, JMC1280 JMC1348 and JMC1304 had the highest leaf dry mass; JMC1339 had the lowest (Fig. 1C). JMC1255 had the thickest leaves, and JMC1325 accession had the thinnest (Fig. 1D). JMC1339 had the highest SLA, and JMC1255, JMC1348, RRS002, JMC1280, JMC1304 and JMC1357 had the lowest (Fig. 1E). The highest trichome densities were found on JMC1306 and JMC1280, the lowest on JMC1208 (Fig. 1F). JMC1264, JMC1325 and JMC1313 had the hardest leaves, and the rest had softer leaves (Fig. 1G).

Physiological traits

The physiological variables also differed significantly among the accessions (Scott-Knott $p < 0.005$). Accessions JMC1273, JMC1325, JMC1264, RRS0002, JMC1245, JMC1288, JMC1313, JMC1304, JMC1280, JMC1357, JMC1270 and JMC1350 had the highest photosynthetic rate (P_N), JMC1336 and JMC1339 the lowest (Fig. 2A). WUE was highest in JMC1337, JMC1245, and JMC1270, and lowest in JMC1336 (Fig. 2B). JMC1364 had the highest g_s , for overall (Fig. 2C). Transpiration was highest in JMC1273 and JMC1364 (Fig. 2D). JMC1336 and JMC1364 had the highest C_i for overall (Fig. 2E).

Leaf damage and yield

The percentage of leaf damage differed significantly among all accessions (Scott-Knott $p < 0.005$) and at DAE (Scott-Knott $p < 0.005$). The highest percentages of damage were found at 45 DAE, followed by 60 and 30 DAE. At 30 DAE, accessions JMC1271, JMC1255 and RRS0001 had the highest percentages of damage, and JMC1325, JMC1336, JMC1297, JMC1313, JMC1288, JMC1270, JMC1364, JMC1348, JMC1337, JMC1245, JMC1339, JMC1306 and JMC1264 had the lowest (Fig. 3A). For the damage index, RRS0001, JMC1271 and JMC1255 had the highest values; JMC1297, JMC1325, JMC1306, JMC1273 and JMC1264 had the lowest (Table 2). At 45 DAE, JMC1273 had the highest percentage

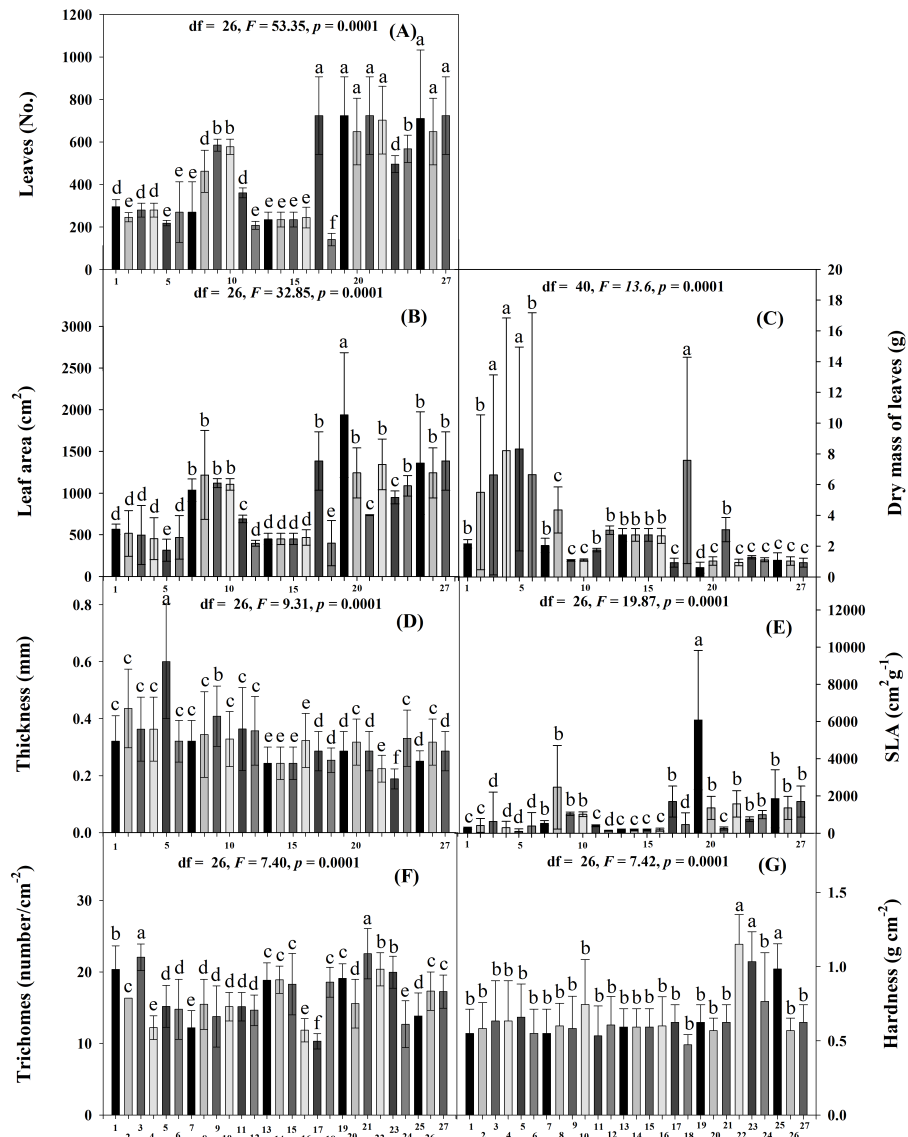


Figure 1 Means (\pm SD) at 60 days after emergence for leaf resistance traits of 27 *Phaseolus lunatus* landrace accessions from southeastern Mexico. Different letters above histograms denote a significant difference among accessions (Scott-Knott, $p < 0.05$). (A) Number of leaves, (B) leaf area, (C) dry mass of leaves, (D) thickness, (E) specific leaf area, (F) number of trichomes, (G) hardness. Accession codes on x-axis: 1 = RRS0001, 2 = JMC1271, 3 = JMC1280, 4 = RRS0002, 5 = JMC1255, 6 = JMC1304, 7 = JMC1240, 8 = JMC1350, 9 = JMC1254, 10 = JMC1327, 11 = JMC1273, 12 = JMC1357, 13 = JMC1345, 14 = JMC1270, 15 = JMC1337, 16 = JMC1245, 17 = JMC1208, 18 = JMC1348, 19 = JMC1339, 20 = JMC1288, 21 = JMC1306, 22 = JMC1264, 23 = JMC1325, 24 = JMC1297, 25 = JMC1313, 26 = JMC1336, 27 = JMC1364. Days after emergence (DAE), specific leaf area (SLA).

Full-size [DOI: 10.7717/peerj.12088/fig-1](https://doi.org/10.7717/peerj.12088/fig-1)

of foliar damage; JMC1306, JMC1264 and JMC1357 had the lowest (Fig. 3A). The damage index at 45 DAE showed that JMC1255, JMC1273, JMC1280 and JMC1339 had the greatest damage; JMC1306, JMC1264, JMC1325 and RRS0002 had the lowest (Table 2). At day 60 DAE, the highest percentages of leaf damage were on JMC1348, JMC1336, JMC1339,

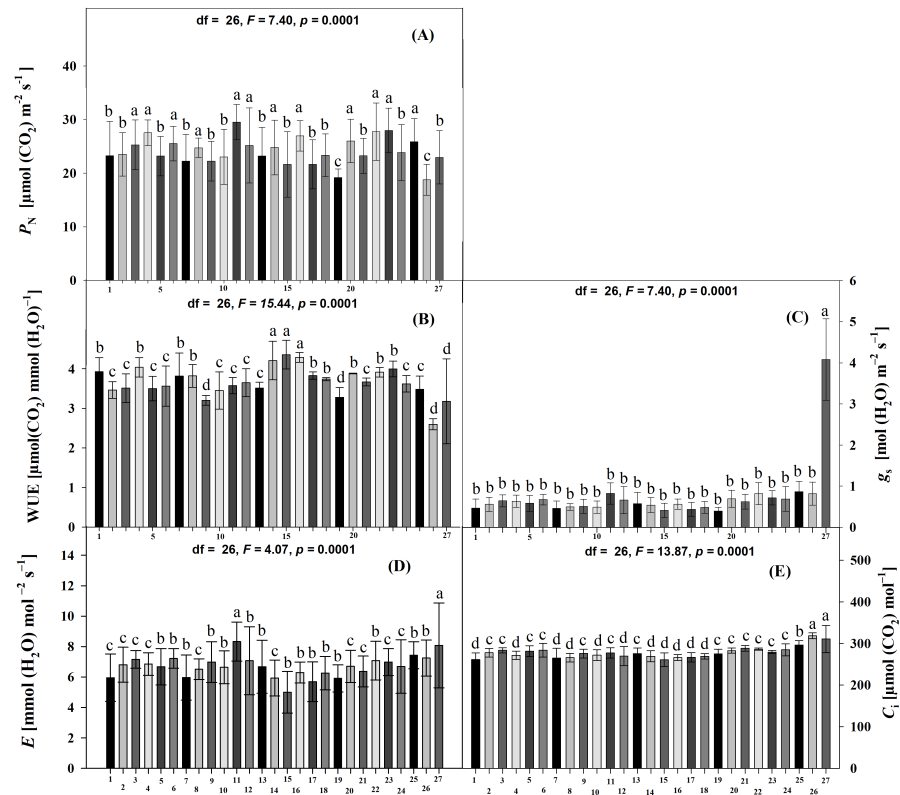


Figure 2 Means (\pm SD) at 60 days after emergence for physiological tolerance traits of 27 *Phaseolus lunatus* landraces accessions from southeastern Mexico. Different letters above histograms denote a significant difference among accessions at 60 DAE (Scott-Knott, $p < 0.05$). (A) Photosynthesis assimilation rate (P_N), (B) water-use efficiency (WUE), (C) stomatal conductance (g_s), (D) transpiration (E), (E) intercellular carbon (C_i). Accession codes on x-axis: 1 = RRS0001, 2 = JMC1271, 3 = JMC1280, 4 = RRS0002, 5 = JMC1255, 6 = JMC1304, 7 = JMC1240, 8 = JMC1350, 9 = JMC1254, 10 = JMC1327, 11 = JMC1273, 12 = JMC1357, 13 = JMC1345, 14 = JMC1270, 15 = JMC1337, 16 = JMC1245, 17 = JMC1208, 18 = JMC1348, 19 = JMC1339, 20 = JMC1288, 21 = JMC1306, 22 = JMC1264, 23 = JMC1325, 24 = JMC1297, 25 = JMC1313, 26 = JMC1336, 27 = JMC1364.

Full-size [DOI: 10.7717/peerj.12088/fig-2](https://doi.org/10.7717/peerj.12088/fig-2)

JMC1288, JMC1327, the lowest were on JMC1297, JMC1357, JMC1306, JMC1304, JMC1255 and JMC1325 (Fig. 3C). In addition, the damage index was notably higher for JMC1336 than for JMC1348, JMC1339, JMC1288, JMC1306, JMC1264, JMC1325, JMC1297 and JMC1313, which had the lowest values (Table 2). Seed yields also differed significantly among accessions (Scott-Knott $p < 0.005$), the accessions JMC1325 and JMC1348 had the highest yield (Table 2).

Variation in resistance and physiological traits

In the PCA for the 12 resistance and gas-exchange traits evaluated, five main components were significant with values >1 . These components together explained 86.72% of the variation. PC1 explained 32.45% of the total variation in the original data, PC2 18.32%, PC3 15.20%, PC4 11.02% and PC5 explained 9.72% (Fig. 4, Table 3). PC1 consisted of leaf

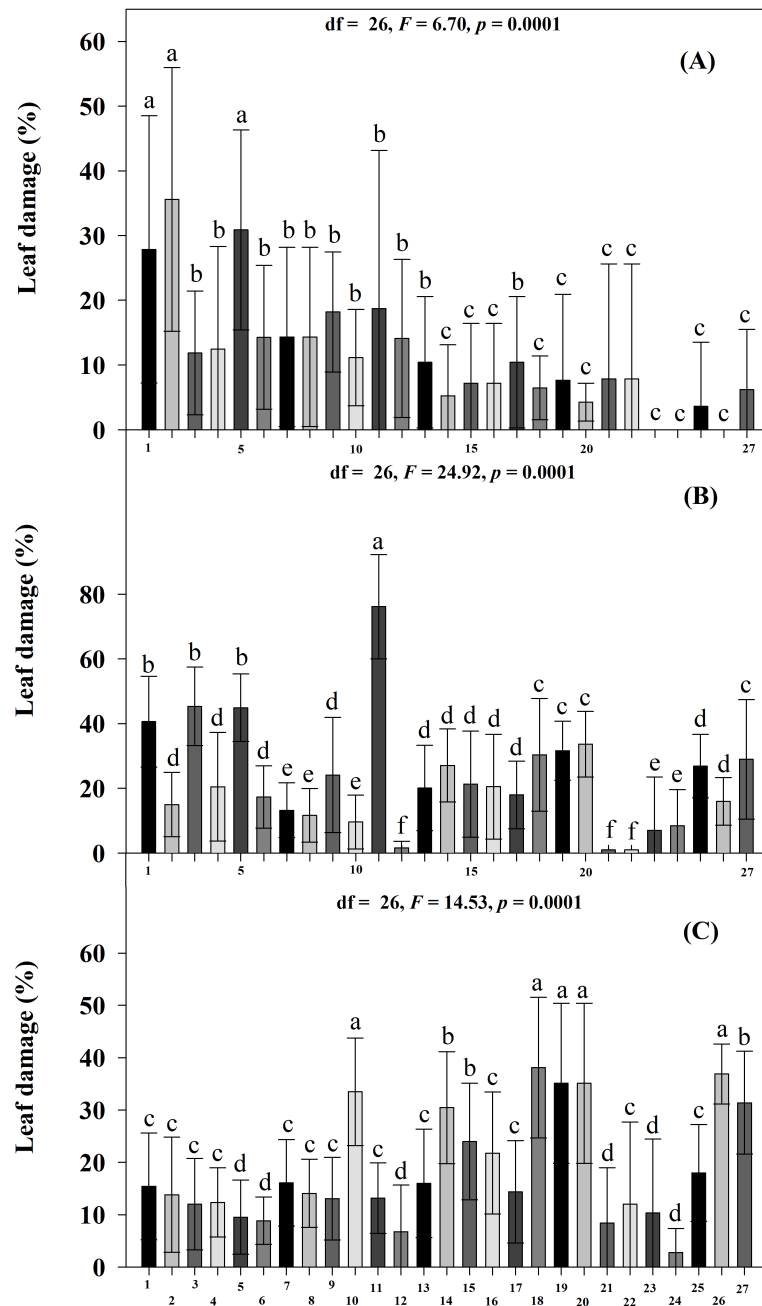


Figure 3 Mean (\pm SD) leaf damage caused by herbivorous insects at three ages of 27 *Phaseolus lunatus* landrace accessions from southeastern Mexico. Different letters above histograms denote a significant difference among accessions at (A) 30, (B) 45 and (C) 60 days after emergence (DAE) (Scott-Knott, $p < 0.05$).

Full-size DOI: 10.7717/peerj.12088/fig-3

Table 2 Estimates of cumulative damage index at three growth ages (30, 45 and 60 days after emergence; DAE) and of seed yield at 60 DAE for lima bean (*P. lunatus*). The index was calculated by dividing the lowest recorded number by the highest recorded number for each accession, at 30, 45 y 60 DAE (days after emergence). For seed yield, different letters within a column denote a significant difference among accessions within the each DAE (Scott-Knott, $p < 0.05$). Means (\pm SD).

Accession code	Accessions	Damage index 30 DAE	Damage index 45 DAE	Damage index 60 DAE	Seed yield (g plant ⁻¹)	
1	RRS0001	0.14	0.24	0.14	53.6 \pm 0.62	c
2	JMC1271	0.11	0.03	0.11	49.7 \pm 0.93	c
3	JMC1280	0.03	0.38	0.09	53.6 \pm 0.62	c
4	RRS0002	0.02	0.02	0.21	49.4 \pm 0.74	d
5	JMC1255	0.14	0.42	0.04	38.5 \pm 0.61	d
6	JMC1304	0.07	0.07	0.18	33.0 \pm 0.75	e
7	JMC1240	0.06	0.11	0.23	28.1 \pm 0.92	e
8	JMC1350	0.06	0.04	0.24	34.5 \pm 0.77	e
9	JMC1254	0.03	0.09	0.11	31.3 \pm 0.92	e
10	JMC1327	0.04	0.03	0.21	34.9 \pm 0.74	e
11	JMC1273	0.01	0.42	0.21	58.8 \pm 1.69	b
12	JMC1357	0.02	0.11	0.03	35.0 \pm 0.74	e
13	JMC1345	0.03	0.14	0.03	32.8 \pm 0.61	e
14	JMC1270	0.03	0.22	0.23	29.0 \pm 0.92	e
15	JMC1337	0.03	0.08	0.25	38.4 \pm 0.92	d
16	JMC1245	0.03	0.08	0.07	30.8 \pm 0.84	e
17	JMC1208	0.03	0.16	0.03	52.4 \pm 0.01	b
18	JMC1348	0.05	0.05	0.18	66.6 \pm 0.92	a
19	JMC1339	0.02	0.33	0.02	54.9 \pm 0.92	c
20	JMC1288	0.09	0.34	0.02	55.9 \pm 2.24	c
21	JMC1306	0.01	0.00	0.03	42.4 \pm 0.43	d
22	JMC1264	0.01	0.00	0.02	51.9 \pm 0.92	c
23	JMC1325	0.00	0.02	0.02	67.0 \pm 0.92	a
24	JMC1297	0.00	0.02	0.06	30.8 \pm 0.92	e
25	JMC1313	0.02	0.20	0.03	39.5 \pm 0.92	d
26	JMC1336	0.00	0.04	0.59	42.1 \pm 0.74	d
27	JMC1364	0.03	0.10	0.27	42.9 \pm 0.61	d

area, SLA, number of leaves and dry mass; PC2 consisted only of P_N , PC3 of WUE, PC4 of g_s and C_i , and PC5 was formed solely by trichome density (Fig. 4, Table 3).

Association of resistance traits and physiology to performance

The RDA showed a reduced separation of the morphological and physiological variables (eigenvalues axis 1 < 0.1; cumulative variance 99.1%), although the axes were marginally significant (axis 1: $F = 2.1$, $p = 0.054$; all axes: $F = 1.06$, $p = 0.056$) (Fig. 5). In addition, the damage index was significantly higher (Monte Carlo test, $F = 2.04$, $p = 0.02$) in accessions JMC1339 and JMC1288, which had the most leaves and greatest SLA and foliar area. Although the difference in seed yield was not significant, it did tend to be higher in these accessions (Fig. 5). In contrast, JMC1270, JMC1245 and JMC1254 had the least damage,

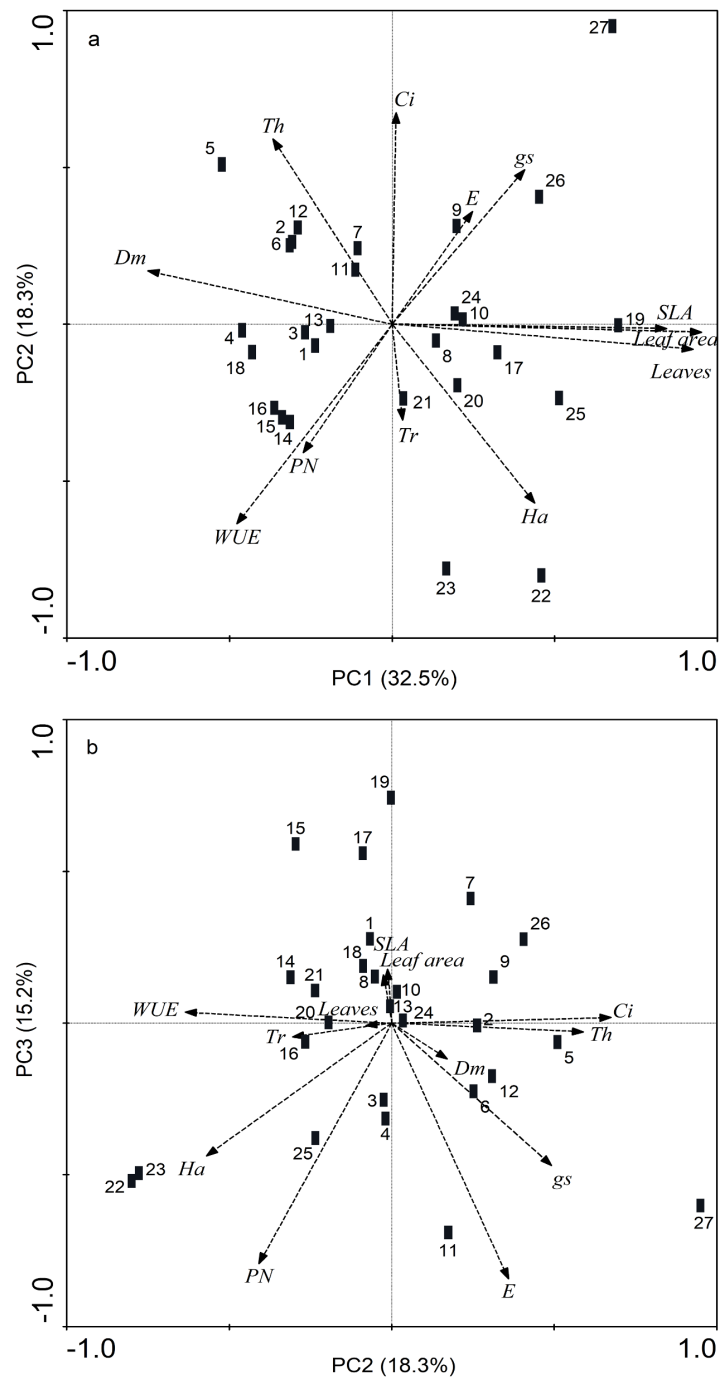


Figure 4 Biplot of principal component analysis of 27 *Phaseolus lunatus* landrace accessions from southeastern Mexico based on 12 morphological and physiological leaf traits. (A) PC1 and PC2, (B) PC2 and PC3. Morphological traits: trichomes (*Tr*), thickness (*Th*), hardness (*Ha*), dry mass of leaves (*Dm*). Physiological traits: photosynthesis assimilation rate (P_N), water-use efficiency (*WUE*), stomatal conductance (g_s), transpiration (*E*), intercellular carbon (C_i).

Full-size DOI: [10.7717/peerj.12088/fig-4](https://doi.org/10.7717/peerj.12088/fig-4)

Table 3 Variance explained in PCA by five principal components derived from 12 leaf traits of lima bean (*P. lunatus*) and contributions of the original variables to each component. Specific leaf area (SLA), photosynthesis assimilation rate (P_N), stomatal conductance (g_s), intercellular carbon (C_i), water-use efficiency (WUE), transpiration (E), days after emergence (DAE).

Axes	Principal component axes				
	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.895	2.199	1.825	1.323	1.167
Explained proportion of variation (%)	32.456	18.322	15.206	11.026	9.722
Cumulative proportion of variation (%)	32.456	50.778	65.984	77.010	86.732
Trait	Correlation matrix				
Leaf area (cm ²)	0.974	-0.053	0.093	0.033	-0.056
Dry mass (g)	-0.776	-0.011	0.267	-0.221	-0.042
SLA (cm ² g ⁻¹)	0.867	-0.085	0.161	-0.06	-0.036
Trichomes (cm ²)	-0.072	-0.023	0.023	-0.078	0.961
Hardness (g cm ⁻²)	0.454	0.656	-0.129	-0.18	0.182
Thickness (mm)	-0.389	-0.162	0.62	-0.123	-0.486
Number of leaves	0.914	0.092	0.162	0.025	0.086
P_N [$\mu\text{mol (CO}_2\text{) m}^{-2}\text{ s}^{-1}$]	-0.283	0.911	-0.14	-0.042	-0.097
g_s [$\text{mol (H}_2\text{O) m}^{-2}\text{ s}^{-1}$]	0.207	0.184	0.248	0.846	0.146
C_i [$\mu\text{mol (CO}_2\text{) mol}^{-1}$]	-0.091	-0.291	-0.044	0.87	-0.24
WUE [$\mu\text{mol (CO}_2\text{) mmol (H}_2\text{O)}^{-1}$]	-0.333	0.248	-0.838	-0.157	-0.107
E [$\text{mmol (CO}_2\text{) mol (H}_2\text{O)}^{-1}$]	0.058	0.612	0.667	0.349	0.017

but the lowest seed yield. In addition, they had the highest values of foliar biomass, for gas-exchange variables (P_N , E , g_s , WUE and C_i) and defense traits (hardness, thickness, and trichome density).

DISCUSSION

The data showed a wide variation in the evaluated foliar characteristics, in line with the high diversity among the evaluated landrace accessions found by *Ballesteros (1999)* and *Martínez-Castillo et al. (2004)* for *P. lunatus* in the Yucatan Peninsula. In the search for pest resistance among select landrace accessions, genetic variation is a key element because the wider genetic pool increases the likelihood of finding highly resistant populations as found for *Vigna umbellata* resistant to *Callosobruchus chinensis* (L.) (*Somta et al., 2008*), *P. vulgaris* resistant to *Callosobruchus chinensis* (*Ku-Hwan et al., 2002*), and *Pisum fulvum* resistant to *Bruchus pisorum* (*Clement, Hardie & Elbersson, 2002*). The effectiveness of species belonging to Fabaceae in resisting damage caused by pest insects is likely a function of multiple defense mechanisms ranging from morphological characteristics to physiological adaptations (*Bonte et al., 2010*).

When evaluating the resistance traits independently, we found distinct differences among the accessions as reported for other traits of native and cultured materials, thus allowing selection of populations with desirable defense characteristics (*Maag et al., 2015*; *Moya-Raygoza, 2016*; *Dos Santos et al., 2020*). Although many studies have evaluated physical resistance traits, chemical defenses, and biological interaction networks in agricultural production systems, these factors can also contribute to germplasm selection and crop

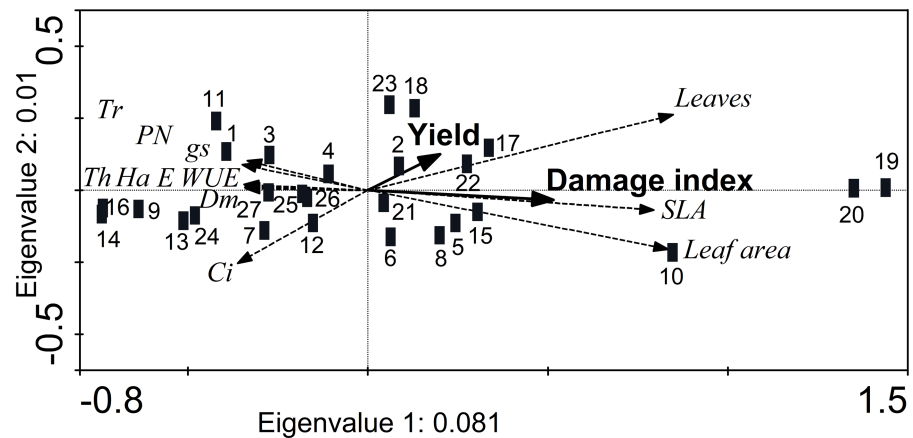


Figure 5 Redundancy analysis showing the ordination of morphological and physiological leaf traits associated with the cumulative damage index and seed yield of 27 landraces accessions of *Phaseolus lunatus* from southeastern Mexico. Accessions: 1 = RRS0001, 2 = JMC1271, 3 = JMC1280, 4 = RRS0002, 5 = JMC1255, 6 = JMC1304, 7 = JMC1240, 8 = JMC1350, 9 = JMC1254, 10 = JMC1327, 11 = JMC1273, 12 = JMC1357, 13 = JMC1345, 14 = JMC1270, 15 = JMC1337, 16 = JMC1245, 17 = JMC1208, 18 = JMC1348, 19 = JMC1339, 20 = JMC1288, 21 = JMC1306, 22 = JMC1264, 23 = JMC1325, 24 = JMC1297, 25 = JMC1313, 26 = JMC1336, 27 = JMC1364. Morphological traits: trichomes (Tr), thickness (Th), hardness (Ha), dry mass of leaves (Dm). Physiological traits: photosynthesis assimilation rate (P_N), water-use efficiency (WUE), stomatal conductance (g_s), transpiration (E), intercellular carbon (C_i).

Full-size DOI: 10.7717/peerj.12088/fig-5

improvement (Chen et al., 2008; Mitchell et al., 2016). Some accessions with larger leaves are likely to have greater vigor and tolerance to damage by herbivorous insects (Ssekandi et al., 2016). Likewise, the thickness and hardness of leaves also have an important impact on resistance; as leaf thickness increases, some sucking insect larvae spend less time on the leaf blade (Rao, 2002; Saheb et al., 2018). To produce large, thick leaves, the plant must also have a greater photosynthetic capacity to generate the necessary photoassimilates. Furthermore, stomatal conductance and transpiration rates play a fundamental role in the WUE of plants with large leaves such as those of *P. lunatus*. Here, the accessions with outstanding physiological traits such photosynthetic assimilation also had outstanding morphological characteristics for resisting insect damage. Plants with harder leaves require greater effort by leaf-eating insects (Schofeld et al., 2011). Accession JMC1325 in our study conditions produced the hardest and thinnest leaves, so it is a good candidate for further studies on resistance to foliar damage since some plants with thinner leaves are less preferred by insects, as in the case of *Vigna radiata* (L.) (Lakshminarayan, Singh & Mishra, 2008), *V. mungo* (Taggar & Gill, 2012), *Gossypium hirsutum* (Butter & Vir, 1989) and *Cucumis sativus* (Shibuya et al., 2009). Leaf hardness has been positively associated with the nutritional quality of the leaf; thus, the insects may be able to evaluate and select their food (Larcher, 2006). Therefore, the harder leaves of some accessions might not always be defensive traits, but rather provide a better food source for certain insects, making the plant more susceptible to defoliation (Schädler et al., 2007; Caldwell, Read & Sanson, 2016). In addition, we found that for another widely studied morphological trait,

trichome density, accession JMC1306 has a high density. This trait and its contribution to plant defense is difficult to generalize among plant species (Dos Santos et al., 2020). For example, in Fabaceae species, trichomes can contribute to resistance against defoliating insects (Oghiakhe, Jackai & Makanjuola, 1992; Veeranna & Hussain, 1997) by hindering pest movement on the plants (Tian et al., 2012; Figueiredo et al., 2013).

Physiological traits also varied among accessions. Groups of accessions with high values for a particular trait were found, for example, for P_N with 13 accessions; for E , C_i and WUE with two; and for g_s with only one variable. For P_N and E , the evaluated accessions had considerably high levels ($P_N = 24 P_N [\mu\text{mol} (\text{CO}_2) \text{m}^{-2} \text{s}^{-1}]$ and $E = 11 [\text{mmol} (\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}]$) (Ribeiro et al. (2004), since values ranged from 25 to 29 $[\mu\text{mol} (\text{CO}_2) \text{m}^{-2} \text{s}^{-1}]$ for P_N and 8 to 9 $[\text{mmol} (\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}]$ for E . Thus, accessions with higher P_N seem to have a greater carboxylation capacity in the environmental conditions of the region, and in the case of accessions with high E values, a greater release of water molecules as result of large stomatal openings (Meneses-Lazo et al., 2018). In addition, in the case of g_s , most accessions have low values, which could be interpreted as an indicator of drought tolerance (Khazaei et al., 2019), although sometimes the ability to regulate g_s can be a better strategy than having low values, as found for *Phaseolus vulgaris* L. (Rosales et al., 2012). For C_i , we only found two accessions at the highest levels, which could be the result of a differential photosynthetic adjustment between accessions, and in the ability to regulate mitochondrial respiration such as its photorespiration, impacting the release of CO_2 (Lawlor & Cornic, 2002). For WUE, accessions JMC1337, JMC1245, and JMC1270 had higher values than the rest and large leaves, a desirable combination of traits for insect resistance.

For all traits described, both resistance and physiological, we highlighted those with the most appropriate values for optimal performance, depending on the type of trait, but an accession should not be selected on the basis of one variable (Capblancq et al., 2018; Vangestel et al., 2018). In this regard, seven accessions—JMC1339, JMC1288, JMC1264, JMC1325, JMC1208 and JMC1313—had the lowest damage indices at 60 DAE. On the basis of leaf damage, they could have a better resistance throughout their ontogeny (Nzungize et al., 2012), but when we also consider higher seed yield, we found that five accessions—JMC1325, JMC1288, JMC1339, JMC1208 and JMC1264—were the best performers. These accessions had the highest yields (67.0 ± 0.92 to $51.9 \pm 0.92 \text{ g plant}^{-1}$) and lowest damage indices (0.02 to 0.03). However, when we analyzed with the RDA all accessions simultaneously with the morphological and physiological traits and cumulative damage index during the experiment, we found that only JMC1339 and JMC1288 maintained an optimum seed yield (54.9 ± 0.92 to $55.9 \pm 2.24 \text{ g plant}^{-1}$), despite having a high cumulative damage index. Interestingly, we found a strong positive association of leaf production, SLA and leaf area with the cumulative damage index. Overall, this finding may not appear to be very surprising since some plants can maintain high yields while being more susceptible to damage by pests (Lale & Kolo, 1998; Kimiti, Odee & Vanlauwe, 2009; Keneni, Bekele & Imtiaz, 2011; Kiptoo et al., 2016), perhaps because plants that allocate more resources to defense will have less to allocate toward growth or reproduction (Gong & Zhang, 2014). However, we emphasize the association of a higher cumulative damage index with greater leaf production, SLA and leaf area because SLA has been suggested as critical driver of

variation in resource availability above ground (Poorter *et al.*, 2012), which might help lima bean compensate for the resource limitation caused by the lost leaf area or even maximize light capture area (high SLA) (Evans & Poorter, 2001; Freschet, Swart & Cornelissen, 2015) through unfolding its leaves in such a way as to avoid leaf overlap (Santiago & Wright, 2007).

Our main results revealed positive correlations between foliar resistance traits and herbivorous insect damage levels but a negative correlation between these traits and yield. Nevertheless, we measured only 45 individuals for each of 27 accessions in only one environment, and the genetic diversity among the accessions has not been assessed. Although it is difficult to generalize the strength of the resistance traits and their possible correlations with insect damage and bean yield, our study highlights the importance of morphological traits such as greater leaf production, leaf area and SLA in relation to increased plant productivity (McNickle & Evans, 2018) through the capture of more light energy and efficient use of available resources in plants (Maschinski & Whitham, 1989).

CONCLUSIONS

Our measurements and comparisons of resistance, physiological and yield traits in landrace accessions of *P. lunatus* in the Yucatan Peninsula highlights the great diversity in germplasm resources. Five accessions, JMC1325, JMC1288, JMC1339, JMC1208 and JMC1264, performed the best in the field in terms of seed yield and lowest cumulative damage index, even though two, JMC1339 and JMC1288, had the greatest damage. Our results found a positive correlation between high values for “resistance traits” and actual resistance to herbivorous insect damages, but a negative correlation between these traits and yield. Our study identifies important morphological (number of leaves, leaf area, and dry mass of leaves; trichome density, specific leaf thickness and hardness) and physiological traits (photosynthetic rate, stomatal conductance, intercellular carbon, water-use efficiency and transpiration) for selecting lima bean accessions belonging to landrace accessions with high yields (regardless of the damage they may suffer) when no agrochemicals are used, despite the limitations of our study. This is the first step toward identifying resistant lines of lima beans for sustainable, safe production in the Yucatan Peninsula.

ACKNOWLEDGEMENTS

The authors thank Ángel Manuel Herrera Gorocica, Leticia Osalde Navarrete and Ramon Vela Solís for their support in fieldwork.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by CONACYT with the doctoral scholarship to Roberto Rafael Ruiz-Santiago (No. 845968), CONACYT project (no. YUC-2018-03-01-119959) and Tecnológico Nacional de México (TECNM) project (no. 8983.20-P): “Selección de variedades locales con características morfológicas y de defensa en contra de insectos

herbívoros en el cultivo de *Phaseolus lunatus* L.”. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

CONACYT: 845968, YUC-2018-03-01-119959.

Tecnológico Nacional de México (TECNM) project: 8983.20-P.

Selección de variedades locales con características morfológicas y de defensa en contra de insectos herbívoros en el cultivo de *Phaseolus lunatus* L.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Roberto Rafael Ruiz-Santiago conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Horacio Salomón Ballina-Gómez conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Esau Ruiz-Sánchez conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Jaime Martínez-Castillo, René Garraña-Hernández and Rubén Humberto Andueza-Noh performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the [Supplementary File](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.12088#supplemental-information>.

REFERENCES

- Agrawal AA. 2011.** Current trends in the evolutionary ecology of plant defence. *Functional Ecology* 25:420–432 DOI 10.1111/j.1365-2435.2010.01796.x.
- Ballesteros GA. 1999.** Ballesteros GA 1999. Contribuciones al conocimiento del frijol Lima (*Phaseolus lunatus* L.) en América Tropical. In: *Colegio De Posgraduados*. Montecillos: Estado De México.
- Blair MW, Wu X, Bhandari D, Zhang X, Hao J. 2016.** Role of legumes for and as horticultural crops in sustainable agriculture. In: *Nandwani D. Organic Farming for Sustainable Agriculture. Sustainable Development and Biodiversity*. DOI 10.1007/978-3-319-26803-3_9.

- Bonte D, De Roissart A, Vandegehuchte ML, Ballhorn DJ, Van Leeuwen T, De la Peña E. 2010.** Local adaptation of aboveground herbivores towards plant phenotypes induced by soil biota. *PLOS ONE* 5:11174 DOI [10.1371/journal.pone.0011174](https://doi.org/10.1371/journal.pone.0011174).
- Butter NS, Vir BK. 1989.** Morphological basis of resistance in cotton to the whitefly *Bemisia tabaci*. *Phytoparasitica* 17:251–561 DOI [10.1007/BF02980754](https://doi.org/10.1007/BF02980754).
- Caldwell E, Read J, Sanson GD. 2016.** Which leaf mechanical traits correlate with insect herbivory among feeding guilds? *Annals of Botany* 117:349–361 DOI [10.1093/aob/mcv178](https://doi.org/10.1093/aob/mcv178).
- Camacho-Pérez L, Martínez-Castillo J, Mijangos-Cortés JO. 2008.** Genetic structure of Lima bean (*Phaseolus lunatus* L.) landraces grown in the Mayan area. *Genetic Resources and Crop Evolution* 65:229–241 DOI [10.1007/s10722-017-0525-1](https://doi.org/10.1007/s10722-017-0525-1).
- Capblancq T, Luu K, Blum MGB, Bazin E. 2018.** Evaluation of redundancy analysis to identify signatures of local adaptation. *Molecular Ecology Resources* 18:1223–1233 DOI [10.1101/258988](https://doi.org/10.1101/258988).
- Chen M, Huang Y, Liu G, Qin F, Yang S, Xu X. 2016.** Effects of enhanced UV-B radiation on morphology, physiology, biomass, leaf anatomy and ultrastructure in male and female mulberry (*Morus alba*) saplings. *Environmental and Experimental Botany* 129:85–93 DOI [10.1016/j.envexpbot.2016.03.006](https://doi.org/10.1016/j.envexpbot.2016.03.006).
- Chen Y, Schmelz EA, Wäckers F, Ruberson JR. 2008.** Cotton plant, *Gossypium hirsutum* L. defense in response to nitrogen fertilization. *Journal of Chemical Ecology* 34:1553–1564 DOI [10.1007/s10886-008-9560-x](https://doi.org/10.1007/s10886-008-9560-x).
- Clement SL, Hardie DC, Elberson LR. 2002.** Variation among accessions of *Pisum fulvum* for resistance to pea weevil. *Crop Science* 42:2167–2173 DOI [10.2135/cropsci2002.2167](https://doi.org/10.2135/cropsci2002.2167).
- Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. 2016.** Grupo InfoStat. Universidad Nacional De Córdoba. Argentina. Available at <http://www.infostat.com.ar>.
- Dien J, Beal DJ, Berg P. 2005.** Optimizing principal components analysis of event-related potential analysis: matrix type, factor loading weighting, extraction, and rotations. *Clinical Neurophysiology* 116:1808–1825 DOI [10.1016/j.clinph.2004.11.025](https://doi.org/10.1016/j.clinph.2004.11.025).
- Dirzo R, Domínguez CA. 1995.** Plant-herbivore interactions in Mesoamerican tropical dry forest. In: Bullock S, Mooney H, Medina E, eds. *Seasonally dry tropical forest*. Cambridge: Cambridge University Press, 305–325.
- Dos Santos LFC, Ruiz-Sánchez E, Andueza-Noh RH, Garruña Hernández R, Latournerie-Moreno L, Mijangos-Cortés JO. 2020.** Leaf damage by *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) and its relation to leaf morphological traits in maize landraces and commercial cultivars. *Journal of Plant Diseases and Protection* 127:103–109 DOI [10.1007/s41348-019-00276-y](https://doi.org/10.1007/s41348-019-00276-y).
- Evans JR, Poorter H. 2001.** Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell & Environment* 24:755–767 DOI [10.1046/j.1365-3040.2001.00724.x](https://doi.org/10.1046/j.1365-3040.2001.00724.x).

- FAO. 2018. *World food and agriculture –Statistical pocketbook*. Rome: FAO. Available at <http://www.fao.org/policy-support/tools-and-publications/resources-details/en/c/1160800/>.
- Figueiredo AST, Resende JTV, Morales RGF, Gonçalves APS, Da Silva PR. 2013. The role of glandular and non-glandular trichomes in the negative interactions between strawberry cultivars and spider mite. *Arthropod-Plant Interactions* 7:53–58 DOI 10.1007/s11829-012-9218-z.
- Freschet GT, Swart EM, Cornelissen JHC. 2015. Integrated plant phenotypic responses to contrasting above- and below-ground resources: key roles of specific leaf area and root mass fraction. *New Phytologist* 206:1247–1260 DOI 10.1111/nph.13352.
- Gong B, Zhang G. 2014. Interactions between plants and herbivores: a review of plant defense. *Ecological Society of China* 34:325–336 DOI 10.1016/j.chnaes.2013.07.010.
- Hill MO. 1979. DECORANA-A FORTRAN program for detrended correspondence analysis and reciprocal averaging. Ithaca: Cornell University, 1–52.
- Keneni G, Bekele E, Imtiaz M. 2011. Breeding chickpea (*Cicer arietinum* [Fabaceae]) for better seed quality inadvertently increased susceptibility to adzuki bean beetle (*Callosobruchus chinensis* [Coleoptera: Bruchidae]). *International Journal of Tropical Insect Science* 31:249–261 DOI 10.1017/S1742758411000373.
- Khazaei H, Wach D, Pecio A, Vandenberg A, Stoddard FL. 2019. Genetic analysis of photosynthesis-related traits in faba bean (*Vicia faba* L.) for crop improvement. *Plant Breeding* 138:761–769 DOI 10.1111/pbr.12716.
- Kimiti JM, Odee DW, Vanlauwe B. 2009. Area under grain legumes cultivation and problems faced by smallholder farmers in legume production in the semi-arid eastern Kenya. *Journal of Sustainable Development in Africa* 11:305–315.
- Kiptoo GJ, Kinyua M, Kiplagat O, Wanjala FME, Kiptoo JJ, Cheboi JJ, Kimno SK, Rotich G, Ngurwe JK. 2016. Evaluation of common bean (*Phaseolus vulgaris* L.) varieties for resistance to bean stem Maggot (*Ophiomyia* spp.) in Kenya. *American Journal of Experimental Agriculture* 12:1–7.
- Ku-Hwan L, Seung-Keun J, Sang IIP, Beom-Heon S, Yong-Gu C, Hong-Sig K. 2002. Agronomic characteristics of resistant mungbean to *Callosobruchus chinensis*. *Journal of Agricultural Science* 19:41–52.
- Lakshminarayan S, Singh PS, Mishra DS. 2008. Relationship between whitefly population, YMV disease and morphological parameters of green gram germplasm.. *Environment and Ecology* 26:978–982.
- Lale NES, Kolo AA. 1998. Susceptibility of eight genetically improved local cultivars of cowpea to *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) in Nigeria. *International Journal of Pest Management* 44:25–27 DOI 10.1080/096708798228482.
- Larcher W. 2006. *Ecofisiologia vegetal*. São Carlo: RiMa, 525–550.
- Lawlor DW, Cornic G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment* 25:275–294 DOI 10.1046/j.0016-8025.2001.00814.
- Legendre P, Legendre L. 1998. *Numerical ecology*. Amsterdam: Elsevier, 380.

- Lynch JP. 2007. Roots of the second green revolution. *Australian Journal of Botany* 55:493–512 DOI 10.1071/bt06118.
- Maag D, Erb M, Bernal JS, Wolfender JL, Turlings TCJ, Glauser G. 2015. Maize domestication and anti-herbivore defences: leaf-specific dynamics during early ontogeny of maize and its wild ancestors. *PLOS ONE* 8:e0135722 DOI 10.1371/journal.pone.0135722.
- Martínez-Castillo J, Colunga-García MP, Zizumbo-Villarreal D. 2008. Genetic erosion and in situ conservation of Lima bean (*Phaseolus lunatus* L.) landraces in its Mesoamerican diversity center. *Genetic Resources and Crop Evolution* 55:1065–1077 DOI 10.1007/s10722-008-9314-1.
- Martínez-Castillo J, Camacho-Perez L, Coello-Coello J, Andueza-Noh R. 2012. Wholesale replacement of Lima bean (*Phaseolus lunatus* L.) landraces over the last 30 years in northeastern Campeche, Mexico. *Genetic Resources and Crop Evolution* 59:191–204 DOI 10.1007/s10722-011-9675-8.
- Martínez-Castillo J, Zizumbo-Villarreal D, Perales-Rivera H, Colunga-García M. 2004. Intraspecific diversity and morpho-phenological variation in *Phaseolus lunatus* L. from the Yucatan Peninsula, Mexico. *Economic Botany* 58:354–380 DOI 10.1663/0013-0001(2004)058[0354:IDAMVI]2.0.CO;2.
- Maschinski J, Whitham TG. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *The American Naturalist* 134:1–19 DOI 10.1086/284962.
- McNickle GG, Evans WD. 2018. Tolerant games: compensatory growth by plants in response to enemy attack is an evolutionarily stable strategy. *AoB Plants* 10:1–14 DOI 10.1093/aobpla/ply035.
- Meneses-Lazo RE, Garruña Hernández R, Latournerie-Moreno L, Andrade-Torres JL, Pérez-Gutiérrez A. 2018. Phenological and physiological characterization of experimental habanero pepper varieties with high agronomic potential. *Revista Fitotecnia Mexicana* 41:67–74 DOI 10.35196/rfm.2018.1.67-74.
- Mitchell C, Brennan RM, Graham J, Karley AJ. 2016. Plant defense against herbivorous pests: exploiting resistance and tolerance traits for sustainable crop protection. *Frontiers in Plant Science* 7:1132 DOI 10.3389/fpls.2016.01132.
- Moya-Raygoza G. 2016. Early development of leaf trichomes is associated with decreased damage in teosinte, compared with maize, by *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 109:737–743 DOI 10.1093/aesa/saw049.
- Nzungize JR, Lyumugabe F, Busogoro JP, Baudoin J-P. 2012. Pythium root rot of common bean: biology and control methods. A review. *Biotechnology, Agronomy, Society and Environment* 16:405–413.
- Oerke EC, Dehne HW. 2004. Safeguarding production-losses in major crops and the role of crop protection. *Crop Protection* 23:275–285 DOI 10.1016/j.cropro.2003.10.001.
- Oghiakhe LEN, Jackai L, Makanjuola WA. 1992. A rapid visual field screening technique for resistance of cowpea (*Vigna unguiculata*) to the legume pod borer, *Maruca*

- testulalis* (Lepidoptera: Pyralidae). *Bulletin of Entomological Research* **82**:507–512 DOI [10.1017/S0007485300042589](https://doi.org/10.1017/S0007485300042589).
- Panda N, Khush GS. 1995.** *Host plant resistance to insects*. Biddles, Guildford: CAB International in Association with International Rice Research Institute.
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012.** Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**:30–50 DOI [10.1111/j.1469-8137.2011.03952](https://doi.org/10.1111/j.1469-8137.2011.03952).
- Rao RVS. 2002.** Method of screening for resistance to leaf miner, *Aproaerema modicella* (Deventer) under greenhouse conditions. *Annals of Plant Protection Sciences* **10**:38–41.
- Ribeiro RV, Dos Santos MG, Souza GM, Machado EC, De Oliveira RF, Angelocci LR, Pimentel C. 2004.** Environmental effects on photosynthetic capacity of bean genotypes. *Pesquisa Agropecuária Brasileira* **39**:615–623 DOI [10.1590/S0100-204X2004000700001](https://doi.org/10.1590/S0100-204X2004000700001).
- Rosales MA, Ocampo E, Rodríguez-Valentín R, Olvera-Carrillo Y, Acosta-Gallegos J, Covarrubias AA. 2012.** Physiological analysis of common bean (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. *Plant Physiology and Biochemistry* **56**:24–34 DOI [10.1016/j.plaphy.2012.04.007](https://doi.org/10.1016/j.plaphy.2012.04.007).
- Saheb YP, Hari-Prasad KV, Swarajya KL, Rani JS. 2018.** Mechanisms of resistance in groundnut genotypes against leaf bud borer, *Anarsia ephippias* (Meyrick). *Journal of Entomology and Zoology Studies* **6**:694–705.
- Santiago LS, Wright SJ. 2007.** Leaf functional traits of tropical forest plants in relation to growth form. *Functional Ecology* **21**:19–27 DOI [10.1111/j.1365-2435.2006.01218](https://doi.org/10.1111/j.1365-2435.2006.01218).
- Schädler M, Roeder M, Brandl R, Matthies D. 2007.** Interacting effects of elevated CO₂, nutrient availability and plant species on a generalist invertebrate herbivore. *Global Change Biology* **13**:1005–1015 DOI [10.1111/j.1365-2486.2007.01319.x](https://doi.org/10.1111/j.1365-2486.2007.01319.x).
- Schofeld RMS, Emmett KD, Niedbala JC, Nesson MH. 2011.** Leafcutter ants with worn mandibles cut half as fast, spend twice the energy, and tend to carry instead of cut. *Behavioral Ecology and Sociobiology* **65**:969–982 DOI [10.1007/s00265-010-1098-6](https://doi.org/10.1007/s00265-010-1098-6).
- Shibuya T, Hirai N, Sakamoto Y, Komuro J. 2009.** Effects of morphological characteristics of *Cucumis sativus* seedlings grown at different vapor pressure deficits on initial colonization of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Journal of Economic Entomology* **102**:2265–2267 DOI [10.1603/029.102.0631](https://doi.org/10.1603/029.102.0631).
- Sohrabi F, Nooryazdan HR, Gharati . 2017.** plant resistance to the moth *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae) in tomato cultivars. *Neotropical Entomology* **46**:203–209 DOI [10.1007/s13744-016-0441-7](https://doi.org/10.1007/s13744-016-0441-7).
- Somta P, Kaga A, Tomooka N, Isemura T, Vaughan DA, Srivines P. 2008.** Mapping of quantitative trait loci for a new source of resistance to bruchids in the wild species *Vigna nepalensis* Tateishi & Maxted (*Vigna subgenus* Ceratotropis). *Theoretical and Applied Genetics* **117**:621–628 DOI [10.1007/s00122-008-0806-3](https://doi.org/10.1007/s00122-008-0806-3).
- Ssekandi JW, Mulumba J, Colangelo P, Nankya R, Fadda C, Karungi J, Otim M, De Santis P, Jarvis DI. 2016.** The use of common bean (*Phaseolus vulgaris*) traditional varieties and their mixtures with commercial varieties to manage bean

- fly (*Ophiomyia* spp.) infestations in Uganda. *Journal of Pest Science* **89**:45–57
[DOI 10.1007/s10340-015-0678-7](https://doi.org/10.1007/s10340-015-0678-7).
- Stinchcombe J. 2002.** Can tolerance traits impose selection on herbivores? *Evolutionary Ecology* **16**:595–602 [DOI 10.1023/A:1021617418037](https://doi.org/10.1023/A:1021617418037).
- Stout MJ. 2013.** Re-evaluating the conceptual framework for applied research on host-plant resistance. *Insect Science* **20**:263–272 [DOI 10.1111/1744-7917.12011](https://doi.org/10.1111/1744-7917.12011).
- Strauss SY, Agrawal AA. 1999.** The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution* **14**:179–185
[DOI 10.1016/s0169-5347\(98\)01576-6](https://doi.org/10.1016/s0169-5347(98)01576-6).
- Taggar GK, Gill RS. 2012.** Preference of whitefly, *Bemisia tabaci*, towards black gram genotypes: role of morphological leaf characteristics. *Phytoparasitica* **40**:461–474
[DOI 10.1007/s12600-012-0247-z](https://doi.org/10.1007/s12600-012-0247-z).
- Ter Braak CJE, Smilauer P. 2002.** *CANOCO reference manual and canodraw for windows user's guide: software for canonical community ordination (version 4.5)*. Ithaca: Microcomputer Power, 500.
- Tian D, Tooker J, Peiffer M, Chung SH, Felton GW. 2012.** Role of trichomes in defense against herbivores: comparison of herbivore response to woolly and hairless trichome mutants in tomato (*Solanum lycopersicum*). *Planta* **236**:1053–1066
[DOI 10.1007/s00425-012-1651-9](https://doi.org/10.1007/s00425-012-1651-9).
- Vangestel C, Andrew JE, Wegrzyn JL, St. Clair JB, Neale DB. 2018.** Linking phenotype, genotype and environment to unravel genetic components underlying cold hardiness in coastal Douglas-fir (*Pseudotsuga Menziesii* Var. *Menziesii*). *Tree Genetics & Genomes* **14**:10 [DOI 10.1007/s11295-017-1225-x](https://doi.org/10.1007/s11295-017-1225-x).
- Veeranna R, Hussain M. 1997.** Trichomes as physical barriers for cowpea podborer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *Insect Environment* **3**:1–15.
- Widstrom NW, McMillian WW, Wiseman BR. 1979.** Ovipositional preference of the corn earworm and the development of trichomes on two exotic corn selections. *Environmental Entomology* **8**:833–839 [DOI 10.1093/ee/8.5.833](https://doi.org/10.1093/ee/8.5.833).