

## SEEDLING AND ADULT PLANT RESISTANCE TO *Uromyces viciae-fabae* (Pers.) J. Schröt. IN MEXICAN FABA BEAN (*Vicia faba* L.) GENOTYPES

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### ABSTRACT

Faba bean rust (*Vicia faba* L.) is a globally significant disease caused by *Uromyces viciae-fabae* (Pers.) J. Schröt, resulting in yield losses of up to 70 %. Genetic resistance, whether specific or non-specific, is viable for reducing losses caused by this fungus. In faba bean, genotypes with specific resistance to *U. viciae-fabae* have been found, although most genotypes allow for some levels of pathogen reproduction. Therefore, the objective was to evaluate the resistance to *U. viciae-fabae* in faba bean lines at the seedling and adult plant stages. Thirty faba bean genotypes were examined as part of two breeding initiatives at Postgraduate College and the Agricultural, Aquaculture, and Forestry Research and Training Institute of the State of Mexico. Adult plants were evaluated in the field at two locations: 1) Palmillas, Texcaltitlán, State of Mexico, and 2) Postgraduate College Montecillo Campus, Texcoco, State of Mexico. A two-factor factorial model was used in the design, which included a generalized randomized block design and four replicates. The variables evaluated were the percentage of disease severity, final severity, and grain yield. Seedlings were inoculated with a concentration of  $3 \times 10^6$  urediniospores mL<sup>-1</sup>, incubated in a growth chamber at  $18 \pm 2$  °C and 100 % relative humidity for 24 h, and transferred to a temperature-controlled greenhouse at  $18 \pm 2$  °C. The variables evaluated included the number of pustules per leaf, the severity percentage, pustule size, latency period (LP1, LP50, and LP100), and the type of infection. Approximately 95 % of the lines tested in seedlings were susceptible to faba bean rust, with line 20CP showing moderate resistance. Incomplete non-hypersensitive resistance was identified in the bean-*U. viciae-fabae* pathosystem.

**Keywords:** incomplete genetic resistance, hypersensitivity reaction, faba bean rust.

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## INTRODUCTION

Faba bean rust is a disease of global importance caused by the biotrophic fungus *U. viciae-fabae* (Pers.) J. Schröt (syn. *U. fabae*) (Ijaz *et al.*, 2017; Rubiales and Khazaei, 2022). It is a macrocyclic and autoecious rust with sexual and asexual phases, but its sexual phase is rarely observed in temperate regions (Rubiales and Khazaei, 2022). This fungus can cause yield losses of up to 70 % when it attacks at early stages and up to 30 % if the infection occurs in adult plants (Terefe *et al.*, 2016).

Genetic resistance is viable to reduce the losses caused by *U. viciae-fabae*. It can be specific (vertical, complete) or non-specific (horizontal, incomplete, partial) (Niks *et al.*, 2011). Specific resistance only confers protection for a short period of time, since it is given only by a major gene. When a fungus acquires a virulence gene, the resistance is broken; therefore, efforts have been made to generate varieties with non-specific resistance that is durable over several crop cycles. In faba bean (*Vicia faba* L.), complete resistance to this pathogen has not been found, but non-specific or incomplete resistance to *U. viciae-fabae* has been observed, since most faba bean genotypes allow certain levels of pathogen reproduction (Sillero *et al.*, 2000; Ijaz *et al.*, 2021).

Sillero *et al.* (2000) first reported the hypersensitivity reaction in the faba bean crop. They observed late-onset necrotic reactions, resulting in small pustules with macroscopically visible necrosis. This hypersensitivity reaction is considered incomplete resistance because it is associated with a longer latency period, a reduction in pustule size, and a lower frequency of infection, with the difference that it presents macroscopically visible necrosis. This hypersensitive response in faba bean is controlled by dominant genes (Ávila *et al.*, 2003; Adhikari *et al.*, 2016).

Non-specific resistance reduces epidemic development despite a high degree of compatibility between the pathogen and the host (Parlevliet, 1975). Several sources of resistance that delay rust disease in faba bean have been detected (Torres *et al.*, 2006). This reduces the potential of reinfections, allowing the plant to overcome the disease with minimal impact on yield. Genetic resistance is a viable strategy to generate rust-resistant or tolerant faba bean genotypes. According to Ávila *et al.* (2017), there are no studies or published information on this subject in Mexico. The resistance of Mexican faba bean genotypes to this pathogen is unknown. Therefore, the hypothesis to be tested in this study is that there are faba bean lines with resistance in both adult plants and seedlings from two improvement programs in Mexico. The objective of this study was to evaluate 30 faba bean lines for their resistance to *U. viciae-fabae*, both at the seedling and adult plant stages, and to understand the behavior of the genotypes to the disease in two environments.

## MATERIALS AND METHODS

### Germplasm

Thirty lines of faba bean (*Vicia faba* L.) were evaluated as part of the Postgraduate College (CP) Montecillo Campus and the Agricultural, Aquaculture, and Forestry

Research and Training Institute of the State of Mexico (ICAMEX) improvement program (Table 1).

**Table 1.** Faba bean (*Vicia faba* L.) genotypes used in the evaluation of resistance in seedlings and adult plants to *Uromyces viciae-fabae*.

Name	Subspecies	Name	Subspecies
1 ICAMEX	<i>major</i>	16 ICAMEX	<i>major</i>
2 ICAMEX	<i>major</i>	17 ICAMEX	<i>major</i>
3 ICAMEX	<i>major</i>	18 ICAMEX	<i>major</i>
4 ICAMEX	<i>major</i>	19 ICAMEX	<i>major</i>
5 ICAMEX	<i>major</i>	20 ICAMEX	<i>major</i>
6 ICAMEX	<i>major</i>	1 CP	<i>minor</i>
7 ICAMEX	<i>major</i>	5 CP	<i>minor</i>
8 ICAMEX	<i>major</i>	9 CP	<i>minor</i>
9 ICAMEX	<i>major</i>	13 CP	<i>minor</i>
10 ICAMEX	<i>major</i>	16 CP	<i>minor</i>
11 ICAMEX	<i>major</i>	17 CP	<i>minor</i>
12 ICAMEX	<i>major</i>	20 CP	<i>minor</i>
13 ICAMEX	<i>major</i>	22 CP	<i>minor</i>
14 ICAMEX	<i>major</i>	46 CP	<i>minor</i>
15 ICAMEX	<i>major</i>	47 CP	<i>minor</i>

ICAMEX: Agricultural, Aquaculture, and Forestry Research and Training Institute of the State of Mexico; CP: Postgraduate College.

### Resistance evaluation in adult plants

The experiment was repeated each year under field conditions, in two locations and over two years: 1) Palmillas, in the municipality of Texcaltitlán, State of Mexico (23° 15' 00" N and 99° 34' 00" W), at an altitude of 2824 m, in 2020, under rainfed conditions in the autumn-winter agricultural cycle; and 2) Colegio de Postgraduados Campus Montecillo, in the municipality of Texcoco, State of Mexico (19° 27' 51" N and 98° 54' 15" W), at an altitude of 2250 m in 2021, under rainfed conditions in the spring-summer agricultural cycle. In both locations, the same faba bean genotypes were planted (Table 1) under a factorial model of two factors with a generalized randomized block experimental design with four replications.

The experimental unit consisted of four 2.8-m-long furrows spaced 40 cm apart. Two weeding operations were performed at 30 and 50 d after emergence (DAE). Fertilization was carried out with a 60-80-40 formula with urea fertilizers (Fertilizante del campo<sup>®</sup>), triple calcium superphosphate (Agrocolmex<sup>®</sup>), and potassium chloride (Agrocolmex<sup>®</sup>). To ensure the presence of the disease in the central part of each plot, a plant was inoculated with *U. viciae-fabae* pathotype CP21, which was morphologically identified using the keys of Cummins and Hiratsuka (2003). Temperature and relative humidity were recorded with the data logger Onset HOBO U23 Pro v2, which was placed 20

DAE. The average temperature in Texcaltitlán during the 130 DAE was 11.68 °C, with a minimum of -1 °C and a maximum of 31.1 °C, and an average relative humidity of 83.76 %, with a minimum of 26.1 % and a maximum of 100 %. In Texcoco, the minimum temperature was 5.4 °C, with an average of 17.14 °C and a maximum of 33.5 °C, and the minimum relative humidity was 29.11 %, with an average of 78.67 % and a maximum of 100 %.

### Recorded variables

Disease severity was assessed as the percentage of the total plant surface covered with pustules, following Villegas-Fernández *et al.* (2011). Evaluations were conducted every 10 d beginning with the appearance of the first pustules, using the severity relative to the entire plant as the reference. A total of six readings were taken. Final severity was defined as the disease severity recorded at the last evaluation (sixth reading). In addition, the Area Under the Disease Progress Curve (AUDPC) was calculated to quantify the cumulative disease pressure over time, using the equation described by Bjarko and Line (1988):

$$AUDPC = \sum_i \frac{Y_i + Y_{i+1}}{2} * (t_{i+1} - t_1)$$

where  $Y$  is the percentage of leaves with pustules at each reading, and  $t$  represents the corresponding time.

Grain yield was recorded as the total grain weight of all plants per plot ( $\text{g plot}^{-1}$ ) and subsequently converted to  $\text{kg ha}^{-1}$ . Data from both locations were analyzed using SAS 9.3 (SAS Institute, 2013), and mean comparisons were performed using the DMS test ( $p \leq 0.05$ ).

### Resistance evaluation in seedlings

The seedling response of the 30 faba bean genotypes was tested at the National Laboratory of Rusts and Other Cereal Diseases (LANAREC) of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP), Valley of Mexico Experimental Field (CEVAMEX), located at 19° 29' 88" N and 98° 54' 22" W, at an altitude of 2260 m. Seeds of each genotype were sown in 295 cm<sup>3</sup> Styrofoam cups with Promix® brand peat substrate. The experimental unit was one seedling, with ten replicates used in total. Eight days after sowing, the seedlings were inoculated with fresh urediniospores of the *U. viciae-fabae* isolate CP21 from the faba bean rust collection at the Genetic Resistance Laboratory of the Plant Pathology Program of the Postgraduate College, with a concentration of  $3 \times 10^6$  urediniospores mL<sup>-1</sup>. Mineral oil (Sotrol® 170) was used as a diluent vehicle. Inoculation was carried out with a sprinkler adapted to a FELISA® brand vacuum pump, model 1500. Once inoculated, all seedlings were transferred to

a bioclimatic chamber with a temperature of  $18 \pm 2$  °C, white light, and 100 % relative humidity for 24 h. After incubation, the seedlings were moved to a greenhouse with a controlled temperature of  $18 \pm 2$  °C.

### Recorded variables

The total number of pustules per leaf (calculated as the average from three leaves taken from the central part of each plant), the percentage of severity according to Villegas-Fernández *et al.* (2011), and the average pustule size (also based on three central leaves) were all recorded 20 d after inoculation. The latency period (LP) was monitored beginning 10 d after inoculation, evaluating LP1, LP50, and LP100, which correspond to the appearance of the first pustule, 50 % of erumpent pustules, and 100 % of erumpent pustules, respectively. The LP50 was estimated as the number of days from inoculation to the time when 50 % of the final number of urediniospores became visible (Niks *et al.*, 2011).

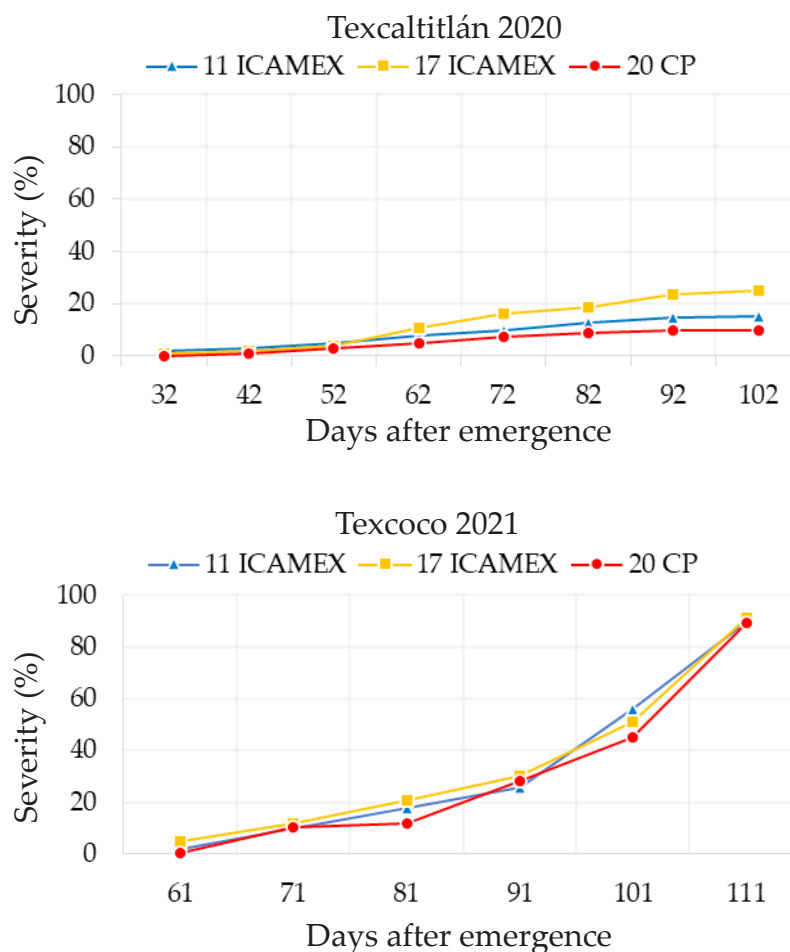
Infection type (IT) was measured using the scale of Stakman *et al.* (1962), adapted for faba bean by Sillero *et al.* (2000). In this scale, IT0 corresponds to asymptomatic reactions, where IT denotes necrotic spots; IT1 indicates tiny pustules with minimal sporulation; IT2 reflects necrosis surrounding small pustules; IT3 represents pustules that sporulate in the presence of chlorosis; and IT4 corresponds to well-formed pustules without chlorosis or necrosis. Data for all evaluated variables were analyzed using SAS 9.3 (SAS Institute, 2013), and mean comparisons were performed using the Tukey test ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

### Adult plant resistance

Urediniospores of *U. viciae-fabae* germinate at temperatures ranging from 5 to 26 °C, with an optimum of 20 °C (Joseph and Hering, 1997). Emeran *et al.* (2011) report that a humidity of 80 % is adequate for the development of the disease. Although the temperature was not optimal in field experiments, it was within the ranges of temperature and relative humidity reported for urediniospore germination. Therefore, field conditions were adequate for the development of the disease.

The first pustules were observed at 32 DAE in the Texcaltitlán area, at an early stage of the crop, while in Texcoco they were observed at 61 DAE (Figure 1). Since 30 genotypes were evaluated, only three genotypes were used as models, as the other materials exhibited similar behavior. Significant differences ( $p \leq 0.05$ ) were observed for the genotype factor in the final severity and yield variables, while there was no significant difference in the AUDPC variable. Regarding the environment, there were highly significant differences ( $p \leq 0.01$ ) in the three measured variables. There was a significant difference ( $p \leq 0.05$ ) in the genotype  $\times$  environment interaction in the final severity and yield variables. The differences for all sources of variation are attributed to the different environmental conditions and genotypes (Table 2).



**Figure 1.** Comparative progression of faba bean rust severity in Texcaltitlán and Texcoco, Mexico.

**Table 2.** Analysis of variance (ANOVA) for final severity, area under the disease progress curve (AUDPC), and yield in 30 faba bean (*Vicia faba* L.) lines evaluated across two locations.

Source of variation	DF	Final severity (%)	AUDPC	Yield (kg ha <sup>-1</sup> )
Genotype	1	23.92 *	845.47 ns	1 438 616.40 **
Environment	29	252 900.05 **	2 419 064.93 **	82 059 756.81 **
Genotype × environment interaction	29	19.01 *	633.376 ns	1 274 390.39 **
Experimental error		11.80	730.66	187 361.2
Mean		53.09	190.97	1498.61
Coefficient of variation (%)		6.46	14.15	28.88

\*, \*\*: significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively. DF: degrees of freedom; ns: not significant.

There were statistical differences found between environments, with the adult plant (Table 3) having the lowest final severity and AUDPC, and higher yield in Texcaltitlán compared to Texcoco. The average yield of all genotypes in Texcaltitlán was 35 % higher than the national average of 1360 kg ha<sup>-1</sup> (SIAP, 2024). Meanwhile, the average yield for all genotypes in Texcoco was 40 % lower than the national average. Texcoco had a higher final rust severity, which limited the grain yield of the genotypes.

**Table 3.** Environmental comparison of mean final severity, area under the disease progress curve (AUDPC), and yield for 30 faba bean (*Vicia faba* L.) genotypes under *Uromyces viciae-fabae* infection.

Environments	Final severity (%)	AUDPC	Yield (kg ha <sup>-1</sup> )
Texcaltitlán	15.61 b	75.04 b	2174.00 a
Texcoco	90.58 a	307.24 a	827.50 b
Mean	53.09	191.14	1500.75

Values with the same letter are significantly equal (Tukey,  $p \leq 0.05$ ).

All genotypes were susceptible in the field, presenting a final severity greater than 49 %, with line 17 ICAMEX being the most susceptible. This is consistent with the findings of Ijaz *et al.* (2021), who reported a severity of 80 to 90 % for the Fiord cultivar that was considered susceptible. Of the 30 genotypes evaluated, materials 17 CP, 20 CP, 1 CP, and 46 CP presented a lower susceptibility according to the statistical differences, but this does not provide evidence that they have a level of resistance in adult plants that can be used for the generation of varieties resistant to rust, at least to the CP21 pathotype.

Regarding yield, the genotypes 17 ICAMEX and 20 ICAMEX had the best performance, both exceeding 2150 kg ha<sup>-1</sup>, while 13 CP and 47 CP showed the lowest yield, which did not exceed 750 kg ha<sup>-1</sup>. It should be noted that the CP genotype belongs to the *minor* subspecies, having a low yield compared to the ICAMEX genotypes of the *major* species (Table 4).

All genotypes evaluated in Texcoco showed a final severity greater than 80 % and lower yields compared to Texcaltitlán (Table 5). For example, genotype 8 ICAMEX had a better yield in Texcaltitlán, while its yield was lower in Texcoco with 86 % severity. While the genotypes exhibited some level of horizontal resistance in Texcaltitlán, they demonstrated susceptibility in Texcoco. This behavior could be due to the fact that the climatic conditions in Texcoco were close to the optimal range of temperature and relative humidity conditions for the development of the disease, allowing a greater number of polycycles.

Sillero *et al.* (2000) mention that susceptibility is very common in faba bean cultivation and that resistance genes have been found in few genotypes. Some can express their

**Table 4.** Mean final severity and yield of 30 adult-plant faba bean (*Vicia faba* L.) genotypes evaluated across two environments in 2020–2021 affected by *Uromyces viciae-fabae*.

Genotype	Final severity %	Yield (kg ha <sup>-1</sup> )
17 ICAMEX	58.33 a	2172.17 a
20 ICAMEX	56.33 ab	2213.33 a
16 ICAMEX	55.00 ab	2009.83 ab
14 ICAMEX	55.50 ab	1635.17 abcde
9 ICAMEX	55.00 ab	1756.83 abc
13 ICAMEX	54.66 ab	2061.83 ab
2 ICAMEX	54.50 ab	1980.50 ab
12 ICAMEX	54.50 ab	1848.17 abc
3 ICAMEX	54.16 ab	1684.83 abcd
10 ICAMEX	53.83 ab	1845.50 abc
5 ICAMEX	53.83 ab	1647.50 abcde
6 ICAMEX	53.66 ab	1668.67 abcd
19 ICAMEX	53.33 ab	1888.50 abc
7 ICAMEX	53.33 ab	1354.83 abcdef
15 ICAMEX	53.33 ab	1183.50 bcdef
4 ICAMEX	53.33 ab	1635.33 abcde
47 CP	53.16 ab	702.00 ef
16 CP	53.00 ab	1002.83 cdef
8 ICAMEX	52.50 ab	2067.50 ab
11 ICAMEX	52.50 ab	1571.67 abcdef
1 ICAMEX	52.50 ab	1177.83 bcdef
18 ICAMEX	52.16 ab	2055.00 ab
13 CP	52.00 ab	639.50 f
5 CP	51.66 ab	1572.83 abcdef
22 CP	50.83ab	972.00 cdef
9 CP	50.83 ab	1112.50 bcdef
17 CP	50.33 b	735.33 def
20 CP	50.00 b	794.17 def
1 CP	50.00 b	791.67 def
46 CP	49.16 b	1177.17 bcdef

Values with the same letter are significantly equal (Tukey,  $p \leq 0.05$ ).

resistance at high or low temperatures depending on the genetic information. The same authors mention that genotype V-313 showed horizontal resistance with 12 and 22 % severity at 10 and 20 °C, respectively, but at 25 °C it had a severity of 70 %, showing susceptibility, while V-1335 showed a severity of 10–17 % at the three temperatures evaluated. According to our results, the genotypes could have resistance genes that interact with temperatures. On the other hand, locations play a fundamental role in the expression and behavior of bean genotypes (González-González *et al.*, 2016). However, to determine whether genotype 20 CP exhibits this type of interaction, it is necessary to study it at different controlled temperatures to observe its response.

**Table 5.** Mean comparison of final severity and yield for 30 adult-plant faba bean (*Vicia faba* L.) genotypes based on the genotype × environment interaction under *Uromyces viciae-fabae* infection.

Genotype	Texcoco		Texcaltitlán	
	Final severity %	Yield (kg ha <sup>-1</sup> )	Final severity %	Yield (kg ha <sup>-1</sup> )
16 CP	95.00 a	851.00 jklmno	11.00 c	1154.67 ghijklmno
13 ICAMEX	93.33 a	997.33 ijklmno	16.00 bc	3126.33 abcd
16 ICAMEX	93.33 a	1011.00 ijklmno	16.66 bc	3008.67 abcd
20 ICAMEX	91.66 a	767.67 lmno	21.00 bc	3659 a
6 ICAMEX	91.66 a	770.67 jklmno	15.66 bc	2566.67 abcdefg
5 CP	91.66 a	1445.00 efghijklmno	11.66 c	1700.67 defghijklmno
19 ICAMEX	91.66 a	1092.67 hijklmno	15.00 bc	2684.33 abcdef
3 ICAMEX	91.66 a	475.67 no	16.66 bc	2894.00 abcde
10 ICAMEX	91.66 a	723.67 mno	16.00 bc	2967.33 abcd
14 ICAMEX	91.66 a	864.33 jklmno	18.33 bc	2406.00 abcdefghi
17 ICAMEX	91.66 a	607.67 lmno	25.00 bc	3736.67 a
47 CP	90.66 a	701.67 lmno	15.66 bc	702.33 lmno
18 ICAMEX	90.00 a	1123.33 ghijklmno	14.33 bc	2986.67 abcd
2 ICAMEX	90.00 a	1022.33 hijklmno	19.00 bc	2938.67 abcd
15 ICAMEX	90.00 a	511.67 no	16.66 bc	1855.33 defghijklmno
11 ICAMEX	90.00 a	1124.00 ghijklmno	15.00 bc	2019.33 cdefghijklm
20 CP	90.00 a	576.67 mno	10.00 c	1011.67 ijklmno
22 CP	90.00 a	843.00 jklmno	11.66 c	1101.00 ghijklmno
5 ICAMEX	90.00 a	1031.67 hijklmno	17.66 bc	2263.33 abcdefghij
7 ICAMEX	90.00 a	604.00 mno	16.66 bc	2105.67 cdefghijkl
12 ICAMEX	90.00 a	794.67 klmno	19.00 bc	2901.67 abcde
9 ICAMEX	90.00 a	1020.00 hijklmno	20.00 bc	2493.67 abcdefg
4 ICAMEX	90.00 a	1035.67 hijklmno	16.66 bc	2235 bcdefghijk
9 CP	90.00 a	955.67 ijklmno	11.66 c	1269.33 fghijklmno
1 ICAMEX	88.33 a	419.67 o	15.00 bc	1936.00 defghijklmn
17 CP	90.00 a	652.67 lmno	10.66 c	818.00 jklmno
8 ICAMEX	88.33 a	711.00 lmno	16.66 bc	3424.00 abc
13 CP	88.33 a	578.67 mno	15.66 bc	700.33 lmno
1 CP	86.66 a	773.67 klmno	11.66 c	809.67 klmno
46 CP	86.66 a	494.33 no	11.66 c	1860.00 defghijklkmno

Values with the same letter are significantly equal (Least significant difference,  $p \leq 0.05$ ).

### Seedling resistance

There was a highly significant difference between the lines evaluated in the seedling stage in all variables (Table 6). For the variable infection type, all genotypes presented infection type III except for 20 CP, which showed infection type I and a longer LP50 and LP100 compared to the other faba bean lines, up to 3 d. In addition, this genotype had a lower number and size of pustules, which was reflected in the severity, and showed incomplete, non-hypersensitive resistance.

**Table 6.** Response of 30 faba bean (*Vicia faba* L.) genotypes to *Uromyces viciae-fabae* at the seedling stage under controlled conditions.

Genotype	Type of infection <sup>†</sup>	LP1 <sup>b</sup>	LP50 <sup>b</sup>	LP100 <sup>b</sup>	Number of pustules <sup>‡</sup>	Severity <sup>‡,b</sup>	Size of pustules <sup>‡,§</sup>
1 ICAMEX	3 a	13 a	14.6 bc	16.7 bcdefg	8.1 abc	10.1 abc	1.2 abcd
1 CP	3 a	11 c	14.7 b	16.6 cdefg	12.3 abc	13.2 abc	1.1 abcdefg
18 ICAMEX	3 a	11.6 b	14.5 bcd	16.6 cdefg	11.6 abc	14.7 abc	0.9 efgh
19 ICAMEX	3 a	11 c	14.3 bcd	17.3 bcd	9.4 abc	11.6 abc	1.2 abcde
5 ICAMEX	3 a	11 c	13.5 defg	16 fg	11.9 abc	14.2 abc	1.3 abc
9 CP	2.7 a	10.8 c	14.3 bcd	17.6 bc	6.9 bc	6.7 c	0.9 gh
13 ICAMEX	3 a	11.1 c	13.8 bcdefg	16 fg	8.1 bc	11 abc	1.3 abc
10 ICAMEX	3 a	11 c	13.7 bcdefg	15.8 g	9.3 abc	12 abc	1.1 bcdef
14 ICAMEX	3 a	11 c	14 bcdef	16 fg	10 abc	12.7 abc	1.4 a
22 CP	3 a	11 c	14.2 bcde	16.6 cdefg	9.7 abc	10.2 abc	1.0 cdefg
8 ICAMEX	3 a	11 c	14.1 bcdef	16.5 defg	15.5 a	18.6 a	1.2 abcd
47 CP	3 a	11 c	14.7 b	17.7 b	7.9 abc	9.6 abc	1.1 cdefg
15 ICAMEX	3 a	11 c	13.7 bcdefg	16.1 fg	13.6 abc	19.3 a	1.3 abc
11 ICAMEX	3 a	11 c	14 bcdefg	16 fg	12.5 abc	17.1 ab	1.4 ab
5 CP	3 a	11 c	14.1 bcdef	16.5 defg	12.7 abc	18 a	0.9 fgh
3 ICAMEX	3 a	11.2 bc	13 g	16f g	10.8 abc	15.1 abc	1.2 abcde
4 ICAMEX	3 a	11 c	13.2 efg	16.2 efg	8.6 abc	12.6 abc	1.2 abcdef
13 CP	3 a	11 c	13.1 fg	16.7 bcdefg	11.9 abc	15 abc	0.9 gh
2 ICAMEX	3 a	11.1 c	13.6 defg	16.2 efg	11.4 abc	15.1 abc	1.2 abcd
7 ICAMEX	2.7 a	11 c	13.5 efg	16.7 bcdefg	10.0 abc	13.7 abc	1.0 cdefg
6 ICAMEX	3 a	11.1 c	13 g	16 fg	11.8 abc	15.2 abc	1.1 cdefg
17 CP	3 a	11 c	14.2 bcde	17 bcdef	9.3 abc	14.3 abc	0.9 defgh
12 ICAMEX	3 a	11 c	14 bcdefg	16 fg	10.2 abc	16.5 abc	1.1 abcdefg
16 CP	3 a	11 c	14 bcdefg	16.7 bcdefg	10.0 abc	13.6 abc	0.9 fgh
17 ICAMEX	3 a	11 c	13.8 cdefg	16 fg	14.6 a	17.1 ab	1.0 cdefg
16 ICAMEX	3 a	11 c	13.2 efg	16 fg	8.7 abc	13.2 abc	1.2 abcdef
20 CP	1.1 b	11.1 c	17.3 a	20 a	8 abc	7.8 bc	0.7 h
9 ICAMEX	3 a	11 c	13.6 cdefg	16.5 defg	6.8 c	11.2 abc	1.1 cdefg
46 CP	3 a	11.2 bc	14.2 bcde	17.2 bcde	7.7 abc	12.4 abc	0.9 efgh
20 ICAMEX	3 a	11 c	13.5 edfg	16.2 efg	8.6 abc	13.2 abc	1.21 abcdefg
p	≤ .0001	≤ .0001	≤ .0001	≤ .0001	≤ 0.0005	≤ .0001	≤ .0001

<sup>†</sup>: qualitative scale according to Stakman *et al.* (1962), adapted to faba bean by Sillero *et al.* (2000); <sup>‡</sup>: average of three leaves from the central part of the plant; <sup>§</sup>: diameter in millimeters; <sup>b</sup>: LP: Latency Period. Values with the same letter are significantly equal (Tukey,  $p \leq 0.05$ ).

According to Parlevliet (1979), an incomplete non-hypersensitive response should be considered partial resistance. The behavior of the 20 CP line can be incorporated into genetic improvement programs aimed at developing varieties with vertical resistance, where the gene-to-gene relationship is applied. For each host resistance gene, there is a corresponding pathogenicity gene in the pathogen (Flor, 1942). Resistance genes are designated as R, and their counterparts in the pathogen are known as avirulence

genes (Avr). When an R gene recognizes an Avr gene in the pathogen, it activates a cell death process in the infected cell, known as the hypersensitive response. This reaction stops infection and colonization entirely, thereby conferring resistance to the plant (Burbano-Figueroa, 2020).

Line 9 CP also had similar behavior in the final severity, AUDPC, and yield variables but presented infection type III, with 97 % of the genotypes exhibiting a susceptible infection type (IT3). Infection type IV was not observed, nor was infection type 0. This aligns with Ijaz *et al.* (2021), who evaluated 40 Australian genotypes used as parents in the faba bean improvement program at the IA Watson Grains Research Center in Narrabri and did not identify any genotypes with infection type 0. In contrast, Sillero *et al.* (2000) reported that several faba bean cultivars exhibited infection type IV in their seedling evaluation, using the same infection type scale applied in this study. According to Stakman *et al.* (1962), Sillero *et al.* (2000), and Ijaz *et al.* (2021), IT0 is classified as resistant, IT1 as somewhat resistant, IT2 as moderately resistant, IT3 as moderately susceptible, and IT4 as very susceptible.

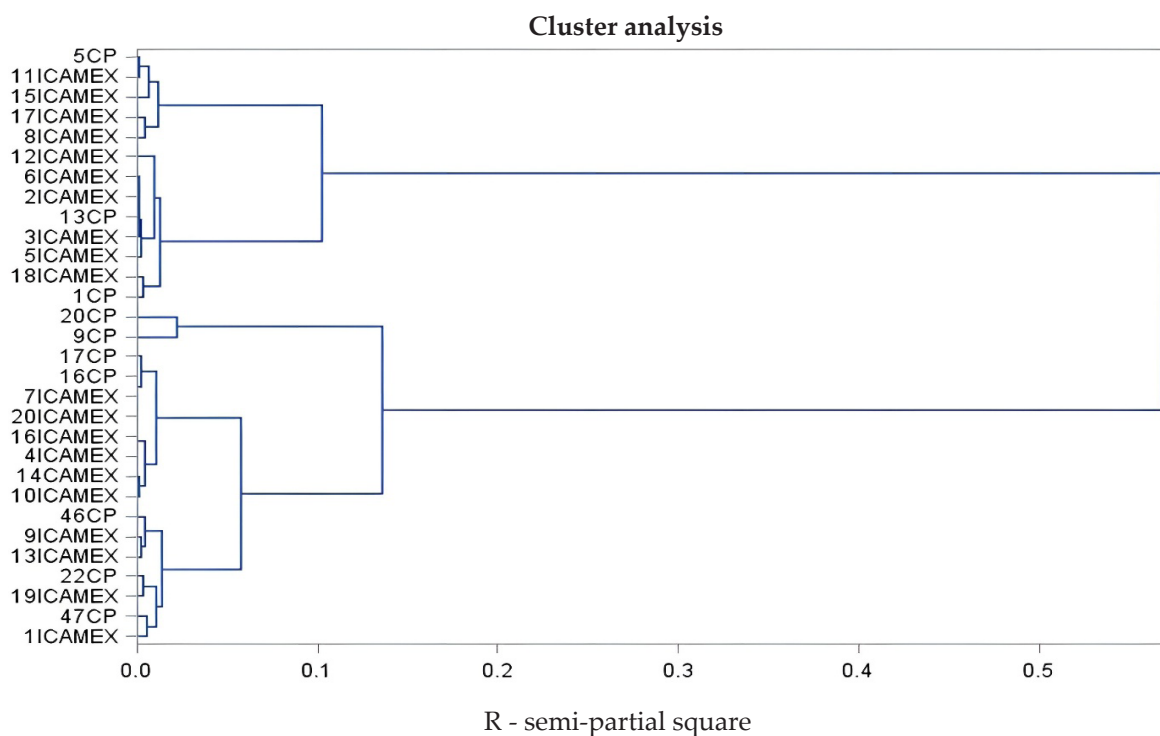
The data obtained show that all cultivars have a degree of resistance in seedlings to the CP21 pathotype. The high percentage of cultivars with a susceptible type of infection in seedlings and high percentages of severity of these cultivars in adult plants (Tables 3 and 4) show that the germplasm that was evaluated presented a low quantitative resistance to faba bean rust. The same results were obtained by Sillero *et al.* (2000), which show that a faba evaluation must be carried out on varieties, lines, and non-advanced materials from the different improvement programs, as well as collections in search of a greater number of materials with incomplete resistance given by major genes and quantitative resistance. Quantitative resistance in varieties allows for a durable resistance to the different existing pathotypes (Vanderplank, 1963; Robinson, 1987). It is also confirmed that incomplete non-hypersensitive resistance exists in the bean-*U. viciae-fabae* pathosystem.

Incomplete resistance is common in faba beans and has been reported by several authors, including Sillero *et al.* (2000) and Herath *et al.* (2001). Hypersensitive resistance was only identified by Sillero *et al.* (2000); however, the necrotic reaction occurs late, resulting in a limited infection size rather than complete resistance (Sillero *et al.*, 2010). Both types of resistance are associated with a longer latency period, a reduction in pustule size, and a lower infection frequency. They only differ in the presence or absence of macroscopically visible necrosis (Sillero *et al.*, 2000); therefore, hypersensitive resistance was not found in the present study.

It was also observed that line 17 ICAMEX was very susceptible both as an adult plant and as a seedling. This is important since it can be used as a susceptibility reference for the development of monopustular strains, inoculum production, plant-pathogen interaction studies, and as a susceptible border to achieve infection in the field in the evaluation of genotypes. It is necessary to continue studying the reaction of faba bean germplasm at the national level, focusing on quantitative and incomplete non-hypersensitive resistance to faba bean rust, in order to use it in genetic improvement

programs, since information on the genetic basis of resistance in faba beans is still scarce, both in Mexico and worldwide.

For the multivariate analysis of all evaluated variables, distinct groups were identified, with line 20CP standing out due to its incomplete resistance (Figure 2). This line clusters with line 9CP, which also shows quantitative resistance characteristics by limiting rust development. This is reflected in its longer LP1, LP50, and LP100 values relative to the other materials (Table 6), as well as its lower number of pustules, reduced severity, and a pustule size of 0.9 mm. Although it did not exhibit a hypersensitive reaction, its infection type was 2.7.



**Figure 2.** Multivariate characterization of genotypes using infection type, latency periods, pustule traits, and disease severity.

Ijaz *et al.* (2021) conducted a plant histology study in which they described the infection process of *U. viciae-fabae* in susceptible genotypes with infection type III. Urediniospores germinate on the leaf surface and form appressoria over the stomata, from which the germ tube penetrates into the leaf apoplast. Within the apoplast, substomatal vesicles are produced, giving rise to intercellular infection hyphae. These hyphae branch throughout the intercellular spaces of the leaf and generate haustorial mother cells, from which haustoria are formed to infect the mesophyll cells.

After 4–5 d, substantial hyphal growth with numerous haustoria is observed, without compromising the integrity of the host cell membrane. Infection sites continue expanding and subsequently form pustules at 7 d postinoculation. Resistant genotypes with infection type I also show notable hyphal growth and haustoria formation on host cells; however, tissue darkening with callose deposition appears at infection sites 4 d postinoculation. Pustules are detected at 11 d and display variable size, remaining smaller than those formed in the susceptible genotype.

### CONCLUSIONS

There is at least one genotype with incomplete hypersensitive resistance among the Mexican faba bean genotypes evaluated. Line 20CP behaved as moderately resistant in seedlings. In the bean-*U. viciae-fabae* pathosystem, incomplete hypersensitive resistance was identified. No complete hypersensitive resistance was observed. Ten genotypes possess quantitative resistance components.

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