Analysis of *Fusarium*-Common Beans Pathosystem in Aguascalientes, Mexico

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Abstract

In Mexico, high incidences of *Fusarium* affect common bean (*Phaseolus vulgaris* L.) production, reducing grain yields due to seedling death and crop standing reductions. Production of resistant germplasm could be an appropriate strategy for grain yield increasing. Bean breeding programs need the former analysis of plant-pathogen pathosystem to perform the selection of segregating populations with improved resistance to root rot pathogens and the best agroecosystem adaptation. Here, we report our results on characterization of genetic variability patterns of *Fusarium solani* f. sp. *phaseoli* (FSP) from Aguascalientes, México; the analysis of *P. vulgaris* germplasm reactions to highly and naturally FSP-infested field and controlled conditions; and the identification of genetic basis of resistance to FSP root rot in segregating common bean populations. Significant genetic variability in FSP isolates from Aguascalientes and other regions of México was found. Also, we found high variation on reactions to FSP root rots, resistance was more frequent on black seed-coated beans, and susceptibility was common in pinto beans. Resistance to FSP in BAT 477 seedlings was associated with one quantitative trait loci (QTL).

**Keywords:** *Fusarium solani* f. sp. *phaseoli* root rots, *Phaseolus vulgaris* L., Aguascalientes, genetic diversity, root rot incidence and severity, genetic resistance, molecular markers

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the second major crop in México. In 2016, approximately 1.63 million hectares were cultivated with common beans and an average grain yield
of 690 kg ha\(^{-1}\) was reported [1]. Grain yields of common bean in México are low since potential yields are estimated to be \(\approx 3\) t h\(^{-1}\). Several factors such as biotic (diseases, insect pest, weeds) and abiotic (drought, freeze, low-fertility soils, high temperatures, salinity) stresses reduce common bean production [2]. Drought stress and root rots caused by *Fusarium solani* f. sp. *phaseoli* (FSP), alone or combined, affect bean grain yield in major regions producing common beans in México. Both stresses reduce grain yields due to increase the percentages of seedling death and, consequently, reduce the crop standing (root rots) or reduce growth and development and seed production (water deficits) [3, 4].

Grain yield reductions decrease crop profits. More than 70% of common bean growers use low inputs for production, or in some cases, common bean is a subsistence crop. We consider that production of common bean germplasm with combined resistance to drought stress and diseases could be an appropriate strategy for grain yield improvement because it is a cheap, sustainable and durable strategy for grain yield stabilization [5]. The control of major root rot pathogens includes chemical, cultural and biological strategies, but most of them are not enough efficient to control pathogens or they have poor possibilities to be applied under Mexican bean grower conditions because they are expensive [6].

Mexican common bean breeding programs need the former analysis of plant-pathogen pathosystem to perform the selection of those genotypes with improved resistance to root rot pathogens and the best environmental adaptation. Another challenge is the characterization of pathogenic variability of root rot pathogen populations to identify molecular genetic factors of parasitic capability of the pathogen, since these characteristics affect the variation on reactions of common bean germplasm to the fungus. Then, the development of molecular marker technologies to improve the evaluation and selection of resistant common bean germplasm under marker-assisted selection strategy is needed [7].

This research includes three objectives: (1) to characterize the genetic variability patterns of *Fusarium* isolates from Aguascalientes and other regions of México; (2) to assess the reactions of each root rot pathogen in *Phaseolus* sp. germplasm under field and controlled conditions and (3) to define the genetic basis of resistance to each root rot pathogen in common beans.

### 2. Materials and methods

Despite the states of Aguascalientes and México, other states do not outstand as bean producers in México that three Mexican northern states (Chihuahua, Zacatecas and Durango) produce 60% of common beans at country while other four southern states (Chiapas, Oaxaca, Veracruz and Puebla) produce 20%, and both groups produce 80% of total beans in Mexico, they are considered by Mexican bean breeders as good locations for germplasm evaluation and/or selection for resistance to drought stress and root rot diseases caused by *Fusarium* sp., *Rhizoctonia solani* and *Pythium* sp., among other diseases such as common blight (*Xanthomonas axonopodis* pv. *phaseoli*) and anthracnose (*Glomerella lindemuthiana*) [8, 9].
Field trials included in this report were conducted in one location of the State of Aguascalientes: Sandovales and one from the State of México: Chapingo. Sandovales is located at 22°09′N, 102°18′W, and 2000 m above sea level and shows dry land conditions with summer rainfall. Annual average precipitations range from 350 to 400 mm, with average temperature ranges from 12 to 18°C. Chapingo is located at 19°28′N, 98°52′W; 2250 m above sea level and has a temperate climate with fresh summer and low variable temperatures (15–18°C) and the annual average precipitations range from 600 to 700 mm [10].

2.1. Variability of FSP isolates from Aguascalientes, México

The procedures for *Fusarium* isolates characterization by using *in vitro*, pathogenicity and AFLP genotype strategies were described when we analyzed the isolates from the State of Aguascalientes [11] and Aguascalientes, México, Guanajuato and Veracruz [4].

2.2. Reactions of common bean germplasm to root rot pathogens under field conditions

Previous works indicated us that soils of Chapingo and Sandovales are highly, naturally and homogeneously infested in most cases by FSP [12, 13]. We divided the characterizations into two groups.

The first group included 6 (experiment I), 75 (experiment II) and 36 (experiment III) (Table 1) common bean genotypes under rainfall conditions at Sandovales, Aguascalientes. Experiments were established on June 27 (E-I and E-II) and July 11 (E-III), 2002 under randomized complete block (RCB) design with four replications (E-I), where experimental unit was three rows 5 m-length. The germplasm of E-II was divided into three groups based on color seed coat: 25 pinto seed-type bean genotypes, 25 Flor de Mayo seed-type and 20 black seed beans. Each group of genotypes was randomized in a RCB design with three replications, and where experimental unit was two rows 6 m-length. Finally, germplasm in E-III was randomized on 6×6 lattice design with three replications and experimental unit of 2 rows 6 m in length.

In the second group of experiments, 49 common bean genotypes (Table 1) were evaluated under two levels of soil moisture: irrigated and rainfed conditions. Germplasm was randomized in 7 × 7 lattice design with four replications. Two replications were carried out under irrigated conditions, while the other two under rainfed conditions (irrigation was stopped when the most of germplasm initiated flowering and no irrigation was supplied until harvest). Experiments were established in Sandovales and Chapingo, México.

In both groups of experiments, FSP root rot severity ratings were determined at 28 and 56 days after sowing. Five plants were randomly picked off from each experimental unit and damage was evaluated by using the scale described by Abawi and Pastor-Corrales [6]. The scale has nine degrees of damage (1–9) where 1 = no symptoms and 9 = more than 75% of root or stem tissues infected by the pathogen. We took the values 1–3 as a reaction of resistance, while
### Experiment I

| Early            | Late                  |
|------------------|-----------------------|
| PT Villa         | PT Zapata            |
| AZ Tapatío       | Tlaxcala 62          |
| FM M38           | BY Criollo del Llano |

### Experiment II

| Pintos          | Flor de Mayo          | Blacks         |
|-----------------|-----------------------|----------------|
| PTD-99036       | FMD-99121             | NGD-99048      |
| PTD-99004       | FMD-99018             | NG-99040       |
| PTD-9903        | FMD-99035             | NG-99010       |
| PTD-99015       | FMD-99004             | NG-99039       |
| PTD-99008       | FMD-99034             | NG-99005       |
| PTD-99002       | FMD-99002             | NG-99025       |
| PTD-99107       | FMD-99033             | NG-99028       |
| PTD-99035       | FMD-99006             | NG-99011       |
| PTD-99043       | FMD-99005             | NG-99004       |
| PTD-99014       | FMD-99008             | NG-99044       |
| PTD-99046       | FMD-99011             | NG-99023       |
| PTD-99034       | FMD-99013             | NG-99012       |
| PTD-99045       | FMD-99019             | NGD-99030      |

### Experiment III

| RAB-608         | RJB                   | SEA 17         | SEA 23         | G 40068 | Tío Canela 75 |
|-----------------|-----------------------|----------------|----------------|---------|---------------|
| RAB-609         | RAB-632               | SEA 18         | INB 35         | G 40159 | DOR 390       |
| RAB-618         | RAB-650               | SEA 19         | INB 36         | G 21212 | PT Villa      |
| RAB-636         | RAB-651               | SEA 20         | INB 37         | G 1977  | Apetito       |
| RAB-619         | SEA 15                | SEA 21         | INB 38         | SEA 5   | FM Sol        |
| RAB-620         | SEA16                 | SEA 22         | INB 39         | BAT 477 | FM 2000       |

### Irrigated-Rainfed Experiment

| G 17427         | G 13637               | G 842          | 97RS110        | PT Zapata | AZ Namiquipa |
|-----------------|-----------------------|----------------|----------------|------------|--------------|
| G 14645         | G 19012               | G 4258         | DON35          | MD 23–24   | ICA Quimbaya |
| G 22923         | G 19953A              | G 801          | DON38          | VAX 2      | NG Veracruz  |
| G 1836          | G 2774                | G 18147        | BY San Luis    | SEA 5      | Black Jack   |
| G 17666         | G 16054               | G 4364         | 97RS101        | TLP 19     | NG Huasteco 81 |
| G 1354          | G 16054               | G 3386         | NG Durango     | MC 6       |              |
| G 6762          | G 21137               | G 1977         | NG 8025        | B98111     |              |
| G 1688          | G 2846                | G 3107         | G 4523         | NG INIFAP  |              |
| G 14538         | G 847                 | SEA10          | PT Villa       |            |              |

Prefix indicates seed coat color or commercial type: PT = ‘Pinto’, AZ = ‘Azufrado’ (Yellow), FM = ‘Flor de Mayo’, BY = ‘Bayo’ (Cream or beige), FJ = ‘Flor de Junio’ and NG = Black.

**Table 1.** Germplasm included on field experiments at Sandovales and Chapingo, México.
values of 4–9 indicated susceptibility. Plants were analyzed at laboratory in order to ratify the infection by FSP [11]. Days to flowering and to maturity were registered in each experimental unit in all experiments, and grain yield (kg h⁻¹) registered after physiological maturity.

Data were subjected to analysis of variance (ANOVA). When ANOVA detected significant (P < 0.05) differences among treatments, Tukey significant difference values (Tukey LSD, P = 0.05) were calculated for mean comparisons. Statistical analysis was performed using Statistical Analysis System version 6.12 and Statistica version 6.0 for Windows.

2.3. Genetic basis of resistance to root rot pathogens in selected common bean cultivars

We selected two common bean genotypes based on their contrasting reaction to FSP under both controlled and field conditions: BAT 477 (resistant) and Pinto UI-114 (susceptible). Crosses between the two parents were carried out under greenhouse conditions at Chapingo, México during 2002. F₁ to F₈ seeds were obtained in successive sowings in different locations of México. Reactions to a highly virulent isolate of FSP were measured in F₉ recombinant inbred lines [3]. A genetic linkage map was built with genotypic data obtained with 30 + 3/+3 AFLP. QTLs associated with resistance to FSP were identified using R software ver. 2.10.1 [14, 15].

3. Results

3.1. Variability of FSP isolates from Aguascalientes, México

Nineteen isolates of Fusarium were obtained from different locations of Aguascalientes, although most of them were collected in Pabellón. Ten isolates were FSP and the other nine were F. oxysporum f. sp. phaseoli (FOP). As controls, isolates from Guanajuato, México and Veracruz were included. FSP and FOP isolates showed a great variability on morphology (Figure 1). Most of the isolates showed radial growth of colony, purple color of colony and variation on mycelial production and conidia size and shapes (Table 2). Most of the common bean cultivars were susceptible to most of FSP isolates, mainly those from Mesoamerican genetic race. AFLP molecular markers clearly separated FSP isolates from FOP isolates, but pathogenicity patterns were not associated with Fusarium species (Table 3) [11].

Significant differences were found in morphology, pathogenicity and AFLP genotype among isolates. Isolates from Veracruz, Guanajuato and Aguascalientes grew faster in vitro than those from México and showed the largest conidia. The most pathogenic isolates were from Aguascalientes and Mexico. Bean cultivars with Flor de Mayo (Jalisco race) and Pinto (Durango race) seed coat showed the highest frequencies of resistance to the most of FSP isolates (Table 2). Isolates from the State of Mexico were genetically different from the other isolates with genetic dissimilarity of >9% [4].
Figure 1. *In vitro* variation of *F. solani* f. sp. *phaseoli* isolates from Aguascalientes, México.

| Isolate   | Origin             | Growth pattern | Color of colony | Conidia (µm) | Aerial mycelium |
|-----------|--------------------|----------------|-----------------|--------------|-----------------|
|           |                    |                | Conidia         | Length  | Width  | L/W |                |
|           |                    |                | Color of colony |          |        |     |                |
| AGS01     | Sta. Rosa          | Radial         | Pink            | 1.42     | 0.48   | 2.97 | +               |
| AGS02     | Pabellón           | "              | Purple          | 1.49     | 0.55   | 2.72 | —               |
| AGS03     | El Novillo         | "              | "              | 1.31     | 0.53   | 2.48 | +               |
| AGS04     | Pabellón           | "              | "              | 0.73     | 0.33   | 2.20 | —               |
| AGS05     | Sta. Rosa          | "              | White           | 1.32     | 0.40   | 3.31 | +               |
| AGS06     | Pabellón           | "              | "              | 1.92     | 0.91   | 2.11 | +               |
| AGS07     | Pabellón           | "              | Purple          | 1.22     | 0.34   | 2.09 | +               |
| AGS08     | Sta.Rosa/Loreto    | "              | White           | 1.38     | 0.52   | 2.68 | +               |
| AGS09     | "                  | "              | Dark purple     | 1.26     | 0.37   | 3.45 | —               |
| AGS10     | Pabellón           | Irregular      | Pink            | 1.19     | 0.46   | 2.61 | +               |
| AGS11     | Pabellón           | Radial         | Purple          | 1.43     | 0.34   | 4.19 | +               |
| AGS12     | El Molino          | "              | Dark purple     | 2.41     | 0.64   | 3.81 | —               |
| AGS13     | Pabellón           | "              | Purple          | 1.33     | 0.32   | 4.23 | —               |
| AGS14     | Pabellón           | "              | White           | 1.20     | 0.45   | 2.67 | +               |
| AGS15     | Pabellón           | "              | Pink            | 1.33     | 0.40   | 3.35 | +               |
| AGS16     | La Luz             | "              | White           | 0.86     | 0.32   | 2.60 | +               |
| Mean      |                    |                |                 | 1.48     | 0.46   | 2.89 |                |
3.2. Reactions of common bean germplasm to root rot pathogens under field conditions

Experiment I. The greatest root rot severity was found in Flor de Mayo M38, Pinto Zapata, and Azufrado Tapatío, while Pinto Villa, Tlaxcala 62 and Bayo Criollo del Llano showed the low severity. Tlaxcala 62, Bayo Criollo del Llano and Flor de Mayo M38 were more later than Pinto Villa, Azufrado Tapatío and Pinto Zapata. Pinto Villa, Azufrado Tapatío and Pinto Zapata exhibited the best agronomic characteristics (Table 4). A negative association between seed yield and root rot severity at vegetative and reproductive stages was found. Seed yield was negatively associated to days to flowering and days to maturity, while phenology was positively related to harvest index. Harvest index was found to be negatively associated to days to flowering and days to maturity. A positive relationship between root rot severity at vegetative and reproductive stage was found (Table 5).

| Isolate | Origin   | Growth pattern | Color of colony | Conidia (μm) | Aerial mycelium |
|---------|----------|----------------|-----------------|--------------|----------------|
| VER01   | Cotaxtla | Radial         | White           | 1.94 0.72    | 2.69 +         |
| GTO01   | Irapuato | Radial         | "              | 1.27 0.48    | 2.65 +         |
| MEX01   | Texcoco  | Irregular      | Yellow          | 1.28 0.47    | 2.72 +         |
| Mean    |          |                |                 | 1.50 0.56    | 2.69           |

Table 2. Morphological in vitro characteristics of F. solani f. sp. phaseoli isolates from Aguascalientes, México.

3.2. Reactions of common bean germplasm to root rot pathogens under field conditions

| Germplasm seed coat color/type (genotypes) | Genetic race       | Resistance (%) | Susceptibility (%) |
|------------------------------------------|--------------------|----------------|--------------------|
| 48 Mexican FSP isolates                  |                    |                |                    |
| Flor de Mayo (FM Sol, FM Bajío, FM M38)  | Jalisco            | 47             | 53                 |
| Pintos (PT Villa, PT Mestizo, PT Zapata) | Durango            | 47             | 53                 |
| Bayos (BY Zacatecas, BY Madero, BY Criollo del Llano) | Durango | 29             | 71                 |
| Black/Yellow (NG Altiplano, NG Vizcaya, Tlaxcala 62) | Durango/Mesoamérica/Jalisco | 7              | 12                 |
| 10 Aguascalientes FSP isolates           |                    |                |                    |
| Mesoamérica (BAT 477, TLP 19, SEQ 12, NG 8025, Rio Tibagi) | Mesoamérica | 32             | 78                 |
| Durango (BY Durango, PT Villa, PT UI-114) | Durango            | 13             | 87                 |
| Jalisco (BY Mecentral, AZ Tapatío)       | Jalisco            | 10             | 90                 |

Table 3. Resistance/susceptibility percentages in common bean germplasm classified by genetic races in response to inoculation with F. solani f. sp. phaseoli isolates.
| Experiment | Classification | Genotype                      | Days to flowering | Days to maturity | Seed yield (kg h\(^{-1}\)) | Root rot severity |
|------------|----------------|-------------------------------|-------------------|------------------|--------------------------|------------------|
| I          | Early          | PT Villa                      | 44                | 89               | 778                      | 4.9              |
|            |                | PT Zapata                     | 42                | 86               | 703                      | 6.6              |
|            |                | AZ Tapatio                    | 43                | 89               | 719                      | 6.2              |
|            |                | **Mean**                      | **43**            | **88**           | **733**                  | **5.9**          |
| Late       |                | FM M38                        | 56                | 98               | 547                      | 6.9              |
|            |                | Tlaxcala 62                   | 56                | 109              | 597                      | 5.0              |
|            |                | BY Criollo del Llano          | 56                | 100              | 546                      | 5.0              |
|            |                | **Mean**                      | **56**            | **102**          | **563**                  | **5.6**          |
|            |                | Tukey (P = 0.05)              | 1                 | 1                | 218                      | 0.9              |
| II         | Pinto-Resistant | PTD-99057                    | 50                | 97               | 782                      | 3.5              |
|            |                | PTD-99092                     | 36                | 88               | 739                      | 3.5              |
|            |                | PTD-99004                     | 46                | 96               | 871                      | 3.6              |
|            |                | **Mean**                      | **44**            | **94**           | **797**                  | **3.5**          |
| Pinto-Susceptible |             | PT Mestizo                   | 38                | 88               | 877                      | 5.8              |
|            |                | PTD-99008                     | 43                | 96               | 1056                     | 5.8              |
|            |                | PT Zapata                     | 36                | 88               | 705                      | 5.7              |
|            |                | **Mean**                      | **41**            | **91**           | **879**                  | **5.8**          |
| Flor de Mayo-Resistant |       | FMD-99033                    | 40                | 91               | 594                      | 3.3              |
|            |                | FMD-99019                     | 42                | 97               | 747                      | 3.7              |
|            |                | FMD-99004                     | 44                | 88               | 884                      | 3.7              |
|            |                | **Mean**                      | **42**            | **92**           | **742**                  | **3.6**          |
| Flor de Mayo-Susceptible |       | FMD-99022                    | 44                | 92               | 810                      | 6.0              |
|            |                | FMD-99013                     | 40                | 88               | 825                      | 5.9              |
|            |                | FMD-99002                     | 39                | 90               | 768                      | 5.9              |
|            |                | **Mean**                      | **41**            | **90**           | **801**                  | **5.9**          |
| Black-Resistant |            | NG Otomí                      | 43                | 98               | 1000                     | 2.3              |
|            |                | NG 8025                       | 54                | 96               | 855                      | 2.4              |
|            |                | NGD-99023                     | 50                | 97               | 1003                     | 2.6              |
|            |                | **Mean**                      | **49**            | **97**           | **953**                  | **2.4**          |
| Black-Susceptible |          | NG Altiplano                  | 51                | 97               | 848                      | 3.9              |
|            |                | NGD-99040                     | 46                | 100              | 1055                     | 3.9              |
|            |                | NGD-99028                     | 44                | 99               | 963                      | 3.8              |
|            |                | **Mean**                      | **47**            | **99**           | **955**                  | **3.9**          |
|            |                | Tukey (P = 0.05)              | 3                 | 2                | 290                      | 1.5              |
Experiment II. No clear relationship between root rot severity and seed yield was found (Figure 2a; Table 5). Grain yields ranged from 500 to 1250 kg h\(^{-1}\), but we found a clear differentiation among cultivars by reaction to root rots on the basis of seed coat color. Resistance was more frequent in black beans while intermediate reactions were found in Flor de Mayo germplasm and susceptibility was found in pinto beans. No differences on grain yield were detected between resistant and susceptible genotypes in any seed color type. Resistance was common in bred cultivars, as can be seen in pinto or Flor de Mayo bean types (Table 4).

### Table 4. Phenology, seed yield and root rot severity in common bean germplasm grown in Aguascalientes, México.

| Experiment | Classification | Genotype | Days to flowering | Days to maturity | Seed yield (kg h\(^{-1}\)) | Root rot severity |
|------------|----------------|----------|-------------------|-----------------|--------------------------|------------------|
| III        | Resistant      | SEA 20   | 46                | 93              | 798                      | 1.6              |
|            |                | RAB 636  | 43                | 91              | 689                      | 2.6              |
|            |                | BAT 477  | 47                | 95              | 1052                     | 2.6              |
|            | Mean           |          | 45                | 93              | 846                      | 2.3              |
|            | Susceptible    | FM 2000  | 47                | 101             | 862                      | 9.0              |
|            |                | SEA 16   | 43                | 91              | 780                      | 5.3              |
|            |                | SEA 15   | 43                | 91              | 688                      | 4.9              |
|            | Mean           |          | 44                | 94              | 777                      | 6.4              |
|            | Tukey (P = 0.05)|        | 4                 | 5               | 504                      | 3                |

Experiment Classification Genotype Days to flowering Days to maturity Seed yield (kg h\(^{-1}\)) Root rot severity
III Resistant SEA 20 46 93 798 1.6
RAB 636 43 91 689 2.6
BAT 477 47 95 1052 2.6
Mean 45 93 846 2.3
Susceptible FM 2000 47 101 862 9.0
SEA 16 43 91 780 5.3
SEA 15 43 91 688 4.9
Mean 44 94 777 6.4
Tukey (P = 0.05) 4 5 504 3

Table 5. Pearson’s correlation coefficients (r) among characteristics of common bean grown in experiments conducted at Sandovales and Chapingo, México.

| Characteristic | Root rot severity (56 d after sowing) | Days to flowering |
|---------------|----------------------------------------|-------------------|
| Experiment I  | Seed yield -0.25NS                      | -0.54**           |
|               | Days to flowering -0.17NS               |                   |
| Experiment II | Seed yield -0.11NS                      | 0.29**            |
|               | Days to flowering -0.10NS               |                   |
| Experiment III| Seed yield -0.07NS                      | 0.02NS            |
|               | Days to flowering -0.09NS               |                   |
| Combined irrigated-rainfed experiment | Seed yield -0.25** | 0.18** |
|               | Days to flowering 0.12*                 |                   |

* (p≤0.05); ** (p≤0.01).
Experiment III. As found in E-II, no clear relationship between root rot severity and seed yield was detected in this experiment (Figure 2b; Table 5). All 36 genotypes showed grain yields ranged from 550 to 1100 kg h\(^{-1}\). Here, we found that most of germplasm showed a root rot severity ranged from 2 to 5.5 (intermediate), while grain yield ranged from 550 to 1000 kg h\(^{-1}\). Only three cultivars were clearly different from all other cultivars: BAT 477 (that showed the highest seed yields), SEA 20 (that exhibited the lowest root rot severity) and Flor de Mayo 2000 (that showed the highest root rot severity). No differences can be appreciated on days to flowering or days to maturity or seed yield between resistant and susceptible cultivars (Table 4).

Rainfed-irrigated experiment. In Chapingo, germplasm showed later biological cycle and greater seed yields and root rot severity than at Sandovales. In both locations, rainfed conditions reduced seed yields and increased root rot severity (Table 6). In this experiment, negative relationship between seed yield and root rot severity was more clear than other experiments and a positive association was found between seed yield and days to flowering and flowering and root rot severity (Table 5). The relationship between root rot severity and grain yield exhibited different patterns across locations. In Sandovales, we found a greater variation on root rot severity on the germplasm, while an opposite response was found at Chapingo.

Figure 2. Relationship between root rot severity caused by *F. solani* f. sp. *phaseoli* and grain yield in common beans: (a) experiment II and (b) experiment III.
An opposite pattern was found in grain yield because higher seed yields were found at Chapingo (50–1880 kg h\(^{-1}\) under rainfed conditions and 230–3300 kg h\(^{-1}\) under irrigated conditions) than Sandovales (150–1150 under rainfed and 500–2300 kg h\(^{-1}\) under irrigated conditions) (Figure 3a and b). No differences on days to flowering are detected between the two groups of genotypes, but resistant germplasm exhibited greater seed yields than susceptible cultivars (Table 4).

### 3.3. Genetic basis of resistance to root rot pathogens in selected common bean cultivars

Genetic analysis identified one QTL significantly associated with resistance to FSP in BAT 477 growing under controlled conditions. This QTL explained 2.7% of variation in response to the disease and the marker was found at LG 5 [16].

|                        | Days to flowering | Seed yield (kg ha\(^{-1}\)) | Root rot severity (56 d after sowing) |
|------------------------|-------------------|-----------------------------|---------------------------------------|
| **Experiment Sandoval**|                   |                             |                                       |
| Rainfed                | 49                | 622                         | 5.7                                   |
| Irrigated              | 48                | 1141                        | 4.6                                   |
| **Experiment Chapingo**|                  |                             |                                       |
| Rainfed                | 53                | 940                         | 6.3                                   |
| Irrigated              | 54                | 1730                        | 5.4                                   |
| Tukey (P = 0.05)       | 1                 | 109                         | 0.7                                   |

**Resistant genotypes**
- G 2494: 53 days, 982 kg, 4.0
- G 4258: 44 days, 1015 kg, 4.1
- 97RS101: 52 days, 1273 kg, 4.3
- NG 8025: 54 days, 1303 kg, 4.6
- PT Zapata: 44 days, 1303 kg, 4.6
- **Mean**: 49 days, 1175 kg, 4.3

**Susceptible genotypes**
- G 801: 52 days, 947 kg, 8.1
- SEA 5: 51 days, 588 kg, 7.7
- G 14538: 58 days, 653 kg, 7.1
- G 4523: 48 days, 1094 kg, 7.0
- G 14645: 46 days, 763 kg, 6.7
- **Mean**: 51 days, 809 kg, 7.3
- Tukey (P = 0.05): 2 days, 556 kg, 2.4

Table 6. Agronomical characteristics on common bean germplasm under rainfed-irrigated conditions in two locations of México.
4. Discussion

4.1. Variability of *Fusarium solani* f. sp. *phaseoli* from Aguascalientes, México

A high morphologic, pathogenic and genetic variability was found in FSP isolates from Aguascalientes, despite the identical host (common beans) and geographical origin. In addition, no relationship among morphology, pathogenicity and genotype was found. Our results indicated the high values of genetic variability in the species due to the presence of heterokaryosis and parasexualism as genetic exchange mechanisms between vegetative compatible isolates. Single members of the same vegetative compatibility group (VCGs) are genetically similar and they are related on basis of genetic lineages [17]. The characterization of VCGs on *Fusarium* isolates from Aguascalientes and other regions of México could clarify the association among *Fusarium* populations and genetic lineages. This research confirmed the diverse and heterogeneous nature on the genus. Host specialization could be useful to establish artificial taxonomic divisions and to perform pathogenic groups and *forme speciales*. However, the host plays an important biological role in selection pressure to the fungus. In addition, the genetic exchange between isolates is supported by the development of VCGs or other strategies for DNA transmission. The evolution of pathogenicity and VCGs contribute to increase in molecular variability. Further research that includes traditional and molecular methodologies will improve the knowledge and understanding of *Fusarium* biodiversity.

The most of common bean cultivars were susceptible to most of FSP isolates, and all isolates were pathogenic to common beans. This result is opposite to Cramer et al. [18]. Most of the resistant germplasm belonged to Mesoamerica of Jalisco genetic races, while susceptible cultivars are classified as Durango race. High frequencies of resistance to other root rot pathogen (*Macrophomina phaseolina*) of common beans were found in Mesoamerican beans [19].
Results suggest that resistance to root rot pathogens in common beans could be operating as a resistance gene cluster that controls similar strategies to defend roots and stems against root rot fungi. Further research could confirm this suggestion. No clear association between host and fungus genotypes was found; this relation was reported in *M. phaseolina*-common beans [20] in contrast with other biotrophic pathogens of common bean as *Colletotrichum lindenuthianum* [21], where a clear formation of genetic lineages based on geographical origin was found.

4.2. Reactions of common bean germplasm to *F. solani* f. sp. *phaseoli* under field conditions

A high variation on reactions to FSP was found in both locations and no immunity was found, while no immunity to root rot pathogens in common bean germplasm was detected previously [8, 22, 23] in Pabellón de Arteaga, Aguascalientes and Chapingo, State of México. No clear association between root rot severity and seed yield or phenology was found in all experiments. However, results indicated that resistance to FSP was more frequent on black beans, while susceptibility was common on pinto beans, which has been found in previous works [20, 22]. Results suggest that black beans from Mesoamerican race could provide resistance to FSP in México. Under rainfed conditions, genotypes as BAT 477 and SEA 20 stood out for their high seed yields and resistance to root rot pathogens. BAT 477 has showed a consistent resistance to root rot pathogens such as *Fusarium*, *Rhizoctonia* and *Macrophomina* [20, 22, 24].

In both locations, rainfed conditions reduced seed yields and increased root rot severity in common bean germplasm. Navarrete-Maya et al. [23] reported a positive relationship between rain precipitation and *Fusarium* severity in Chapingo. We suggest that low water availability increased physiological stress in the host. Therefore, host defense mechanisms are not efficient to arrest fungal infection or for slow pathogenesis. The relationship between root rot severity and grain yield exhibited different patterns, since a broad variation on root rot severity on the germplasm (Sandovales) or an opposite response (Chapingo). Opposite patterns in grain yields were found, the highest seed yields were found in Chapingo and the lowest in Sandovales. Our data suggested that climate and fungi conditions of Sandovales are more appropriate for common bean germplasm screening for resistance to root rot pathogens under field conditions than Chapingo.

4.3. Genetics of resistance to *Fusarium solani* f. sp. *phaseoli* in common bean cv. BAT 477

Genetic analysis identified one QTL significantly associated with resistance to FSP in BAT 477 growing under controlled conditions. This QTL explained 2.7% of variation in response to the disease and the marker was found at LG 5 [16]. Identification of few QTLs with high effects on explanation of phenotypic variation is important and promising to simplify the
introgression of resistance genes to susceptible germplasm. However, our results indicated a low genetic effect of the QTL detected in BAT 477. Therefore, a more intensive searching of significant QTLs is needed. Genetic map development allows identification and use of genes and genomic regions (QTLs) with economic interest and then develops marker-assisted selection (MAS) strategies [25]. Vallejos et al. [26] performed the first gene map of common beans using morphologic, isozymes and RFLP markers. Then, Schneider et al. [27] identified 16 QTLs associated with *F. solani* f. sp. *phaseoli* resistance in *F* 4:6 RILs derived from Montcalm (susceptible) x FR266 (resistant). These QTLs were mainly found on LGs 2 and 5, and seven QTLs explained 64% of disease resistance. Chowdhury et al. [28] identified two QTLs associated with FSP resistance and explaining 50% of phenotypic variance using *F* 2:6 RILs from AC Compass (susceptible) x NY2114–12 (resistant), while Román-Avilés et al. [29] identified nine QTLs associated with resistance to FSP in *F* 4:5 inbred backcross populations from Red Hawk (susceptible) x NG San Luis (Resistant) and C97407 (susceptible) x NG San Luis, which explained from 5 to 53% of phenotype variation and located mainly at LGs 1 and 7.

5. Conclusions

We found significant genetic variability in FSP isolates from Aguascalientes and other regions of México although no clear association among morphology, pathogenicity or AFLP genotype was detected.

Under field conditions, we found high variation on reactions to FSP root rots; resistance was more frequent on black seed-coated beans, while susceptibility was common in pinto beans. We found a greater variation on root rot severity disease in Aguascalientes when compared with State of México, while an opposite response on grain yields was found across locations.

One QTL with low variance explanation of FSP resistance in BAT 477 was found; therefore, more intensive searching of significant QTLs is needed to improve marker-assisted selection strategies in common beans for México.

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References

[1] Servicio de Información Agroalimentaria y Pesquera (SIAP). Estadísticas de Producción Agrícola 2016. Available in http://www.siap.gob.mx (date of consulting: September 30, 2017)

[2] Acosta-Gallegos JA. In: Lépiz-Ildefonso R, editor. Taller de Mejoramiento de Frijol Negro Mesoamericano. PROFRIJOL; Veracruz, México; 1998

[3] Méndez-Aguilar R, Reyes-Valdés MH, Mayek-Pérez N. Avances y perspectivas sobre el mapeo genético de la resistencia a las pudriciones de la raíz en frijol común. Φyton, International Journal of Experimental Botany. 2013;82:215-226

[4] Martínez-Garnica M, Nieto-Muñoz F, Hernández-Delgado S, Mayek-Pérez N. Pathogenic and genetic characterization of Mexican isolates of Fusarium solani f. sp. phaseoli (Burk.) Snyd. & Hans. Revista de la Facultad de Agronomía (Universidad del Zulia, Venezuela). 2014;31:539-557

[5] Mayek-Pérez N, Hernández-Delgado S. Charcoal rot or ashy stem blight. Research Techniques. Bean Improvement Cooperative. 2010. Available in http://www.bic.css.msu.edu/ResearchTechniques.cfm [date of consulting: August 12, 2017]

[6] Abawi GS, Pastor-Corrales MA. Root Rots of Beans in Latin America and Africa: Diagnosis, Research Methodologies, and Management Strategies. Cali, Colombia: CIAT; 1990. 114 p

[7] Hernández-Delgado S, Lira-Méndez K, Mayek-Pérez N. Analysis of Macrophomina phaseolina-common beans pathosystem. In: Rodríguez-Herrera R, Aguilar CN, Simpson-Williamson JK, Gutierrez-Sanchez G, editors. Phytopathology in the Omics Era. Kerala, India: Research Ringspot; 2011. pp. 231-250
[8] Acosta-Gallegos JA, Navarrete-Maya R, Ochoa-Márquez R. Identification of tolerant materials to root rots in rainfed beans. Phaseolus, Publicación Especial. 1993;8:103-108

[9] Acosta-Gallegos JA, Acosta-Díaz E, Padilla-Ramírez JS, Goytia-Jiménez MA, Rosales-Serna R, López-Salinas E. Mejoramiento de la resistencia a la sequía del frijol común en México. Agronomía Mesoamericana. 1999;10:83-90

[10] Servicio Meteorológico Nacional. Constantes climatológicas de México. México: Comisión Nacional del Agua; 2017. Available in http://www.smn.cna.gob.mx [date of consulting: August 22, 2017]

[11] Martínez-Garnica M, Hernández-Delgado S, Padilla-Ramírez JS, Mayek-Pérez N. Diversidad patogénica y genética de aislamientos de Fusarium de Aguascalientes, México. Revista Mexicana de Fitopatología. 2004;22:321-327

[12] López-Frías LC. Definición de prioridades de investigación fitopatológica para la zona templada del Altiplano Central de México. Agricultura Técnica en México. 1991;17:17-54

[13] Navarrete-Maya R, Acosta-Gallegos JA. Reacción de variedades de frijol común a Fusarium spp. y Rhizoctonia solani en el altiplano de México. Agronomía Mesoamericana. 1999;10:37-46

[14] R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2012. Available in http://www.R-project.org/

[15] Méndez-Aguilar R, Reyes-Valdés MH, Hernández-Delgado S, López-Salinas E, Cumpián-Gutiérrez J, Cantú-Almaguer MA, Mayek-Pérez N. Identification and mapping of QTLs associated with resistance to Macrophomina phaseolina and drought stress in common beans. Bean Improvement Cooperative. 2017;60:23-24

[16] Méndez-Aguilar R. Loci de Caracteres Cuantitativos Ligados con la Resistencia a Macrophomina phaseolina, Fusarium sp. y Sequía en Frijol Común [Tesis de Doctorado]. Altamira, Tamaulipas: Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, Unidad Altamira-Instituto Politécnico Nacional. 2013. 98 p

[17] Woo SL, Noviello C, Lorito M. Sources of molecular variability and applications in characterization of the plant pathogen Fusarium oxysporum. In: Bridge P, Couteaudier Y, Clarkson J. editors. Molecular Variability of Fungal Pathogens. CAB International. Oxon, United Kingdom. 1998. pp. 187-208

[18] Cramer RA, Brick MA, Byrne PF, Schwartz HF, Wickliffe E. Characterization of Fusarium wilt isolates collected in the central high plains. Bean Improvement Cooperative. 2002;45:38-39

[19] Mayek-Pérez N, López-Castañeda C, y Acosta-Gallegos JA. Reacción de germoplasma de Phaseolus sp. a Macrophomina phaseolina. Revista Fitotecnia Mexicana 2002;25:35-42

[20] Mayek-Pérez N, López-Castañeda C, López-Salinas E, Cumpián-Gutiérrez J, Acosta-Gallegos JA. Resistencia a Macrophomina phaseolina en frijol común bajo condiciones de campo en México. Agrociencia. 2001;35:649-661
[21] González M, Rodríguez R, Zavala ME, Jacobo JL, Hernández F, Acosta J, Martínez O, Simpson J. Characterization of Mexican isolates of Colletotrichum lindemuthianum by using differential cultivars and molecular markers. Phytopathology. 1998;88:292-299

[22] Mayek-Pérez N, Acosta-Gallegos JA, Esquivel-Esquivel G, Rosales-Serna R. Resistencia a patógenos de la raíz en frijol. Memorias del XXIV Congreso Nacional de Fitopatología; Ciudad Obregón, México. 1997. Resumen 99

[23] Navarrete-Maya R, Trejo-Albarrán E, Navarrete-Maya J, Prudencio-Sáinz P, Acosta-Gallegos JA. Reaction of bean genotypes to Fusarium sp. and Rhizoctonia solani in central Mexico. Annual Report Bean Improvement Cooperative. 2002;45:154-155

[24] Pastor-Corrales MA, Abawi GS. Reactions of selected bean germplasm to infection by Fusarium oxysporum f. Sp. phaseoli. Plant Disease. 1987;71:990-993

[25] Danzmann RG, Gharbi K. Gene mapping in fishes: A means to an end. Genetics. 2001;111:3-23

[26] Vallejos CE, Sakiyama NE, Chase CD. A molecular marker based linkage map of Phaseolus vulgaris L. Genetics. 1992;131:733-740

[27] Schneider KA, Grafton KF, Kelly JD. QTL analysis of resistance to Fusarium root rot in bean. Crop Science. 2001;41:535-542

[28] Chowdhury MA, Yu K, Park SJ. Molecular mapping of root rot resistance in common beans. Bean Improvement Cooperative. 2002;45:96-97

[29] Román-Avilés B, Kelly JD. Identification of quantitative trait loci conditioning resistance to Fusarium root rot in common bean. Crop Science. 2005;45:1881-1890
