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Screening of USDA Onion Germplasm for Fusarium Basal Rot Resistance

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Abstract: Fusarium basal rot (FBR) caused by *Fusarium oxysporum* f. sp. *cepae* (FOC) is a major threat to onion (*Allium cepa* L.) production and marketing worldwide. Finding new sources of FBR-resistance to develop synthetic cultivars is a priority for onion breeders. As there are no FBR-resistant short-day onion cultivars, 85 U.S. National Plant Germplasm System plant introduction onion accessions that originated from 23 different countries were screened for their FBR susceptibility. To compare FBR susceptibility of these accessions at their seedling and mature bulb stages, a susceptible check cultivar, NuMex Crimson, a partially resistant check cultivar, Serrana and its selected population, Serrana-sel, were included in the study. The seedling screening was performed after inoculating silica-sand media with a virulent FOC isolate 'CSC-515' at 1.0×10^4 macroconidia g^{-1} of sand. Each entry was evaluated twice in growth chambers, and percent survival was adjusted to the number of seedlings that germinated in the uninoculated trays. Mature bulbs produced in the field were screened by inoculating transversely cut basal plates with potato dextrose agar plugs containing spores of the same isolate at 3.0×10^4 macroconidia mL^{-1}. FBR severity and incidence were then calculated after 20 days of incubation. Significant variation was found among the accessions for FBR-susceptibility ($p < 0.001$) at both the seedling and the mature bulb stages. Two sets of 18 accessions were identified either for their higher seedling survival or higher mature bulb FBR-resistance compared to the checks. Among them, PI 256326 ('Baia Periforme', the originator cultivar of 'Serrana') had a higher seedling survival than both the checks, and a lower mature bulb severity than the susceptible check. Another accession, PI 656956 ('S015'), exhibited higher seedling survival than the susceptible check and a low FBR severity (4.3 on a 1 to 9 scale) and incidence (41.7%). These two accessions, which were known previously for their high intra-population heterogeneity and root or bulb resistance for FBR, respectively, show promise for incorporating FBR-resistance into short-day onion cultivars. The cultivar rankings could vary in future studies with a range of FOC isolates due to a high cultivar × isolate interaction as observed in past studies.

Keywords: disease incidence; disease severity; *Fusarium oxysporum* f. sp. *cepae*; mature bulb screening; plant introduction accession; seedling survival

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1. Introduction

Fusarium basal rot (FBR) is a major disease of onion (*Allium cepa* L.) worldwide caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cepae* (FOC). Severe crop loss of up to 60% has been reported in FOC-infested organic soils of the midwestern USA [1]. This disease is considered a significant threat to the summer non-storage onion production that occurs in the state of New Mexico, USA [2]. Development of FBR-resistant cultivars is a viable alternative to circumvent the use of large land acreage needed for crop rotation or costly soil fumigation, which is detrimental for beneficial soil microorganisms [3,4]. Resistance screening during the seedling stage is predominant in onion breeding programs [5–9]. This method could be accomplished by either inoculation of seed [5,6] or growing media [7–9]. In many studies, seedling screening was a reliable indicator of mature bulb resistance [5,8] and

moderate to highly FBR-resistant inbred populations were developed based on selection using seedling screening under controlled conditions [7,10].

As FOC is saprophytic in nature, it survives in field residue for a significant amount of its life cycle [2]. Screening resistant germplasm in fields infested with the pathogen is an important method in addition to the seedling screening [11,12]. However, field screening is prone to disease escape caused by the sporadic distribution of FOC in a field [13] or due to microbial antagonism and suppressive soil conditions created by non-pathogenic isolates [14]. These disease escapes result in little to no breeding gains for FBR resistance [15,16]. Moreover, FOC does not always produce visible symptoms during the field growing stage and more often causes damage of marketable bulbs during storage [2]. A mature bulb screening method was developed recently that can select FBR-resistant bulbs by creating ample infection in genetically different onion cultivars [17]. As two different host plant resistance responses against FOC were reported at both growth stages in the past [15,18], screening germplasm at the seedling and the mature bulb stages is necessary for a complete FBR resistance profile.

Although long-day and intermediate-day onion cultivars have exhibited FBR resistance, commercially available short-day cultivars do not possess any resistance to this disease [17,19]. As onion is a highly cross-pollinated species [20], finding different sources of FBR resistance is critical to develop FBR-resistant synthetic cultivars [7]. Transferring resistance between contrasting daylength categories or related *Allium* species is challenging due to a smaller bulb size as a result of insufficient daylength and sterile F_1 hybrids, respectively [20,21]. As a result, onion researchers are limited to only daylength specific cultivated onion germplasm. Moderate FBR resistance was reported for a single short-day cultivar, Serrana (Monsanto Vegetable Seeds, Woodland, CA, USA) after seedling screenings [6,22], therefore it was included as a check cultivar in a recent study [17]. However, FBR resistance in this cultivar is conditional, as it behaves similar to susceptible cultivars when exposed to a virulent FOC isolate during mature bulb inoculation [17].

Gene banks around the world play a pivotal role for researchers who want to incorporate valuable germplasm into their breeding programs. The National Plant Germplasm System (NPGS) of the United State Department of Agriculture (USDA), a part of the Global Germplasm Resource Information Network (https://www.grin-global.org/, accessed on 26 June 2021), is a collaborative effort to maintain genetic diversities of agriculturally important crops. Seeds of these crops, which are collected from countries around the world, are stored for long-term preservation in the gene banks across 20 locations of the United States. Seeds of onion are stored in the National Germplasm Repository located at Geneva, NY, USA. This repository holds a total of 1864 accessions of onion. Although 284 accessions were previously evaluated for FBR, we found 85 accessions that had not been evaluated for this trait previously. Due to the highly outcrossing nature of this crop, we hypothesized that there should be abundant variability present in these accessions for FBR susceptibility. Therefore, the objective of this study was to identify USDA plant introduction accessions that possess a higher seedling and mature bulb FBR-resistance than a few commercially available cultivars of short-day onion.

2. Materials and Methods

2.1. Plant Materials

Depending upon the availability of seeds, 85 plant introduction (PI) accessions of onion were identified from the NPGS, USDA, to evaluate for their FBR resistance at the seedling and mature bulb stages (Table S1). These accessions were located at the USDA—Agricultural Research Service, Northeast Regional PI Station, Plant Genetic Resources Unit, Geneva, NY, USA, and were not screened for FBR resistance previously. To ensure proper disease development and to compare an accession's susceptibility reactions to FBR, a susceptible check cultivar, NuMex Crimson [23], a partially resistant check cultivar, Serrana and its selected population, Serrana-sel, were included in the evaluations. FBR susceptibility reactions of the check cultivars and the selected population were solely based

on their mature bulb FBR resistance for multiple seasons (unpublished data) as seedling susceptibility information was only reported for Serrana [6,22].

2.2. Seedling Inoculation Screening

A standard seedling screening procedure was followed for inoculum preparation and inoculation with slight modification [24]. Starting in December 2018, fresh macroconidia suspensions of a highly virulent isolate of FOC, 'CSC 515', which was recommended for its ability to separate FBR susceptible and resistant individuals efficiently [6], were prepared for inoculation. Small potato dextrose agar (PDA) plugs containing FOC mycelia were placed in 250 mL potato dextrose broth located inside conical flasks. The conical flasks were then covered with aluminum foil and rotated with 90 rpm for two weeks at ambient temperature (~25–28 °C). After two weeks, the mixture was sieved through multiple layers of cheesecloth to eliminate fungal mycelia. The spore concentration in this inoculum suspension was calculated using a hemocytometer. Distilled water (1 L) containing a FOC spore suspension was mixed thoroughly with 13 kg of sterilized silica sand in four separate trays before planting seeds. Final spore concentrations of these trays were adjusted to 1.0×10^4 spores g^{-1} of sand. A comparable amount of only distilled water was applied to a fifth uninoculated control tray to observe seed germination. Onion seeds were surface sterilized in buckets with a 15% bleach (containing 8.25% NaOCl) solution in water for five minutes, rinsed three times with water, and air-dried before planting to remove any external source of pathogens. In each of the five trays, 75 seeds of eight entries (including accessions, one susceptible checks, 'NuMex Crimson', one partially resistant check, 'Serrana' or 'Serrana-sel') were sown 1.5 cm deep. The 'Serrana-sel' population was recently selected from 'Serrana' using a conidial inoculation method [17] and showed a higher level of mature bulb FBR resistance compared to 'Serrana' (unpublished data). This selected version was included into the study after the availability of its seeds. These trays were then placed inside a growth chamber in a randomized complete block design, where rows of each of the eight entries were randomized in every tray (to be considered as a replication or block), and were adjusted to an initial temperature of 22 °C, which was raised to 28 °C after seedlings emergence. In order to provide an adequate amount of light period for the accessions that came from different parts of the world and could belong to different daylength categories, a 16 h light period was maintained throughout the experiment as used in earlier onion seedling inoculation screening studies [8,15]. After four weeks of inoculation, the percent survival was determined by the number of seedlings alive in the inoculated tray divided by the total number of seedlings germinated in the uninoculated control tray. Each entry was screened twice (two runs), and the mean percentage of survival was calculated. Entries with low germination (<50%) in the uninoculated trays at least in one run were eliminated for the final survival percentage result.

2.3. Mature Bulb Inoculation Screening

Due to limited amounts of seed and low germination, all accessions were sown during August 2018 in black plastic trays containing a soilless growing medium in the greenhouse. Once plants were large enough by October 2018, the seedlings were field transplanted into raised beds (5.5 m × 0.56 m) arranged in a randomized complete block design with three replications per accession. Two rows per bed were transplanted and 10 cm plant-to-plant distance was established at the 4–5 vegetative leaves stage. Standard cultural practices of growing onions for the southern New Mexico were followed [25]. During the summer of 2019, mature bulbs were harvested when 80% of plant tops had lodged.

FOC inoculum was prepared using PDA with suspended 'CSC 515' spores (3.0×10^4 spores mL^{-1}) a night before inoculation to minimize spore germination [17]. Individual bulbs were inoculated by placing a 1 cm diameter PDA plug on the top of the transversely-cut (≈0.25–0.30 mm) basal plate. All of the inoculated bulbs of an entry were placed in a single black plastic bulb crate ($58 \times 37 \times 22$ cm^3) (Bekuplast GmbH, Ringe, Germany) with the inoculated surface facing upwards. High humidity (≈85%) conditions were

created for 24 h in these crates by covering the inoculated crates with black polyethylene bags (83.8 × 104 × 0.00254 cm^3, Poly-America Grand Prairie, TX, USA). These bulbs were then incubated in an open-air environment on shelves with proper ventilation to facilitate disease development at ambient summer conditions. After 20 days of incubation, 20 bulbs were selected arbitrarily from each crate, cut transversely again (≈0.25–0.30 mm depth), and visually rated for FBR disease severity on a scale of 1 to 9 [11,17]. In this scale, 'one' represented no FBR discoloration in the basal plate, 'two' indicated 1–10% of the basal plate was showing FBR discoloration, and 10% increments in disease symptoms for every subsequent increase in the scale. Scale 'nine' indicated most of the basal plate was symptomatic for FBR (>70% basal plate area). Disease incidence was calculated from the percentage of inoculated bulbs exhibiting FBR symptoms.

2.4. Statistical Analysis

Data analysis was performed using SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) of the accessions and the checks was conducted for seedling survival, mature bulb FBR severity, and mature bulb FBR incidence using the Proc GLM statement of SAS. For the seedling inoculation screening experiment, the main and the interaction effects of the factors, viz., PI accessions or checks, replications, and the runs, were studied on seedling survival. For the mature bulb inoculation screening experiment, the main and the interaction effects of the classification variables, viz., PI accessions or checks and replications, were studied either on the mature bulb FBR severity or incidence. Means and other statistics (standard deviations, standard errors, maximum values, and minimum values) were calculated for each of the accessions. Mean separation was performed using Dunnett's multiple comparison test to identify the accessions with less FBR at the seedling and mature bulb stages against the three checks. The Proc CORR statement was used to find any correlations between seedling survival, mature bulb FBR severity, and incidence of the accessions with higher FBR-resistance during the early stage of their growth. For the correlation analysis between the seedling survival, mature bulb FBR severity, and incidence, three individual values per accession were used.

3. Results

3.1. Seedling FBR Susceptibility

Germination rate was low for 55 accessions in the uninoculated trays, and therefore they were eliminated from the seedling screening result. Lower germination could be attributed to seed age and low seedling vigor. Survival percentages of 25 accessions were reported based on their higher germination (>50%) in the uninoculated control trays (Table 1).

Table 1. Mean percent survival (in descending order) of USDA onion PI accessions evaluated in the seedling stage after inoculation with *Fusarium oxysporum* f. sp. *cepae* during 2018–2019.

Accessions/Checks [x]	Uninoculated Seedling Germ % [y]	Adjusted Seedling Survival % [z] ± SE
PI 639911	54.7	38.5 ± 7.4
PI 656956	54.6	37.4 ± 4.6
PI 288275	64	34.7 ± 5.3
PI 576900	58	29.8 ± 3.3
PI 656922	86	29.7 ± 3.8
PI 656921	72	28.5 ± 4.4
PI 288079	80.7	27.8 ± 9.2
PI 256326	84	25.6 ± 9.9
PI 546110	78	24.4 ± 4.0
PI 261767	67.3	18.0 ± 7.3
PI 546109	61.3	17.7 ± 5.6
PI 174019	58	16.9 ± 7.3
PI 656897	51.3	16.9 ± 2.9
PI 656955	52.6	15.0 ± 4.8
PI 174018	78	12.9 ± 6.1

Table 1. *Cont.*

Accessions/Checks [x]	Uninoculated Seedling Germ % [y]	Adjusted Seedling Survival % [z] ± SE
PI 272255	61.3	11.4 ± 3.1
PI 656958	77.3	9.3 ± 4.4
PI 662405	87.3	8.9 ± 2.4
PI 656905	58	8.4 ± 2.0
PI 662393	57.3	7.0 ± 2.3
PI 662461	78	6.6 ± 1.8
PI 656942	61.3	2.4 ± 1.4
PI 656920	69.3	1.9 ± 1.1
PI 656947	74	1.7 ± 0.8
PI 662391	59.3	0.6 ± 0.4
Serrana-sel	79.3	23.8 ± 2.7
Serrana	41	8.0 ± 3.2
NuMex Crimson	36.1	4.2 ± 1.6

[x] The highlighted accessions had significantly higher (>Dunnett's$^{0.05}$) seedling survival compared to 'NuMex Crimson' (susceptible check) and/or 'Serrana' (partial resistant check). The three checks were also highlighted to indicate as references to compare seedling survival of the accessions. [y] Seedling germination in the uninoculated trays, averaged over two runs. [z] Mean seedling survival adjusted by the number of seedlings alive in the inoculated tray divided by the total number of seedlings germinated in the uninoculated control tray in two runs. Accessions with low germination (<50%) of the uninoculated trays at least in one run were eliminated for the final survival percentage result. SE is standard error of the mean.

Dunnett's *t*-test identified eight accessions, PI 288079, PI 288275, PI 256326, PI 576900, PI 639911, PI 656921, PI 656922, and PI 656956, with higher (>Dunnett's$^{0.05}$) seedling survival than 'NuMex Crimson' (4.2%). Out of these eight accessions, only PI 639911 and PI 656956 had higher survival than 'Serrana' (8.0%). No accessions showed higher survival than 'Serrana-sel' (23.8%), which demonstrated low seedling survival as well. The accessions with poor seedling germination in the uninoculated tray that produced mature bulb in the field, were evaluated only for their mature bulb severity and incidence.

There was significant variation ($p < 0.001$) among the USDA accessions for FBR susceptibility during the seedling stage (Table 2). No significant main effects of runs, replications, or the interactions between accessions, run, and replications were found in the seedling survival results (Table 2).

Table 2. Analysis of variance of percent survival of short-day USDA onion PI accessions and check seedlings at four weeks after inoculation with *Fusarium oxysporum* f. sp. *cepae* inside a growth chamber in two runs during 2019.

Sources [x]	df	Seedling Survival [y]	
	p	Mean Squares	*p*-Values
Accession	27	1519.3 ***	<0.0001
Run	1	443 [NS]	0.2443
Replication (Run)	6	351 [NS]	0.3754
Accession × Run	27	389.8 [NS]	0.2446
Accession × Rep. (Run)	162	155.7 [NS]	1
Error	120	323.5	

[x] Each of the short-day USDA accessions and three checks was evaluated for its seedling survival after inoculating with *Fusarium oxysporum* f. sp. *cepae* conidia (3.0×10^4 spores mL^{-1}) with four replications for two runs. [y] Mean seedling survival with respect to seedling germination of the uninoculated trays in two runs. Entries with low germination (<50%) of the uninoculated trays at least in one run were eliminated for the final survival percentage result. [NS, ***] Not significant, significant at $p < 0.001$, respectively.

3.2. Mature Bulb FBR Susceptibility

For the accessions evaluated for FBR resistance in both seedling and mature bulb stages (Table 1), only PI 656956 had lower (<Dunnett's$^{0.05}$) mature bulb severity than 'NuMex Crimson'. For the accessions that were evaluated only for their mature bulb FBR resistance, a paired mean comparison with Dunnett's *t*-test identified PI 222765, PI 289688,

PI 377901, PI 430372, PI 433311, and PI 639912 with lower (<Dunnett's$^{0.05}$) severity than 'NuMex Crimson' (sev. 6.3. inc. 73.3%) (Table 3). These and four more accessions, PI 286413, PI 344388, PI 441970, PI 433313, had a lower incidence than 'NuMex Crimson' (Table 3). None of the ten accessions were different than 'Serrana' for either severity or incidence (Table 3).

Table 3. Mean FBR severity (in ascending order) and incidence (%) of USDA onion PI accessions evaluated in the mature bulb stage after inoculation with *Fusarium oxysporum* f. sp. *cepae* during 2018–2019.

Accessions/Checks [x]	Mature Bulb Severity [y] ± SE	Mature Bulb Incidence [z] (%) ± SE
PI 289688	1.9 ± 0.2	11.7 ± 1.7
PI 430372	2.0 ± 0.1	13.3 ± 1.7
PI 433311	2.0 ± 0.9	18.3 ± 10.9
PI 377901	2.2 ± 0.1	20.0 ± 2.9
PI 222765	2.6 ± 0.4	25.0 ± 5.8
PI 639912	2.9 ± 0.5	33.3 ± 10.1
PI 656956	2.8 ± 0.3	33.3 ± 6.0
PI 433313	3.1 ± 0.2	27.0 ± 3.0
PI 286413	3.1 ± 1.1	26.7 ± 13.3
PI 344388	3.2 ± 0.1	31.7 ± 1.7
PI 441970	3.4 ± 0.4	30.0 ± 5.0
PI 656905	3.5 ± 0.8	33.3 ± 10.1
PI 174018	3.6 ± 0.3	36.7 ± 6.0
PI 656942	3.7 ± 0.4	36.7 ± 7.3
PI 656955	3.7 ± 0.4	53.3 ± 4.4
PI 344259	3.7 ± 0.6	45.0 ± 5.8
PI 656958	3.8 ± 0.6	45.8 ± 3.0
PI 272257	3.8 ± 0.9	40.0 ± 12.6
PI 344257	3.9 ± 0.2	41.7 ± 3
PI 414933	3.9 ± 2.0	80.0 ± 5.0
PI 274781	4.0 ± 0.6	41.7 ± 9.3
PI 656920	4.2 ± 0.6	41.7 ± 8.3
PI 273626	4.2 ± 0.7	43.3 ± 9.3
PI 272255	4.3 ± 0.5	41.7 ± 6.7
PI 656897	4.3 ± 0.8	43.3 ± 9.3
PI 656954	4.3 ± 0.9	48.3 ± 10.9
PI 256326	4.3 ± 1.9	41.7 ± 23.3
PI 662461	4.4 ± 0.6	45.5 ± 7.1
PI 656957	4.4 ± 0.8	53.3 ± 14.2
PI 344387	4.5 ± 1.3	45.0 ± 15.0
PI 546110	4.6 ± 0.8	46.7 ± 9.3
PI 639257	4.7 ± 1.5	48.3 ± 19.6
PI 264319	5.0 ± 0.7	52.6 ± 9.1
PI 288272	5.0 ± 1.2	52.3 ± 16.4
PI 546087	5.0 ± 1.0	50.0 ± 12.6
PI 433314	5.2 ± 0.3	53.3 ± 4.4
PI 546109	5.3 ± 0.6	55.0 ± 5.8
PI 656921	5.4 ± 0.6	58.3 ± 10.1
PI 656947	5.4 ± 1.4	54.6 ± 17.2
PI 433345	5.5 ± 1.7	56.7 ± 21.9
PI 639258	5.6 ± 1.4	58.3 ± 16.4
PI 639251	5.7 ± 0.7	60.0 ± 7.6
PI 354086	5.8 ± 0.2	63.3 ± 1.7
PI 288079	5.9 ± 1.6	65.0 ± 20.2
PI 248753	6.1 ± 1.0	67.5 ± 13.8

Table 3. *Cont.*

Accessions/Checks [x]	Mature Bulb Severity [y] ± SE	Mature Bulb Incidence [z] (%) ± SE
PI 546127	6.1 ± 0.1	65.0 ± 0.0
PI 639913	6.2 ± 1.1	68.2 ± 12.9
PI 344260	6.3 ± 0.5	71.1 ± 4.5
PI 344261	6.4 ± 0.1	68.3 ± 1.7
PI 288273	6.5 ± 0.8	71.7 ± 10.1
PI 656922	6.7 ± 1.2	73.3 ± 13.3
PI 656898	6.7 ± 1.2	76.2 ± 13.1
PI 656880	6.8 ± 0.2	78.3 ± 1.7
PI 639916	7.1 ± 0.5	80.0 ± 5.0
PI 639915	7.3 ± 0.6	90.0 ± 2.9
PI 662405	7.7 ± 0.4	87.2 ± 2.0
PI 639911	7.7 ± 0.6	88.3 ± 6.0
PI 342943	7.7 ± 0.8	83.3 ± 10.1
PI 414932	8.0 ± 0.3	88.3 ± 3.3
PI 576900	8.1 ± 0.7	90.0 ± 7.6
PI 255462	8.2 ± 0.8	93.3 ± 6.7
PI 662393	8.4 ± 0.3	93.3 ± 4.4
PI 662424	8.6 ± 0.2	94.6 ± 2.9
PI 233190	8.6 ± 0.2	94.8 ± 3.0
PI 261767	8.6 ± 0.2	96.4 ± 1.8
NuMex Crimson	**6.3 ± 1.1**	**73.3 ± 13.0**
Serrana	**4.6 ± 0.4**	**45.0 ± 5.0**

[x] The highlighted accessions had significantly lower (<Dunnett's$^{0.05}$) mature bulb FBR severity and/or incidence compared to 'NuMex Crimson' (susceptible check). The three checks were also highlighted as references. PI 288275, PI 174019, and PI 662391, which were evaluated in seedling screening, did not produce sufficient bulbs for the mature bulb screening. 'Serrana-sel' was only included in the seedling screening study and not the mature bulb screening study. [y] After 20 days of disease development 20 arbitrarily-selected, inoculated bulbs were rated individually for FBR severity on a scale of 1 to 9, where 1 = no symptoms, 2 ≤ 10% basal plate decay, 3 = 11–20%, 4 = 21–30%, 5 = 31–40%, 6 = 41–50%, 7 = 51–60%, 8 =61–70%, 9 > 70% of the basal plate symptomatic. SE is standard error of the mean [11,17]. [z] Percentage of 20 inoculated bulbs showing FBR symptoms after 20 days post inoculation.

Significant variation ($p < 0.001$) was also found in FBR-susceptibility of the accessions at their mature bulb stage (Table 4). The replication effect was also significant for both severity and incidence, which could be explained several ways. The accessions were inoculated replication-wise, i.e., replication-1 of all the accessions and the checks were inoculated first before moving into the next replication. Since the bulb maturity of the accessions varied widely and there was a large volume of bulbs to be inoculated manually, there were replications that were inoculated very late in summer and into autumn. As a result, the replication difference most likely reflects the differences of the fungal growth in the basal plate caused by the changing ambient temperatures during the incubation period in the shed. Another possible explanation for the replication variation could be variation in virulence from multiple batches of the FOC isolate 'CSC-515' culture being generated during the period of this study.

3.3. Correlation between the Seedling and Mature Bulb FBR Susceptibilities

For the accessions that were evaluated for FBR resistance in both the seedling and mature bulb stages, a very low and non-significant correlation ($r = 0.20$ with severity and $r = 0.19$ with incidence) was observed between the two growth stages. Among the eight accessions with higher seedling survival compared to 'NuMex Crimson', only PI 656956 (Sev. 2.83, Inc. 33.33%) had a lower mature bulb severity than the check cultivars (Table 2). PI 256326 (Sev. 4.3, Inc. 41.7%) exhibited a low mature bulb FBR severity and incidence though not different from the checks. These two accessions should be a possible choice for further FBR resistance evaluations.

Table 4. ANOVA of visual FBR severity and incidence (%) in mature bulbs of USDA PI accessions evaluated in 2018–2019 season.

Sources [x]	df	Visual Severity [y]		Visual Incidence [z]	
		Mean Squares	*p*-Values	Mean Squares	*p*-Values
Accession	66	10.2 ***	<0.0001	3639.0 ***	<0.0001
Replication	2	14.7 ***	0.0003	1537.8 ***	<0.0001
Error	132	1.7		241.4	

[x] Entries were tested in a randomized complete block design with three replications. [y] After 20 days of disease development 20 arbitrarily-selected, inoculated bulbs were rated individually for FBR severity on a scale of 1 to 9, where 1 = no symptoms, 2 ≤ 10% basal plate decay, 3 = 11–20%, 4 = 21–30%, 5 = 31–40%, 6 = 41–50%, 7 = 51–60%, 8 =61–70%, 9 > 70% of the basal plate symptomatic. [z] Percentage of 20 inoculated bulbs showing FBR symptoms after 20 days post inoculation. *** Significantly different at $p < 0.001$.

4. Discussion

4.1. Response to Seedling and Bulb Inoculation

In this study, overall mean seedling survival (%) of the USDA NPGS accessions was very low. The partial resistant checks that had high mature bulb FBR severity and incidence in our trials (unpublished data), exhibited low seedling resistance in the growth chamber study. This result demonstrated that both 'Serrana' and its selection, possess mature bulb FBR resistance and not seedling resistance. A low seedling survival after inoculation of USDA NPGS accessions has been observed also by earlier seedling screening trials in growth chambers for FBR resistance [7,24]. Growth stage specific FBR resistance of eight accessions that exhibited higher seedling survival than the check cultivars, was confirmed by the very low correlation between seedling survival (%) and mature bulb infection. This result is in agreement with our earlier finding which indicated that there are two different mechanisms of resistance that confer seedling and mature bulb resistance, and FBR resistance of the seedling stage might not translate into the mature bulb stage [15].

Grouping cultivar populations based on their reactions to FOC is an efficient way to make progress for FBR resistance breeding as no known mechanism of resistance has been identified [6]. Recently, quantitative trait loci (QTL) for FBR resistance have been identified using a putative marker-trait association study utilizing different daylength-adapted germplasm [26]. This result is in agreement with earlier findings that suggest that multiple nuclear and cytoplasmic genes interact together to develop FBR-resistant phenotypes [7]. Since these gene pools comprise both minor and major genes [27] and are daylength specific [26], selections for locally adapted cultivars are crucial. The two short-day accessions, PI 256326 and PI 656956, with higher seedling survival than the check cultivars and moderate to high mature bulb FBR-resistance, are suitable candidates for FBR-resistant cultivar development trials intended for the southwestern USA.

4.2. PI 256326 ('Baia Periforme')

This germplasm originated from Brazil, one of the top ten producers of onion in the world [28]. The pedigree of 'Baia Periforme' indicated that it is part of a Portuguese introduction 'Garrafal' [28]. Starting from the 1970s, 'Baia Periforme' germplasm was heavily used by the onion breeding program of the Agronomic Institute of Pernambuco, Brazil, for selecting several varieties with superior breeding traits, such as high temperature adaptation, bulb yield, earliness, and tolerance to major biotic stresses [28]. Good bulb production of this accession in the research fields of New Mexico is due to the fact that it is well adopted into the irrigated agricultural practices of the semi-arid regions of Northeast Brazil [28].

FBR performance of 'Baia Periforme' has been recognized as moderately susceptible at the early growth stage by others [29]. They suggested that early susceptibility against FOC is due to effective root colonization by the fungus [29]. Unlike our study that identified the mature bulb FBR resistance of 'Baia Periforme' as partially resistant based on its mature bulb FBR severity and incidence compared with the partial-resistant check 'Serrana' (Table 3),

they categorized the cultivar as 'susceptible' (51% disease index) or 'moderately susceptible' (60% incidence) [30]. The first reason for the discrepancy of FBR resistance behavior of the 'Baia Periforme' population used in the study could be related to the high intra-population heterogeneity of FBR resistance of this cultivar [30]. The population used in this study could be a subset of the original population comprising a majority of FBR resistant bulbs, as earlier study showed that FBR resistant bulbs of this cultivar could survive a three months storage period [30]. Secondly, because of the high isolate-cultivar interaction [6], FBR resistance behavior of an onion cultivar could differ in two different geographical regions based on the difference of the respective FOC isolate virulence [19]. Being a landrace cultivar with high genetic variability, 'Baia Periforme' generated numerous strains after selection by individual growers in different areas of Brazil [31]. Interestingly, our partially resistant check, 'Serrana, originated from a strain of 'Baia Periforme' and was selected for uniform characteristics for many generations at Paulinia, Brazil [31]. Consequently, these two cultivars very closely resemble each other in many characters [31]. As with 'Serrana-sel', which was selected from 'Serrana' and showed very high mature bulb resistance (unpublished data), our results in this study suggest an opportunity for further selection to reduce within-population variability of 'Baia Periforme' to be used as potential breeding line in the New Mexico State University (NMSU) onion breeding program.

4.3. PI 656956 ('S015')

The cultivar information provided for this accession in the USDA NPGS is limited. The passport data indicated that this accession produces aggregated bulbs, an indication that it could belong to the shallot group (*Allium cepa* L. var. *aggregatum* G. Don) of cultivated onion. This group is very close to the single bulb producing group *Allium cepa* L. var. *cepa* and cultivated worldwide. Information about FBR resistance mechanisms about shallot is lacking and has been investigated only recently [32–34].

An investigation of the mechanisms of shallot resistance against FOC showed that actively growing shallot plants with bulbs and roots when challenged by FOC produced no symptoms of FBR in the above and below ground plant parts [32]. Even though the roots can harbor the pathogen, fungal penetration is prevented due to increases in the total saponin and phenolic compound concentration after inoculation [32]. A subsequent biochemical analysis indicated that FBR resistance of this species is due to spirostanol saponins, alliospiroside A and alliospiroside B, that were highly effective in controlling FOC and a number of other fungal species [35]. Together with these studies, we hypothesize that FBR resistance of shallot, that is moderate in the seedling stage (Table 1), increases with plant age [2] and become highest in the mature bulb stage (Table 3) [32,35]. High FBR resistance in the basal plate tissue of PI 656956 (Table 3) in the present study could be explained by the fact that levels of alliospiroside A and alliospiroside B were found highest in this tissue [35].

FBR resistance in cultivated onion is quantitative in nature [26] and the inheritance of this resistance was studied in interspecific hybrids of *Allium* [26,36]. FBR resistance genes were recently located on chromosome 2A of shallot [32]. As shallot is very closely related to single bulb producing cultivated onion, PI 656956 could be a valuable asset in the NMSU onion breeding program to investigate the heredity of the FBR resistance genes in reciprocal crosses between cultivated onion and shallot. Shallot and onion both possess antifungal furostanol saponins, ceposides, that vary in their antifungal activities against FOC [35,37]. Due to the outcrossing nature of this crop and high cultivar-FOC isolates interaction [6], onion cultivars are expected to differ in their antifungal saponin contents. As saponins are constitutive or pre-existing compounds in plants including *Allium* [38], comparisons of NMSU cultivars with PI 656956 for antifungal saponin could be a possible criterion for a biochemical selection strategy in the near future.

5. Conclusions

Seedling and mature bulb screenings of 85 USDA short-day onion germplasm accessions indicated differences in their FBR-resistance at young and adult plant growth stages after inoculating with a virulent FOC isolate 'CSC-515'. Two sets of 18 accessions were identified for either their higher seedling survival or higher mature bulb FBR-resistance. Among them, two accessions, PI 256326 and PI 656956, had a higher seedling survival, and moderate to high mature bulb FBR-resistance compared with the check cultivars. These accessions should be included for further trials at onion breeding program at NMSU to develop FBR-resistant synthetic cultivars. The cultivar rankings could vary in future studies with a range of FOC isolates due to a high cultivar × isolate interaction as observed in past studies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7070174/s1, Table S1: USDA onion accessions evaluated for Fusarium basal rot resistance at seedling stage.

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