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Selection Progress for Resistance to Fusarium Basal Rot in Short-Day Onions Using Artificial Inoculation Mature Bulb Screening

Suman Sharma and Christopher S. Cramer *

Department of Plant and Environmental Sciences, New Mexico State University, N127 Skeen Hall, MSC 3Q, Las Cruces, NM 88003-8003, USA
* Correspondence: cscramer@nmsu.edu; Tel.: +1-575-646-2657

Abstract: Fusarium basal rot (FBR), caused by a soil-borne fungus, *Fusarium oxysporum* f. sp. *cepae* (FOC), is a major disease hindering onion production worldwide. This study was conducted to evaluate the initial and the most advanced selected populations of seven open-pollinated short-day onion cultivars for FBR susceptibility, along with two check cultivars using the conidial inoculation of mature bulbs for two consecutive years. The artificial inoculation of mature bulbs was carried out by applying a virulent FOC isolate 'CSC 515' at a final concentration of 3.0×10^4 spores mL^{-1} to the transversely cut basal plates of onion bulbs. The basal plates of 20 arbitrarily chosen bulbs per plot were recut after 20 days of incubation and then were rated for FBR severity using a rating scale of 1 (no disease) to 9 (≥70% of the basal plate is infected). The bulbs with a rating of 1 were saved and then bulked to form the seeds for the next generation. The selected populations exhibited a variable response for FBR severity when evaluated over two years, with an improvement in the most advanced selections observed for a majority of the cultivars. For example, the advanced selections of 'NuMex Sweetpak' exceeded the partially resistant check 'Serrana' in their levels of resistance when both were evaluated in the second year. A conidial inoculation can be effective in the development of FBR-resistant cultivars. In addition, this inoculation method can accelerate breeding efforts by determining the genetic mechanism(s) responsible for FBR resistance, locating quantitative trait loci, and facilitating marker-assisted selection.

Keywords: *Allium cepa*; *Fusarium oxysporum* f. sp. *cepae*; disease severity; conidial inoculation; quantitative resistance

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1. Introduction

Onion (*Allium cepa* L.) is an important vegetable and spice crop grown worldwide. It is a biennial crop that produces bulbs in the first year and seeds from the planted bulbs in the second year. It is a cross-pollinated crop with a diploid chromosome number (2n = 2x = 16) [1]. Onion is an economically important crop in the United States, with a total of 55,000 harvested hectares and an economic value of 1 billion USD in 2021 [2]. In New Mexico, 2600 hectares of dry onions were harvested in 2021 with a value of 130 million USD [3].

Bulb initiation in onions is influenced by daylength, with lengthening days promoting bulb development. Based on the daylength requirement, onions have been divided into three major categories: long-day onions, which require > 16 h of daylength for bulb formation, while intermediate-day and short-day ones need 13–14 and 11-12 h of daylength, respectively [4]. In New Mexico, short-day and intermediate-day onions are grown, and onion bulb production faces several biotic and abiotic challenges during the growing season that can lead to reduced yield.

Fusarium basal rot (FBR) is one of the most devastating diseases in onions and is caused by a soil-borne fungal species, *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cepae*

(H.N. Hans.) W.C. Snyder & H. N. Hans. (FOC) [5]. This pathogen can attack at any stage during the life cycle of an onion plant; however, seedlings and mature bulbs are the most susceptible stages [6]. It can either directly penetrate the basal plate area [7] or invade through the roots [8]. The symptoms of the disease include the damping-off of seedlings, the chlorosis and necrosis of leaves, brown discoloration of the basal plate area of mature bulbs, root death, root abscission, and, eventually, plant death [6]. The exact losses due to FBR have not been documented well; however, a ~45% yield loss after harvesting and 12–30% bulb loss in storage have been observed in shallots (*Allium cepa* L. var. *aggregatum* G. Don), which is a close relative of common bulb onion [9]. In southern New Mexico, a disease incidence of 40% for autumn-sown and 29% for winter-sown cultivars has been reported [10]. The control measures for FBR include soil solarization [11], crop rotation [7,12], use of soil fumigants such as methyl bromide or metam sodium [13] and fungicides such as benomyl [14,15], biological control [16], and host plant resistance. Host plant resistance is the most effective and economical approach to combat FBR [7] because of the limitations of other control measures. Moderate to very high levels of resistance have already been incorporated into intermediate- and long-day commercial hybrid onion cultivars [6]. However, FBR-resistant cultivars still need to be developed for short-day onions. A good screening method is a prerequisite for the development of resistant cultivars. Different screening procedures have been evaluated for their ability to distinguish between susceptible and resistant individual plants. For example, a seedling screening has been quite effective for developing disease-resistant germplasm for intermediate- and long-day onions [6,17,18]. However, this method has not worked well for the improvement of short-day onions [19]. Under natural field conditions, the non-uniform distribution of the pathogen [20] can make it difficult to achieve a substantial selection gain [19,21]. Additionally, a strong genotype by isolate interaction can cause varying FBR susceptibility responses [22]. These challenges can make selection progress difficult to achieve.

A successful artificial inoculation mature bulb screening (AIMBS) method has been developed by the onion breeding program at New Mexico State University (NMSU) in which a conidial inoculation is used to artificially inoculate mature bulbs with a virulent isolate of the pathogen in order to select disease-resistant bulbs [23]. Phenotypic recurrent selection has been used for the improvement of cross-pollinated crops in order to increase the frequency of desirable alleles through repeated selection [24]. Based on past studies at NMSU, it was observed that FBR severity is a moderately heritable trait [25]; therefore, selection can be effectively used for improving FBR resistance. Based on this information, we hypothesized that a conidial inoculation would be a reliable screening method to discriminate FBR-susceptible and FBR-resistant cultivars, which is an important quality for any screening method. We also hypothesized that screening using a conidial inoculation would reduce FBR severity of short-day onion cultivars after a few generations of recurrent phenotypic selection. The objective of this study was formulated to assess our efforts to reduce the FBR severity by evaluating an initial and the most advanced selected populations of seven autumn-sown, short-day, New Mexican onion cultivar populations generated at NMSU along with two check cultivars using a conidial inoculation of mature bulbs.

2. Materials and Methods

2.1. Location of Study and Plant Material

The study was conducted at the Fabian Garcia Science Center (FGSC) in Las Cruces, NM, USA (32.2799° N, 106.7725° W) for the growing season of 2019–20, and the Leyendecker Plant Science Research Center (LPSRC), La Mesa, NM, USA (32°11′54.7″ N 106°44′25.9″ W) for 2020–21. The selected populations of seven autumn-sown, Grano-type, open-pollinated short-day onion cultivars belonging to three different maturity groups [Early maturity: 'NuMex Camino', 'NuMex Sweetpak'; Intermediate maturity: 'NuMex Chaco', 'NuMex Crispy', 'NuMex Mesa'; Late maturity: 'NuMex Luna', 'NuMex Vado'] were field planted [26–31]. These selected cultivars have shown less FBR disease incidence in field trials [32,33], and are resistant to the pink root fungal disease (caused by *Phoma terrestris* E.M. Hans.), which is presumed to be

correlated with FBR [6]. Moreover, these cultivars were not developed for FBR resistance, so the levels of resistance were expected to be low. Two check cultivars, a partially resistant, 'Serrana', [34] and a susceptible, 'NuMex Crimson' [35], were also included in this study to assess the reliability of the screening method.

2.2. Onion Bulb Production and Selection of Fusarium Basal Rot (FBR)-Resistant Bulbs via Conidial Inoculation

The selected populations of seven cultivars were planted on raised beds and arranged in a randomized complete block design with four replications for two consecutive years in the autumn of 2019 and 2020. Two rows were direct seeded on each bed, and the plant-to-plant distance was maintained at ~10 cm. Subsurface drip irrigation tape (T-Tape; T-Systems International, San Diego, CA, USA) with 20 cm emitter distances was placed 10 cm below the soil surface and was used to apply fish fertilizer (2N-3 P-1 K; Neptune's Harvest, Gloucester, MA, USA) and acid-based liquid fertilizer (26 N-0 P-0 K-6 S; Western Blend, Inc., Las Cruces, NM, USA). The harvesting was undertaken in the summer (May–July) of 2020 and 2021 for the trials sown in October 2019 and 2020, respectively, when 80% of the tops were lodged.

The evaluation of the selected populations for FBR resistance was conducted through conidial inoculation using a virulent FOC isolate 'CSC-515' [Figure 1A] [23,36]. The highly virulent local isolate was selected for the screening of FBR-resistant germplasm based on its ability to effectively distinguish between FBR-resistant and FBR-susceptible individuals [22]. In the conidial inoculation, a spore suspension was prepared, and a day before the artificial inoculation of the mature bulbs, the plates were prepared by mixing the spore suspension with prepared potato dextrose agar (PDA) to make a final concentration of 3.0×10^4 spores mL^{-1}.

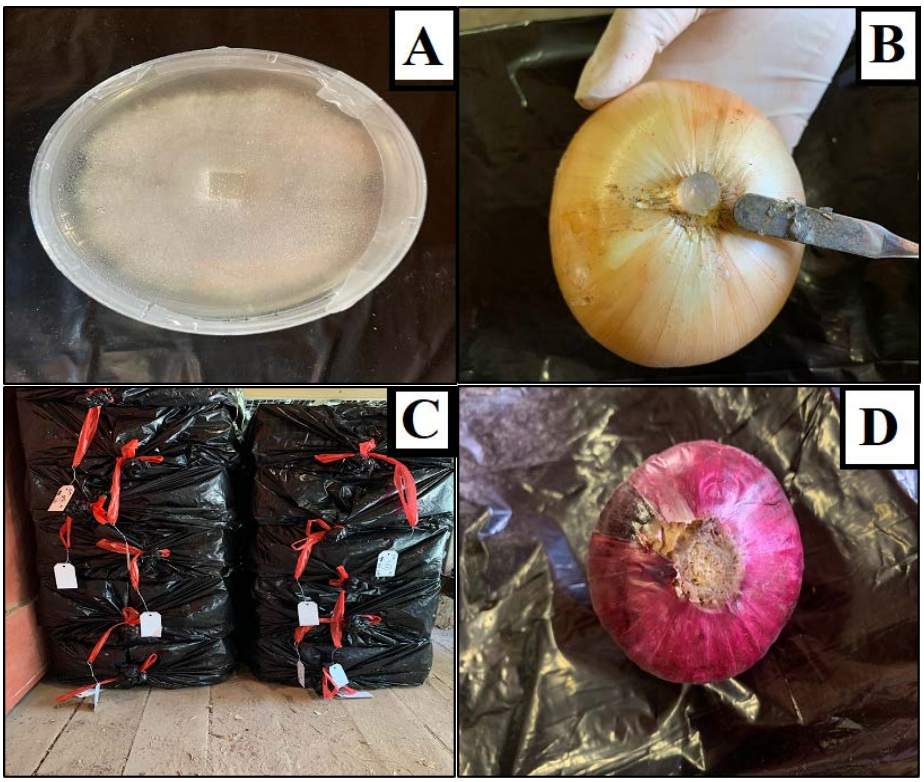

Figure 1. Conidial inoculation procedure for selection of Fusarium basal rot (FBR)-resistant onion bulbs (**A**) a culture of *Fusarium oxysporum* f. sp. *cepae* (FOC), (**B**) artificial inoculation of a transversely cut basal plate with conidia suspended potato dextrose agar (PDA) plug, (**C**) incubation of inoculated bulbs for ~24 h to create high humidity for disease development, (**D**) a susceptible onion bulb with a rating of 9.

For inoculation, 1-cm-diameter plugs from the spore-suspended PDA plates were used to inoculate the transversely cut (~0.25–0.30 mm) basal plates of the mature onion bulbs. Afterward, the inoculated bulbs were placed in a plastic crate and covered with a polyethylene bag to generate high humidity conditions for ~24 h [Figure 1B,C]. After 20 days of incubation, the basal plates of the bulbs were recut transversely to rate them for FBR disease severity using a severity rating scale of 1 (no disease) to 9 [≥70% of the basal plate is infected as shown in Figure 1D] [37]. The diseased bulbs were discarded, and the bulbs with a rating of 1 were saved and advanced to produce the seeds for the next generation.

2.3. Development of Different Populations of Each Cultivar

The starting generation (FBR1) was advanced through selection to generate advanced generations (FBR5 and FBR1-4) for each cultivar (Figure 2). Through previous research, the populations were evaluated and selected for FBR resistance using two different artificial inoculation mature bulb screening methods (mycelium and conidial inoculation) [23]. Mycelium inoculation uses actively growing fungal mycelium for the artificial inoculation of mature bulbs.

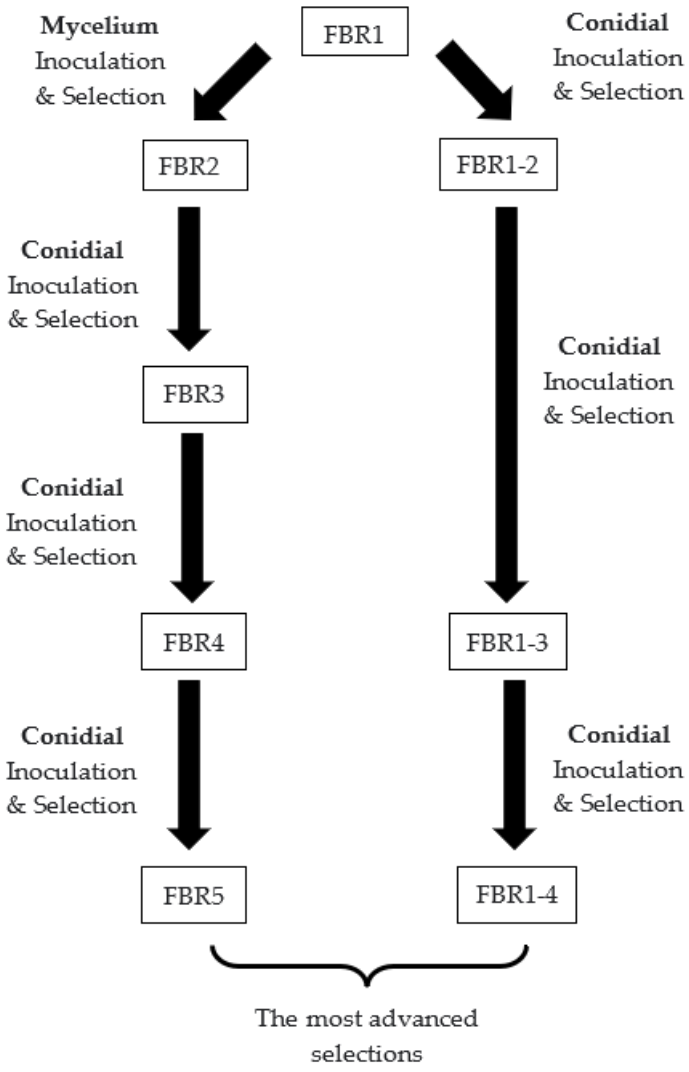

Figure 2. Selection scheme for development of cultivar populations using artificial inoculation mature bulb screening (AIMBS).

Previous research determined that this method was not entirely successful in obtaining an equal pathogen delivery for all of the inoculated bulbs [23]. The method resulted in the possibility of disease escape because it was difficult to deliver the same number of fungal spores to each bulb using mycelium. This method was later replaced with the conidial inoculation in which a quantified number of spores were delivered so that an equal number of pathogen spores could be placed on each bulb [23,36,38].

2.4. Evaluation of Mature Bulbs for FBR Susceptibility

In the summers of 2020 and 2021, the selected populations of seven cultivars, along with two checks, were evaluated for their FBR susceptibility. After the mature bulbs were harvested, they were placed in a well-ventilated onion shed for almost a month to allow for any disease development caused by natural FOC infection. Afterward, the healthy bulbs were artificially inoculated using the conidial inoculation procedure described previously. Twenty arbitrarily selected bulbs from each plot were rated for FBR severity by looking at the extent of disease symptoms in the basal plate tissue [23,36,37].

2.5. Seed Production of Selected Bulbs to Form Next Generation

At the end of the storage period in September–October, the remaining bulbs for each cultivar population were placed in a seed production cage that was covered with polyester fabric nets to maintain purity. During the flowering period in May, European honeybees (*Apis mellifera* L.) and blue bottle flies (*Calliphora vomitoria* L.) were placed in the cages for pollination. Umbels were harvested upon maturity when the seed capsules had turned brown.

2.6. Statistical Analysis

The statistical analysis was performed using SAS® Studio in a web-based environment known as SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). For each cultivar population/generation, mean plot severity was calculated from twenty bulbs per plot. Then, the plot means were used to calculate the mean FBR severity. Proc MEANS was used to calculate the plot means along with lower and upper limits for 95% confidence intervals. Proc MIXED statement was used in which replication was considered as a random effect. Pair-wise comparisons were also estimated for the populations of each cultivar to assess the improvement in reducing the FBR severity of the selected populations using the contrast statement.

3. Results

The artificial inoculation mature bulb screening via conidial inoculation was successful in producing disease for the evaluation and selection of the seven short-day onion cultivars. The selected cultivar populations exhibited differential expression to artificial inoculation depending upon the year of evaluation because the FBR severity of all the cultivars was less in 2021 than in 2020.

3.1. Reduction of FBR Severity of Advanced Selected Populations via Conidial Inoculation

The success of the conidial inoculation was observed in the performance of two check cultivars. The partially resistant check 'Serrana' exhibited less disease severity (5.53 in 2020; 3.24 in 2021) than the susceptible check 'NuMex Crimson' (8.15 in 2020; 8.85 in 2021) (Figure 3). Moreover, the selected populations of all of the cultivars exhibited a reduced disease severity in 2021 when compared to the susceptible check (Figure 4).

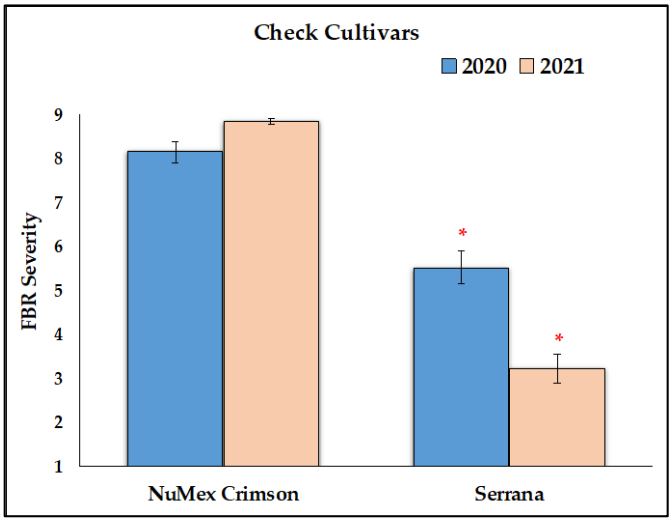

Figure 3. FBR severity of check cultivars in 2020 and 2021, where 'NuMex Crimson' is the susceptible check, and 'Serrana' is the partially resistant check. Error bars are the standard errors of the mean for FBR severity. * refers to means different from 'NuMex Crimson' at $p < 0.05$.

In the early maturity group, both 'NuMex Camino' [FBR5 (4.98) and FBR1-4 (6.11) in 2020; FBR5 (2.32) and FBR1-4 (1.98) in 2021] and 'NuMex Sweetpak' [FBR5 (4.81) and FBR1-4 (5.58) in 2020; FBR5 (1.71) and FBR1-4 (2.20) in 2021] exhibited no difference in FBR severity between two advanced selected populations. For 'NuMex Camino', there was no difference between the initial population, FBR1 (5.45 in 2020; 1.69 in 2021), and the two advanced selected populations (FBR5 and FBR1-4) in both years (Figure 4A). However, the advanced selections for 'NuMex Sweetpak' exhibited lower disease severity as compared to the initial population, FBR1 (7.85 in 2020; 2.89 in 2021), except for FBR1-4 in 2021 (Figure 4B).

For the intermediate maturity group, all three cultivars, 'NuMex Chaco' [FBR5 (5.00) and FBR1-4 (4.40) in 2020; FBR5 (1.64) and FBR1-4 (2.28) in 2021], 'NuMex Crispy' [FBR5 (5.04) and FBR1-4 (5.08) in 2020; FBR5 (4.68) and FBR1-4 (4.77) in 2021] and 'NuMex Mesa' [FBR5 (4.94) and FBR1-4 (4.26) in 2020; FBR5 (2.89) and FBR1-4 (2.44) in 2021] exhibited no difference in FBR severity between the two advanced selected populations. In 2020, the most advanced selections of all three cultivars exhibited less disease when compared to the initial population, FBR1 ['NuMex Chaco' (7.84) (Figure 4C), 'NuMex Crispy' (6.60) (Figure 4D), 'NuMex Mesa' (6.28) (Figure 4E)]. However, in 2021, only the most advanced selection (FBR5) of 'NuMex Chaco' (Figure 4C) exhibited a reduced disease severity as compared to its initial population, FBR1 (3.28), while no difference was observed for 'NuMex Crispy' [FBR1 (4.86)] (Figure 4D) and 'NuMex Mesa' [FBR1 (3.01)] (Figure 4E).

For the late maturity group, a difference was observed between FBR5 (6.14) and FBR1-4 (4.68) for 'NuMex Luna', and a comparison can be noticed by looking at the 95% error bars for average FBR severity (Figure 4F); however, no difference was observed between FBR5 (4.41) and FBR1-4 (4.69) for 'NuMex Vado' in 2020 (Figure 4G). In addition, no difference was observed between the most advanced selections for both cultivars, 'NuMex Luna' [FBR5 (1.90) and FBR1-4 (2.95)] and 'NuMex Vado' [FBR5 (3.49) and FBR1-4 (3.03)] in 2021. A reduced FBR severity was observed in the most advanced selections as compared to the initial population (FBR1) for 'NuMex Luna' [(7.46) in 2020; (5.00) in 2021] (Figure 4F) and for 'NuMex Vado' [(6.53) in 2020; (4.35) in 2021] (Figure 4G), except for FBR5 in 2021.

We also compared the advanced selected populations for each cultivar with the performance of the resistant check 'Serrana', and none of them were different except for one. The most advanced selections (FBR5 and FBR1-4) of 'NuMex Sweetpak' exhibited a disease severity that was less than the partially resistant check 'Serrana' in 2021 (Figure 5).

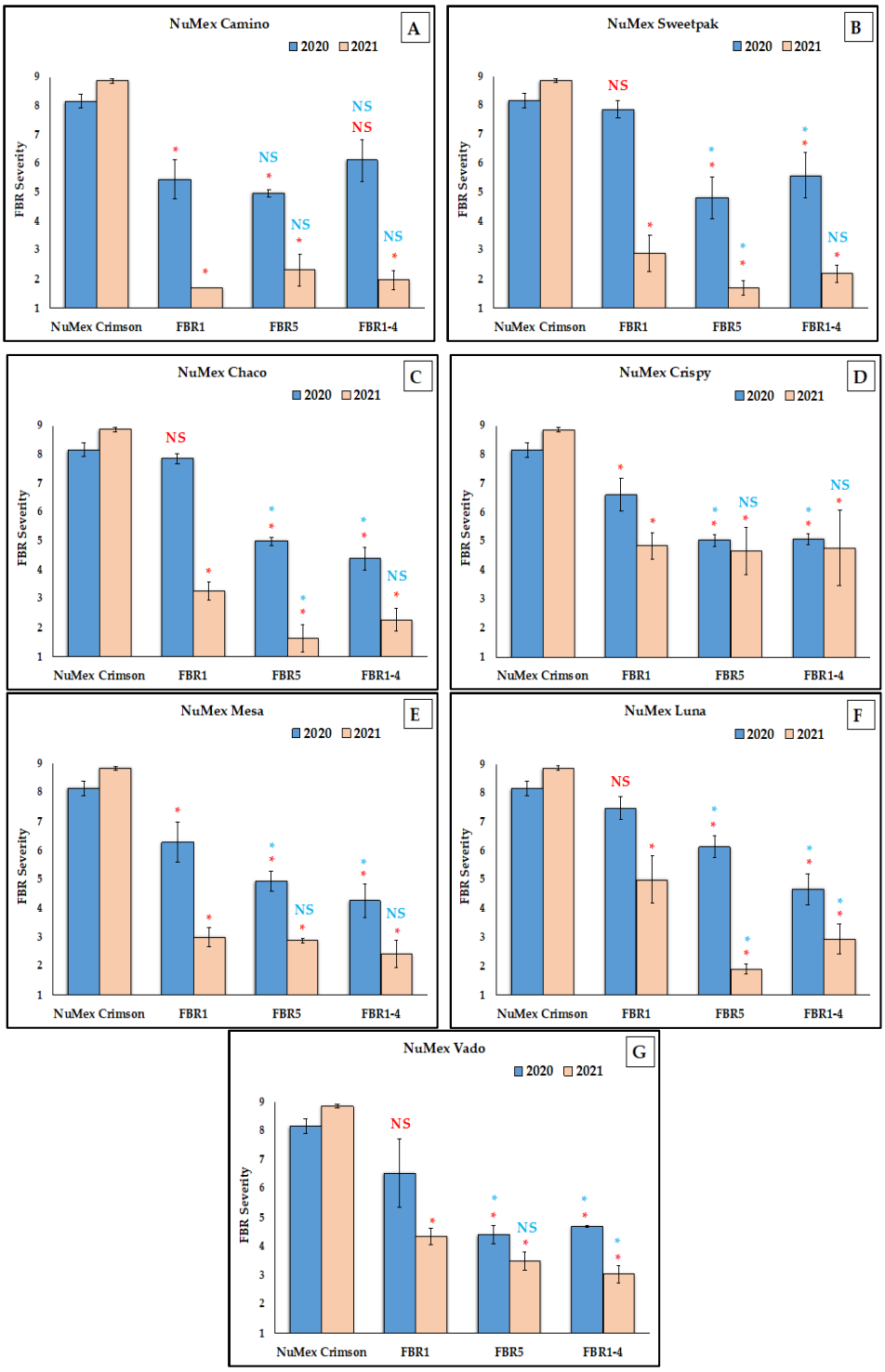

Figure 4. Mean FBR severity of seven short-day onion cultivars, 'NuMex Camino' (**A**), 'NuMex Sweetpak' (**B**), 'NuMex Chaco' (**C**), 'NuMex Crispy' (**D**), 'NuMex Mesa' (**E**), 'NuMex Luna' (**F**), and 'NuMex Vado' (**G**) in 2020 and 2021, where comparisons were made between the susceptible check 'NuMex Crimson' and three populations of each cultivar and between the initial population and the most advanced selected populations of each cultivar. Error bars are the standard errors of the mean FBR severity of each cultivar population. * refers to differences from 'NuMex Crimson', and NS means not different from 'NuMex Crimson' at $p < 0.05$. * refers to means of advanced selections different from FBR1, and NS means not different from FBR1 at $p < 0.05$.

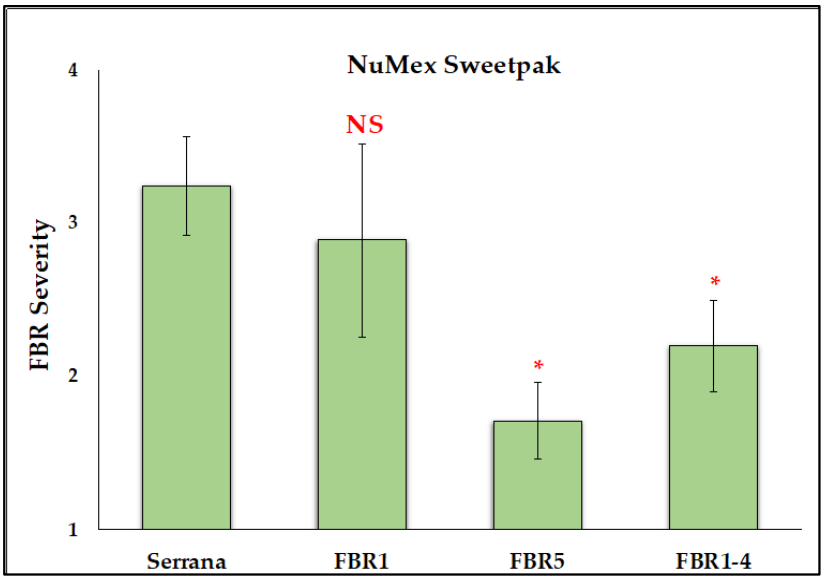

Figure 5. Increased levels of FBR resistance in the most advanced selections of 'NuMex Sweetpak' in comparison to the partially resistant check 'Serrana' in the year 2021. Error bars are the standard errors of the mean FBR severity. * refers to different from 'Serrana' and ^{NS} means not different from 'Serrana' at $p < 0.05$.

3.2. Within Population Variability for FBR Severity

To visualize the variation in FBR severity within a population, 95% confidence intervals (CIs) for all three populations of 'NuMex Vado' were determined for the years 2020 and 2021. All three generations of 'NuMex Vado' exhibited a higher disease severity in 2020 than in 2021 (Figure 6). In 2020, a reduced variation with a narrow range of severity ratings around the mean was observed for the most advanced selected populations. This result might suggest that selection has reduced the variation in rating values from bulb to bulb while also reducing the mean severity.

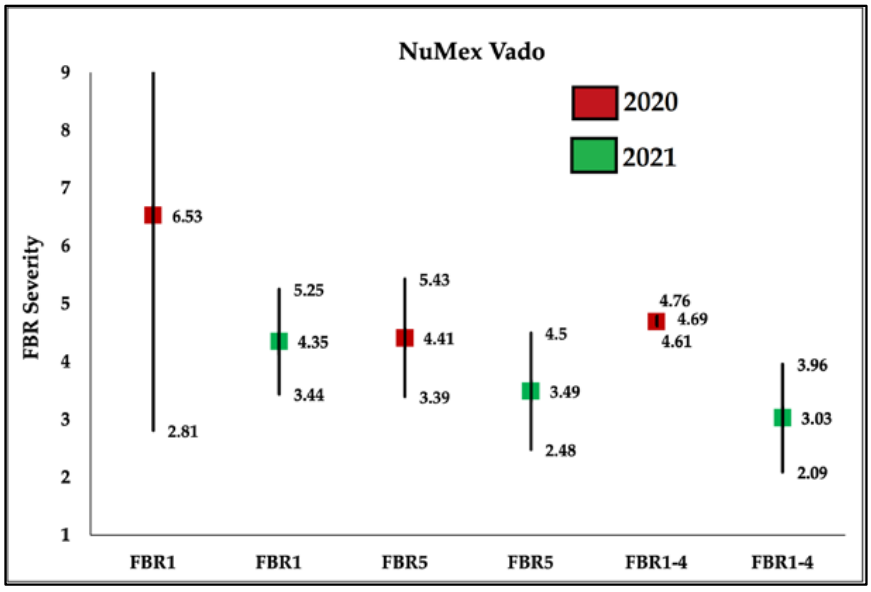

Figure 6. 95% confidence intervals (CIs) providing the range of possible values around point estimates for FBR severity of three selected populations (FBR1, FBR5, and FBR1-4) of 'NuMex Vado' in 2020 and 2021.

4. Discussion

4.1. No Difference between Two Advanced Selected Populations

The two most advanced selected populations (FBR5 and FBR1-4) developed after three cycles of conidial inoculation for each cultivar were compared to evaluate if there is any difference because the FBR5 generation has an additional cycle of mycelium inoculation. Despite an additional selection cycle, the FBR5 generation did not show any improvement as compared to FBR1-4. Therefore, an extra cycle of mycelium inoculation did not help to improve resistance. This result confirms our assertion that a mycelium inoculation is not effective for selecting FBR-resistant bulbs. Although higher disease pressure had been observed using mycelium inoculation as compared to the conidial inoculation [39,40], it has been recommended to use conidial because mycelium is hard to quantify, and a standardized spore concentration is difficult to obtain [40,41].

4.2. Germplasm with Lesser FBR Severity Than the Susceptible Check

The susceptible check 'NuMex Crimson' exhibited a severity rating of more than 8 in both years. Our selected cultivar populations exhibited less disease than the susceptible check, which suggests the presence of some level of resistance in the starting population (FBR1). This resistance could be improved by fixing the alleles responsible for FBR resistance using the phenotypic recurrent selection of the resistant bulbs via conidial inoculation. The selection can fix a higher proportion of the desirable alleles for the cumulative genetic progress over multiple cycles of selection [42].

4.3. More FBR Resistance in the Most Advanced Selected Populations

The most advanced selected populations (FBR5 and FBR1-4) for most of the cultivars had less FBR than the starting population (FBR1), suggesting that selection progress with increased FBR resistance had been accomplished in the advanced selections. Mandal and Cramer (2021) also used conidial inoculation screening for the evaluation of the same cultivar populations and observed an improvement in the FBR resistance of the most advanced selected populations [36]. Similarly, advanced selections of pearl millet (*Pennisetum glaucum* (L.) R. Br.), which is also a cross-pollinated crop, exhibited a greater disease resistance than the parental lines after five selection cycles [43]. It is clear that phenotypic recurrent selection has been successful in improving the FBR resistance trait due to its moderate heritability [25,36]. However, some of the advanced selections, for instance, 'NuMex Camino' in both years, did not exhibit a significant improvement over the initial population in our results which could be due to less change in the allelic frequency leading to less gain per selection [44,45].

4.4. Higher FBR Resistance Than the Partially Resistant Check

The partially resistant check 'Serrana' is not completely resistant to FBR because some of the bulbs of this cultivar also express disease symptoms. One of the cultivars, 'NuMex Sweetpak', exhibited more resistance in its most advanced selected populations (FBR5 and FBR1-4) in comparison to 'Serrana'. This might be due to an abundance of FBR-resistant alleles in the most advanced selected populations of the 'NuMex Sweetpak'. So, an elite population can be developed by selecting superior genotypes in every cycle of selection and discarding susceptible ones from further breeding cycles in a series of intermediate populations [46].

4.5. Reduction in within Population Variation

In addition to decreasing mean FBR severity, another goal is to reduce variation within a population after multiple cycles of selection by fixing the desirable alleles. Based on 95% CIs, it was observed that within-population variation decreased in the most advanced selected populations. However, some cultivar populations exhibited a greater variation depending upon arbitrarily selected bulbs for severity rating. Here, the sampling of the bulbs for rating (1–9) plays an important role because onion cultivars are highly heterozygous

and heterogenous, with each plant possessing a unique genotypic constitution contributing towards the huge variation within a population [47].

4.6. Yearly Differences of Same Cultivar Populations

The susceptible check 'NuMex Crimson' exhibited almost the same disease severity in both years; however, the partially resistant check 'Serrana' exhibited a reduced disease severity in 2021. Moreover, the FBR severity was reduced in the second year for our selected populations and populations of the same cultivar exhibited differences in improvement over the starting population (FBR1). For example, two of the cultivars from the intermediate maturity group did not exhibit any improvement over the starting population in the second year, but they did in their first year of evaluation. In addition, 95% CIs showing variation about the point estimate also presented yearly variation for our selected cultivar populations. Several factors might be responsible for these differences in our results. One of these factors might be the variation in the spore concentration of the spore suspension. Even a slight difference in spores counting and sporulation due to culture-to-culture variability can make a difference in imposing higher or lower disease pressure. It has been observed that spore counting using a hemacytometer can be variable because of the differences among individual hemacytometer squares that accounts for ~51% to 91% of the total variance, and it also depends on the uniformity of the spore suspension [48]. In our study, every onion bulb did not provide the same disease rating showing some bulb-to-bulb variability. So, there is an equal probability of selecting a bulb with a rating of 1 through 9 since each plant in a population has a different genotype for an open-pollinated cultivar of onion. In addition, the crop's cross-pollinated behavior could also be responsible for differences because of the variable distribution of the resistance genes among individual bulbs in a population [49].

4.7. Quantitative Nature of FBR Resistance

A reduced disease severity over multiple cycles of selection is an indication of additive gene effects that suggests a quantitative nature of FBR resistance controlled by quantitative trait loci (QTLs), with each locus providing a partial increase in resistance [19,50]. Studies related to QTL identification have been conducted recently [47,50]. Two QTLs conditioning FBR resistance have been identified on chromosomes 2 and 4, and another QTL on chromosome 4 has been found to be involved in increasing FBR susceptibility in a recent study [50]. Another study by Taylor et al. [47] identified that chromosomes 1, 6, and 8 were involved in FBR resistance. The results for these two studies differ in explaining the involvement of several QTLs in controlling FBR resistance which requires confirmation in other genetic backgrounds. Usually, multiple factors are considered to be involved in imparting horizontal resistance [51], which can be improved via recurrent selection. Although quantitative or horizontal resistance cannot impart complete resistance, it has been identified as more durable than qualitative or vertical resistance because the latter is controlled by a single or a few genes with large effects, which can be easily overcome by the pathogen. Moreover, the sustainability of disease resistance depends on population genetics and the dynamics of the pathogen. Horizontal resistance is long-lasting because it exerts less intense selection pressure due to the involvement of multiple genes with small incremental effects. Conversely, vertical resistance challenges a pathogen population with more intense selection pressure that requires a single gene mutation in the pathogen that can make it virulent in killing the host. However, the breakdown of horizontal resistance can also happen due to multiple mutations in the pathogen or the simultaneous loss of several independent genes. [52]

After a pathogen attack, the defensive system of a plant is activated and pathogenesis-related (PR) protein genes are upregulated, which triggers pathways for several chemical compounds such as salicylic acid, jasmonic acid, and ethylene or activates other immune responses [53]. Particularly, a group of secondary metabolites, known as saponins, have been identified in onion bulbs for their antifungal activity [54]. Two steroidal saponins

(alliospiroside A and alliospiroside B) were identified in shallot for their antifungal properties against soil-borne fungi via a loss of fungal cell membrane integrity caused by saponins that led to cell death [55]. Alliospiroside A was isolated from Japanese bunching onion, *Allium fistulosum* L., with an extra chromosome 2A from shallot, and its antifungal properties were studied against *Fusarium* species [56]. The levels of a particular saponin compound could be responsible for imparting some level of resistance against FBR in onions, which needs further exploration to investigate a possible correlation between the degree of FBR resistance and the amounts of a particular saponin compound. This can be used to develop a biochemical screening procedure to identify plants with higher amounts of antifungal saponins.

4.8. Successful Screening Method to Improve Resistance in Short-Day Onion Cultivars

The AIMBS method was highly successful in developing FBR disease in the mature bulbs of all the cultivars, including checks. The effectiveness of an artificial screening procedure is very imperative to select resistant individuals from the population by avoiding the chances of disease escape [57]. Most importantly, the AIMBS was able to distinguish between two check cultivars and also revealed the differences present between the starting and the advanced selected populations. Based on our results, conidial inoculation can be successfully employed in the future to select FBR-resistant genotypes to develop elite onion germplasms.

5. Conclusions

The selected populations of seven New Mexican short-day onion cultivars were evaluated for their FBR susceptibility after multiple cycles of phenotypic recurrent selection using a conidial inoculation to assess the selection progress. The populations exhibited a variable response in FBR severity over two consecutive years, with more improvement in the advanced selections of the cultivars. Even the advanced selections of 'NuMex Sweetpak' exceeded the partially resistant check in levels of FBR resistance. The quantitative nature of FBR resistance, onion's cross-pollinated behavior, variability in spore counting, and sampling effect make it difficult to achieve the same response every year. Moreover, no improvement in some of the cultivars might indicate less or slower fixation of desirable resistant alleles in the population. The development of FBR-resistant cultivars using a conidial inoculation can help to accelerate breeding efforts by determining the genetic mechanism(s) responsible for FBR resistance, locating QTLs, and facilitating marker-assisted selection.

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