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Genetic Analyses of Resistance to Fusarium Basal Rot in Onion

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Abstract: Fusarium basal rot (FBR) is a serious disease of onion (*Allium cepa*). We identified sources of FBR resistance, assessed efficacy of selection for increased resistance, and investigated its genetic control. Onion accessions were evaluated for FBR resistance, and percentage survival ranged from 0% to 78%. Survivors were intercrossed, and progenies from one cycle of selection showed increased survival by 18% to 52%. Selections were crossed to male-sterile lines, and hybrids showed specific combining ability for FBR resistance. Segregating families were produced, and quantitative trait loci (QTLs) were identified on chromosomes 2 and 4 conditioning FBR resistance. A second QTL on chromosome 4 was identified that decreased FBR resistance. Plants from families with different genotypes across the 1.5 logarithm of odds (LOD) regions on chromosomes 2 and 4 were self-pollinated, and resulting families were evaluated for FBR survival. Genomic regions on chromosomes 2 and 4 associated with resistance were validated at $p = 0.05$ and 0.10, respectively. The region on chromosome 4 associated with increased susceptibility was validated at $p = 0.05$. These results are in agreement with previous studies reporting high heritability and specific combining ability for FBR resistance and should be useful for selection of FBR-resistant onion.

Keywords: disease resistance; quantitative trait locus; single-nucleotide polymorphism

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1. Introduction

Fusarium basal rot (FBR) is caused by *Fusarium oxysporum* f. sp. *cepae* (FOC) and is a serious soil-borne disease of onion (*Allium cepa*) worldwide [1–3]. FBR was reported in onion fields as early as 1910 [4], and significant losses routinely occur [1,5–8]. In the USA, yield losses due to FBR vary between 3% to 35%, and storage losses have been reported as high as 75% [7,9,10]. FOC-infected seedlings show symptoms of damping off, stunted growth, or death, and FBR symptoms on adult plants include browning of the basal plate, separation of roots from the stem plate, chlorotic leaves progressing to tip necrosis, and eventually death of the plant [5]. Methods to control FBR include crop rotation, soil solarization, chemical treatments, and resistant cultivars [5]. However, crop rotation can be difficult if land availability becomes limited, and soil treatments are expensive; therefore, planting of FBR-resistant cultivars is the most desirable control option.

Factors affecting evaluations for FBR resistance include FOC virulence, inoculum concentration, temperature, and plant wounding [4,9,11]. Evaluations conducted between 28 to 32 °C often result in rapid FBR development, and little disease pressure develops below 12 °C [4]. FOC isolates show varying degrees of virulence and ability to incite FBR [6,8,11,12]. Higher concentrations of FOC conidia using virulent isolates are more lethal than lower concentrations [11]. Wounding of plants results in higher FOC infection relative to noninjured plants [4,13].

Several FBR screening protocols exist, including seedling assays in controlled environments, bulb inoculations, or evaluations in fields either artificially or naturally infested

with FOC [4,8,11,14–18]. Seedling assays in controlled environments often involve mixing a suspension of FOC conidia with sand or soil, planting seeds, and evaluating for severity or survival [11,16]. Onion bulbs have been evaluated by piercing of the basal plate with a sterile needle, soaking in a FOC conidial suspension, and planting in the greenhouse [4,5]. Field evaluations involved planting into infested fields [2,15,19–21]. Retig et al. [16] reported high positive correlations between field and seedling assays of onion for FBR resistance.

Several inheritance modes for FBR resistance have been proposed. Holz and Knox-Davies [22] reported FBR resistance as polygenic. Bacher [23] proposed that resistance was conditioned by partially dominant alleles at two independently segregating loci (*FOC1* and *FOC2*); however, an unexpected result from this study was the 89% reduction in the level of FBR resistance after three cycles of self-pollination and selection for FBR resistance, which was attributed to inbreeding depression. Tsutsui [18] proposed that a dominant allele at one locus conditioned resistance to FBR; however, this dominant allele had variable expression, and additional loci may control FBR resistance. Krueger et al. [24] generated a complete diallel by crossing among onion inbreds with varying levels of FBR resistance and showed that general (GCA) and specific combining abilities (SCA), as well as reciprocal effects, were significant for FBR resistance. Galvan Vivero [2] evaluated a tri-hybrid mapping population from *A. cepa* × (*A. roylei* × *A. fistulosum*) for reaction to FOC by transplanting adult plants into infested soil, and resistances from *A. roylei* and *A. fistulosum* mapped to chromosomes 2 and 8, respectively. Vu et al. [25] studied FBR resistance using *A. fistulosum*, shallot (*A. cepa* var. *aggregatum*), and eight monosomic addition lines developed by the addition of one chromosome from shallot (1A to 8A) to the complete genome of recipient *A. fistulosum* (FF). The monosomic line FF+2A showed the highest resistance to FOC, indicating that chromosome 2 of shallot conditions resistance to FBR. This same monosomic addition line accumulated a saponin associated with *Fusarium* resistance [26].

Diverse modes of inheritance from different onion germplasm or *Allium* species suggest that independent sources of FBR resistance may exist, which could be combined to increase overall levels of resistance in commercial cultivars. In this research, we evaluated onion accessions for reaction to FOC, assessed response to selection for increased FBR resistance, identified sources of FBR resistance that showed dominance, and developed segregating families to map FBR resistance and estimate genetic effects.

2. Materials and Methods

Isolate ID4ss2 of FOC was isolated from an FBR infected onion bulb from Payette, ID USA, and was used for all evaluations. The isolate was cultured from a single conidium and was chosen on the basis of relatively high virulence and ability to consistently produce mostly microconidia, which allow for more accurate inoculum quantification as compared to macroconidia which have variable numbers of cells per conidium. The isolate was preserved on silica gel (Sigma-Aldrich, St. Louis, MO, USA) as described by Dhingra and Sinclair [27]. For inoculum preparation, silica crystals infested with FOC were transferred to petri plates with potato dextrose agar (PDA) and grown for 7 days at 24 C with 12 h fluorescent light. Using a sterile cork borer, 5 mm diameter plugs were aseptically cut from the PDA colonies, and 10 plugs were transferred into a 500 mL flask containing 250 mL of sterile potato dextrose broth (PDB). The FOC-inoculated PDB was incubated at room temperature on a rotary shaker at 160 rpm for 7 days. Conidia were harvested by filtering the PDB through eight layers of cheesecloth, and filtrate was centrifuged at 2000× *g* for 10 min. The supernatant was discarded, and the conidial pellet was resuspended in reverse-osmosis water. Conidial concentration was determined using a hemocytometer and adjusted to 0.5×10^6 conidia/mL.

The FBR screening protocol was described by Krueger [14], Retig et al. [16], and Tsutsui [18] with modifications. Disease evaluations used randomized complete block designs (RCBDs) with three or four replications of 50 seeds. Because onion seed can be naturally infested with FOC [28], seeds were placed into mesh bags, soaked in 0.5% Virkon S (DuPont, Wilmington, DE, USA) for 15 min with constant agitation, and drained. Seeds

were then rinsed under running water for 30 min and dried at 35 C for 4 h. Germination rates were determined by planting 50 Virkon-treated seeds and counting the numbers of plants 21 days after sowing.

Five hundred milliliters of the conidial suspension was mixed with 1000 mL of Hoagland's solution (Sigma-Aldrich), placed in a 3.8 L pressurized sprayer, and sprayed into 15 kg (dry weight) of silica sand (Industrial Quartz 4030, Unim Corp., Portage, WI, USA) in a running concrete mixer for 5 min. The purpose of this step was to ensure that the sides of the mixer were evenly coated with infested sand, and this initial infestation was discarded. Afterward, 500 mL of the conidial suspension was mixed with 1000 mL of Hoagland's solution and sprayed into 15 kg (dry weight) of silica sand in a running concrete mixer for 10 min including spray time. Infested sand was dispensed into 30 × 50 × 10 cm stainless-steel pans. The sand was firmly packed, and a row template was pressed into the sand to form eight rows divided into two 14 cm long and 1.25 cm deep plots. Fifty seeds were sown per replication, and seeds were covered by pinching sand from each side of the furrow. USDA inbred B5351 was included as the susceptible check. Trays were covered with stainless-steel lids and placed in water tanks in controlled environment rooms. The temperature was maintained at 19 °C to allow germination and emergence of onion seedlings with minimal development of FBR. Ten days after seed sowing, covers were removed, and the temperature was increased to 28 °C to allow development of FBR. Plants were watered daily with reverse-osmosis water. At 18 to 34 days after sowing, when the susceptible check had approximately 5% survival, numbers of surviving plants in each replication were counted. Mean survival values were calculated and adjusted by dividing by the respective germination proportion for each entry.

Initial FBR screening was conducted using a publicly available onion germplasm previously reported as FBR-resistant (W440 [29]) or in the United States Department of Agriculture (USDA) Germplasm Resources Information Network (GRIN) (plant introductions (PIs) 171,473, 249,539, 264,326, 354,088, and 368,359). Survivors were transplanted into 2.54 cm Styrofoam flats with a potting mix (Fafard 4P, Sun-Gro Horticulture, Agawam, MA, USA) and grown in a greenhouse at 21 °C without additional lighting until transplanting to the field for bulb production. Bulbs were harvested and vernalized at 4 °C; then, surviving plants from each selection were intercrossed in cages to generate FBR-selected progenies. The original unselected (per se) and FBR-selected populations were evaluated for FBR resistance using RCBD with four replications as described above.

FBR-selected W440 was crossed to three male-sterile lines (Ski-A, B2113A, and MSU611-1AxMSU611B) with low to intermediate levels of FBR resistance. 'Ski-A' was selected from the open-pollinated population 'Sapporo-Ki'. 'B2113A' is an unreleased USDA male-sterile inbred selected from a cross between the open-pollinated cultivars Early Yellow Globe and New Mexico Yellow Grano. 'MSU611-1AxMSU611B' is a male-sterile seed parent released by the USDA in 1979 [30]. Seeds of the hybrids and parental lines were evaluated for FBR survival in a RCBD with four replications as described above.

FBR-susceptible inbred B5351 was crossed as the female with the FBR-selected W440, and a seed-to-seed method was used to induce flowering of the hybrid plants [31]. In September, seeds from the B5351 × W440 cross were planted in a soil mix and plants grown in a greenhouse at 21 °C with 12 h lighting. B5351 and W440 have semi-glossy and waxy foliage, respectively, and hybrid plants were identified by waxy foliage [32]. In the following January, plants were transferred to a cold room at 4 °C with 12 h lighting. In May, plants were transplanted into field plots at the UW Horticulture Research Farm (Arlington, WI, USA), and two flowering hybrids were paired and intercrossed to produce a segregating F_2) family. Leaf tissue was harvested from 33 F_2 plants for DNA isolation, and single-nucleotide polymorphisms (SNPs) were genotyped as described below. These same F_2 plants were self-pollinated to produce 33 F_3 families which were evaluated for FBR survival using four replications as described above.

After initial mapping of FBR resistance, additional segregating families were developed using five F_3 families with contrasting genotypes for SNPs across the 1.5 LOD

intervals associated with resistance or susceptibility to FBR (described below). Randomly selected plants from each F_3 family were self-pollinated to produce 91 F_4 families which were evaluated for FBR survival as described above. DNA was isolated from the F_3 parents of the F_4 families and genotyped for SNPs across the 1.5 LOD intervals on chromosomes 2 and 4.

Leaf samples were collected from segregating progenies, and DNA was extracted using a minipreparation (NucleoSpin Plant II Midi DNA Purification kit, Macherey-Nagel, Duren, Germany). DNA quantity was measured spectrophotometrically (NanoDrop, Thermo-Fisher Scientific, Waltham, MA, USA), and adjusted to 50 ng/µL. DNA quality was assessed by electrophoresis of 500 ng of DNA through 1% agarose gels, stained, and visually examined for clear bands migrating with uncut λ DNA. SNP markers evenly spread across the eight chromosomes of onion [33] were genotyped using the KASPar platform (LGC Genomics, Boston, MA). Goodness of fit to expected 1:2:1 segregation was determined using chi-square tests, and SNPs with goodness of fit at $p < 0.001$ were removed. Genetic mapping was performed using JoinMap 4.1 [34], and linkage groups were constructed using maximum likelihood mapping at LOD 6.0. Linkage groups were assigned to chromosomes using previously mapped SNPs [32,33].

Analyses of variance (ANOVAs) were performed using RStudio V1.0.136 (R foundation for Statistical Computing, Vienna, Austria) or Statistical Analysis System (SAS) version 9.4 (SAS Institute, Cary, NC, USA). Mean germination-adjusted percentage survival ± standard deviations for populations or crosses was calculated and compared using least significant difference (LSD) with the Bonferroni adjustment at $p = 0.05$ using SAS or Rstudio. For mapping of FBR resistance, SNP genotypes of 124 (33 + 91) segregating progenies and germination-adjusted percent FBR survival of their respective families from self-pollinations were analyzed by imputation (sim.geno) and forward/backward selection (stepwiseqtl) with the R/qtl and R/broman packages in R Studio [35,36]. Multiple imputation was used because not all SNPs segregated in both families, and this approach is better with missing scores due to monomorphic markers. For all analyses, 1000 permutations were completed to determine the LOD significance threshold at $p = 0.05$. After identifying a QTL, the maximum LOD score, additive and dominance effects, and percentage phenotypic variation explained by the QTL were calculated.

3. Results and Discussion

3.1. Sources of FBR Resistance

We completed one cycle of selection for FBR resistance using onion germplasm previously reported as showing some level of resistance. The original unselected and FBR-selected populations were evaluated using the seedling screen, and the mean percentage survival was calculated. One cycle of selection successfully increased FBR resistance in PIs 171,473, 249,539, 264,326, 354,088, and 368,359 (Table 1). The original population of PI 249,539 was susceptible at 4% survival, and one cycle of selection significantly increased survival to 76%. These results are in agreement with Cramer [37], who also reported significant response to a single cycle of selection for FBR resistance. One cycle of selection did not significantly increase the level of FBR resistance for W440, possibly because resistant individuals already predominated in this inbred or due to the negative effect of inbreeding [23].

Table 1. Mean percentage (%) survival ± standard deviations (SD) and level of significant differences between original unselected plant introductions (PIs) or inbreds of onion versus progenies from one cycle of selection for resistance to Fusarium basal rot (FBR).

Accession	Mean % FBR Survival ± SD		Level of Significance [z]
	Original	Selection	
PI 171473	44.0 ± 13.4	64.5 ± 1.8	*
PI 249539	3.8 ± 2.5	76.0 ± 5.5	***
PI 264326	7.1 ± 5.4	48.7 ± 14.3	***
PI 354088	36.0 ± 22.5	54.4 ± 23.8	*
PI 368359	6.6 ± 5.4	44.0 ± 14.1	***
W440	66.2 ± 16.3	76.1 ± 15.3	ns
S [y]	5.0 ± 4.9		-

[z] ns, *, and *** = nonsignificant or significant at $p < 0.05$, and 0.001, respectively, according to the least significant difference. [y] Susceptible (S) check.

The FBR-resistant selection of W440 was crossed as the male parent to three male sterile lines; 'B2113A' and 'Ski-A' are susceptible to FBR, and 'MSU611-1AxMSU611BA' shows an intermediate level of resistance (Table 2). The survival of hybrid families was significantly different (Table 2), and none showed a higher mean survival than W440. Of the three hybrid families, progenies of Ski-A × W440 had significantly higher FBR resistance compared to the other two families, even though Ski-A was the most susceptible to FBR (Table 2). This result is in agreement with Krueger et al. [24], who reported significant specific combining ability for FBR resistance, which will be important when choosing parents to produce FBR-resistant hybrids.

Table 2. Mean percentage (%) survival ± standard deviation (SD) for testcross progenies, male-sterile lines, and inbred W440 selected for resistance to Fusarium basal rot (FBR) in a randomized complete block design with four replications.

Pedigree	% FBR Survival ± SD [z]
Ski-A × W440	66.9 ± 12.4 a
FBR-selected W440	60.9 ± 12.5 a
B2113A × W440	46.2 ± 9.7 b
(MSU611-1AxMSU611B) × W440	37.8 ± 4.5 bc
MSU611-1AxMSU611B	38.7 ± 9.9 b c
Ski-A	32.0 ± 7.2 c
B2113A	2.4 ± 3.0 d
S [y]	4.9 ± 2.5 d

[z] Means followed by same letter were not significantly different at $p < 0.05$ according to the least significant difference with Bonferroni adjustment. [y] Susceptible (S) check.

3.2. Genetic Analysis and Mapping of FBR Resistance

The 33 F_2 progenies from B5351 × W440 were segregated for 116 SNPs that fit the expected 1:2:1 segregation ratio at $p > 0.001$. Fourteen linkage groups were constructed at LOD 6.0 and were assigned to chromosomes using previously mapped SNPs [32,33]. Mapping of FBR resistance in this family revealed three quantitative trait loci (QTLs), one on chromosome 2 and two on chromosome 4 (Table 3, Supplemental Table S1). Although the two regions on chromosome 4 (referred to as 4A and 4C) segregated independently in the F_2 family, they mapped 31 cM apart in the genetic map reported by Damon and Havey [32]. Marker isotig38484_281 on chromosome 2B was most significantly associated with a codominant FBR resistance, and, for every W440 allele added, there was a 21% increase in survival (Table 3). Marker isotig44683_192 on chromosome 4A was significantly associated with FBR resistance, and a dominant allele from W440 at this marker increased survival by 17% (Table 3). The W440 allele at isotig31106_505 on chromosome 4C had the additive effect of decreasing survival by 15% (Table 3).

Table 3. Quantitative trait loci (QTLs) for resistance to Fusarium basal rot (FBR) in the segregating F_2 family of onion, single-nucleotide polymorphisms (SNPs) and 1.5 logarithm of odds (LOD) confidence interval associated with each QTL, LOD score and threshold, additive and dominance effects, and percentage (%) phenotypic variation explained by QTL.

Chrom [z]	Most Significant SNP	SNP Flanking 1.5 LOD Confidence Interval	LOD Score	LOD Threshold	Additive Effect [y]	Dominance Effect [y]	% Variation Explained
2B	isotig38484_281	isotig36256_344 to isotig32786_424	7.6	3.6	20.7	0.8	41.1
4A	isotig44683_192	isotig33399_1211 to isotig35268_1082	8.0	3.6	17.2	19.4	44.2
4C	isotig31106_505	isotig45610_340 to isotig31106_505	5.6	3.6	−14.9	−10.3	25.3

[z] Two linkage groups (4A and 4C) segregated independently and were assigned to chromosome 4 on the basis of common markers in the map of Damon and Havey [32]. [y] Positive effects indicate an increase in percentage survival conditioned by the chromosome region from W440.

Due to the small (33) size of the segregating family, the genetic effects of the three QTLs associated with FBR resistance may be overestimated [38]. We increased numbers of segregating progenies by self-pollinating random plants from five F_3 families that were homozygous or heterozygous for the SNPs most significantly associated with FBR resistance in the F_2 family (Table 4), and these F_4 families were screened for FBR survival. Mean percentage survival was significantly different ($p < 0.05$) among families, and survival was highest for families homozygous for the W440 alleles on chromosomes 2B and 4A and the B5351 allele on chromosome 4C (Table 4). Regions on chromosomes 2B and 4C affecting survival were validated at $p < 0.05$ (Table 5); the region on chromosome 4A was significant at $p = 0.10$. For chromosome 2, the region from the resistant parent W440 codominantly increased survival by 17% (Table 5), in agreement with the original family which showed an additive effect to increase seedling survival by 21% (Table 3). Segregations in the F_4 families confirmed that the region on chromosome 4C from W440 significantly reduced seedling survival (Table 5).

Table 4. Genotypes at single-nucleotide polymorphisms (SNPs) segregating with the highest significance for resistance to Fusarium basal rot (FBR) in F_3 families of onion, numbers of F_4 families derived from each F_3 family, and mean percentage (%) survival ± standard deviations (SD) of the F_4 families determined using a seedling evaluation for FBR.

F_3	Genotypes at SNP [z]			F4 Families	Mean %
Family	Isotig 38484_281	Isotig 44683_192	Isotig 31106_505	Evaluated	Survival ± SD [y]
24,602	H	A	H	17	28.7 ± 20.0 a
24,622	H	H	H	10	44.2 ± 32.9 a
24,620	H	H	A	11	51.6 ± 29.3 ab
24,648	B	H	A	17	77.1 ± 16.5 bc
24,628	B	B	H	36	78.2 ± 21.6 c

[z] SNPs are listed in Table 3. A = allele from susceptible B5351; H = heterozygous; B = allele from resistant W440. [y] Means followed by the same letter were not significantly different according to the least significant difference with Bonferroni adjustment at $p = 0.05$.

Table 5. Quantitative trait loci (QTLs) for resistance to Fusarium Basal Rot (FBR) in segregating F_4 families of onion, chromosome (Chrom), most significant single-nucleotide polymorphism (SNP), logarithm of odds (LOD) score and threshold at $p = 0.05$, additive and dominance effects, and percentage (%) phenotypic variation explained by QTL.

Chrom	Most Significant SNP [y]	LOD Score	LOD Threshold	Additive Effect [z]	Dominance Effect [z]	% Variation Explained
2B	isotig30461_1472	3.26	2.35	16.9	9.1	14.1
4C	isotig31106_505	2.88	2.35	−3.7	−23.7	12.4

[z] Positive effects indicate the increase in percentage survival conditioned by allele from W440. [y] SNP marker within the 1.5 LOD interval shown in Table 3.

These mapping results are consistent with previous analyses reporting high heritabilities [24,37] and one or two gene models for FBR resistance [18,23]. These results are also consistent with observations by Vu et al. [25] and Galvan Vivero [2] of FBR resistance associated with chromosome 2 of shallot and *A. roylei*, respectively. In a patent application, Black et al. [39] identified a region on their linkage group 2 that carried a codominantly inherited FBR resistance with the allele from the resistant parent increasing seedling survival by ~7%. We previously demonstrated that linkage group 2 in this patent application corresponds to chromosome 4 of onion [40]. SNP marker NQ0257570 reported in this patent mapped 6.2 cM from the most significant SNP (isotig44683_192) on chromosome 4 for resistance to FBR (Supplemental Table S1), indicating that the region on chromosome 4 was detected in two independent sources of FBR resistance (W440 (Table 3) and the cultivar 'Serrana' [39]). On the basis of results of this study (Tables 3 and 5) and those of previous researchers [18,23,39], we recommend that the locus names of *FOC1* and *FOC2* proposed by Bacher [23] be assigned to FBR resistances on chromosomes 2 and 4, respectively. The significant reduction in seedling survival associated with the heterozygous genotype at the region on chromosome 4C was unexpected; however, it is consistent with significant specific combining abilities for FBR phenotypes reported by Krueger et al. [24]. Our results indicate that significantly greater FBR resistance should result from selection for the regions on chromosomes 2 and 4 associated with resistance, together with selection against the susceptibility region on chromosome 4 (Table 3).

Taylor et al. [41] used association analysis of a diverse onion germplasm and identified five SNPs associated with FBR resistance, three of which were mapped to chromosomes 1, 6, and 8. However these regions were not significantly associated with FBR resistance in our study, indicating that there may be numerous QTLs conditioning FBR resistance in onion. FOC isolates from different geographic regions varied from mildly to highly virulent [8,11,12,42]. Phylogenetic and vegetative-compatibility-group studies revealed a high level of diversity among FOC populations and a relationship between FOC virulence and phylogenetic clade [42]. Diversity among FOC isolates may complicate the selection of FBR-resistant germplasms and subsequent genetic studies. Nevertheless, the SNP markers identified in this study and those of Taylor et al. [41] should be useful to combine independently inherited resistances to minimize losses due to FBR in onion.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7120538/s1: Table S1. Chromosome (Chrom), position in centiMorgans (cM), and goodness of fit (Prob.) to the expected 1:2:1 segregation ratio for single-nucleotide polymorphisms (SNPs) used for mapping of resistance to Fusarium basal rot of onion.

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