Genetic Analyses and Mapping of Pink-Root Resistance in Onion

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ABSTRACT. Pink root [PR (caused by *Phoma terrestris*)] is a major soil-borne disease of onion (*Allium cepa*) and reduces both yield and quality of bulbs. PR-resistant cultivars offer the best control option for this disease. The objectives of this study were to complete genetic analyses and mapping of PR resistances from independent sources. Segregating families were developed from different sources of PR resistance and evaluated using a seedling screen. PR severity in two segregating families from the same source of resistance mapped to one position on chromosome 4 with logarithm of odds (LOD) scores of 8.0 and 10.3, and explained 28% and 35% of the phenotypic variation, respectively. Estimates of additive and dominance effects revealed this source of PR resistance is codominantly inherited. PR resistance from a second source was assessed by percent survival in the seedling evaluation, showed codominance, and mapped to the same region on chromosome 4 at LOD 12.5 and explained 54% of the phenotypic variation. This research demonstrates that PR resistance from different sources mapped to the same chromosome region and showed similar modes of inheritance.

Pink root [PR, caused by *Phoma terrestris* (Pt), synonym *Pyrenochaeta terrestris*] is a major soil-borne disease of onion (Entwistle, 1990). The disease was first reported in Texas (Tabenhaus, 1917) and has since been identified in onion growing regions worldwide (Chaves and Erickson, 1960; Klingner and Pontis-Videla, 1961; Pflieger and Vaughan, 1972). The characteristic symptom of Pt-infected onion roots is a pink color that progresses to dark purple and eventually the roots disintegrate (Summer, 1995). PR significantly reduces the weight, quality, and yield of onion bulbs (Coleman and Ellerbrock, 1992; Lacy and Roberts, 1982; Perry and Jones, 1955) and can become a severe problem especially during seasons of high soil temperatures (Lacy and Roberts, 1982). Methods to control PR include long crop rotations (Entwistle, 1990), soil solarization or fumigation (Ahmed and Harrington, 1974; Hartz et al., 1989; Katan, 1980; Lee et al., 2007; Porter et al., 1989; Rabinowitch et al., 1981), and genetic resistance (Jones and Perry, 1956; Levy and Gornik, 1981; Nichols et al., 1960, 1965).

Numerous studies evaluated onion populations for PR resistance using seedling screens, bulb inoculations, or survival of plants in fields either artificially or naturally infested with Pt. Tabenhaus and Mally (1921) reported that the short-day

cultivars Crystal Wax and Yellow Bermuda (YB) were resistant to PR. The PR-resistant cultivar Excel 986 was selected from YB and later was used as a parent of other PR-resistant cultivars such as L36, Eclipse (L303), and L365 (Perry and Jones, 1955). Levy and Gornik (1981) screened for resistance to PR using field and greenhouse assays and reported that cultivars Dessex, Granex, Laredo, Grano, and Grano 502 showed PR resistance, while Golden and Yellow Creole were susceptible. In a greenhouse test, Netzer et al. (1985) classified ‘Granex’ as susceptible to PR, even though the female parent of ‘Granex’ showed resistance to PR (Perry et al., 1964). Using a seedling test, Nichols (1963) reported low PR infection in ‘Excel’ and ‘Texas Hybrid 28’ and high PR infection in ‘Texas Grano 502’. PR resistance has also been identified in long-day onions, including cultivars Brigham Yellow Globe, Downing Yellow Globe, and Southport White Globe (Lacy and Roberts, 1982; Nichols, 1963). Using different sources of resistance, Jones and Perry (1956) and Nichols et al. (1965) proposed that PR resistance is conditioned by a single recessive locus.

The goals of this study were to develop segregating families for genetic analyses and mapping of PR resistance in onion. We demonstrate that PR resistances from different sources are conditioned by the same chromosomal region and show codominance.

Materials and Methods

SEEDLING EVALUATION FOR PR RESISTANCE. Segregating families and parents (described below) were evaluated in a seedling screen for PR severity or survival. The isolate of Pt (*Tx1c*) was obtained from PR-infected onion bulbs from Texas and used for all evaluations. The isolate was preserved in sterile soil (Dhingra and Sinclair, 1985), sprinkled onto V8 agar

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Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Most significant single nucleotide polymorphism (SNP), SNPs flanking the 1.5 logarithm of odds (LOD) confidence interval, LOD score, LOD significance threshold at $P = 0.05$, additive and dominance effects, and percent phenotypic variation explained for evaluations of pink root severity in segregating families PR1 and PR2 of onion.

Family	Most significant SNP ^a	1.5 LOD interval ^a	LOD score	LOD threshold	Additive effect ^y	Dominance effect	Variation explained (%)
PR1	i32829_1057	i38830_623 to i21519_664	8.03	3.74	-2.05	0.35	27.6
PR2	i29175_343	i28612_1057 to i35268_1082	10.1	4.02	-2.33	-0.32	35.1

^aSNP markers described by Duangjit et al. (2013); i = isotig.

^yNegative additive effect indicates a reduction in pink-root severity conditioned by the resistant (DehyA) parent.

Fig. 1. Additive effects of alleles at isotig32829_1057 (A) and isotig29175_343 (B) on pink-root (PR) severity (y-axis) on a 1 (resistant) to 9 (susceptible) scale for the PR1 (A) and PR2 (B) families of onion, respectively. Allele B from the resistant parent decreased disease severity relative to allele A from the susceptible parent.

plates, and incubated at 24 °C with 12-h fluorescent lighting for 7 d. Eight 10-mm-diameter plugs were transferred to a 1000-mL bottle containing 110 mL of sterile Czapek Dox Broth (Gorenz et al., 1949). The bottles were placed horizontally inside an incubator at 24 °C with 12-h fluorescent lighting for 10 d with gentle mixing every third day. After 10 d of incubation, the mycelium with broth was chopped for 2 min in a blender (Jarden Corp., New York, NY), and diluted at a ratio of 100 mL of inoculum to 400 mL of non-sterile reverse-osmosis (RO) water. This mycelial suspension was mixed with 1000 mL Hoagland's solution (Sigma, St. Louis, MO) and sprayed into 15 kg (dry weight) of silica sand [30% retained on 40 mesh (Industrial Quartz 4030; Unimin Corp., Portage, WI)] in a running concrete mixer. The mixer was allowed to run for 10 min including the spray time. The first batch of infested sand was used to coat the mixer walls and was discarded. Subsequent batches of infested sand were firmly packed into 30 × 50 × 10-cm

stainless steel pans, and a row template was pressed into the sand to form eight rows divided into two 14-cm sections at a depth of 1.25 cm. Fifty onion seeds were sown per section and covered by pinching sand from each side of the row. Trays were covered with stainless steel lids and placed in water tanks in controlled environment rooms or greenhouses. The temperature was maintained at 19 °C to allow germination and emergence of onion seedlings with minimal development of the disease. Ten days after sowing (DAS), the temperature was increased to 28 °C to allow development of PR. Pans were lightly watered along the rows with RO-purified water when the sand was dry and crumbled easily. PR severity was assessed or numbers of survivors were counted at 25 to 34 DAS when the susceptible check (B5351) had ≈15% survival.

DEVELOPMENT AND GENOTYPING OF SEGREGATING FAMILIES. Two segregating families were developed by crossing the male-sterile inbred DehyA (mean PR survival of 66.2% ± 12.5%) as the female with inbreds B5351C (mean PR survival of 15.2% ± 14.8%) and Ski-MsMs (mean PR survival of 76.3% ± 11.0%). Both of these male parents are homozygous dominant at the nuclear male-fertility restoration (*Ms*) locus and therefore hybrids were male fertile. Single F_1 plants from each cross were self-pollinated to produce two F_2 families (Damon and Havey, 2014). At least 50 random F_2 progenies from each family were intercrossed in separate cages using flies and seed was bulked from all plants in each cage to produce F_2 -massed (M) families (PR1 and PR2 for DehyA crossed with B5351C or Ski-MsMs, respectively). Because plants in the PR1 and PR2 families segregated for male fertility, one fourth of plants would be male sterile and not possible to self pollinate to produce progenies for replicated PR evaluations. Therefore, F_2 -M progenies from both families were evaluated for PR severity using the seedling screen. PR severities for 93 and 92 progenies from PR1 and PR2 families, respectively, were scored 25 to 34 DAS when the susceptible parent (B5351C) had ≈15% survival using a 1 to 9 scale, in which 1 = seedling healthy, leaves showing no necrosis; 3 = 1% to 25% of leaves necrotic; 5 = seedling stunted, 26% to 50% of leaves necrotic; 7 = seedling severely stunted, 51% to 75% of leaves necrotic; and 9 = seedling severely stunted, 76% to 100% of leaves necrotic or plant dead (photographs of phenotypes available in Marzu, 2015).

PR-susceptible inbred B5351C was crossed as the female with inbred W446B [mean PR survival of 88.8% ± 15.4% (Goldman, 1996)]. Groups of three to four hybrid plants were planted together and intercrossed using flies to produce two F_1 -massed (F_1 M) families. Plants from these F_1 M families were randomly chosen and self pollinated to produce 54 and 39 F_1 -massed-selfed (F_1 MS) families (PR3 and PR4, respectively). Four 50-seed replications from each F_1 MS family and parental inbreds were evaluated in a completely randomized design using the seedling screen, and numbers of survivors were counted 25

Table 2. Most significant single nucleotide polymorphism (SNP), SNPs flanking the 1.5 logarithm of odds (LOD) confidence interval, LOD score, LOD significance threshold at $P = 0.05$, additive and dominance effects, and percent phenotypic variation explained for evaluations of seedling survival in the presence of the pink-root fungus for combined analysis of segregating families PR3 and PR4 of onion.

Most significant SNP ^z	1.5 LOD interval ^z	LOD score	LOD threshold	Additive effect ^y	Dominance effect	Variation explained (%)
i26045_1046	i38830_623 to i35268_1082	12.5	3.2	23.9	1.9	54

^zSNP markers were described by Duangjit et al. (2013); i = isotig.

^yPositive additive effect indicates increased % survival in pink-root evaluations conditioned by the resistant (W446) parent.

Fig. 2. Additive effects of alleles at marker isotig26045_1046 on percent survival of onion seedlings (y-axis) in pink root evaluations from the PR3 and PR4 families. Allele B from the resistant parent increased % survival relative to allele A from the susceptible parent.

to 34 DAS when the susceptible parent (B5351C) had $\approx 15\%$ survival. To account for germination differences, 30 seeds from each family were planted in the greenhouse and resulting plants counted 30 DAS to estimate the germination rate. The mean percent PR survival was adjusted by the respective germination rate for each family.

DNA was isolated using a mini-preparation (Nucleospin 96 Plant II; Macherey-Nagel, Bethlehem, PA) from 93 and 92 segregating F_2 M plants from the PR1 and PR2 families and from the 54 and 39 F_1 M plants used to produce the PR3 and PR4 families, respectively. DNA quantities were determined using a spectrophotometer (Nanodrop ND-1000; Thermo Fisher Scientific, Waltham, MA) and qualities were assessed by electrophoresis through 1% agarose gels. For the F_2 M progenies from the PR1 and PR2 families, insufficient DNA was isolated from seedlings for genotyping and a whole-genome amplification (LGC Genomics, Boston, MA) was used to increase DNA quantity. For the PR1 and PR2 families, 395 single nucleotide polymorphisms (SNP) spread across the eight chromosomes of onion were genotyped using the KASPar assay (Duangjit et al., 2013). For the PR3 and PR4 families, 169 SNPs (Duangjit et al., 2013) and two SNP markers reported as linked to PR resistance in a patent application (Black et al., 2015) were genotyped using the KASPar assay. Segregations from the PR3 and PR4 families were combined after establishing homogeneity of errors (Gomez and Gomez, 1984). Goodness-of-fit to the expected 1:2:1 ratio and genetic mapping as F_2 families were completed using Joinmap 4.0 and the Kosambi mapping function (Van Ooijen, 2006). Genetic distances in centimorgans will be larger for the segregating families used in this research than for F_2 families due to the two generations of recombination.

QUANTITATIVE TRAIT ANALYSES. For the PR1 and PR2 families, severity scores were not normally distributed and

log and square-root transformations were calculated. R/qtl (Broman et al., 2003; R Core Team, 2013) was used for composite interval mapping (CIM) to identify quantitative trait loci (QTL) conferring PR resistance, and two covariates and a 10-cM window size were used in the CIM mapping. For the PR3 and PR4 families, the Shapiro-Wilk test confirmed a normal distribution of the survival data and analysis used imputation and forward/backward selection in the R/qtl (Broman et al., 2003) and R/broman (Broman and Broman, 2016) packages in R Studio V1.0.136 (R Studio Team, 2016). Multiple imputation was used because not all SNPs segregated in both the PR3 and PR4 families, resulting in missing data, and this approach is better with missing scores due to monomorphic markers. For all analyses, 1000 permutations were completed to determine the 0.05 significance logarithm of odds (LOD) threshold. After identifying a QTL, the maximum LOD score, additive and dominance effects, and percent phenotypic variation explained by the QTL were calculated. The most significant SNP and loci flanking the 1.5 LOD intervals were identified.

Results and Discussion

The PR1 and PR2 segregating families were developed from crosses of a seed parent (DehyA) with an intermediate level of PR resistance (mean PR survival of $66.2\% \pm 12.5\%$) with B5351 (mean PR survival of $15.2\% \pm 14.8\%$) and Ski-MSMS (mean PR survival of $76.3\% \pm 11.0\%$) male parents, respectively. Both families segregated for PR resistance and were not significantly different for mean survival at $50.0\% \pm 22.6\%$ and $50.0\% \pm 19.5\%$, respectively. Although the cross of DehyA and Ski-MSMS was between two inbred lines showing PR resistance, one of the plants used for crossing must have been susceptible to PR and the PR2 family segregated for resistance. Both the PR1 and PR2 families were evaluated using the seedling screen and individual progenies scored for PR severity on the 1 to 9 scale. A total of 111 SNPs were mapped using 93 F_2 M progenies from the PR1 family and 162 SNPs with 92 F_2 M progenies from the PR2 family (SNPs and segregations reported in Marzu, 2015). Using non-transformed severity scores, PR resistance in both families mapped to the same position on chromosome 4 at LOD scores of 8.0 and 10.1 and explained 28% and 35% of the phenotypic variation for PR1 and PR2 families, respectively (Table 1). The same chromosome region was detected using log and square-root transformations of severity scores. Additive effects in both families were larger than dominance effects (Table 1), and in both families PR resistance from the DehyA parent decreased disease severity and was codominantly inherited (Fig. 1).

For the PR3 and PR4 families, 54 and 39 F_1 MS families, respectively, were screened in replication for percent survival. After establishing homogeneity of errors, 151 SNPs were used for mapping of PR survival in these two families (SNPs and segregations reported in Straley, 2018). One highly significant

Table 3. Sequences of single nucleotide polymorphisms (SNP) on chromosome 4 of onion mapping within the 1.5 logarithm of odds (LOD) confidence interval for pink root-resistance in three segregating families.

SNP	Sequence flanking SNP
isotig21519_664	TGATGTATCGTACCGTGCTCTTGAANTTTCATCAGAANGCACCTCATAGGCCAGACGAAT[A/G] NNCTTGAATACCTCACTGGCCTGTGAATCTTGACTGACATCAGGATGNTANTTTCTAGCA
isotig26045_1046	GGCGCAAGGCCTTGCAGTGGGTCTTCCCTTGTGGAAGAAGACAAGCTAGAGCTTGCCAC[A/G] TCTCTTCTTGCAGAAAGCCAAAGCTAAAGGTGTTTCTTTGTTGCTTCCCACTGATGTTATT
isotig29175_343	GTATATGGAGACTGGAAAGCTTCTAGCAGTTGTTCACTCAGGTGGAGAGTTTGTACCAA[C/T] AAAGATGGAACAATATCTTATAGTGGTGGTGAAGCACATGCTCTTGATATTGGCCGTGAT
isotig32829_1057	AAATCACCACNNCCACTCCCCACATTTTGCAAATCGCCACTTCCACTCCCCACATTTTGC[A/G] AATCGCCGCTTCCACTTCCCATATTTTGCAAACACTCCCTCCACTACCTGCATTCTGCA
isotig33399_1211	TCTATCAGCTGGATGATTAACCTGCATGTCATTCCAAACCTGCAAATCCCCGTGCGCAT[A/G] TAATATGAGCAGTCTGGCTCGCCAGGCCCTCTCTGGATATTGCCCACTTCCCATATCTTCG
isotig35268_1082	CTTAGGAGGTCAGCTGATACTCTTTGGCAGATCCAGCAGCAGCTTGATCTGTAATCAGTT[C/T] TNAACTTTTTGAGTTATTGTACGGACACATATTGTGTTTTGTCGTNCNNNNNNNNNNNNNN
isotig38830_623	TTTGACAACCTCAACTTCTTCCCTACTTCCATCNCATCTTCTTCATCGCTATTATTACT[A/G] NNGTTACTTTCTCCAGTGCTCTTTTCCACTTNGCAAGCTTGTAATCGTACTGTGTGGA

*Sequences were previously reported by Duangjit et al. (2013) and are listed here for convenience only. SNP shown in brackets. N indicates nucleotides not scored due to low confidence.

Fig. 3. Genetic linkages on chromosome 4 of onion among single nucleotide polymorphisms (SNPs) across the 1.5 logarithm of odds (LOD) confidence interval (black bars) for pink-root resistance for segregating families PR1 (Dehya × B5351), PR2 (Dehya × Ski-MsMs), and the combined analysis of PR3 and PR4 (B5351 × W446) families. SNP names are shown on right (Duangjit et al., 2013) and genetic distances in centimorgans are shown on left. Genetic map was developed using an F₂ family from Dehya × B5351 as reported by Damon and Havey (2014).

QTL explained 54% of the phenotypic variability for PR survival (Table 2) and mapped to the same region on chromosome 4 as the PR1 and PR2 families. The PR resistance QTL was located closest to SNP marker isotig26045_1046, and the positive additive effect of the region tagged by marker isotig26045_1046 indicated that for every W446B allele added, PR survival increased by 24% (Fig. 2).

We attempted to join the genetic maps from the PR1, PR2, PR3, and PR4 families, but too few SNPs segregated across families to construct a consensus map. However SNPs within the 1.5 LOD confidence intervals for the PR1, PR2, and PR3/PR4 families (Table 3) were previously mapped by Damon and Havey (2014) using a segregating F₂ family from the same parents as the PR1 family, and positions of the 1.5 LOD confidence intervals are shown in Fig. 3. In a patent application, Black et al. (2015) mapped PR resistance from the short-day population SYG-75-1706 and reported that resistance showed a significant additive effect of 32% increased seedling survival. We determined the segregations of two SNP markers (NQ0257962 and NQ0257570) reported in this patent application as linked to PR resistance, and both mapped within the 1.5 LOD interval on chromosome 4 for PR resistance in the PR3 and PR4 families. NQ0257962 mapped between SNPs isotig26045_1046 and isotig38830_623 and NQ0257570 between SNPs isotig35268_1082 and isotig33399_1211. The results of our study and that of Black et al. (2015) reveal that three sources of PR resistance show codominance in seedling evaluations and map to the same region on chromosome 4 (Fig. 3). Earlier studies evaluated segregating families in Pt-infested field plots (Jones and Perry, 1956) and in a seedling assay (Nichols et al., 1965) similar to the one used in this study, and both concluded that PR resistance is recessively inherited. These recessive PR resistances may represent different resistance mechanism(s) to PR. The SNPs identified in this study (Table 3) associated with PR resistance will be useful for comparative mapping of different sources of resistance, and allow independent resistances to be combined with the codominant source identified by this study to potentially increase overall PR resistance. The SNP markers identified in this study will also be useful for the introgression of the PR resistance on chromosome 4 into diverse onion populations.

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