Article

Quantitative Trait Loci for Resistance to Potato Dry Rot Caused by Fusarium sambucinum

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Abstract: Tuber dry rot is an important disease of potato caused by soil and seed-borne pathogens of the Fusarium genus leading to losses that may reach 60% of the yield. The goal of this work was to study the inheritance of the dry rot resistance in two diploid potato hybrid populations (11–36 and 12–3) with complex pedigrees, including several wild Solanum spp. We used an aggressive isolate of F. sambucinum for phenotyping both progenies, parents, and standard potato cultivars in laboratory tuber tests, in three subsequent years. The QTL for dry rot resistance were mapped by interval mapping on existing genetic maps of both mapping populations. The most important and reproducible QTL for this trait was mapped on chromosome I and additional year- and population-specific QTL were mapped on chromosomes II, VII, IX, XI, and XII, confirming polygenic control of this resistance. This is the first study mapping the loci affecting tuber dry rot resistance in potato genome that can contribute to better understanding of potato-F. sambucinum interaction and to more efficient breeding of resistant potato cultivars.

Keywords: diversity array technology; mapping; Solanum tuberosum; tuber rot; wild crop relatives

1. Introduction

Potato (Solanum tuberosum L.) is the third most important humans’ food crop, with global annual production of 359 million tons (data from 2020, FAOSTAT http://www.fao.org/faostat/en/#data, accessed on 13 October 2021). With more than 70% of water content, potato tubers are vulnerable to many diseases and rots which contribute to food losses and waste occurring at all stages from field to fork. The losses and waste of root and tuber crops, including potato, are estimated at 40–50% and are greater than the average losses of 1/3 (1.3 billion tons) of the global food production (FAO Key Facts, http://www.fao.org/food-loss-and-food-waste/flw-data, accessed on 13 October 2021).

Tuber dry rot is an important disease of potato caused by soil and seed-borne pathogens of the Fusarium genus. These ascomycetes can infect tubers in the field, but the disease develops mainly in the storage, leading to losses that may reach 60% of the yield [1]. A risk of mycotoxin contamination raises further concerns about Fusarium-infected tubers. Fusarium sambucinum Fuckel, F. coeruleum Lib. ex Sacc., F. graminearum Schwabe, and F. oxysporum Schltdl. are most common among the 13 species described as causal agents of the potato tuber dry rot in different parts of the world [1,2]. Fusarium taxonomy, however, has lately been very dynamic. Species complexes, new species and even genera, such as Neocosmospora, are distinguished [3]. Naming the dry rot pathogens is a challenge because some Fusarium spp. found in potato tubers may play the roles of secondary pathogens or saprophytic colonizers [4,5].

Chemical control of the dry rot in potato tubers is limited, mostly to tuber treatments of the seed materials, either after harvest or before planting. Frequent appearance of fungicide-resistant Fusarium spp. strains, as well as concerns about human and ecosystem...
health, have resulted in search for environment-friendly chemicals (salts, plant extracts, essential oils, etc.) and biocontrol means against the disease [1]. Antagonistic bacteria, such as Bacillus subtilis and Pseudomonas spp. [6,7] or chitosan [8], can serve as examples here. Proper cultivation and storage practices are essential for limiting dry rot losses in ware potato production. Among them, using healthy seed tubers, harvest timing ensuring tuber maturity and good skin set, avoiding bruises and wounding during harvest, allowing wound healing after harvest, disinfection and ventilation of storage facilities are most effective [1]. Due to long-term survival of the Fusarium spp. in the soil and their broad host ranges, crop rotation is not helpful in potato dry rot management [9].

Breeding potatoes for dry rot resistance is a disease control strategy with a great potential, and little exploited so far. Growing resistant cultivars would allow cutting carbon footprint of potato cultivation by minimizing losses without application of any protection means, which leave carbon footprint, too. Breeding for resistance starts with search for resistance sources. Screenings for resistance to dry rot have been performed in different countries and have generally demonstrated a lack of resistance to this disease among tested potato cultivars and breeding lines. The Fusarium species and isolates used, as well as the potato material, varied between the studies. In Poland, potato cultivars Arran Banner, Great Scot, Bem, and Flora have been evaluated as most resistant to both F. sambucinum and F. coeruleum, among 61 cultivars tested in laboratory dry rot tuber tests [10]. An extensive screening of 1000 potato clones from Lüsewitz Potato Collection has revealed only 29 as having low susceptibility to dry rot of tubers [11]. One of them, cultivar Edzell Blue, also showed moderate resistance to dry rot in a later study in the UK, where it was tested together with 13 other potato cultivars [12]. A study in China has described all 67 tested potato clones as susceptible to F. sambucinum, with cultivar Désirée showing the smallest dry rot lesions [13]. Heltoft et al. [14] have shown some variation in susceptibility to dry rot among 10 potato cultivars commonly grown in Norway, as well as significant interaction between potato cultivars and tested Fusarium spp. (F. coeruleum, F. culmorum, and F. sambucinum), indicating existence of pathogen species-specific resistance to dry rot. Aydin and Inal [15] have divided 17 potato cultivars into three categories of moderately susceptible, susceptible, and highly susceptible to tuber dry rot, after testing them with three isolates of F. sambucinum and one isolate of F. solani. This study has also demonstrated a significant effect of the pathogen species/isolate × potato cultivar interaction on the results of dry rot resistance evaluation.

Low level of dry rot resistance in cultivated S. tuberosum gene pool has triggered wider search among other tuber-bearing Solanum spp. Sources of dry rot resistance have been found in S. chacoense, S. ehrenbergii, S. specazzini, and S. vernei [16]. Long-day adapted S. tuberosum subsp. andigena (Neotuberosum) lines have been screened for resistance to F. coeruleum and F. sulphureum (syn. F. sambucinum), with some clones found more resistant than the control cultivar Désirée [17]. Lynch et al. [18] tested from one to three accessions of 31 Solanum species for resistance to F. sambucinum and have described S. boliviense, S. gourlayi, S. microdontum, S. sancta-rosae, S. kurtzianum, S. fendleri, S. gandarillasii, S. oplocense, and S. vidaurrei as highly resistant. Assessment of 10 progenies from intra- and inter-specific crosses between resistant and susceptible genotypes from this study indicated complex inheritance of dry rot resistance in seven cases, while, in three crosses, single recessive genes have been proposed as underlying the trait [18]. Other studies on inheritance of resistance to dry rot in tetraploid potatoes have shown that the resistance to different Fusarium spp. is inherited independently [19] and that it has a large genetic component [20]. In a diploid hybrid potato population, the genetic factors affecting the resistance to tuber dry rot have been described as lacking the additive effects [21].

The goal of this work was to study the inheritance of the dry rot resistance in two diploid potato hybrid populations with complex pedigrees, including several wild Solanum spp. We used an aggressive isolate of F. sambucinum for phenotyping both progenies in three subsequent years and Diversity Array Technology (DArT) markers for genotyping.
Using interval mapping, we mapped the Quantitative Trait Loci (QTL) for resistance to tuber dry rot on the potato genetic map.

2. Materials and Methods

2.1. Plant Material

Two F1 populations of diploid potato were used in this study. The population 11–36 (N = 149), originated from cross between diploid potato clones DG 06-5 and DG 03-226, was sown in 2011 and was used to map QTL for tuber morphology traits, as well as for tuber blackspot bruise and enzymatic discoloration susceptibilities [22,23]. The population 12–3 (N = 183) obtained from a cross between DG 00-683 and DG 08-28/13 and sown in 2012, served for mapping QTL for tuber starch content and chip color [24–26]. The parental forms of both populations resulted from a recombinant breeding program at IHAR-PIB that included in their pedigree a number of Solanum species: S. tuberosum, S. acaule, S. chacoense, S. demissum, S. gourlayi, S. microdontum, S. phureja, S. stenotonum, S. verrucosum, and S. yungasense. Individuals of both populations have been genotyped using Diversity Array Technology (DArT) and a number of PCR markers. The genetic maps have been constructed by regression mapping as described earlier [23,24]. As standards, we used a set of Polish potato cultivars described in earlier studies, where they were scored as resistant to tuber dry rot (Bartek, Gawin, Kuba) or susceptible (Harpun, Hinga) using a number of isolates from several Fusarium spp. [4,5].

2.2. Fusarium sambucinum

A highly aggressive isolate MF1 of *F. sambucinum* from the IHAR-PIB Młochów collection was used in all tests. It originates from diseased potato tuber collected in 1999 in Poland, and it was used as reference in other works [4,5]. The pathogen was stored in liquid nitrogen and propagated for the tests on potato sucrose agar (PSA) medium [27].

2.3. Dry Rot Resistance Tests

Due to losses of individuals from both mapping populations, 120 individuals of the population 11–36 and 178 individuals of the population 12–3 were tested for tuber dry rot resistance in laboratory tests. Tubers of mapping populations, parental forms and standard cultivars, were planted in the field at the end of April and harvested at the end of September in 3 consecutive years (2017, 2018, and 2019). The field was fertilized with 90, 90, and 170 kg·ha⁻¹ of N, P, and K, respectively, and plants were chemically protected against pests and pathogens. Harvested tubers were stored for 2.5 months at 5–10 °C in standard potato storage, and the dry rot resistance tests were performed in December–January after each growing season according to the scheme: 3 years × 2 different inoculation dates × 2 replications × 5 tubers per genotype. The year of harvest was used as a testing season identifier. The tests were performed as described by Reference [28]: potato tubers were wounded on the apical ends with a steel rod leaving a hole 10 mm deep and 5 mm wide. The wounds were inoculated with 50 μL of inoculum (*F. sambucinum* isolate MF1 spore suspension in sterile tap water, 2.5 spores × 10⁶ mL⁻¹, washed off from 3-week-old fungal cultures on PSA medium). Tubers were incubated for 3 weeks at 16 °C, in darkness and under glass cover to maintain elevated air humidity. Then, they were cut along the longer axes, and two perpendicular diameters of the dry rot lesions were measured using a ruler (2017, 2018) or electronic calipers (2019) CD-1.

AX (Mitutoyo Poland Sp. z o.o., Wroclaw, Polska). Lesion size was calculated as an arithmetic mean of the two diameters measured for each tuber and treated as inverse estimation of dry rot resistance.

2.4. Genetic Maps and QTL Analysis

For both populations, genetic maps have been constructed earlier using JoinMap® 4.0 (Kyazma B. V., Wageningen, The Netherlands) [29] software. The following parameters
were used: CP (cross pollination/outbreeder full-sib family) population type, and indepen-
dence LOD (significance cut-off, LOD score >3) as a grouping parameter. Linkage maps
were calculated using a regression method (linkage significance cut-off, LOD score >3) and
the Haldane’s mapping function for the calculation of map distances.

A genetic map of the population 11–36 contained 1359 Diversity Array Technology
(DArT) markers and 9 sequence characterized amplified regions (SCAR) and cleaved
amplified polymorphic sequence (CAPS) markers [23]. Genetic map of the population 12–3
consisted of 1597 markers, including 1584 DArT markers, 11 CAPS markers, one SCAR
marker, and one phenotypic (purple flower color) marker [24].

Interval mapping was applied to locate QTL for dry rot resistance using both mapping
populations and MapQTL®6 software (Kyazma B. V., Wageningen, The Netherlands) [30],
as described previously [23,24]. QTLs were detected using an LOD threshold ≥3 estimated
from the cumulative distribution function of the maximum LOD on a chromosome for QTL
analysis based on four QTL genotypes [31].

2.5. Statistical Analyses

The normality of the distribution of phenotypic data was tested with the Kolmogorov-
Smirnov, Lilliefors, and Shapiro–Wilk tests. The reproducibility of analyzed traits between
years was estimated by calculating linear Pearson’s correlation coefficients. Duncan’s
multiple range test was used to assess significance of differences in dry rot lesion sizes
between the parental forms and standard potato cultivars. Analysis of variance was
performed to assess the significance of the genotype, year, and their interaction effects
on the dry rot resistance in the mapping populations. The broad-sense heritability was
calculated according to Domański et al. [32]. All statistical analyses were performed using
STATISTICA for Windows (StatSoft Polska, Krakow, Poland).

3. Results

3.1. Dry Rot Resistance Tests

The parents of the population 11–36 differed strongly in their tuber dry rot resistance
evaluated in a laboratory tuber test with *F. sambucinum* isolate MF1 (Table 1). The three-year
mean lesion size for the resistant parent DG 06-5 was 12.2 mm, and it was the smallest
one among the results obtained for the parental forms and standard cultivars. DG 06-5
did not differ significantly in dry rot resistance from the most resistant standard cultivar
Bartek with the lesion size 14.1 mm. The other parent, DG 03-226, was susceptible to dry rot
and with the mean lesion size of 23.7 mm, and it did not differ significantly from the most
susceptible standard cultivar Hinga. Both parents of the population 12–3 were moderately
resistant with mean lesion sizes 19.9 and 21.4 mm for the DG 08-28/13 and DG 00-683,
respectively (Table 1).

The mean results of dry rot lesion size evaluations were distributed normally among
the tested 120 individuals of the population 11–36 and 178 individuals of the population
12–3, according to the Kolmogorov-Smirnov test (Figure 1). However, other tests indicated
deviation from normality of the mean dry rot lesion size distribution in the population
12–3 (Figure 1b). The range of mean lesion sizes of individuals from the population 11–36
was wider, and the population mean was lower than in the population 12–3 (Table 1). In
particular years, the number of the individual progeny clones tested for dry rot resistance
varied between 109 and 119 in the population 11–36 and between 107 and 177 in the
population 12–3. The dry rot resistance test results from different years of evaluation
were significantly correlated with each other in both populations, with one exception: in
the population 12–3, datasets from 2017 and 2019 were not correlated (Table 1). Potato
genotype and year of testing had significant effects on the dry rot resistance tests result
in both populations, as shown by analyses of variance (Table 2). Heritability in the broad
sense in the population 11–36 was 0.58, and it was higher than in the population 12–3
(Hb = 0.22; Table 2). There was a weak negative correlation between mean tuber starch
content measured in a previous study in years 2012–2014 [23] and mean (2017–2019)
dry rot lesion size in the population 11–36 (Pearson’s correlation coefficient $r = -0.227$, $p = 0.013$). No significant correlation was detected between tuber starch content and dry rot lesion size in the population 12–3, which was also evaluated for tuber starch content in years 2012–2014 [24].

**Table 1.** Evaluation for *Fusarium* dry rot resistance of the standard potato cultivars, parental lines and individuals of the diploid potato populations 11–36 and 12–3. Mean results of three-year (2017–2019) measurements of a lesion sizes (±SD) caused by the *F. sambucinum* isolate MF1.

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Mean Lesion Size in mm (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard cultivars</strong></td>
<td></td>
</tr>
<tr>
<td>cv. Bartek</td>
<td>14.1 (±5.1) a</td>
</tr>
<tr>
<td>cv. Kuba</td>
<td>18.2 (±8.1) b</td>
</tr>
<tr>
<td>cv. Harpun</td>
<td>20.3 (±5.3) c</td>
</tr>
<tr>
<td>cv. Gawin</td>
<td>20.4 (±6.6) c</td>
</tr>
<tr>
<td>cv. Hinga</td>
<td>24.8 (±8.5) d</td>
</tr>
<tr>
<td><strong>Parental forms</strong></td>
<td></td>
</tr>
<tr>
<td>DG 06-5</td>
<td>12.2 (±3.8) a</td>
</tr>
<tr>
<td>DG 08-28/13</td>
<td>19.9 (±4.9) bc</td>
</tr>
<tr>
<td>DG 00-683</td>
<td>21.4 (±4.5) c</td>
</tr>
<tr>
<td>DG 03-226</td>
<td>23.7 (±7.2) d</td>
</tr>
<tr>
<td><strong>Population 11–36</strong></td>
<td></td>
</tr>
<tr>
<td>Population mean</td>
<td>18.0</td>
</tr>
<tr>
<td>Population median</td>
<td>18.0</td>
</tr>
<tr>
<td>Mean lesion size range</td>
<td>12.9–24.7</td>
</tr>
<tr>
<td>Range of Pearson’s coefficients between results from years 2017–2019</td>
<td>0.55–0.67</td>
</tr>
<tr>
<td><strong>Population 12–3</strong></td>
<td></td>
</tr>
<tr>
<td>Population mean</td>
<td>18.6 (±1.8)</td>
</tr>
<tr>
<td>Population median</td>
<td>18.8</td>
</tr>
<tr>
<td>Mean lesion size range</td>
<td>12.7–22.2</td>
</tr>
<tr>
<td>Range of Pearson’s coefficients between results from years 2017–2019</td>
<td>0.05–0.37</td>
</tr>
</tbody>
</table>

Values marked with the same letter do not differ significantly according to Duncan’s multiple range test with a significance level of 0.05. All Pearson’s correlation coefficients were significant at $p < 0.01$, except the correlation coefficient between results obtained for the 12–3 population in years 2017 and 2019 ($p = 0.64$).

**Table 2.** Analysis of variance for tuber dry rot lesion size, evaluated in three years in the diploid potato mapping populations 11–36 (a) and 12–3 (b).

<table>
<thead>
<tr>
<th>Factor</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F Value</th>
<th>p</th>
<th>Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 11–36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1] genotype</td>
<td>3566.9</td>
<td>112</td>
<td>31.8</td>
<td>5.42</td>
<td>&lt;0.001</td>
<td>0.58</td>
</tr>
<tr>
<td>[2] year</td>
<td>15357.1</td>
<td>2</td>
<td>7678.5</td>
<td>1306.97</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>[1] × [2]</td>
<td>1400.2</td>
<td>224</td>
<td>6.3</td>
<td>1.06</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>1991.6</td>
<td>339</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) 12–3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1] genotype</td>
<td>1343.2</td>
<td>107</td>
<td>12.6</td>
<td>2.26</td>
<td>&lt;0.001</td>
<td>0.22</td>
</tr>
<tr>
<td>[2] year</td>
<td>6210.4</td>
<td>2</td>
<td>3105.2</td>
<td>557.95</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>[1] × [2]</td>
<td>1445.6</td>
<td>214</td>
<td>6.8</td>
<td>1.21</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>1803.2</td>
<td>324</td>
<td>5.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SS, sum of squares; df, degrees of freedom; MS, mean square; Hb, heritability in the broad sense. $Hb = \sigma^2_g / (\sigma^2_g + \sigma^2_{ge} + \sigma^2_e)$, $\sigma^2_g = (M_1 - M_2) / L$, $\sigma^2_{ge} = M_2 - \sigma^2_e$, where $M_1$ = mean square of effect of genotype, $M_2$ = mean square of effect of genotype × year interaction, $L$ = number of years, $\sigma^2_e$ = mean square of error [32].
The QTL for dry rot resistance were mapped by interval mapping on existing genetic maps of both mapping populations 11–36 and 12–3 (Table 3). The QTL analyses were performed for the mean phenotypic data sets obtained in each year of testing and for the three-year mean data sets (17–19).
Table 3. QTL for dry rot resistance detected by interval mapping in datasets from the individual years of phenotypic assessments (2017–2019) and in the mean dataset (17–19, in bold) of the diploid potato populations 11–36 and 12–3.

<table>
<thead>
<tr>
<th>Trait and Dataset</th>
<th>Chromosome</th>
<th>QTL Peak (cM)</th>
<th>Marker or Marker Interval at Peak</th>
<th>LOD</th>
<th>$R^2$ (%)</th>
<th>Marker Origin$^a$</th>
<th>Peak Position DM1-3 v6.1$^b$</th>
<th>QTL Range (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population 11–36</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017 I</td>
<td>74.9</td>
<td>pPt-537438</td>
<td>3.65</td>
<td>14.3</td>
<td>P2</td>
<td>01</td>
<td>84651175–84651556</td>
<td>69.4–81.5</td>
</tr>
<tr>
<td>2018 I</td>
<td>66.8</td>
<td>pPt-533988</td>
<td>3.66</td>
<td>13.3</td>
<td>P2</td>
<td>01</td>
<td>78163480–78164064</td>
<td>50.7–71.5</td>
</tr>
<tr>
<td>2019 I</td>
<td>71.274</td>
<td>toPt-440651</td>
<td>6.05</td>
<td>20.9</td>
<td>P2</td>
<td>01</td>
<td>81329689–81330960</td>
<td>50.7–108.8</td>
</tr>
<tr>
<td>17–19 I</td>
<td>81.5–84.5</td>
<td>pPt-535624–pPt-652982</td>
<td>4.89</td>
<td>17.1</td>
<td>P1–P2</td>
<td>01</td>
<td>87666433–87667054</td>
<td>54.1–108.1</td>
</tr>
<tr>
<td>2019 VII</td>
<td>21.1</td>
<td>pPt-471208</td>
<td>3.60</td>
<td>13.0</td>
<td>H</td>
<td>07</td>
<td>3733794–3734717</td>
<td>20.8–21.4</td>
</tr>
<tr>
<td>2019 VII</td>
<td>38.6</td>
<td>pPt-457469</td>
<td>3.28</td>
<td>11.9</td>
<td>H</td>
<td>07</td>
<td>48569422–48569808</td>
<td>37.3–38.6</td>
</tr>
<tr>
<td>2019 IX</td>
<td>42.6</td>
<td>pPt-472930</td>
<td>5.20</td>
<td>18.2</td>
<td>P1</td>
<td>09</td>
<td>59439764–59440711</td>
<td>27.3–49.0</td>
</tr>
<tr>
<td>2017 XI</td>
<td>37.6</td>
<td>pPt-456657</td>
<td>3.42</td>
<td>13.5</td>
<td>H</td>
<td>11</td>
<td>39659759–39660130</td>
<td>37.6–38.3</td>
</tr>
<tr>
<td><strong>Population 12–3</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2018 I</td>
<td>19.5</td>
<td>pPt-539549</td>
<td>3.5</td>
<td>9.0</td>
<td>P2</td>
<td>01</td>
<td>1255475–1255976</td>
<td>19.0–26.6</td>
</tr>
<tr>
<td>2019 I</td>
<td>67.7</td>
<td>pPt-558970–pPt-473819</td>
<td>4.87</td>
<td>11.9</td>
<td>P2H</td>
<td>01</td>
<td>73152139–7315272674921680–74922384</td>
<td>61.2–71.7</td>
</tr>
<tr>
<td>17–19 I</td>
<td>62.5</td>
<td>capPt-672722</td>
<td>3.78</td>
<td>9.3</td>
<td>P1</td>
<td>01</td>
<td>73308821–73309676</td>
<td>58.1–69.8</td>
</tr>
<tr>
<td>2018 II</td>
<td>77.9</td>
<td>pPt-536805</td>
<td>3.43</td>
<td>8.8</td>
<td>P1</td>
<td>02</td>
<td>14674331–14674870</td>
<td>77.3–77.9</td>
</tr>
<tr>
<td>2019 II</td>
<td>4.5</td>
<td>pPt-536998</td>
<td>3.31</td>
<td>8.3</td>
<td>P2</td>
<td>02</td>
<td>42632179–42624277</td>
<td>4.4–6.5</td>
</tr>
<tr>
<td>2019 II</td>
<td>15.2</td>
<td>pPt-538350</td>
<td>3.42</td>
<td>8.5</td>
<td>H</td>
<td>02</td>
<td>41355373–41356084</td>
<td>15.2–15.3</td>
</tr>
<tr>
<td>2019 II</td>
<td>74.7</td>
<td>pPt-456871</td>
<td>3.27</td>
<td>8.2</td>
<td>H</td>
<td>02</td>
<td>14674331–14674875</td>
<td>74.6–75.7</td>
</tr>
<tr>
<td>17–19 II</td>
<td>52.5</td>
<td>pPt-457616</td>
<td>3.99</td>
<td>9.8</td>
<td>P2</td>
<td>02</td>
<td>32260675–32262674</td>
<td>52.0–52.7</td>
</tr>
<tr>
<td>17–19 II</td>
<td>43.0</td>
<td>pPt-552007</td>
<td>4.39</td>
<td>10.7</td>
<td>H</td>
<td>07</td>
<td>32689974–32690374</td>
<td>36.8–53.9</td>
</tr>
<tr>
<td>2019 IX</td>
<td>17.7</td>
<td>pPt-653179</td>
<td>3.72</td>
<td>9.2</td>
<td>H</td>
<td>09</td>
<td>8316412–8316923</td>
<td>16.9–18.8</td>
</tr>
<tr>
<td>2018 XII</td>
<td>125.3</td>
<td>pPt-655073–pPt-456622</td>
<td>3.28</td>
<td>8.5</td>
<td>P1</td>
<td>02</td>
<td>29779568–29780095</td>
<td>121.6–128.6</td>
</tr>
</tbody>
</table>

$^a$ P1—inherited from DG 03-226, P2—DG 08-05–5 (population 11–36), P3—DG 00-683 and P4—DG 08-28/13 (population 12–3); H—descended from both parents of given population;

$^b$ position in the reference genome *S. tuberosum* Group Phureja DM1-3 (v6.1 [33]) defined by BLAST search results; if the particular marker was not found in the reference genome, the interval limited by positions of its flanking markers is given.
3.2. QTL Analysis in Population 11–36

In the population 11–36, the most important QTL for dry rot resistance was detected on chromosome I. It was significant in the three data sets from the different years of testing and in the mean data set. Its peak shifted between 66.8 and 81.5–84.5 cM in different data sets. From 13.3% (2018; LOD = 3.66) to 20.9% (2019; LOD = 6.05) of variance could be ascribed to the markers at peak of QTL detected in data sets from particular years. In the mean (17–19) data set, this QTL spanned over a large region of chromosome I (54.1–108.1 cM) and explained up to 17.1% (LOD = 4.89) of variance in dry rot resistance (lesion sizes). The markers at the peak of the QTL originated from the resistant parent DG 06-5 (Table 3). Four other QTL for dry resistance in the population 11–36 were detected in single data sets. In 2017, a QTL explaining 13.5% (LOD = 3.42) of variance in dry rot resistance was identified on chromosome XI (37.6–38.3 cM). In the data set from 2019, two QTL on chromosome VII (20.8–21.4, 37.3–38.6 cM) and one QTL on chromosome IX (27.3–49.0 cM) were detected. They explained up to 13.0%, 11.9% (chromosome VII, LOD = 3.60 and 3.28, respectively), and 18.2% (chromosome IX, LOD = 5.20) of variance in dry rot resistance (Table 3).

3.3. QTL Analysis in Population 12–3

In the population 12–3, two QTL for dry rot resistance were reproducible between different data sets (Table 3). A QTL identified on chromosome I was significant in a data set from 2019 (peak at 67.7 cM) and in the mean (17–19) data set (peak at 62.5 cM), explaining, respectively, up to 11.9% (LOD = 4.87) and 9.3% (LOD = 3.78) of variance in the results of phenotypic tests. The second reproducible QTL was located on chromosome VII with peak detected at 36.8 cM in 2018 and at 43.0 in the mean data set. The percentage of explained variance in dry rot resistance that could be ascribed to this region reached 16.2 (LOD = 6.58) in 2018 and 10.7 (LOD = 4.39) in the mean data set (Table 3).

Additional QTL for dry rot resistance in population 12–3, that were not reproducible between the data sets, were located on chromosomes I, II, IX, and XII. The QTL on chromosome I explained up to 9.0% of variance (LOD = 3.5) and it was detected in the 2018 data set at 19.0–26.6 cM, in a region not overlapping with the locations of QTL identified in 2019 and mean data sets on the same chromosome. On chromosome II, one QTL was detected in the 2018 data set, three QTL in the 2019 data set, and one in the mean data set (17–19). None of their positions overlapped, and they all spanned over narrow regions not exceeding 2.1 cM. The most significant of them was detected in the mean data set and explained 9.8% of variance in dry rot resistance (LOD = 3.99). In the 2019 data set, a QTL explaining 9.2% of variance (LOD = 3.72) was identified on chromosome IX, and, in the 2018 data set, a QTL explaining 8.5% of variance (LOD = 3.28) was identified on chromosome XII (Table 3).

3.4. QTL Location in the Reference Genome

The markers at QTL peak were located in the reference genome of *S. tuberosum* Group Phureja DM1-3 v4.03 using Genome Browser at the Solanaceae Genomics Resource (http://spuddb.uga.edu/), accessed on 13 October 2021, and then their sequences were found by BLAST search in the v6.1 [33] of the reference genome (Table 3). By the same approach, the locations of the DArT markers flanking the most important QTL for dry rot resistance were identified in the reference potato genome DM1-3 v6.1 (Table S1). The projections of the QTL locations on chromosomes I and VII in the reference genome are shown in Figure 2. On chromosome I, the QTL detected in population 11–36 in all four data sets overlapped with each other and partially, also, with a QTL detected in population 12–3, data sets 2019 and mean 17–19, while another QTL identified in population 12–3 (data set 2018) was located in a different region. The range of QTL detected in the mean 17–19 data set in the population 11–36 corresponded to the 77.6–87.6 Mbp region of chromosome I in the reference genome DM1-3 v6.1 (Table S1). Among the four QTL on chromosome VII, the one detected in data set 2019 in population 11–36 overlapped with the QTL identified in the population 12–3, mean data set (Figure 2), projected to the region 48.3–57.6 Mbp of chromosome VII in the reference genome DM1-3 v6.1 (Table S1).
4. Discussion

This is the first study mapping the loci affecting tuber dry rot resistance in potato genome. Using two mapping populations of diploid potato that were phenotyped with highly aggressive isolate of *F. sambucinum*, we mapped the most important QTL for this trait on chromosome I and year- and population-specific QTL on chromosomes II, VII, IX, XI, and XII, confirming polygenic control of this resistance.

In contrast to earlier studies, the incubation period in our dry rot resistance tests was shortened to 3 weeks at 16 °C. After this period, due to high aggressiveness of the *F. sambucinum* isolate MFI and the small sizes of the tested tubers, we were running into a risk that the most susceptible tubers get completely infected, and we are unable to measure lesion sizes. In exemplary earlier studies, the incubation period has varied: 4–5 months at 3.85–6.85 °C [34], 8–10 weeks at 10 °C [17,19], 5–6 weeks at 10.85–14.85 °C [35], 5 weeks at 15–20 °C [15], and 4 weeks at 10 °C [12]. In 2019, we modified the lesion size measurement method by introducing electronic calipers. It reduced the amount of labor and improved the precision of measurement, which may have resulted in a bigger number of QTL detected in the 2019 data set (Table 3).

The most important QTL for dry rot resistance was reproducibly detected in the data sets from different years and was in regions partially overlapping between the two populations, which may be caused by a widespread presence of the resistance alleles in this locus and its universal character. Other explanation could be related to common pedigree
elements between the parental forms of the two populations that all were derived from IHAR-PIB, Młochów Research Center, diploid potato breeding program. The reproducibility of the phenotyping tests was affected by the age of the mapping populations that were sown in 2011 (population 11–36) and in 2012 (population 12–3). Since then, they were field and greenhouse-propagated, which resulted in possible virus accumulation, loss of some individuals each year, smaller tubers and insufficient yields, and, in turn, various and decreasing numbers of individuals tested for dry rot resistance. In years of testing 2017–2019, the mapping populations were field-propagated, which was an important source of variation in the dry rot resistance tests, as the uncontrolled weather and especially rainfall affected the condition, size, and maturity of tubers. Despite these limitations that are reflected in rather low broad sense heritability coefficients of the dry rot resistance and lack of strong correlations between the results of testing in different years, the QTL on chromosome I was detected in most of the phenotypic data sets showing the strength of this genetic component of the resistance. In a study of Valluru et al. [20], broad-sense heritability of dry rot resistance in a tetraploid *S. tuberosum* progeny has been estimated as very high (H coefficients: 0.77–0.84). Slightly lower broad-sense heritability coefficients (0.63–0.81) have been described for a diploid *S. phureja*-*S. stenotomum* population [21]. However, in these two works, the broad-sense heritability has been calculated for each year of testing by an approach different than ours.

Additional QTL for dry rot resistance were identified less reproducibly among the data sets, and, with the exception of the partially overlapping QTL on chromosome VII, they were unique for each mapping population, indicating the presence of complex resistance inherited from different sources and effect of growth environment on the resistance. The sources of resistance to *F. sambucinum* in our material cannot be identified precisely, as the data on dry rot resistance of all the ancestors of the mapping populations is not available. The theoretical contributions of *S. tuberosum* in the origins of the four parental forms varied from 64 to 70% [23,24], and, as the vast majority of the tested *S. tuberosum* cultivars have been described as susceptible, we found this species rather unlikely a source of the dry rot resistance in our materials. Standard cultivar Bartek (registered in Poland in 2003, Zamarte Potato Breeding Ltd.—IHAR Group) showed high resistance to *F. sambucinum* in this study and in an earlier work of Azil et al. [5], but it may be attributed to *S. vernei* and *S. demissum* in its pedigree (Wageningen Potato Pedigree Database https://www.plantbreeding.wur.nl/PotatoPedigree/index.html, accessed on 13 October 2021). *Solanum chacoense*, *S. microdontum*, and *S. gourlayi*, that are present in pedigrees of the four parental forms in this study, have been described earlier as highly resistant to tuber dry rot caused by *F. sambucinum* [16,18]. These species might have contributed to the resistance of our materials, although we used different accessions than the authors of the earlier works [16,18]. Potential sources of dry rot resistance in both mapping populations could have been also *S. verrucosum*, *S. acaule*, *S. demissum*, *S. phureja*, and *S. yungasense*, and, in the population 11–36, additionally, *S. stenotomum* [23,24].

In both mapping populations, the QTL for dry rot resistance on chromosome I overlapped with QTL for tuber starch content described earlier [23,24]. In the population 11–36, dry rot resistance was weakly correlated to tuber starch content, while no such correlation was detected in the population 12–3. Dry rot resistant parent of the population 11–3, DG 06-5, was also the one with high tuber starch content, which could support a hypothesis that the genes affecting tuber starch content and those controlling dry rot resistance are linked and co-inherited in this material. In the population 11–36, the same region of chromosome I harbored a QTL for enzymatic discoloration of tuber flesh [23,24]. We cannot exclude that the genes and alleles shaping chemical composition of tuber have pleiotropic effects on resistance to *F. sambucinum* infestation. In the population 11–36, QTL for morphological traits (tuber shape, regularity of tuber shape, eye depth, and mean tuber weight) have been identified on chromosome I in the regions partially overlapping with the QTL for dry rot resistance [22]. However, in laboratory test for dry rot resistance, performed with
controlled and standardized inoculation of each tuber, such traits should not be relevant for dry rot resistance.

The research on potato resistance against pathogens has been dominated by mapping and identification of the genes and QTL for resistance to *Phytophthora infestans*. Knowledge on resistance to fungal pathogens has been limited. Resistance to *Synchytrium endobioticum*, an obligate biotroph from *Chytridiomycota* phylum, has been one of the best studied cases with several major genes and QTL mapped [36]. QTL and two underlying genes against *Verticillium dahliae* (*Ascomycota*) causing Verticillium wilt and potato early dying have been mapped on chromosome IX [37] and used in potato breeding [38]. A resistance gene against related pathogen *V. albo-atrum* has been found in *S. chacoense* [39] and QTL for the resistance to this pathogen have been identified on potato chromosomes II, VI, IX, and XII [40]. Massa et al. [41] have mapped a QTL on chromosome V, explaining 46.5% of variance in resistance to *Verticillium* wilt and 41.3% of variance in resistance to early blight caused by *Alternaria tenuis* (*Ascomycota*) using a tetraploid russet potato population. In a different tetraploid potato population, numerous QTL for resistance to *A. solani* in foliage and in tubers, as well as QTL for resistance to defoliation, have been mapped [42]. Our study characterized new sources of resistance to *F. sambucinum*, and it provided knowledge on QTL for dry rot resistance that can be exploited in further studies on plant-fungus interactions and identification of genes involved in it. Our findings can be implemented in breeding of potato cultivars resistant to dry rot that would improve the crop resilience and decrease storage losses, thus contributing to sustainable potato production.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12010203/s1, Table S1: Locations of the DArT markers flanking the most important QTL for dry rot in the reference potato genome DM1-3 v6.1.

**Author Contributions:** All authors contributed to the study conception and design. S.S. maintained *F. sambucinum*, prepared inocula and participated in tuber dry rot resistance tests, M.J. participated in tuber dry rot resistance tests, E.S. participated in tuber dry rot resistance tests and data management, I.W.-F. propagated plant material and analyzed the potato pedigrees, J.´S. performed data analyses and interpretation and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Mapping data has been published earlier [23,24]. Selected potato breeding lines are available at Plant Breeding and Acclimatization Institute—National Research Institute in National Centre for Plant Genetic Resources: Polish Genebank. *Fusarium sambucinum* isolate MF1 is available from IHAR-PIB Młochów pathogen collection.

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