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Identification of Soybean Germplasm and Associated Molecular Markers with Resistance to *Fusarium graminearum*

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Abstract: Soybean ranks second by total production of all crops grown in the United States. From surveys of soybean production regions in the US and Canada, seedling diseases have been consistently identified as one of the top five biotic limitations on yield for over two decades. The role of *Fusarium graminearum* as an aggressive member of this complex was unknown until relatively recently and, consequently, publicly and commercially available varieties with resistance to this pathogen are unavailable. To address the need for resistant germplasm and to improve our understanding of the genetic basis underlying the resistance, we screened a set of 208 accessions of soybean from the United States Department of Agriculture Soybean Germplasm Collection (USDA-SGC) under controlled greenhouse conditions. A ratio of the root weight of inoculated plants compared to mock-inoculated controls was used to evaluate the degree of resistance. A linear mixed model identified eight resistant accessions (PI 548311, PI 438500, PI 561318 A, PI 547690, PI 391577, PI 157484, PI 632418, and PI 70466 -3) with significantly higher resistance than the population mean. Previous genotyping publicly available through the SoyBase database was used in a genome-wide association study (GWAS) to determine single nucleotide polymorphism (SNP) markers associated with resistant and susceptible phenotypes. A total of five significant marker-trait associations (MTAs) were discovered on chromosomes Gm02, Gm03, Gm06, Gm07, and Gm13, each accounting for 4.8, 4.3, 3.8, 4.1, and 3.0% of the phenotypic variance, respectively. This study, thus, lays a foundation for the better dissection of germplasm resistant to *F. graminearum*.

Keywords: soybean; *Fusarium graminearum*; seedling disease; host resistance; genome-wide association study

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1. Introduction

Seedling diseases, alternatively damping-off or seedling blights, are a broad diagnosis encompassing the symptoms of pre-emergence seed rots and post-emergence seedling decay caused by biotic pathogens [1,2]. Seedling diseases threaten yield by reducing plant stands when favorable environmental conditions for the pathogen combine with vulnerable seeds and seedlings. Soilborne pathogens in the genera *Fusarium*, *Pythium*, *Phytophthora*, and *Rhizoctonia* are often the major drivers of seedling diseases in soybean [3–5]. For the past two decades, soybean yield losses due to major diseases have been extensively monitored across the soybean production regions of the US and Canada by various extension services [6–9]. From the most recently published survey, soybean seedling diseases have consistently ranked as the second highest biotic yield limitation behind soybean cyst nematode [7]. Current management strategies for soybean seedling diseases primarily target prevention and include practices relating to the reduction of excess moisture at planting, planting in warm conditions, and pre-emptive deployment of fungicidal seed treatments when disease pressure is expected to be high [10]. The use of seed-applied

fungicides further increases the cost of soybean production and has questionable efficacy at mitigating seedling disease-associated losses and improving yield [11]. Worse yet, evidence of the development of fungicide insensitivity raises the question of whether increased dependence on chemical control will be sustainable for managing these pathogens in the future [12–15]. Continued research into the management of seedling diseases through nonfungicidal means is, therefore, critical to prevent severe yield and profit losses.

Pythium spp., *Rhizoctonia solani*, and *Phytophthora sojae* infections of soybean have been extensively documented. Many species in the genus *Fusarium* are associated with seedling diseases, while a relatively newly documented soybean pathogen, *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch), has now been implicated as a primary cause of *Fusarium*-derived seedling diseases [16]. *F. graminearum* has long been known in soybean production regions as a poaceous pathogen causing head blight of many cereal crops and ear and stalk rot of maize. In 2004, its pathogenic association with soybean was demonstrated conclusively for the first time [16]. Since 2007, reports from around the United States of highly pathogenic *F. graminearum* isolates from soybean seedlings have been increasing [3,13,17,18]. In South Dakota, *F. graminearum* was recently identified as the primary *Fusarium* species from a survey of seedling disease samples taken from across the state [19]. *F. graminearum* is a predominant driver of soybean seedling diseases, especially in the Midwest, and should be targeted to reduce the incidence and severity of disease.

Genetic resistance offers a reliable and affordable option for growers to help manage losses caused by *F. graminearum* infections. Several resistant accessions and quantitative trait loci (QTL) have been reported to confer partial resistance and tolerance to *F. graminearum*. Zhang et al. reported cultivar differences shortly after *F. graminearum* pathogenicity on soybean was confirmed in North America [20]. Ellis et al. reported resistance from accession PI 525453 and identified four QTL conferring resistance, each accounting for approximately 3 to 11% of the phenotypic variation observed in their experiments [21]. A follow-up attempt at fine-mapping using recombinant inbred lines (RILs) descended from PI 525453 identified three QTL conferring 3.1–8.6% of the phenotypic variation of the resistance, only one of which was previously identified in PI 525453 in the original study [21,22]. Several other accessions, including PI 424354, were identified with greater resistance to *F. graminearum* than PI 525453 in the first study by Ellis et al.; however, they were not included in the QTL mapping studies [21]. Acharya et al. identified two QTL from PI 567301 B accounting for 38.5 and 8.1% of the phenotypic variation under the conditions of their experiments [23]. A follow-up study fine-mapped the major QTL and predicted candidate genes related to seed coat properties [24]. When the seed coat of PI 567301 B was mechanically removed, the resistant phenotype was lost, suggesting a potential role of seed coat properties in soybean resistance to *F. graminearum*. Cheng et al. also identified a major QTL from PI 567516 C accounting for 40.2% of the phenotypic variation in *F. graminearum* resistance on the same chromosome as the major QTL discovered by Acharya et al.; however, it was concluded that they were distinct from one another [23,25]. Zhang et al. conducted a GWAS on a panel of 314 accessions from the Chinese National Soybean GeneBank and found 12 significant marker-trait associations (MTAs) explaining 5.5–14.7% of the phenotypic variation [26]. A follow-up QTL mapping study identified seven QTL underlying resistance in a cross between 'Hefeng-25' and PI 525453 [27]. The lack of consensus among the loci reported between studies and the small effect sizes of many of the QTL indicate that resistance to *F. graminearum* in soybean is complex and likely controlled by multiple loci across different genetic backgrounds.

Despite knowledge of genetic loci conferring resistance to *F. graminearum*, no commercial or public varieties with resistance have been developed. An alternative approach to breeding for major genes and QTL is to use marker-assisted selection to select for multiple MTAs at once. This may result in cumulative effects that are large enough to warrant the breeding efforts. Therefore, it is important to continue improving our knowledge of genetic resistance against *F. graminearum* by identifying resistant germplasm and the markers linked to resistance loci. With the overall purpose of trying to increase the availability

of resources for soybean resistance to *F. graminearum*, the objectives of this study were to (a) screen a diverse panel of soybean accessions of diverse international origin, including land races, cultivars, and elite varieties (hereafter referred to as accessions) from maturity groups 0-VI adapted to the North Central USA for phenotypic resistance to *F. graminearum* and to (b) perform a GWAS to identify molecular markers associated with the observed *F. graminearum* resistance. In this study, we screened a panel of 208 soybean accessions for resistance against two soybean-derived *F. graminearum* isolates using a greenhouse assay and then performed a GWAS by integrating our phenotypic data with previously curated and publicly available genotypic data from SoyBase (www.soybase.org accessed on 8 February 2023).

2. Materials and Methods

2.1. Preparation of Inoculum

Two isolates of *Fusarium graminearum*, *Fay11* and *AC7T1-1*, collected from soybean hosts, were obtained for use in resistance screening. *Fay11* was collected in Ohio for a previous study [14], whereas isolate *AC7T1-1* was obtained from a field scouting for soybean seedling disease samples in Indiana in 2019 [28] and was used for the first time in this study. The isolates were preserved for long-term storage in 20% glycerol both in liquid nitrogen and within a −80 °C freezer and regularly maintained on potato dextrose agar (PDA) on the benchtop (21 °C ± 2°).

Subcultures of the isolates were grown on 100 × 15 mm PDA plates for 7 days before transferring to a rice medium for inoculum increase. To a 12 L mycological spawn bag (0.44 µm filter), parboiled brown rice and distilled water were added at a 1:1 ratio. The rice and water were autoclaved twice for 40 min each at 121 °C with a 24 h period in between sterilizations. For every 700 g of rice, one fully colonized PDA plate was aseptically cut into approximately 1 cm^2 pieces and added to the sterile rice medium in a biological safety cabinet. For the control treatments, sterile PDA plates were added to the rice medium instead. The inoculums were grown for 14 days on the benchtop and shaken every other day to ensure even colonization. Prior to plant inoculation, spawn bags were bulked for each treatment to ensure uniformity of the inoculum.

2.2. Plant Material

A total of 207 *Glycine max* and a single *Glycine soja* accession were selected from the USDA-SGC based on their adaptation to the North Central USA growing region (maturity groups 0-VI) and obtained through the USDA-ARS Germplasm Resources Information Network (GRIN) (Table 1). All 208 accessions were grown at the Purdue University Agronomy Center for Research and Education (ACRE) in 2020 and harvested using a single plant belt thresher with thorough cleanings between each threshing. These accessions were previously genotyped using the SoySNP50K BeadChip [29] and the corresponding SNP data are publicly available through the SoyBase database. Two of these accessions, PI 525453 and PI 424354, which were previously identified as resistant to *F. graminearum* [21], served as benchmarks throughout the analysis to compare their relative resistance under our experimental conditions. PI 548631 (cv. 'Williams') had previously been identified as susceptible in the same study and served as the susceptible check throughout our experiments [21]. Other resistant accessions referenced in the introduction were unavailable from GRIN at the time of this study.

Table 1. Mean RRW and stand counts of the soybean accessions after inoculation with *Fusarium graminearum* isolate *AC7T1-1*.

Variety [a]	RRW [b]	Stand Count [c]	Seed Coat Color [d]	Origin [d]
PI 548311	0.823 ± 0.46	1 ± 0.16	Yellow	Ontario, Canada
PI 438500	0.7 ± 0.13	0.833 ± 0.08	Greenish brown	United States
PI 561318 A	0.631 ± 0.27	0.857 ± 0.06	Black	Beijing city, China
PI 547690	0.587 ± 0.05	1.222 ± 0.14	Yellow	Illinois, United States
PI 391577	0.586 ± 0.11	1 ± 0.13	Brown	Jilin Province, China
PI 157484	0.568 ± 0.17	0.833 ± 0.08	Light green	South Korea
PI 632418	0.564 ± 0	0.722 ± 0.12	Yellow	Maryland, United States
PI 70466 -3	0.515 ± 0.1	0.611 ± 0.12	Yellow	Jilin Province, China
PI 54615 -1	0.495 ± 0.22	0.545 ± 0.31	Yellow	Heilongjiang Province, China
PI 549040	0.494 ± 0.19	0.846 ± 0.11	Green	Liaoning Province, China
PI 81785	0.492 ± 0.13	1 ± 0	Brown	Hokkaidô, Japan
PI 603420	0.476 ± 0.01	0.867 ± 0.27	Black	China
PI 578375 B	0.465 ± 0.08	0.833 ± 0	Black	China
PI 378680 E	0.46 ± 0.19	0.667 ± 0.27	Yellow	Russian Federation
PI 594451	0.46 ± 0.12	0.667 ± 0.14	Yellow	Sichuan Province, China
PI 639528 B	0.444 ± 0.11	0.765 ± 0.04	Brown	Primorsky krai, Russian Federation
PI 89005 -5	0.437 ± 0.12	0.688 ± 0.12	Yellow	China
PI 547779	0.429 ± 0.14	0.75 ± 0.15	Yellow	Illinois, United States
PI 548540	0.422 ± 0.13	0.778 ± 0.12	Yellow	Iowa, United States
PI 592937	0.422 ± 0.19	0.563 ± 0.21	Yellow	China
PI 567262 A	0.419 ± 0.17	0.611 ± 0.18	Yellow	Fujian Province, China
PI 458505	0.419 ± 0.02	0.75 ± 0.19	Yellow	Liaoning Province, China
PI 507088	0.418 ± 0.15	0.688 ± 0.23	Yellow	Japan
PI 567,685	0.412 ± 0.08	0.786 ± 0.08	Yellow	Henan Province, China
PI 518750	0.41 ± 0.15	0.722 ± 0.05	Yellow	Former Serbia and Montenegro
PI 68604 -1	0.405 ± 0.1	0.727 ± 0.24	Yellow	China
PI 567361	0.395 ± 0.11	0.813 ± 0.01	Yellow	Ningxia Hui Autonomous Region, China
PI 548634	0.391 ± 0.11	0.765 ± 0.12	Yellow	Ohio, United States
PI 518751	0.391 ± 0.25	0.556 ± 0.09	Yellow	Former Serbia and Montenegro
PI 84987 A	0.385 ± 0.1	0.647 ± 0.17	Yellow	Saitama, Japan
PI 594301	0.385 ± 0.12	0.667 ± 0.14	Yellow	Japan
PI 594777	0.384 ± 0.07	0.706 ± 0.14	Yellow	Yunnan Province, China
PI 84987	0.376 ± 0.13	0.667 ± 0.21	Yellow	Saitama, Japan
PI 154189	0.373 ± 0.05	0.643 ± 0.07	Yellow	Netherlands
PI 603424 A	0.367 ± 0.1	0.8 ± 0	Yellow	China
PI 567353	0.362 ± 0.04	0.667 ± 0.12	Brown	Gansu Province, China
PI 514671	0.362 ± 0.19	0.556 ± 0.12	Yellow	Heilongjiang Province, China
PI 567346	0.361 ± 0.14	0.75 ± 0.12	Yellow	Gansu Province, China
PI 567558	0.357 ± 0.15	0.688 ± 0.3	Yellow	Shandong Province, China
PI 639543	0.356 ± 0.04	0.929 ± 0.05	Greenish brown	Primorsky krai, Russian Federation
PI 603399	0.353 ± 0.16	0.611 ± 0.25	Yellow	China
PI 437991 B	0.347 ± 0.14	0.625 ± 0.21	Yellow	China
PI 475820	0.346 ± 0.12	0.722 ± 0.16	Yellow	Xinjiang Uygur Autonomous Region, China
PI 567604 A	0.339 ± 0.16	0.529 ± 0.13	Yellow	Shandong Province, China
PI 437110 A	0.336 ± 0.13	0.667 ± 0.21	Brown	Russian Federation
PI 479735	0.333 ± 0.16	0.556 ± 0.24	Yellow	Jilin Province, China
PI 437505	0.33 ± 0.13	0.765 ± 0.22	Black	Primorsky krai, Russian Federation
PI 603494	0.33 ± 0.15	0.471 ± 0.13	Greenish brown	China
PI 605765 B	0.326 ± 0.04	0.889 ± 0.05	Greenish brown	Tuyên Quang, Vietnam
PI 437169 B	0.325 ± 0.1	0.778 ± 0.12	Yellow	Krasnodar, Russian Federation

Table 1. *Cont.*

Variety [a]	RRW [b]	Stand Count [c]	Seed Coat Color [d]	Origin [d]
PI 603426 G	0.323 ± 0.06	0.8 ± 0.17	Yellow	China
PI 567418 A	0.304 ± 0.02	0.625 ± 0.02	Yellow	Shanxi Province, China
PI 171451	0.303 ± 0.02	0.8 ± 0.28	Yellow	Kanagawa, Japan
PI 603442	0.303 ± 0.1	0.75 ± 0.1	Black	China
PI 567416	0.302 ± 0.2	0.5 ± 0.21	Yellow	Shanxi Province, China
PI 591431	0.301 ± 0.12	0.625 ± 0.15	Yellow	Ontario, Canada
PI 157421	0.291 ± 0.17	0.412 ± 0.2	Black	South Korea
PI 547460	0.285 ± 0.11	0.5 ± 0.14	Yellow	Illinois, United States
PI 438239 B	0.283 ± 0.06	0.765 ± 0.09	Brown	China
PI 437776	0.281 ± 0.12	0.588 ± 0.24	Yellow	China
PI 548512	0.28 ± 0.09	0.444 ± 0.12	Yellow	Indiana, United States
PI 424391	0.28 ± 0.12	0.688 ± 0.16	Light green	Jeollabuk-do, South Korea
PI 437788 A	0.279 ± 0.03	0.778 ± 0.05	Black	China
PI 209334	0.277 ± 0.12	0.5 ± 0.21	Brown	Hokkaidô, Japan
PI 507681 B	0.275 ± 0.12	0.389 ± 0.16	Yellow	Uzbekistan
PI 548561	0.273 ± 0.09	0.444 ± 0.12	Yellow	Minnesota, United States
PI 437160	0.268 ± 0.11	0.556 ± 0.2	Yellow	Krasnodar, Russian Federation
PI 483464 A	0.265 ± 0.01	0.722 ± 0.05	Black	Ningxia Hui Autonomous Region, China
PI 437485	0.262 ± 0.06	0.647 ± 0.1	Green	Primorsky krai, Russian Federation
PI 398296	0.26 ± 0.07	0.529 ± 0.06	Yellow	Kyonggi, South Korea
PI 603345	0.258 ± 0.03	0.667 ± 0.08	Yellow	China
PI 378663	0.256 ± 0.17	0.625 ± 0.32	Greenish brown	Russian Federation
PI 438323	0.249 ± 0.14	0.375 ± 0.16	Yellow	France
PI 87620	0.248 ± 0.08	0.5 ± 0.14	Yellow	Hamkyeongpukto, North Korea
PI 438112 B	0.247 ± 0.08	0.6 ± 0.16	Yellow	China
PI 91160	0.246 ± 0.11	0.444 ± 0.2	Yellow	Liaoning Province, China
PI 603290	0.238 ± 0.09	0.444 ± 0.12	Yellow	China
PI 591432	0.236 ± 0.09	0.5 ± 0.12	Yellow	Ontario, Canada
PI 437653	0.236 ± 0.08	0.529 ± 0.16	Yellow	China
PI 561701	0.235 ± 0.12	0.357 ± 0.12	Yellow	Georgia, United States
PI 468408 B	0.235 ± 0.1	0.588 ± 0.25	Yellow	China
PI 561389 B	0.234 ± 0.04	0.429 ± 0.08	Yellow	Japan
PI 437695 A	0.233 ± 0.1	0.529 ± 0.21	Yellow	China
PI 567525	0.231 ± 0.08	0.5 ± 0.08	Yellow	Shandong Province, China
PI 548182	0.228 ± 0.09	0.588 ± 0.23	Imperfect black	Illinois, United States
PI 548198	0.225 ± 0.09	0.556 ± 0.16	Gray	Illinois, United States
PI 548427	0.225 ± 0.06	0.733 ± 0.27	Black	Liaoning Province, China
PI 578493	0.22 ± 0.08	0.5 ± 0.16	Yellow	China
PI 88479	0.217 ± 0.11	0.444 ± 0.18	Yellow	Jilin Province, China
PI 548356	0.217 ± 0.06	0.625 ± 0.21	Yellow	Pyongyang, North Korea
PI 490766	0.216 ± 0.12	0.444 ± 0.2	Black	Hebei Province, China
PI 591541	0.215 ± 0.03	0.556 ± 0.16	Yellow	Illinois, United States
PI 639559 B	0.206 ± 0.04	0.389 ± 0.05	Black	Ukraine
PI 567298	0.206 ± 0.09	0.471 ± 0.24	Yellow	Gansu Province, China
PI 416751	0.204 ± 0.08	0.5 ± 0.21	Yellow	Japan
PI 567293	0.202 ± 0.04	0.438 ± 0.03	Yellow	Gansu Province, China
PI 89138	0.201 ± 0.08	0.5 ± 0.21	Yellow	Hamkyeongpukto, North Korea
PI 567780 B	0.201 ± 0.08	0.529 ± 0.13	Yellow	Jiangsu Province, China
PI 467343	0.2 ± 0.1	0.556 ± 0.23	Yellow	Jilin Province, China
PI 88468	0.199 ± 0.04	0.563 ± 0.11	Black	Liaoning Province, China
PI 508083	0.199 ± 0.03	0.588 ± 0.04	Yellow	Minnesota, United States
PI 567258	0.198 ± 0.07	0.5 ± 0.16	Brown	Jiangxi Province, China
PI 597464	0.198 ± 0.07	0.611 ± 0.18	Yellow	Zhejiang Province, China

Table 1. *Cont.*

Variety [a]	RRW [b]	Stand Count [c]	Seed Coat Color [d]	Origin [d]
PI 542403	0.196 ± 0.08	0.556 ± 0.12	Yellow	Minnesota, United States
PI 548402 S	0.194 ± 0.08	0.294 ± 0.09	Black	Beijing city, China
PI 54614	0.193 ± 0.08	0.444 ± 0.2	Yellow	Jilin Province, China
PI 567439	0.193 ± 0.07	0.529 ± 0.06	Yellow	Shanxi Province, China
PI 603549	0.191 ± 0.05	0.444 ± 0.16	Black	China
PI 81041 -1	0.187 ± 0.11	0.471 ± 0.06	Reddish brown	Hokkaidô, Japan
PI 603389	0.187 ± 0.08	0.5 ± 0.21	Yellow	China
PI 548402	0.187 ± 0.05	0.4 ± 0.14	Black	Beijing city, China
PI 291310 C	0.185 ± 0.03	0.5 ± 0.06	Yellow	Heilongjiang Province, China
PI 506933	0.181 ± 0.07	0.471 ± 0.13	Yellow	Japan
PI 507467	0.18 ± 0.1	0.375 ± 0.15	Yellow	Japan
PI 438309	0.18 ± 0.05	0.625 ± 0.14	Yellow	China
PI 548298	0.179 ± 0.09	0.389 ± 0.2	Yellow	China
PI 103088	0.177 ± 0.08	0.444 ± 0.18	Yellow	Henan Province, China
PI 476352 B	0.176 ± 0.12	0.444 ± 0.24	Yellow	Kyrgyzstan
PI 438230 A	0.175 ± 0.07	0.556 ± 0.24	Yellow	China
PI 438019 B	0.173 ± 0.07	0.333 ± 0.16	Yellow	China
PI 404188 A	0.17 ± 0.07	0.222 ± 0.09	Yellow	China
PI 84631	0.17 ± 0.08	0.375 ± 0.15	Green	Kyonggi, South Korea
PI 548521	0.17 ± 0.08	0.412 ± 0.11	Yellow	Iowa, United States
PI 612730	0.169 ± 0.06	0.333 ± 0.08	Yellow	China
PI 603675	0.167 ± 0.09	0.529 ± 0.24	Yellow	China
PI 81041	0.167 ± 0.12	0.357 ± 0.17	Yellow	Hokkaidô, Japan
PI 549021 A	0.162 ± 0.05	0.333 ± 0.04	Black	Liaoning Province, China
PI 86904 -1	0.161 ± 0.07	0.5 ± 0.21	Yellow	Chungcheongbuk-do, South Korea
PI 417398	0.16 ± 0.12	0.333 ± 0.21	Yellow	China
PI 68521 -1	0.157 ± 0.11	0.412 ± 0.2	Yellow	China
PI 547686	0.157 ± 0.07	0.353 ± 0.15	Yellow	Illinois, United States
PI 84946 -2	0.157 ± 0.09	0.471 ± 0.2	Yellow	Busan-gwangyeoksi, South Korea
PI 437793	0.155 ± 0.06	0.563 ± 0.15	Yellow	China
PI 88313	0.155 ± 0.09	0.235 ± 0.15	Yellow	China
PI 567395	0.155 ± 0.08	0.4 ± 0.2	Grayish green	Shaanxi Province, China
PI 497953	0.155 ± 0.03	0.333 ± 0.08	Yellow	Bihar, India
PI 548391	0.154 ± 0.07	0.471 ± 0.2	Yellow	Liaoning Province, China
PI 404182	0.153 ± 0.07	0.333 ± 0.16	Yellow	China
PI 592960	0.151 ± 0.02	0.235 ± 0.06	Yellow	Heilongjiang Province, China
PI 62203	0.148 ± 0.06	0.444 ± 0.12	Yellow	Hebei Province, China
PI 567782	0.146 ± 0.04	0.471 ± 0.11	Yellow	Ontario, Canada
PI 548411	0.146 ± 0.07	0.389 ± 0.16	Yellow	China
PI 438496 C	0.139 ± 0.05	0.389 ± 0.12	Black	United States
PI 567225	0.138 ± 0.06	0.563 ± 0.24	Yellow	Moldova
FC 33243 -1	0.132 ± 0.05	0.5 ± 0.21	Yellow	Unknown
PI 547716	0.13 ± 0.05	0.444 ± 0.2	Yellow	Illinois, United States
PI 54591	0.13 ± 0.07	0.429 ± 0.2	Yellow	Liaoning Province, China
PI 548360	0.129 ± 0.05	0.333 ± 0.08	Yellow	North Korea
PI 547459	0.129 ± 0.05	0.333 ± 0.08	Yellow	Illinois, United States
PI 548571	0.128 ± 0.05	0.353 ± 0.15	Yellow	Ontario, Canada
PI 592940	0.127 ± 0.06	0.222 ± 0.12	Yellow	China
PI 548193	0.127 ± 0.02	0.5 ± 0.14	Yellow	Iowa, United States
PI 437685 D	0.126 ± 0.06	0.278 ± 0.12	Yellow	China
PI 437814 A	0.123 ± 0.06	0.267 ± 0.11	Yellow	China

Table 1. *Cont.*

Variety [a]	RRW [b]	Stand Count [c]	Seed Coat Color [d]	Origin [d]
PI 253661 B	0.12 ± 0.04	0.5 ± 0.16	Yellow	China
PI 417381	0.119 ± 0.08	0.286 ± 0.14	Yellow	Hokkaidô, Japan
PI 58955	0.118 ± 0.05	0.444 ± 0.18	Yellow	Shandong Province, China
PI 525453	0.118 ± 0.01	0.471 ± 0.15	Yellow	Iowa, United States
PI 86024	0.116 ± 0.05	0.471 ± 0.2	Grayish green	Hokkaidô, Japan
PI 464896	0.109 ± 0.05	0.412 ± 0.17	Yellow	Jilin Province, China
PI 567698 A	0.108 ± 0.02	0.5 ± 0.16	Yellow	Anhui Province, China
PI 438498	0.105 ± 0.03	0.462 ± 0.2	Black	United States
PI 533655	0.103 ± 0.07	0.235 ± 0.1	Yellow	Illinois, United States
PI 291294	0.102 ± 0.02	0.375 ± 0.1	Yellow	Heilongjiang Province, China
PI 424354	0.102 ± 0.04	0.182 ± 0.06	Black	Chungcheongnam-do, South Korea
PI 603463	0.101 ± 0.03	0.529 ± 0.06	Yellow	China
PI 464912	0.101 ± 0.04	0.294 ± 0.16	Green	Liaoning Province, China
PI 507293 B	0.099 ± 0.04	0.278 ± 0.09	Yellow	Japan
PI 68732 -1	0.098 ± 0.04	0.353 ± 0.15	Yellow	Heilongjiang Province, China
PI 587588 A	0.095 ± 0.06	0.294 ± 0.12	Yellow	Jiangsu Province, China
PI 437127 A	0.091 ± 0.05	0.222 ± 0.12	Yellow	Georgia
PI 407701	0.09 ± 0.05	0.25 ± 0.12	Yellow	China
PI 361066 B	0.089 ± 0.03	0.333 ± 0.14	Yellow	Romania
PI 83881 A	0.088 ± 0.03	0.235 ± 0.04	Yellow	Kangweonto, North Korea
PI 54608 -1	0.082 ± 0.06	0.294 ± 0.16	Yellow	Liaoning Province, China
PI 90479 P	0.082 ± 0.06	0.167 ± 0.08	Yellow	China
PI 548362	0.081 ± 0.06	0.222 ± 0.12	Yellow	Illinois, United States
PI 594456 A	0.078 ± 0.02	0.412 ± 0.18	Yellow	Sichuan Province, China
PI 437265 D	0.074 ± 0.05	0.294 ± 0.16	Yellow	Moldova
PI 417242	0.074 ± 0.04	0.353 ± 0.16	Green	China
PI 578503	0.074 ± 0.03	0.235 ± 0.12	Yellow	China
PI 417479	0.073 ± 0.06	0.222 ± 0.18	Yellow	Japan
PI 437838	0.071 ± 0.05	0.313 ± 0.16	Yellow	Russian Federation
PI 587804	0.07 ± 0.04	0.5 ± 0.18	Yellow	Hubei Province, China
PI 407716	0.067 ± 0.01	0.222 ± 0.05	Yellow	Jilin Province, China
PI 232992	0.067 ± 0.05	0.188 ± 0.12	Black	Saga, Japan
PI 548631	0.066 ± 0.04	0.294 ± 0.12	Yellow	Illinois, United States
PI 391583	0.064 ± 0.05	0.063 ± 0.05	Yellow	Jilin Province, China
FC 29333	0.064 ± 0.03	0.222 ± 0.09	Yellow	Unknown
PI 438083	0.061 ± 0.03	0.333 ± 0.16	Yellow	China
PI 464923	0.061 ± 0.03	0.176 ± 0.09	Yellow	Liaoning Province, China
PI 79862 -1	0.058 ± 0.03	0.118 ± 0.05	Yellow	China
PI 548190	0.056 ± 0.01	0.313 ± 0.08	Yellow	Illinois, United States
PI 592954	0.051 ± 0.02	0.294 ± 0.12	Yellow	China
PI 578412	0.049 ± 0.03	0.222 ± 0.12	Yellow	China
PI 548520	0.048 ± 0.03	0.235 ± 0.12	Yellow	Iowa, United States
PI 398881	0.039 ± 0.03	0.176 ± 0.14	Yellow	Kyonggi, South Korea
PI 548565	0.037 ± 0.01	0.222 ± 0.05	Yellow	Ohio, United States
PI 567532	0.036 ± 0.02	0.333 ± 0.16	Yellow	Shandong Province, China
PI 594880	0.035 ± 0.02	0.188 ± 0.09	Reddish brown	Yunnan Province, China
PI 506942	0.032 ± 0.03	0.111 ± 0.09	Yellow	Japan
PI 458510	0.025 ± 0.01	0.222 ± 0.09	Yellow	Liaoning Province, China
PI 567307	0.024 ± 0.02	0.143 ± 0.27	Black	Gansu Province, China
PI 83881	0.02 ± 0.01	0.188 ± 0.08	Black	Kangweonto, North Korea
PI 591511	0.019 ± 0.01	0.111 ± 0.05	Yellow	Illinois, United States
PI 567722	0.01 ± 0.01	0.056 ± 0.05	Yellow	Anhui Province, China
PI 548348	0.01 ± 0.01	0.056 ± 0.05	Yellow	China
PI 548162	0 ± 0	0 ± 0	Yellow	Illinois, United States

[a] Accession identifier in the USDA-ARS-GRIN database. [b] Mean RRW score of the accession across three replicates ± standard error. [c] Mean adjusted stand count of the accession across three replicates ± standard error. [d] Accession data retrieved from the USDA-ARS-GRIN database (www.ars-grin.gov accessed on 8 February 2023).

2.3. Phenotyping

Phenotyping was conducted in a greenhouse in the spring of 2021 using a previously described procedure [30]. Seeds were selected from each accession that were uniform in size and color, as well as free from visible damage or disease symptoms. Resistance screening was performed in 53 × 27 cm plastic greenhouse flats with 48-cell inserts, with each cell measuring 3.8 × 6 × 5.8 cm. The trays were filled with twice steam-sterilized, fine-grade vermiculite and placed on flood benches filled with tap water to a height of 2 cm. A planting hole was placed 4 cm in depth and filled with 1.5 mL of either inoculated or mock-inoculated rice medium. Six seeds per accession were placed individually into separate cells of both treatments in direct contact with the inoculum and covered with sterile vermiculite. The accessions were arranged according to a randomized block design. The experiment was conducted three times between January and March 2021. The temperature was maintained at 24–28 °C with a 16:8 photoperiod with overhead lighting. The water level was maintained at 2 cm throughout the duration of each experiment.

Measurements were made 14 days after planting (DAP) and included the fresh root weight and stand count of each accession in both treatments. The ratio of root weight (RRW) score, proposed by Lin et al., was selected to detect small effect differences in root rot severity, which are difficult to discern visually [30]. Furthermore, this score incorporates the stand count and root weight data in a single unified disease measure thereby reducing the number of input phenotype variables in our GWAS (i.e., in the case of running a GWAS separately on stand counts and root weight). Briefly, the baseline germination of each accession was first estimated as the average stand count across all mock-inoculated control treatments. Then, the RRW scores for each accession in each replicate were calculated as follows:

$$\mathrm{RRW} = \frac{\left[\frac{\text{Total fresh root weight of the inoculated replicate}}{\text{Baseline germination of all three controls}}\right]}{\left[\frac{\text{Total fresh root weight of the control replicate}}{\text{Stand count of the same control replicate}}\right]}$$

To test if the RRW, as a unified disease measure, was an accurate reflection of an accession's resistance to *F. graminearum*, correlations were tested between the RRW and two previously validated disease indices for seedling diseases, adjusted stand count, and standardized root weight [31,32]. The adjusted stand count was calculated by taking the number of germinated seeds in an inoculated replicate divided by the number of seeds germinated in its matched control. Seeds were considered to have germinated if their cotyledons were fully emerged above the surface of the vermiculite. The standardized root weight was calculated by dividing the fresh root weight of an inoculated replicate by the fresh root weight of its matched mock-inoculated control. Pearson's correlation coefficients were calculated between the averages of the RRW scores, adjusted stand count, and standardized root weight measures using 'cor.test' in base R [33]

All accessions were tested first against isolate *AC7T1-1* collected in Indiana. A subset of the accessions with the top 30 highest RRW scores and bottom 10 RRW scores, as well as the resistant checks, were then screened against *Fay11* using the same procedures as above.

2.4. Genome-Wide Association Study

The SNP files were downloaded from SoyBase for each of the 208 accessions. A GWAS was performed using the GAPIT3 (version 3.1.0) package in R with the statistical procedure 'BLINK' [34,35]. Pedigree data for this population are largely unknown, since the majority of the accessions screened here were land races. Principal component analysis (PCA) was performed on the SNP data using the function 'prcomp' in base R to identify the population structure among our accessions. Principal components explaining more than 5% of the variance of the SNPs inherited by each of the 208 accessions were included in the GWAS to control for population stratification [33]. The mean RRW score for each accession across all three replicates was used as the phenotype.

2.5. Statistical Analysis

A linear mixed model was fit with accessions set as fixed effect and replicates assigned as random effect using the package 'lme4' [36] in R as follows:

$$Y_{ij} = \mu + Acc_i + Rep_j + \varepsilon_{ij}$$

where Y_{ij} is the observed RRW score of the ith accession in the jth replicate, μ is the intercept and estimated baseline mean before any effects, Acc_i is the ith accessional fixed effect, Rep_j is the jth replicate random effect, and ε_{ij} is the residual variance associated with the observation of the ith accession in the jth replicate. The p-values for each genotype's estimated effect were calculated using 'lmerTest' with Satterthwaite's approximation of degrees of freedom at an alpha of 0.05 [37]. Variance components were generated by decomposing the above model with both accession and replicate as random effects to calculate broad-sense heritability (H^2) as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

where σ_g^2 is the genotypic variance, σ_e^2 is the error variance, and r is the number of replicates.

3. Results

3.1. Soybean Accessions Resistant to Fusarium graminearum

The panel of soybean accessions tested represent diverse geographic origins and are all of suitable maturity groups for growth in various locations of the North Central USA where most soybeans are produced (Table 1). A mixture of seed coat colors was included, although no correlation was found between significantly resistant accessions and the seed coat color trait. The symptoms of seedling diseases, including damping-off, root discoloration, necrotic lesions, and root system stunting, compared to the mock-inoculated controls were abundantly evident in the inoculated treatments by 14 DAP. No accession showed a symptomless immune-type reaction to inoculation, but visual differences in disease severity for the symptoms listed above were clear across accessions.

Isolate *Fay11*, which was reported to be highly virulent in previous publications [14,21–23,38,39] showed reduced virulence across all replicates, even in the susceptible check PI 548631 (Supplementary Materials Figure S1). As such, data from this isolate were excluded, and all statistical analyses were performed using only the three replicates inoculated with the *AC7T1-1* isolate. Pearson's correlation tests indicated that the RRW score was significantly correlated with both standardized root weight and adjusted stand count ($p < 0.001$) but more closely for the standardized root weight (Figure 1). The accessions showed a continuous distribution of the mean RRW scores ranging from 0 to 0.823 (Table 1). The mean RRW scores were left-skewed, with most accessions scoring below 0.50 (Figure 2). The mean RRW score was 0.224, with a standard deviation of 0.14 across accessions. The two resistant check accessions, PI 525453 and PI 424354, had average RRW scores of 0.118 and 0.102, respectively, which were lower but nonsignificantly than the population average. The susceptible check had a mean RRW of 0.066 and was the 22nd most susceptible accession of the 208. The adjusted stand counts followed a normal distribution (Figure 2) with a mean of 0.488 and standard deviation of 0.21. Only one accession, PI 548162, failed to germinate even a single seed after inoculation, despite an average control germination of 5.333 seeds.

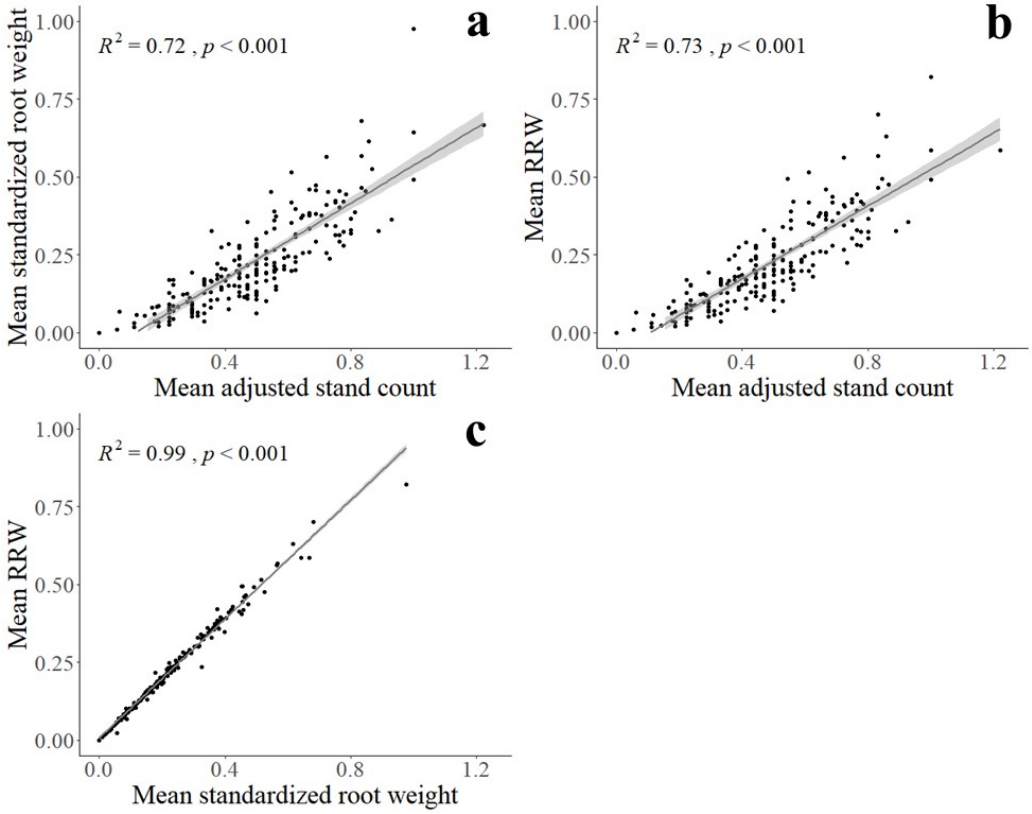

Figure 1. Correlation plots of phenotypic measures for a fitted linear regression model and 95% confidence intervals in gray for (**a**) mean standardized root weight versus mean adjusted stand count; (**b**) mean RRW versus mean adjusted stand count; (**c**) mean RRW versus mean standardized root weight.

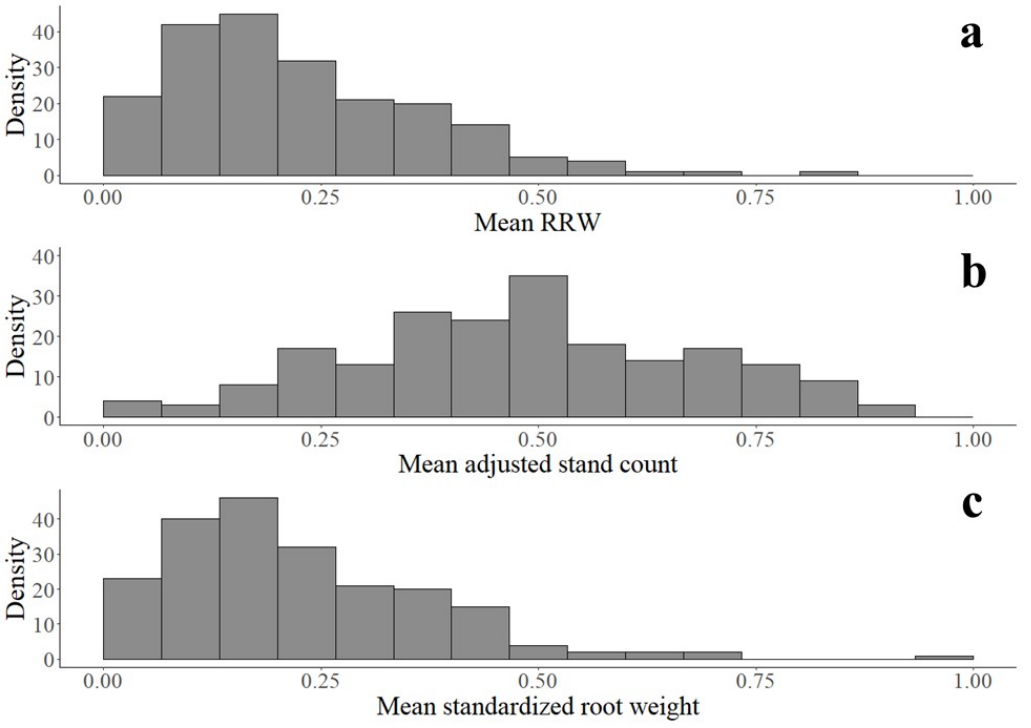

Figure 2. Frequency histograms of the mean phenotypic responses: (**a**) mean RRW score; (**b**) mean-adjusted stand count; (**c**) mean standardized root weight.

A linear mixed model was fitted to reduce the variance introduced by replicate differences through introducing replicates as random effect. The estimated variance for replicate was 0.013. The model's intercept was estimated at an RRW score of 0.224 prior to introducing accessional effects. The estimated accessional fixed effects ranged from −0.225 to 0.598 in this population. The accession effects were found to be significantly correlated with the RRW score as a dependent measure ($p < 0.001$) (Table 2). PI 548311, PI 438500, PI 561318 A, PI 547690, PI 391577, PI 157484, PI 632418, and PI 70466 -3 all had significant positive estimated effects on the model's mean (Table 3). Their mean RRW scores ranged from 0.495 to 0.823, while the population's mean was 0.224. PI 525453 and PI 424354, which were previously reported as resistant, had nonsignificant but negative estimated effects on the mean, indicating that they were not resistant under the conditions of our experiment. No accessions were identified as significantly more susceptible than the mean. The full random model estimated the genotypic variance to be 0.011 and the error variance to be 0.028, and the heritability of the RRW score was calculated to be 54.1%.

Table 2. One-way analysis of variance (ANOVA) table for the RRW score by accession.

Predictor	SS	MS	Numerator DF	Denominator DF	F-Value	*p*-Value [a]
Accession	12.89	0.062271	207	414	2.1874	9.06×10^{-12} *

[a] *p*-Values calculated using Satterthwaite's approximation of the denominator degrees of freedom. Estimated effects significantly ($p < 0.05$) greater than zero are marked with an asterisk.

Table 3. Summary table for the linear mixed model regression of the RRW scores by accession for the accessions with statistically significant ($p < 0.05$) estimated effects.

Estimator	Estimated Effect	T-Value	*p*-Value [a]
Intercept	0.225	1.9115	7.07×10^{-2}
PI 548311	0.598	4.3395	1.80×10^{-5} *
PI 438500	0.469	3.4058	7.24×10^{-4} *
PI 561318 A	0.406	2.9492	3.37×10^{-3} *
PI 547690	0.362	2.6272	8.93×10^{-3} *
PI 391577	0.362	2.6247	8.99×10^{-3} *
PI 157484	0.343	2.4898	1.32×10^{-2} *
PI 632418	0.339	2.4633	1.42×10^{-2} *
PI 70466 -3	0.291	2.1096	3.55×10^{-2} *

[a] *p*-Values calculated using Satterthwaite's approximation of the denominator degrees of freedom. Estimated effects significantly ($p < 0.05$) greater than zero are marked with an asterisk.

3.2. Genome-Wide Association of SNP Markers with Fusarium graminearum Resistance

PCA was used to estimate the relatedness among accessions, and two components contributing more than 5% of the variance in the SNP data for the population were identified (Figure 3). Component 1 explained 15.78% of the variance, and component 2 explained 6.14%. The number of principal components called in the subsequent GWAS was correspondingly set to two.

Five significant (false discovery rate (FDR)-adjusted *p*-value < 0.05) MTAs were discovered on chromosomes Gm02, Gm03, Gm06, Gm07, and Gm13 (Figure 4). Each MTA contributed between 3.0 and 4.8% of the variance in the mean RRW score, with an average effect size of 3.7% per locus (Table 4). The minor allele variant of each MTA was responsible for positive effects on chromosomes Gm02, Gm06, and Gm13 and negative effects on Gm03 and Gm13. The lowest minor allele frequency observed across these significant associations was 0.24. The ranked RRW scores and estimated effects in the model of the eight resistant accessions closely mirrored the estimated cumulative allelic effects for each accession at the five significant MTA loci (Table 5). PI 438500 was the exception to this trend, having the lowest estimated allelic effects but having the second highest mean RRW score and estimated effect in the model. Additionally, 29 markers were identified as associated with

the RRW score before applying the FDR adjustment but failed to reach the significance threshold after adjustment (Supplementary Materials Table S1).

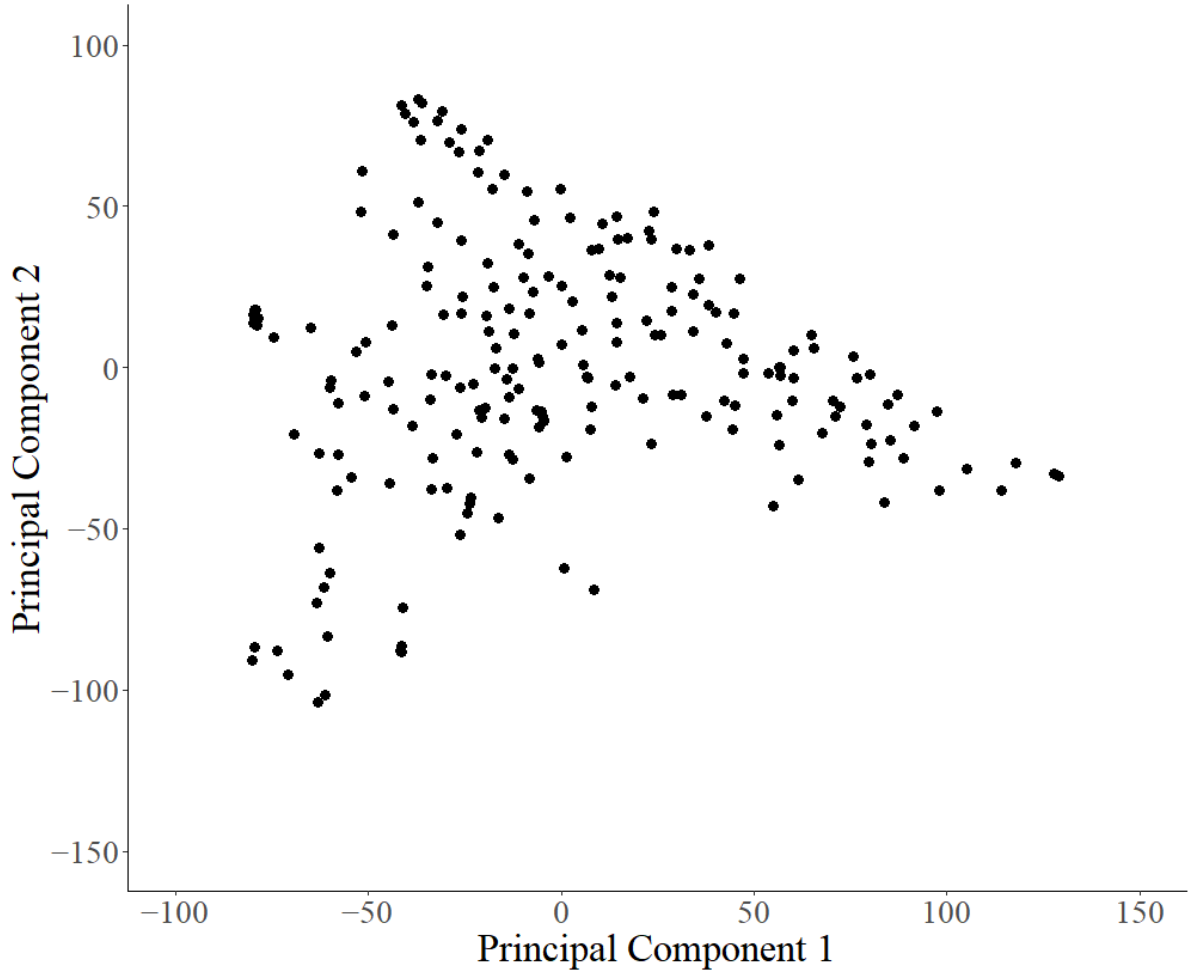

Figure 3. Principal component analysis plot showing all 208 accessions across two dimensions of the SNP-based relatedness.

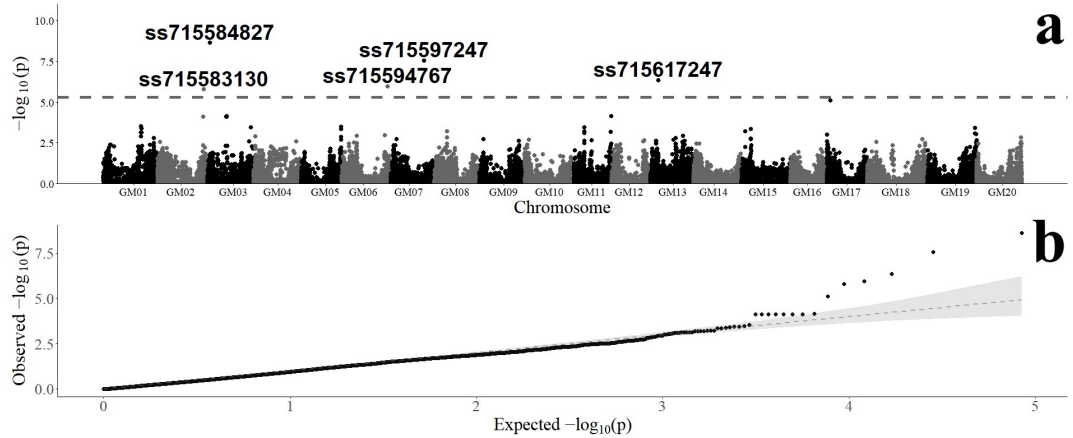

Figure 4. (**a**) Genome-wide Manhattan plot of the SNP marker associations with *Fusarium graminearum* resistance with five significant marker names annotated with a dashed line representing the significance threshold for SNP-trait associations, and a(**b**) Q–Q plot of the *p*-values from the association tests of each SNP marker with 95% confidence intervals of the relationship between expected and observed *p*-values in gray.

Table 4. SNP markers associated with the RRW score to *Fusarium graminearum*, genome position, and their estimated effects.

SNP Marker	Chromosome	Position (bp) [a]	Variants	Minor Allele	MAF [b]	B&H *p*-Value [c]	Effect [d]
ss715583130	2	46,379,738	A/G	G	0.48	1.41×10^{-2}	0.0301
ss715584827	3	2,309,023	A/G	G	0.39	1.05×10^{-4}	−0.0484
ss715594767	6	47,356,804	C/A	A	0.24	1.18×10^{-2}	0.0418
ss715597247	7	34,593,871	C/T	T	0.37	6.15×10^{-4}	−0.0431
ss715617247	13	12,663,715	C/A	A	0.34	6.60×10^{-3}	0.0381

[a] Williams 82 version 4 reference genome. [b] MAF, minor allele frequency. [c] B&H *p*-value, FDR-adjusted *p*-value using the Benjamini and Hochberg method. [d] Estimated effect size of the minor allele variant.

Table 5. Summary of the favorable SNP marker alleles for each of the five significant markers and alleles present in each of the eight significantly resistant accessions. Favorable alleles present in each accession are in bold and underlined.

SNP Marker	Favorable Allele	Effect	PI 548311	PI 438500	PI 561318A	PI 547690	PI 391577	PI 157484	PI 632418	PI 70466-3
ss715583130	G	3.00%	**G**	**G**	**G**	A	**G**	A	A	**G**
ss715584827	A	4.80%	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
ss715594767	A	4.20%	**A**	C	**A**	**A**	C	C	**A**	**A**
ss715597247	C	4.30%	**C**	T	**C**	**C**	**C**	**C**	**C**	**C**
ss715617247	A	3.80%	**A**	**A**	**A**	**A**	**A**	**A**	C	C
Sum of allelic effects:		20.20%	20.2%	11.7%	20.2%	17.1%	16.0%	13.0%	13.3%	16.3%
Mean RRW score:		-	0.823	0.7	0.631	0.587	0.586	0.568	0.564	0.515

4. Conclusions and Discussion

In this study, a total of 208 soybean accessions from the USDA-SGC were evaluated for resistance to *Fusarium graminearum*. Eight accessions were identified as significantly resistant to the pathogen based on a unified RRW score encompassing quantitative measures of fresh root weight and stand count. A GWAS revealed five significantly associated markers underlying the phenotypic resistance. These findings and the corresponding MTAs underlying the phenotypes of the examined accessions contribute valuable knowledge and genetic resources for resistance to *F. graminearum* that are potentially useful for researchers, breeders, and growers.

The eight resistant accessions identified in this study are of immediate utility for further genetic dissection, including for mapping of the QTL detected by our GWAS. Two of the accessions (PI 548311 and PI 632418) are improved cultivars bred for desirable agronomic performance, making them particularly useful for breeders looking to perform introgression of *F. graminearum* resistance with minimal or no drag of undesirable agronomic qualities. Five of the accessions (PI 157484, PI 391577, PI 438500, PI 70466 -3, and PI 561318 A) are collections of land races with unknown improvement status, suggesting that soybean varieties grown in the past may have had better genetic resistance to *F. graminearum*. This could help to explain *F. graminearum*'s relatively unknown role in soybean disease until 2004. However, a much more comprehensive screening would be needed to confirm this trend. The remaining resistant accession (PI 547690) is a progeny line from a genetic cross of 'Harosoy (6)' x PI 548195. Neither parent was tested for resistance in this screening. Future work should include evaluating these two parental lines, as well as other accessions from their pedigree, to identify the donor of the resistance found here.

Variation in the standard error across the replicates among the eight resistant accessions was observed. This could indicate that factors underlying some of the phenotypic resistance may be sensitive to discrete environmental variation not fully controlled in a greenhouse environment. This is consistent with previous findings that quantitative resistance is much more likely to show sensitivity to environmental factors than major R-gene-mediated resistance [1]. It is unknown what role confounding and discrete environmental factors may play in the efficacy of resistance of the eight accessions or any other accession carrying the resistance markers uncovered here. As with all greenhouse findings, field trials under

typical soybean growing conditions should be conducted to confirm the durability of the sources of resistance discovered here.

The eight accessions identified as resistant for the first time by this study were significantly more resistant than two previously reported accessions (PI 525453 and PI 424354) when screened under the conditions of our experiment [14]. This could suggest that either the accessions screened in this study are more resistant than the two previously reported accessions included as checks or that resistance may be isolate specific. Additionally, the differences in our germplasm assay procedures and measurements versus the original procedures under which resistance was first identified may have led to the nonsignificant performance of the two check accessions. In the case of the first hypothesis, there is now evidence that germplasm more resistant to *F. graminearum* exists in the USDA-SGC that may have a larger phenotypic effect than previously reported resistant accessions. Therefore, it is justified to continue screening germplasm from the collection, as more highly resistant accessions may still be uncovered.

Because of the inability of *Fay11* to infect even strongly susceptible accessions from this population, we were unable to compare our resistance to the check accessions using the isolate they were originally screened against. The pathogenicity of *Fay11* has been confirmed on Petri plate assays and rolled towel assays in numerous publications since its isolation in 2007 [14,22,23,38,39]. Whether the hypovirulence was due to our specific screening procedure (i.e., infested layer versus rolled towel assay) or due to a loss of virulence during repeated subculturing is unknown. Regardless, data were excluded from all replicates inoculated with *Fay11*, and resistance is only confidently reported here against isolate *AC7T1_1.* Further work is needed to confirm the presence of isolate-specific resistance in any of the accessions reported so far.

Seed coat properties, such as permeability and pigments indicating high flavonoid concentrations, have previously been linked to resistance against seed rot pathogens, particularly in soybean [24]. The eight significantly resistant accessions included a mixture of four yellow, one brown, one greenish brown, one light green, and one black seed coat accessions. While the seed coat color could play a role in some of the resistance discovered here, there was no obvious trend between resistance to the isolate *AC7T1_1* and seed coat color that would implicate it as a primary factor in the resistance of the accessions reported here.

To the best of our knowledge, the five significant SNP markers and their association with *F. graminearum* resistance have not been reported elsewhere. Despite being detected from a different population of soybean accessions, several of the markers were near MTAs and QTL reported in other studies. The marker on chromosome 2 is 4.6 mega base pairs (mbp) away from an MTA correlated with *F. graminearum* resistance uncovered in another study that is currently in press [38]. These separate detections may corroborate the same resistance loci in the same genomic region or represent two unique loci on the same chromosome. Interestingly both the marker from this study and that reported by Okello et al. are not within the only other reported QTL associated with *F. graminearum* resistance on chromosome 2 [27]. Both markers lie more than 10 mbp away from the nearest flanking marker of the QTL and are separated by multiple recombination hot spots [40]. The MTA detected on chromosome 13 lies roughly 4.1 mbp from the QTL for *F. graminearum* resistance independently identified in two different studies [21,22]. The MTA also lies within the *F. graminearum* resistance QTL reported by Zhang et al.; however, the marker interval is reported to span over 28 mbp, which may encompass regions well beyond the true QTL responsible for the observed resistance [27]. The MTAs on chromosomes 6 and 7 appear to represent novel QTL and are over a minimum of 19 mbp and 79 mbp, respectively, from any reported QTL or MTA on those chromosomes [23,25–27]. Additionally, several markers were in genomic regions reportedly carrying resistance loci to other root rot pathogens. The marker on chromosome 6 was within a region believed to contain the independently validated *F. virguliforme* quantitative resistance locus *qRfv06-01*, the marker on chromosome 7 was within the region reported for a putative QTL for *Pythium ultimum* var. *ultimum* resistance, and the marker on chromosome 13 was within a region for partial resistance to

Phytophthora sojae containing the *qRps13-01* locus. Additionally, the marker on chromosome 3 was 600 kbp away from the major R gene *Rps9* governing *P. sojae* resistance [41,42].

Many more markers tentatively associated to *F. graminearum* resistance were eliminated in the FDR-adjustment of the *p*-values. FDR adjustments significantly reduce the number of type I errors (i.e., false positives) but simultaneously increase the chance of type II errors (i.e., false negatives). Our heritability estimate indicated a 54.1% heritability of the variation in the RRW score, but our five significant loci had a combined estimated effect explaining only 20.2% of the variation (Table 5). Furthermore, one accession (PI 438500) contained the fewest of our significant MTAs but had the second-best resistance of the eight significantly resistant accessions. One or several more markers responsible for the missing heritability in this study may have been lost as false negatives. Interestingly, a marker on chromosome 17 nearly made the FDR-adjusted cutoff (FDR-adjusted $p = 0.0564$) and is within 5 and 6 mbp of two predicted defense genes annotated on that chromosome in the 'Williams82' reference genome, which were identified through a meta-analysis of GWAS and transcriptome data [43]. It is also only 3.7 mbp away from another MTA, detected by Zhang et al. [26]. The marginally nonsignificant MTA on chromosome 17 could be a detection of one or both of the predicted loci reported by Almeida-Silva and Venancio or the same region linked to the MTA identified by Zhang et al. However, the predicted effect size of the MTA on chromosome 17 does not fully explain the missing percentage of the heritability calculation. Other loci affecting *F. graminearum* resistance may or may not be present in conjunction with this potentially false negative (Supplementary Materials Table S1).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13092376/s1, Figure S1: Mean RRW scores ± standard error after inoculation with *Fay11* of the 30 highest and 10 lowest RRW scoring accessions identified after inoculation with *AC7T1_1*.; Table S1: SNP markers associated with susceptible and resistant phenotypes to *Fusarium graminearum* before FDR-adjustment.

Author Contributions: Conceptualization: G.C.; methodology: G.C. and C.D; investigation: C.D. and G.C.; analysis: C.D. and G.C.; writing—original draft preparation: C.D.; writing—review and editing: G.C. and J.M.; project administration: G.C.; funding acquisition: G.C. and J.M. All authors have read and agreed to the published version of the manuscript.

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