Identification of New Isolates of *Phytophthora sojae* and Selection of Resistant Soybean Genotypes

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Phytophthora root and stem rot (PRR), caused by *Phytophthora sojae*, can occur at any growth stage under poorly drained and humid conditions. The expansion of soybean cultivation in South Korean paddy fields has increased the frequency of PRR outbreaks. This study aimed to identify four *P. sojae* isolates newly collected from domestic fields and evaluate race-specific resistance using the hypocotyl inoculation technique. The four isolates exhibited various pathotypes, with GJ3053 exhibiting the highest virulence complexity. Two isolates, GJ3053 and AD3617, were screened from 205 soybeans, and 182 and 190 genotypes (88.8 and 92.7%, respectively) were susceptible to each isolate. Among these accessions, five genotypes resistant to both isolates were selected. These promising genotypes are candidates for the development of resistant soybean cultivars that can effectively control PRR through gene stacking.

**Keywords**: pathotype diversity, *Phytophthora sojae*, *R*-gene mediated resistance

Phytophthora root and stem rot (PRR) caused by *Phytophthora sojae* Kaufmann & Gerdemann is considered one of the most destructive diseases affecting soybean (*Glycine max* (L.) Merr.) in the world. PRR was first reported in Indiana (Kaufmann and Gerdean, 1958), and the average annual yield loss due to the disease was more than 1.0 million tons in the United States and Canada, from 1996 to 2019 (Bradley et al., 2021). In susceptible cultivars, *P. sojae* causes seedling damping-off in the early stages of growth, and root and stem rot, wilting, brown stem lesions, and death, in severe cases, during the later stages of growth (Schmitthenner, 1985). *P. sojae*, a soil-borne pathogen, overwinters as oospores in soil and plant debris. When the soil becomes saturated with moisture, oospores produce sporangia filled with zoospores, which are released and infect plant roots (Dorrance et al., 2007).

Complete resistance is managed by the *Rps* (resistance to *Phytophthora sojae*) gene, which is race-specific and single dominant (Sugimoto et al., 2012). Since the first resistance gene (*R* gene), *Rps1a*, was reported in the 1950s (Bernard et al., 1957), more than 30 *Rps* genes have been identified on 10 loci (Chandra et al., 2022; Jang and Lee, 2020): *Rps1b*, *Rps1c*, *Rps1d*, *Rps1k*, and *Rps7* on chromosome 3 (Anderson and Buzzell, 1992; Bernard and Cremeens, 1981; Buzzell and Anderson, 1992; Mueller et al., 1978);
Rps2 on chromosome 16 (Kilen et al., 1974); Rps3a, Rps3b, Rps3c, and Rps8 on chromosome 13 (Gordon et al., 2006; Ploper et al., 1985; Sandhu et al., 2005).

Soybean PRR was first identified in Chungnam province in South Korea in 1996 (Jee et al., 1998). As the soybean production area in paddy fields increased from 4,422 ha in 2016 to 18,314 ha in 2023 (Statistics Korea, 2023), there are concerns about the damage of PRR in humid conditions. Research on soybean PRR in South Korea began in earnest, utilizing a modified hypocotyl inoculation method (Dorrance et al., 2008). Four isolates of *P. sojae*, isolate P-9662 (Korean Agricultural Culture Collection [KACC] no. 40412), P-98145 (KACC no. 40468), 2457, and 3444-1, were identified, and major Korean soybean cultivars were tested in 2019 (Kang et al., 2019). For the Daepung/Sochenong2 RIL population, the single nucleotide polymorphisms on chromosome 3 (36.2-37.4 Mbp) and chromosome 18 (2.1-2.6 Mbp) were significantly associated with resistance to isolate P-9662 and 2457, respectively (Jang et al., 2020b). On chromosome 3, a genomic region for resistance to isolate 2457 in the Daepung/Daewon RIL population was searched in the 3.8-4.5 Mbp region (Jang et al., 2020a), and a locus of resistance to isolate 2858 in the Daepung/Saedenbaek RIL population was identified in the 3.3-4.5 Mbp region (You et al., 2023b). On chromosome 3, a genomic region for resistance to isolate 2457 in the Daepung/Daewon RIL population was searched in the 3.8-4.5 Mbp region (Jang et al., 2020a), and a locus of resistance to isolate 2858 in the Daepung/Saedenbaek RIL population was identified in the 3.3-4.5 Mbp region (You et al., 2023b). Recently, a significant resistance locus for another isolate P-98145 (KACC no. 40468) was reported on chromosome 18 (55.9-56.4 Mbp) using the Daepung/Cheonal RIL population (You et al., 2023a).

Soybean PRR research has increased in recent years, primarily due to the increased threat posed by *P. sojae* outbreaks. However, there remains a shortage of resistant resources, and limited research has focused on identifying *Rps* genes. One effective strategy for managing PRR is to develop resistant cultivars with *R* genes (Jang and Lee, 2020) capable of sustaining stable production even in humid fields. It is essential to identify the pathotype of the isolates and discover resistant genotypes. The objectives of this study were (1) to report new isolates of *P. sojae* and (2) to identify resistant genotypes by screening soybean varieties.

A total of four *P. sojae* isolates were used in this study. Three isolates, GJ3053 (KACC no. 48989), AD3617 (KACC no. 48988), and WJ3624, were collected in 2019 from symptomatic soybean plants growing in converted-paddy fields in Gimje (35°77′N, 126°82′E), Andong (36°63′N, 128°66′E), and Wanju (35°90′N, 127°15′E), South Korea, respectively. DG3968, the fourth, was isolated from diseased soybean at the Daegu experiment station of National Institute of Crop Science (NICS) (35°91′N, 128°45′E) in 2020. Isolate WJ3624 and DG3968 have not been officially registered with KACC. The four isolates grew well on 10% V8 media (17 g of agar per liter) at 28°C, especially isolate 3053 could cover the surface of media 9 cm in diameter in 7 days, while the other three isolates covered more than half of media (Fig. 1). These isolates were identified using polymerase chain reaction (PCR). Three other *Phytophthora* species, *P. capsici* (KACC no. 44716), *P. infestans* (KACC no. 47707), and *P. nicotianae* (KACC no. 48120), were obtained from the KACC and used as negative controls. To identify *P. sojae*,
specific primers, PSOJF1 (5′-GCCTGCTCTGTGTTGCT-3′) and PSOJR1 (5′-GGTTTAAAAAGTGGGCT-CATGATC-3′), were used for PCR (Bienapfl et al., 2011). PCR products were detected using gel electrophoresis. Amplification was performed using a GB/GBox iChemi XL gel documentation system (Syngene, Frederick, MD, USA). Clear bands were amplified for all four isolates of P. sojae, whereas three negative controls were not detected (Fig. 2). The size of the PCR product was approximately 120 bp and the isolates were identified as P. sojae (Bienapfl et al., 2011; Kang et al., 2019).

In this study, a set of 16 differentials was used to evaluate the pathotypes of the four isolates of P. sojae (Dorrance et al., 2004). Nine differentials were obtained from the Genebank of National Agrobiodiversity Center of the Rural Development Administration of South Korea, and seven were collected from the USDA-ARS (Agricultural Research Service) Germplasm Resources Information Network (Table 1). Two universally susceptible differentials, “Williams” and “Zhonghuang 13,” were used as susceptible controls (Zhong et al., 2019). The evaluation was conducted by the hypocotyl inoculation (Dorrance et al., 2008), which is the most common method for evaluating Rps-mediated resistance to PRR. For preparation of inoculum, a 5.5 mm piece of media with mycelium was cultured on 10% V8 media at 28°C for 7-10 days. Twelve seeds of each genotype were planted in a 13 cm plastic pot filled with bed soil in a greenhouse. A 1 cm slit was made in the center of the hypocotyl of 7-10 day seedlings, and a mycelial slurry (0.3 ml) was injected into the slit using a 10 ml syringe with an 18 guage needle. The inoculated seedlings were incubated under humid conditions for one day. Seven days after inoculation, the number of dead (brown stem lesions or rot) and survived (no symptoms) seedlings was counted per genotype. The reactions of the genotypes were determined as susceptible (S) if less than 30% of the seedlings survived, intermediate (I) if 30-70% survived,
or resistant (R) if more than 70% survived (Fig. 3). The experiment was repeated at least three times, and the phenotypic data were determined using the average survival rate. Pathotypes of *P. sojae* isolates were determined by susceptibility to inoculation with a set of 16 differentials (Cerritos-Garcia et al., 2023). The virulence pathotype was as follows (Table 2): GJ3053 (vir 1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8), AD3617 (vir 1a, 1b, 1d, 3a, 3b, 3c, 4, 5, 6, 7, 8), WJ3624 (vir 1a, 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 8), and DG3968 (vir 1a, 1b, 1c, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7). The pathotype complexity, the number of *Rps* genes for which an isolate is virulent (Cerritos-Garcia et al., 2023), was highest for isolate GJ3053, as it was compatible to all differentials. Williams 82 with *Rps1k* showed resistant and intermediate reaction to three isolates, except for isolate GJ3053. *Rps1k* had possessed stable PRR resistance over a long period of time but it is not effective any more in the United States (McCoy et al. 2023). In South Korea, unlike, *Rps1k* could be still useful in providing protection against *P. sojae*.

GJ3053 and AD3617 were used in phenotypic assays for 205 soybean genotypes consisting of 170 domestic cultivars, 15 breeding lines, and 20 landraces. Seeds were harvested from NICS field in Miryang (35°29′N, 128°44′E), South Korea, in 2019. They were evaluated by hypocotyl inoculation (Dorrance et al., 2008) and reactions were determined as the criteria mentioned above. Twelve (5.9%), 11 (5.4%), and 182 (88.8%) genotypes were resistant, intermediate, and susceptible to GJ3053, respectively (Fig. 4A). Eight (3.9%), seven (3.4%), and 190 (92.7%) genotypes were resistant, intermediate, and susceptible to isolate AD3617, respectively (Fig. 4B). The number of genotypes with survival rates <10% was 160 (78.0%) and 168 (82.0%) for isolates GJ3053 and AD3617, respectively (Fig. 4C and D). The phenotypic frequency distribution of survival rate was similar between the two isolates. This similarity was attributed to the shared parent lines of the cultivars used in screening. Genotypes were classified by type and color in the scatter plot indicating the survival rate of each *P. sojae* (Fig. 4E). None of the breeding lines was resistant, and only two of the landraces were resistant to more than one isolate. Domestic cultivars, with the largest proportion, were mainly distributed with a survival rate of 30% or less for each isolate. Among them, the resistant cultivars to previously reported isolates in South Korea had different reactions to the isolates used in this study. For example, Cheongja, which was resistant to isolate P-98145 and 3444-1, and Daewon, Daepung 2, Pungwon, and Hwangkeum, which were resistant to isolate 2457, showed susceptible reaction to both GJ3053 and AD3617, the isolates used in this study (Kang et al., 2019). Saedanbaek resistant to isolate 2858 was intermediate to GJ3053 and susceptible to AD3617 (You et al., 2023b).

The five genotypes, Heugmi, Jungmo3009, Namcheon, Blackhawk, and Cheongja2, were resistant to the both isolates, which is only 2.4% of all screened genotypes (Table 3). Five genotypes, including Taechong, were only resistant to isolate GJ3053, while all differentials were susceptible to the GJ3053 (Table 2), suggesting that they may possess unknown *Rps* alleles or genes that were not included in the differentials. Cheongja2 is the parental line of Jungmo3009 and Taechong (Baek et al., 2004; Han et al., 2016; Seo et al., 2020). Cheongja2 was developed
Resistant Soybeans for New Isolates of Phytophthora sojae

Since Ilpumgeomjeong was susceptible to both isolates (data not shown), the given resistance to both isolates in Jungmo3009 and Taecheong was probably inherited from Milyang70, which is an elite line developed in the past.

Five resistant genotypes selected after screening and two susceptible elite cultivars were further inoculated with isolate WJ3624 and DG3968 (Supplementary Table 1). Resistant genotypes Manpung, Jungmo3009, and Namcheon were susceptible to more than one isolate. Due to such race-specificity, gene stacking is necessary in use of R-gene mediated resistance for the long-term management of PRR in soybeans. Gene stacking is the process of accumulating R genes resistant to pathogenic variants (Zhu et al., 2012). The integration of multiple Rps genes into a cultivar can confer resistance to a broad range of P. sojae pathotypes that are dominant in local soybean fields. This is important because the intensive use of a few Rps genes can increase the selection pressure on certain isolates and eventually accelerate the loss of functionality of the heavily used Rps genes (Schmitthenner, 1985). A recent analysis showed a decline in the efficacy of specific Rps genes, such as Rps1a, Rps1c, and Rps1k, in soybean PRR control and a significant increase in pathotype complexity of isolates over time (McCoy et al., 2023). To enhance the effectiveness of PRR management, it is imperative to develop cultivars with multiple Rps genes and to continually monitor changes in the pathotypes of numerous P. sojae isolates.

In summary, with the expansion of soybean cultivation in paddy fields in South Korea, there has been an escalating occurrence of PRR, which is more likely to occur under warm and humid conditions. The present study was conducted using four isolates of P. sojae collected from soybean production areas in South Korea. All four isolates were identified as P. sojae using PCR and exhibited different pathotypes. Pathotype assessment of isolates reported in South Korea has not been conducted until recently, leading to ambiguous distinctions among pathotypes. However, a set of 16 differentials was used to evaluate the pathotypes of the four isolates of P. sojae in the study. Therefore, the results of this study will contribute to the identification of pathotype diversity of P. sojae in South Korea and the monitoring of changes in virulence. When screening soybean genotypes for isolates GJ3053 and AD3617, most genotypes were susceptible, with a survival rate of less than 30% for both isolates. Current domestic cultivars and breeding lines are vulnerable to P. sojae. Only five genotypes were identified as sources of resistance in the two tested isolates. These results can be used to develop PRR-resistant cultivars in South Korea.

### Table 2. Reactions of soybean differentials to four isolates of Phytophthora sojae

<table>
<thead>
<tr>
<th>Differentials</th>
<th>Rps(^a) gene</th>
<th>Reaction by isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GJ3053</td>
</tr>
<tr>
<td>Williams rps</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Zhonghuang 13 lps</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Harlon la</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>L77-1863 lb</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Williams 79 lc</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>PI 103091 ld</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Williams 82 lK</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>L82-1449 2</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>L83-570 3a</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>L91-8347 3b</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>L92-7857 3c</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>L85-2352 4</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>L85-3059 5</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>L89-1581 6</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>L93-3258 7</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>PI 399073 8</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

\(a\) Rps: resistance gene to Phytophthora sojae.

### Table 3. Reactions of selected soybean genotypes to resistance against either or both of the tested Phytophthora sojae isolates

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Isolate GJ3053</th>
<th>Isolate AD3617</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heugmi</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Jungmo3009</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Namcheon</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Blackhawk</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cheongja2</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Socheong2</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>Saeal</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>Taecheong</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Sobaegnamul</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Kwangkyo</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>PI 82183</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Jonam</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Manpung</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Miso</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Heukseong</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>

\(S\) (susceptible: <30% survival), \(I\) (intermediate: 30-70% survival), and \(R\) (resistant: >70% survival).
Heo et al.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ijpponline.org/).

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Resistant Soybeans for New Isolates of Phytophthora sojae


