

Review

Current Status and Future Prospects in Genomic Research and Breeding for Resistance to *Xanthomonas citri* pv. *glycines* in Soybean

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Abstract: Soybean [*Glycine max* (L.) Merr.] is an economically important crop with high protein and oil contents. A range of biotic stresses constantly threaten soybean production and lead to decreases in yield and quality, but bacterial pustule caused by *Xanthomonas citri* pv. *glycines* (Xcg) is one of the most destructive diseases affecting worldwide soybean production. This review provides an extensive summary of multidisciplinary research on the soybean–Xcg interaction. First, we introduce general biological features of the causal agent Xcg as well as symptoms of the bacterial pustule disease it causes. Second, we review the geographic distribution of and genetic changes in the Xcg population over time, based on molecular evidence from recent studies. Third, we integrate several published studies to identify resistance loci against Xcg using bi-parental mapping populations and collections of germplasm along with genetic sources and molecular markers associated with resistance. Fourth, we summarize the molecular interactions between soybean and Xcg. Lastly, we discuss perspectives on future genomic research and breeding for improved resistance to Xcg in soybean.

Keywords: soybean; bacterial pustule; disease resistance; genomics-assisted breeding; diversity of *Xanthomonas citri* pv. *glycines* (Xcg)



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1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the most economically important legume crop and provides edible oil and protein used in food, feed, and industrial products worldwide [1–3]. The percentages of oil and protein in soybean seeds are almost 18.0% and 38.0%, respectively [4]. As estimated, almost 40.0% of the edible vegetable oil consumed worldwide is produced from soybeans [5]. Unfortunately, soybean is susceptible to numerous pests and pathogens, including aphids, beetles, mites, stinkbugs, nematodes, viruses, bacteria, oomycetes, and fungi, resulting in decreased yield and quality [6–8]. For example, the cumulative economic loss from 1996 to 2016 due to 23 diseases across 28 states in the United States was \$73,535 per hectare [9]. These 23 diseases can be grouped into six categories: stem/root, nematode, foliar, bacteria, virus, and other diseases. Of these, infection with bacterial diseases specifically results in a \$529 per hectare loss at the national level (across 28 states and 21 years) [9].

Bacterial pustule (BP) caused by *Xanthomonas citri* pv. *glycines* (Xcg) is one of the most significant bacterial diseases in susceptible soybean genotypes worldwide [10]. Over the last 120 years, the identification and distribution of Xcg has been continuously documented around the world [11–18]. Several genetic studies have identified genomic regions associated with resistance to Xcg using mapping populations and collections of soybean accessions [10,19–27], with a 33-kb region located on chromosome 17 often identified as

a strong candidate locus [20]. Molecular markers associated with resistance to *Xcg* have also been reported in a few studies. Genome-wide gene expression was compared between soybean near-isogenic lines carrying either a BP-resistant or -susceptible allele following inoculation with *Xcg* [28]. However, resistance genes for *Xcg* have yet to be cloned, and much remains unknown despite these efforts. Recent research has revealed that the genetic diversity of BP isolates is increasing, and molecular evidence suggests that a population shift has occurred in *Xcg* during the past 20 years [13,16,29]. Climate change also increases the possibility of changes in the *Xcg* population, but little work has been done in the past decade to mine new genetic sources and identify resistance genes/quantitative trait loci (QTLs). Therefore, the objectives of this review are (1) to summarize the biology of *Xcg* and its population diversity, (2) to integrate the current understanding of genetic and genomic research regarding resistance to *Xcg* in soybean, and (3) to suggest possible future work in genomics-assisted breeding of soybean for more effective protection against *Xcg*.

2. Bacterial Pustule and Its Causal Agent *Xanthomonas citri* pv. *glycines*

Xanthomonas citri pv. *glycines* (*Xcg*) is the causal agent of BP [30]. Hedges [31] first identified the typical pustule symptoms caused by this disease and named the causal agent *Bacterium phaseoli* var. *sojense*. The pathogen was later reclassified to *Xanthomonas campestris* pv. *glycines* [32] and then *Xanthomonas axonopodis* pv. *glycines* through DNA–DNA hybridization analysis [33] before finally receiving the name *Xanthomonas citri* pv. *glycines* [30].

Xcg is a Gram-negative, aerobic, and motile bacterium with a single polar flagellum. It is rod shaped, with sizes of 0.5–0.9 µm (width) × 1.4–2.3 µm (length). This bacterium can grow at 10–40 °C (but grows optimally at 30–33 °C in wet conditions) and at a pH of 4.0–8.0 (optimal pH = 7.0). Colonies on tryptic soy agar are small, circular, and smooth, and have entire margins. The bacterium grows slowly in culture and is known to produce auxins, cytokinin, bacteriocins, toxins, and extracellular polysaccharides, among other molecules [34–37].

Several molecular biology tools and techniques, including polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR), have been used to detect *Xcg* directly in plant tissues and seeds. Primers for DNA hybridization and conventional PCR have been developed for the glycinecin A (*glyA*) gene [38,39] and the *PstI* fragment containing the open reading frame of the *TAL* gene [40]. Real-time PCR methods based on the *rpoD* gene can also detect the presence of *Xcg* and quantify the degree of *Xcg* contamination in seeds [41].

DNA sequences of various genes have been utilized to analyze the genetic relatedness of different species within the genus *Xanthomonas*, as well as that of different strains within the species *X. citri*. According to Constantin et al. [30], a *gyrB* fragment was selected as an effective target gene for the identification of *Xanthomonas* taxa [42].

For differentiation within *Xanthomonas* species, multi-locus sequence analysis (MLSA) has been used in phylogenetic analysis, and recently, 109 *Xanthomonas* strains were distinguished based on seven housekeeping genes (*atpD*, *dnaK*, *efp*, *glnA*, *gyrB*, *rpoD*, *lepA*, and *lrp*) [30,43]. These analyses showed that *Xcg* is closely related to some pathogenic bacterial species on different hosts, namely *X. axonopodis* pv. *citri* and *X. citri* pv. *mangiferaeindicae*, but it is relatively distant from *X. campestris* pv. *syngonii* and *X. axonopodis* pv. *axonopodis* [30].

Key symptoms of BP, shown in Figure 1, include small, yellowish spots that later turn reddish-brown and slightly raised pustules in the center of the lesions [44]. The pustules often arise through hypertrophic changes in the parenchymatous tissues of the host responding to the bacterial infection. Pustule production on soybean leaves is known to be accompanied by a drastic increase in indole-3-acetic acid content in host tissues [45]. These lesions often occur on the abaxial side of leaves and vary from specks to large, irregularly shaped, and mottled-brown areas, which develop when the lesions coalesce. The leaf spots can form without developing pustules.



Figure 1. Bacterial pustule symptom caused by *Xanthomonas citri* pv. *glycines* on a susceptible soybean genotype.

The etiology of BP is fairly well understood. *Xcg* can infect common bean and cowpea as well as soybean [46]. It overwinters in seeds, on plant debris in the soil, and in the soil itself. *Xcg* enters the plant through natural openings and wounds, where it multiplies intercellularly. *Xcg* invades and multiplies within the apoplast, causing localized leaf spots or leaf streaks [47]. Plant-to-seed infection occurs via the vascular system through the funiculus as well as through infected pods. Seeds can also get contaminated with the bacterium during threshing after harvest. Infections are more common on younger leaves, which are more susceptible than older leaves, although the disease appears at all stages of plant growth [22,48,49]. Disease incidence depends on the susceptibility of the soybean genotype, virulence of the pathogen, and favorability of weather conditions. Bacterial cells are spread by wind-blown rain, by rain splashing up from old crop residue, and during field work when the canopy is wet. For the disease to occur, soil- and seed-borne inocula must be spread during warm and humid weather, which promotes fast multiplication of the bacterium. Kang et al. [29] reported that disease severity of BP in Korea is often correlated with the amount of precipitation in August when soybean plants are at the flowering stage. Thus, especially wet conditions are likely to be one of the main factors contributing to a high incidence of BP.

3. Distribution and Population Diversity of *Xcg*

Xcg is widely distributed and commonly occurs in major soybean-producing countries such as Brazil, the United States, China, Korea, Thailand, Australia, and Benin [50]. In recent years, disease severities of up to 26% and incidences of up to 70% have been reported in several countries [18,51]. In addition, the bacterium was reported to be present in areas where it had not previously been found. For instance, in the United States, the disease had commonly been found in the southern region, but more recently it was also reported as far north as North Dakota [15]. In China, the disease is most severe in South China, especially in the Yangtze and Huai River Basins. The incidence of BP has been increasing due to global warming and the more frequent occurrence of storms [16,52]. It was also reported that BP occurred during all four seasons in the northern coastal region of New South Wales, Australia [53]. In Benin, Africa, BP was present in 33 of 34 sites in the Guinea Savanna, with mean incidence varying from 15 to 70% [18]. In Korea, BP occurs nationwide and can be observed from July to September. During the 1990s and 2000s, BP was so severe that it could be observed in 86–89% of all soybean fields in Korea [54].

Strains of the BP pathogen have been shown to fall into distinct races [29,42,55,56] that differ in aggressiveness [57]. Hwang et al. [42] evaluated the pathogenic variability of 63 *Xcg* isolates in a set of 11 differential soybean cultivars, including Chippewa, Harosoy, Mukden, Pella, and Williams. Based on the results of this experiment, they classified the isolates into five races, designated 1, 2, 3, 4, and 5. Similarly, Kaewnum et al. [57]

demonstrated that 26 *Xcg* isolates from Thailand differed in their capability to induce disease on soybean cultivars and in their ability to induce a hypersensitive response (HR) in a range of plant species, including tobacco (*Nicotiana tabacum*), cucumber (*Cucumis sativus*), pea (*Pisum sativum*), and sesame (*Sesamum indicum*). They classified these Thai *Xcg* isolates into three races based on the presence of an *avr* gene that contributes to differential virulence on soybean cultivars. Park et al. [56] classified 155 *Xcg* isolates from Korea into six groups based on the number and size of genomic fragments hybridizing with an *avrBs3* gene family probe, as differences in *avrBs3* content are correlated with resistance and/or aggressiveness. They also identified six type strains that represent these groups. As a follow-up to that study, Kang et al. [29] characterized the diversity of a nationwide collection of 106 Korean *Xcg* isolates based on *avrBs3* banding patterns and reported that the diversity of *Xcg* strains increased during the last two decades. They also documented the emergence of new type strains along with new dominant strains.

4. Resistance Genes/QTLs for Bacterial Pustule of Soybean

To date, several soybean genotypes have been reported as resistant to BP, including ‘CNS (PI 548445)’, PI 416937, PI 96188, ‘Danbaekkong’, ‘Sinpaldalkong’, ‘SS2-2’, and ‘Meng8206’ [10,20,22–24,27]. Table 1 summarizes the genomic loci associated with a resistance to BP as identified by linkage mapping using bi-parental populations. CNS was the first U.S. soybean variety reported to resist *X. phaseoli* in field conditions, thanks to the single recessive gene *rxp* (resistance to *X. phaseoli*) [58]. However, artificially inoculated CNS plants showed substantial pathogen growth and became severely diseased under controlled conditions [59]. Soybean lines P-4-2 and P-169-3 were found to be completely immune to *Xcg* even when inoculated with a high concentration of 10^9 colony-forming units (cfu)/mL of the *Xcg* isolate AM2 under ambient conditions [60]. The genetic mechanism underlying this resistance in P-4-2 was evaluated in F_1 , F_2 , and F_3 progeny from reciprocal crosses between Monetta (susceptible) and P-4-2 (resistant). The results of this experiment showed that resistance to *Xcg* in P-4-2 was controlled by duplicated recessive genes [61]. Thus, BP resistance in P-4-2 is due to resistance genes other than *rxp*.

Narvel et al. [22] mapped the dominant locus *Rxp* to the genomic region between Satt372 and Satt014 on Chr. 17 (linkage group, LG, D2) in a ‘Young’ (R) × ‘PI 416937’ population, and the mapped locus *rxp* was confirmed by genotyping 106 F_2 -derived lines for the molecular markers Satt372 and Satt135 in a ‘PI 97100’ × ‘Coaker237’ (R) population. Kim et al. [21] mapped the recessive *rxp* locus to the genomic region between Satt372 and McctEact 97 in a SS2-2 (R) × Jangyeobkong population. Through fine mapping, the recessive locus *rxp* was mapped to a 33-kb region on Chr. 17 (D2), flanked by the molecular markers SNUSSR17_9 and SNUSNP17_12, where two candidate genes are located [20,21]. Although the genotypes carrying the resistance gene have been widely used in breeding for resistance against *Xcg*, the actual *rxp* gene has yet to be cloned.

Table 1. Genomic regions associated with resistance to bacterial pustule identified by bi-parental linkage mapping.

	Reference	Source of Resistance	Infection Type	Xcg Strain	Chr. (LG) ^a	Position 1 (bp) ^b	Position 2 (BP) ^b	Flanking Marker 1	Flanking Marker 2	PVE (%) ^c
1	Narvel et al. 2001 [22]	Young Coaker 237	Natural	-	17 (D2)	6,475,946	7,542,029	Satt014	Satt372	-
			Natural	-	17 (D2)	5,891,979	7,542,029	Satt135	Satt372	-
2	Van et al. 2004 [24]	Danbaekkong	Artificial	SDL2178	4 (C1)	43,305,171	-	Satt294	-	7
				LMG7403	5 (A1)	29,529,125	-	Satt155	-	13
				8ra, Mixed	10 (O)	46,657,863	-	Satt243	-	11–15
				Mixed	10 (O)	-	-	Satt259	-	19
				LMG7403	13 (F)	15,306,234	-	Satt269	-	8
				6 strains, Mixed	17 (D2)	7,542,029	-	Satt372	-	9–43
				OCS-G	19 (L)	-	-	Satt143	-	6
				LMG7403	19 (L)	40,637,071	-	Satt156	-	11
3	Kim et al. 2004 [21]	SS2-2	Artificial	8ra	17 (D2)	7,542,029	-	Satt372	McctEact 97	10–15
4	Seo et al. 2009 [23]	Shinpaldalkong	Artificial	8ra	4 (C1)	16,738,759	-	Satt190	-	9
					9 (K)	* 5,753,983	-	Satt137	-	6
					14 (B2)	38,859,467	-	Satt556	-	7
					17 (D2)	5,891,979	-	Satt135	-	11–21
					20 (I)	27,664,504	-	Satt496	-	3
5	Kim et al. 2010 [20]	Danbaekkong	Artificial	8ra	17 (D2)	7,005,804	7,038,893	SNUSNP17_12	SNUSSR17_9	-
6	Kim et al. 2011 [27]	PI 96188	Artificial	8ra	10 (O)	48,199,089	-	Sat_108	-	-
7	Zhang et al. 2018 [62]	Dongnong594	Artificial	Xagneau001	2 (D1b)	-	-	Mark1046791	Mark1018851	-
					17 (D2)	-	-	Mark1406417	Mark1409648	-
					19 (L)	-	-	Mark926558	Mark961660	-
8	Zhao et al. 2022 [10]	Meng8206	Artificial	C5	5 (A1)	* 1	* 1,169,356	-	-	7
					17 (D2)	* 5,158,677	* 5,994,063	-	-	22
					17 (D2)	* 6,777,393	* 6,883,854	-	-	74
					17 (D2)	* 6,293,843	* 6,883,854	-	-	35

^a Chr—Chromosome; LG—Linkage group. ^b Physical positions (in base pair) are based on the reference genome Glyma.Wm82.a2 (Glyma2), while those with an asterisk (*) are based on Glyma.Wm82.a1 (Glyma1.1). ^c Phenotypic variance (%) explained by marker. Unknown information is indicated with a hyphen (-).

Additional QTLs on Chrs. 4 (C1), 5 (A1), 9 (K), 10 (O), 13 (F), 14 (B2), 17 (D2), 19 (L), and 20 (I) have been reported to be associated with BP resistance [23,24,27]. Van et al. [24] detected eight QTLs for BP resistance on Chrs. 4 (C1), 5 (A1), 10 (O), 13 (F), 17 (D2), and 19 (L) in the ‘Suwon157’ × ‘Danbaekkong’ (R) 75 RIL population using six isolates in both greenhouse and field conditions. Field resistance was evaluated using a mixture of three isolates (OCS-F, SDL2178, and LMG7404), while greenhouse resistance was evaluated using individual inoculations with six *Xcg* isolates (OCS-F, OCS-G, SDL2178, LMG7403, LMG7404, and 8ra). Seven QTLs (Satt294, Satt155, Satt243, Satt269, Satt372, Satt143, and Satt156) were found to condition resistance to each isolate separately, with a phenotype variance of 6–43%. Three QTLs were associated with a resistance to the mixture of three isolates (OCS-F, SDL2178, and LMG7404) in field conditions, with a phenotype variance of 9–19%. One of these QTLs, Satt372 on LG D2, was identified in both greenhouse and field conditions and accounted for 9–43% of the phenotypic variance. Seo et al. [23] identified five QTLs associated with BP resistance on Chrs. 4 (C1), 9 (K), 14 (B2), 17 (D2), and 20 (I) in a ‘Keunolkong’ × ‘Shinpaldalkong’ (R) F₁₀ RIL population grown in both field and greenhouse conditions. Four QTLs (Satt556, Satt135, Satt496, and Satt137) accounted for 36.4% of the phenotypic variance in field-grown soybean, while two QTLs (Satt190 and Satt135) accounted for 19.6%. One of these QTLs, Satt135 on LG D2, was identified in both field and greenhouse experiments. The BP resistance gene from PI 96188, which exhibits only pustules without chlorotic haloes, was mapped to chromosome Chr. 10 (O) in the ‘PI 96188’ × ‘Jinjoo1’ F₇ RIL population, being linked with the SSR marker Sat_108 at the distal end of Chr. 10 [27]. Zhang et al. [62] identified three QTLs (QTL2, QTL17, and QTL19) associated with BP resistance on Chrs. 2 (D1b), 17 (D2), and 19 (L) in the ‘Charleston’ × ‘Dongnong594’ (R) RIL population grown in phytotron conditions. Four (Glyma.02g108700, Glyma.02g110500, Glyma.02g112300, and Glyma.02g120800), two (Glyma.17g204600 and Glyma.17g204300), and one (Glyma.19g074900) candidate genes were found in these three QTL regions, located on Chrs. 2, 17, and 19, respectively. Zhao et al. [10] reported three BP-resistance QTLs on Chrs. 5 (*qrxp_05_1*) and 17 (*qrxp_17_2* and *qrxp_17_1*), which explained 7.3–74.3% of the phenotypic variance in a ‘Meng8206’ (R) × ‘Zhengyang’ 126 F_{2:9} population grown and artificially inoculated in the field. More recently, genome-wide association analysis (GWAS) was employed to investigate BP resistance in collections of diverse soybean genotypes (Table 2) [10,19,26]. A study using a collection of >3000 soybean accessions detected three significant SNPs (ss715580342, ss715609404, and ss715628133) on Chrs. 1 (D1a), 11 (B1), and 17 (D2), respectively. Two LRR-RLK resistance gene candidates (Glyma.01g197600 and Glyma.01g197800), one LRR-RLK gene (Glyma.11g196800), and an RLK gene (Glyma.17g090400) were found in the same linkage disequilibrium (LD) blocks as three significant SNPs. Of these three SNPs, one (ss715628133) was located both in the coding region of an RLK gene (Glyma.17g090400) and in the previously reported *rxp* locus where two candidate genes are located (Glyma.17g090100 and Glyma.17g090200), which suggests that Glyma.17g090400 may contribute to the resistance associated with the *rxp* locus, along with Glyma.17g090100 and Glyma.17g090200 [26].

Eleven and five SNPs for resistance against *Xcg* strain 2440^P and strain 2447 were identified in the GWAS of 118 soybean genotypes. For *Xcg* strain 2440^P, five significant SNP markers were detected on Chr. 3 (N); one each on Chrs. 5 (A1), 8 (G), and 10 (O); and three on Chr. 13 (F). The most significant SNP marker (Gm03_46214163_C_T) associated with a resistance to BP caused by strain 2440^P was located on Chr. 3 (N). For strain 2447, 52 SNPs were identified as being significantly associated with BP resistance. Of the 52 SNPs, three markers located on Chr. 15 (E) and one on Chrs. 6 (C2) and 17 (D2) had $-\log_{10}(P)$ higher than 22 and were considered for further analysis. As a result of candidate gene analysis, two significant SNPs (Gm03_46214163_C_T and Gm15_5097389_T_C) were predicted to result in amino acid changes in the benzyl alcohol *O*-benzoyltransferase-like and MAIN-LIKE 1-like proteins, respectively [19].

Table 2. Single nucleotide polymorphisms (SNPs) associated with resistance to bacterial pustule in the previous genome-wide association study (GWAS).

	Reference	Plant Material	No. of SNPs	Infection Type	Xcg Strain	Chr. (LG) ^a	Position (bp) ^b	SNP ID	Allele	−Log ₁₀ P	PVE (%) ^c
1	Chang et al. 2016 [26]	Germplasm (<i>n</i> = 3173)	37,659	Natural	-	1 (D1a)	53,136,582	ss715580342	T/G	7.1	-
						11(B1)	26,963,752	ss715609404	A/G	6.9	-
						17 (D2)	7,042,685	ss715628133	G/A	6.6	-
2	Capobiango da Fonseca et al. 2021 [19]	Germplasm (<i>n</i> = 118)	3807	Artificial	2440 ^P	3 (N)	34,416,830	ss715585454	G/T	3.1	13
						3 (N)	34,612,476	ss715585486	G/A	3.2	14
						3 (N)	36,042,575	ss715585676	A/G	3.3	14
						3 (N)	44,055,029	ss715586464	C/T	3.5	15
						3 (N)	44,213,517	ss715586487	C/T	3.6	16
						5 (A1)	2,357,871	ss715592433	A/G	3.3	14
						8 (A2)	43,619,289	ss715602088	A/G	3.6	15
						10 (O)	2,445,007	ss715605954	A/G	3.3	14
						13 (F)	28,859,734	ss715614710	A/G	3.2	14
						13 (F)	30,875,555	ss715615049	G/T	3.3	14
						13 (F)	34,087,365	ss715615474	C/A	3.2	14
				Artificial	2447	6 (C2)	9,453,068	ss715595677	T/C	23.0	94
						15 (E)	5,116,201	ss715622817	T/C	23.0	94
						15 (E)	5,381,724	ss715622835	C/T	23.0	94
						15 (E)	5,457,236	ss715622838	G/A	23.0	94
						17 (D2)	7,015,860	ss715628131	C/T	24.0	94
3	Zhao et al. 2022 [10]	Germplasm (<i>n</i> = 476)	61,166	Both	C5	5 (A1)	* 7,667,820	Gm05_7667820	G/A	4.1–4.2	3.3–3.4
				Both		5 (A1)	* 7668047	Gm05_7668047	-	4.1–4.2	3.3–3.4
				Artificial		9 (K)	* 36,501,019	Gm09_36501019	-	4.2–4.7	3.5–3.9
				Both		17 (D2)	* 5,628,119	Gm17_5628119	T/C	4.5–4.6	3.7–3.9
				Both		17 (D2)	* 5628133	Gm17_5628133	-	4.5–4.6	3.7–3.9
				Both		17 (D2)	* 7603802	Gm17_7603802	T/C	4.1–8.7	3.4–7.9
				Artificial		17 (D2)	* 7603992	Gm17_7603992	-	4.1–6.9	3.4–6.0
				Both		17 (D2)	* 7604008	Gm17_7604008	-	5.0–6.8	4.2–5.9
				Both		17 (D2)	* 7712768	Gm17_7712768	-	4.1–8.2	3.3–7.3
				Both		17 (D2)	* 7721556	Gm17_7721556	-	5.0–8.3	4.2–7.5
				Both		17 (D2)	* 7736150	Gm17_7736150	-	4.3–6.7	3.6–5.8
				Both		17 (D2)	* 7754016	Gm17_7754016	-	4.1–8.1	3.3–7.3
				Both		17 (D2)	* 7754048	Gm17_7754048	-	3.3–8.1	3.3–7.3

^a Chr.—Chromosome; LG—Linkage group. ^b Physical positions (in base pair) are based on the reference genome Glyma.Wm82.a2 (Glyma2), while those with an asterisk (*) are based on the Glyma.Wm82.a1 (Glyma1.1). ^c Phenotypic variance (%) explained. Unknown information was indicated with a hyphen (-).

Thirteen quantitative trait nucleotides (QTNs) associated with BP resistance were identified in a GWAS performed on 476 cultivars in a soybean breeding germplasm population. Eleven out of the thirteen steady QTNs were identified in both artificial and natural conditions. In this study, three BP resistance QTLs on Chrs. 5 (*qrxp_05_1*) and 17 (*qrxp_17_2* and *qrxp_17_1*) in a ‘Meng8206’ (R) × ‘Zhengyang’ 126 F_{2.9} population were also detected. Through further analysis, the three BP-resistance QTLs and the eleven stable BP resistance QTNs co-localized to three genomic regions. Four genes (Glyma.17g086300, Glyma.17g090100, Glyma.17g090200, and Glyma.17g090400) located in genomic region 3, which covers the *rxp* gene and Glyma.05g040500 (the homolog of Glyma.17g086300), were predicted to be the candidate genes [10].

5. Molecular Mechanisms Underlying Bacterial Pustule Formation in Soybean

During disease establishment, *Xanthomonas* pathogens such as *Xcg* employ various virulence factors, including adhesins, polysaccharides, lipopolysaccharides, and degradative enzymes. One key pathogenicity factor is the type III secretion system (T3SS), which enables bacteria to directly inject effector proteins into the host cell cytosol. The type III (T3) effectors are translocated into the host cells, where they interfere with host immunity responses or facilitate the nutritional or virulence processes of the pathogen [47,63,64]. The T3SS is encoded by the hypersensitive reaction and pathogenicity (*hrp*), *hrp*-conserved (*hrc*), and avirulence (*avr*) genes and reacts with specific resistance (R) receptors. Effector proteins are encoded by *hrp*-dependent out protein (*hop*) genes [65].

Kim et al. [66] characterized the *HrpT3SS* and *hpa* (*hrp*-associated) virulence genes and identified HR-eliciting proteins such as HpaG in the *Xcg* genome. The pathogenicity island (PAI) contained seven plant-inducible promoter boxes and was composed of nine *hrp*, nine *hrc*, and eight *hpa* genes that are regulated by HrpG and HrpX. The Hrp PAI in *Xcg* resembled that of other *Xanthomonas* species, and the Hrp PAI core region was highly conserved.

The largest effector family found in the *Xanthomonas* spp. is the AvrBs3/PthA or TAL family. *Xanthomonas* strains express a combination of typically 20–40 T3 effectors. *Xcg* strains possess more than 30 T3 effectors and contain at least four homologs of *avrBs3*. It was reported that Korean *Xcg* strains display the greatest variation in *avrBs3* homolog numbers, and their plasmids also carry several *avrBs3* homologs [13]. According to Athinuwat et al. [55], the *avrBs3* homolog *avrXg1* contributes to increased bacterial establishment in soybean leaves and acts as a determinant for virulence specificity. Similarly, several TAL effectors from *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) are essential virulence factors for the infection of rice (*Oryza sativa*) [47]. Meanwhile, a variety of transcription activator-like (TAL) effectors are known to be associated with pathogen virulence and disease symptoms, and the presence or absence of specific effectors can contribute to the pathogenicity on specific hosts [67]. The specificity of TAL effectors is determined by repeat-variable diresidues (RVDs), which allow binding to the promoter of the host plant [68]. Recently, whole-genome sequencing of *Xcg* strains revealed that the diversity and size of RVDs are limited within *Xcg*, and also predicted that the effector binding elements of the TAL effectors fall into six groups and are strongly overlapping in sequence [11]. This suggests that the target binding domains in soybean cultivars may evolve specifically. However, despite these studies, little is known about the corresponding host virulence targets of TAL effectors and their role in pathogenicity.

Previous research has identified candidate genes in soybean that relate to BP resistance (Table 3) [10,19,25,26,69,70]. Gene-encoding membrane proteins, zinc finger family proteins, LRR-RLK resistance proteins, Benzyl alcohol O-benzoyl transferase-like protein, MAIN-LIKE 1-like protein, Lateral organ boundaries (LOB), CASP-like protein 4A3, E3 ubiquitin-protein ligase SIS3-like, LATERAL ORGAN BOUNDARY1 and a defective hydroperoxide lyase (*HPL*) gene, among others, are related to BP resistance. The control of BP is complicated because many small QTLs influence plant resistance. However, soybean breeders could adopt gene cloning using the genes implicated in the disease resistance.

Table 3. Candidate genes for resistance to Xcg in the previous studies.

	Reference	Chr. (LG) ^a	Gene ID	Position ^b	Functional Annotation
1	Kim et al. 2010 [20]	17 (D2)	Glyma.17g090100	7,020,522 ... 7,022,065	Membrane protein At2g36330; <i>Arabidopsis thaliana</i>
		17 (D2)	Glyma.17g090200	7,028,352 ... 7,034,934	Zinc finger (C3HC4-type RING finger) family protein; <i>Arabidopsis thaliana</i>
2	Chang et al. 2016 [26]	1 (D1a)	Glyma.01g197600	53,149,380 ... 53,153,676	LRR-RLK resistance gene
		1 (D1a)	Glyma.01g197800	53,170,021 ... 53,173,875	LRR-RLK resistance gene
		11 (B1)	Glyma.11g196800	27,106,029 ... 27,107,951	LRR-RLK gene
		17 (D2)	Glyma.17g090400	7,040,797 ... 7,042,768	RLK gene
3	Zhang et al. 2018 [62]	2 (D1b)	Glyma.02g108700	10,404,064 ... 10,404,874	Calcium-binding EF-hand family protein
		2 (D1b)	Glyma.02g110500	10,655,319 ... 10,660,151	NB-ARC domain-containing disease resistance protein
		2 (D1b)	Glyma.02g112300	10,900,201 ... 10,902,765	NB-ARC domain-containing disease resistance protein
		2 (D1b)	Glyma.02g120800	11,926,840 ... 11,931,251	Leucine-rich repeat receptor-like protein kinase family protein
		17 (D2)	Glyma.17g204600	33,223,552 ... 33,226,195	Receptor-like protein 12
		17 (D2)	Glyma.17g204300	33,083,927 ... 33,090,560	Enhancer of polycomb-like transcription factor protein
		19 (L)	Glyma.19g074900	27,195,325 ... 27,202,148	LRR protein kinase family protein
4	Capobianco da Fonseca et al. 2021 [19]	3 (N)	-	-	Benzyl alcohol O-benzoyl transferase-like (LOC100793892)
		15 (E)	-	-	Protein MAIN-LIKE 1-like (LOC102667247)
5	Wang et al. 2020 [25]	12 (H)	Glyma.12g191400	35,295,823 ... 35,301,648	Defective hydroperoxide lyase (HPL) gene
6	Zhao et al. 2022 [10]	5 (A1)	Glyma.05g040500	3,625,982 ... 3,628,389	LBD domain-containing transcription factor
		17 (D2)	Glyma.17g086300	6,660,761 ... 6,663,277	Lateral organ boundaries (LOB) domain-containing protein 25
		17 (D2)	Glyma.17g090100	7,020,522 ... 7,022,065	CASP-like protein 4A3
		17 (D2)	Glyma.17g090200	7,028,352 ... 7,034,934	E3 ubiquitin-protein ligase SIS3-like
		17 (D2)	Glyma.17g090400	7,040,797 ... 7,042,768	Uncharacterized

^a Chr., Chromosome; LG, Linkage group. ^b Physical positions (in base pairs) are based on the reference genome Glyma.Wm82.a2 (Glyma2).

6. Future Prospects in Genomic Research and Breeding for Improved Resistance to Bacterial Pustule

As described above, many studies have identified genetic sources of resistance to BP and have characterized this resistance using genetic and genomic analyses. In spite of such efforts, the major resistance gene on Chr. 17 has yet to be cloned, and much remains unknown. Although BP occurrence fluctuates based on geographic regions and timing, BP still remains one of the major yield-limiting factors in soybean-growing countries, including Korea, China, and Brazil [10,20,22–24,27,58,71–74].

Genetic diversity and changes in the *Xcg* population over time are important factors that should be considered by soybean researchers and breeders with regard to disease management because the pathogens co-evolve as plant defenses develop [75]. Strains of *Xcg* have been reported to have distinct races [42,55,56] that differ in their aggressiveness [57]. Kang et al. [29] characterized the genetic diversity of a Korean collection of 106 *Xcg* isolates based on *avrBs3* banding patterns. The diversity of *Xcg* strains increased, and the dominant strain changed between 1999 and 2017, with three new type strains comprising 44% of the isolates examined from 2012 to 2017 [29]. Over 30 Korean cultivars and breeding lines were inoculated with three representative *Xcg* strains, implicating race-specific resistance or susceptibility [75]. Therefore, it is strongly recommended that *Xcg* isolates be collected continuously from fields and monitored periodically for changes.

Large-scale screening of unexplored soybean germplasm is also required to discover new genetic resources for resistance against newly emerging strains of *Xcg*, since new strains of a pathogen can often overcome known resistance genes. This work will assist in the selection of appropriate resistance sources and help respond to newly emerging or dominant *Xcg* strains in soybean fields. Wild soybean (*Glycine soja* Siebold and Zucc.) accessions have a much wider variation in their gene pools than cultivated soybean, providing more possibilities for favorable alleles or genes related to targeted traits [76,77]. To take advantage of this resource, the development and implementation of precise high-throughput disease screening methods will need to use phenotypic evaluations and appropriate environmental conditions, experimental designs, and statistical analyses, leading to higher accuracy in selecting germplasm.

In addition to large-scale germplasm screenings, the identification of novel genes/QTLs is a key step in breeding for improved resistance to a pathogen with high levels of genetic diversity [78–80]. Linkage analysis via bi-parental populations and GWAS experiments using collections of accessions will allow for the detection of plausible resistance loci and the subsequent development of molecular markers for efficient selection for resistance to BP. Various types of plant materials are currently being used for such research, including the core collection, the nested association mapping population, and multiparent advanced generation intercross and diversity panels (MAGIC) [81–84]. Fine mapping of identified resistance loci with large effects using backcrosses will facilitate their employment in soybean breeding programs via marker-assisted selection (MAS). Pyramiding multiple genes resistant to different strains of *Xcg* through MAS will provide durable and broad-spectrum resistance [85,86].

Molecular approaches can be applied to better understand soybean–*Xcg* interactions at the molecular level. First, genome-editing technologies, such as CRISPR/Cas9 editing, can lead to improved disease resistance by enabling targeted genome modifications [87–92]. Soybean is a fairly recalcitrant species to genetic transformation technology; thus, additional efforts may be necessary to apply these tools to soybean research. A combined approach of both traditional breeding and genetic engineering could be essential for studying the underlying molecular mechanisms of soybean–*Xcg* interactions in the future. Second, multi-omics, such as combined transcriptomics, proteomics, and metabolomics, can also play an important role in plant research. Deep transcriptome analysis (RNA-seq) has been widely used to investigate how plants respond to diseases such as soybean cyst nematode [93,94], soybean brown stem rot [95], and even BP [28,96,97]. Proteomic studies in soybean have mainly focused on traits related to abiotic stresses, such as salinity [98], flooding [99], and

low temperature [100]. In addition, metabolite profiling can be used as a tool to compare gene function and provide deeper insights into complex regulatory processes [101,102]. The integration of multi-omics data has recently been applied to advanced analyses in plant research. For instance, the integration of transcriptomic and proteomics was employed to reveal host genes that were modulated after an infection of yellow mosaic virus under natural conditions [103]. Combined transcriptomics and metabolomic data have been analyzed to investigate how soybean responds to *P. sojae* infection [104]. Multi-dimensional data analysis can provide new insights into the interpreting of big multi-omics data. To do this, more reference-quality genomes, improved genome annotation, and advanced analytical algorithms may be necessary.

In this review, we have discussed the biological features and population genetics of the causal agent of BP, *Xcg*, and integrated findings from genetic and genomic studies. Much of the soybean germplasm remains unexplored with regard to resistance to *Xcg*. The characterization of these genotypes and their responses to diverse *Xcg* strains will provide a framework for developing efficient breeding systems and the sustainable disease management of BP. Previous studies have reported candidate genes on several chromosomes, but no follow-up studies have further characterized or cloned these genes/QTLs. Implementing high-throughput sequencing technology and applying advanced analytic approaches such as multi-omics will also facilitate a deeper understanding of soybean–*Xcg* interactions in the future.

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