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Phenotypic and genotypic diversity of potato late blight pathogen (*Phytophthora infestans*) in South Korea

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SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
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ABSTRACT

Potato is one of the major staple food crops in the world. Potato production is severely damaged by the late blight caused by *Phytophtora infestans*. To develop management strategies for the late blight disease, understanding the characteristics of *P. infestans* populations is essential. In several studies have been performed using phenotypic and genetic markers to analyze the *P. infestans* characteristics for more precise disease management. However, little is known about the genetic characteristics of *P. infestans* in South Korea. Here, I characterized a total of 86 *P. infestans* isolates collected in South Korea using phenotypic (mating type, virulence, and fungicide resistance) and genotypic markers (SSRs and next-generation sequencing). As a result, about 90% of isolates were A1 mating type and sensitive to

dimethomorph fungicide. Moreover, about 50% of isolates were pathogenic to differential potato lines containing the resistance gene *R2*, *R4*, *R6*, and *R8*. These results suggest that using the fungicide dimethomorph for disease control is still effective and the sexual reproduction of *P. infestans* was limited. The genotypic analysis revealed that six clonal lineages were distributed in South Korea. Among them, SIB-1 like and KR_2 like isolates were newly identified. Whole-genome resequencing and population analysis elucidated that KR_2 like isolates was clearly distinguished from other groups. Moreover, non-synonymous mutations in both genome-wide and *Avirulence* gene regions were occurred more frequently than in other clonal lineages. These results indicated that KR_2 like was migrated from other countries or that genetic variation of KR_2 like was recently evolved. Taken together, these results could contribute to developing the efficient management system of *P. infestans* in South Korea as well as breeding late blight-resistant potato cultivars.

Keywords: Potato late blight, *Phytophthora infestans*, genotype, phenotype

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LIST OF ABBREVIATIONS

Avr	Avirulence
CAPS	Cleaved amplified polymorphic sequences
DAPC	Discriminant analysis of principal component
ETI	Effector-triggered immunity
MLG	Multi locus genotype
MSN	Minimum spanning network
NGS	Next generation sequencing
NLR	Nucleotide-binding leucine rich repeat
PCA	Principal component analysis
PCR	Polymerase chain reaction
R	Resistance
SNPs	Single nucleotide polymorphisms
SSRs	Simple sequence repeats

INTRODUCTION

Potato (*Solanum tuberosum*) is one of the most significant staple food crop in the world with a production of 338 million tons and planted area of 17 million hectares (FAO, 2019). In South Korea, potato is cultivated in around 25,000 hectares representing an average annual yield of 60-kilo tons (KOSIS, 2020). At a global level, 17 pests and pathogens contribute to a 17.2% estimated yield loss in potatoes (Savary *et al*., 2019). Late blight, caused by the oomycete pathogen *Phytophthora infestans*, is the most devastating disease in potatoes, accounting for about 6% yield loss worldwide. If disease management does not occur appropriately, late blight can destroy the fields within a week (Fry *et al*., 2008). Given the importance of controlling potato disease, it is necessary to understand the phenotypic and genotypic characteristics of *P. infestans*.

P. infestans is a heterothallic pathogen with two mating types, designated as A1 and A2. Oospores results from sexual reproduction between A1 and A2 mating types can survive long-term harsh environmental conditions such as drying and low temperatures in soil (Turkensteen *et al*., 2000). Only the A1 mating type was widespread all over the world except Mexico until the 1980s. However, the A2 mating type was discovered in 1984 among isolates obtained in Switzerland in 1981, the first documented mention of the A2 mating-type presence outside of Mexico. Genetic recombination occurs due to the presence of the A2 mating type, resulting in a population with stronger pathogenicity or more adaptability to the environment (Guha Roy *et al*., 2021). Metalaxyl is a phenylamide fungicide that was developed in the 1970s to

control oomycete. Resistance to the fungicide metalaxyl in *P. infestans* field isolates was first documented in the Netherlands in 1981. With the development of the A2 mating-type in Europe. However, as the increase in the occurrence of metalaxyl resistance, there is a growing fear of sexual recombination and resulting progeny with higher pathogenicity and decreased sensitivity to metalaxyl (Chen *et al*., 2018). With the advent of metalaxyl-resistant isolates, fungicide with different mechanisms of action was required. Dimethomorph, one of the fungicides developed in response to metalaxyl-resistance is a cinnamic acid derivative with high specificity against *Phytophthora* spp. Dimethomorph has a long retention period on the leaf surface and has excellent medicinal effects on metalaxyl-resistant isolates. In South Korea, dimethomorph has been used for potato late blight control since 1993. Fungicides containing metalaxyl or dimethomorph were often applied 5-10 times to prevent or cure the blight infection in South Korea (Kim *et al*., 2014).

P. infestans secretes a diverse set of effector proteins carrying the conserved RxLR (Arg-any-Lys-Arg) motif that is essential for the effective infection and proliferation in plants (Birch *et al*., 2009; Dou *et al.,* 2008). As a counterpart, host plant has evolved Resistance (R) genes that encode nucleotide-binding leucine-rich repeat (NLR) proteins, which act as intracellular receptors for avirulence effector of pathogen. Avirulence (Avr) proteins induce effector-triggered immunity (ETI) in the host and inhibit pathogens from infecting the plant successfully. Mutation of *Avr* genes enables *P. infestans* to escape recognition by the cognate R proteins (Zhang *et al*., 2021). The *P. infestans* genome has been characterized as being a two-speed genome

in which repeat-rich regions enable rapid evolution of effectors and promote virulence gains. *P. infestans* has relatively large genome compare to other *Phytpphthora* spp. It contains 240 megabases of DNA. Among them, transposable elements and other repetitive sequences accounting for 74% of the genome. Effector genes are disproportionately found in gene-poor, repeat-rich regions. Positive selection, which refers to an excess of base mutations that alter the corresponding amino acid, was also more common in these repeat regions (Raffaele *et al*., 2010b; Dong *et al*., 2015). *P. infestans* in which one or few amino acid substitutions may be responsible for the change between virulence and avirulence phenotypes. For example, $AVR3a^{EM}$ was changed by $AVR3a^{KI}$ enable to evade recognition of *R3a* (Armstrong *et al*., 2005). The *P. infestans* effector genes evolve to escape recognition by corresponding host *R* genes. Therefore, it is important to detect effector variation not only to guide the breeding of resistance genes but also to integrate disease management strategies for crop protection.

A variety of genotypic markers have been developed to examine the diversity of *P. infestans*. During the last decades, the genetic diversity of *P. infestans* populations has been elucidated using molecular markers such as simple sequence repeats (SSRs) and effector genes (Arafa *et al*., 2020). A set of 12 SSRs markers developed by Li *et al*. (2013) was used globally for genotyping and classifying clonal lineages of *P. infestans*. Clonal lineages were composed of clonal descendants of one common ancestor. Novel clonal lineages are thought to migrate from asexual or sexual populations somewhere else such as Mexico that can admix with or displace currently existing lineages

(Goss *et al*., 2014). It is well-documented that *P. infestans* individuals belonging to a clonal lineage tend to share phenotypic characteristics, such as fungicide sensitivity or mating type (Danies *et al*., 2013). In a previous study, 172 *P. infestans* isolates from 2009 to 2016 in South Korea were tested, using a set of 12 SSRs markers. It was found that there were four clonal lineages in South Korea, and the full genome sequences of both KR_1_A1 and KR_2_A2 were released (Choi *et al*., 2020; Yoon *et al*., 2020).

Furthermore, phenotypic markers have been developed based on mating type, fungicide resistance, and virulence (Cooke & Lees, 2004). In *Solanum demissum*, a Mexican wild *Solanum* species, eleven genes for hypersensitivity-type resistance (*R* gene) have been characterized. Eleven *R* genes introgressed into cultivated potato from *S. demissum* in various combinations have been gathered by Masternbroek and were called the Masternbroek differential set: from Ma*R*1 to Ma*R*11 (Black *et al*., 1953; Malcolmson *et al*., 1966). The Masternbroek differential set was used to identify pathogen virulence race based on susceptibility and resistance with these potatoes.

The analysis of single nucleotide polymorphisms (SNPs) which can be used as the basis for molecular diagnostic assays has become possible due to advances in sequencing techniques based on next-generation sequencing (NGS) technology. Moreover, according to the decrease in the cost of NGS, whole-genome resequencing analysis has also enabled the elucidation of the diversity of *P. infestans* populations on a genome-wide level (Arafa *et al*., 2020). For example, within the US23 clonal lineage which identified using SSRs

analysis contain at least three distinct sub lineage (Fry *et al*., 2015). However, there has been no prior study about genotypic characteristics of *P. infestans* with NGS data in South Korea.

Here, I performed a population analysis of phenotypic and genotypic traits of 86 *P. infestans* isolates collected in South Korea from 2009 to 2020. Phenotypes including mating type, dimethomorph resistance, and response to the Masternbroek differential set were examined. Moreover, clonal lineages were identified using SSR markers. Among them, SIB-1 like and KR_2 like genotypes were newly identified in South Korea. Using the NGS data, whole-genome resequencing and population analysis were performed and identified genetic variation between KR_2 like and other clonal lineages suggesting emergence of new *P. infestans* lineage. These results provide a valuable resource for developing late blight-resistant potato cultivars and the efficient management system for *P. infestans* in South Korea.

MATERIALS AND METHODS

Collection of *P. infestans* isolates

A total of 86 *P. infestans* isolates were collected from 6 regions in 11 locations in the major potato-growing areas of South Korea, from 2009 to 2020 (Table 1). *P. infestans* were named as followed by Highland agriculture research institute; Kpi indicated Korean *P. infestans*, two number after hyphen indicated year of collection, and the two number behind hyphen indicated the order of collection. Of the 86 isolates, 35 isolates from 2009 to 2017 were received by Choi (Choi *et al*., 2020). Isolates were grown on a V8-agar medium (50 ml V8 juice, 1.5 g calcium carbonate, 0.025 g β-sitosterol, 8.5 g agar, and 450 ml distilled water) and incubated at 18 °C, dark, for 7 days.

Virulence assays

A detached leaf assay was used to assess the virulence phenotypes of 86 *P. infestans* isolates on a set of 11 potato near-isogenic differential lines (Black and Mastenbroek's differential sets), each possessing *R*-gene (R1-R11) from *Solanum demissum* (provided by the International Potato Center) and potato cultivar Superior, which has no known *R* genes, was used as a susceptible control (Black *et al*., 1953; Malcolmson and Black, 1966). Detached potato leaves laid on a wet filter paper in Petri dish. Sporangial suspension at 4.0×10^4 sporangia mL^{-1} concentration of *P. infestans* were prepared. Inoculation to all leaflets was performed by giving 15 μL sporangial suspension on side of

the leaflet and then incubated at 18 ℃ in a 16 h light / 8 h dark cycle. All tests were repeated at least twice for each isolate.

Mating type

a) Pairing test

The *P. infestans* isolate with unknown mating-type was crossed with either a known A1 or A2 reference isolate (KA-2 and BC-3, respectively) on the 10% V8 agar medium (Tian *et al*., 2015). Mycelial plugs from unknown mating-types were placed on the opposite side (4 cm apart) either KA-2 or BC-3 isolates. After 10 days of incubation in the dark at 15 °C, plates were checked for the presence or absence of oospores in the hyphal interaction area between the isolates paired under a light microscope.

b) Cleaved amplified polymorphism sequence (CAPS) marker

CAPS marker W16-1 (5'-AACACGCACAAGGCATATAAAT GTA-3') and W16-2 (5'-GCGTAATGTAGCGTAACAGCTCTC-3') were used according to Judelson et al. (1995). The PCR (Polymerase chain reaction) was conducted in a 20 µl reaction mixture consisting of 10 µl of 2X TOPsimple^{TM} PreMIX-nTaq (Enzynomics, Inc., South Korea), 0.4 mM of each primer, and 50 ng of template DNA. PCR amplification was as follows: initial denaturation for 5 min at 94℃, followed by 30 cycles of denaturation for 1 min at 94℃, primer annealing for 1 min at 53℃, primer elongation for 1 min at 72℃, and a

final extension step of 72°C for 10 min. The PCR product was cleaved using restriction enzyme *HaeIII* (Thermo Fisher Scientific, USA) for 3 hours at 37°C. The recognition site is 5'-GGCC-3'. Electrophoresis was carried out for 1 hour on a 1.8% agarose gel.

Fungicide response

Isolates were grown on Rye A agar plates to which 0, 5, and 100 μg/ml metalaxyl (Syngenta Crop, Cambridge, UK) had been sprayed (Dimethomorph was sprayed in concentrations of 0, 0.5, and 5 μg/ml). All isolates were incubated at 18°C for 10 days. Radial growth of mycelia was measured, compared with the control culture plate. Sensitive, intermediate, and resistant isolates were classified as exhibiting <40% on 5 μg/ml, >40% on 5 μg/ml, >40% on 5 μg/ml and >40% on 100 μg/ml growth, respectively. Dimethomorph was used as the standard by substituting 0, 0.5, and 5 instead of 0, 5, and 100 as the same standard as metalaxyl (Kim *et al*., 2014).

SSRs genotyping analysis

Genotyping using SSRs markers in a multiplex PCR technique was performed according to the method described by Li *et al.* (2013). Twelve markers were used in this study (Table 2). PCR was conducted using the QIAGEN multiplex PCR kit (QIAGEN, Germany). PCR amplification was run under the following conditions: 15 min at 95°C; 33 cycles of 20 s at 95°C, 90 s at 58°C, and 60 s at 72°C, and a final extension 20 min at 72°C. PCR products

were analyzed with an ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Raw data and sizing determination were analyzed in GeneMapper software v. 5 (Applied Biosystems, USA). For population genetic analysis and the computation of the genotype accumulation curve, the R package poppr v.2.4.1 (Kamvar *et al*., 2014) was used. Basic parameters for this analysis was according to Choi *et al.* (2020). Using the phylogenetic software program PAUP v.4.0 (Cummings *et al*., 2004) in combination with FIGTREE v. 1.4 (Rambaut *et al*., 2012) and 1,000 bootstrap permutation, a phylogenetic tree based on the genetic matrix with UPGMA clustering was generated. A minimum spanning network was produced based on the Bruvo genetic distances calculated using poppr.

DNA extraction, sequencing, and SNPs calling

P. infestans isolates were grown on a V8-agar medium for 14 days at 18°C. Mycelium was scraped from the edge of the colony. After freeze-drying with liquid nitrogen, it was ground using a pestle. DNA of *P. infestans* was extracted from 100 mg of freeze-dried mycelium using the Exgene Plant SV mini Kit (Geneall Corp, South Korea) following the manufacturer's instructions. All samples were sequenced using Illumina Hiseq X-ten platform (Illumina, USA). The FASTQ format files were aligned to the *P. infestans* KR_1_A1 reference genome (Lee *et al*., 2020) using the BURROWS-Wheeler Aligner (v0.7.17-r1198) program. Using the SAMtools (v1.11) program, raw SNPs were detected and consensus sequences were retrieved from the BAM format file produced by the mapping procedure. By comparing each sample to the

reference genome, the raw SNPs location was acquired, which was then utilized as a candidate for generating a list of unions.

Population diversity and structure analyses

STRUCTURE v2.3.4 software was used to estimate population structure. With an admixture model and an independent allele frequency model, the number of population (*K*) was set from 1 to 10 with 20 replications for each K, with the length of the burn-in period set to 10,000 and the number of Monte Carlo Markov Chain replications set to 10,000. Principal Component Analysis (PCA) was conducted using the SNPRelate R package (Zheng *et al*., 2012). Discriminant Analysis of Principal Component (DAPC) plot was created using R package adegenet (Jombart and Collins, 2015). With DAPC, the optimal number of clusters (*K*) was determined by the lowest Bayesian information criterion. A phylogenetic tree was constructed using MEGA7 software (Kumar *et al*., 2016) with the maximum-likelihood method with the following parameters: a test of phylogeny, bootstrap method; no. of bootstrap replications, 1000; model/method, general time reversible model; rates among sites, gamma distributed with invariant sites (G+I). Based on the hierarchically grouped genotyping profiles, a cluster was manually separated into subclades.

Table 1. List of *P. infestans* isolates used in this study

Region(Province)	Location	2009-2017	2019	2020	Total	Note[a]
Gangwon	Gangneung	6	5	8	19	Spring[b]
	Pyeongchang	9	10	5	24	Summer[c]
	Hongcheon	-	3	-	3	Summer
	Jeongseon	4	2	-	6	Summer
Chungnam	Seosan	-	-	2	2	Spring
	Dangjin	-	-	1	1	Spring
Jeonbuk	Muju	-	-	1	1	Spring
	Buan	-	-	2	2	Winter[d]
Jeonnam	Boseong	3	-	1	4	Spring
Gyeongnam	Miryang	6	-	3	9	Winter
	Namwon	5	-	1	6	Winter
Jeju	Jeju	2	-	7	9	Fall[e]
		35	20	31	86	

[a] Cropping season when isolates was collected

[b] March to June

[c] May to October

[d] grown under plastic film house in November to March

[e] August to November

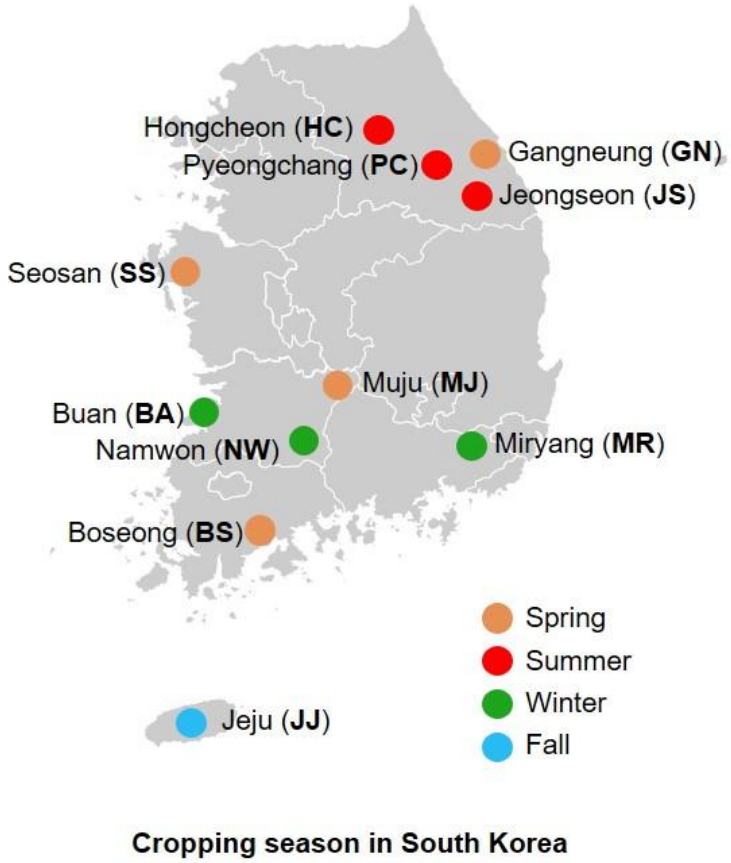

Figure 1. Geographical distribution of *P. infestans* isolates used in this study

Sampling locations which late blight outbreak in potato production areas. *P. infestans* was collected one isolate per village that is small administrative divisions of South Korea. Each color indicates sampling locations according to potato cropping season.

Table 2. Primers used for multiplex PCR in this study

Primer name	Sequence (5'-3')
G11_F	TGCTATTTATCAAGCGTGGG
G11_R	GTTTCAATCTGCAGCCGTAAGA
SSR3_F	ACTTGCAGAACTACCGCCC
SSR3_R	GTTTGACCACTTTCCTCGGTTC
SSR11_F	TTAAGCCACGACATGAGCTG
SSR11_R	GTTTAGACAATTGTTTTGTGGTCGC
D13_F	TGCCCCCTGCTCACTC
D13_R	GCTCGAATTCATTTTACAGACTTG
SSR8_F	AATCTGATCGCAACTGAGGG
SSR8_R	GTTTACAAGATACACACGTCGCTCC
SSR4_F	TCTTGTTCGAGTATGCGACG
SSR4_R	GTTTCACTTCGGGAGAAAGGCTTC
Pi04_F	AGCGGCTTACCGATGG
Pi04_R	GTTTCAGCGGCTGTTTCGAC
Pi70_F	ATGAAAATACGTCAATGCTCG
Pi70_R	CGTTGGATATTTCTATTTCTTCG
PinfSSR6_F	GTTTTGGTGGGGCTGAAGTTTT
PinfSSR6_R	TCGCCACAAGATTTATTCCG
Pi63_F	ATGACGAAGATGAAAGTGAGG
Pi63_R	CGTATTTTCCTGTTTATCTAACACC
PinfSSR2_F	CGACTTCTACATCAACCGGC
PinfSSR2_R	GTTTGCTTGGACTGCGTCTTTAGC
Pi4B_F	AAAATAAAGCCTTTGGTTCA
Pi4B_R	GCAAGCGAGGTTTGTAGATT

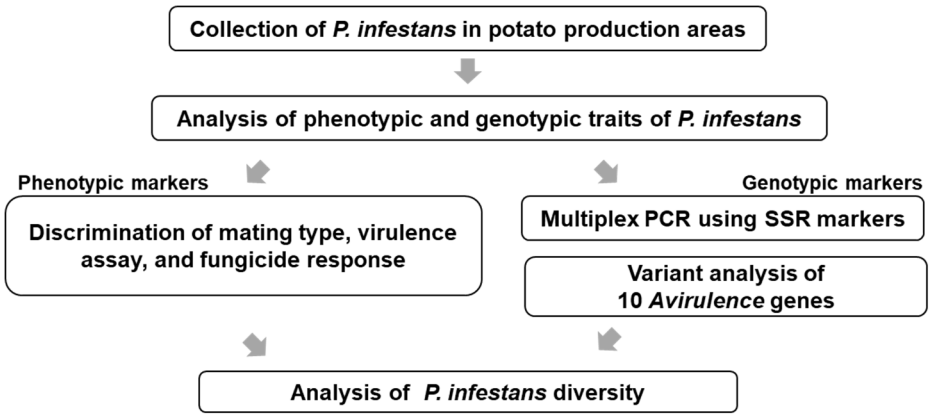

Figure 2. Workflow of this study

RESULTS

The A1 mating-type and dimethomorph-sensitive isolates were mainly distributed in South Korea

In a previous study, mating type and fungicide response were studied to gain insights into the genetic diversity of *P. infestans* populations. I investigated phenotypic characteristics for mating type and sensitivity to the fungicide of metalaxyl and dimethomorph. A total of 86 *P. infestans* isolates were collected during 2009-2020 from six regions and 12 locations. Among these 86 isolates, A1 mating-type individuals were detected in 78 isolates (90%), and A2 mating-type individuals were identified in 8 isolates (10%) (Figure 3A). The A1 mating type was the most common. However, the A2 mating type was found only in Miryung and Buan region, where winter cultivation is mainly carried out (Figure 3B). The metalaxyl response test showed that 20% of the isolates were resistant, 48% were intermediate, and 33% sensitive. The dimethomorph response test indicated that the majority (95%) of isolates were sensitive, while 5% were intermediate (Figure 3C). The isolates identified as A2 mating-type were 60% resistant and 40% intermediate to metalaxyl response(Figure 3D). The PCR test using CAPS markers was used to validate the pairing test for mating-type determination of *P. infestans* (Figure 4). Taken together, these results indicated that *P. infestans* isolates with the characteristics of the A1 mating-type and dimethomorph-sensitive are mainly distributed in

South Korea.

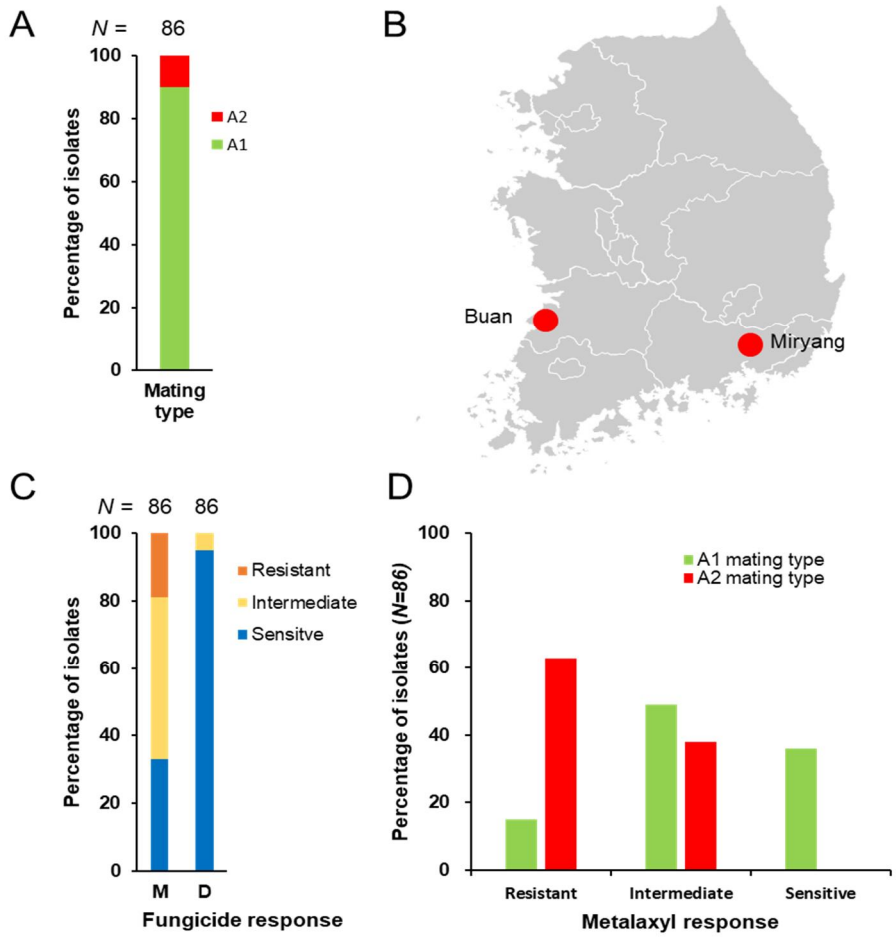

Figure 3. Phenotypic variation of *P. infestans* field isolates.

(A) The percentage of A1 and A2 mating types of total 86 *P. infestans* isolates. (B) The red dot indicates the location where isolates with A2 mating-type were found and where winter potato production under plastic film house is carried out. (C) The percentage of fungicide response. M and D indicates metalaxyl and dimethomorph, respectively. (D) The percentage of A1 and A2 isolates of *P. infestans* classified as resistant, sensitive, or intermediate in response to metalaxyl.

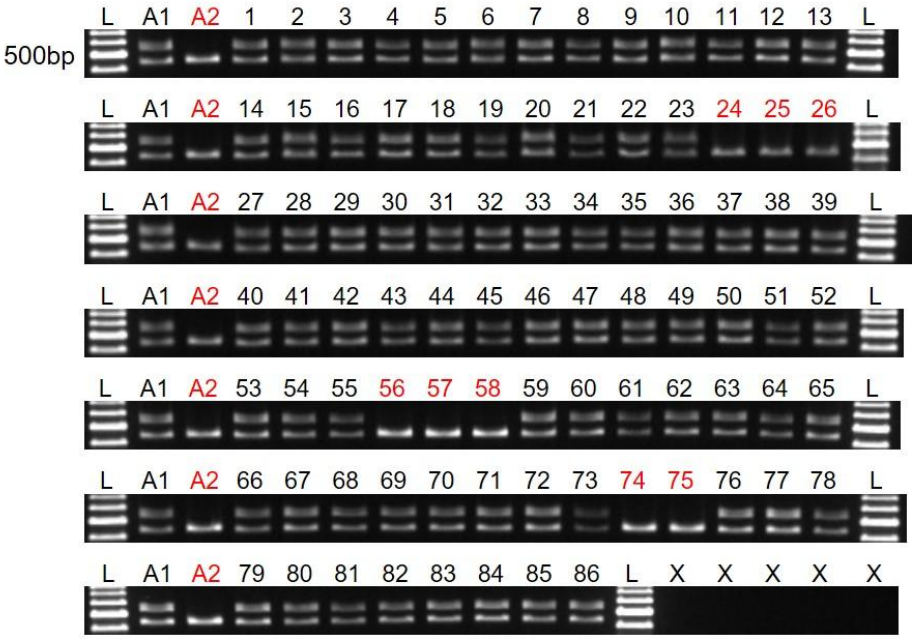

Figure 4. PCR analysis of mating type of *P. infestans* field isolates.

L means 100 base pairs ladder. A1 and A2 are reference isolates for the determination of mating-type. Two fragments (557 bp and 457 bp) are characteristic of the A1 mating type. One fragment (457 bp) is characteristic of the A2 mating type. Number of 1 to 86 indicates samples. 78 of 86 isolates are the A1 mating type. Eight of total 86 isolates are the A2 mating type.

Majority of isolates were virulent against Ma*R2*, Ma*R4,* Ma*R6*, and Ma*R8* potato lines.

P. infestans resistance genes were introduced into potato cultivars from the wild species *Solanum demissum* to confer resistance to *P. infestans* in the cultivated potato *S. tuberosum* around the last of the century. This resulted in the establishment of Ma*R1-R11* differential potato lines, which have 11 distinct recognition specificity. Therefore, differential potato lines have been used worldwide to determine the virulence phenotypes occurring in the pathogen population. To test the virulence of 86 isolates, races were investigated using masternbroek differentials set. The virulence test showed variation among isolates. The mean frequency of virulence against Ma*R2*, Ma*R4*, Ma*R6,* and Ma*R8* during 2009-2020 was 56, 71, 58, and 54%, respectively. Only 11 isolates (2%) overcome the resistance gene Ma*R9* (Figure 5A). Among the 86 isolates, 38 race combination were found. Race combination of Ma*R2*, Ma*R4*, Ma*R6*, and Ma*R8* (25 isolates) was predominant, followed by Ma*R2*, Ma*R4*, and Ma*R6* (12 isolates) and Ma*R4*, Ma*R6*, and Ma*R8* (4 isolates) (Figure 5B). These results suggest that *P. infestans* in South Korea were mostly pathogenic against differential potato lines containing *R2*, *R4*, *R6*, and *R8*.

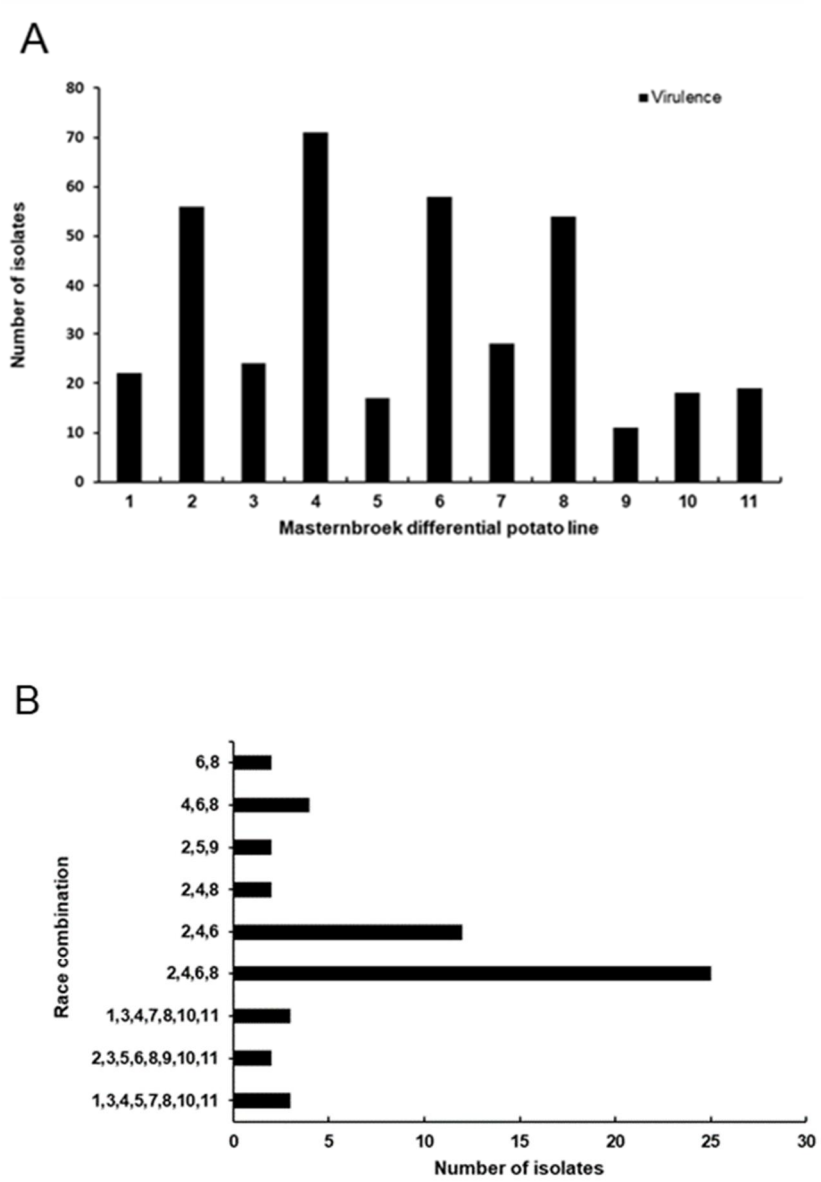

Figure 5. Race structure of *P. infestans* field isolates

(A) The number of isolates that carry virulence factor 1-11 that overcome potato resistance genes *R1-R11*. (B) The number of the most common (≥ 2.0%) races combination of *P. infestans*

Six genotypes occurred in South Korea

To examine genotypic diversity in the *P. infestans* populations used in this study, multiplex PCR was performed using 12 SSRs markers with 86 isolates. SSRs genotyping in this study was compared with a previous study (Choi *et al*., 2020), and the specific genotypes were assigned based on the same or close genotypes. The SSRs genotyping of the 86 isolates of *P. infestans* subject to SSRs analysis were compared to profiles from other lineages and categorized into six major groups (Li *et al*., 2013). As a result of SSRs marker data indicated 51 distinct alleles, with an average of 4.25 alleles per marker ranging from 2 (at loci Pi70 and SSR2) to 13 (at locus G11) according to the analysis. Hexp (Nei, 1978), the anticipated heterozygosity per locus that accounts for genetic richness and evenness, varied from 0.377 for SSR8 to 0.767 for SSR4. An average index of evenness (Grünwald *et al*., 2003) of 0.768 showed that alleles were uniformly distributed, with a high of 0.974 for SSR2 and a low of 0.326 for Pi70. The 86 *P. infestans* isolates could be grouped in 43 multilocus genotypes grouped into six clades, from here on described as clonal genotypes KR_1_A1, KR_2_A2, SIB-1, US-11, SIB-1 like, and KR_2 like (Figure 6A). The proportion of groups were KR_1_A1 (35%), SIB-1 like (45%), KR_2_A2 (3%), KR_2 like (6%), SIB-11 (5%), and US-11 (6%). Lineages other than SIB-1 like and KR_2 like were reported to exist in South Korea before 2016 (Choi *et al*., 2020). Both SIB-1 like and KR_2 like were the first discovered in this study. SIB-1 like and KR_2 like were genetically closed when

considering in terms of a phylogenetic tree. However, All SIB-1 like were A1 mating type like KR_1_A1, and all KR_2 like were A2 mating type like KR_2_A2. The 86 isolates were included in the minimum spanning network (MSN) analysis. Figure 6B depicted dominant MLGs creating bigger nodes that were connected to other nodes of the same color by strong black lines, indicating smaller genetic distances and minimal SSRs changes within a common clonal genotype. The narrower the lines, the greater the genetic distance and the weaker the relationships. In comparison to the US-11 cluster, which showed a higher genetic distance from these two clones, SIB-1 like formed the biggest cluster and showed some similarities with SIB-1. Taken together, six clonal lineages existed in South Korea from 2009 to 2020, and SIB-1 like and KR_2 like were newly identified in 2017 and 2020, respectively.

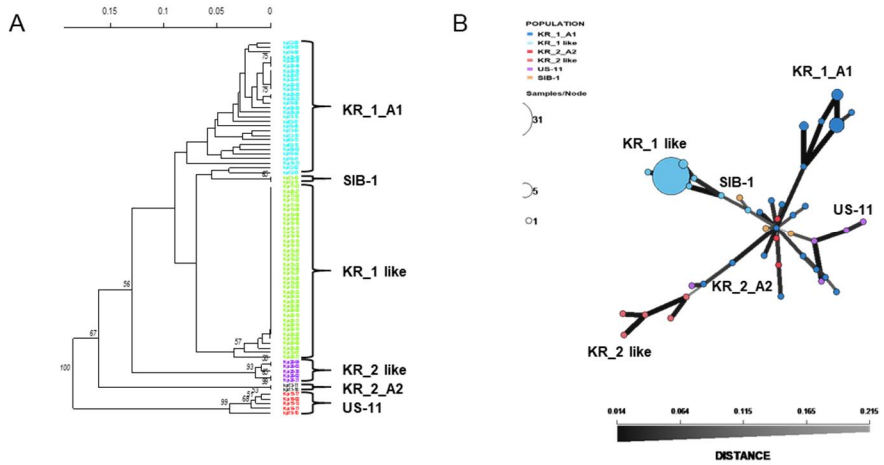

Figure 6. Genotype analysis of *P. infestans* field isolates using SSR markers.

(A) Phylogenetic analysis of the South Korean *P. infestans* population, 2009-2020. UPGMA phylogeny tree inferred from 12 SSRs markers using PAUP v3.0 by MLGs. Bootstrap values >50% are given at the nodes. Genotypes are colored according to the 12 SSRs markers shown in Table 2. (B) Minimum spanning network based on the Bruvo's distance of the 86 isolates using SSRs genotyping data. Nodes represent each multilocus genotype, with the center of each circle. The thicker and darker lines linking nodes with the shortest genetic distance are displayed.

Genome resequencing and SNPs calling to understand the genetic basis of *Avr* genes

To understand the genetic variation of *P. infestans* population, genome re-sequencing was performed. A total of 650.53 Gb pair-end data were generated with sequencing depth (X) of 21.8 to 42.4. An average of 7.5 Gb sequence data per sample (31.4 X) was obtained. After trimming low-quality reads, about 541.59 Gb high-quality reads were mapped to the KR_1_A1 reference genome. The average mapping depth was 28.6 X (Table 3). A total of 3,308,187 SNPs were initially obtained. After filtering, 1,673,046 SNPs remained for further analysis. The largest number of SNPs was found on US-11 group (776,843 SNPs), followed by KR_2 like (717,710 SNPs), and whereas the smallest number of SNPs was found in SIB-1 (425,694 SNPs). Analysis of the functional effects of SNPs revealed a total of 88,471 synonymous and 106,316 non-synonymous polymorphisms.

Table 3. Statistics of *P. infestans* genome sequencing used in this study.

Isolate name	Raw data		Trimmed data [a]		Coverage (X)[b]
	Reads	Bases	Reads	Bases	%
Kpi09-03	40.39 Mb [c]	6.01 Gb [d]	35.39 Mb	5.01 Gb	24.97
Kpi09-05	39.96 Mb	6.03 Gb	35.75 Mb	5.11 Gb	25.44
Kpi09-07	40.49 Mb	6.11 Gb	35.47 Mb	5.02 Gb	25.01
Kpi09-18	40.26 Mb	6.08 Gb	35.36 Mb	5.01 Gb	24.96
Kpi09-79	42.16 Mb	6.37 Gb	36.73 Mb	5.19 Gb	25.86
Kpi09-81	45.12 Mb	6.81 Gb	39.88 Mb	5.67 Gb	28.25
Kpi09-90	44.69 Mb	6.75 Gb	39.14 Mb	5.53 Gb	27.55
Kpi10-19	50.06 Mb	7.56 Gb	44.91 Mb	6.41 Gb	31.97
Kpi10-40	43.68 Mb	6.60 Gb	38.70 Mb	5.51 Gb	27.46
Kpi10-41	49.93 Mb	7.54 Gb	44.07 Mb	6.26 Gb	31.19
Kpi10-44	37.78 Mb	5.71 Gb	32.96 Mb	4.66 Gb	23.24
Kpi10-47	40.78 Mb	6.16 Gb	35.86 Mb	5.09 Gb	25.35
Kpi11-18	39.76 Mb	6.00 Gb	34.55 Mb	4.87 Gb	24.26
Kpi12-01	34.10 Mb	5.15 Gb	30.59 Mb	4.38 Gb	21.83
Kpi12-09	37.74 Mb	5.70 Gb	33.60 Mb	4.79 Gb	23.89
Kpi12-18	42.14 Mb	6.36 Gb	37.41 Mb	5.33 Gb	26.57
Kpi12-82	45.41 Mb	6.86 Gb	40.59 Mb	5.79 Gb	28.87
Kpi12-83	41.60 Mb	6.28 Gb	36.88 Mb	5.24 Gb	26.12
Kpi13-10	44.19 Mb	6.67 Gb	38.81 Mb	5.49 Gb	27.38
Kpi13-11	40.82 Mb	6.16 Gb	35.98 Mb	5.12 Gb	25.50
Kpi13-43	41.93 Mb	6.34 Gb	36.48 Mb	5.16 Gb	25.71
Kpi13-83	44.56 Mb	6.73 Gb	39.72 Mb	5.64 Gb	28.22
Kpi14-11	47.19 Mb	7.13 Gb	41.45 Mb	5.87 Gb	29.23
Kpi14-13	41.21 Mb	6.22 Gb	36.40 Mb	5.17 Gb	25.77
Kpi15-10	42.63 Mb	6.44 Gb	37.49 Mb	5.32 Gb	26.53
Kpi15-13	43.15 Mb	6.52 Gb	38.36 Mb	5.47 Gb	27.27
Kpi15-17	51.33 Mb	7.75 Gb	46.14 Mb	6.61 Gb	32.93
Kpi15-18	43.53 Mb	6.57 Gb	38.67 Mb	5.51 Gb	27.45
Kpi16-02	38.63 Mb	5.83 Gb	33.92 Mb	4.78 Gb	23.85
Kpi16-06	43.15 Mb	6.52 Gb	37.89 Mb	5.35 Gb	26.70

Kpi16-08	42.26 Mb	6.38 Gb	37.16 Mb	5.27 Gb	26.29
Kpi17-01	42.24 Mb	6.38 Gb	37.36 Mb	5.31 Gb	26.51
Kpi17-02	41.23 Mb	6.23 Gb	36.59 Mb	5.20 Gb	25.94
Kpi17-03	40.76 Mb	6.15 Gb	35.49 Mb	5.01 Gb	24.96
Kpi17-04	42.61 Mb	6.43 Gb	37.60 Mb	5.33 Gb	26.57
Kpi19-05	52.44 Mb	7.92 Gb	48.73 Mb	7.12 Gb	35.47
Kpi19-06	57.01 Mb	8.61 Gb	49.91 Mb	7.21 Gb	35.95
Kpi19-07	62.56 Mb	9.45 Gb	55.60 Mb	8.06 Gb	40.15
Kpi19-08	60.26 Mb	9.10 Gb	53.10 Mb	7.69 Gb	38.31
Kpi19-09	58.92 Mb	8.90 Gb	51.63 Mb	7.46 Gb	37.17
Kpi19-10	56.10 Mb	8.47 Gb	49.50 Mb	7.17 Gb	35.75
Kpi19-11	62.82 Mb	9.49 Gb	55.41 Mb	8.03 Gb	40.00
Kpi19-12	62.96 Mb	9.51 Gb	55.75 Mb	8.08 Gb	40.26
Kpi19-16	63.89 Mb	9.65 Gb	56.86 Mb	8.25 Gb	41.13
Kpi19-18	60.24 Mb	9.10 Gb	53.12 Mb	7.69 Gb	38.31
Kpi19-19	65.83 Mb	9.94 Gb	58.67 Mb	8.51 Gb	42.42
Kpi19-20	61.77 Mb	9.33 Gb	54.44 Mb	7.89 Gb	39.31
Kpi19-21	60.38 Mb	9.12 Gb	53.84 Mb	7.82 Gb	38.96
Kpi19-22	59.38 Mb	8.97 Gb	51.86 Mb	7.50 Gb	37.38
Kpi19-23	60.51 Mb	9.14 Gb	53.42 Mb	7.74 Gb	38.57
Kpi19-26	55.64 Mb	8.40 Gb	51.43 Mb	7.51 Gb	37.43
Kpi19-27	53.52 Mb	8.08 Gb	49.80 Mb	7.27 Gb	36.25
Kpi19-28	60.96 Mb	9.20 Gb	55.81 Mb	8.13 Gb	40.53
Kpi19-29	54.22 Mb	8.19 Gb	49.79 Mb	7.26 Gb	36.20
Kpi19-30	55.42 Mb	8.37 Gb	50.97 Mb	7.44 Gb	37.06
Kpi20-02	50.86 Mb	7.68 Gb	44.49 Mb	6.43 Gb	32.06
Kpi20-03	58.16 Mb	8.78 Gb	49.17 Mb	7.09 Gb	35.31
Kpi20-04	41.97 Mb	6.34 Gb	37.05 Mb	5.36 Gb	26.72
Kpi20-07	57.35 Mb	8.66 Gb	48.91 Mb	7.06 Gb	35.20
Kpi20-08	53.89 Mb	8.14 Gb	44.84 Mb	6.42 Gb	32.00
Kpi20-09	56.41 Mb	8.52 Gb	47.31 Mb	6.80 Gb	33.89
Kpi20-10	56.96 Mb	8.60 Gb	49.10 Mb	7.09 Gb	35.33
Kpi20-11	57.89 Mb	8.74 Gb	48.65 Mb	7.01 Gb	34.91

Kpi20-13	57.35 Mb	8.66 Gb	48.00 Mb	6.90 Gb	34.39
Kpi20-14	56.53 Mb	8.54 Gb	48.76 Mb	7.06 Gb	35.18
Kpi20-19	41.63 Mb	6.29 Gb	36.81 Mb	5.32 Gb	26.49
Kpi20-20	54.89 Mb	8.24 Gb	44.26 Mb	6.41 Gb	31.94
Kpi20-23	55.04 Mb	8.31 Gb	48.01 Mb	6.95 Gb	34.65
Kpi20-24	55.44 Mb	8.07 Gb	46.45 Mb	6.73 Gb	33.52
Kpi20-25	54.60 Mb	8.24 Gb	47.49 Mb	6.90 Gb	34.38
Kpi20-27	51.17 Mb	7.73 Gb	44.48 Mb	6.45 Gb	32.14
Kpi20-28	57.41 Mb	8.67 Gb	49.52 Mb	7.14 Gb	35.58
Kpi20-30	54.51 Mb	8.23 Gb	46.38 Mb	6.69 Gb	33.36
Kpi20-31	57.46 Mb	8.68 Gb	50.33 Mb	7.29 Gb	36.35
Kpi20-33	49.54 Mb	7.48 Gb	43.44 Mb	6.28 Gb	31.32
Kpi20-34	45.25 Mb	6.83 Gb	40.35 Mb	5.88 Gb	29.30
Kpi20-35	44.68 Mb	6.75 Gb	39.66 Mb	5.77 Gb	28.74
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Kpi20-39	55.67 Mb	8.41 Gb	44.63 Mb	6.42 Gb	31.98
Kpi20-41	43.87 Mb	6.62 Gb	36.84 Mb	5.26 Gb	26.21
Kpi20-42	57.15 Mb	8.63 Gb	45.70 Mb	6.57 Gb	32.76
Kpi20-43	55.07 Mb	8.32 Gb	47.21 Mb	6.83 Gb	34.03
Kpi20-45	56.52 Mb	8.53 Gb	48.90 Mb	7.07 Gb	35.25
Kpi20-46	40.02 Mb	6.04 Gb	35.03 Mb	5.06 Gb	25.22

[a]Number of total trimmed reads. [b]Trimmed data bases / reference genome size.

[c]Maga (1,000,000) base [d]Giga (1,000,000,000) base

Three subgroups were divided by SNPs of *Avr* genes

To explore the variation of *Avr* genes in *P. infestans* isolates, SNPs of 10 effector genes were investigated. 10 effectors were *Avr*1, *Avr*2, *Avr*3a, *Avr*4, *Avr*10, *Avrblb*1, *Avrblb*2, *AvrSmira*1, *AvrSmira*2, and PexRD24. I found 18 putative *Avr* genes in KR_1_A1 genome and detected 372 SNPs of *Avr* gene regions among 86 isolates. To figure out how populations were organized, the STRUCTURE program was used, and the deviance information criterion (DIC) value showed the maximum value when the delta *K* was 3, so the group was classified using *K*=3 as the optimal cluster model. These results suggested that the Korean *P. infestans* genotypes should be categorized into subgroup 1 (40 isolates), subgroup 2 (10 isolates), and subgroup 3 (36 isolates) (Figure 7A). To further verify the grouping observed in the population structure, DAPC was performed on SNPs and showed a clear pattern of grouping according to the field of origin (Figure 7B), suggesting a strong population structure. Subgroup 1 comprised SIB-1 like and 2 KR_2 genotypes while subgroup 3 contained KR_1_A1 and SIB-1 genotypes. Subgroup 2 included KR_2 like and US-11. As a result, genetic population analysis based on SNPs and clonal lineage from SSRs genotyping were enable an understanding of population dynamics which has been useful for disease management.

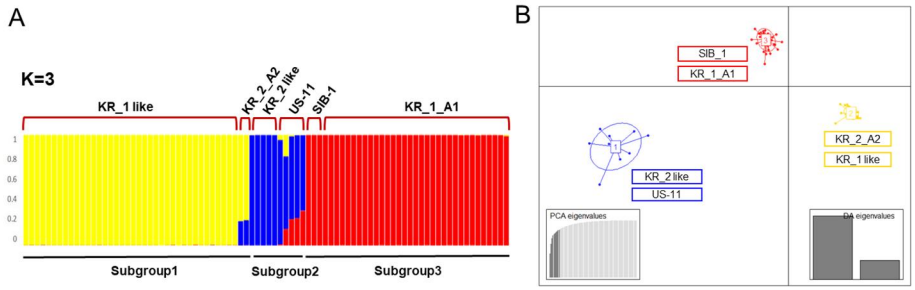

Figure 7. STRUCTURE analysis and DAPC using 372 SNPs of 86 *P. infestans* isolates.

(A) STRUCTURE results in three groups. Each color represents a subpopulation based on STRUCTURE results. The horizontal coordinate of each subgroup represents the clonal lineage. (B) Scatterplots represent the distribution of individuals (dots) and fields (colors) as inertia ellipses. The insets indicate the eigenvalues of the DAPC analysis, with dark bars representing axis 1 and 2 of the plots.

KR_2 like was A2 mating type and resistant to metalaxyl

To investigate the evolutionary diversity of *P. infestans* isolates, a phylogenetic analysis was performed. A phylogenetic tree comprising 86 *P. infestans* genotypes was constructed based on 372 SNPs of *Avirulence* genes. These 86 isolates were divided into two clusters. The first cluster comprised SIB-1 like, KR_2_A2, KR_2 like, and US-11. The second cluster comprised SIB-1 and KR_1_A1. In the first cluster, isolates of KR_2 like were diverse but strongly related to each other. Next, the results obtained by analyzing the phenotype and genotype were integrated based on the phylogenetic tree. In subgroup 1 (SIB-1 like and KR_2_A2), isolates with resistance or intermediate-resistance to metalaxyl were distributed. The group with KR_2 like and US-11 had more isolates resistant to metalaxyl than subgroup 1. Although KR_2 like was composed of A2 mating type, it can be seen that it was distinguished from KR_2_A2, which has A2 mating type known to be in South Korea. Also, it was found that KR_2 like had more variation than other isolates. Thus, it was clearly distinguished on a phylogenetic tree. It was known that the isolate with the A2 mating-type is mainly resistant to metalaxyl. From this data, it was identified that isolates with the A2 mating-type showed resistance to metalaxyl, and the *Avirulence* gene variation was markedly found on the KR_2 like genotype.

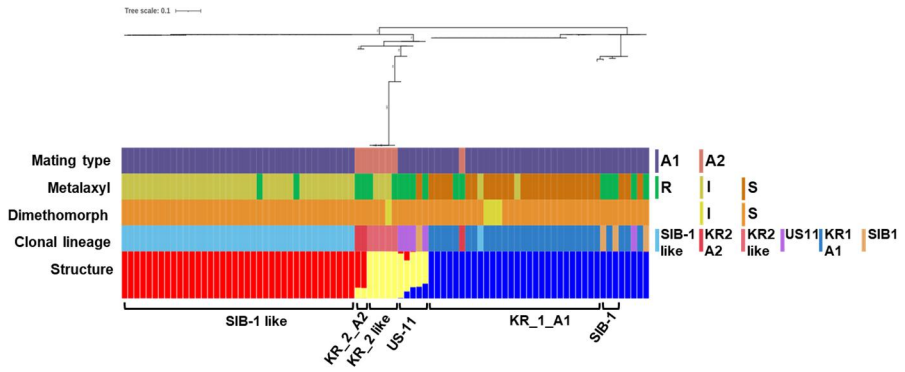

Figure 8. Phylogenetic analysis and summary of phenotypic and genotypic patterns of *P. infestans* field isolates.

Phylogenetic tree based on neighbor-joining was constructed based on 372 SNPs in *Avirulence* genes. Bootstrap analysis with 1,000 replicates were performed. Clusters determined using mating type, metalaxyl response, dimethomorph response, clonal linege, and structure are shown as colored chart; same colors indicates the members of the same clusters. A1 and A2 indicates mating type. R, I, and S indicates the response of resistant, intermediate, and sensitive to metalaxyl or dimethomorph.

Genetic polymorphisms were observed within the certain clonal lineage

Through structural analysis, it was confirmed that *P. infestans* isolates were divided into three subgroups based on 372 SNPs of the *Avirulence* gene. To understand how the SNPs of the *Avirulence* gene are consisted, the heatmap method was used to visualize diversity and population structure within each of the 6 clonal lineages. 10 putative *Avirulence* gene regions found in the reference genome. More specifically, non-synonymous mutations affecting protein expression were investigated. For the individual *Avirulence* genes, the different genotype profiles were observed; the *Avr*3a and *Avr*2 gene displayed homozygous genotype, whereas the *AvrSmira*1, *Avr*1, and *Avr*10 showed heterozygous genotype. Furthermore, the *Avr*8 gene displayed different genotype profiles from the rest of the *Avr* genes. For *Avr*8, SIB-1 and US-11 showed similar heterozygous profiles while the other isolates showed homozygous profiles. KR_2 like showed heterozygous profiles in *Avr3a.* Whereas the other isolates showed the homozygous profiles in *Avr3a*. Taken together, suggested that the KR_1_A1 and SIB-1 like isolates quite similar in the *Avr*8. In addition, SNPs mutation of *Avr3a* in KR_2 like was found to be differentiated from other isolates.

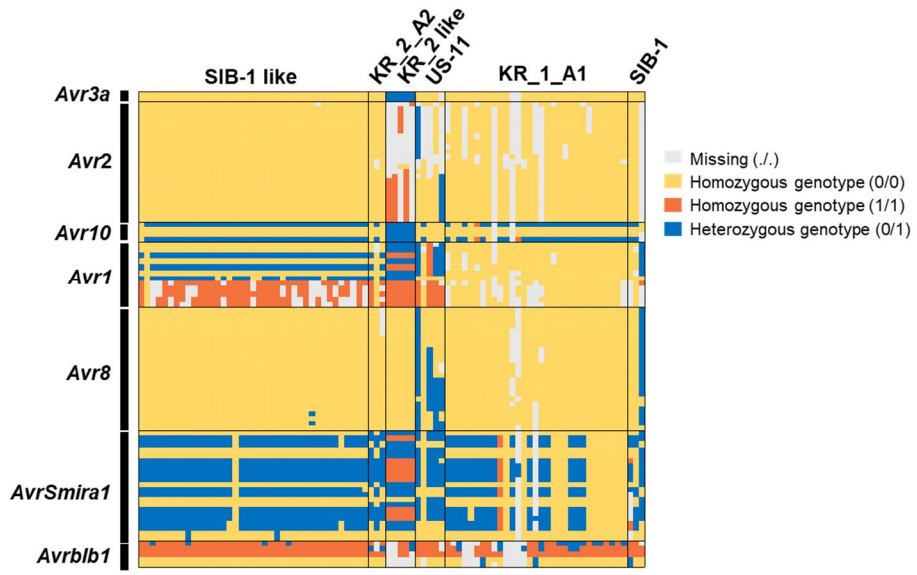

Figure 9. Heatmap based on non-synonymous SNPs of *Avirulecne* gene.

The color indicates the genotype of non-synonymous SNPs in the *Avirulence* genes. The X-axis indicates the clonal lineage of *P. infestans*. The Y-axis indicates variant loci. Bold lines indicate the range of SNPs position in *Avirulence* genes.

DISCUSSION

Potato late blight caused by *P. infestans*, is an important plant pathogen in the potato industry. The sustainable management strategies of potato late blight disease are clearly based on an understanding of the pathogen and its population dynamics. Therefore, the study of genetic diversity in *P. infestans* has allowed for a better understanding of population dynamics and effective disease management. In this study, the diversity of phenotypic and genotypic characteristics was investigated in 86 isolates collected in South Korea.

The A1 mating type was predominant (90%) among Korean isolates tested in this study. In contrast, A2 mating type was found in a limited number (10%), years (2011, 2013, and 2020), and location. All isolates with A2 mating-type were resistant or intermediate to metalaxyl. Choi *et al*., 2020 reported that the *P. infestans* A2 mating-type was found in Miryang locations in 2011, 2013, and 2015. In my study, isolates of A2 mating-type were also found in Miryang in 2020 and were first identified in Buan in 2020. In Miryang and Buan, potatoes are cultivated in the plastic-film houses during the winter season when late blight was more outbreak than in other locations due to high humidity and low temperatures (Kim *et al*., 2014). Because of the frequent outbreaks, chemical control methods, such as the usage of metalaxyl, may have created

enough diverse selection pressure to result in a slight increase in genetic diversity. However, in contrast to metalaxyl responses, it was found that 95% of isolates were sensitive to dimethomorph regardless of the mating type. This implies that isolates with A2 mating-type in South Korea were expected to occur mainly in locations where winter film-house cultivation is performed. It suggested that there is a possibility that the A2 mating type will appear steadily in the Buan location as in the Miryang location.

In the virulence test, *P. infestans* was predominantly pathogenic against Ma*R2,* Ma*R4,* Ma*R6*, and Ma*R8* differential potato lines. In addition, 38 race combinations were detected. In more detail, the most common the race combination identified in the isolate population was race combination of Ma*R2*, Ma*R4*, Ma*R6*, and Ma*R8* (25 isolates), followed by Ma*R2*, Ma*R4*, and Ma*R6* (12 isolates), and Ma*R4*, Ma*R6*, and Ma*R8* (4 isolates). This suggests that the potato variety with *R2, R4, R6*, and *R8* genes might be susceptible to potato late blight. For example, potato varieties carrying the *R2* gene may be attacked by *P. infestans* race 2 or any of a number of related race structures (e.g., 2.4, 2.6, and 2.4.6.8), but they cannot be attacked by races 1, 1.3, 1.4, and so on. According to the report of Kim *et al*., 2007, a total of 261 isolates collected in South Korea from 2003 to 2005 represented the races with 18 complex virulence spectra, and there were no isolates that could virulence to Ma*R8* and Ma*R9*. The results suggest that the race structure of Korean *P. infestans* was more diverse and that isolates that are virulent to Ma*R8* and Ma*R9* have

emerged. Virulence phenotype has proved less effective than other markers in *P. infestans* population characterization because the study has shown that some the differential lines actually possess combinations of *R* genes. For example, the *R3a* and *R3b* genes were present in the Ma*R3* differential (Huang *et al*., 2004). Thus, a differential set of genetically modified plants harboring single late blight *R* genes was developed (Zhu *et al*., 2015). But, due to the GMO issue, the domestic introduction is not permitted. Therefore, virulence determination is critical for breeders and plant pathologists who want to use race-specific *R*-genes in breeding programs and management strategies.

Genotyping using SSRs markers to genotype 86 isolates collected from 2009 to 2020 was performed to investigate the South Korean *P. infestans* population. Four clonal lineages including KR_1_A1, KR_2_A2, SIB-1, and US-11 were identified in a previous study (Choi *et al*., 2020). In this study, two more clonal lineages of SIB-1 like and KR_2 like were identified. SIB-1 like and SIB-1 had a common ancestor (Figure 6A). This suggested that SIB-1 like could possibly be similar to SIB-1. In addition, PCA analysis based on whole SNPs showed SIB-1 and SIB-1 like were grouped together. The SIB-1 genotype was firstly found in Siberia, and suggesting later migration of this genotype from either Russia or Japan into China (Guha Roy *et al*., 2021). There was some polymorphism for metalaxyl resistance in the SIB-1 clonal lineage. The SIB-1 genotypes from Sakhalin Island were all resistant to metalaxyl, but the SIB-1 genotypes from Irkutsk were sensitive (Elansky *et al*., 2001). Among isolates

collected in South Korea, SIB-1 had a chracteristics of metalaxyl resistance. However, SIB-1 like genotypes were sensitive to metalaxyl (Figure 8). This implies that SIB-1 like undefined clonal lineage may be a SIB-1 genotype. Further studies are needed with reference isolates. If SIB-1 like is not SIB-1 and other lineages, then it can be suspected that movement of the pathogen into South Korea via infected seed tubers from other counties (Choi *et al*., 2020). Occurrence of KR_2 like was clearly identified with well-supported phylogenetic analysis. Among the Korean *P. infestans*, genotypes of KR_2_A2 and KR_2 like existed with the A2 mating-type. *P. infestans* isolates with A2 mating-type were rarely found in Miryang until 2015, but have not been found since then. However, in 2020, A2 mating-type isolates were found in Miryang and Buan, which were different from the KR_2_A2 based on. This suggested that a novel clonal lineage with A2 mating-type appeared. Additionally, isolates collected in both Miryang and Buan were KR_2 like. It may be due to the migration of infected potato tuber between the two locations.

There were several nucleotide polymorphisms in effector genes in Korean *P. infestans*. However, it is not certain whether this effector gene is an *Avirulence* gene that interacts with the R gene or not. This is because the functional analysis of the candidate effector gene found in the KR_1_A1 reference genome was not performed. Therefore, to clarify whether the untested effectors might be putative *Avr* genes, transient assays such as agroinfiltration and agroinfection fulfill the demand for efficient functional analysis. These

methods facilitate the delivery of several transgenes into the same cell with simultaneous expression of interacting proteins. This is effective to go over R-AVR interactions associated with a specific host and pathogen interactions by co-infiltrating the agent of *Agrobacterium tumefaciens* strains that express *Avr* genes with strains expressing the corresponding *R* genes. Expression of *Avr* genes in plant cells carrying *R* genes generally resulted in the hypersensitive cell death response (Vleeshouwers *et al*., 2014). Diversity study of *P. infestans* that consider both phenotypic and genotypic traits have enabled an understanding of population dynamics which has been useful for disease management and potato breeding. However, lack of knowledge about *P. infestans* jeopardizes an apt response to resistance breakdown. The characteristic of *P. infestans* in South Korea could provide insight to the understanding of distribution of *P. infestans* and control.

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ABSTRACT IN KOREAN

감자는 전세계적으로 중요한 식량작물 중 하나이다. 감자 역병균(*Phytophthora infestans)*에 의해 발병하는 감자 역병은 감자 생산에 가장 심각한 피해를 끼치는 병이다. 감자 역병에 대한 효율적인 방제 전략을 세우기 위해서는 *P. infestans* 집단에 대한 이해가 필요하다. *P. infestans* 의 특성에 대해 분석하기 위해 다양한 유전적 및 표현적 마커들이 개발되어 왔다. 최근에는 더 정밀한 병 관리를 위해서 유전적 마커를 이용한 *P. infestans* 의 특징들을 분석하는 연구들이 많이 수행되고 있다. 하지만 한국에서는 유전형 마커를 이용한 감자 역병균에 대한 연구는 부족한 실정이다. 본 연구에서는 한국 전 지역에서 최근 3 년간 수집한 감자 역병균 86 점에 대해 표현형 마커(교배형, 병원성, 약제 반응)와 유전형 마커(SSRs, 차세대염기서열분석)를 이용하여 특징을 분석하였다. 표현적 특징들로는 교배형 A1 와 dimethomorph 에 감수성을 가진 균들이 90% 정도 분포하였다. 또한, R2, R4, R6, R8 을 가지고 있는 감자 판별 품종에 대해 병원성을 가진 균주들이 약 50%정도 분포하였다. 이를 통해, 병 방제에 dimethomorph 약제를 사용하는 것이 효과적일 수 있음을 시사한다. 유전적 분석을 통해 한국 전역에 6 개의 계통이 분포함을 확인하였으며 이들 중 SIB-1 과 KR_2 like 유전형은 한국에서 새롭게 확인되었다. NGS 데이터를 기반으로 한 SNPs 분석에서는 KR_2 like 가 계통수적으로 다른 그룹과 확연히 구분되었다. 더

욱이, 전체 유전체와 비병원성 유전자의 SNPs 변이가 다른 균주에 비해 더 많이 존재함을 확인했다. 이것은 KR_2 like 가 국외에서 한국으로 이동하였거나 KR_2 like 의 유전적 변이가 빠르게 일어날 수 있음을 나타낸다. 이러한 결과들을 종합했을 때, 본 연구에서 수행한 *P. infestans* 의 특성 조사는 한국의 감자 역병균의 효율적인 방제를 위한 방법 개발뿐만 아니라 저항성 감자 품종을 개발하는데 유용하게 사용될 것이다.

주요어: 감자 역병, *Phytophthora infestans*, 유전형, 표현형

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