

## Assessment of the resistance of bacterial soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* KACC 21701 in Kimchi cabbage genetic resources

Parthiban Subramanian, Ho Chul Ko, Seong-Hoon Kim, Jae Eun Lee, Aejin Hwang, Bichsaem Kim, Yoon-Jung Lee, Awraris Derbie Assefa, Onsook Hur, Nayoung Ro, Jung Sook Sung, Ju Hee Rhee, Ho-Sun Lee<sup>1</sup> and Bum-Soo Hahn\*

National Agrobiodiversity Center, National Institute of Agricultural Sciences, Rural Development Administration, Jeonju 54874, Republic of Korea

<sup>1</sup>International Technology Cooperation Center, Rural Development Administration, Jeonju 54875, Republic of Korea

### \*Corresponding author

Bum-Soo Hahn  
Tel. 063-238-4930  
E-mail. bshahn@korea.kr

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**Abstract:** Bacterial phytopathogen *Pectobacterium* causes soft rot disease in several vegetable crops globally, resulting in heavy agricultural losses at both the pre and post-harvest stages. The present work was carried out to screen Kimchi cabbage genetic resources conserved at the National Agrobiodiversity Center, Rural Development Administration, Korea, for resistance against the soft rot pathogen *Pectobacterium carotovorum* subsp. *carotovorum* KACC 21701 over a period of three years (from 2020 to 2022). Infection of the phytopathogen was carried out at four-leaf stage and for each accession, twenty-five plants per germplasm were infected with KACC 21701. Kimchi cabbage cultivars Wangmatbaechu, Seoulbaechu, and CR Kiyoshi were used as control. Seven-days post-infection, the Disease Index (DI) values were manually recorded from zero to four, zero matched perfectly healthy plants and four completely dead plants. The 682 accessions of Kimchi cabbage exhibited varying degrees of disease resistance to KACC 21701 and thirty accessions, exhibiting a  $DI \leq 2$ , were considered for replication studies. During the replication studies, four landrace germplasms (IT102883, IT120036, IT120044, and IT120048) and one cultivar (IT187919) were confirmed to be moderately susceptible to KACC 21701. Results of the preliminary screening as well as replication studies were documented for the all the 682 germplasms. Addition of such information to the passport data of stored germplasms might serve as potential bio-resource for future breeders and researchers to develop resistant varieties or study the mechanisms involved in resistance of plants to such phytopathogen.

**Keywords:** soft rot, *Pectobacterium carotovorum*, genetic resource, Kimchi cabbage, screening

## INTRODUCTION

Agricultural productivity faces a multitude of challenges among which, pests and pathogens are major factors that can significantly affect both quality and yield of

crops during growth as well post-harvest, making them significant elements in modern day agriculture (Savary *et al.* 2019). While pests are eukaryotic organisms such as insects and worms, plant pathogens are usually microorganisms which could be fungi, bacteria or viruses. Among

them, bacterial pathogens can cause several plant-detrimental diseases, including spots, abscesses and rotting of several parts of the plant leading to plant death. The pectobacteria causing bacterial soft rot are phytopathogens with a broad host range and affect several economically important crop plants. The major pectobacteria that cause soft rot belong to genera *Pectobacterium* and *Dickeya*, and are well documented to cause necrotrophic rotting of roots (Davidsson *et al.* 2013).

The bacterium *P. carotovorum* causing soft rot has been considered as one of the top ten plant pathogenic bacteria due to huge economic losses resulting from their infection (Mansfield *et al.* 2012). It was originally classified as *Erwinia carotovora* and was known to be geographically globally distributed (Mansfield *et al.* 2012). The recognized hosts of the pathogen include Kimchi cabbage, cabbage, radish, carrot, potato, and lettuce, etc., which are widely cultivated across China, Japan, and South Korea (Leite *et al.* 2014; Kim *et al.* 2016; Meng *et al.* 2017; Jeon *et al.* 2018; Liu *et al.* 2019a; Afroz *et al.* 2021). The phytopathogen *P. carotovorum* subsp. *carotovorum* causes soft rot in plants by degrading plant tissues. Mutagenic approaches to understand its pathogenicity have confirmed the role of its extracellular plant cell wall-degrading enzymes (PCWDE, exoenzymes) including pectate lyases (Pel), polygalacturonases (PGs), proteases (Prt), and cellulases (Cel) (Lee *et al.* 2013). The pathogen primarily causes damage of the cell membrane and thereby leading to leakage of electrolytes, extensive tissue maceration, rotting, and subsequent plant death (Davidsson *et al.* 2013). This ultimately results in losses in marketable yield in the field and also during transit, and storage.

The plant family Brassicaceae is one of the largest plant families that naturally inhabit several parts of Asia, especially Korea (Kim *et al.* 2019; Lee *et al.* 2020). Members of this family are economically important and their production often affected by diseases and pests leading to huge agricultural losses (Ko *et al.* 2021a, 2021b). Among them, Kimchi cabbage (*Brassica rapa* subsp. *pekinensis*) is a plant that have been historically used in the culinary processes in parts of Asia and are widely grown and consumed in Korea, China, and Taiwan (Lee *et al.* 2020). The plants occur both as annual and biennial forms as headed or loose-headed types and are economically important crops (Fordham and Hadley 2003). The current study was aimed at screening of the Kimchi cabbage genetic resources collected and stored at the National Agrobiodiversity Center, Rural development Administration, Korea to identify any resistant accessions

against soft rot causing *P. carotovorum* subsp. *carotovorum* for their characterization which can serve as a potential resource for future studies aimed at understanding the plant immune responses and gaps in the available knowledge in molecular basis of disease resistance.

## MATERIALS AND METHODS

The germplasms for screening against bacterial soft rot were obtained from National Agrobiodiversity Center, RDA Genebank. 682 accessions were screened over a period of 3 years from 2020 to 2022. The strain *Pectobacterium carotovorum* subsp. *carotovorum* (KACC 21701) was obtained from Korean Agricultural Culture Collection (KACC) and used for infection of the Kimchi cabbage germplasms.

Twenty-five healthy Kimchi cabbage seedlings at the four-leaf stage were used during experiments. For inoculation of 14 days old seedlings, a fresh batch of the strain *P. carotovorum* subsp. *carotovorum* KACC 21701 was cultured on tryptic soy agar (TSA) at 28°C followed by transfer to tryptic soy broth where it was grown for 24 h at 150 RPM, 28°C. Cells were then centrifuged at 4,000 rpm for 10 mins and the harvested cells were adjusted for a final O.D. of 0.1. For infecting the seedlings, the bacterial suspension was mixed with mineral oil at a ratio of 4 : 1 and added on the surface of the soil (Chung *et al.* 2003). The cultivars including Wangmatbaechu, Seoulbaechu and CR-Kiyoshi were used as control. The infected seedlings were then transferred to plant growth chamber maintained at 30°C, 90% relative humidity (RH), no light for one day after which the conditions were changed to 30°C, 80% relative humidity and 12 h light/dark cycle for six more days. The disease index (DI) of at least ten infected plants was examined and recorded at the end of seven days post infection. The values of disease index were determined according to the severity of the disease and are explained in Figure 1. Seedlings with DI = 0 were considered highly resistant, 0.1 to 1.0 to be resistant, 1.1 to 2.0 moderately resistant, 2.1 to 3.0 to be moderately susceptible 3.1–4.0 were considered susceptible.

For the replication experiments of the resistance to KACC 21701, the primary accessions during initial screening with a DI value of less than or equal to two were selected and the experiments repeated. The replication experiments were repeated twice independently to find any consistency to primary screening data. The results obtained



**Fig. 1.** Visual reference for recording disease index (DI) values after infection of Kimchi cabbage germplasms with soft rot causing *Pectobacterium carotovorum* subsp. *carotovorum* KACC 21701.

**Table 1.** Year wide status of Kimchi cabbage germplasms considered after screening

Kimchi cabbage status	2020	2021	2022
Landrace	253	165	0
Cultivar	0	29	104
Unassigned	0	18	113

from initial screening and replication testes were analyzed using Microsoft Excel (2016) and SigmaPlot ver. 15.0.

## RESULTS AND DISCUSSION

Six hundred and eighty-two Kimchi cabbage germplasms were selected between 2020 and 2022 (253, 212, and 217 accessions, respectively) for screening of resistance against soft rot caused by *P. carotovorum* subsp. *carotovorum*. Disease index was recorded for a minimum of at least 10 seedlings in all the 682 germplasms screened (Table 1). These studied germplasms included landraces and cultivars as well as unassigned germplasms with origins from Asia, Australia, Americas, Russia, Europe and Africa (Table 1; Fig. 2). Among them about 50% of the germplasm were originating from Korea, followed by China (14.8%), Taiwan (13.6%) and Japan (11%) (Fig. 2).

Exponential increase in food production around the world in the past five decades at the expense of environmental degradation has led to several problems including deficiency in particular nutrients, reduced biodiversity and even emergence of susceptibility to diseases at a large scale (Pathirana and Carimi 2022). Genebanks that conserve plant genetic resources play a major role in preserving this depleting biodiversity as well as play a complementary role

between currently practiced agriculture and nature. In this context, proper documentation of the various properties of the conserved germplasms in a GenBank is vital as to understand the value of a particular bioresource for breeding and research (Weise *et al.* 2020). Studies to elucidate such properties of plant genetic resources are necessary and continuous diversification of available data followed by structured documentation and information sharing are essential for successful functioning of genetic resource conservatories (Weise *et al.* 2020; Pathirana and Carimi 2022). To supply the data available on Kimchi cabbage accessions at the National Agrobiodiversity Center, Rural Development Administration, the present study involved large-scale screening and replication experiments of resistance to KACC 21701 in randomly chosen Kimchi cabbage genetic resource.

Screening of germplasms from South Korea for resistance to bacterial soft rot disease has been earlier reported in Kimchi cabbage (Chung *et al.* 2003). However, the report of Chung and coworkers only involved screening of only forty-three cultivars or line of Kimchi cabbage. With a DI range of 0–3, they observed four out of forty-three cultivars to exhibit  $DI < 1$  which they reported to be resistant to KACC 21701. In our screening of Kimchi cabbage germplasms, we recorded DI values at a range of 0 to 4 (Fig. 1). The six hundred and eighty-two germplasms in the current study displayed different degrees of resistance to KACC 21701 observable by their varying DI values and only one accession IT120033 (Pin Chong 1) indicated  $DI < 1$  (Table 2; Fig. 3A).

As explained above, the present study differs from Chung *et al.* (2003) by the total number of germplasms studied and the range of DI values recorded. Therefore, their suggestion of cultivars exhibiting  $DI < 1$  to be resistant to KACC 21701 cannot be strictly followed. During



**Fig. 2.** The highlighted areas on the map on the left indicate the regions or parts of the world where the germplasms screened in this study originated from. The table on the right provides country-wise data on the origin of the studied Kimchi cabbage germplasms. Country codes are as follows: KOR - South Korea; CHN - China; TWN - Taiwan; JPN - Japan; HKG - Hongkong; UNK - United Kingdom; PRK - North Korea; UZB - Uzbekistan; AUT - Austria; USA - United States; RUS - Russia; KAZ - Kazakhstan; MMR - Myanmar; AUS - Australia; BRA - Brazil; CAN - Canada; ETH - Ethiopia; IDN - Indonesia; KGZ - Kyrgyzstan; MUS - Mauritius; NLD - Netherlands; PER - Peru.

our studies, overall, we found a total of one germplasm to have less than DI 1, twenty-nine germplasms to have DI at a range of 1.01–2.00, ninety-nine germplasms to have DI at a range of 2.01–3.00 and five hundred and fifty-three germplasms to have DI range of 3.01–4.00 (Fig. 3A). This distribution of various levels of susceptibility to KACC 21701 was found to occur in landraces, cultivars as well as unassigned germplasms. For our replication studies, among the 682 germplasms showing various levels of susceptibility to KACC 21701, we choose 30 accessions that exhibited a DI value of less than or equal to 2. These accessions consisted of predominantly landraces (23) followed by unassigned (4) and cultivars (3) of Kimchi cabbage. Landraces are understood to be better suited for their environment and in the present study seven of the accessions 23 germplasms with  $DI \leq 2$  have originated from Korea (KOR) followed by 6 from Taiwan (TWN) and 5 from Hong Kong (HKG) (Fig. 3B). Infection by soft rot causing *P. carotovorum* has been well studied and reported from members of Brassicaceae, Solanaceae and Cucurbitaceae (Meng *et al.* 2017; Lee *et al.* 2018, 2020; Afroz *et al.* 2021). Previous studies conducted in Korea involved screening of commercial cultivars of radish and Kimchi cabbage whereas the current study included Korean landraces and unassigned germplasms in addition to cultivars.

Our initial screening of the accessions resulted in thirty accessions of Kimchi cabbage to exhibit  $DI \leq 2.0$  (Figs. 3A

and 4). Also, these germplasms were found to have high ratio of resistant individuals to KACC 21701 (Table 2). These 30 germplasms contributed to 5.5% of landraces (23 of 418), 2.3% in case of cultivars (3 of 133) and 3.1% in case of unassigned germplasms (4 of 131) (Fig. 3A) and had their origins as KOR (23%) followed by TWN (20%), HKG (17%), JPN (17%), CHN (7%), USA (7%), NLD (3%), MUS (3%) and IDN (3%) (Table 2; Fig. 3B). Cultivated varieties and hybrids have been generally proved to be competitively superior compared to their wild relatives in terms of biomass and resistance to microbial diseases (Schröder and Prasse 2013; Okoń *et al.* 2021). However, in the current study landrace germplasms indicated significantly high numbers of disease resistance compared to cultivated or unassigned varieties.

In the replication experiments, it was observed that all of the selected thirty accessions were affected by KACC 21701 at the end of seven days (Table 3). Among them, five of the germplasms consisting of four landraces (IT102883, IT120036, IT120044, and IT120048) and one cultivar (IT187919) were observed to be moderately susceptible to KACC 21701. We observed that one of our controls CR Kiyoshi which was earlier reported to be moderately resistant to soft rot causing *P. carotovorum* (Lee *et al.* 2020) but was severely affected in this study (Fig. 4). This could be attributed to the difference in the pathogenic *Pectobacterium* strains (KACC 21701 used in the current study vs KACC



**Table 2.** List of Kimchi cabbage (*Brassica rapa* subsp. *pekinensis*) accessions exhibiting disease index (DI) values less than 2.0 during preliminary screening

S. No	IT number	Resource name	Origin	Status	Disease Index (DI) Mean ± SE	Ratio of resistant individuals (%)
1	100480	Seoul	KOR	Landrace	1.96 ± 0.38	56
2	102883	Seoul cabbage	KOR	Landrace	1.79 ± 0.41	64.3
3	102903	Suncheon Seoul cabbage	KOR	Landrace	1.39 ± 0.30	77.8
4	102907	Tatekcha	KOR	Landrace	1.41 ± 0.28	81.8
5	103000	Woldongchu	KOR	Landrace	1.57 ± 0.26	81
6	103065	Gyeoulchu	KOR	Landrace	1.77 ± 0.35	63.6
7	112954	Jeonbuk Gimje-1985-12954	KOR	Landrace	1.24 ± 0.26	84
8	120021	Mikado Santo-Hakusai	JPN	Landrace	1.58 ± 0.45	66.7
9	120022	Granat	NLD	Landrace	1.78 ± 0.31	72.2
10	120030	Michihli	USA	Landrace	1.56 ± 0.30	81.3
11	120031	Granat	IDN	Landrace	1.59 ± 0.29	77.3
12	120033	Pin Chong 1	HKG	Landrace	0.86 ± 0.24	90.9
13	120034	31464	HKG	Landrace	1.54 ± 0.31	76.9
14	120036	Super Early No 1	HKG	Landrace	1.18 ± 0.18	94.1
15	120040	31470	HKG	Landrace	1.20 ± 0.24	85
16	120042	Wu Chao Chia 45 Days	HKG	Landrace	1.33 ± 0.43	75
17	120043	Hsia Sheng 35 Days	TWN	Landrace	1.93 ± 0.37	64.3
18	120044	Tieh Chia No 2	TWN	Landrace	1.42 ± 0.27	84.2
19	120047	Yamato-Noen Emperor	TWN	Landrace	1.24 ± 0.27	71.4
20	120048	Wong Bok(Mandarin)	USA	Landrace	1.11 ± 0.28	83.3
21	120053	Ogon	JPN	Landrace	1.20 ± 0.26	86.7
22	120062	Zenkyu	JPN	Landrace	1.24 ± 0.27	88.2
23	221743	Chang Pu Wan Sheng Hei Yeh	TWN	Landrace	1.44 ± 0.23	84
24	160327	Hangzhou xiao baicai	CHN	Cultivar	1.85 ± 0.27	60
25	160329	Zaoshu 5 hao baicai	CHN	Cultivar	1.83 ± 0.29	69.6
26	187919	Can-tong White	JPN	Cultivar	2.00 ± 0.39	58.3
27	100355	AVRDC-KJH-1985-100355	TWN	Unassigned	1.71 ± 0.32	76.5
28	100356	AVRDC-KJH-1985-100356	TWN	Unassigned	1.60 ± 0.25	76
29	135431	Meike Komatsuna 030001	JPN	Unassigned	2.00 ± 0.23	44
30	135457	ABD 4 Brede Di Baton Blauc	MUS	Unassigned	1.13 ± 0.20	93.8

Ratio of resistant individuals (%) = (number of individual plants with DI < 1) / number of infected individuals × 100; SE - standard error

10225) which indicates high pathogenicity of the current strain as well as difference in the methods employed. In the earlier study where CR Kiyoshi was found to be moderately resistant, the age of seedlings was much higher (24-days old seedling vs 14 days old seedlings in the present study) and methods of infection were different (transient dipping vs soil inoculation of pathogen). These factors combined could have produced such results in our studies. We identified five germplasms among the thirty to be moderately susceptible to KACC 21701 and exhibiting high levels of variance in disease resistance (Table 3). Taking into consideration that the moderately resistant CR Kiyoshi con-

trol seedlings were completely dead (Fig. 4) during our experiments, it can be understood that our strain of choice (KACC 21701) and experimental conditions led to high rates of infection and disease in the studied Kimchi cabbage genetic resources. Moreover, presence of individual seedlings with no symptoms of KACC 21701 infection can still be observed in the thirty germplasms from the percentage ratio of resistant individuals (Table 3).

In screening-based studies such as the present one, despite the fact that all the seedlings tested originated from same batch of seeds of a germplasm, few of the seedlings survive the infection whereas others perish. Although this

**Table 3.** Replication results of disease resistance in the selected Kimchi cabbage (*Brassica rapa* subsp. *pekinensis*) germplasms

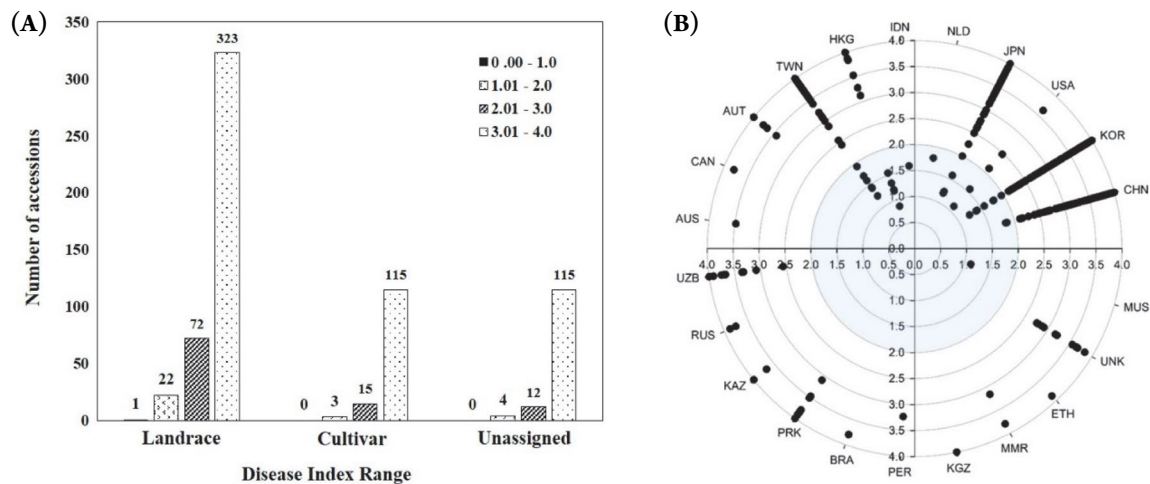
S. No	IT number	Resource name	Disease index (DI) Mean $\pm$ S.E.	Ratio of resistant individuals (%)
1	100480	Seoul	4 $\pm$ 0.2	9.7
2	102883	Seoul cabbage	3 $\pm$ 0.2	13.6
3	102903	Suncheon Seoul cabbage	4 $\pm$ 0.1	2.0
4	102907	Tatekcha	4 $\pm$ 0.1	4.0
5	103000	Woldongchu	4 $\pm$ 0.1	2.0
6	103065	Gyeoulchu	4 $\pm$ 0.1	5.8
7	112954	Jeonbuk Gimje-1985-12954	4 $\pm$ 0.1	5.3
8	120021	Mikado Santo-Hakusai	4 $\pm$ 0.2	8.8
9	120022	Granat	4 $\pm$ 0.1	2.0
10	120030	Michihli	4 $\pm$ 0.1	3.9
11	120031	Granat	4 $\pm$ 0.1	2.5
12	120033	Pin Chong 1	4 $\pm$ 0.1	12.9
13	120034	31464	4 $\pm$ 0.1	8.3
14	120036	Super Early No 1	3 $\pm$ 0.2	3.9
15	120040	31470	4 $\pm$ 0.0	2.0
16	120042	Wu Chao Chia 45 Days	4 $\pm$ 0.1	8.8
17	120043	Hsia Sheng 35 Days	4 $\pm$ 0.1	0.0
18	120044	Tieh Chia No 2	3 $\pm$ 0.2	5.8
19	120047	Yamato-Noen Emperor	4 $\pm$ 0.1	2.0
20	120048	Wong Bok (Mandarin)	3 $\pm$ 0.3	13.6
21	120053	Ogon	4 $\pm$ 0.1	0.0
22	120062	Zenkyu	4 $\pm$ 0.1	9.7
23	221743	Chang Pu Wan Sheng Hei Yeh	4 $\pm$ 0.1	0.0
24	160327	Hangzhou xiao baicai	4 $\pm$ 0.2	10.0
25	160329	Zaoshu 5 hao baicai	4 $\pm$ 0.0	0.0
26	187919	Can-tong White	3 $\pm$ 0.2	13.7
27	100355	AVRDC-KJH-1985-100355	4 $\pm$ 0.1	4.0
28	100356	AVRDC-KJH-1985-100356	4 $\pm$ 0.1	0.0
29	135431	Meike Komatsuna 030001	4 $\pm$ 0.1	1.7
30	135457	ABD 4 Brede Di Baton Blauc	4 $\pm$ 0.1	14.8

Ratio of resistant individuals (%) = (number of individual plants with DI < 1) / number of infected individuals  $\times$  100; SE-standard error

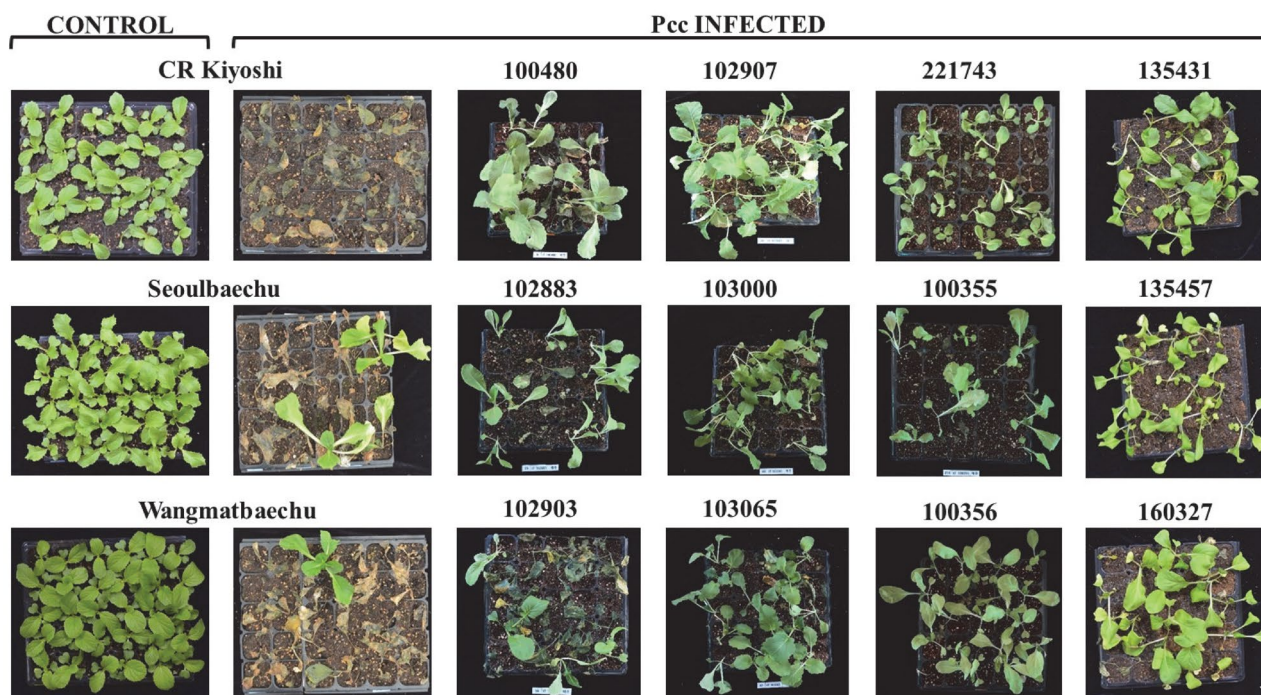
is a common phenomenon accounting for the deviancy of disease index (DI) values in screening-based studies, it fascinating to see how few seedlings stand out of the homogeneity. A recent study on resistance of *Solanum chilense* germplasms to fungal pathogen *Phytophthora infestans* has established that the relative changes in resistance phenotypes that occur among individuals were mainly determined by the plant genotype (Kahlon *et al.* 2021). Although the seedlings during the screening studies were raised from a single batch of seeds collected from a specific germplasm, they may not genotypically identical as they are collected from plants grown *ex-situ* which are vulnerable to pollination by wild plants or other relatives. In addition to the

genetic characteristic, ecological aspects have also been reported to cause such results in case of fungal pathogens studied on field scale (Davelos *et al.* 1996). Though such bias in *in-situ* studies is diminutive, minute differences in age of the plant, small changes in environmental conditions and spontaneous changes in plants can also be suggested to contribute to the resistance or susceptibility of the seedlings.

Generally, glucosinolates and lignin biosynthesis has been reportedly increased in Kimchi cabbage cultivars resistant to soft rot causing *P. carotovorum* which might help the plant to play a defensive role against the pathogen (Liu *et al.* 2019b). Also, the pathogen-pattern triggered immu-



**Fig. 3.** A. Statistics of resistant and susceptible cultivars of the studied accessions to *Pcc*. Resistance to *Pcc* was considered if the DI was  $\leq 2.0$ . B. Country-wise distribution of Kimchi cabbage accessions combined with their disease index. The axis indicates the range of disease index values observed during the experiments.



**Fig. 4.** Control and studied germplasm seven days post infection. The first column presents uninfected control varieties of the same age (21 days). Columns 2–6 presents infected seedlings seven days post infection.

nity (PTI) has been reported to be strongly triggered in resistant mutant lines, leading to expression of defense response genes including calcium dependent protein kinases (CDPK), calcium binding proteins (CaBP), respiratory burst oxidase homologs (RBOH) and mitogen activated

protein kinases (MAPK3 and MAPK4) (Liu *et al.* 2019b). Overexpression of a polygalacturonase-inhibiting protein gene *BrPGIP2* has been observed to play a significant role in resistance of Kimchi cabbage against soft rot causing *Pectobacterium* (Hwang *et al.* 2010). Though the molecular

mechanisms involved in the pathogenicity of *P. carotovorum* and resistance in plants has been studied, elucidation of the molecular details involved in signaling networks in plant cells is essential to understand the necrotrophic nature of the phytopathogen (Davidsson *et al.* 2013). While this of interest to scientists, breeders may be interested to develop soft rot resistant cultivars with desired traits that would economically benefit farmers from Korea at a large scale. In the current study, the studied germplasm exhibited various degrees of resistance or susceptibility to the phytopathogen *P. carotovorum* and we identified five germplasms to be moderately susceptible to its infection. This data was documented and amended to already available passport information on these 682 germplasms currently conserved at RDA Genebank, the National Agrobiodiversity Center.

### CRedit authorship contribution statement

Parthiban Subramanian: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing. Ho Chul Ko: Resources, Data Curation. Seong-Hoon Kim: Software, Visualization. Jae Eun Lee: Resources, Data Curation. Aejin Hwang: Resources, Software. Bichsaem Kim: Validation, Resources. Yoon-Jung Lee: Data Curation. Awraris Derby Assefa: Formal analysis. Onsook Hur: Resources. Nayoung Ro: Writing - Review & Editing. Jung Sook Sung: Investigation. Ju Hee Rhee: Resources. Ho-Sun Lee: Validation. Bum-Soo Hahn: Conceptualization, Data Curation, Supervision, Project administration, Funding acquisition.

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