



Outbreak of Rice Blast Disease at Yeosu of Korea in 2020

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Rice blast is the most destructive disease threatening stable rice production in rice-growing areas. Cultivation of disease-resistant rice cultivars is the most effective way to control rice blast disease. However, the rice blast resistance is easy to breakdown within years by blast fungus that continually changes to adapt to new cultivars. Therefore, it is important to continuously monitor the incidence of rice blast disease and race differentiation of rice blast fungus in fields. In 2020, a severe rice blast disease occurred nationwide in Korea. We evaluated the incidence of rice blast disease in Yeosu and compared the weather conditions at the periods of rice blast disease in 2019 and 2020. We investigated the races and avirulence genes of rice blast isolates in Yeosu to identify race diversity and genetic characteristics of the isolates. This study will provide empirical support for rice blast control and the breeding of blast-resistant rice cultivars.

Keywords : avirulence gene, race diversity, rice blast

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Rice is a staple food crop for more than half of the world's population, and 90% of rice production is cultivated in Asia (Fairhurst and Dobermann, 2002). Rice production accounts for the highest proportion in the agricultural and forestry industry in Korea, and 58% of all farm households cultivate rice (Jeong et al., 2017, 2021). Rice blast disease is one of the most devastating fungal diseases of rice, reducing the annual yields of rice cultivated globally by 10-30% (Fernandez and Orth, 2018; Talbot, 2003). The disease highly occurred in rice fields with the cultivation of susceptible rice cultivars, high inputs of nitrogen fertilizer, and favorable weather conditions (Luo et al., 1998). Under the optimal conditions for occurring the disease, rice blast fungus, *Magnaporthe oryzae*, causes great damage to rice by infecting leaves, stems, nodes, necks, and panicles through the rice-growing period (Dean et al., 2005).

Research efforts on rice blast disease and breeding of the disease-resistant cultivars have been made to prevent the outbreak of rice blast disease in Korea since the 1960s (Cho et al., 2007; Han, 1995; Han et al., 1994, 2001). In the early 1970s, the Indica and Japonica hybrid rice cultivar, Tongil, was developed with improved grain yield and enhanced disease resistance, and diverse Tongil-type rice cultivars were released nationwide in Korea. However, the proliferation of various genotypic rice cultivars led to the differentiation of rice blast fungus that resulted in the breakdown and loss of blast resistance in Tongil-type rice cultivars (Cho et al., 2007; Chung and Heu, 1991; Han, 1995; Kim, 2018). Since 2000, the outbreak of rice blast disease had gradually declined by the climate changes, the dissemination of cultivation technology with less nitrogen fertilizer, agronomical control such as early rice planting, and the breeding of rice cultivars with improved rice blast resistance. Nevertheless, rice blast disease occurred by the emergence of a new rice blast race and highly increase of the compatible rice blast fungus (Han, 1995; Han et al., 1994, 2001; Lee et

al., 2010). Therefore, continuous monitoring of rice blast disease and race diversity are required for disease control during the rice cultivation period.

Race diversity of rice blast fungus in Korea has been investigated using the Korean differential race system to develop disease-resistant rice and prevent the incidence of rice blast. Korean differential race system developed in 1987 comprises Indica-, Tongil-, and Japonica-type rice cultivars and differentiates the rice blast fungus as KJ- and KI-races according to the patterns of the resistant reactions (Lee et al., 1987). However, it is difficult to understand the genetic characteristics of the rice blast fungus only with the Korean differential race system as there is little research on the genetic background of the Korean differential rice cultivars. Rice blast resistance is explained by the dynamic interaction between a resistance gene of rice and the corresponding avirulence gene of rice blast fungus based on the gene-for-gene theory (Flor, 1971; Silué et al., 1992). The International Rice Research Institute (IRRI)-Japan Collaborative research project developed monogenic lines for rice blast resistance to identify the race of rice blast fungus (Fukuta et al., 2004; Telebanco-Yanoria et al., 2010; Tsunematsu et al., 2000). The monogenic lines contain 24 resistance genes in the genetic background of a susceptible Japonica-type rice cultivar, Lijiangxintuanheigu (LTH). Thus, recent research on race diversity in Korea has been conducted in parallel with the monogenic lines to identify the genetic characteristics of the rice blast fungus. In 2020, rice blast disease severely occurred on the susceptible rice cultivars, Jinsang and Yeonghojinmi, cultivated in more than 68% of the total rice cultivation area in Yeosu. Rice blast continued to progress from leaf to neck and panicles during the rice cultivation period (Supplementary Fig. 1). This study evaluated the incidence of rice blast disease in Yeosu in 2020 and analyzed the meteorological factors and the races of rice blast isolates to determine the causes.

The disease incidence area of rice leaf blast at rice paddy fields in Yeosu was estimated to investigate the incident rate of rice blast. The incidence area of rice leaf blast at 3,285 rice paddy fields corresponding to an area of 1,084 ha was investigated in Yeosu according to the manual of crop diseases and pests monitoring and control established by the Rural Development Administration (2013). Disease

incidence level was divided into low (<5%), medium (6-10%), high (11-20%), and severe (>20%) according to diseased leaf area, and the total incidence area was estimated by the following formula:

$$\begin{aligned} & \text{Incidence area of rice leaf blast (ha)} \\ &= \frac{\text{Incidence area of paddy field (ha)}}{\text{Investigated area of paddy field (ha)}} \\ & \times \text{total area of paddy fields (ha)} \end{aligned}$$

The disease incidence area of rice leaf blast was 67 ha, 6.2% of the investigated area. When applied to the total rice cultivation area in Yeosu, the disease incidence area was approximately 439 ha corresponding to 1,463 paddy fields (Table 1). The rice paddy fields showing severe and high incidence levels were 32.4% of the total disease incidence area.

Rice blast development is intimately related to meteorological factors that include air temperature, accumulated rainfall, and amount of sunshine (Kang et al., 2019; Katsantonis et al., 2017; Kim et al., 2018; Lee and Park, 1979). Meteorological factors including the average air temperature, rainfall, rainy days, and the average amount of sunshine at Yeosu from June to July in 2019 and 2020 were compared with mean values of climate parameters (climatological normals) from 1981 to 2010 to investigate the meteorological factors affecting the incidence of rice leaf blast. The average air temperature, total rainfall, rainy days, and the average amount of sunshine were obtained through daily weather observation data and daily climatological normals data provided by the Rural Development Administration (<https://weather.rda.go.kr>) and the Meteorological Administration (<https://data.kma.go.kr>). The average air temperatures of June and July in 2020 were 0.3°C and 4.1°C lower than in 2019, respectively, and 0.3°C and 3.4°C lower than the climatological normals, respectively (Table 2). In 2019, the average air temperature in June was close to the climatological normals but was 0.7°C higher in July. Rainy days were similar in both years, but the accumulated rainfall from June to July in 2020 was 58% higher than in 2019. However, the accumulated rainfall in 2019 and 2020 was 50.4% and 21.6% less than the climatological normals. The average daily sunshine duration of June and July in 2020 was 0.8 h and 1.4 h shorter than in 2019, 0.4 h longer

Table 1. Incidence of rice leaf blast at Yeosu in 2020

Rice cultivation area (ha)	Disease incidence area according to disease severity ^a (ha)					Proportion of disease incidence area (%)
	Low	Medium	High	Severe	Total	
7,078	183	114	72	70	439	6.2

^aDisease severity is divided into low (<5%), medium (6-10%), high (11-20%), and severe (>20%) levels.

Table 2. Meteorological characteristics in June and July at Yeosu

Meteorological factors	2019		2020		Climatological normals ^a	
	June	July	June	July	June	July
Average of air temperature (°C)	21.7 ± 1.7	25.1 ± 1.9	21.4 ± 2.1	21.0 ± 1.8	21.7 ± 0.9	24.4 ± 0.9
Total rainfall (mm)	68.0	188.0	72.0	332.5	146.0	370.2
Rainy days (day)	10.0	16.0	12.0	14.0	17.1	27.4
Average amount of sunshine (h/day)	7.4 ± 4.5	5.0 ± 3.8	6.6 ± 4.1	3.6 ± 3.5	6.3 ± 1.1	4.6 ± 0.6

^aClimatological normals indicate the average of climate parameters from 1981 to 2010.

than in June of the climatological normals, and 1 h shorter than in July of the climatological normals. In contrast, the average daily sunshine duration of June and July in 2019 was 1.2 h and 0.4 h longer than the climatological normals, respectively. These results indicate that low air temperature, high rate of total rainfall, and short daily sunshine from June to July in 2020 compared to 2019 would affect the outbreak of the rice leaf blast in Yeosu. Therefore, rice blast disease in Yeosu occurred by the optimal weather conditions as well as the cultivation of the susceptible rice cultivars and excessive fertilization.

To investigate the diversity of rice blast fungus in Yeosu, a total of 66 rice blast isolates were collected from the infected leaf samples of Jinsang and Yeonghojinmi (Supplementary Table 1). Single spores were isolated from each lesion of the infected rice leaves. Spores were induced on the infected rice leaves by placing on water agar medium (15 g agar powder in 1 liter) and incubated at room temperature for 24 h. Induced spores were spread on water agar medium using the loop and incubated at room temperature for 24 h. Germinated single spores were isolated on potato dextrose agar (PDA) or rice bran agar medium (2 g rice bran, 2 g sucrose, 2 g agar powder in 1 liter) under the microscope and incubated at 26°C. The rice blast isolates were identified as KJ- and KI-races according to the Korean race differential system. Conidial suspension of the isolates were inoculated on Korean differential rice cultivars. The Korean differential rice cultivars were composed of one Indica-type (Tetep), three Tongil-types (Taebaeg, Tongil, and Yushin), and four Japonica-types (Kanto51, Nongbaeg, Jinheung, and Nakdong) (Supplementary Table 2) (Lee et al., 1987). Conidia were harvested from 10-day-old rice bran agar plates that induce conidia by removing the aerial mycelia and further incubating for three days. A volume of 25 ml of conidia suspension adjusted to 10⁵ conidia per ml containing Tween20 (250 ppm, a final concentration) was sprayed onto 4-5 leaf stage of rice seedlings. Inoculated rice seedlings were moved into the greenhouse after incubating in a dew chamber at 26°C for 24 h under a dark condition. Disease severity was evaluated by scoring

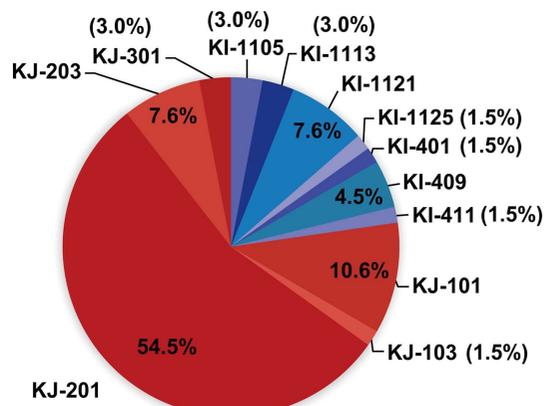


Fig. 1. KJ and KI race differentiation of the Yeosu isolates. Race diversity of the Yeosu isolates belonging to KJ race and KI race.

from 0 to 5 following the IRRI standard evaluation method at seven days after incubation (Bonman et al. 1986). The isolates were divided into 12 races that were five different KJ-races and seven different KI-races, and the ratio of KJ- and KI-races in the isolates was 77.3 to 22.7, with KJ-races approximately 3.4 times more than KI-races (Supplementary Table 3). It suggests that KJ-race isolates were much more than KI-race isolates, but the isolates of KI-race were more diverse than those in KJ-race in the fields. Among the isolates, the predominant isolates were KJ-201 (54.5%), KJ-101 (10.6%), KJ-203 (7.6%), and KI-1121 (7.6%) (Fig. 1). The KJ-201 race accounting for more than half of all isolates was first identified in 1981 and had been steadily distributed at a high rate in Korea (Han, 1995; Han et al., 1994).

The distribution of avirulence genes in 61 isolates was also identified to understand the genetic characteristics of the isolates in Yeosu. We analyzed the resistant reactions of the monogenic resistant lines infected by the isolates. Pathogenicity assay was performed by spraying conidial suspension of the isolates on monogenic resistant lines. The monogenic resistant lines were rice varieties introducing 19 different resistant genes (*Pish*, *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5(t)*, *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Pi9(t)*, *Piz-5*,

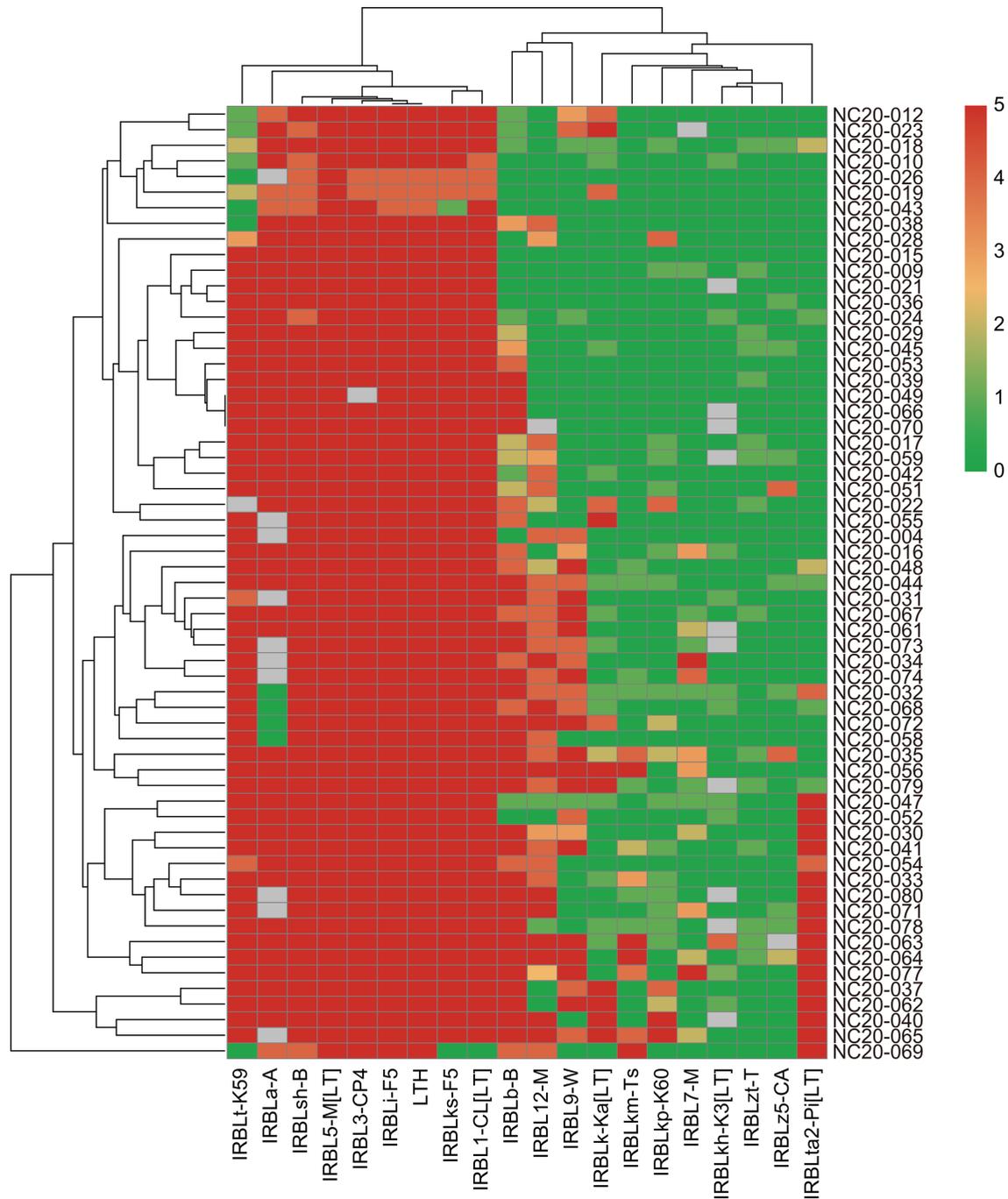


Fig. 2. Reaction of monogenic resistant lines against the Yeoju isolates in 2020. Heatmap of resistant reactions of monogenic lines against the Yeoju isolates.

Piz-t, *Pita-2*, and *Pi12(t)* based on a susceptible Japonica-type cultivar, LTH (Supplementary Table 4) (Fukuta et al., 2004; Telebanco-Yanoria et al., 2010; Tsunematsu et al., 2000). The monogenic resistant lines introducing *Piz-t* (100.0%), *Pik-h* (98.0%), *Piz-5* (96.7%), *Pi7(t)* (95.0%), *Pik-p* (91.8%), *Pik-m* (88.5%), *Pik* (80.3%), *Pita-2* (70.5%), *Pi9(t)* (60.7%), and *Pi12(t)* (51.7%) showed resistant reac-

tions against more than half of the isolates (Fig. 2). When inoculated the predominant KJ-201 isolates among the isolates, the monogenic resistant lines introducing *Piz-t* (100.0%), *Pik-h* (100.0%), *Piz-5* (97.1%), *Pi7(t)* (94.3%), *Pik-p* (91.4%), *Pik-m* (91.4%), and *Pik* (85.7%) showed highly resistant reactions. In addition, we also analyzed the presence and absence of the avirulence genes using

the specific molecular markers (Supplementary Table 5). Genomic DNA of the isolates was extracted from mycelia cultivated on PDA. The mycelial block was ground in extraction buffer (1 M KCl, 100 mM Tris-HCl, 10 mM EDTA) using a sterilized grind stick. The genomic DNA was extracted following a quick and safe method (Chi et al., 2009) and used for screening of avirulence genes by PCR. More than 70% of the isolates had *Avr-Pi9*, *Avr-Pita1*, *Avr-Pita2*, *Avr-Pita3*, *AvrPiz-t*, *Avr-Pik*, *Avr-Pikm*, *Avr-Pib*, *PWL2*, and *ACE1*, but none of them had *Avr-Pia*, *Avr-Pii*, and *Avr1-CO39* (Supplementary Table 6). These results revealed that the isolates mainly have the avirulence genes corresponding to *Piz-t*, *Pik-h*, *Piz-5*, *Pi7(t)*, *Pik-p*, *Pik-m*, and *Pik* and those seven resistance genes would be effective in preventing rice blast in Yeosu. Therefore, introducing *Piz-t*, *Pik-h*, *Piz-5*, *Pi7(t)*, *Pik-p*, *Pik-m*, and *Pik* into rice cultivars would help prevent rice blast disease in Yeosu. These results will provide useful information to control rice blast disease and for the breeding of resistant rice cultivars.

Conflicts of Interest

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

Acknowledgments

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Electronic Supplementary Material

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

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