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Evaluation of Sequential Planting Method for Screening of Durable Resistance against Rice Blast in Rice Breeding Program

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A sequential planting method was developed to screen rice plants with durable resistance against rice blast in a short time, and applied for several years in Korean rice breeding program. In this study, we showed the advantages of a sequential planting method compared to other pathogenicity tests. The correlation analysis among three pathogenicity tests and other factors demonstrated that durable resistance depended on the average of diseased leaf area and the number of compatible pathogens. Significant correlations were found in the nursery test but not in the field test result. In addition, we traced changes in the pathogen population during sequential planting stages through re-isolation of the pathogen. The portion of compatible pathogens was increased during sequential planting. Through this study, we provide an effective sequential planting method and direction of durable resistance in a breeding program.

Keywords : Durable resistance, Pathogen population, Rice blast, Sequential planting

Rice blast caused by *Magnaporthe oryzae* is one of the most serious diseases that affect the quantity and quality of rice production throughout the rice-growing season (Kiyosawa, 1972). Because rice blast deleteriously affects rice production worldwide, development of control methods has been demanded for many years, and several have been applied, such as cultural and chemical controls and the use of resistant cultivar. Of these methods, the use of resistant cultivar has been regarded as the most effective method. To examine the rice blast resistance of new cultivars, several pathogenicity tests such as the true resistance test, the nursery test, and the field test have been performed. Although a lot of resistant rice cultivars have been developed, breakdown of resistance has occasionally been reported after 2–5 years of field cultivation (Ryu *et al*., 1987). Han *et al.* (2001) suggested that these phenomena could be due to breeding that has focused on vertical resistance of rice blast. To solve this problem, the developmental strategy of resistance breeding has been focusing on durable resistance that remains effective in a large growing area over a long period in favorable environments for disease development (Johnson, 1984). However, tests of durable resistance require a lot of time, extensive area, and the resulting costs of time and labor. To resolve this issue, sequential planting method has been developed as an effective method over a short period (Kim *et al*., 2004; Roh *et al*., 2009).

In this study, we compared a sequential planting method with other pathogenicity tests for screening rice plant resistance in fields, and demonstrated the advantages of the sequential planting method in reducing the time and scale of experiments.

Sequential planting method were followed by Kim *et al.* (2004). Prior to full-scale sequential planting method, we determined representative *Magnaporthe* isolates among library of *Magnaporthe* isolates collected for decades through the individual true pathogenicity test. After individual true resistance test according to the cultivars, a total set of representative isolates were fixed to contain more than one strain that is compatible with each rice cultivar. The first step in the sequential planting method was artificial inoculation with a mixture of representa-

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tive *Magnaporthe* isolates. Spore suspensions (1×10^5 spores/ml) were sprayed on 2 or 3-week-old plants, and incubated under humid conditions overnight. Each test cultivar was boxed off from other test cultivars in greenhouse. After two weeks, diseased leaf area (DLA, %) was evaluated. Healthy rice plants were then placed beside the diseased plants from the previous step to infect the healthy plants naturally with rice blast. Disease evaluation was repeated seven times at 2-week intervals after the initial inoculation.

Nursery test was performed at seven hot spot places including Cheolwon, Jinbu, Iksan, Milyang, Sangju, Icheon and Jecheon in Korea from 2011 to 2013, and degree of nursery test was followed by International Rice Research Institute (IRRI) (IRRI, 1988). Field test was performed at three legions such as Jecheon, Icheon and Iksan, and DLA (%) was examined from 2011 to 2013. Correlation analysis between three pathogenicity tests and factors of sequential planting methods were performed by R statistic program (R Development Core Team, 2008). The results of pathogenicity tests and correlation analysis were presented in upper and lower section of Table 1, respectively.

The results of sequential planting method suggested that the average percentage of DLA at all stages was highly correlated with the degree of durability (correlation coefficient $r = 0.891$; Table 1). In addition, the numbers of compatible blast isolates (CI) showed a strong positive correlation with the degree of

Table 1. Pathogenicity tests from 2011 to 2013 and correlation analysis

Cultivar (or lines)	Sequential planting			Ns[d]	Fd[e]
	DD[a]	CI[b]	DLA (%)[c]		
Hwasung	6	19	38.02 ± 19	5.74 ± 0.54	0.37 ± 0.27
Dongan	7	7	43.45 ± 13.82	2.95 ± 0.15	0.00
Jinbu	0	1	0.71 ± 0.58	2.38 ± 0.44	0.00
Suwond547	5	13	24.03 ± 24.26	3.92 ± 0.26	0.06 ± 0.09
Suwon548	0	9	12.45 ± 12.04	4.47 ± 1.03	0.00
Iksan537	7	16	46.3 ± 26.5	5.03 ± 0.78	0.01 ± 0.01
Milyang265	7	24	43.72 ± 22.04	4.12 ± 1.3	0.02 ± 0.02
Youngduk55	0	5	5.14 ± 6.33	3.49 ± 0.9	0.01 ± 0.01
Jinbu53	0	1	0.31 ± 0.25	2.18 ± 0.32	0.00
Chulwon84	0	1	0.38 ± 0.39	2.81 ± 0.29	0 ± 0.01
Sangju46	7	12	46.74 ± 12.34	3.04 ± 0.82	0.00
Iksan541	5	19	27.98 ± 19.76	4.64 ± 0.82	0.00
Milyang255	7	18	66.6 ± 15.91	5.69 ± 3.09	0.67 ± 1.15
Milyang261	7	27	69.5 ± 12.74	6.81 ± 0.75	6.6 ± 2.55
Milyang271	7	12	44.73 ± 18.43	5.27 ± 0.93	0.27 ± 0.12
Milyang268	0	9	3.03 ± 2.37	1.49 ± 0.1	0 ± 0.01
Suwon552	5	11	40.33 ± 19.97	5.07 ± 0.58	3.59 ± 3.65
Boramchan	4	10	19.99 ± 26.47	3.92 ± 2.29	0.00
Hanareum2	0	2	0.27 ± 0.26	2.31 ± 2.5	0.00
Ilpum	6	6	34.01 ± 18.88	4.27 ± 1.7	1.42 ± 0.84
Suwon574	5	8	38.74 ± 6.2	5.77 ± 3.06	3.67 ± 6.35
Correalation Analysis	**DD[a]**	**CI[b]**	**DLA[c]**	**Ns[d]**	**Fd[e]**
DD	–	–	–	–	–
CI	0.761	–	–	–	–
DLA	0.891	0.791	–	–	–
Ns	0.664	0.709	0.712	–	–
Fd	ND[f]	ND	0.493	0.621	–

[a] Degree of durable resistance. Maximum degree of susceptibility in this test is 7.

[b] Number of compatible rice blast isolates among representative isolates.

[c] Average diseased leaf area (DLA, %) of each stage in the sequential planting method.

[d] Average degree of the nursery test in seven hot spots from 2011 to 2013. Maximum degree of susceptibility is 9.

[e] Average DLA percentage (%) in field tests from 2011 to 2013.

[f] ND indicates "No Determined" because $P > 0.05$.

durable resistance (DD; correlation coefficient *r* = 0.761), while the correlation coefficient between the CI and DLA was 0.791 (Table 1). The results indicated that DLA is the primary factor in determining durable resistance, since the DLA value at each step is the standard of durable resistance. Intriguingly, both Suwon548 and Milyang268 showed a low degree of durable resistance although they had enough number of compatible blast isolates. We inferred that these lines might have strong durable resistance to prevent spreading of pathogens in next step, or be resistant enough to coexist with compatible pathogens.

In comparison of the nursery and sequential planting method, the correlation coefficient between DD and disease degree in the nursery test (Ns) was 0.604 while the correlation coefficient between Ns and CI was 0.706, which suggests that in the nursery tests a wide range of pathogen isolates existed in hot spots (Table 1). The results of field tests showed lower correlation with DD than those of the nursery tests. Taken together, the sequential planting method is required to determine durable resistance since it includes aspects that other tests cannot cover. Moreover, the strong positive correlation between CI and DD suggested that securing as many different compatible blast isolates as possible is necessary for successful screening of durable resistance.

Finally, we examined how pathogen population changes and how selection pressure occurs during sequential planting and disease development. For this study, we designed a re-isolation and origin trace experiment during the sequential planting method. Twenty-six isolates of 23 pathotypes were used as inocula for the pathogenicity test on the first plants. These isolates were selected on the basis of the need to include a wide range of pathotypes and a distinguishable pattern of the *Pot2* genotype. Re-isolation of pathogens from diseased leaves and *Pot2* rep-PCR were performed for tracing origins of pathogen population at each sequential planting stage. *Pot2* rep-PCR was followed by previously described (George *et al.*, 1998). At the initial planting, the mixed pathogens were artificially inoculated onto 4 rice cultivars: IRBL9-W (*Pi-9(t)*), IRBL3-CP4 (*Pi-3*), Palgong, and Ilpum. Pathogens were re-isolated from the diseased plants of the first, third, fifth, and seventh stage. Population of pathogens on IRBL9-W was dramatically increased with a breakdown of resistance (Fig. 1). At the first stage, only four isolates were found to be compatible pathogens to IRBL9-W, whereas from the third stage to seventh stage most of the re-isolates were compatible (Fig. 1A). As the ratio of compatible isolates was increased, DLA of IRBL9-W was also increased until breakdown of resistance (Fig. 1B). However, Palgong and IRBL3-CP4 showed strong durable resistance although many compatible pathogens were isolated on these cultivars. At the initial stage, over one third of the pathogens were compatible with Palgong and IRBL3-CP4. Such as IRBL9-W, ratio of compatible pathogens increased, and in the end most of re-isolates were compatible with Palgong and IRBL3-CP4 compared to initial inocula. However, DLA was not increased accordingly throughout the sequential planting stages (Fig.1 B). The results indicated that Palgong and IRBL3-CP4 had excellent durable resistance, whereas the resistance of IRBL9-W and Ilpum broke down. The results also showed that some cultivars may offer durable resistance despite being compatible pathogens. Taken together, we inferred that selection pressure of resistant cultivars might be higher than those of susceptible cultivars.

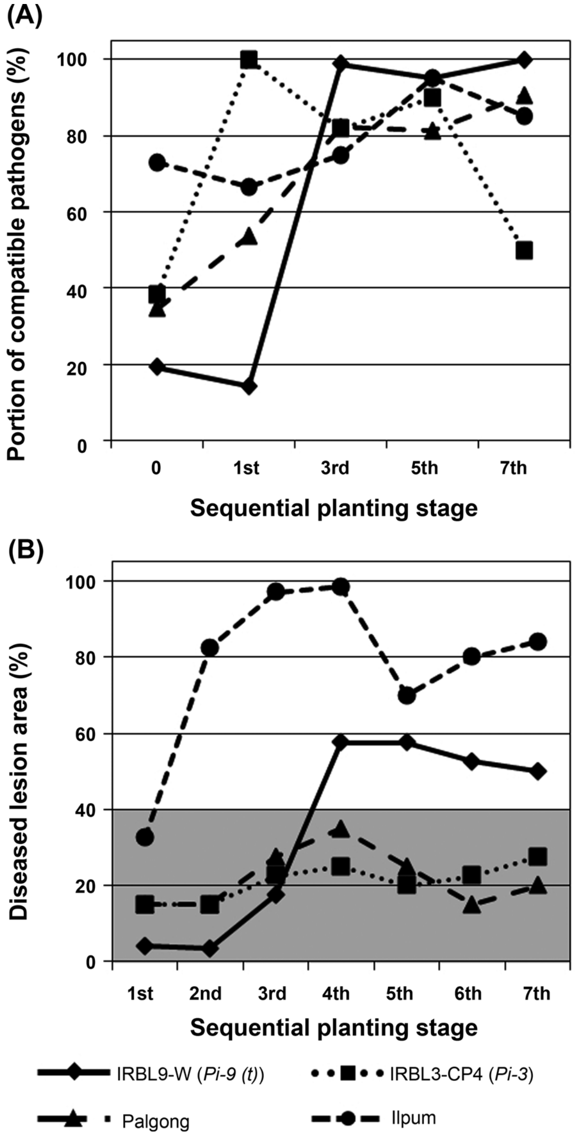

Fig.1. Portion of compatible pathogens (**A**) and diseased leaf area (**B**) at each sequential planting stages. (**A**) Percentage of compatible pathogens at stage 0 is based on number of compatible pathogens among total inoculated pathogens. Pathogens were re-isolated from lesion at each stage, and origins of the re-isolates were confirmed using *Pot2* rep-PCR, (**B**) Changes in diseased leaf area (%) during sequential planting stages. Data were from Kim *et al*. (2004).

The sequential planting method has been developed to determine durable resistance easily in a short period (Kim *et al.*, 2004; Suh *et al.*, 2009). The critical importance of sequential planting is to optimize the pathogen populations to the test cultivars through sequential inducement of rice blast. In this test, there are two main factors to determine pathogenicity of rice plants. One is criteria of durable resistance degree. The degree of durability in this test can be determined based on 40% DLA, the threshold that could cause economic damage in real fields (Yeh *et al.*, 2008). The other is initial inoculums consisted of many pathotypes. So, we have chosen representative rice blast isolates every year (Goh *et al.*, 2013). Through correlation analyses of the sequential planting method and other pathogenicity tests in rice fields, we found that determination of durable resistance in sequential planting method depends on the average DLA and the number of compatible pathogens. These results indicated that it is important to secure strong and diverse pathotypes continuously from all over the country. In addition, we found low correlations between Fd and sequential planting factors (DD, CI, DLA) except for Ns. These findings indicated that results of field test provided variable values for comparative analysis compared to other tests. By tracking changes in pathogen populations during the sequential planting method, we found that population of compatible pathogens increased during the sequential planting. Even though the number of compatible pathogens is often an important factor in determining durable resistance, some rice cultivars did show durable resistance despite having a high number of compatible pathogens. The final goal of sequential planting method is to screen for durable resistance like these plants. Despite of many advantages in the sequential planting method, there are some problems in evaluation of durable resistance such as deficiency of compatible blast isolates or fluctuated results of pathogenicity. Thus in further study, we are going to improve the sequential planting method with more reliability.

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