International Journal of Agricultural Technology 2020 Vol. 16(1): 49-54
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Blast resistance evaluation and genetic inheritance of gene controlling leaf blast resistance in Dawk Pa-yawm Rai variety (GS23774)

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Janthasri, S. and Parinthawong, N. (2020). Blast resistance evaluation and genetic inheritance of gene controlling leaf blast resistance in Dawk Pa-yawm Rai variety (GS23774). International Journal of Agricultural Technology 16(1): 49-54.

Abstract The landrace rice Dawk Pa-yawm Rai variety was highly resistant to 18 blast isolates, moderately resistant to 5 and susceptible to 2 blast isolates. Therefore, Dawk Pa-yawm Rai variety was broad-spectrum resistance to many blast isolates and can be used as genetic resource in breeding programs for blast disease resistance. The genetic inheritance of blast resistance in F_2 population was identified. Seven hundred and thirty F_2 plants from a cross between KDML105 (recipient) and Dawk Pa-yawm Rai (donor) were generated. The disease assessment revealed 524 F_2 plants were resistant and 206 F_2 plants were susceptible. The segregation of resistance and susceptible phenotypes showed a goodness of fit to the ratio 3:1 (R:S). The result found is important factor and useful in mapping of blast resistant gene in Dawk Pa-yawm Rai variety.

Keywords: rice blast disease, *Pyricularia oryzae*, genetic inheritance

Introduction

Rice blast disease, caused by plant pathogenic fungi, *Pyricularia oryzae*, is one of the most economically important diseases of rice worldwide (Ou, 1985) because its widespread distribution and destructiveness. A continuous effort has been made to control the disease, especially by using fungicides and breeding program against the disease.

Thai jasmine rice or Khao Dawk Mali 105 is popular to both Thai and foreign consumers alike. The dominant feature is its low amylose content about 10.5% and the smell is unique but is susceptible to blast disease (Rice Department, 2011). Controlling rice blast disease using resistant varieties is the most appropriate way because it provides long-term value and reduces the use of chemicals to prevent disease. This may also decrease cost of rice production

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and environmental pollutions. The improved rice resistant varieties to blast disease in Thailand is therefore recommended (Mekwatanakarn *et al.*, 2007).

The Dawk Pa-yawm Rai variety (GS23774) used in this study was derived from screening of the landrace rice cultivars for a blast-resistant phenotype using 29 blast isolates collected from the disease epidemic areas in Thailand (Salih *et al.*, 2013). Dawk Pa-yawm Rai was selected from the screening for broad-spectrum resistance to rice blast disease and therefore can be used as a good genetic resource in the breeding program.

Thus, this study was aimed to determine the reaction and genetic inheritance of disease resistance in Dawk Pa-yawm Rai variety (upland rice variety). The results from this study is important and useful for further analysis in finding and mapping of blast resistant gene in Dawk Pa-yawm Rai variety.

Materials and Methods

Rice varieties

Dawk Pa-yawm Rai variety (GS23774) used in this study was derived from screening of the landrace rice cultivars for a blast-resistant phenotype using 29 blast isolates collected from the disease epidemic areas in Thailand, as described in Salih *et al*. (2013). The F_1 obtained from the cross between Dawk Pa-yawm Rai as donor and Khao Dawk Mali 105 (KDML105), a well-known aromatic rice variety which is highly susceptible to leaf and neck blast diseases (as recipient), were grown for F_2 seed production. The resistant Jao Hom Nin (JHN) and IR64 and the susceptible KDML105 were control cultivars used as standard check varieties in all experiments.

Pathogen isolates

The 25 blast fungal isolates were collected from different blast disease epidemic areas of Thailand (Table 1). The blast fungi isolated from leaf or neck with blast symptoms were cultured on rice-flour agar medium (20 g/L rice flour, 20 g/L agar and 2 g/L yeast extract) at 25℃ and stored as dried mycelium on filter paper at -20℃ as described by Sirithunya *et al*. (2008). These were used for further experiments.

Inoculation and evaluation of blast resistance on Dawk Pa-yawm Rai (GS23774)

Each of the 25 blast isolates (Table 1) was inoculated on Dawk Pa-yawm Rai variety and 3 rice control cultivars (KDML105 (susceptible, S), Jao

Hom Nin (JHN) and IR64 (resistance, R). Tested rice seeds were sown individually in plastic trays (42 x 28 cm) where JHN, IR64 and KDML105 were planted at both sides of the end rows as resistant and susceptible check varieties respectively. Five grams and 1 gram of urea fertilizer was added after planting and 7 days prior to inoculation, respectively. Inoculation of the blast isolates was performed following the method described by Sreewongchai *et al*. (2009). Each of 25 blast isolates was grown on rice flour agar medium and incubated at 25℃. Sporulation was induced by scraping 8 to 10-day-old mycelium and allowing growth for another 2 days. Spores were harvested and the concentration of spores was adjusted to 1×10^5 spores/mL in 0.5% gelatin. Inoculum was sprayed onto 14-day-old seedlings using an air-brush pressure pump. The inoculated seedlings were placed in a high-humidity chamber for 18 hours at 25℃ and were then transferred to a greenhouse. The degree of infection of each seedling was evaluated 7 days after inoculation by a standard reference scale for rice blast, scoring 0 – 2, 3 – 4, and 5 – 6, for resistant, moderate resistant and susceptible, respectively (Roumen *et al*., 1997).

Table 1. Twenty-five isolates of *P. oryzae* used in this phenotypic analysis and their collection locations

Area	Province	Number of isolates
North	Chiang Rai, Phitsanulok	3
	Chaiyaphum, Khon Kaen, Nong Khai	
North-east	Surin, Ubon Ratchathani	13
Central	Bangkok, Chachoengsao, Ratchaburi	7
South	Phatthalung	2

Genetic inheritance of blast resistance in F_2 population

The genetic inheritance of blast resistance in F_2 population was identified. Seven hundred and thirty F_2 plants from a cross between KDML105 (susceptible) and Dawk Pa-yawm Rai (resistance) were generated and sown individually in plastic trays (42 x 28 cm) and 5 g and 1 g of urea fertilizer were added after planting and 7 days prior to inoculation, respectively. A mix of 25 blast isolates was inoculated on Dawk Pa-yawm Rai (GS23774) and 3 rice control cultivars (KDML105, Jao Hom Nin and IR64) using the method described by Sreewongchai *et al*. (2009). Each of the blast isolates was cultured on rice flour agar medium, culture plate was incubated at 25℃ and the spore production was induced. After spore formation, the spores were harvested using sterile distilled water. Each isolate was adjusted to a concentration of 1×10^5

spore/ml using sterilized distilled water with 0.5% gelatin and mixed together equally. The inocula prepared was sprayed onto 14-day-old seedlings using an air-brush pressure pump and the disease reactions were scored 7 days after inoculation, as described by Roumen *et al*. (1997).

Results

Blast disease resistance of Dawk Pa-yawm Rai (GS23774)

The disease resistance evaluation of Dawk Pa-yawm Rai variety to 25 blast isolates showed that Dawk Pa-yawm Rai variety was highly resistant to 18 blast isolates, moderately resistant to 5 and susceptible to 2 blast isolates, respectively. The rice control cultivar KDML105 was highly susceptible to 21 isolates, moderately resistant to 2 and resistant to 2 isolates. Jao Hom Nin and IR64 were highly resistant to all 25 blast isolates (Table 2). Dawk Pa-yawm Rai variety showed broad-spectrum resistance to many blast isolates and can be used as genetic resource in breeding programs.

Table 2. The disease assessment to blast resistance of Dawk Pa-yawm Rai and control varieties after inoculated with each of 25 blast isolates collected from different blast disease epidemic areas of Thailand

Name	Resistance	Moderately resistant	Susceptible
Dawk Pa-yawm Rai	18	5	2
KDML105	2	2	21
JHN	25	0	0
IR64	25	0	0

Genetic inheritance and phenotypic analysis of blast resistance in F_2 population

The genetic inheritance of blast resistance in F_2 population was identified. Seven hundred and thirty F_2 plants from a cross between KDML105 (susceptible) and Dawk Pa-yawm Rai (resistance) plus three rice control cultivars were inoculated with a mixture of 25 blast isolates and the disease score of each plant was evaluated 7 days after inoculation. The disease assessment revealed 317 F_2 plants were resistant, 207 and 206 F_2 plants were moderately resistant and susceptible, respectively (Table 3). The segregation of resistance (317 and 207 plants) and susceptible (206 plants) phenotypes showed a goodness of fit to the ratio 3:1 (R:S). The obtained Chi-square data on the segregation analysis was 3.735, with a *p* value was less than 0.045 ($p < 0.045$) (Table 4), suggested that the resistant phenotype of the Dawk Pa-yawm Rai variety against blast disease was controlled by single dominant gene.

Table 3. Reactions of F_2 population obtained from the cross between KDML105 and Dawk Pa-yawm Rai varieties to the mixed 25 isolates of *P. oryzae*

Number of Evaluated (Seedlings)	Resistance	Moderately resistant	Susceptible
	0-2*	3-4	5-6
730	317	207	206

*0 – 2 = resistant; 3 – 4 = moderately resistant; 5 – 6 = susceptible

Table 4. Segregation of F_2 population obtained from the cross between KDML105 and Dawk Pa-yawm Rai varieties after inoculated with the mixed 25 isolates of *P. oryzae*

Total no. of seedlings	Expected Radio	Expected No.		Observed No.		X^{2*}	*P*
		R**	S	R	S		
730 (F_2)	3:1	547.5	182.5	524	206	3.735	0.045

*X^2 (0.05, 1) = 3.84, df =1.0

**R = resistant; S = susceptible

Discussion

In this study, disease assessment to blast resistance of Dawk Pa-yawm Rai variety was conducted using 25 blast fungal isolates collected from different blast disease epidemic areas of Thailand. Each fungal isolate was inoculated on Dawk Pa-yawm Rai variety and 3 rice control cultivars (KDML105 (susceptible), Jao Hom Nin and IR64 (resistance) and the disease reactions were scored 7 days after inoculation. The results showed that Dawk Pa-yawm Rai variety (GS23774) was broad-spectrum resistance to blast disease. This confirmed the research of Salih *et al*. (2013), that Dawk Pa-yawm Rai variety (GS23774) is one of four landrace rice cultivars which appeared broad-spectrum resistance to many blast isolates.

Seven hundred and thirty F_2 plants from a cross between KDML105 (susceptible) and Dawk Pa-yawm Rai (resistance) were evaluated for resistance phenotype where 524 F_2 plants were resistant and 206 F_2 plants were susceptible. The segregation of resistance and susceptible phenotypes showed a goodness of fit to the ratio 3:1 (R:S). The Chi-square data on the segregation analysis suggest that the resistant phenotype of Dawk Pa-yawm Rai variety against blast disease was controlled by single dominant gene. The identification of blast resistant genes has been reported in landrace Thai rice cultivars by Phaitreejit *et al*. (2011). Total of 203 landrace rice cultivars were identified using gene specific markers for blast resistant gene *Pi9*, *Pi36* and *Pigm(t)*. The authors showed that all 203 cultivars have at least one blast resistance gene and

42 cultivars have all the three blast resistant genes. Moreover, Parinthawong *et al*. (2015) reported the genetic inheritance of Huai (GS19769) variety, 1 of 4 varieties obtained from screening of the landrace rice cultivars for a blast-resistant phenotype using 29 blast isolates collected from the disease epidemic areas in Thailand by Salih *et al*. (2013) which at least 1 blast resistance gene was mapped on the chromosome 11. The outcome of this study revealed a new blast resistance gene discovered in Thai landrace rice variety which will be used as blast resistance gene source in the future breeding programs.

Acknowledgement

This research was done at the rice blast laboratory at KMITL and supported by the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang.

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(Received: 10 August 2019, accepted: 28 December 2019)