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# Investigation of blast resistance genes in a broad-spectrum resistant indigenous rice, Yang Mawng variety

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**Abstract** The indigenous rice Yang Mawng variety was highly resistant to 24 isolates and moderate resistant to 3 isolates of *P. oryzae*. The results confirmed the broad-spectrum resistance to blast disease of Yang Mawng variety. The pathogenicity test of 27 isolates of *P. oryzae* was conducted on 31 Near Isogenic Lines (NILs). The result showed that virulence index (VI) was valued from 0 to 0.50 and the most pathogenic isolate on NILs was UBN2010 13515. The investigation for 8 rice blast disease resistance genes of Yang Mawng variety was done using blast resistance gene specific primers and PCR technique. The result showed that 5 blast resistance genes including *Pi9, Pigm(t), Pi54, Pib* and *Pita*, were detectable. The information of this research is a preliminary data for study of resistance genes present in Yang Mawng variety.

**Keywords:** *Pyricularia oryzae*, rice blast disease, Pathogenicity, Yang Mawng variety

## Introduction

Rice blast disease caused by *Pyricularia oryzae* is important fungal disease of rice and also is one of the most important disease infecting rice plants. The disease results in low grain yield. Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses up to 90 % (He *et al.*, 2012; Singh *et al.*, 2015). *P. oryzae* could infect the rice plants from the seedling to maturity stages. The pathogen is the most common on leaves, causing leaf blast during the vegetative stage of growth or on neck nodes panicle and culms (Srivastava *et al.*, 2014).

Rice resistant improvement have become good potential approach for farmers to protect rice from blast disease because using rice resistant variety can decrease fungicide application, reduce the production costs and is environmentally friendly (Ashkani *et al.*, 2015). Therefore, identifying and using broad-spectrum resistance genes in rice breeding have been considered as the most economical strategy to control the disease. Thai

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indigenous rice variety, Yang Mawng, has been reported for being highly and broad-spectrum resistant to all tested 29 isolates of *P. oryzae* (Salih *et al.*, 2013). This study presents the information of resistance genes found in indigenous rice Yang Mawng variety.

## Materials and methods

### *Pyricularia oryzae isolates*

The 27 isolates of *P. oryzae* were isolated from different regions of Thailand including Northern, North-eastern, Central and Eastern. Some *P. oryzae* isolates were obtained from difference providers; Ubon Ratchathani Rice Research Center; Department of Agronomy, Faculty of Agriculture, Kasetsart University; Rice Science Center & Rice Gene Discovery Unit, Kasetsart University Kamphaengsaen Campus; Plant Pathology Laboratory, Department of Plant Production Technology, Faculty Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (Table 1).

**Table 1.** List, origin, and given isolate name of *Pyricularia oryzae* being used in the study

| Region | Province | Isolate name |
|---|---|---|
| **North-eastern** | Ubon Ratchathani | UBN2010 11351[/1] |
| | | UBN2010 13515[/1] |
| | | UBN2009 207129[/1] |
| | | UBN2010 195167[/1] |
| | | UBN2010 195171[/1] |
| | Nong Khai | NKI2010 47181[/1] |
| | Sakon Nakon | SKN2008 60867[/1] |
| | Khon Kaen | KKN2008 7357[/1] |
| | | KKN2009 61067[/1] |
| | Sakon Nakhon | SKN61009[/4] |
| | Surin | SRN54007[/4] |
| **Northern** | Chiang Rai | Chiangrai34.1[/2] |
| | Chiang Mai | THL794[/3] |
| | Lampang | LPG61004[/4] LPG61005[/4] |
| | | LPG61011[/4] |
| | Tak | TAK61002[/4] |
| **Central** | Ratch buri | RBR55001[/4] RBR55003[/4] |
| | Bangkok | BKK55001[/4] BKK55003[/4] |
| | Phetchabun | PNB61005[/4] PNB61008[/4] |
| | Phitsanulok | Phitsanulok40.4[/2] |
| **Eastern** | Chachoengsao | CCO56004[/4] |
| **Southern** | Phatthalung | PL61003[/4] PL61017[/4] |

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[2/] Department of Agronomy, Faculty of Agriculture, Kasetsart University

[3/] Rice Science Center & Rice Gene Discovery Unit, Kasetsart University Kamphaengsaen Campus.

[4/] Plant pathology Laboratory, Faculty Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang

***Pathogenicity evaluation***

The fungal isolates used in the pathogenicity test were grown on RFA medium and incubated at 25$^{o}$C. After 8 – 10 days, sporulation was induced by scraping all mycelium in each plate and the plates were incubated for another 2 days. The fungal spores were then harvested in sterile distilled water and the concentration of spore suspension was adjusted to $10^5$ spores/ml in 0.5% gelatin. Each isolate was sprayed onto 2-week-old rice seedlings including Yang Mawng (YM), 31 Near Isogenic Lines (NILs), Khao Dawk Mali 105 (KDML105) and Jao Hom Nin (JHN). The inoculated plants were placed in a high humidity chamber for 18 h at 25$^{o}$C and were subsequently transferred to green house. The development of symptoms on the rice leaves was observed 7 days after inoculation and scored using the rating described by Roumen *et al.* (1997). Score 0 – 2 were resistant, 3 – 4 were moderate resistant and 5 – 6 were susceptible.

The virulence index (VI) of *P. oryzae* was calculated following formula:

$$\text{VI} = \frac{\text{No. of susceptible variety}}{\text{No. of tested variety}}$$

***DNA extraction and molecular identification using PCR***

Leaves of Yang Mawng variety were ground into powder with liquid nitrogen. Seven NILs (IRBL9-W, IRBLkh-K3, IRBLa-A, IRBLta-K1, IRBLk-Ka, IRBLb-B) and standard resistance varieties JHN and IR64were used as positive control, while Lijiangxintuanheigu (LTH) was selected to be the negative control. DNA was extracted from leaves using Plant DNA Extraction Kit (VIVANTIS, Malaysia) and kept at 4 ℃. The presence of 8 resistance genes was determined by PCR technique using gene specific primers (Table 2). The PCR was performed using 20 µl reaction mixtures contained sterile water, 5 µM of each primer, 10 ng of genomic DNA and 10 µl GeneDireX (Bio-Helix, Taiwan). The PCR amplification conditions consisted of an initial denaturation at 95 ℃ for 5 min followed by 30 cycles of denaturation at 94 ℃ for 30 seconds (sec), annealing for 40 sec according to primers annealing temperatures, extension at 72 ℃ for 30 sec and a final extension at 72 ℃ for 5 min. All PCR products were verified using 2% agarose gel electrophoresis analysis.

**Table 2.** List of gene specific primers of rice blast resistant genes used in this study

| Gene | Primer (5'>>>3') | Annelling temp (°C) | Size range (bp) | Reference |
|---|---|---|---|---|
| *Pi9* | F' CCCAATCTCCAATGACCCATAAC<br>R' CCGGACTAAGTACTGGCTTCGATA | 56 | 500 | Liu *et al.*, 2002 |
| *Pi54* | F' CAATCTCCAAAGTTTTCAGG<br>R' GCTTCAATCACTGCTAGACC | 55 | 261 | Ramkumar *et al.*,2011 |
| *Pia* | F' GAGCAATGCCCAATCTCCAG<br>R' TTTACCGTTCACTGACGCAG | 60 | 906 | - |
| *Pi50* | F' CTTGACATCCAAACCGCACC<br>R' TAGGCCTAGCCAATTTTTGCC | 60 | 1172 | Xiao *et al.*, 2017 |
| *Pigm (t)* | F' CAGTGAAACGAACGCTATG<br>R' AATAGGAAGGGTTGATGTTG | 56 | 555 | Deng *et al.*, 2006 |
| *Pi-ta* | F' AGCAGGTTATAAGCTAGGCC<br>R' CTACCAACAAGTTCATCAAA | 58 | 1024 | Jia *et al.*, 2002 |
| *Pik* | F' GGAAAGCTGATATGTTGTCG<br>R' ACTCGGAGTCGGAGAGTCAG | 58 | 1144 | - |
| *Pib* | F' ATCAACTCTGCCACAAAAATCC<br>R' CCCATATCACCACTTGTTCCCC | 57 | 629 | Cho *et al.*, 2007 |

## Results

### *Pyricularia oryzae isolates*

The 27 isolates of *P. oryzae* were obtained from 16 provinces of different regions including Northern, North-eastern, Central Eastern and Southern region of Thailand, as listed in Table 1.

### *Resistance degree of Yang Mawng variety to P. oryzae isolates infection*

Each isolate of *P. oryzae* was inoculated on Yang Mawng and disease severity was scored 7 days after inoculation. The result showed that Yang Mawng was high resistant to 24 isolates included CCO56004, KKN2009 61067, KKN2008 7537, RBR55001, RBR55003, SRN54007, THL794, UBN2010 13515, UBN2009 207129, UBN2010 195167, NKI2010 47181, BKK55001, BKK55003, UBN2010 11351, LPG61004, LPG61005, LPG61011, Chiangrai34.1, TAK61002, PNB61005, PNB61008, PL61003, PL61017 and SKN61009, and moderate resistant to 3 isolates included SKN2008 60867, UBN2010 196171 and PLK40.4. The results indicated that Yang Mawng variety has broad-spectrum resistance to many isolates of *P. oryzae*. KDML105 and Jao Hom Nin were used as control and were found to be susceptible and resistant, respectively, to the infection of all tested *P. oryzae* isolates (Figure 1A-D).

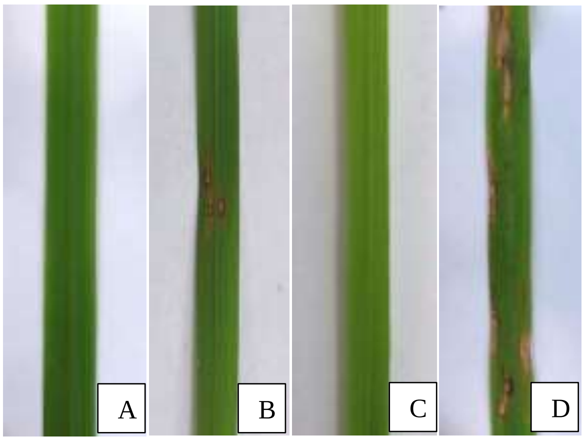

**Figure 1**. Blast symptoms on Yang Mawng (YM), Jao Hom Nin (JHN) and KDML105 varieties upon infection by *P. oryzae* isolates included YM after inoculated with isolate RBR55001 (A) and PLK40.4 (B), where JHN (C) and KDML105 (D) were used as control

### *Pathotype of P. oryzae on Near Isogenic Lines (NILs)*

The pathotype of 27 isolate of *P. oryzae* was evaluated by inoculating each isolate on 31 Near Isogenic Lines (NILs) and disease severity was scored 7 days after inoculation with score 0 – 6. The score 0 – 2; rice were resistance resulting from compatible reaction between R gene of rice plant and the Avr gene of *P. oryzae*, the score 3 – 4; rice were moderate resistance and score 5 – 6; rice were susceptible indicating incompatible interaction between the R gene and the Avr gene. The result showed that virulence index (VI) were valued from 0 to 0.50. The most pathogenic isolate on NILs was UBN2010 13515 (VI = 0.50) and 6 isolates included RBR55001, UBN2009 207129, BKK55001, PL61003, SKN2008 60867 and LPG61005, were not able to cause symptoms on NILs (VI = 0) (Table 3). The highest virulent isolate, UBN2010 13515, could infect numbers of tested variety but not Yang Mawng variety. The pathotypic evaluation results on NILs revealed that UBN2010 13515 contained *Avr-Piks, Avr-Pik, Avr-Pi7(t), Avr-Pita, Avr-Pikm, Avr-Pikp, Avr-Pikh, Avr-Pish* and *Avr-Pi20,* which indicated the corresponding R genes might present in Yang Mawng variety. Therefore, the fungi were unable to cause disease on Yang Mawng variety.

***Identification of blat resistance genes in Yang Mawng variety***

Identification of 8 blast resistance genes, including *Pia*, *Pi9*, *Pigm(t)*, *Pik*, *Pi50*, *Pi54*, *Pib* and *Pita* was done using PCR technique and gene specific primers. The results showed that Yang Mawng variety contained *Pi9*, *Pigm(t)*, *Pi54*, *Pib* and *Pita*, with the PCR product size of 500, 555, 261, 629 and 1024 bp, respectively (Figure 2A-H).

**Table 3**. Virulence index (VI) of each 27 *P. oryzae* isolates analyzed on NILs

| No. | Isolate | VI | No. | Isolate | VI |
|---|---|---|---|---|---|
| **1** | CCO56004 | 0.03 | **15** | LPG61005 | 0.00 |
| **2** | KKN2009 61067 | 0.06 | **16** | LPG61011 | 0.06 |
| **3** | KKN2008 7537 | 0.03 | **17** | UBN2010 11351 | 0.09 |
| **4** | RBR55001 | 0.00 | **18** | LPG61004 | 0.22 |
| **5** | RBR55003 | 0.09 | **19** | TAK61002 | 0.35 |
| **6** | SRN54007 | 0.06 | **20** | PNB61005 | 0.13 |
| **7** | THL794 | 0.29 | **21** | PL61003 | 0.00 |
| **8** | UBN2010 13515 | 0.50 | **22** | PL61017 | 0.06 |
| **9** | UBN2009 207129 | 0.00 | **23** | SKN61009 | 0.03 |
| **10** | UBN2010 195167 | 0.06 | **24** | SKN2008 60867 | 0.00 |
| **11** | NKI2010 47181 | 0.29 | **25** | UBN2010 195171 | 0.03 |
| **12** | BKK55001 | 0.00 | **26** | PLK40.4 | 0.40 |
| **13** | BKK55003 | 0.06 | **27** | PNB61008 | 0.06 |
| **14** | Chiangrai34.1 | 0.12 | | | |

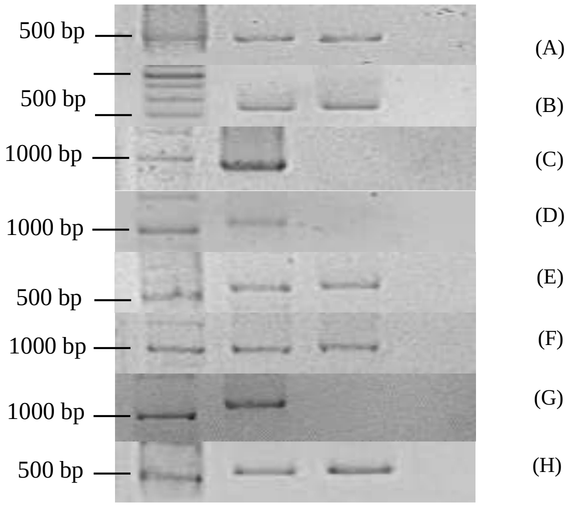

**Figure 2.** Identification of 8 blast resistance genes included *Pi9* (A), *Pi54* (B), *Pia* (C), *Pi50* (D), *Pigm(t)* (E), *Pita* (F), *Pik* (G) and *Pib* (H) using PCR technique and gene specific primers. (YM = Yang Mawng variety; P = Positive control was IRBL9-W, IRBLkh-K3, IRBLa-A, JHN, IR64, IRBLta-K1, IRBLk-Ka and IRBLb-B, corresponding to the resistance gene *Pi9, Pi54, Pia, Pi50, Pigm(t), Pita, Pik* and *Pib,* Respectively; N = Negative control (LTH); M = DNA ladder)

## Discussion

In this study, Yang Mawng variety was highly resistant to 24 blast isolates including CCO56004, KKN2009 61067, KKN2008 7537, RBR55001, RBR55003, SRN54007, THL794, UBN2010 13515, UBN2009 207129, UBN2010 195167, NKI2010 47181, BKK55001, BKK55003, UBN2010 11351, LPG61004, LPG61005, LPG61011, Chiangrai34.1, TAK61002, PNB61005, PNB61008, PL61003, PL61017 and SKN61009, moderately resistant to 3 isolates including SKN2008 60867, UBN2010 196171 and PLK40.4. The results indicated that Yang Mawng variety has broad spectrum resistance to many isolates of *P. oryzae.* This observation confirmed previous report of Salih *et al.* (2013) that Yang Mawng (GS20874) was one of the four landrace varieties which appeared to have broad-spectrum resistance with no blast symptom detected after the inoculation of the mixed 29 *P. oryzae* isolates collected from seven provinces of Thailand.

Investigation of 8 resistance genes using gene specific primers and PCR technique showed that Yang Mawng variety contained *Pi9*, *Pigm(t)*, *Pib*, *Pita* and *Pi54*. It has been reported that *Pi9* is present in 64 Thai landrace rice varieties and 1 among them was southern landrace Thai rice, 16 varieties from northern, and 47 varieties from north-eastern of Thailand regions and *Pigm(t)* is present in 201 Thai landrace rice varieties (Phaitreejit *et al*., 2011). One hundred and ten Thai landrace rice varieties have been analyzed using molecular markers specific to rice blast resistant genes. The resistance gene *Pita*, *Pib* and *Pi2t* were found in 78 Thai landrace rice varieties. *Pita* is present in 29 Thai landrace rice varieties, *Pib* is present in 7 Thai landrace rice varieties and *Pi2t* is present in 76 Thai landrace rice varieties. (Kladmook *et al.,* 2009). The blast resistance gene *Pi54* was initially identified and cloned from the indica rice. The gene conferred broad-spectrum resistance against Indian rice blast isolates (Rai *et al.,* 2011). Nowadays, many blast resistance alleles of *Pi54* were also cloned from various wild rice species providing a high degree of resistance to *P. oryzae* (Das *et al.,* 2012; Devanna *et al.,* 2014).

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