



Agriculture and Natural Resources

journal homepage: <http://www.journals.elsevier.com/agriculture-and-natural-resources/>

Original Article

Detection and allele identification of rice blast resistance gene, *Pik*, in Thai rice germplasm



Kasirapat Ariya-anandech,^a Chaivarakun Chaipanya,^a Wattanaporn Teerasan,^a Sureeporn Kate-Ngam,^b Chatchawan Jantasuriyarat^{a, c, *}

^a Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand

^b Department of Agronomy, Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani, 34190, Thailand

^c Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University (CASTNAR, NRU-KU), Kasetsart University, Bangkok, 10900, Thailand

ARTICLE INFO

Article history:

Received 16 February 2018

Accepted 5 May 2018

Available online 22 November 2018

Keywords:

DNA marker
Rice blast fungus
Rice blast disease

ABSTRACT

The objectives of this study were to detect the rice blast resistance gene, *Pik*, in Thai rice germplasm and to identify their alleles. Rice blast resistance gene specific primers were designed and used to screen for the presence of the *Pik* resistance gene in 373 Thai rice varieties. The results showed that 29 out of the 373 Thai rice varieties contained the *Pik* gene. All 29 polymerase chain reaction (PCR) products were digested by the *Pst*I restriction enzyme. PCR products from 25 rice varieties could be digested by the *Pst*I enzyme, indicating that they contained the *Pikp* resistance group, which has a narrow disease spectrum. The PCR products from four rice varieties could not be digested by the *Pst*I enzyme, suggesting that they contained the desirable *Pikm* resistance group, which has a broad disease spectrum. The nucleotide sequences of these four rice varieties in the *Pikm* resistance group revealed that the Khaw reng rice variety had the *Pikm* allele and the other three rice varieties had the *Piks* allele. Khaw reng can be used as an elite resistant donor in conventional and molecular breeding programs for blast disease resistance in Thailand.

Copyright © 2018, Kasetsart University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Rice is the most important crop as it serves as a source of food and energy to humans with approximately 90% of world's rice production coming from Asia (Ito et al., 1989). Rice blast disease, caused by the fungus *Magnaporthe oryzae*, is one of the most devastating diseases of rice (Disthaporn, 1994). The symptoms or lesions can be found on all parts of the plant including the leaves, collar, neck and panicle (Ou, 1985). Rice blast epidemics have occurred in most provinces in north and northeast Thailand, where the most effective management and control of rice blast disease is the use of resistant rice varieties (Noenplab et al., 2015). Thailand rice germplasm has been reported to have a high level of genetic diversity (Harlan, 1992). Many landrace rice varieties in Thailand harbor exotic genetic materials, which help adapt to unfavorable environment and provide resistance to insects and diseases

including blast disease (Chang, 1976). Information about disease resistance genes in rice germplasm is important for the development of resistant rice varieties and many rice blast-resistance genes have been identified and mapped in rice genome (Ashkani et al., 2014; Sharma et al., 2012), of which 22 genes have been successfully cloned and molecularly characterized (Wang et al., 2014). Many rice blast resistance genes including *Pik* have been shown to be effective against the rice blast population in Thailand (Chaipanya et al., 2017). During the past decade, breeders from the Rice Department of Thailand used the *Pik* gene as a source of rice blast resistance to develop several improved rice varieties, for example Bahng Taen (Kaewcheenchai et al., 2014), IR77955-24-75-284 (Kotchastit, 2013) and Jao Hawm Phitsanulok51 (Noenplab et al., 2015). The rice blast resistance gene, *Pik*, located on rice chromosome 11, encodes for the nucleotide binding site-leucine rich repeat (NBS-LRR) resistance protein (Belkhadir et al., 2004; Gururani et al., 2012). The *Pik* resistance protein directly interacts with the corresponding *Avr-Pik* effector protein, produced by the pathogen, leading to a host defense response. Both the *Pik* and *Avr-Pik* genes have variable multiple alleles (Yoshida et al., 2009). There are seven

* Corresponding author. Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand.

E-mail address: fscicwj@ku.ac.th (C. Jantasuriyarat).

Table 1
Thai rice germplasm (373) used in the experiment.

GS. Number	Name	Category	Pik gene
137	Pha leuod khway	Northern lowland rice	–
143	Dor e hen	Northern lowland rice	–
155	Nang phim pha	Northern lowland rice	–
158	Kaew peing	Northern lowland rice	–
168	Pha peing	Northern lowland rice	<i>Pikp group</i>
215	Leung pin thong	Northern lowland rice	–
231	Hom thu rein	Northern lowland rice	–
238	Lam pang	Northern lowland rice	–
239	Leung aon	Northern lowland rice	–
243	Khaw leb	Northern lowland rice	–
249	Leung aon	Northern lowland rice	–
257	Mae paed	Northern lowland rice	–
259	Sa mer jai	Northern lowland rice	–
334	Khaw jan	Northern lowland rice	–
337	Nhew leuod raed	Northern lowland rice	–
1409	Kaew	Northern lowland rice	–
1411	Wang hin	Northern lowland rice	–
1412	Kab sang	Northern lowland rice	–
1413	Joa hom	Northern lowland rice	<i>Piks</i>
1418	Dor dork praw	Northern lowland rice	–
1424	Leb chang	Northern lowland rice	–
1425	Nang nooan	Northern lowland rice	–
1433	Kaew noi	Northern lowland rice	–
1438	Mhon	Northern lowland rice	–
1439	Kaew nam phung	Northern lowland rice	–
1442	Nam pak kood	Northern lowland rice	–
1443	Taeng aon	Northern lowland rice	–
1446	Nhew sam phiw	Northern lowland rice	–
1448	Man tun	Northern lowland rice	–
1449	Feung leung	Northern lowland rice	–
1451	Ka long	Northern lowland rice	–
1454	Lang kay mhon	Northern lowland rice	–
1455	Lang kea lae	Northern lowland rice	–
1458	Kab kaew	Northern lowland rice	–
1468	Dor nok kaen	Northern lowland rice	–
1471	Hwi	Northern lowland rice	–
1473	Dok pud yang	Northern lowland rice	–
1484	Nhew san pha tong	Northern lowland rice	<i>Pikp group</i>
1487	Ma tan	Northern lowland rice	–
2120	Lay hla	Northern lowland rice	–
2934	Lab pe	Northern lowland rice	–
3101	Dor ma na	Northern lowland rice	–
3105	Kae meaw	Northern lowland rice	–
3131	Leung hom	Northern lowland rice	–
3134	Khaw ti	Northern lowland rice	–
3141	Mae hang	Northern lowland rice	–
3167	Lay loung	Northern lowland rice	–
3173	Ma ha wong	Northern lowland rice	–
3177	Pha kong noi	Northern lowland rice	–
3178	Dor hom	Northern lowland rice	–
607	Nhew khaw	Northeastern lowland rice	–
2720	Khi tum hom	Northeastern lowland rice	–
3247	Kaen jan	Northeastern lowland rice	–
3249	Leung tong	Northeastern lowland rice	–
3268	Ku kum	Northeastern lowland rice	–
3269	Dor hom	Northeastern lowland rice	–
3270	Hom nang nooan	Northeastern lowland rice	–
3327	Ai pun	Northeastern lowland rice	–
3358	Khaw krung	Northeastern lowland rice	–
3361	Khaw krung	Northeastern lowland rice	–
3366	Khaw set thi	Northeastern lowland rice	–
3369	Khaw kam	Northeastern lowland rice	–
4068	Hom hud	Northeastern lowland rice	–
4087	Hom ka set	Northeastern lowland rice	–
4489	Nhew hom ma li	Northeastern lowland rice	–
4496	Nhew loy	Northeastern lowland rice	–
4813	Khaw hom	Northeastern lowland rice	–
4819	Khaw hom	Northeastern lowland rice	–
4842	Khaw hom	Northeastern lowland rice	–
5601	Ai dang	Northeastern lowland rice	–
5624	Hom tung	Northeastern lowland rice	–
5648	Dor dang	Northeastern lowland rice	–

Table 1 (continued)

GS. Number	Name	Category	Pik gene
5671	Khaw kung	Northeastern lowland rice	–
5729	Nang nooan	Northeastern lowland rice	–
5808	Leung aon	Northeastern lowland rice	–
5826	Khaw ma li	Northeastern lowland rice	–
6721	Ai khaw hom	Northeastern lowland rice	–
6735	Hom in tok	Northeastern lowland rice	–
7613	Hang yi 71	Northeastern lowland rice	–
7627	Khi tum kam nan	Northeastern lowland rice	–
7655	Hom pla siw	Northeastern lowland rice	–
7672	Hom phae pha lo	Northeastern lowland rice	–
7971	Khaw nak	Northeastern lowland rice	–
7983	Leung lay	Northeastern lowland rice	–
7997	Bak mouy	Northeastern lowland rice	–
10669	Dang hloung	Northeastern lowland rice	–
10670	Khaw pha ma	Northeastern lowland rice	–
10677	Pra in	Northeastern lowland rice	–
10684	Khaw krung	Northeastern lowland rice	–
10689	Ma ou	Northeastern lowland rice	–
10693	Ai ko	Northeastern lowland rice	–
12154	Dok rim	Northeastern lowland rice	–
12155	Dor khaw	Northeastern lowland rice	–
12498	Leung klang	Northeastern lowland rice	–
13996	Mang dan	Northeastern lowland rice	–
788	Khaw sa ong	Central lowland rice	–
789	Khaw phoung	Central lowland rice	–
1626	Khaw hom	Central lowland rice	–
1628	Hom tung	Central lowland rice	–
1629	Hom tung	Central lowland rice	–
1751	Leung hom	Central lowland rice	–
2018	Hom in dia	Central lowland rice	–
2747	Hom ma li	Central lowland rice	–
3683	Khaw cha wat	Central lowland rice	–
3686	Ta pi	Central lowland rice	–
3688	Khaw sa wad	Central lowland rice	–
6155	Khaw ta hang	Central lowland rice	–
6162	Khaw kaew	Central lowland rice	–
6426	Hom ma li	Central lowland rice	–
6444	Leung hom	Central lowland rice	–
6455	Leung teiy	Central lowland rice	–
6465	Leung hom	Central lowland rice	–
6480	Bao hom	Central lowland rice	–
6515	Nang yuon	Central lowland rice	–
6517	Bang ka pi	Central lowland rice	–
6543	Kaen taeng	Central lowland rice	–
6887	Nang pa ya	Central lowland rice	–
9169	Khaw luong	Central lowland rice	–
9554	Leung ra hang	Central lowland rice	–
9559	Leung tong kum	Central lowland rice	–
9565	Khaw e jeek	Central lowland rice	–
9574	Leung pra tiw	Central lowland rice	–
9583	Khaw kor dew	Central lowland rice	–
9598	Khaw set thi	Central lowland rice	–
10775	Khaw luong	Central lowland rice	–
10858	Luong pra taan teiy	Central lowland rice	–
11427	Leung aon	Central lowland rice	–
14474	Khaw sa mer	Central lowland rice	–
14484	Leung nooan	Central lowland rice	–
15954	Ti si	Central lowland rice	–
15957	Leung roi et	Central lowland rice	–
15971	Leung tong kum	Central lowland rice	–
15974	Ha roun hom	Central lowland rice	–
18203	Nang dam	Central lowland rice	–
19860	Poung pa yeom	Central lowland rice	Pikp group
20960	Khaw pra koud	Central lowland rice	–
20987	Khaw ta hang rang si	Central lowland rice	–
21347	Leung pin kaew	Central lowland rice	–
21920	Khaw sa aad	Central lowland rice	–
22041	Hom ku laab	Central lowland rice	–
22042	Hom ta kraï	Central lowland rice	Pikp group
22043	Pin kaew bao	Central lowland rice	–
23898	Pra tun ta ni 1	Central lowland rice	–
1769	Tul	Southern lowland rice	–

(continued on next page)

Table 1 (continued)

GS. Number	Name	Category	Pik gene
4105	Dang chai ta lae	Southern lowland rice	–
4175	Sai khaw	Southern lowland rice	–
4208	Kor lae	Southern lowland rice	–
4219	Chong re	Southern lowland rice	<i>Pikp group</i>
4254	Ya sai	Southern lowland rice	–
4295	Khaw bao	Southern lowland rice	–
4313	Khaw kerd	Southern lowland rice	–
4315	Khaw look wein	Southern lowland rice	–
4318	Nhew look phung	Southern lowland rice	–
4371	Hom jan	Southern lowland rice	–
4524	Hoa na	Southern lowland rice	–
4539	Bao nam kaang	Southern lowland rice	<i>Pikp group</i>
4561	Hom jan	Southern lowland rice	–
4663	pa ya yud chang	Southern lowland rice	–
5206	Hoa na	Southern lowland rice	<i>Pikp group</i>
5209	Look chang	Southern lowland rice	–
5212	Unknown	Southern lowland rice	–
7000	Sang yood	Southern lowland rice	–
7009	Hom jan	Southern lowland rice	–
7032	Hom jan	Southern lowland rice	–
7064	Khaw hom	Southern lowland rice	–
7065	Hom jan	Southern lowland rice	–
7401	Khae nok	Southern lowland rice	–
9673	Hong tong	Southern lowland rice	–
9747	Nhew hom dam	Southern lowland rice	<i>Pikp group</i>
9862	Hom	Southern lowland rice	<i>Pikp group</i>
9943	Puong waii	Southern lowland rice	–
9969	Look dang	Southern lowland rice	–
10251	Nuoy keua	Southern lowland rice	–
10396	Bao hom	Southern lowland rice	–
10406	Pin kaew	Southern lowland rice	–
14441	Pin kaew bao	Southern lowland rice	–
14450	Nhew tan	Southern lowland rice	–
15541	Hom ma li	Southern lowland rice	–
16737	Chang oum	Southern lowland rice	–
16898	Bao look wai	Southern lowland rice	–
17339	Hom look kery	Southern lowland rice	–
19544	Leung aon	Southern lowland rice	–
20318	Tong hom	Southern lowland rice	<i>Pikp group</i>
20811	Hom chai ya	Southern lowland rice	–
21962	Lab nok pad ta ni	Southern lowland rice	–
21963	Look dang pad ta ni	Southern lowland rice	–
21964	Cheang pad ta lung	Southern lowland rice	–
21976	Cheang	Southern lowland rice	–
421	Dor hom 26	Northern upland rice	–
1370	Nhew san pha tong	Northern upland rice	–
1385	Haw	Northern upland rice	–
1452	Dok pud	Northern upland rice	–
1501	Ai lung	Northern upland rice	–
1502	Kai hai	Northern upland rice	–
1831	Paer nai khaw	Northern upland rice	<i>Pikp group</i>
1836	Unknown	Northern upland rice	<i>Piks</i>
1840	Lor	Northern upland rice	–
3084	Khaw reng	Northern upland rice	<i>Pikm</i>
4446	Hom mae jo	Northern upland rice	–
4457	Ma waii	Northern upland rice	<i>Piks</i>
5127	Feung kum	Northern upland rice	<i>Pikp group</i>
5133	Dor laos	Northern upland rice	–
5147	Long khi kai	Northern upland rice	–
5149	Hom tong	Northern upland rice	–
5151	Feung ngam	Northern upland rice	–
5152	Unknown	Northern upland rice	–
5158	Dor hom	Northern upland rice	–
5171	Dor hom	Northern upland rice	–
5249	Dor hom	Northern upland rice	–
6652	Siw dang	Northern upland rice	<i>Pikp group</i>
6981	Jao hom	Northern upland rice	–
7529	Kra teun dang	Northern upland rice	–
11702	Se sa	Northern upland rice	–
11736	Long khi kay	Northern upland rice	–
17656	Lab chang	Northern upland rice	–
17739	Leung	Northern upland rice	<i>Pikp group</i>
19676	Nhew lai	Northern upland rice	–

Table 1 (continued)

GS. Number	Name	Category	Pik gene
19739	Unknown	Northern upland rice	–
19753	Khaw haw	Northern upland rice	Pikp group
19774	Unknown	Northern upland rice	–
21512	Ja tae	Northern upland rice	–
21514	Or mae ma	Northern upland rice	Pikp group
21524	Ja nor beu	Northern upland rice	–
21543	Beeaw keem seeaw	Northern upland rice	Pikp group
21548	Blae blood	Northern upland rice	–
21549	Blae la goo goh	Northern upland rice	–
21557	Blae kor	Northern upland rice	–
22359	Leung pra tan yery wai	Northern upland rice	–
22365	Ma li 3	Northern upland rice	–
22399	Lee sor	Northern upland rice	–
22416	Kam	Northern upland rice	–
23012	Blae jai	Northern upland rice	–
23145	Khaw pai	Northern upland rice	–
23163	Ja nu nair nair	Northern upland rice	Pikp group
1664	Khaw how	Northeastern upland rice	–
1872	Nhew dam	Northeastern upland rice	–
1891	Hom	Northeastern upland rice	Pikp group
1895	Ai non	Northeastern upland rice	Pikp group
3219	Khaw how	Northeastern upland rice	–
4009	Leung chang	Northeastern upland rice	–
4476	Ai neung	Northeastern upland rice	–
5787	Hom dong	Northeastern upland rice	–
6718	Dor hom	Northeastern upland rice	–
6734	Hom dong	Northeastern upland rice	–
7932	Rak hairng	Northeastern upland rice	–
7956	Jao dang	Northeastern upland rice	–
7962	Khaw rai	Northeastern upland rice	–
7975	Chao Kao	Northeastern upland rice	–
10164	Krim kra chai	Northeastern upland rice	–
13585	Khaw	Northeastern upland rice	Pikp group
13594	Dang	Northeastern upland rice	–
14037	Lai	Northeastern upland rice	–
14464	Khaw ta pae	Northeastern upland rice	–
14539	Hom dong	Northeastern upland rice	–
15125	Chao kao	Northeastern upland rice	–
17632	Nang nooan	Northeastern upland rice	–
17640	Nang sa oad	Northeastern upland rice	–
17679	Kam	Northeastern upland rice	–
17683	Hang moo	Northeastern upland rice	–
20281	Plong airw	Northeastern upland rice	–
20282	Dok pra doo	Northeastern upland rice	–
20283	Ai leung	Northeastern upland rice	–
21696	Khaw saw	Northeastern upland rice	–
22718	Khaw tud ngon	Northeastern upland rice	–
22724	Pra siw	Northeastern upland rice	–
22809	Long na	Northeastern upland rice	–
667	PW 237	Central upland rice	–
886	Khaw pa ma	Central upland rice	–
1503	55NM S-4Neu	Central upland rice	–
3668	Khaw dang	Central upland rice	–
3765	Leung	Central upland rice	–
4410	Hom aum	Central upland rice	–
5459	Hom bai	Central upland rice	–
6230	Lory ha rooang	Central upland rice	–
6390	Leung kra bang	Central upland rice	–
6544	Puong tong	Central upland rice	–
6871	Khaw dang	Central upland rice	–
6925	Khaw ka men	Central upland rice	–
8077	Eree wong pu dong	Central upland rice	–
8082	Pery ter	Central upland rice	–
8088	Beung wo	Central upland rice	–
8100	Ai thai	Central upland rice	–
8104	Beung eang wong oa	Central upland rice	–
8135	Bae ooa poo	Central upland rice	–
8145	Neua paer	Central upland rice	–
8155	Bae bong	Central upland rice	–
8163	Ai kao pae	Central upland rice	–
8944	Khaw ah kad	Central upland rice	–
11069	Khaw dok ma li	Central upland rice	–

(continued on next page)

Table 1 (continued)

GS. Number	Name	Category	Pik gene
11457	Pra perng	Central upland rice	–
11565	Leung hai	Central upland rice	–
14296	Hin kong	Central upland rice	–
14297	Ta pao lom	Central upland rice	–
15833	Nam sa kuy 19	Central upland rice	–
19681	Pleuak khaw	Central upland rice	–
19684	Unknown	Central upland rice	–
21418	Yao hai	Central upland rice	–
21825	Beu ner ber	Central upland rice	–
21829	Oay leeaw	Central upland rice	–
22327	Jao khaw	Central upland rice	–
22931	Leung rai	Central upland rice	–
22932	Pooh yai lee	Central upland rice	–
23073	Hom ma li rai	Central upland rice	–
23083	Pa per	Central upland rice	–
23084	Khaw dang	Central upland rice	–
23087	Unknown	Central upland rice	–
23088	Unknown	Central upland rice	–
1911	Dok hom	Southern upland rice	–
1931	Sai	Southern upland rice	–
1947	Dok pa yeom	Southern upland rice	–
8118	Hom poang	Southern upland rice	–
11131	Lab nok	Southern upland rice	–
12763	Ya mor	Southern upland rice	–
12764	Ya yor	Southern upland rice	–
12811	Leung	Southern upland rice	–
12813	Soo por ming	Southern upland rice	–
12815	Nang muy dok kairk	Southern upland rice	–
12817	Hom	Southern upland rice	–
12818	Feun din	Southern upland rice	–
12823	Pa sae	Southern upland rice	–
12848	Mornng	Southern upland rice	–
12852	Ar nor ma dor	Southern upland rice	–
12854	Seu ra	Southern upland rice	–
12856	Ya bo je nor	Southern upland rice	–
12866	Mor me ngeng	Southern upland rice	–
12872	Leung	Southern upland rice	–
14330	Bao leung	Southern upland rice	–
14363	Book yah	Southern upland rice	–
15524	Keeaw	Southern upland rice	–
15536	Je san	Southern upland rice	–
15727	Dok praw	Southern upland rice	–
15775	Ya lam pae	Southern upland rice	–
15789	Yi tair sa ming	Southern upland rice	–
16461	Dok pud	Southern upland rice	–
16691	Dok mud	Southern upland rice	–
17655	Bao ti	Southern upland rice	–
17764	Aun lee ba ba	Southern upland rice	–
19248	Pae nae	Southern upland rice	–
19271	Nang rung	Southern upland rice	–
20348	Khaw ma lay	Southern upland rice	–
23795	Khaw rai	Southern upland rice	–
23798	Kem ngern rai	Southern upland rice	–
23810	Nhew dam pleuak dam	Southern upland rice	–
23813	Nhew dam pleuak dam	Southern upland rice	–
23819	Nhew dang	Southern upland rice	–
23837	Nhew dam pleuak khaw	Southern upland rice	–
23840	Dam nok la	Southern upland rice	–
23845	Dok mud	Southern upland rice	–
23846	Dok pa yeom rai	Southern upland rice	–
23856	Dok pa yeom	Southern upland rice	–
23859	Nhew dam rai	Southern upland rice	–
23860	Lab nok rai	Southern upland rice	–
7125	RD27	Improved rice	–
16233	Phitsanulok 60-1	Improved rice	–
16235	Chum phae 60	Improved rice	–
16410	RD13	Improved rice	–
19326	RD15	Improved rice	–
20864	Plai ngahm prachin buri	Improved rice	–
22051	RD8	Improved rice	–
22788	RD6	Improved rice	–
23406	Khaw jao prachin buri 1	Improved rice	–
23407	Nhew ubon1	Improved rice	–

Table 1 (continued)

GS. Number	Name	Category	<i>Pik</i> gene
23408	Nhew ubon 2	Improved rice	<i>Pikp</i> group
23409	Khaw jao hom phitsanulok 1	Improved rice	<i>Pikp</i> group
24546	Phitsanulok 3	Improved rice	–
24547	Phitsanulok 80	Improved rice	<i>Pikp</i> group
24595	Ayudtaya 1	Improved rice	–
24597	RD12	Improved rice	–
24600	RD35	Improved rice	<i>Pikp</i> group
24608	RD45	Improved rice	–
24625	Sang yod phattalung	Improved rice	–
15262	Leung	Improved rice	–
21857	Khaw pong kraai	Improved rice	–

– = Thai rice varieties lacking the *Pik* resistance gene.

Pik alleles from the host (*Pik*, *Pikm*, *Pikp*, *Piks*, *Pikh*, *Pik1* and *Pi7*) and five *Avr-Pik* alleles from the pathogen (*Avr-PikA*, *Avr-PikB*, *Avr-PikC*, *Avr-PikD* and *Avr-PikE*) (Constanzo and Jia, 2010; Kanzaki et al., 2012; Yoshida et al., 2009). The recognition specificity of each *Pik* allele to its *Avr-Pik* allele differs from one allele to another. The *Pikp* allele from the Japanese rice variety K60 recognizes only the *Avr-PikD* allele. The *Pik* allele from the Japanese rice variety Kanto51 recognizes the *Avr-PikD* and *Avr-PikE* alleles but not the *Avr-PikA*, *Avr-PikB* and *Avr-PikC* alleles. The *Pikm* allele from the Japanese rice variety Tsuyuake recognizes the *Avr-PikA*, *Avr-PikD* and *Avr-PikE* alleles but not the *Avr-PikB* and *Avr-PikC* alleles. The *Pi7* allele from the Thai rice variety Jao Hom Nin recognizes only the *Avr-PikD* allele (Chaipanya et al., 2017; Kanzaki et al., 2012). From the specific interaction pattern between the *Pik* and *Avr-Pik* alleles, the *Pik* alleles can be classified into three groups based on their disease spectra. The *Pikm* group including *Pikm*, *Pikh* and *Pi1* shows a broad disease spectrum. The *Pikp* group including *Pikp*, *Pik* and *Pi7* shows a narrow disease spectrum, while the *Piks* group is susceptible to all blast fungal isolates harboring any of the five *Avr-Pik* alleles (Chaipanya et al., 2017). The objectives of this study were to detect the rice blast resistance gene, *Pik*, in Thai rice germplasm and to identify their *Pik* alleles. The information generated from this research can be used by breeders and researchers to identify parental lines for rice blast resistance breeding programs and rice blast research in Thailand.

Materials and methods

Plant materials

In total, 373 rice varieties were examined from Thai rice germplasm, consisting of 96 rice varieties from Northern Thailand, 90 rice varieties from Southern Thailand, 89 rice varieties from Central Thailand, 77 rice varieties from Northeastern Thailand and 21 improved rice varieties (Table 1).

Genomic DNA extraction

Genomic DNA was extracted from immature leaf samples using the modified protocol from Doyle and Doyle (1987). Each leaf sample was ground in liquid nitrogen and then cetyltrimethylammonium bromide buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 100 mM NaCl, 2% SDS) was added and incubated at 65 °C for 60 min. Chloroform-isoamyl alcohol (24:1 ratio) was added to fill up the tube and mixed by shaking for 2–3 min, then centrifuged at 12,000 rpm and 25 °C for 10 min. Next, the aqueous portion (upper layer) was transferred to a new tube and 1 mL of ice-cold isopropanol added and with the tube being gently inverted several times and then incubated overnight at 4 °C. After incubation, the sample was centrifuged at 12,000 rpm and 25 °C for 10 min and

washed with 70% ethanol. The solution was discarded and the DNA was allowed to dry for 5 min, before adding 100 µL of TE buffer and storing the DNA sample at 4 °C. Each DNA sample was quantified using a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific Inc.; Waltham, MA, USA) and the DNA quality was checked on a 1% agarose gel electrophoresis, stained with ethidium bromide, visualized and photographed using an infinity 3000 gel photographic system (Vilber Lourmat; Eberhardzell, Germany).

Rice blast resistance gene, *Pik*, screening with *Pik* gene specific primers

The genomic DNA samples from the 373 Thai rice varieties were checked for the presence/absence of the *Pik* gene with gene specific primers designed in this study: *RGA4_F3* (5'-GGAAAGCTGATATGTTGTCG-3') and *RGA4_R3* (5'-ACTCGGAGTCGGAGAGTCAG-3'). Polymerase chain reaction (PCR) was performed in a total volume of 10 µL reaction, consisting of 6.65 µL of sterile distilled water, 1 µL of 10xPCR buffer A, 0.25 µL of 50 mM MgCl₂, 1 µL of 1 mM dNTPs mix, 0.1 µL of 5 units/µL Taq DNA polymerase enzyme (Vivantis; Subang Jaya, Selangor, Malaysia) and 0.5 µL of 50 ng/µL rice genomic DNA. The PCR cycling program was initiated by denaturation for 2 min at 94 °C, followed by 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 55 °C, 50 s of extension at 72 °C and a final extension at 72 °C for 5 min. The PCR products were determined using 1% agarose gel electrophoresis at 100 V for 30 min, stained with ethidium bromide and photographed under ultraviolet light using an infinity 3000 gel photographic system (Vilber Lourmat; Eberhardzell, Germany).

Allele identification of *Pik* rice blast resistance gene

The amplified PCR products were digested by the restriction enzyme *Pst*I (New England Biolabs; Ipswich, MA, USA) for identification of the *Pik* resistance gene group. The recognition site of the *Pst*I enzyme is present in the *Pik* resistance gene *Pikp* group (*Pik*, *Pikp* and *Pi7* alleles) but not present in the *Pik* resistance gene *Pikm* group (*Pikm*, *Pi1* and *Pikh* alleles) and the *Piks* group. If the amplified PCR products can be digested with the *Pst*I enzyme, the *Pik* resistance gene belongs to the *Pikp* group. If the amplified PCR products cannot be digested with the *Pst*I enzyme, the *Pik* resistance gene belongs to either the *Pikm* group or the *Piks* group. The total volume of the digestion reaction was 10 µL, which consisted of 8 µL of PCR products, 1 µL of digestion buffer and 1 µL of the *Pst*I restriction enzyme. The digestion reaction was incubated at room temperature for 30 min and determined using 1% agarose gel electrophoresis at 100 V for 30 min, stained with ethidium bromide and photographed under ultraviolet light using an infinity 3000 gel photographic system (Vilber Lourmat; Eberhardzell, Germany).

In this study, the *Pik* resistance gene *Pikm* group, which shows broad disease spectrum, was the main interest, so the PCR products from rice samples, which could not be digested by the restriction enzyme *Pst*I were sent for sequencing at MacroGen, Korea to confirm the nucleotide sequence of the *Pik* allele. For the sequencing step, the primers *Pikm_F* (5'-CGTGTTCAGATTCCCATGG-3') and *Pikm_R* (5'-GGAAAGCTGATATGTTGTCG-3') were designed in this study. The PCR reaction was performed in a total volume of 10 μ L reaction, consisting of 6.65 μ L of sterile distilled water, 1 μ L of 10xPCR buffer A, 0.25 μ L of 50 mM MgCl₂, 1 μ L of 1 mM dNTPs mix, 0.1 μ L of 5 units/ μ L Taq DNA polymerase and 0.5 μ L of 50 ng/ μ L rice genomic DNA. The PCR cycling program was initiated by denaturation for 2 min at 94 °C, followed by 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 58 °C, 50 s of extension at 72 °C and a final extension at 72 °C for 5 min. The PCR products were determined using 1% agarose gel electrophoresis at 100 V for 30 min, stained with ethidium bromide and photographed under ultraviolet light using an infinity 3000 gel photographic system (Vilber Lourmat; Eberhardzell, Germany). The PCR product was then sent out for sequencing at MacroGen, Korea. The obtained nucleotide sequences were used to determine the *Pik* allele by comparison with the *Pik* allele reference sequences *Pi1-5* (GenBank accession number: HQ606329.1), *Pikm-1* (GenBank accession number: KU365330.1) and *Piks-1* (GenBank accession number: HQ662329.1) using MAFFT v7.380.

Results

Distribution of *Pik* resistance gene in Thai rice germplasm

Twenty-nine out of the 373 Thai rice varieties gave the amplification product from the *Pik* gene specific primer, which was an approximately 1200 bp PCR product (Table 1). Fourteen rice varieties were from Northern Thailand: Pha peing, Joa hom, Nheu san pha tong, Paer nai khaw, U8 unknown, Khaw reng, Ma waii, Feung kum, Siw dang, Leung, Khaw haw, Or mae ma, Beeaw keem seeaw and Ja nu nair nair. Six rice varieties were from Southern Thailand: Chong re, Bao nam kaang, Hoa na, Nheu hom dam, Hom and Tong hom. Three rice varieties were from North-eastern Thailand: Hom, Ai non and Khaw. Two rice varieties were from Central Thailand: Pong pa yeom and Hom ta kraai and four rice varieties were improved rice varieties: Nheu ubon 2, Khaw jao hom Phitsanulok 1, Phitsanulok 80 and RD35. The Jao Hom Nin rice variety, known to contain the *Pik* gene, was used as the positive control.

Analysis of *Pik* resistance gene

To determine the allele of the *Pik* resistance gene, all 29 PCR products from the Thai rice varieties were digested with the *Pst*I restriction enzyme. The results showed that the PCR products from 25 Thai rice varieties could be digested by the *Pst*I restriction

enzyme giving product sizes of 1000 and 200 bp (Fig. 1). These 25 Thai rice varieties were: Pha peing, Nheu san pha tong, Pong pa yeom, Hom ta kraai, Chong re, Bao nam kaang, Hoa na, Nheu hom dam, Hom, Tong hom, Paer nai khaw, Feung kum, Siw dang, Leung, Khaw haw, Or mae ma, Beeaw keem seea, Ja nu nair nair, Hom, Ai non, Khaw, Nheu ubon 2, Khaw jao hom Phitsanulok 1, Phitsanulok 80 and RD35. PCR product amplification from four rice varieties (Jao hom, U8 unknown, Khaw reng and Ma waii) could not be digested by the *Pst*I restriction enzyme. These four rice varieties contain either the *Pik* resistance gene *Pikm* group (*Pikm*, *Pi1* and *Pikh* alleles) or the *Piks* group.

In order to determine the *Pik* resistance allele in these four rice varieties, genomic DNA samples were used to amplify by the *Pikm* and *Piks* group specific primer using a PCR technique. All four rice varieties gave an amplification product size of 525 bp. The nucleotide sequences were compared with reference sequences of *Pik* alleles using MAFFT v7.380. The results showed that nucleotide sequence from the Khaw reng rice variety was identical to the *Pikm* allele and the nucleotide sequences from the U8 Unknown, Ma waii and Jao hom were identical to the *Piks* allele (Figs. 2 and 3).

Discussion

This study screened the presence of the rice blast resistance gene, *Pik*, in 373 Thai rice varieties. Less than 10% of the Thai rice varieties (29 out of 373) had the *Pik* gene (Table 2). Rice varieties containing the *Pik* gene were distributed in all four regions of Thailand. However, the distribution rates were less than 10% except in Northern Thailand (14.58%). Even in the 21 improved rice varieties, only four rice varieties (19.04%) contained the *Pik* gene. This result indicated that the rice blast resistance gene, *Pik*, had a low occurrence in the Thai rice gene pool. The rice blast resistance gene, *Pik*, has been shown to have broad-spectrum resistance against rice blast populations in Thailand (Chaipanya et al., 2017; Sreewongchai, 2008). The *Pik* gene is widely used in both conventional and molecular breeding programs against rice blast disease in Thailand and the Thai rice variety, Jao Hom Nin (JHN), containing *Pik* gene was famously used as a resistant donor to introgress the *Pik* resistance gene from JHN into commercial Thai rice varieties, which are susceptible to rice blast fungus, with examples of these varieties being RD6 (Wongsaprom et al., 2010), Khoa Dok Mali 105 (Noenplab, 2011), IR77955-24-75-284 (Kotchawatit, 2013), Bahng Taen (Kaewcheenchai et al., 2014), Jao Hawm Phitsanulok51 (Noenplab et al., 2015) and San Par Tong1 (Yajai and Ketsuwan, 2015). Recently, Chaipanya et al. (2017) reported the presence of several rice blast isolates, which can break down *Pik* resistance in Thailand. The investigation of the *Pik* allele in JHN showed that JHN contains the *Pikp* allele, which can recognize only the *Avr-PikD* allele of the fungus. The need is high to identify new rice varieties that have a broader disease spectrum for rice blast disease. Since the *Pikm* resistance group including *Pikm*, *Pikh*, and *Pi1* is more effective than the *Pikp* resistance group,

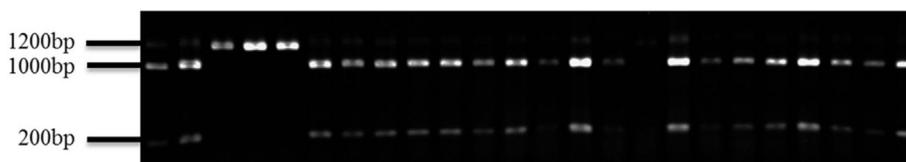


Fig. 1. Example of polymerase chain reaction products with RGA4 primer after digestion with *Pst*I restriction enzyme of Thai rice varieties: lane 1, JHN (positive control); lane 2, Paer nai khaw; lane 3, U8 unknown; lane 4, Khaw reng; lane 5, Ma waii; lane 6, Feung kum; lane 7, Siw deng; lane 8, Leung; lane 9, Khaw haw; lane 10, Or mae ma; lane 11, Ja nu nair nair; lane 12, Hom; lane 13, E nnon; lane 14, Khaw; lane 15, Pha peing; lane 16, Jao hom; lane 17, Nheu san pha tong; lane 18, Pong pa yeom; lane 19, Hom ta kraai; lane 20, Chong re; lane 21, Bao nam kaang; lane 22, Hoa na; lane 23, Nheu hom dam; lane 24, Hom.

```

1829                                     1888
U8 Unknown atggtggacgataagagccgtacaaaagcaatgtcattggttgcaagtacggttggagt
Khaw reng atggtggacgataagagccgtacaaaagcaatgtcattggttgcaagcacggttggagt
Ma waii atggtggacgataagagccgtacaaaagcaatgtcattggttgcaagtacggttggagt
Jao hom atggtggacgataagagccgtacaaaagcaatgtcattggttgcaagtacggttggagt
Pil-5 atggtggacgataagagccgtacaaaagcaatgtcattggttgcaagtacggttggagt
Pikm-1 atggtggacgataagagccgtacaaaagcaatgtcattggttgcaagcacggttggagt
Piks-1 atggtggacgataagagccgtacaaaagcaatgtcattggttgcaagtacggttggagt
*****.*****
1889                                     1948
U8 Unknown cactcgggtgcaatcgccggtgacctaaagagacgaggttgggtggtcggtgatggcatt
Khaw reng cactcgggtgcaatcgccggtgacctaaagagacgaggttgggtggtcggtgatggcatt
Ma waii cactcgggtgcaatcgccggtgacctaaagagacgaggttgggtggtcggtgatggcatt
Jao hom cactcgggtgcaatcgccggtgacctaaagagacgaggttgggtggtcggtgatggcatt
Pil-5 cactcgggtgcaatcgccggtgacctaaagagacgaggttgggtggtcggtgatggcatt
Pikm-1 cactcgggtgcaatcgccggtgacctaaagagacgaggttgggtggtcggtgatggcatt
Piks-1 cactcgggtgcaatcgccggtgacctaaagagacgaggttgggtggtcggtgatggcatt
*****
1949                                     2008
U8 Unknown gactccatcaatctggtctctgcgctccggaagaaggtgggcccctgcgatgtttctggag
Khaw reng gactccatcaatctggtctctgcgctccggaagaaggtgggcccctgcgatgtttctggag
Ma waii gactccatcaatctggtctctgcgctccggaagaaggtgggcccctgcgatgtttctggag
Jao hom gactccatcaatctggtctctgcgctccggaagaaggtgggcccctgcgatgtttctggag
Pil-5 gactccatcaatctggtctctgcgctccggaagaaggtgggcccctgcgatgtttctggag
Pikm-1 gactccatcaatctggtctctgcgctccggaagaaggtgggcccctgcgatgtttctggag
Piks-1 gactccatcaatctggtctctgcgctccggaagaaggtgggcccctgcgatgtttctggag
*****
2009                                     2068
U8 Unknown gtcagccaagcaaaaggaagatgtgaaggagataacggcgatgcttgcgcccgtgaaatcc
Khaw reng gtcagccaagcaaaaggaagatgtgaaggagataacggcgatgcttgcgcccgtgaaatcc
Ma waii gtcagccaagcaaaaggaagatgtgaaggagataacggcgatgcttgcgcccgtgaaatcc
Jao hom gtcagccaagcaaaaggaagatgtgaaggagataacggcgatgcttgcgcccgtgaaatcc
Pil-5 gtcagccaagcaaaaggaagatgtgaaggagataacggcgatgcttgcgcccgtgaaatcc
Pikm-1 gtcagccaagcaaaaggaagatgtgaaggagataacggcgatgcttgcgcccgtgaaatcc
Piks-1 gtcagccaagcaaaaggaagatgtgaaggagataacggcgatgcttgcgcccgtgaaatcc
*****
2069                                     2128
U8 Unknown atatgtgaatttcacgaggtcaaaaacaatttgcatccttggattgccagggggaggcaaa
Khaw reng atatgtgaatttcacgaggtcaaaaacaatttgcatccttggattgccagggggaggcaaa
Ma waii atatgtgaatttcacgaggtcaaaaacaatttgcatccttggattgccagggggaggcaaa
Jao hom atatgtgaatttcacgaggtcaaaaacaatttgcatccttggattgccagggggaggcaaa
Pil-5 atatgtgaatttcacgaggtcaaaaacaatttgcatccttggattgccagggggaggcaaa
Pikm-1 atatgtgaatttcacgaggtcaaaaacaatttgcatccttggattgccagggggaggcaaa
Piks-1 atatgtgaatttcacgaggtcaaaaacaatttgcatccttggattgccagggggaggcaaa
*****
2129                                     2188
U8 Unknown acaacgattgcccagtgactatatacatgcatggtggaacgcagttccaatgcccgggttttc
Khaw reng acaacgattgcccagtgactatatacatgcatggtggaacgcagttccaatgcccgggttttc
Ma waii acaacgattgcccagtgactatatacatgcatggtggaacgcagttccaatgcccgggttttc
Jao hom acaacgattgcccagtgactatatacatgcatggtggaacgcagttccaatgcccgggttttc
Pil-5 acaacgattgcccagtgactatatacatgcatggtggaacgcagttccaatgcccgggttttc
Pikm-1 acaacgattgcccagtgactatatacatgcatggtggaacgcagttccaatgcccgggttttc
Piks-1 acaacgattgcccagtgactatatacatgcatggtggaacgcagttccaatgcccgggttttc
*****
2189                                     2248
U8 Unknown gcatcaatctctccaagttccagccccagttcccaatctaacagagactcttgcagacatt
Khaw reng gcatcaatctctccaagttccagccccagttcccaatctaacagagactcttgcagacatt
Ma waii gcatcaatctctccaagttccagccccagttcccaatctaacagagactcttgcagacatt
Jao hom gcatcaatctctccaagttccagccccagttcccaatctaacagagactcttgcagacatt
Pil-5 gcatcaatctctccaagttccagccccagttcccaatctaacagagactcttgcagacatt
Pikm-1 gcatcaatctctccaagttccagccccagttcccaatctaacagagactcttgcagacatt
Piks-1 gcatcaatctctccaagttccagccccagttcccaatctaacagagactcttgcagacatt
*****
2249                                     2298
U8 Unknown ttcgctcaagcacaactaggagtaactgatacacttagcacaccataatgg
Khaw reng ttcgctcaagcacaactaggagtaactgatacacttagcacaccataatgg
Ma waii ttcgctcaagcacaactaggagtaactgatacacttagcacaccataatgg
Jao hom ttcgctcaagcacaactaggagtaactgatacacttagcacaccataatgg
Pil-5 ttcgctcaagcacaactaggagtaactgatacacttagcacaccataatgg
Pikm-1 ttcgctcaagcacaactaggagtaactgatacacttagcacaccataatgg
Piks-1 ttcgctcaagcacaactaggagtaactgatacacttagcacaccataatgg
*****

```

Fig. 2. Alignment of multiple sequences using MAFFT v7.380 to compare the nucleotide sequences from four Thai rice germplasm samples (U8 unknown, Khaw reng, Ma waii, Jao hom) with the *Pikm* group: *Pik1-5* (GenBank accession number: HQ606329.1), *Pikm-1* (GenBank accession number: KU365330.1), *Piks-1* (GenBank accession number: HQ62329.1).

U8 Unknown	MVDDKSR TKAMSLVASTVGVH SVA IAGDLRDEVV VVVDGID SINLVSALRKKVGFAMFLE	60
Khaw reng	MVDDKSR TKAMSLVASTVGVH SVA IAGDLRDEVV VVVDGID SINLVSALRKKVGFAMFLE	60
Ma waii	MVDDKSR TKAMSLVASTVGVH SVA IAGDLRDEVV VVVDGID SINLVSALRKKVGFAMFLE	60
Jao hom	MVDDKSR TKAMSLVASTVGVH SVA IAGDLRDEVV VVVDGID SINLVSALRKKVGFAMFLE	60
Pik1-5	MVDDKSR TKAMSLVASTVGVH SVA IAGDLRDEVV VVVDGID SINLVSALRKKVGFAMFLE	60
Pikm-1	MVDDKSR TKAMSLVASTVGVH SVA IAGDLRDEVV VVVDGID SINLVSALRKKVGFAMFLE	60
Piks-1	MVDDKSR TKAMSLVASTVGVH SVA IAGDLRDEVV VVVDGID SINLVSALRKKVGFAMFLE	60
	*****;*****.*****	
U8 Unknown	VSQAKEDVKEITAMLAPVKS ICEFHEVKTICILGLPGGGKTTIARVLYHALGTQFQCRVF	120
Khaw reng	VSQAKEDVKEITAMLAPVKS ICEFHEVKTICILGLPGGGKTTIARVLYHALGTQFQCRVF	120
Ma waii	VSQAKEDVKEITAMLAPVKS ICEFHEVKTICILGLPGGGKTTIARVLYHALGTQFQCRVF	120
Jao hom	VSQAKEDVKEITAMLAPVKS ICEFHEVKTICILGLPGGGKTTIARVLYHALGTQFQCRVF	120
Pik1-5	VSQAKEDVKEITAMLAPVKS ICEFHEVKTICILGLPGGGKTTIARVLYHALGTQFQCRVF	120
Pikm-1	VSQAKEDVKEITAMLAPVKS ICEFHEVKTICILGLPGGGKTTIARVLYHALGTQFQCRVF	120
Piks-1	VSQAKEDVKEITAMLAPVKS ICEFHEVKTICILGLPGGGKTTIARVLYHALGTQFQCRVF	120
	.**	
U8 Unknown	ASISPSSSPSPNLTETLADIFAQAQLGVTDTLSTPY	156
Khaw reng	ASISPSSSPSPNLTETLADIFAQAQLGVTDTLSTPY	156
Ma waii	ASISPSSSPSPNLTETLADIFAQAQLGVTDTLSTPY	156
Jao hom	ASISPSSSPSPNLTETLADIFAQAQLGVTDTLSTPY	156
Pik1-5	ASISPSSSPSPNLTETLADIFAQAQLGVTDTLSTPY	156
Pikm-1	ASISPSSSPSPNLTETLADIFAQAQLGVTDTLSTPY	156
Piks-1	ASISPSSSPSPNLTETLADIFAQAQLGVTDTLSTPY	156

Fig. 3. Alignment of multiple amino acid sequences using MAFFT v7.380. to compare the amino acid sequences from four Thai rice germplasm samples (U8 unknown, Khaw reng, Ma waii, Jao hom) with the *Pikm* group: *Pik1-5* (GenBank accession number: AEB00617.1), *Pikm-1* (GenBank accession number: ANO81528.1), *Piks-1* (GenBank accession number: AET36547.1).

Table 2
Distribution of *Pik* resistance gene in Thai rice germplasm.

Category	Number of rice varieties	Number of rice varieties with <i>Pik</i> gene (%)
Northern Thailand	96	14 (14.58)
Northeastern Thailand	77	3 (3.90)
Central Thailand	89	2 (2.25)
Southern Thailand	90	6 (6.67)
Improved rice	21	4 (19.04)
Total	373	29 (7.77)

identification of rice varieties harboring the *Pikm* resistance group is necessary for rice blast resistance breeding in Thailand.

The current study successfully found one rice variety (Khaw reng) out of 373 screened Thai rice varieties, containing the *Pikm* allele. This rice variety can be used as an elite resistant donor in conventional and molecular breeding programs for blast disease resistance in Thailand. However, since there is a low frequency of the *Pik* gene in Thai rice germplasm, Thai breeders may need to find new sources of the *Pik* rice blast resistance gene from other countries to strengthen the genetic diversity of *Pik* gene in Thailand rice gene pool. The information generated from this study should be useful to alert breeders and researchers in Thailand to identify other sources of rice blast resistance gene for rice blast resistance breeding programs.

Conflict of interest

The authors declare that there is no conflicts of interest.

Acknowledgements

The authors thank Mr. Robert Harman II for grammatically reviewing an earlier version of the manuscript. This research was supported by the Kasetsart University Research and Development Institute, the Graduate Program Scholarship from the Graduate School of Kasetsart University, the Foundation of Kasetsart

University and The University Consortium Student Travel Grant for Research (UC).

References

- Ashkani, S., Rafii, M., Shabanimofrad, M., Ghasemzadeh, A., Ravanfar, S., Latif, M., 2014. Molecular progress on the mapping and cloning of functional genes for blast disease in rice (*Oryza sativa* L.): current status and future considerations. *Crit. Rev. Biotechnol.* 36, 353–367.
- Belkadir, Y., Subramaniam, R., Dangl, J.L., 2004. Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Curr. Opin. Plant Biol.* 7, 391–399.
- Chaipanya, C., Telebanco-Yanoria, M.J., Quime, B., et al., 2017. Dissection of broad-spectrum resistance of the Thai rice variety Jao hom nin conferred by two resistance genes against rice blast. *Rice* 10, 18. <https://doi.org/10.1186/s12284-017-0159-0>.
- Chang, T.T., 1976. The origin, evolution, cultivation, dissemination and diversification of Asia and African rice. *Euphytica* 25, 425–441.
- Constanzo, S., Jia, Y., 2010. Sequence variation at the rice blast gene *Pi-km* locus: implications for the development of allele specific markers. *Plant Sci.* 178, 523–530.
- Disthaporn, S., 1994. Current rice blast epidemics and their management in Thailand. In: Zeigler, R.S., Leong, S.A., Teng, P.S. (Eds.), *Rice Blast Disease*. CAB International, Wallingford, UK.
- Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19, 11–15.
- Gururani, M.A., Venkatesh, J., Upadhyaya, C.P., Nookaraju, A., Pandey, S.K., Park, S.W., 2012. Plant disease resistance genes: current status and future directions. *Physiol. Mol. Plant Pathol.* 78, 51–65.
- Harlan, J.R., 1992. *Crop and Man*, second ed. American Society of Agronomy and Crop Science Society of America, Madison, WI, USA.
- Ito, S., Peterson, E.W.F., Grant, W.R., 1989. Rice in Asia: is it becoming an inferior good? *Am. J. Agric. Econ.* 71, 32–42.
- Kaewcheenchai, R., Sriwisut, S., Noenplab, A., et al., 2014. Improving blast resistance in a rice variety Bahng Taen by using marker-assisted backcrossing. In: *Proceeding of the 32nd Rice and Temperate Cereal Crop Annual Conference*. Petchaburi, Thailand, pp. 158–170.
- Kanzaki, H., Yoshida, K., Saitoh, H., et al., 2012. Arms race co-evolution of *Magnaporthe oryzae* AVR-Pik and rice *Pik* genes driven by their physical interactions. *Plant J.* 72, 894–907.
- Kotchasatit, A., 2013. UBN03007-47-7-26-35-19: an early maturing, non-glutinous promising rice line resistant to blast. In: *Proceedings of the 30th Rice and Temperate Cereal Crops Annual Conference 2013*. Bangkok, Thailand, pp. 56–71.
- Noenplab, A., 2011. Minimization of rice blast severity by means of multilines in the lower north. In: *Proceeding of Rice Research Symposium 2011: Rice Research Center Groups in Upper and Lower Northern Region*. Phrae, Thailand, pp. 225–241.
- Noenplab, A., Noenplab, A., Palawisut, S., 2015. Increasing blast resistance in HPSP 1 using marker assisted selection. In: *Proceedings of the 8th Rice Research*

- Conference 2015: Rice Research Center Groups in Upper and Lower Northern Region. Chiang Rai, Thailand, pp. 3–16.
- Ou, S.H., 1985. Rice Diseases. Commonwealth Mycological Institute, Kew, UK.
- Sharma, T.R., Rai, A.K., Gupta, S.K., Vijayan, J., Devanna, B., Ray, S., 2012. Rice blast management through host-plant resistance: retrospect and prospects. *Agri. Res.* 1, 37–52.
- Sreewongchai, T., 2008. Identification of *Magnaporthe grisea* Avirulence Genes Specific to Rice Blast Resistance Genes. Ph.D. thesis. Kasetsart University, Bangkok, Thailand.
- Wang, X., Lee, S., Wang, J., Ma, J., Bianco, T., Jia, Y., 2014. Current advances on genetic resistance to rice blast disease. In: Yan, W., Bao, J. (Eds.), *Rice-germplasm, Genetics and Improvement*. Intech, Rijeka, Croatia, pp. 195–217.
- Wongsaprom, C., Sirithunya, P., Vanavichit, A., et al., 2010. Two introgressed quantitative trait loci confer a broad-spectrum resistance to blast disease in the genetic background of the cultivar RD6 a Thai glutinous jasmine rice. *Field Crop. Res.* 119, 245–251.
- Yajai, P., Ketsuwan, K., 2015. The use of anther culture for developing rice blast resistant true breeding lines. In: *Proceedings of the 8th Rice Research Conference 2015: Rice Research Center Groups in Upper and Lower Northern Region*. Chiang Rai, Thailand, pp. 47–52.
- Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Yoshida, K., Tosa, Y., Chuma, I., Takano, Y., Win, J., Kamoun, S., Terauchi, R., 2009. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. *Plant Cell* 21, 1573–1591.