SOUPHANOUVONG JOURNAL 1 (2015) 21-25
SU: FAF/SJ.1.5.004

ການສຳຫຼວດ ລັກສະນະພັນຕ້ານທານພະຍາດໃບໄໝ້ຂອງເຂົ້າ ທີ່ປູກໃນພື້ນທີ່ແຂວງຫຼວງພະບາງ

ວິລະພອນ ກັນຍາສອນ* ແລະ ຫຼຸຍ ວັນນະມະຫາໄຊ

ພາກວິຊາ ວິທະຍາສາດພືດ, ຄະນະກະເສດສາດ ແລະ ຊັບພະຍາກອນປ່າໄມ້ ມະຫາວິທະຍາໄລສຸພານຸວົງ, ສປປ ລາວ

ບົດຄັດຫຍໍ້

ພະຍາດໄໝ້ຂອງເຂົ້າທີ່ເກີດຈາກເຊື້ອຣາ "*Pyricularia Oryzea*" ຈັດເປັນພະຍາດອັນດັບໜຶ່ງ ທີ່ມີໃນນາເຂົ້າ ໃນ ໂລກ ແລະ ສາມາດພົບເຫັນຢູ່ໃນທຸກໆ ລະບົບນິເວດວິທະຍາການປູກເຂົ້າໃນ ສປປ ລາວ . ນອກຈາກເປັນພະຍາດທີ່ສ້າງ ຄວາມເສຍຫາຍໂດຍກົງ ຕໍ່ກັບການຜະລິດເຂົ້າແລ້ວ ມັນຍັງເປັນຂໍ້ກີດໜ່ວງ ໃນການຍົກສະມັດຕະພາບຜົນຜະລິດເຂົ້າອີກ. ຜົນການຄົ້ນຄ້ວາພົບວ່າ ມີເຊື້ອພະຍາດ ୨ ຊີວະຊະນິດພັນ ທີ່ພົບເຫັນ ໃນພື້ນທີ່ການປູກເຂົ້າ ຢູ່ໃນ ୩ ບ້ານ ຂອງ ແຂວງ ຫຼວງພະບາງ ສປປ ລາວ . ຈາກປະເມີນການສຳຫຼວດ ລັກສະນະພັນຕ້ານທານພະຍາດໃບໄໝ້ ໃນພື້ນທີ່ປູກເຂົ້າພົບວ່າ : ແນວພັນ TDK1, TDK5, TDK11, RD6 ແລະ PNG5 ອ່ອນແອຕໍ່ກັບເຊື້ອພະຍາດເຖິງ ୮ ຊີວະຊະນິດຄື: BMK-001, BMK-002, BMK-003, BMK-004. BOU001, BPS001, BPS002 ແລະ BPS003. ມີ ୩ ແນວພັນ TDK11,Takiet ແລະ RD6 ອ່ອນແອຕໍ່ກັບເຊື້ອພະຍາດທັງໝົດ.

ຄຳສຳຄັນ: ລັກຊະນະ,ຊີວະຊະນິດ,ພະຍາດໄໝ້,ຄວາມຮ້າຍແຮງຂອງເຊື້ອພະຍາດ, ແນວພັນທົນທານ ແລະ ບໍ່ທົນ ທານ.

Investigation on Genetic Characterization of Blast Disease Resistance in Rice Fields: A Case Study of Three Villages in Luang Prabang Province .

Vilaphone Kanyasone* and Louis Vannamahaxay

Department of Plant Science, Souphanouvong University, LuangPrabang, Lao PDR

Abstract:

Rice blast (*Pyricularia Oryzea*) is one of the most important diseases affecting rice worldwide and in Laos. Under favorable environmental conditions, the losses inflicted by this disease are enormous. A sample of nine *Pyricularia Oryzea* were collected from the rice growing areas of 3 villages in Luang Prabang Province, Laos. To begin the investigation on genetic characterization of blast disease resistance in rice fields, single conidium isolates were inoculated into 15 commercial rice varieties. Results from the research shows that: TDK1, TDK5, TDK11, RD6 and PNG5 were susceptible to 8 blast isolates BMK-001, BMK-002, BMK-003 and BMK-004. BOU001, BPS001, BPS002 and BPS003. In addition, 13 varieties of TDK11 Takiet and RD6 were susceptible to all blast isolates.

Keywords: Genetic characterization, *pyricularia oryzae*, resistance, rice.

* *Corresponding Author: Vilaphone Kanyasone, Tel: +865 20 54947776, E-mail: vilaphone.su@yahoo.com*
Received: 28 July 2015; Accepted: 29 September 2015.

1. Introduction

Rice blast disease, caused by *Pyricularia oryzae* is one of the most important and widely distributed plant diseases. The rice blast disease has spread to some 85 countries worldwide and including the Lao PDR. The economic loss of rice production caused by rice blast disease worldwide and in Laos is nearly US$ 5 billion per year (Schiller et al., 2000).

The economic importance of blast can be seen recently by the panicle blast epidemic in Thailand. This was reported in 1992 affecting over 200,000 ha in 12 provinces of the northern part of Thailand. The result of disease diagnosis confirmed that about 60% of diseased plants had panicle blast caused by *P. oryzae* and the rest were infected by other fungi. Total yield loss of 60% was confirmed to be due to the disease, approximately equivalent to 65,000 tons of paddy rice (Disthaporn, 1994). Despite the magnitude of the problem, satisfactory control of blast is achieved in tropical irrigated rice primarily through the use of resistant varieties. The use of resistant cultivars has been recognized as the most practical and economical method by which the farmers could control the blast disease (Correa – Victoria et al., 1994).

Ban Ou, Ban Moukhy and Ban Paksee in the Luang Prabang district, are major rice production areas in the Luang Prabang province, Laos. Irrigated lowland rice systems and rain fed lowland rice are preferably practiced by farmers. Rice blast is one of the major rice diseases in this region because of the wide distribution and favorable conditions in the rainy season. However, farmers are ignorant and unconcerned about it. They have continuously used old varieties for more than 10 years that had been affected by blast diseases and the yield is reduced dramatically during the rainy season. In the worst cases, for some farmers, yields were completely lost due to rice blast. This study, aims evaluate 15 commercial rice varieties for resistance to 9 blast isolates from different hosts. The objective of this study is to survey and investigate the resistant blast disease rice and to investigate the cause of blast disease in the case of 3 villages in Luang Prabang.

2. Materials and Methods

2.1. Survey and collection of blast simples

Sampling of *Pyricularia oryzae* was done during the rice growing season of 2013 using two ecologies (rain lowland rice and irrigated lowland rice). Isolates were collected from different hosts in the rice fields, before harvesting time, of the rain fed lowland rice fields in 3 villages of the Luang Prabang district. The main rice growing stages were also subject to sampling during field trips at the appropriate times of the rice crop, during leaf blast epidemics. Each sample was collected and kept in a paper envelope. Samples were brought to the laboratory.

2.2. Isolation from infected rice leaves

Each diseased leaf sample was cut into small pieces 1 cm long including the lesions and were incubated in plates having filter paper moistened with sterile, distilled water and kept at 25-28^{o}C for 1 to 2 days. Conidia were taken from the lesions and spread on 4% water agar medium. After germination, single conidial isolates were obtained and transferred to a Petri dish with RFA medium. The dishes were incubated at 28^{o}C for 4 days. A single identified colony was transferred using a sterile scalpel to cut out a small piece from the colony edge. After that, each colony was removed and dried in desiccators for 14 days. The completely dried, small cuts were kept inside plastic bags. The mycelium was collected in filter papers after filtration and stored at -20^{o}C for long term use.

2.3. Isolates used for screening test varieties

Fifteen blast isolates were collected from different hosts from 3 villages of Luang Prabang district for screening of 15 test varieties, including resistance IR64 and susceptible KDML105 were used as check varieties based on infection pattern. Inoculums were prepared for the rice seedlings at 14 days. Inoculation preparation consisted of, sterilization with distilled water and adjusted to the concentration of $5x10^4$ conidia /ml. The suspension with 0.5 % gelatin was sprayed on each seeded tray for inoculation. Then, inoculated seedlings were incubated in a plastic cover at 25^{o}C in a high humidity chamber for 16 hours after the cover

was opened. The leaf blast was recorded 7 days after inoculation. Evaluations of blast symptoms were done according to a standard reference scale with 7-lesion type categories.

2.4. Statistical analysis

The data on disease severity score of 2 cultivars and 15 test varieties screened against 9 blast isolates were analyzed using the average of 2replications. The resistance index (RI) was analyzed using the virulent.

3. Results

3.1. Survey and collection of *Pyricularia oryzae* isolates.

A total of 32 mono conidial *Pyricularia oryzae* isolates were obtained from infected rice leaves collected in Luang Prabang district, Lao PDR. They were collected from 3 villages namely: Ban Moukhy, Ban Ou and Ban Paksee.

3.2. Test varieties screening

Data from 15 test varieties screening using 09 isolates showed that IR64 are resistance to all isolates.

The results of virulence testing of 9 isolates with 15 test varieties were summarized in Table 2. The data were classified into 2 groups, a virulent (0, 1, 2, 3) and Virulent (4, 5, 6). From 9 blast isolates, 2 were virulent indicating 22% over the total. 7 blast isolates were not virulent against the 15 test varieties. None of blast isolates were virulent to IR64, Kainoi and TDK8 varieties.

Table 1: Nine isolates of *P. oryzae* collected from rice growing in 3 villages of Luang Prabang district.

No	Villages	Sampled fields	Rice isolates
1	Ban Moukhy (BMK)	15	04
2	Ban Ou (BOU)	17	02
3	Ban Paksee (BPS)	22	03
Total		54	09

Table 2: Fifteen rice varieties used to identify compatible host-pathogen interaction among various blast isolates.

No	Variety name	Pedigree	Origin
1	Thadokkham1 (TDK1)	IR43069-UBN-507-12-2	LAOS
2	Thadokkham3 (TDK3)	IR71510-UBN-TDK-6-1-1-2	LAOS
3	Thadokkham5 (TDK5)	L161-7-3-2-1	LAOS
4	Thadokkham6 (TDK6)	SK12-117-2-3	LAOS
5	Thadokkham7 (TDK7)	IR70824-TDK-5-13-1	LAOS
6	Thadokkham8 (TDK8)	IR-71516-UBN-TDK-6-1-1-2	LAOS
7	Thadokkham10 (TDK10)	BKNLR78015-R-PSL-3-1	LAOS
8	Thadokkham11 (TDK11)	IR4308-TDK-1-2-11	LAOS
9	Thasano1(TSN1)	IR46463-CPA-5-2-1-1	LAOS
10	Thasano3(TSN3)	TDK10027-TSN-13-111-5-1	LAOS
11	PhoneNgram1(PNG1)	IR43086-UBN	LAOS
12	IR64		Philippines
13	RD6		Thailand
14	Takiet		Laos
15	Kainoi		Laos

Table 3. Nine *Pyriculariaoryzae* isolates used for testing reaction to rice blast of rice cultivars.

Isolates Code	Reaction of rice cultivars														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
BMK-001	R	S	S	S	S	R	S	S	S	S	S	R	S	S	R
BMK-002	S	S	S	S	S	R	S	S	S	S	S	R	S	S	R
BMK-003	R	S	S	S	S	R	S	S	S	S	S	R	S	S	R
BMK-004	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
BPS-001	S	S	S	S	S	R	S	S	S	S	S	R	S	S	R
BPS-002	R	S	S	S	S	R	S	S	S	S	S	R	S	S	R
BOU-001	S	S	S	S	S	R	S	S	S	S	S	R	S	S	R
BOU-002	S	S	S	S	S	R	S	S	S	S	S	R	S	S	R
BOU-003	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S

A: TDK1, B: TDK3, C: TDK5, D: TDK6, E: TDK7, F: TDK8, G: TDK10, H: TDK11, I: TSN1, J: TSN3, K: PNG1, L: IR64, M: RD6, N: Takiet, O: Kainoi; R: Resistant, S: Susceptible.

Table 4. Nine blast isolation

No	Isolates Code	Avirulent	Virulence	% of virulence
1	BMK-001	13	2	13
2	BMK-002	10	5	33
3	BMK-003	11	4	27
4	BMK-004	11	4	27
5	BPS-001	5	10	67
6	BPS-002	9	6	40
7	BOU-001	10	5	33
8	BOU-002	11	4	27
9	BOU-003	6	9	60
	Total	7	2	22

4. Discussion

The objective of this study was to test for genetic differentiation among *P. oryzae* sub-populations in 3 villages in Luang Prabang, Laos and on different rice cultivars. In some areas where rice and, presumably, *Pyricularia grisea* rice pathogens, have been introduced only within a few hundred years, pathotypic diversity has been reported to be low (Levy et al., 1991; Roumen et al., 1997). In contrast, the high diverse *P. grisea* population in Laos is probably very old, as the oldest archaeological evidence for rice cultivation in the region dates to about 11,000 years B.C. Chang (1997).

Samples of the blast isolates in the 3 villages in Luang Prabang, Laos were collected from similar geographical locations during harvesting in the dry season. Five blast isolates were collected from rice paddies damaged by blast disease, while the rice areas of Ou and Paksee villages were less damaged, hence less blast isolates were obtained and only four isolates were from Moukhy village.

The 15 test varieties were screened using 9 isolates and showed that IR64, TDK8 and Kainoi were resistant to all isolates. From 9 blast isolates, 2 were virulent (22%) and 7 were not against the 15 test varieties

5. Conclusion

Data on effect of inoculation methods showed significant differences on lesion length and running lesion length produced by various methods of inoculation. Round to spindle shaped lesions with gray centers and brown margins were produced by micropipette inoculation, while small brown necrotic spots were produced by spraying. Two blast isolates were virulent (22%) against the 15 varieties and 7 blast isolates were avirulent(78%).

6. Acknowledgement

This study was financially supported by Asian Development Bank (ADB), Grant 0166-Lao (SF): Strengthening Higher Education Project). Component 4, Category 6.

7. References

Chang, T. T. (1996). Rice Oryza sativa and Oryzaglaberrima (Gramineae-Oryzeae).. In Simmonds NW (ed.). Evaluation of crop plants. pp 98-104 Longman Inc., New York.

Correa-Victoria, F. J., Zeigler, R.S., & Levy, M. (1994). Virulence characteristics of genetic families of Pyriculariagrisea in Colombia, pp. 211-230. In Zeigler, R.S., Leong, S.A.&Teng,P.S. (Eds). Rice blast disease. CAB International and IRRI.

Disthaporn, S. (1994). Current rice blast epidemics and their management in Thailand, pp. 333-342.*In* Zeigler, R.S.. Leong, S.A.&Teng, P.S. (Eds). Rice blast disease. CAB International and IRRI.

Levy, M. J., Romao, M., Marchetti, A. & Hamer, J. E. (1991). DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell. 3: 95-102.

Roumen, E., Levy, M. & Notteghem, J. L. (1997). Characterization of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. Eur. J. Plant Pathol. 103: 363-371.

Schiller, S. Appa Rao, S., Hatsadong & Inthapanya, P. (2000). Glutinous rice varieties of Laos, Their improvement, cultivation, processing and consumption, Chatter written for proposed FAO publication, Special rice in the world, Breeding, production and marketing.