Kasetsart J. (Nat. Sci.) 49 : 327 - 334 (2015)

Mapping of Blast Disease Resistance Genes in BC_2F_6 Population of the Cross KDML105 × IR64

Aussanee Waiyalert, Tanee Sreewongchai*, Tanapon Chaisan and Prapa Sripichitt

ABSTRACT

The IR64 rice variety has a broad spectrum resistance to the blast pathogen in Thailand. Polygenic resistance genes have been identified in this variety. To detect quantitative trait loci (QTLs) controlling blast disease resistance, 192 BC_2F_6 lines (backcross inbred lines; BILs) derived from a cross between IR64 and KDML105 were used for gene mapping. The BILs were examined for the location of resistance genes using six virulent blast isolates. The presence was discovered of broad spectrum resistance loci in the IR64 variety positioned on chromosomes 2, 3, 8 and 12. Interestingly, two loci located on chromosomes 3 and 8 were new QTLs for blast disease resistance. These loci could be exploited in rice breeding programs for the development of broad spectrum and/or durable blast resistant rice varieties after being validated. According to the results of this study, the IR64 variety could be utilized as a donor parent of blast resistance loci to develop durable blast resistant varieties.

Keywords: blast disease resistance, gene mapping, near isogenic lines, backcross, rice

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop in the world with approximately half of the world's population consuming rice as a staple food, especially in Asian countries (Lu and Chang, 1980) Thai jasmine rice, called Khao Dawk Mali 105 (KDML 105), is the most famous and popular aromatic rice variety in Thailand and many other countries (Pongtongkam *et al.*, 2004). The advantages of this variety are its good cooking qualities and aroma (Yoshihashi *et al.*, 2002). However, the variety has some disadvantages such as being photoperiod sensitive, prone to severe lodging and being susceptible to insects and diseases (Sriboonjit and Viboonpong, 2000) Among the abiotic and biotic stresses, blast disease is the most serious problem for rice production (Loan *et al.*, 2003).

Blast is the major fungal disease of rice found all over the world in rice growing areas and is estimated to cause production losses of USD 55 million each year in South and Southeast Asia, with the losses being even higher in East Asia and other temperate rice growing regions around the world (Bonman, 1992) In Thailand, Sriboonjit and Viboonpong (2000) evaluated yield losses due to rice blast disease in KDML 105 and RD6 rice varieties and reported that the average yield loss was up to 50% in the disease-infected areas. The rice blast disease is caused by the fungus *Pyricularia oryzae*, which, in its sexual state, is known as *Magnaporthe oryzae* (Hebert, 1971). While blast disease can infect all aerial parts of

Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: taneesree@yahoo.com

Received date : 02/04/14 Accepted date : 24/12/14

the plant such as the leaf, node, collar and neck, most infections occur on the leaves, causing spindle-shaped lesions with a gray or white center (Bonman, 1992). Blast is more of a problem in cooler climates, rainy periods or periods of high humidity which also favor the disease (Ou, 1972). Sporulation increases with high relative humidity and at 28 °C (Ou, 1972).

The rice variety IR64 possesses many positive agronomic characteristics such as high yield, strong and semi dwarf plant type and resistance to diseases and insects (Wu *et al.*, 2005). Compared to other rice varieties grown in Thailand, IR64 has physical limitations due to much of its seed being short and having chalky grain (Wu *et al.*, 2005). However, it has broad spectrum resistance to blast isolates from more than 20 countries including Latin America, Africa and Asia (Sallaud *et al.*, 2003) In Thailand, IR64 has shown broad-spectrum resistance against various blast pathogen isolates across the country and is being used in breeding programs for blast disease resistance (Sreewongchai *et al.*, 2010). Sirithunya *et al*. (2004) reported that blast resistance genes in this cultivar suggested two major QTLs available on chromosomes 2 and 12 near the simple sequence repeat (SSR) markers RM208 and RM179, respectively. A major gene is also located on chromosome 12. By employing backcross breeding, these resistance genes could be transferred to the susceptible variety, KDML105.

Advanced backcross populations have been developed and used, with near-isogenic lines (NILs) being the most representative type (Takai *et al.*, 2007) NILs have distinct advantages for QTL identification as genetic background noise can be eliminated and a QTL can be visualized as a single Mendelian factor (Xing *et al.*, 2008). Ebitani *et al.* (2005) reported that each NIL carries either one or more donor segments in the near-isogenic background of the recurrent parent, which reduces the effects of interference from the genetic background and while several QTLs have been fine-mapped or cloned on the basis of the NILs, the development is laborious and time-consuming, preventing many researchers from performing map-based cloning of QTLs.

This study developed backcross inbred lines (BILs) of a cross between IR64 and KDML105 by using KDML105 as a recurrent parent. The BC_2F_6 population was used in this study. This type of population is the most effective to identify the QTL location because it has a homogenous genetic background (Fakuoka *et al.*, 2010). The analysis of blast disease-resistant genes in IR64, using a chromosome segment substitution line (CSSL), will enable the generation of genetic markers associated with genes that can be applied in a rice breeding program for broader spectrum resistance to blast pathogen.

MATERIALS AND METHODS

Plant materials

The plant materials used for this study were KDML105, IR64 and the BC_2F_6 population of the cross KDML105 × IR64. The BC_2F_2 population was initially received from a rice breeding project for blast resistance in the KDML105 variety. The BC_2F_2 seeds were grown in 108-hole seed trays and put in 38 × 53 × 12.5 cm (width × length × depth) plastic trays at Kasetsart University, Bangkok, Thailand. BC_2F_6 populations were obtained by self-pollination of each BC_2F_2 line at the flowering stage and were grown under the same conditions as the BC_2F_2 population. The BC_2F_6 populations were evaluated by inoculation of selected virulent blast pathogen isolates.

Rice blast preparation, inoculation and disease evaluation

Disease evaluations were performed in the selected BC_2F_6 lines at the seedling stage. The parental varieties, IR64 and KDML105, were introduced as negative and positive checks. The BC_2F_6 lines were examined to identify the resistance gene using six blast isolates. These six

isolates were collected from different locations covering northern, northeastern and central Thailand (Table 1) and were selected based on genotypic and pathotypic profiles to evaluate levels of resistance of the parents and the BILs.

Inoculum preparation and disease evaluation were performed by following the method of Roumen *et al.* (1997). The isolates were re-grown from the stock cultures, cultured on rice flour agar medium (2.0% of rice flour, 0.2% of yeast extract and 2.0% of agar), and kept at 25 °C under fluorescent light for 12 hr.d^{-1}. After 8–10 d, sporulation was induced by scraping all mycelia in each plate followed by incubation at room temperature for 2 d. The conidia were washed with sterile, distilled water and the concentration of conidia was adjusted to 5×10^4 conidia.mL^{-1} supported with 0.5% gelatin. Inoculations were performed by spraying 100 mL of inocula onto the leaves of 2 wk-old plants, check rowed in plastic trays. Inoculations were done with two replications to assure the accuracy of resistance measurement. The inoculated plants were incubated under high humidity conditions overnight before transferring into a greenhouse. Disease evaluations were done 7 d after inoculation according to a 0–6 scale, where infection types 0–2 were considered resistant, 3–4 were intermediate and 5–6 were susceptible reactions (Sallaud *et al.*, 2003).

Genotyping and marker-assisted selections

DNA extractions of leaf samples were carried out using a DNA Trap_kit (DNA Technology Laboratory, BIOTEC, Bangkok, Thailand). In order to screen polymorphism between the parents, at least 20 SSR markers per chromosome were used. The two microsatellite markers tightly linked to the QTLs identified by Sirithunya *et al.* (2004) were also included in the polymerase chain reaction (PCR) analysis. The tightly linked markers to the two blast resistance QTLs of IR64 on chromosomes 2 and 12 were RM208 and RM179, respectively. Bulked segregant analysis (Michelmore *et al.*, 1991) was used to identify candidate SSR markers linked to blast resistance genes using the polymorphic markers between the parents. Based on the results of the blast resistance evaluation, two different bulks, each containing an equal amount of DNA from 10 blast-resistant and susceptible BC_2F_6plants, were developed and screened for polymorphism together with the parents. Depending on the results of the segregation analysis, four candidate markers were identified and used for individual BC_2F_6genotyping. All SSR markers were assayed on the rice population as described by Panaud *et al.* (1996). The PCR products were separated in 6% polyacrylamide gel electrophoresis. DNA profiles of each marker were scored by comparison with their parents as "1" for alleles of KDML105 (recurrent parent) and "2" for alleles derived from IR64 (donor parent).

Table 1 Place of collection, variety and plant parts from which the six blast isolates used in this experiment were collected.

Entry	Isolate code	Province	Variety	Plant part
1	BAG1.1	Phitsanulok	KDML105	Leaf
2	BAG7.1	Nong Khai	KDML105	Leaf
3	BAG6.4	Chaiyaphum	KDML105	Leaf
4	BAG8.2	Ubon Ratchathani	KDML105	Neck
5	BAG43.2	Lop Buri	KDML105	Leaf
6	THL196	Khon Kaen	KDML105	Neck

RESULTS

Backcross inbred line development

The F_1 plants were generated from KDML105 (recurrent) and IR64 (donor) parents. The F_1 plants were backcrossed to KDML105 to produce the BC_1F_1 generation. These BC_1F_1 plants were again back-crossed with KDML105 to produce BC_2F_1 plants. The BC_2F_1 were self-pollinated to produce 192 BC_2F_6 populations.

Development of BC_2F_6 lines with broad spectrum resistance to Thai blast isolates and marker-assisted selection

The results of the pathotype data, after evaluation of the BILs using six blast isolates from different locations, indicated that IR64 (the resistant parent) showed resistance to all blast isolates with broad spectrum resistance (BSR=1) and KDML105 (the susceptible parent) was susceptible to all isolates (BSR=0). From the total of 192 BILs screened for resistance to six blast isolates, 11 lines (5.7%) were found to be resistant to all the isolates, 4 lines were resistant to 3 of the blast isolates while 1 and 3 lines were resistant to 2 and 1 isolates, respectively. The rest of the lines were susceptible (a score between 4 and 6) to all of the isolates (Table 2).

The bulked segregant analysis using the two contrasting phenotypic groups and the parents resulted in four polymorphic SSR markers—RM208, RM85, RM38 and RM179—situated on four different chromosomes. Out of the 11 BC_2F_6lines with broad spectrum resistance, 10 had two alleles in common introgressed from IR64 and linked to the SSR markers RM85 and RM179, which were located on chromosomes 3 and 12, respectively. Out of the 11 lines, 3 had 4 alleles inherited from IR64 and linked to the SSR markers RM208, RM85, RM38 and RM179, which were located on chromosomes 2, 3, 8 and 12, respectively (Figure 1). Similarly, 3 lines had 3 alleles inherited from IR64 and linked to the SSR markers RM85, RM38 and RM179, which were located on chromosomes 3, 8 and 12, respectively (Table 2).

DISCUSSION

KDML 105 is the most popular jasmine rice in Thailand because of its good cooking and eating qualities (Yoshihashi *et al.,* 2002). Despite its qualities, a sizable amount of yield is lost due to its susceptibility to the rice blast *Magnaporthe oryzae*. In order to improve its resistance to this damaging disease, backcross breeding was attempted using IR64 as a donor and KDML105 as a recipient parent. Consequently, 192 BILs were developed and the BILs were evaluated for resistance to blast, and marker-assisted selection was also performed.

The results of the pathotype data revealed that IR64 was able to resist all the blast isolates with a broad spectrum resistance score of 1. From the total of 192 BILs screened for blast resistance, 11 were found to be as resistant as IR64. This indicates the successful transfer of the resistance QTLs to KDML105. Furthermore, 4 lines were resistant to 3 of the blast isolates while 1 and 3 lines were resistant to 2 and 1 isolates, respectively; indicating specific resistance of the QTLs involved in the lines. Sallaud *et al.* (2003) reported the isolate-specific nature of the blast resistance genes.

The bulked segregant analysis identified four polymorphic SSR markers situated on 4 different chromosomes. All the broad spectrum resistant lines, except one, had 2 alleles in common inherited from IR64 and linked to the SSR markers RM85 and RM179. All the 11 lines had at least 2 alleles inherited from IR64 and linked to 2 of the 4 SSR markers.

The two markers RM208 and RM179 were effective in identifying the loci responsible for the blast resistance and were also used by Sreewongchai *et al.* (2010) in the pyramiding of QTLs derived from IR64 and JaoHom Nin (JHN). One BIL—68-3(R4)—had two alleles

inherited from the resistant parent, each linked to RM85 and RM38. This line was broad-spectrum resistant even in the absence of the QTLs tightly linked to the SSR markers RM208 and RM179. Thus, the two new loci linked to RM85 and RM38 are effective in the absence of the other two loci. However, Langridge *et al.* (2001) recommended that markers should detect polymorphism in different populations derived from various parental genotypes in order to be utilized in routine breeding programs. It is, therefore, necessary to validate these markers in other populations.

Table 2 Comparisons of genotype score and disease resistance scores between the parents (KDML 105, IR64) and BC_2F_6 lines against four SSR markers and six single spore isolates of blast pathogen found in Thailand.

Identifier	Genotype score				Disease resistance score						BSR
	RM208 (Chr.2)	RM85 (Chr.3)	RM38 (Chr.8)	RM179 (Chr.12)	THl196	1.1	7.1	8.2	43	6.4	
KDML (S)	1	1	1	1	6	5	6	6	6	6	0
IR64 (R)	2	2	2	2	0	0	0	0	0	0	1
1-1 (R1)	2	2	2	2	0	0	0	0	0	0	1
2-1 (R1)	2	2	2	2	0	0	0	0	0	0	1
34-1 (R1)	2	2	2	2	0	0	0	0	0	0	1
61-1 (R2)	1	2	2	2	0	0	0	0	0	0	1
62-2 (R2)	1	2	2	2	0	0	0	0	0	0	1
67-1 (R2)	1	2	2	2	0	0	0	0	0	0	1
62-3 (R3)	1	2	1	2	0	0	0	0	0	0	1
64-3 (R3)	1	2	1	2	0	0	0	0	0	0	1
68-2 (R3)	1	2	1	2	0	0	0	0	0	0	1
70-3 (R3)	1	2	1	2	0	0	0	0	0	0	1
68-3 (R4)	1	2	2	1	0	0	0	0	0	1	1
66-2	2	2	1	1	1	0	-	-	-	0	0.50
57-2	2	2	1	1	-	0	-	-	0	0	0.50
56-2	2	2	1	1	-	0	1	-	-	0	0.50
70-1	2	1	2	1	-	-	1	1	-	0	0.50
69-2	1	2	1	1	-	0	-	-	0	-	0.33
6-1	2	1	1	1	-	1	-	-	-	-	0.17
55-1	1	2	1	1	-	-	-	-	0	-	0.17
5-1	1	1	1	1	-	-	-	-	0	-	0.17
8-2	1	1	1	1	6	5	5	5	5	6	0
22-3	1	1	1	1	5	6	6	6	5	5	0
37-2	1	1	1	1	5	6	5	6	5	4	0
43-2	1	1	1	1	5	6	5	5	5	4	0

Chr. = Chromosome; BSR= Broad spectrum resistance.

Genotype score = 1 for KDML105 and 2 for IR64 allele for each SSR marker genotyping.

Disease resistance score = 0 for resistance through to 6 for susceptible to each testing blast isolate.

CONCLUSION

The study confirmed the presence of broad-spectrum resistance loci in the IR64 variety in chromosomes 2, 3, 8 and 12. Two of the loci which were located on chromosomes 3 and 8 were newly discovered QTLs. These loci could be exploited in the development of broad-spectrum or durable blast-resistant rice varieties or both after being validated. Moreover, IR64 could be utilized as a donor parent of blast resistance loci to develop durable blast resistant varieties.

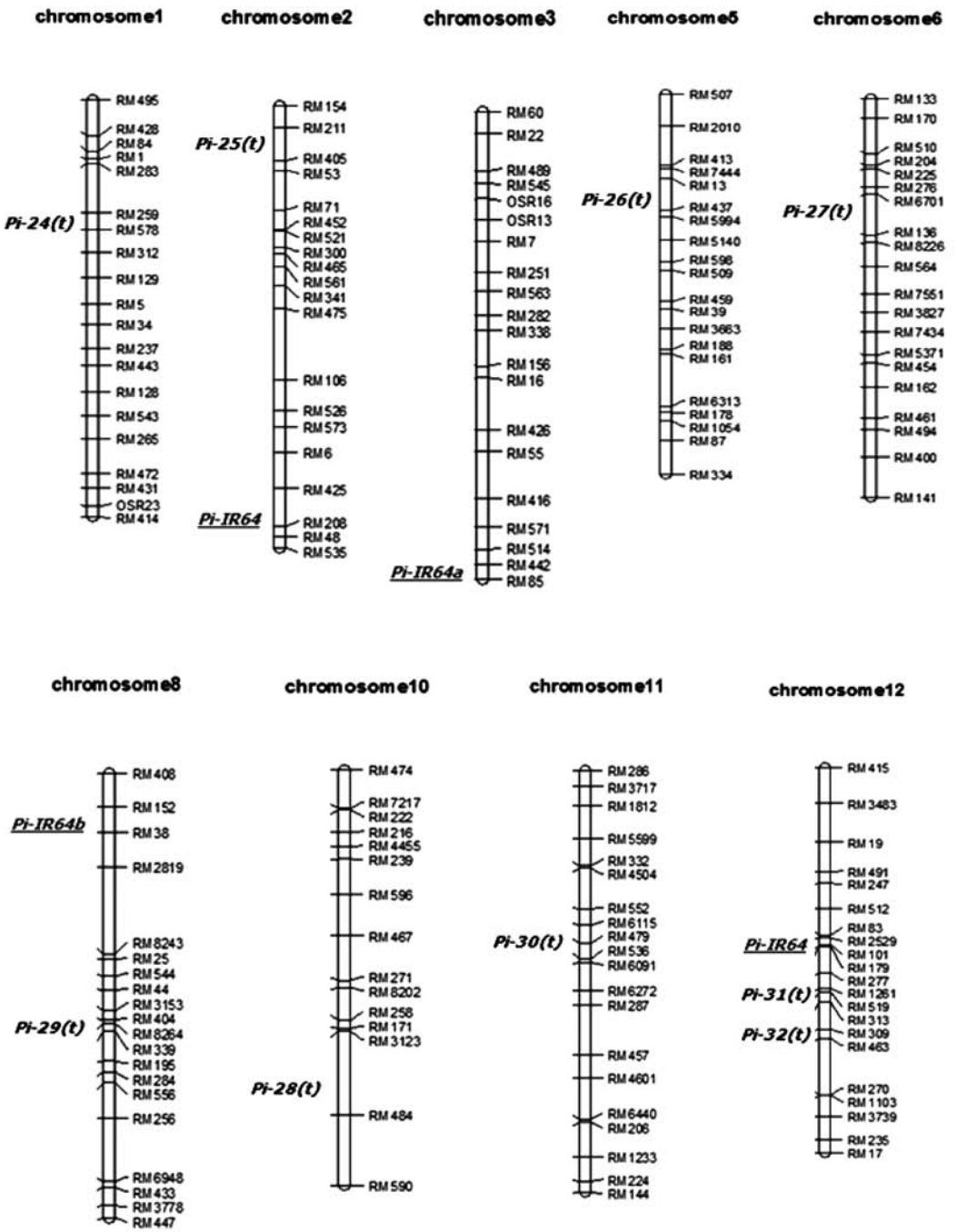

Figure 1 Blast resistance genes on each chromosome of IR64 variety.

ACKNOWLEDGEMENTS

This work was supported by the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Bangkok, Thailand. Research and study by the first author was partially funded by the Graduate School of Kasetsart University.

LITERATURE CITED

Bonman, J.M. 1992. Rice Blast. R.K. Webster and P.S. Gunnel. (eds.). *In* **Compendium of Rice Diseases.** American Phytopathological Society Press. St. Paul, Minnesota. USA. Pages 14–18.

Ebitani, T., Y. Takeuchi, Y. Nonoue, T. Yamamoto, K. Takeuchi and M. Yano. 2005. Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of indica rice cultivar 'Kasalath' in a genetic background of japonica elite cultivar 'Koshihikari'. **Breed. Sci.** 55: 65–73.

Fukuoka, S., K. Ebana, T. Yamamoto and M. Yano. 2010. Integration of genomics into rice breeding. **Rice** 3: 131–137.

Hebert, T.T. 1971. The perfect stage of *Pyricularia grisea*. **Phytopathology** 61: 83–87.

Langridge, P., E. Lagudah, T. Holton, R. Appels, P. Sharp and K. Chalmers. 2001. Trends in genetic and genome analyses in wheat: A review. **Aust. J. Agric. Res.** 52: 1043–1077.

Loan, L.C., P.V. Duand Z. Li. 2003. Identification of genes conferring resistance to some Philippine and Vietnamese races of blast. **Omon. Rice** 11: 49–62.

Lu, J.J. and T.T. Chang. 1980. Rice in its temporal and spatial perspective. *In*: Luh BS (ed) **Rice. Production and Utilization.** AVI Publishing Co. Westport, CT, USA.

Michelmore, R.W., I. Paran and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. **Proc. Natl. Acad. Sci. USA.** 88: 9828–9832.

Ou, S.H. 1972. **Rice Disease.** Commonwealth Mycological Institute. Kew, UK.

Panaud, O., X. Chen and S.R. McCouch. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphisms (SSLP) in rice (*Oryza sativa* L.). **Mol. Gen. Genet.** 252: 597–607.

Pongtongkam P., S. Peyachoknagul, P. Sripichit, A. Thongpan, K. Klakkhaeng, S. Ketsagul and K. Lertsirirungson. 2004. Effects of llysine on callus formation, plant regeneration and flowering of Thai rice c.v. KDML105. **Kasetsart J. Nat. Sci.** 38: 190–195.

Roumen, E., M. Levy and J.L. Nottegham. 1997. Characterization of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. **European J. Pl. Path.**103: 363–371.

Sallaud, C., M. Lorieux, E. Roumen, D. Tharreau, R. Berruyer, P. Svestasrani, O. Garsmeur, A. Ghesquiere and J.L. Notteghem. 2003. Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. **Theor. Appl. Genet.** 106: 794–803.

Sirithunya, P., S. Sriprakhon, C. Wongsaprom, T. Sreewongchai, A. Vanavichit and T. Toojinda. 2004. Discovery of broad spectrum blast resistance in rice. *In* **Proceedings of the 1st International Conference on Rice for the Future, Kasetsart University.** 31 August–3 September 2004. Bangkok, Thailand.

Sreewongchai, T., T. Toojinda, N. Thanintorn, C. Kosawang, A. Vanavichit, D. Tharreau and P. Sirithunya. 2010. Development of elite indica rice lines with wide spectrum of resistance to Thai blast isolates by pyramiding multiple resistance QTLs. **Plant Breeding** 129: 176–180.

Sriboonjit, J. and A. Viboonpong. 2000. Evaluation

of neck blast disease affected to KDML105 rice production by stochastic frontier method. **Econ. J.** 3: 39–52. [in Thai]

Takai T, Y. Nonoue, S. Yamamoto, U. Yamanouchi, K. Matsubara, Z.W. Liang, H.X. Lin, N. Ono Y. Uga and M. Yano. 2007. Development of chromosome segment substitution lines derived from backcross between indica donor cultivar 'Nona bokra' and japonica recipient cultivar 'Koshihikari'. **Breed Sci.** 57: 257–261.

Wu, J.L., C. Wu, C. Lei, M. Baraoidan, A. Bordeos, M.R. Madamba, M. Ramos-Pamplona, R. Mauleon, A. Portugal and V.J. Ulat. 2005. Chemical- and irradiation-induced mutants of indica Rice IR64 for forward and reverse genetics. **Plant Mol. Biol.** 59: 85–97.

Xing, Y.Z., W.J. Tang, W.Y. Xue, C.G. Xuand and Q.F. Zhang. 2008. Fine mapping of a major quantitative trait loci, qSSP7, controlling the number of spikelets per panicle as a single Mendelian factor in rice. **Theor. Appl. Genet.** 116: 789–796.

Yoshihashi, T., N.T.T Houng and N. Kabaki. 2002. Quality evaluation of Khao Dawk Mali 105, an aromatic rice variety of Northeast Thailand. **JIRCAS Work. Rep.** 30: 151–160.