Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol.07, Issue, 02, pp.2358-2363, February, 2016

RESEARCH ARTICLE

IMPROVING THE RESISTANCE TO BROWN PLANTHOPPER OF A RICE RESTORER LINE CHENGHUI 727 BY MOLECULAR-ASSISTED SELECTION

[1,*]Alberto Leonel Alberto and [2]MOU Tong Min

[1]Zambeze University, College of Agronomy and Forest, Mozambique
[2]National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, 430007, China

ARTICLE INFO

Article History:
Received 16th November, 2015
Received in revised form
21st December, 2015
Accepted 28th January, 2016
Published online 29th February, 2016

Key words:
Nilaparvatalugens,
Bph14,
Bph15,
Rice,
Marker-Assisted Selection.

ABSTRACT

Five new breeding lines designed as HWQ13001-4-1, HWQ13002-16-1, HWQ13003-4-1, HWQ13004-37-1 and HWQ13005-55-2 harboring *Bph14* and *Bph15* have been developed from BC_3F_2 segregated population of Chenghui727[3]/B5 by Molecular marker-assistant selection (MAS) and gene pyramiding since September 2012 upto July 2015 in Wuhan and Hainan. These developed new lines were evaluated for their resistance to brown plant hopper (BPH) in 2013 and 2014. The results showed that the developed five breeding lines under genetic background of Chenghui727 were resistant against BPH. The yield, main agronomic traits and grain quality of breeding lines were tested and analyzed. The line HWQ13002-16-1 showed positive IYR compared to breeding lines. Genetic background analysis was carried out to verify the extent of recurrent parent genome recovery in each line. Results showed that recurrent parent genome recovery ranged from 11.875% to 88.9% in BC3F5 generation. HWQ13002-16-1 also showed high RPG recovery (85%) and lowest heterozygous segments.

INTRODUCTION

Rice (*Oryza sativa* L.) is the food source for billions of people in the world (Normile, 2008), which rely on this crop for more than 20% of daily calorie intake (IRRI, Africa Rice and CIAT, 2010). To guarantee global food security for continuing population expansion it is crucial to control the different insect pests that harm rice crop (Normile, 2008) leading to influential and unpredictable decrease of yield (Jairin *et al.* 2007). The Brown planthopper (BPH), *Nilaparvata lugens* Stâl, is one of the most important devastating insects in Asia where rice is widely produced (Hu *et al.,* 2010). The BPH obtained the nutrients from the phloem sap of rice plant through its stylet mouth parts (Huang, 2001). So the heavy infestation of BPH causes complete drying of plants to the field known as "hopperburn", whereas the light reduces growth vigor, plant weight and number of productive tillers (Sogawa, 1982). Popular varieties are almost susceptible to BPH and control methods are dependent on insecticides, which is expensive in terms of demanding more labor, money and unfavorable environmental effects (Tanaka *et al.*, 2000; Heinrichs *et al.*, 1982). Several sprayings upset natural balance between the BPH and its natural enemies enhancing, in the other side, its resistance to insecticides, which lead to BPH resurgence (Heinrichs and Mochida, 1984).

Corresponding author: Alberto Leonel Alberto,
Zambeze University, College of Agronomy and Forest, Mozambique.

To grow genetically rice resistant variety is seen as the most economical and affective method for controlling the BPH. Nowadays, genetic basis of quantitative and qualitative BPH resistance is studied and, to date, at least 25 BPH-resistance genes have been discovered from *indica* cultivars and five wild *Oryza* species (Chen *et al.,* 2006; Jena *et al.,* 2006, Zhang, 2007; Rahman *et al.,* 2009; Myint *et al.,* 2012). Ten of these genes have been fine mapped and one has been cloned (Suh *et al.,* 2011). Molecular marker-assisted selection is recognized to be the highly efficient breeding method because can offer rapid and precise selection of the target gene (Tanksley *et al.,* 1989). Varieties harboring two or more genes for BPH resistance offer more durable resistance than those harboring single major genes (Heinrichs, 1986).In the present study are presented results of improvement for BPH resistance in restorer line Chenghui727 by MAS followed by identification of parental lines with good agronomic traits and quality performances.

MATERIALS AND METHODS

The rice line B5, harboring *Bph14* and *Bph15,* is resistant to brown planthopper, was used as the donor parent for BPH resistance genes and was crossed with Chenghui 727, an elite CMS restorer line used as the recipient parent. The true F_1 plants were continuously backcrossed with Chenghui 727 to produce BC_1F_1. The BC_1F_1 plants with *Bph14* and *Bph15* were further backcrossed with Chenghui 727 to produce BC_2F_1.

Consecutive backcrossing and MAS were employed in each generation. Then, at BC_3F_1 generation, plants were self-crossed to produce BC_3F_2 generation. Homozygous individuals carrying *Bph14* and *Bph15* were selected in BC_3F_2 to originate BC_3F_3 families. At this generation, twenty plants of each breeding line were transplanted with a distance of 16 cm by 20 cm. Therefore, to confirm the homozygous target genes PCR analyses were done again. Five breeding lines designed as HWQ13001-40-1, HWQ13002-16-1, HWQ13003-4-1, HWQ13004-37-1 and HWQ13005-55-2 with a phenotype similar to recurrent parent (Chenghui727) were selected in BC_3F_5 (Figure 1). These lines were tested for BPH resistance.

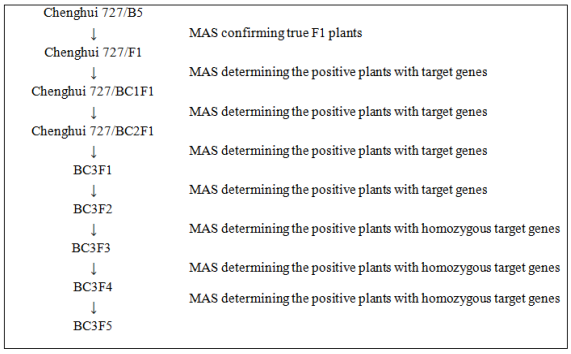
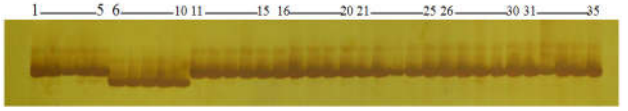


Figure 1 Scheme showing the selecting procedure using Marker Assisted Selection (MAS) in Chenghui 727 recipient parental background

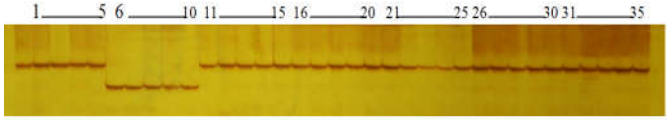
Molecular marker-assistant selection for all resistance genes

In the MAS system, *Bph14* gene was identified using the InDel marker 76-2 and *Bph15* gene was detected using simple sequence repeat (SSR) marker MS5 (Table 1). The SSR marker was designed according to the primary mapping of the resistance genes (Du *et al.* 2009; Huang *et al.* 2001 and Yang *et al.* 2004). The InDel marker was designed based on sequence alignments of the two genome references of Nipponbare and 93-11. The SSR followed the procedures described by Wu and Tanksley (1993).

The SSR method was used to test the offspring per plant genotypes. Genomic DNA was extracted from freshly collected leaf tissue (McCouch *et al.,* 1988). The 800µl 1.5x CTAB method was used to extract DNA from younger leaves. PCR reaction system needed a total volume of 20µl, which included 2µl template DNA, sterilized water 15.6µl, Buffer (10 times, containing magnesium 2+) 2µl, dNTP (5 mm/L) 1.8µl, each 0.24µl and reverse primer, 0.12µl rTag enzyme. Before taking into PCR, 20µl mineral oil was added, to protect the reaction system. According to different primers amplification program and its slightly different annealing temperature the PCR reaction procedure was: 95□ pre modified 4 min; Then each cycle: (1) modified 95^{O}/ 30s, (2) annealing 56□/30min or 58□/30min, (3) extends 72^{O}/ 45s, cycle number for 35 times, and finally 72□ heat preservation 7min. After PCR amplification, was added the indicator and then centrifuged in low-speed, followed by saving at 4^{O} in a refrigerator. The last step consisted of using polyacrylamide gel electrophoresis (PAGE).



Note : Gene : Bph14, primer : 76-2



Note : Gene : Bph15, primer : MS5.1-5 : B5 ; 6-10 : Chenghui727 ; 11-15 : HWQ13001-40-1 ; 16-20 : HWQ13002-16-1 ; 21-25 : HWQ13003-4-1 ; 26-30 : HWQ13004-37-1 ; 31-35 : HWQ13005-55-2

Figure 2. PCR analysis of resistant genes

Evaluation of BPH resistance

To evaluate the resistance against BPH of breeding lines, a seedling test was carried out to phenotype plant reaction to BPH feeding. To ensure all the seedlings at the same growth stage at the moment of BPH infestation, about 50 seeds of each line to be tested were sunk in water for 48 hours for pre-germination. B5 (*Bph14* and *Bph15*), Rathu Heenati (RH, *Bph3*) and TN1 (none resistance genes) were used as controls.

Table 1. Primer used for gene screening

Genes	Primers	Forward primers	Reverse primers
Bph14	76-2	CTGCTGCTGCTCTCGTATTG	CAGGGAAGCTCCAAGAACAG
Bph15	MS5	TTGTGGGTCCTCATCTCCTC	TGACAACTTTGTGCAAGATCAAA

Table 2. Criteria for scoring brown plant-hopper resistance used in the experiment

Resistance score	Plant status(investigated when most of TN1 plants died)	Resistance level
0	None of the leaves shrank and the plant is healthy	HR
1	One leaf showed yellowing	R
3	One to two leaves showed yellowing or one leaf shrank	MR
5	One to two leaves shrank or one leaf shriveled	MS
7	Three to four leaves shrank or two to four leaves shriveled, the Plant is still alive	S
9	The plant died	HS

Note: HR= high resistance; MR= Moderate resistance; MS= moderate susceptibility; HS= high susceptibility

A week after sowing, at the third leaf stage, the seedlings for each line were thinned to 12-15 and infested with second- or third-instar nymphs at a density of 10-12 nymphs per seedling. When all the seedling of TN1 control died, plants from each line were observed and attributed a score of 0, 1, 3, 5, 7 or 9 in accordance to the criteria adapted from IRRI (1996). The low

score means the line is higher resistance and the high score indicates the low resistance to BPH (Table 2).

Evaluation of agronomic and grain quality traits

The breeding restorer lines, were grown in experimental farm of HZAU, Wuhan, 2014. Hundred plants from each breeding lines were planted over three and ten rows, respectively, with the space plant to row 16 cm by 20 cm. All plant protection practices and standard agronomic management were taken on to guarantee a good crop development. Information on agronomic characteristics such as heading date, plant height, productive panicles, average panicle length, spikelet per plant, spikelet fertility percent and 1000-grain weight were recorded as per the standard evaluation system (SES) (IRRI, 1996). Grain quality traits were measured after harvest at 12% to 14% moisture content. Grain quality characters further analyzed were brown rice recovery rate-BRR (%), head rice recovery-RR rate (%), milled head rice length (mm), milled rice recovery rate-MRR (%), length and breadth ratio-LB, chalkiness degree (%), alkali spreading (score) gel consistency-GC (mm) and amylase content-AC (%). The grain samples were dehulled to brown rice with a Satake Rice Machine (Satake Corp. Japan) and milled rice recovery rate-MRR, head rice recovery rate, length and breadth ratio-LB, chalkiness degree were calculated with an automatic machine (JMWT 12, China). Milled rice was ground to pass throughout a 100-mesh sieve on a Cyclone Sample Mill (UDY Corp., Fort Collins, CO), and GC was determined by the method of Cagampang *et al.* (1973). Alkali spreading (AVE) was assayed using the procedure of Bhattacharya (1979). Amylase content (AC) measurement was done using the method reported by Tan *et al.* (1999). A standard curve made using rice samples of known amylase content was used to estimate the AC for each sample, the measurements were taken in triplicate and their averages were determined.

Molecular marker-assistant selection for all resistance genes

The study was conducted to develop lines under background of Chenghui727 and crosses were made between Chenghui727 and B5. In 2014, at the generation BC_3F_5 five plants were selected from five groups of segregating progenies composed by 100 plants and individuals were homozygous for *Bph14* and *Bph15*, namely HWQ13001-40-1, HWQ13002-16-1, HWQ13003-4-1, HWQ13004-37-1 and HWQ13005-55-2 (Figure 2).

RESULTS AND DISCUSSION

Among all the insect pest, Brown planthopper is one of the most devastating pests of rice being responsible for huge yield losses in the field. Physiological, biological and biochemical characteristics of this pest are greatly affected by rice varieties possessing different levels of resistance (Bae, 1970; Rubia-Sanchez, 1999). Recent research of BPH resistance has converged on incorporating single genes, *Bph1, Bph2, Bph3* and *Bph18*. Developing lines harboring single genes have a natural tendency to lose their resistance against BPH as the result of evolution of new biotypes. Gene stacking, which introgresses two or more BPH resistance genes is an effective tool to overcome the rapid evolution of new biotypes due the high variability of the population of BPH (Velusamy *et al.*, 1995). To identify donors for resistance and screening techniques producing direct effect for evaluating breeding lines plays an important role to introgress BPH resistance genes into high popular varieties. Having high level of genetic diversity reduces the risk of a great extent of pests and diseases epidemics (Zhu *et al.,* 2000; Newton *et al.,* 2008). In this research, after introgression of BPH resistance genes, *Bph14* and *Bph15* into Chenghui727, pyramided lines showed high resistance to BPH in the seedling bulk test in comparison to elite line Chenghui727, possessing no *Bph* genes. All of breeding lines HWQ13001-40-1, HWQ13002-16-1, HWQ13003-4-1, HWQ13004-37-1 and HWQ13005-55-2 were resistant against BPH. Hu *et al.*, (2010) in BPH bioassay of lines harboring *Bph* genes showed that pyramided lines with *Bph14* and *Bph15* had high resistance level than *Bph14*-single or *Bph15*-single introgression lines.

On the genetic background of elite line Chenghui727 is evident that pyramided lines harboring *Bph14* and *Bph15* showed higher levels of resistance than lines without resistance genes (Table 3). Li *et al.* (2011) showed that rice lines harboring *Bph15* play important role on delaying BPH male development and decrease the female ratio and copulation rate. Breeding lines were also tested resistant to blast (unpublished results) development of rice varieties resistant to prevalent and destructive diseases is necessary for sustainability of rice grain yields (Sreewongchai *et al.,* 2010).
A whole-genome SNP array, designated RICE6K, was used to examine the genetic background of the developed breeding lines, which provided a graphic genotype for each plant (Figure 3). The figure shows similarities of genetic background of tested individuals to the recurrent parent Chenghui727 but the regions surrounding the transferred genes has dragged regions (red lines) from B5, which can bring possible negative effects due to the undesirable genes linked to target genes.

A number of identified high-quality markers, 640 out of 5102, showed polymosphism between recurrent parent, Chenghui727, and the donor parent, B5. In Table 4 is presented the result of Chenghui727 recovery and heterozygous of breeding lines in BC3F5 population. Results showed that the genetic backgroung recovery of Chenghui727 in HWQ13001-40-1, HWQ13002-16-1, HWQ13003-4-1, HWQ13004-37-1 and HWQ13005-55-2 were 85.63%, 89.22%, 77.34%, 31.33% and 83.67%, respectively (Table 4). Chromosomes 6 and 10 were fully recovered in HWQ13001-40-1, HWQ13002-16-1, HWQ13003-4-1 and HWQ13005-55-2 compared to other chromosomes (Figure 3). The best breeding line was HWQ13002-16-1, showing the highest recurrent parent genome recovery (89.22%) and lowest heterozygous segments. Results obtained by Rajpurohit *et al.* 2011, the maximum recurrent parent genetic background recovery in a short time, breeders would require a large population and proceed background selection atop of foreground check for the gene of interest. The aim of this study was to develop breeding lines with resistance against BPH and with better agronomic characteristics to increase their hybrids yields. The selected breeding lines on the target traits showed existence of genetic drags in their background.

Table 3. Results of BPH resistance evaluation in 2014

Line	Pedigree	Generation	Target gene	Evaluation score		Resistance
				Rep 1	Rep 2	level
Chenghui 727			/	9.0	9.0	HS
B5			*Bph14,Bph15*	1.8	1.9	R
TN1			/	9.0	9.0	HS
HWQ13001-40-1	Chenghui727^4/B5	BC3F5	*Bph14,Bph15*	1.5	3.0	R
HWQ13002-16-1	Chenghui727^4/B5	BC3F5	*Bph14,Bph15*	4.5	2.8	R
HWQ13003-04-1	Chenghui727^4/B5	BC3F5	*Bph14,Bph15*	3.1	4.5	R
HWQ13004-37-1	Chenghui727^4/B5	BC3F5	*Bph14,Bph15*	2.6	1.1	R
HWQ13005-55-2	Chenghui727^4/B5	BC3F5	*Bph14,Bph15*	1.5	1.9	R

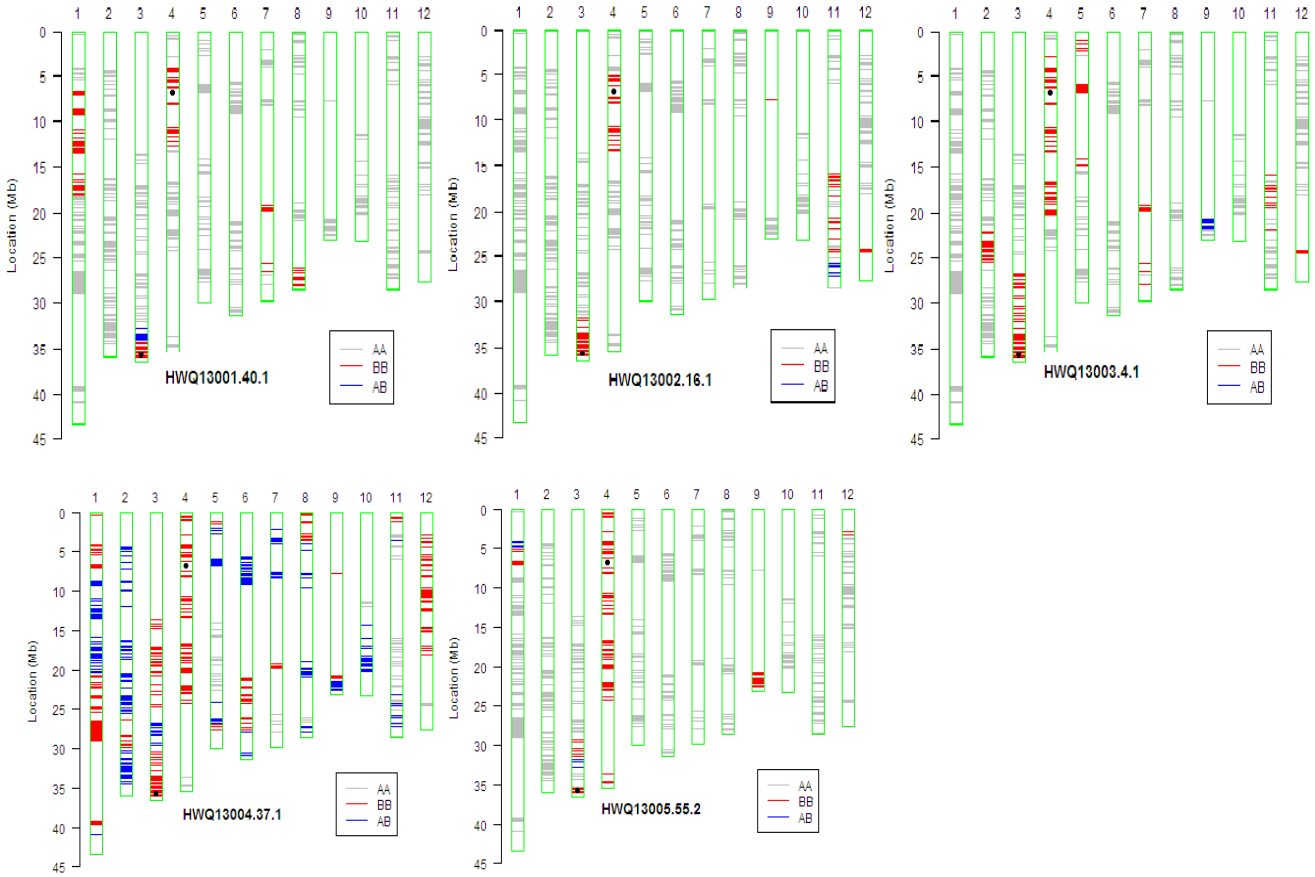


Figure 3. Recurrent parent genome recovery of five selected plants in BC_3F_5
AA=Chenghui727, BB=B5 and AB=Chenghui727/B5

Table 4. Background and Introgressed segments analysis in selected lines of BC3F5

Selected individuals	Number of markers			Recovery (%)
	A	B	H-segments	
HWQ13001-40-1	544	88	8	85.63
HWQ13002-16-1	569	64	4	89.22
HWQ13003-04-1	491	139	8	77.34
HWQ13004-37-1	76	302	249	31.33
HWQ13005-55-2	531	89	9	83.67

Table 5. Yield performance and major agronomic traits for breeding lines

Line	HD (D)	PH(cm)	PP	PL(cm)	SP	SSR (%)	1000GW (g)	Y/P (g)
B5	80	112.0bc	9.2a	29.24a	137.5c	68.97de	28.26bc	22.21
Chenghui727	83	109.5c	7.2b	24.23c	172.8b	78.72ab	28.33abc	25.85
HWQ13001-40-1	79	108.6c	7.4b	24.25c	152.5c	83.33a	27.52c	24.32
HWQ13002-16-1	81	113.9b	7.4b	24.5c	177.4ab	77.98b	29.27a	26.14
HWQ13003-4-1	81	112.7bc	7.0b	27.49b	177.9ab	72.53cd	28.74ab	24.16
HWQ13004-37-1	81	119.2a	7.0b	27.95ab	158.6bc	64.27e	27.75bc	22.47
HWQ13005-55-2	84	111.4bc	6.0b	27.02b	186.8a	75.40bc	26.81d	21.59

HD: heading date, PH: plant height. PP: pancile per plant, SP: spikelets per panicle, SSR: seed set rate, 1000GW: 1000 grain weight, Y/P: yield per plant

Table 6. Rice quality performance of breeding lines

Line	BRR (%)	MRR (%)	HRR (%)	ChGR (%)	Ch (%)	AC (%)	GC (mm)	AVE
B5	78.45a	68.98a	66.57a	12.10a	3.40a	11.17	53.0a	2.00c
Chenghui727	76.99ab	68.43a	65.25ab	2.60b	0.70b	13.79	49.3a	6.5a
HWQ13001-40-1	76.80ab	66.32a	62.97bc	1.30b	0.40b	13.98	52.7a	5.83ab
HWQ13002-16-1	78.06a	68.08a	59.69c	4.80b	1.03b	13.09	51.3a	5.5b
HWQ13003-4-1	76.93ab	68.12a	62.11bc	1.65b	0.40b	12.81	52.7a	5.67ab
HWQ13004-37-1	77.19ab	68.52a	63.79ab	12.95a	3.60a	12.06	50.0a	2.00c
HWQ13005-55-2	76.02b	66.27a	63.08bc	2.25b	0.35b	12.95	53.7a	6.00ab

BRR=Brown rice rate, MRR= Milled Rice rate, HRR= Head rice rate, ChGR= Chalky grain rate, CH= Chalkiness, AC= Amylose content, GC= Gel consistency, AVE= Alkali value elimination

According to Yu *et al.* (2014) this is one of the biggest challenges that plant breeders have to face to stack multiple target genes in a favorable genetic background. For chromosomes that comprise a locus of interest, the recovery of the genome of receiver parent which is linked to target locus is slower in comparison to that do not include target loci (Stam & Zeven, 1981). As result of this inconvenience, analysis of the breeding lines genome showed that donor chromosomal regions surrounding the target locus (red lines) was difficult to eliminate compared to unlinked donor region (Figure 3). According to Collard *et al.* (2008) these fragments from the donor parents can affect negatively the crop performance due the many undesirable genes linked to the target gene, and additional backcross generations may be required.

To analyze the performance of newly developed breeding lines, evaluation for agronomic traits and rice quality was carried on. The main agronomic traits of HWQ13001-40-1, HWQ13002-16-1 and HWQ13003-4-1 were similar to those of Chenghui727 (Table 5), nevertheless the reduction on SP and 1000GW (HWQ13001-40-1), SSR (%) and YP (HWQ13001-40-1 and HWQ13003-4-1). The increased grain production of HWQ13002-16-1 is probably associated to the increasing number of grains per panicle and 1000GW. In a similar study Jairin *et al.* (2009) showed that the increase in grain yield of the ILs was probably because of increase in number of panicles per plant, number of grains per panicle and plant height but not because of 1000-grain weight. However, Kim (1985) cited by Virman (1994) mentioned a higher grain weight contributing for high yield.

The grain quality of the selected lines was almost the same as those of Chenghui727. The Gel consistence (GC) of all five developed breeding lines increased 0.7-4.4 mm compared to those of Chenghui727. Results in the table show reduction of Amylose content (AC), an important trait to increase the taste of rice (Table 6).The success of any plant breeding program relies on the choice of good genotypes as parents in hybridization. So, combining ability analysis can provide information to decide about parents, crosses and appropriate breeding procedure to follow in order to choose suitable segregants (Salgotra *et al.* 2009).

Recently, rice breeding has been concerned on grain quality such as appearance of the cooking rice, texture and aroma. So, the selection of breeding lines is generally centered on the long and slender grain, soft to semi-hard cooked rice (Sharifi *et al.,* 2009). For instance, breeding lines HWQ13001-40-1, HWQ13002-16-1 and HWQ13003-4-1 were the breeding lines resistant to blast and BPH with high recovery of RP background but HWQ13002-16-1 is seen as the promising male parent for hybrid production. Nevertheless, results showed that further self crossings are needed to increase the recovery of RP background since several fragments in each line were still heterozygous. In the other hand, the use of both male and female lines harboring *Bph* resistance genes is essential. Hybrids resulted from cross between Hua1165S and breeding lines showed resistance BPH, Blast and expressed relatively good performance for agronomic traits and grain quality.

REFERENCES

Bae, S.H. and Pathak, M.D. 1970. Life history of Nilaparvata lugens (Hemiptera: Delphacidae) and susceptibility of rice cultivars to its attacks. *Ann Entomol Soc. Am*., 63: 149-155

Bhattacharya, K.R. 1979. Gelatinization temperature of rice starch and its determination. In: Proc of the workshop on chemical aspects of rice grain quality. I*nt Rice Res Inst*, Los Banos, Laguna, Phillippines, pp 231-249

Chen, J.W., Wang, L., Pang, X.F. and Pan, Q.H. 2006. Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene bph19(t). *Mol Gen Genom* 275:321-329

Collard, B.C.Y., Iftekharuddaula, K.M., Pamplona, A.M., Thomson and Mackill D.J. 2008. Cultivating Plant diversity for the resource poor

Du, B., Zhang, W.L., Liu, B.F., Hu, J., Wei, Z., Shi, Z.Y., He, R.F., Zhu, L.L., Chen, R.Z., Han, B. and He, G.C. 2009. Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. *Proc Natl Acad Sci.* USA, 106:22163-22168

Heinrichs, E.A., Aquino, G.B. Chelliah, S., Valencia, S.L. and Reissing, W.H. 1982. Resurgence of *Nilaparvata lugens* (Stâl) populations as influenced by method and timing of insecticide applications in lowland rice. *Environ Entomol* 11(1): 78-84

Heinrichs, E.A. and Mochida, O. 1984. From second to major pest status: the case of insecticide-induce rice brown planthopper, *Nilaparvata lugens*, resurgence. *Protection Ecol* 7: 201-218

Hu, J., Li, X., Wu, X., Wu, C., Yang, C., Hua, H., Gao, G., Xiao, J. and He. Y. 2010. Pyramiding and evaluation of Brown Planthopper resistance genes *Bph14* and *Bph15* in Hybrid rice. *Mol Breeding*

Huang, Z., He, G.C., Shu, L.H., Li, X.H. and Zhang, Q.F. 2001. Identification and mapping of two brown planthopper resistance genes in rice. *Theor Appl Genet* 102:929-934

IRRI (International Rice Research Institute). Standard evaluation system for rice. International Rice Re -search Institute, 2010, Manila, Philippines. Available in Home

page: http://beta.irri.org/index.php/Home/Welcome/ Frontpage.

IRRI. 1996. Standard evaluation systems for Rice. 4th ed. Manila: IRRI, 40-47

Jairin, J., Phengrat, K., Teangdeerith, S., Vanavichit, A. and Toojinda, T. Mapping of a broad-spectrum brown planthopper resistance gene, Bph3, on rice chromosome 6. *Mol Breed*, 2007, 19:35-44

Jairin, J., Teangdeerit, S., Leelagud, P., Kothcharerk, J., Sansen, K., Yi, M., Vanavichit, A. and Toojinda, T. 2009. Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker-assisted selection. *Field Crops Research* 110 (2009) 263–271

Jena, K.K., Jeung, J.U., Lee, J.H., Choi, H.C. and Brar, D.S. 2006. Highresolution mapping of a new brown planthopper (BPH) resistance gene, Bph18(t). and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). *Theor Appl Genet* 112:288-297

Kim, C.H. 1985. Studies on heterosis in F1 rice hybris using cytoplasmic-genetic male steril lines of rice (*Oryza sativa* L.). *res Rep Rural Dev Administration* Suweon, Korea, 27(1): 1-33

Li, J., Chen, Q., Wang, L., Liu, J., Shang K. and Hua, X. 2011. Biological effects of harboring Bph14 and Bph15 on Brown Planthopper, *Nilaparvata lugens. Pest Man Sci* 67: 528-534

Myint, K.K., Fujita, D., Matsumura, M., Sonoda, T., Yoshimura, A. and Yasui, H. 2012. Mapping and pyramiding of two major genes for resistance to brown planthopper (*Nilaparvata lugens* Stâl) in the rice cultivar ADR52. *Theor and Appl Genet* 124(3): 495-504

Normile, D. 2008. Reinventing rice to feed the world. *Science* 321: 330-333

Pathak, P.K. and Heinrichs, E.A. 1982. Selection of biotype populations 2 and 3 of *Nilaparvata lugens* by exposure to resistant rice varieties. *Environ Entomol* 11:85-90

Rahman, M.L., Jiang, W.Z., Chu, S.H., Qiao, Y.L., Ham, T.H., Woo, M.O., Lee, J., Khanam, M.S., Chin, J.H., Jeung, J.U., Brar, D.S., Jena, K.K. and Koh, H.J. 2009. High-resolution mapping of two rice brown planthopper resistance genes, Bph20 (t) and Bph21(t), originating from Oryza minuta. *Theor Appl Genet* 119:1237-1246

Rajpurohit, D., Kumar, R., Kumar, M., Paul, P., Awasthi, A.A., Basha, P.O., Puri, A., Jhang, T., Singh, K. and Dhaliwal, H.S. 2011. Pyramiding of two bacterial blight resistance and a semi dwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica* 178:111–126

Heinrichs, E.A. 1986. Perspectives and directions for the continued soydevelopment of insect-resistant rice varieties. *Agric Ecosyst Environ.* 18:9–36

Rubia-Sanchez, E., Suzuki, Y., Miyamoto, K. and Watanabe, T. 1999. The potential for compensation of the effects of the brown planthopper *Nilaparvata lugens* Stal (Homoptera: Delphacidae) feeding on rice. *Crop Prot* 18:39–45

Salgotra, R.K., Gupta, B.B. and Praveen, S. 2009. Combining ability studies for yield and yield components in Basmati rice. *Oryza* 46 (1): 12-16

Sharifi, P., Dehghani, H. and Moghddam, M. 2009. Genetic and genotype x environment interaction effects for Appearance quality of rice. *Agri Sci* China, 8(8): 891-901

Sogawa, K. 1982. The rice brown planthopper: Feeding physiology and host plant interactions. *Ann Rev Entomol* 27: 49:73

Sreewongchai, T., Toojinda, T., Thanintorn, N., Kosawang C., Vanavichit A., harreau, D. and Sirithunya, P. 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

Stam, P. and Zeven, A.C.1981. The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. *Euphytica* 30: 227-238

Tanaka, K. and Matsumura, M. 2000. Development of virulence to resistant rice varieties in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), immigrating into Japan. *Appl Entomol Zool* 35:529-533

Velusamy, R., Ganesh, M. and Johnson, Y.S. 1995. Mechanism of resistance to Brown Planthopper Nilaparvata lugens in wild rice. (*Oryza ssp*.) cultivars. *Entomol Exp Appl* 74: 245-251

Virmani, S.S. 1994. Heterosis and hybrid rice breeding (IRRI). *Springer-Verlag,* pp7

Wu, K.S. and Tanksley, S.D. 1993. Abundance, polymorphism and genetic mapping of microsatellite in rice. *Mol Gen Genet* 241:225-235

Yang, H.Y., You, A.Q., Yang, Z.F., Zhang, F.T., He, R.F., Zhu, L.L. and He, G.C. 2004. High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.). *Theor Appl Genet* 110:182-191

Yu, H.H., Xie, W., Li, J., Zhou, F.S. and Zhang, Q.F. 2014. A whole-geneme SNP array (Rice6k) for genomic breeding in rice. *Plant Biotech* 12: 28-37

Zhang, Q.F. 2007. Strategies for developing green super rice. *Proc Natl Acad Sci*, USA, 104:16402-16409

Zhu, Y., Chen, H., Fan, J., Wang, Y. and Li, Y. 2000. Genetic diversity and disease control in rice. *Nature* 406: 718-726

McCouch, S.R., Kochert, G., Yu, Z.H. 1988. Molecular Mapping of rice chromosome. *Theor Appl genet,* 76:815-829

Cagampang, G.B., C.M. Perez and B.O. Juliano, 1973. A gel consistency test for eating quality of rice. *J. Sci. Food Agr*. 24: 1589-1594

****