Breeding for Heat Tolerance Rice Based on Marker-Assisted Backcrossing in Vietnam

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ABSTRACT A total of six markers RM3586 and RM160 on chromosome 3 and RM3735, RM3471, RM3687 and RM3536 on chromosome 4 were used to select promising lines in backcrossing populations for heat tolerance at flowering stage in rice. Fifty lines selected in BC3F2, BC4F1, and BC4F2 and parents were planted in 2013, and 2014 dry seasons at the CLRRI field under natural heat stress and greenhouse to evaluate heat tolerance at the reproductive period. Heat tolerance scoring under field condition was based on percentage of unfilled grains. All selected lines exhibited their homozygous alleles with two heat tolerance germplasm N22 or Dular in QTL loci. Twelve lines harboring homozygous alleles to QTL loci RM3586 on chromosome 3 and RM3735 on chromosome 4, respectively were selected and evaluated to agronomic traits and yield potential. Four lines BC4-1-10-1 from OM5930/N22//4*OM5930, BC4-5-8 from OM5930/Dular//4*OM5930, BC4-5-9-4 from AS996/N22//4*AS996, and BC4-6-3 from AS996/Dular//4*AS996, respectively were finally selected to would be for regional adaptable test in Central Coast of Vietnam under heat stress condition to release to rice farmers.

Keywords Rice, Heat tolerance, Marker-assisted selection, Backcross breeding

INTRODUCTION

Temperature is a highly significant environmental factor in the growth and development of plants. Climate change will lead to the change of damages by drought, high temperature and spectrum of insect damage in Vietnam. The heat stress especially occurring during flowering stage in rice growth period results in low yield by low seed setting rate (Morita et al. 2005; Peng et al. 2004). So the research on the genetic mechanism of heat tolerance is getting more and more important to the utilization of heat tolerant gene and the development of new rice varieties with heat tolerance. The most sensitive growth stages of rice to heat stress are flowering time (Mackill et al. 1982; Kuang et al. 2002). In the Central Coast of Southern Vietnam, heat stress at the flowering stage has become a big problem in recent years. High percentage of unfilled spikelets and low grain filling rate (GFR) were obtained due to heat stress at flowering stage (Lang et al. 2015).

High temperature, 35°C beyond threshold level, at flowering stage is considered as a critical level to harm floret fertility and grain yield (Matsushima et al. 1982; Li 2003). Two germplasm N22 and Dular were considered as highly tolerance genotypes to heat stress at flowering stage (Jagadish et al. 2010; Buu et al. 2012, 2013).

Development of rice varieties with tolerance to high temperature stress at the flowering stage is therefore essential for maintaining rice production as the climate continues to normal condition. Two major QTLs that affected heat stress tolerance were detected in the interval between RM5687 and RM471 on chromosome 4, and between RM6132 and RM6100 on chromosome 10 (Xiao...
Marker assisted selection (MAS) is a process whereby a molecular marker is used for indirect selection of a genetic determinant or determinants of a trait of interest (e.g. abiotic stress tolerance).

To identify transcripts induced by heat stress, twenty-day-old rice seedlings of different rice cultivars suffering from heat stress were treated at different times, and differential gene expression analyses in leaves were performed by cDNA-AFLP and further verified by real-time RT-PCR (Cao et al. 2013). More than three thousand different fragments were indentified, and 49 fragments were selected for the sequence and differential expressed genes were classified functionally into different groups.

Grain filling and amylose in the starch were strongly affected by high temperature. Four QTLs, qHAC4, qHAC8a, qHAC8b and qHAC10, which can reduce the deleterious effects of amylose content at high temperature, were identified and mapped to chromosome 4, 8, 8 and 10, respectively. The major QTL qHAC8a, with the highest LOD score of 6.196, was physically mapped to a small chromosome segment (~300 kb) (Zhang et al. 2014).

Development in genomics has provided new tools for discovering and tagging novel alleles and genes. These tools can enhance the efficiency of breeding programs through their use in MAS (Xu and Couch 2008). The use of the genomic tools is more effectively identifying, quantifying, and characterizing genetic variation from all available germplasm resources ( Tanksley et al. 1989; Tanksley and McCouch 1997; Gur and Zamir 2004). The rate of success is likely to increase in gene-base marker development, with more efficient quantitative trait locus (QTL) mapping procedures and lower cost genotyping systems. They need available computational tools, which tailored molecular breeding programs (Xu and Couch 2008). Many reviews comparing the molecular genetic issues related to different types of markers assay, which can significantly affect the success of MAS (Avise 2004). Marker assisted introgression has been shown as an effective approach for precise transfer of genes from wild species with minimum linkage drag (Young and Tanksley 1989) and also it could help in identifying genotypes containing the target gene in early generations even if it is suppressed in a particular genetic background (Hurni et al. 2014).

A total of 310 lines (BC2F2) derived from the cross of OM5930*3/N22 has been evaluated for heat stress at flowering stage. Genetic map has been set up with 264 polymorphic SSR markers to detect linkage to the target traits (Buu et al. 2004). The map covers 2,741.63 cM with an average interval of 10.55 cM between marker loci. Markers associated with heat tolerance have been located mostly on chromosomes 3, 4, 6, 8, 10, and 11. The proportions of phenotypic variation explained by each QTL of markers RM3586 and RM160 on chromosome 3 and RM3735 on chromosome 4 were 36.2, 17.1 and 32.6%, respectively. Two QTLs for filled grains per panicle have been detected at the interval of RM468-RM7076 and RM241–RM26212 on chromosome 4, explaining 13.1% and 31.0% of the total phenotypic variation, respectively. Two QTLs controlling unfilled grain percentage have been also detected at loci RM554, RM3686 on chromosome 3 explaining only 25.0% and 11.2% of the total phenotypic variation. One QTL has been detected for 1,000-grain weight located at the locus RM103 on chromosome 6, explaining 30.6% of the total phenotypic variation. A single QTL at the locus RM5749 on chromosome 4 was identified explaining 10.8% of the total phenotypic variance of grain yield. Attentions have been paid to the interval of RM3586-RM160 at the range of 8.1 cM on chromosome 3 for heat tolerance score (Buu et al. 2014). The subsequent backcross populations derived from the cross of OM5930/N22 have been continued to select via conventional procedures and MAS based on the selected markers.

The heat tolerance rice breeding has been continued through the collaborative research project between NICS, RDA (Korea) and CLRRI, IAS (Vietnam); we conducted the breeding program assisted by molecular markers, which were previously identified in QTL mapping and analysis (Buu et al. 2013, 2014). The study aims to select new promising lines adaptable to heat stress in the central coast of Southern Vietnam from the crosses with heat tolerance germplasm N22 and Dular based on marker-assisted selection using SSR markers.
MATERIALS AND METHODS

Plant materials

Five indica varieties, OM5939, AS996, IR66 (leading varieties of high yielding, susceptible to heat), Gayabyeo (Tongil-type from indica/japonica, high yielding, susceptible to heat) and IKO547 (resistance to brown planthopper possessing Bph18) were used as recurrent parents to develop heat tolerance lines. Two germplasm N22 and Dular were used as donor of QTLs for heat tolerance. The QTL-NIL populations were developed by backcross method based on marker-assisted selection (MAS) (Fig. 1). Twelve QTL-NIL lines of BC$_3$F$_2$, BC$_4$F$_2$, and BC$_5$F$_2$ were finally selected and evaluated to agronomic traits and heat tolerance with check variety OM4900 (Table 1 and 3).

![Fig. 1. Scheme for the development of backcross breeding lines for heat tolerant QTL using marker-assisted foreground and background selection.]

| Line no. | Cross combination                  | Gen. | Designation |
|----------|-----------------------------------|------|-------------|
| HTL 1    | OM5930/N22//4*OM5930               | BC$_3$F$_2$ | BC4-1-10-1  |
| HTL 2    | OM5930/Dular//4*OM5930             | BC$_4$F$_2$ | BC4-5-8     |
| HTL 3    | AS996/N22//4*AS996                 | BC$_3$F$_2$ | BC4-5-9-4   |
| HTL 4    | AS996/Dular//4*AS996               | BC$_3$F$_2$ | BC4-6-3     |
| HTL 5    | Gayabyeo/N22//4*Gayabyeo           | BC$_3$F$_2$ | BC4-5-6     |
| HTL 6    | HT114/4*IR66                       | BC$_3$F$_2$ | BC3-4       |
| HTL 7    | HT111//4*IKO547                    | BC$_3$F$_2$ | BC3-21      |
| HTL 10   | AS996/Dular//2*AS996               | BC$_3$F$_2$ | BC3-2-49    |
| HTL 12   | AS996/Dular//2*AS996               | BC$_3$F$_2$ | BC3-4-48    |
| HTL 13   | AS996/Dular//2*AS996               | BC$_3$F$_2$ | BC3-4-49    |
| HTL 14   | OM5930/N22//4*OM5930               | BC$_3$F$_3$ | BC4-5-8-1   |
| HTL 15   | OM5930/N22//5*OM5930               | BC$_3$F$_2$ | BC5-9-4     |
Evaluation of QTL-NILs under heat stress

Twelve lines of BC$_3$F$_2$, BC$_4$F$_1$ and BC$_4$F$_2$, and parents were planted at the CLRRI field and green house to evaluate heat tolerance during the reproductive period in dry season during 2012 and 2013. To ensure the lines head at the same time all were exposed to the same high temperature stress conditions at heading. Daily average temperature was automatically monitored via meteorological tool from flowering to harvest stages in the rice field. The daily temperature was noticed from 35°C to 40°C at the flowering - harvesting stages in the greenhouse. Heat tolerance (HT) scoring under field condition was based on percentage of unfilled grains as follows: 0 (0-10%), 1 (10-15%), 3 (15-20%), 5 (20-25%), 7 (25-30%) and 9 (>30%).

DNA extraction and PCR amplification

Genomic DNA was extracted using CTAB method as described in Lang (2002). The young leaf was ground using a polished glass rod in well of a Spot Test plate (Thomas Scientific) after adding 400 µl of extraction buffer (50 mM tris-HCl pH 8.0, 25 mM EDTA, 300 mM NaCl and 1% SDS). Additional 400 µl of the extraction buffer was added and mixed into the well by pipetting. Thereafter, 400 µl of lysate was transferred to the original tube of leaf sample. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA was precipitated using absolute ethanol. DNA was then air-dried and resuspended in 50 µl of TE buffer. An aliquot of 1 µl was used for PCR analysis. DNA quality and quantity were spectrophotometrically determined.

PCR amplification was performed in 10 mM Tris-HCl (pH=8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 1 unit of TAKARA Taq, 4 nmole of dNTP, 10 pmole of primer, with 30 ng of genomic DNA per 25 µl using a thermal cycler 9,600 (Perkin-Elmer). The PCR reactions were denatured at 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. The final extension was set at 72°C for 5 min. After PCR, 13 µl of loading buffer (98% formamide, 10 mm EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) were added to the total volume of PCR product. The samples were electrophoresed on 3% agarose gel and consequently stained in ethidium bromide and viewed under UV light for band detection.

Marker-assisted selection to QTLs

Two QTL loci related to heat tolerance were selected using RM3586 and RM160 on chromosome 3, and RM3735, RM3471 and RM3687 on chromosome 4 (Buu et al. 2014). These markers were used to select plants possessing QTL in every backcross (Fig. 1). The homozygous plants were finally selected and used to evaluate agronomic traits and heat tolerance in the field and greenhouse.

RESULTS

Marker-assisted selection on chromosome 4

The PCR products at locus RM3735 linked to QTL for heat tolerance on chromosome 4 (Buu et al. 2014) in BC$_3$F$_1$ population of OM5930/N22//4*OM5930 indicated that twelve plants homozygous alleles like N22 (Supplementary Fig. 1); similarly 15 plants in BC$_4$F$_1$ of Gayabeyo/N22//4*Gayabyeo were homozygous alleles like N22 (Supplementary Fig. 2). This marker was linked to QTL for heat tolerance from N22. The selected plants would be heat tolerance by possessing QTL. Two markers RM3687 and RM3471 on chromosome 4 were used to identify the homozygous for recurrent parents (Supplementary Fig. 3-5). In BC$_3$F$_1$ population of OM5930/N22//4*OM5930, only seven plants of 23, 24, 25, 41, 43, 44 and 45 were introduced to QTL allele of N22 in RM3735 and allele of recurrent parent OM5930 in RM3687 and RM3471 (Supplementary Fig. 1 and 3). Eight plants of 15, 16, 17, 18, 19, 20, 21 and 33 in BC$_4$F$_1$ of Gayabeyo/N22//4*Gayabyeo possessed only QTL allele (Supplementary Fig. 2, 4 and 5). These plants were advanced to generation by self-pollination and used to evaluate major agronomic traits and heat tolerance in the field and greenhouse.

Marker-assisted selection on chromosome 3

Two markers RM3586 and RM160 linked to QTLs for heat tolerance on chromosome 3 (Buu et al. 2014) were screened to select plants possessing QTLs. The PCR products at locus RM3586 on chromosome 3 in BC$_4$F$_1$ of
AS996/N22//4*AS996 were detected to heterozygous QTL allele in 33 plants of 1-32 and 44 (Supplementary Fig. 6). In this population, 30 plants of 1, 2, 4, 5, 7-32 were to harbor hetero allele to QTL for heat tolerance (Supplementary Fig. 9). Eleven plants of 1, 2, 4, 5, 8-11, 24, 27 and 32 harboring N22 allele in two loci of RM3586 and RM160 were advanced the generation to BC4F2 and finally selected homozygous plants for QTL loci. In the population of BC3F1 of AS996/Dular//4*AS996, only six plants, 1, 28-32 harboring Dular allele in two loci in RM3586 and RM160 were selected (Supplementary Fig. 7 and 9) and advanced the generation to select homozygous allele in QTL loci.

**Evaluation for agronomic traits and yield**

A total of fifty lines selected to heat tolerance in eight cross combinations based on MAS (Table 1) were evaluated to major agronomic traits and heat tolerance in the CLRRI field. Only twelve lines were phenotypical acceptability and high percentage of filled grain under field heat stress over 35°C at reproductive stage. The twelve lines were transplanted in the field and greenhouse in two replications for evaluating agronomic traits and yield potential in 2014 dry season. The growth durations from transplant to harvest were 90-105 days. Out of a line BC4-5-8, the growth durations of four lines were same with a leading variety OM4900, and others have two to five days of difference to OM4900. Plant height showed to similar tendency with the growth duration (Table 3). In number of filled grains per panicle, except two lines BC4-5-8 and BC4-6-3, all lines were 85-93 similar with 90 of OM4900. The 1,000-grain weights were 26-29 g except BC4-5-6 of 24.8 g. The grain yields of BC4-1-10-1, BC4-5-8, BC4-5-9-4, BC4-6-3 and BC4-5-8-1 were 6.21-7.63 t/ha significantly higher than other lines and leading variety OM4900. BC4-4-10-1 obtained the lowest grain yield (5.0 t/ha) in the OYT nursery 2013 (data not shown), however high yield of 6.21 t/ha in PYT nursery 2014. BC4-5-8 obtained the highest yield of 7.63 t/ha in the PYT 2014, and two lines BC4-5-9-4 and BC4-6-3 were 6.93 t/ha and 6.90 t/ha, respectively.

Based on MAS result (Table 2), phenotypical acceptability, yield components and yield, we finally selected four lines BC4-1-10-1 from OM5930/N22//4*OM5930, BC4-5-8 from OM5930/Dular//4*OM5930, BC4-5-9-4 from AS996/N22//4*AS996, and BC4-6-3 from AS996/Dular//4*AS996, respectively. These lines would be evaluated in regional adaptable test in Central Coast of Vietnam under heat stress condition for releasing to rice farmer.

**Table 2.** Selection of five promising lines containing heat tolerant QTL on chromosome 3 and 4 based on MAS in BC4F2 population from the crosses among three recurrent and two donor parents.

| Lines\(^{2}\) | Chr. 3 | Allele type (bp)\(^{3}\) | Chr. 4 | Allele type (bp)\(^{3}\) | Response to heat stress\(^{3}\) |
|---|---|---|---|---|---|
| | RM3586 | RM3471 | RM3735 | RM3687 | |
| OM5930 (RP) | 220 | 215 | 215 | 215 | S |
| AS996 (RP) | 220 | 215 | 200 | 215 | S |
| Gayabyeo (RP) | 220 | 215 | 200 | 215 | S |
| N22 (DP) | 210 | 200 | 220 | 200 | T |
| Dular (DP) | 200 | 210 | 210 | 210 | T |
| BC4-1-10-1 | OM | OM | N22 | N22 | MT |
| BC4-5-9-4 | AS | AS | N22 | AS | T |
| BC4-5-6 | Gaya | Gaya | N22 | Gaya | T |
| BC4-5-8 | OM | OM | Dular | OM | T |
| BC4-6-3 | AS | AS | Dular | AS | T |

\(^{2}\)RP: recurrent parent; DP: donor parent to heat tolerance

\(^{3}\)OM: OM5930 allele; AS: AS996 allele; Gaya: Gayabyeo allele; N22: N22 allele; Dular: Dular allele

\(^{3}\)S: susceptible, MT: moderate tolerance; T: tolerance
Table 3. Major agronomic traits and grain yield of promising lines selected via MAS in CLRRI experimental field in dry season 2014.

| Lines       | Growth duration (Day) | Plant height (cm) | No. of panicle/m² | No. of filled grains/panicle | 1,000-grain weight (g) | Grain yield (t/ha) |
|-------------|-----------------------|-------------------|-------------------|------------------------------|------------------------|-------------------|
| BC4-1-10-1  | 98                    | 96 c-d            | 340 b-e           | 91 ab                        | 28.0 ab                | 6.21 c            |
| BC4-5-8     | 97                    | 97 c-d            | 350 a-d           | 80 e                         | 29.0 a                 | 7.63 a            |
| BC4-5-9-4   | 100                   | 99 b-c            | 370 ab            | 93 ab                        | 26.5 ab                | 6.93 b            |
| BC4-6-3     | 105                   | 105 a             | 380 a             | 98 a                         | 26.5 ab                | 6.90 b            |
| BC4-5-6     | 98                    | 97 c-d            | 350 a-d           | 85 bc                        | 24.8 b                 | 5.62 de           |
| BC3-4       | 95                    | 94 d-e            | 330 cde           | 92 ab                        | 28.5 a                 | 5.20 e            |
| BC3-21      | 96                    | 97 c-d            | 320 de            | 89 ab                        | 26.0 ab                | 5.61 de           |
| BC3-2-49    | 100                   | 101 b-c           | 310 e             | 86 bc                        | 26.0 ab                | 5.44 de           |
| BC3-4-48    | 100                   | 99 b-c            | 350 a-d           | 87 bc                        | 27.0 ab                | 4.51 f            |
| BC3-4-49    | 100                   | 99 b-c            | 360 abc           | 93 ab                        | 26.0 ab                | 5.61 de           |
| BC4-5-8-1   | 90                    | 90 e              | 370 ab            | 92 ab                        | 27.0 ab                | 6.53 bc           |
| BC5-9-4     | 103                   | 103 b-e           | 340 b-e           | 89 ab                        | 28.0 ab                | 5.72 d            |
| OM4900 (CK) | 100                   | 94 d-e            | 360 abc           | 90 ab                        | 26.0 ab                | 5.64 de           |

*Days from transplant to harvest
Grain yield is rough rice
a-f: DMRT at 0.05 with three replications

DISCUSSIONS

Heat stress at the flowering stage leads to loss rice grain yield enormous by low seed setting in tropical region under climate change (Morita et al. 2005; Peng et al. 2004). Recently, a few articles were studied to identify QTLs related to heat tolerance (Xiao et al. 2011; Cao et al. 2013; Zhang et al. 2014). These QTLs related to heat tolerance were used to develop the promising lines of heat tolerance based on marker-assisted selection (MAS) (Xu and Couch 2008, Avise 2004, Hurni et al. 2014). A variety OM8608 genotype, which was selected for QTL related to heat tolerance by MAS is well adaptable to Ninh Thuan Province of hot spot for drought and heat stress in Vietnam. The heat tolerance rice breeding has been continued through the collaborative research project between NICS, RDA (Korea) and CLRRI, IAS (Vietnam). We developed eight advanced backcross populations among leading varieties and core germplasm N22 and Dular for heat tolerance via MAS system using five molecular markers, RM3586 and RM160 on chromosome 3 and RM3471, RM3735 and RM3687 on chromosome 4. A marker RM3586 on chromosome 3 could detect promising progenies, which inherited from both N22 and Dular. The QTL controlling unfilled grain percentage has been also detected at locus RM3686 on chromosome 3 explaining 11.2% of the total phenotypic variation (Buu et al. 2014). Attentions have been paid to the interval of RM3586 - RM160 on chromosome 3 at the range of 8.1 cM for QTL of heat tolerance score. A total of fifty QTN-NILs selected to heat tolerance in eight cross combinations based on MAS (Table 1) were evaluated to major agronomic traits and heat tolerance in the CLRRI field. Especially, there was considered to phenotypical acceptability. Twelve lines were finally selected for the evaluation of agronomic traits and yield potential. Most lines were similar to a leading variety OM4900 for the growth duration from transplant to harvest, plant height, number of panicle per m², number of filled grains per panicle and 1,000-grain weight. Five promising lines increased to 10.1-35.3% of grain yield.
compared to leading variety OM4900. Especially, the percentage of unfilled grain per panicle in QTL-NIL harboring QTL related to heat tolerance was lower than leading variety OM4900 and their recurrent parents (data not shown).

Based on phenotypical acceptability and data for agronomic traits and yield potential, four lines were finally selected to BC4-1-10-1 from OM5930/N22/4*OM5930, BC4-5-8 from OM5930/Dular/4*OM5930, BC4-5-9-4 from AS996/N22/4*AS996, and BC4-6-3 from AS996/Dular/4*AS996, respectively. These lines were homozygous in QTL locus of N22 with heat tolerance varieties OM8108 and OM10040, and lines TLR391, TLR392 and TLR394 by RM3735 on chromosome 4 (Fig. 2). In grain filling rate (GFR) observed beside unfilled grain percentage, two-day GFR collected data were analyzed to conclude that OM8108 and BC4-1-10-1 were over 110 mg dry matter/day/main panicle (unpublished data). The QTL linked to RM3735 on chromosome 4 explained 32.3%, 20.7% and 10% of the total phenotypic variation in BC2F2 populations of OM5930/N22/2*OM5930, AS996/N22/2*AS996, and AS996/Dular/2*AS996, respectively, for heat tolerance with the acceptable value of LOD (Buu et al. 2014). Four promising lines BC4-1-10-1, BC4-5-8, BC4-5-9-4 and BC4-6-3 would be evaluated in regional adaptable test in Central Coast of Vietnam under heat stress condition. The best line will be released as rice variety for reducing the damage by high temperature during reproductive stage in dry season. Also, the backcross breeding program via marker-assisted selection was helpful to reduce the duration of two to three seasons for developing promising lines.

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REFERENCES

Avise JC. 2004. Molecular markers, natural history, and evolution. 2nd ed. Sinauer Associates, Sunderland, MA.
Guimares EP, Ruane J, Scherf BD, Sonnino A, Dargie JD. 2007 Marker-assisted selection current status, and future perspectives in crops, livestock, forestry, and fish. FAO, Rome.
Buu BC, Nguyen VH, Vo TT, Bui PT, Vo TTM, Chau TN, Lang NT. 2012. Assessment of breeding materials for heat tolerance rice breeding (Oryza sativa L.). J. Agric. Rural Dev. (Vietnamese, English Abstract) 12: 38-46.
Buu BC, Pham TTH, Bui PT, Tran TN, Nguyen VH, Nguyen TP, Luong TM, Ly HG, Lang NT. 2014. Quantitative Trait Loci Associated with Heat Tolerance in Rice (Oryza sativa L.). Plant Breeding and Biotechnology 2: 14-24.
Buu BC, Thu Ha PT, Tam BP, Nha CT, Lang NT. 2013. Study on genetic variation of heat tolerance trait in BC population of rice (Oryza sativa L.). J. Agric. Rural Dev. (Vietnamese; English Abstract) 2: 10-15.
Cao YY, Zhang Q, Chen YH, Zhao H, Lang YZ, Yu CM, Yan JC. 2013. Identification of differential expression genes in leaves of rice (Oryza sativa L.) in response to heat stress by cDNA-AFLP analysis. BioMed Research Res. International. Epub 2013 Feb 17. Volume 2013, Article ID

Fig. 2. PCR products for identifying homozygous at locus RM3735 linked to QTL for heat tolerance from N22 on chromosome 4.
Gur A, Zamir D. 2004. Unused natural variation can lift yield barriers in plant breeding. PLoS Biol. 2: e245.

Hurni S, Brunner S, Stirnweis D, Herren G, Peditto D, McIntosh RA, et al. 2014. The powdery mildew resistance gene Pm8 derived from rye is suppressed by its wheat ortholog Pm3. Plant J 79: 904-913.

Jagadish SVK, Muthurajan R, Oane R, Wheeler TR, Heuer S, Bennett J, Craufurd PQ. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (Oryza sativa L.). Journal of Experimental Botany 61: 143-156.

Kuang HC, Wen SS, Liu GM. 2002. Studies on the heat tolerance of Luhui 17 and its cross II You 7 at head sprouting. Southwest China Journal of Agricultural Sciences, 15: 106-108. (in Chinese)

Lang NT, Pham TTH, Pham CT, Tran TQ, Luong TM, Ly HG, Hoang VB, Tran TDP, Nguyen TG, Nguyen TH, Truong VH, Mai BN, Buu BC. 2015. Rice lines with heat tolerance via marker-assisted selection. Journal of Science and Technology in Vietnam. (Vietnamese, English abstract) 1: 53-59.

Li CD. 2003. Analysis on a large of empty grains of rice due to high temperature. Shanxi J Agric Sci. 49: 45-47.

Mackill DJ, Coffman WR, Rutger JN. 1982. Pollen shedding and combining ability for high temperature tolerance in rice. Crop Science, 22: 730-733.

Matsushima S, Ikewada H, Maeda A, Honma S, Niki N. 1982. Studies on rice cultivation in the tropics 1: yielding and ripening responses of the rice plant to the extremely hot and dry climate in Sudan. Japan J Trop Agri. 26: 19-25.

Morita S, Ji Yonemaru, Ji Takanashi. 2005. Grain growth and endosperm cell size under high night temperatures in rice (Oryza sativa L.). Annals of Botany, 95: 695-701.

Peng SB, Huang JL, Sheehy JE, Laza RC, Vissers RM, Zhong XM, Centeno GS, Khush GS, Cassman KG. 2004. Rice yields decline with high temperature from global warming. Proceedings of the National Academy of Sciences of the USA, 101, 9971-9975.

Tanksley SD, McCouch SR. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277: 1063-1066.

Tanksley SD, Young ND, Paterson AH, Bonierbale MW. 1989. RFLP mapping in plant breeding: New tools for an old science. Biotechnology (N.Y.) 7: 257-263.

Xiao YH, Pan Y, Luo LH, Deng HB, Zhang GL, Tang WB, Chen LY. 2011. Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (Oryza sativa L.). Euphytica. 178: 331-338.

Xu Y, Couch JH. 2008. Marker-assisted selection in plant breeding: from publication to practice. Crop Sci. 48: 391-407.

Young ND, Tanksley SD. 1989. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. Theor Appl Genet 77: 353-359.

Zhang H, Duan L, Dai JS, Zhang CQ, Li J, Gu MH, Liu QQ, Zhu Y. 2014. Major QTLs reduce the deleterious effects of high temperature on rice amylose content by increasing splicing efficiency of Wx pre-mRNA. Theor Appl Genet 127: 273-282.