

**DETECTION OF HEAT RELATED QTL IN RICE AT FLOWERING  
STAGE USING SELECTED INTROGRESSED LINES \***

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**INTRODUCTION**

A quantitative trait locus is a position in a chromosome that contains one or more genes involved in the determination of a quantitative trait. The theory of quantitative trait loci (QTL) mapping was first described in 1923 by Sax and further elaborated by Thoday in 1961. Since the advent of DNA marker technology in 1980s, it has been dramatically enhanced the efficiency of plant breeding. Single- marker analysis is the simplest method for detecting QTLs associated with single markers. Individuals in a mapping population are analyzed in terms of DNA marker genotypes and the phenotype of interest. The individuals are split into classes according to marker genotypes for each DNA marker. Mean and variance parameters for the phenotype of interest are calculated and compared among the marker classes. A significant difference between the DNA marker genotype classes for the trait of interest indicates a linkage between the DNA marker and the trait of interest (Singh, 2013). QTL mapping studies for heat tolerance have been conducted on various rice populations at flowering stage (Xiao *et al.*, 2011; Ye *et al.*, 2015). The main objective of this research was to improve the heat tolerant trait of MH86 restorer and identify major QTLs responsible for heat tolerant trait.

**MATERIALS AND METHODS;**

In this study, Minghui86 (MH86) as the recurrent parent (RP), an *indica* restorer which is the male parent of many elite *indica* hybrid cultivars commercially grown in South China were used. Four *indica* type donor parents (DP) were used including two Chinese varieties (JiangxiSimiao and Hanhui10) one

\* See "Tropical Agriculturist" Volume 164 for details.

variety (IR65600-27-1-2-2) from Philippines and Bala variety originated from India. Initial crosses were made using RP with 4 donors winter season of 2004 in Hainan to develop 4  $F_1$ s. The 4  $F_1$ s were backcrossed to the recurrent parent, MH86 in the summer 2004 to produce  $BC_1F_1$  seeds, from which 25 random  $BC_1F_1$  plants were backcrossed to the RP to produce 25  $BC_2F_1$  lines. All  $BC_2F_1$  lines were planted in the field and allowed to self-produce  $BC_2F_2$  seeds which were bulk harvested as random as 4  $BC_1F_2$  populations. The seeds were cold stored with the cooling storage facility of Chinese Academy of Agricultural Sciences (CAAS), Institute of Crop Sciences, in Beijing.

Preliminary screening of the  $BC_2F_2$  bulk population for Heat Tolerant (HT) was conducted in Hainan, in summer of 2008 under the green house where temperature maintained around 40 °C at flowering stage. A total of 400 random plants from each of the bulk  $BC_2F_2$  populations were transplanted into a field under a structure of iron bars. The plants were allowed to grow under normal management practices until start flowering. The structure was covered with a polythene material at the time of flowering to make heat stress treatment. The polythene cover was removed after flowering and grain filling was allowed under normal condition. At maturity, individual plants were selected which were having good seed set in comparison to the recurrent parent, MH86. The selected heat tolerant individuals have shown spikelet fertility (63-77%) while recurrent parent, MH86 showing 29%.

Selected  $BC_2F_{2.3}$  plants from each population had few numbers of seed per plant therefore, each selected plants were grown in the winter nursery for multiplication of seed in Hainan during the 2008/09 winter season. Progeny test was conducted in Hainan 2009 Summer and verified the HT performance of selected lines. At the same time, genotype evaluation was done with SSR markers in Institute of Crop Sciences, CAAS, using 20-30g of  $BC_2F_{2.4}$  seeds of each line.  $BC_2F_{2.5}$  and  $BC_2F_{2.6}$  introgressed lines were used for collecting phenotypic data growing them during two summer seasons of the year 2011 and 2012 respectively in Hunan at Agricultural Research Station at Liu yang, China (28° 09' N, 113° 37' E). Seeds were sown (2011 May) in the nursery prepared in the field, and 28 days old seedlings were transplanted in plastic sheds, with two replications, at the same time open field with 3 replications having 10-12 plants in a row with 20×17 cm space with Randomized Complete Block Design (RCBD). The RP, DP and heat tolerant check (CK), N22 were planted repeatedly after each set of

introgressed lines. The field was managed according to standard practice in the area. Plants were grown under flooded condition for the whole growth period. Ventilation mechanisms in the plastic shed were kept open to make minimize the heat stress during the vegetative growth period and ripening period. Heat stress was created closing the side walls and top vent during the flowering period. The maximum temperature inside the shed was maintained within 37-40 °C and no control for night temperature (23-28 °C) during flowering period. Daily maximum and minimum air temperature and relative humidity at the canopy level were recorded with a digital electronic thermometer (Oregon, Scientific, IN-OUT Thermo-Hygrometer and Clock, Model: THG312). Heading date (HD, days) was recorded when 50% of the plants in each line at heading. Plant height (PH, cm) and panicle number (PN) were measured three middle plants in each line at maturity and harvested including RP, DP, and CK. Filled grain number per panicle (FGN), spikelet number per panicle (SNP), filled grain rate (FGR, %) calculated as (FGN/SNP)\*100, thousand grain weight (TGW, g), yield (GY, g/plant) were evaluated under normal and heat stress conditions. Heat tolerant is evaluated using heat stress index, HSI= (FGR under normal condition - FGR under heat stress condition)/FGR under normal condition.

The experiment was continued with BC<sub>2</sub>F<sub>2.6</sub> populations for the year 2012 in the same field. All the ILs were grouped into 5, (104-100d, 99-96d, 95-92d, 91-88d and 87-82d) based on heading date observed in 2011 and sown 5 different days, transplanted with two times to ensure that all lines to be headed at the same time and exposed to the same heat stress condition at the flowering stage. All the other management practices and data collection were same as the previous year.

DNA was extracted from bulked seedlings from BC<sub>2</sub>F<sub>2.4</sub> populations using the CTAB method with slight modification. SSR markers of RM series from 1 to 600 and some markers above RM 1000 (<http://www.gramene.org>) were used for the parents polymorphism and afterword for the populations. Mapchart 2.0 software was used to draw genetic linkage map with polymorphism markers reference to Cornell University (<http://www.gramene.org>) marker distance. One-way ANOVA performed with SAS 8.1 software. QTLs were identified for heat stress index and yield related traits under high temperature stress condition and the normal condition for two years with the significant level of P<0.05. When a QTL was detected by two or more linked markers, the one with the highest F-value was presented.

## RESULTS AND DISCUSSION

Phenotypic variation explains (PVE) for each trait for the all four populations is comparatively higher in this analysis data. The reason might be directional selection under strong selection pressure and selected lines may belong to the upper tail of the normal distribution population for the heat tolerant trait. So, these QTLs might be the major QTLs for the relevant traits. Moreover, small population size also may lead to loss of detection of minor QTLs and thereby increase the PVE for the detected QTLs.

Twelve BC<sub>2</sub>F<sub>2</sub> plants for the population MH86/JianxiSimiao (J), Ten BC<sub>2</sub>F<sub>2</sub> plants for the population MH86/IR65600-27-1-2-2 (K), twelve BC<sub>2</sub>F<sub>2</sub> plants for the population MH86/Hanhui10 (L) and thirteen BC<sub>2</sub>F<sub>2</sub> plants for the population MH86/Bala (M) were selected during the screening under ~ 40 °C temperatures at flowering stage. All the selected plants showed average spikelet fertility (SF) 70.8% (range, 50-91%) while recurrent parent MH86 showing SF as 29% with the range of 5-67% at flowering stage (Table 1). Polymorphism Simple Sequence Repeats (SSR) markers were found as 59, 62, 63 and 71 for the population J, K, L and M respectively distribution among 12 rice chromosomes.

**Table 1. Screening results of 4 Minghui86 (RP) BC<sub>2</sub>F<sub>2</sub> backcross populations for heat tolerance at the flowering stage in Hainan, 2008**

Shuhui527/ Donor Parents	Origin	Population code & size	No. of selected plants	SI <sup>1</sup> (%)	SF (%)	
					Mean± SD	Range
Minghui86/Donor parents						
JiangxiSimiao	China	J, 400	12	3.0	67.9±9.0	50.0-81.3
IR65600-27-1-2-2	Philippines	K, 400	10	2.5	63.4±10.4	50.0-81.4
Hanhui10	China	L, 400	12	3.0	75.0±10.5	50.0-90.9
Bala	India	M, 400	13	3.3	77.0±11.9	54.5-90.7
Mean		400	12	3.0	70.8±6.3	
<b>Minghui86</b>					<b>28.7±22.8</b>	<b>5.0-66.7</b>

Note: All populations belongs to sub species *indica*; <sup>1</sup>SI= selection intensity, SF= spikelet fertility.

There was no significant yield improvement of four populations under normal conditions in 2011 and 2012 except population MH86/Bala. Ranking was done according to the average HSI and grain yield (GY) for two years under stress condition. The best population was MH86/IR65600-27-1-2-2 which shows the lowest HSI (0.43) and the highest GY (10 g/plant). The second one was the population MH86/Hanhui10 (9.65 g/plant, HSI 0.44), Population MH86/Bala (9 g/plant, HSI 0.57) and population MH86/Jianxisimiao (6.95 g/plant, HSI 0.62) were third and fourth respectively according to the ranking results.

The QTL associated with RM180 was found from 3 populations except MH86/Hanhui10. In addition to that RM473, RM474, RM202 and RM237 were detected at least from two populations suggesting that these are important and stable QTLs in MH86 background.

## CONCLUSIONS

A total of 13 QTLs were detected for HSI, including three for population J, six for K and two for L and two for population M. QTL associated with SSR marker RM180 was detected from 3 populations except population L. RM473, RM474, RM202 and RM237 were found at least from two populations suggesting that these are important in selection of heat tolerance QTLs in *indica* background.

## REFERENCES

- Singh, B.D. 2013. Plant Breeding Principles and Methods. Kalyani Publishers, New Delhi, India. 752-753.
- Xiao, Y., L. Yp, G. Luo, H. Zhang, L. Deng, X. Dai, W. Liu, L. Tang, Chen G. Wang. 2011. Quantitative trait loci associated with seed set under high temperature stress at flowering stage in rice. *Euphytica* 178: 331-338.
- Ye, C., F.A. Tenorio, M.A. Argayoso, M.A. Laza, H.J. Koh, E.D. Redona, S.V. Krishna, Jagadish and G.B. Gregorio. 2015. Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics* 16: 41.