RICE BREEDING FOR HEAT TOLERANCE AT INITIAL STAGE

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ABSTRACT

Rice is sensitive to high-temperature stress at almost all the growth stages of its, this stress is major factor causing poor grain filling. The study aims are: (1) to identify genotype and phenotype of heat tolerance breeding lines under 37-40^{0}C condition at flowering stage; (2) to identify some promising lines adapted to Southern Vietnam via marker-assisted selection and genotype-by environment interaction (GxE) analysis. Twenty two crosses were made by the IAS and CLRRI. F_4, F_5 and BC_2 were developed to set up RIL and BC mapping populations. Backcrossing was done with the donors as N22 and Dular, and recurrent parents as OM5930, AS996. Heat treatment of 36-40^{0}C at heading stage under field and phytotron conditions. DNA survey with 45 SSRs among 218 rice accessions was implemented to identify the polymorphic markers. Five characters were selected to assess the progenies of two BC_2F_2 of AS996 x Dular (1080 lines) and OM5930 x N22 (310 lines) as X1: Tolerance score, X2: Plant height, X3: Unfilled grain percentage, X4: 1,000-grain weight, X5: grain yield. Unfilled grain percentage must be considered as the target trait, which strongly related to heat tolerance. RM3586 on the chromosome 3 could be used for MAB to quickly develop BC_4.

Keywords: *grain filling, heat tolerance, marker assisted backcrossing*

INTRODUCTION

Rice is sensitive to high-temperature stress at almost all the stages of its growth and development. Heat tolerance stress influencing at heading (T > 35^{0}C) are recognized, then flowering, pollination and pollen tube development are inhibited and influenced to grain filling (Morita *et al*., 2005; Peng *et al*., 2004; Zhu *et al*., 2005). Three QTLs located on chromosome 1, 4, and 7 are known to relating to heat tolerance (Zhu *et al*., 2005). Interval marker analysis between **C1100-R1783** on chromosome 4 is pinpointed (Zhu *et al*., 2005). Two SSRs are reported as RM3735 on chromosome 4, and RM3586 on chromosome 3 closely linked to the heat tolerance QTL (Zhang *et al*., 2009). *OsWRKY11* and *OsHsfA2e* transgenes related to heat shock protein were reported by Wu *et al*., (2009) and Yokotani *et al*., (2008). The crucial role of heat shock protein 101 (Hsp101) in imparting thermotolerance to cells was noticed. Jagadish *et al*., (2007, 2008, and 2010) proposed standard evaluation of heat tolerance phenotyping.

Study objectives:

- To identify genotype and phenotype of heat tolerance breeding lines under 37-40^{0}C condition at flowering stage
- To identify some promising lines adapted to Southern Vietnam via marker-assisted selection and GxE analysis

The team who works for heat tolerance rice breeding included the Institute of Agricultural Sciences for Southern Vietnam (IAS), Cuu Long Delta Rice Research Institute (CLRRI), and Agricultural Science Institute for

Southern Coastal Central of Vietnam (ASISOV). Thanks are due to the fund supported by Vietnam Government (2010-2014) and Korea project (2010-2012). The initial stage is with the following activities:

- Activity 1: DNA survey with given SSR markers & clustering breeding materials
- Activity 2:
  - ✓ Single crosses, marker assisted backcrosses with male parents: N22, Dular and female parents: OM5930, AS996.
  - ✓ Sharing the breeding materials to project members (Korea, Cambodia, Thailand, Philippines, Malaysia)
- Activity 3:
  - ✓ DNA extraction,
  - ✓ Genotyping with confirmed polymorphic SSRs and putative QTLs.
- Activity 4:
  - ✓ Grain filling phenotyping under both phytotron and field conditions
  - ✓ Critical temperature at heading, unfilled grain percentage and grain yield.
  - ✓ GxE interaction analysis through multi-locational yield trials

MATERIALS AND METHODS

Twenty two crosses were made by the IAS and CLRRI. F_4, F_5 and BC_2 were developed to set up RIL and BC mapping populations. Combined heat tolerance, drought, high yielding and resistance to brown plant hopper (BPH) and blast were designed by CLRRI team.

Breeding materials

- 146 rice accessions were collected to assess their heat tolerance including 7 *japonica* cultivars, and 139 *indica*; there are 50 improved genotypes and 96 landraces.
- Varieties for control treatments were used as AS996 (Mekong Delta, Southeastern regions), VNĐ 95-20 (Dong Thap Muoi), ML48 (Ninh Thuan, Coastal regions) under field conditions.
- Susceptible control variety: Moroberekan
- Donor varieties: Gayabeo, Hanareum, **N22**, Dular, Nipponbare
- Recurrent parents: OM5930, AS996

Phenotyping in phytotron

Rice samples at initiation stage were sampled and kept 24-48 hours before sending to growth chamber with the treatments as followed:

- 7- 8 am: 29^{o}C at phytotron
- 8-10 am: 34^{o}C
- 10-12 am: 37^{o}C
- 12-14 pm: 39^{o}C
- 14-15 pm: 37^{o}C
- 15-16 pm: 34^{o}C
- 16-18 pm: 30^{o}C
- 20pm-7am overnight: 24^{o}C (dark condition)

Maintaining relative humidity of 75%. Pollen grains were treated by 1% iodine potassium iodide.

Table 1. Scoring of heat tolerance based on unfertile grain percentage (UGP)

Score 0	Score 1	Score 3	Score 5	Score 7	Score 9
0-10%	10-15%	15-20%	20-25%	25-30%	>30%

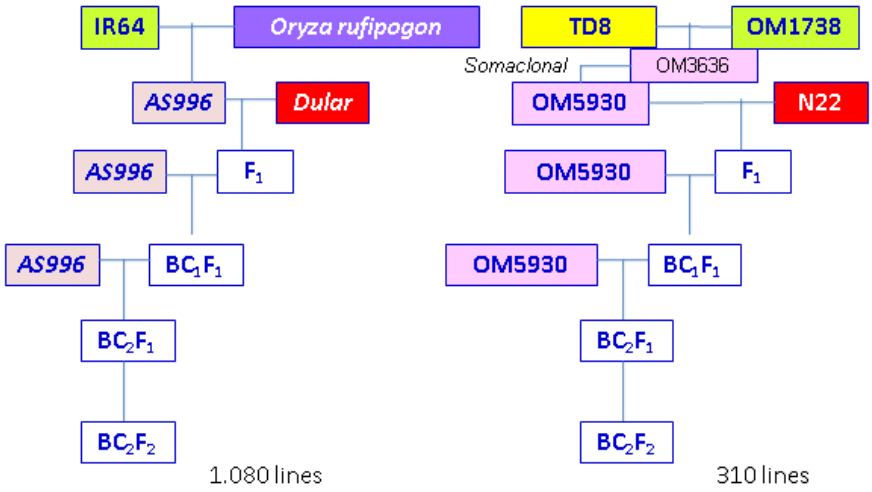

Figure 1. Marker assisted backcrossing scheme of AS996 x Dular and OM5930 x N22

N22 offered the less unfertiled grain percentage (5%), then Dular, Gayabyeo.

- Mong chim roi, Tai nguyen exhibited score 1 (with UGP of 8,3-8,8%)
- Chet Xanh, Tau Huong, Nho Thom, Nanh Chon, Sori do, Nang Huong Cho Dao exhibited score 5 (with UGP of 21-25%)
- Lua Huong, Nang Huong Cho Dao, Re Hanh, Nang Tay Do, Tau Huong, Tieu Chet, Soc Ran, Nho Thom exhibited score 9 (with UGP of # 40%)

RESULTS & DISCUSSIONS

DNA survey

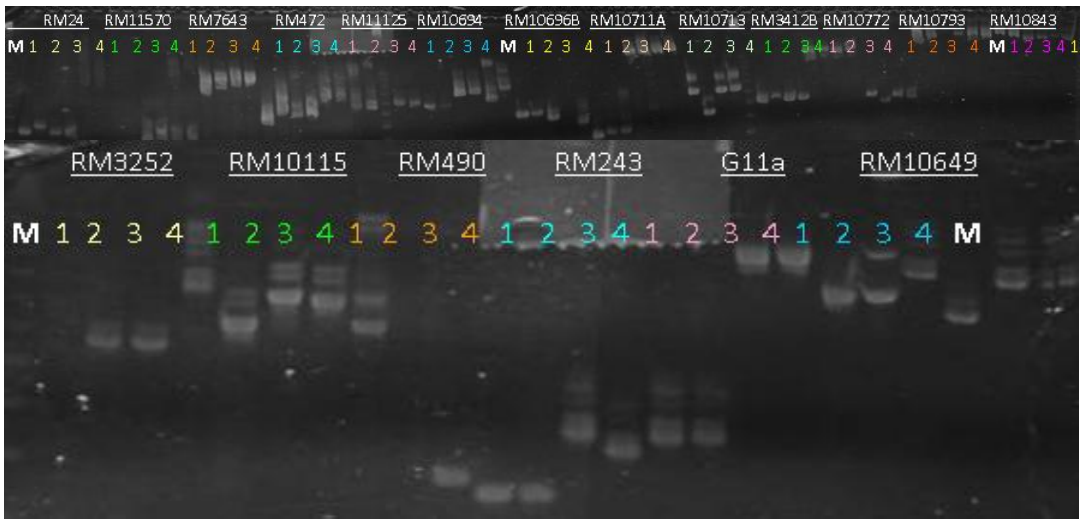

Figure 2. DNA survey with 45 SSRs among 218 rice accessions

Phenotyping of two BC populations under field stress at CLRRI

Fig. 3 shows that the critical temperature of more than 40^0C at flowering stage with pink color linear. Phenotypic evaluation of the backcross lines along with the parents for heat at flowering stage was done by using wax-petrolatum layers in the greenhouse of CLRRI from 2007-2012.

The experiment was laid out by randomized complete block design with three replications. Each experimental plot was 300 m^2. The BC_2F_2 lines and parents were soaked, germinated in incubator, and sowed into plastic trays. After 15 days, they were transplanted into basins which were made of cement. Ten-day after transplanting, the growing rice was developed under field temperature condition until harvesting. Subsequently, the samples were obtained to analyze agronomic characters and grain yield.

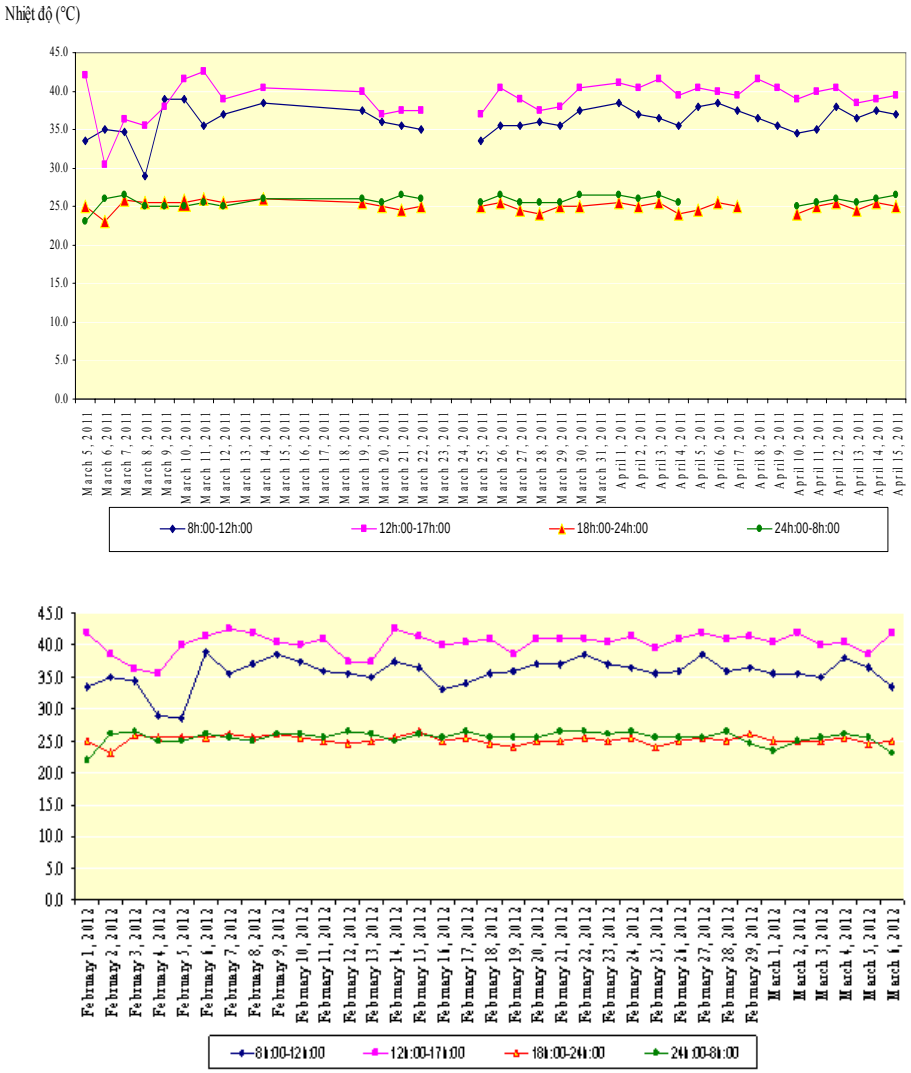

Figure 3. Temperature dynamic at BC_1F_1 (above) and BC_2F_2 (below) under field condition (Meteo-database by CLRRI)

Genotyping through QTL analysis on chromosomes 3 and 4

Four characters were selected to assess the progenies of two BC2F2 of AS996 x Dular (1080 lines) and OM5930 x N22 (310 lines). DNAs for PCR were prepared by using DNA extraction procedure with CTAB method.

- X1: Tolerance score
- X2: Plant height
- X3: Unfilled grain percentage
- X4: 1,000-grain weight
- X5: grain yield

The data in table 2 indicate that unfilled grain percentage must be considered as the target trait, which strongly related to heat tolerance

Table 2. Correlation coefficient between heat tolerance score and some agronomical traits

N=168	Growth duration	Plant height	Panicle length	Panicle/hill	Total grains / panicle	Filled grains / panicle	% unfilled grain	Grain yield
R	0.218 Ns	0.113 ns	0.148 Ns	0.073 ns	0.086 ns	0.142 ns	0.929 **	0.106 ns

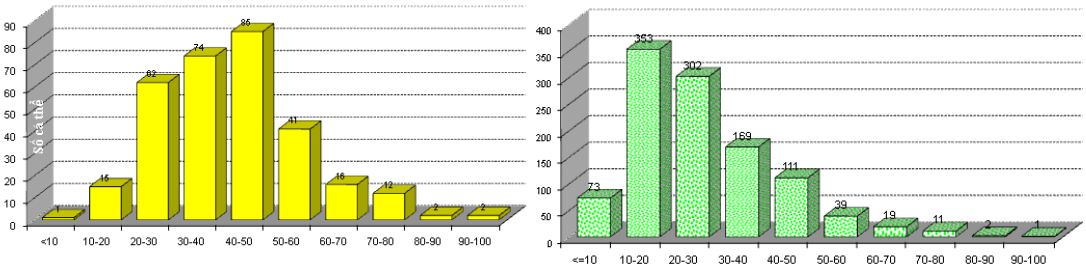

Figure 4. Unfilled grain percentage exhibited the normal distribution in the BC_2F_2 of OM5930/N22 and AS6996/Dular with 310 and 1080 individuals, respectively

QTL analysis at the locus RM3538 on chromosome 3

Table 5 and 6 indicated that RM3586 and RM3735 linked to the QTL related to heat tolerance on chromosome 3 and 4, respectively. Two markers were recommended as

RM3586GAAGAGAGAGCCAGAGCCAG
ACACGATCGAGCTAGAAGACG
chromosome 3, motif (GA)12, PCR products: 118 bp - 142 bp

RM3735GCGACCGATCAGCTAGCTAG
ATAACTCCTCCCTTGCTGCC
chromosome 4, motif (GA)16, PCR: 138 bp – 170 bp

Table 3. ANOVA - Single marker analysis of RM3586 (BC_2F_2 population of OM5930 / N22)

Variable	TMS	Df	EMS	Df	F-RATIO	P-PROB
X1	250.68	2	1.6825	307	148.99	0.000
X2	1558.5	2	689.81	307	2.26	0.104
X3	5337.4	2	197.62	307	27.01	0.000
X4	14.099	2	5.0692	307	2.78	0.062
X5	40.126	2	55.012	307	0.73	0.487

X1:, X2: Plant Hgt, X3: % unfilled Gr, X4: 1,000 Gr Wgt, X5: Gr Yiled

Table 4. ANOVA –Single marker analysis of RM3586 (BC_2F_2 population of AS996 / Dular)

Variable	TMS	Df	EMS	Df	F-RATIO	P-PROB
X1	3646.4	2	3.4267	1077	1064.11	0.000
X2	0.10814E+06	2	980.43	1077	110.30	0.000
X3	47539	2	116.26	1077	408.91	0.000
X4	11.434	2	5.5626	1077	2.06	0.126
X5	3035.4	2	90.361	1077	33.59	0.000

X1: heat Tol score, X2: Plant Hgt, X3: % unfilled Gr, X4: 1,000 Gr Wgt, X5: Gr Yiled

Table 5. Single marker analysis on 1080 lines of OM5930 / N22 (BC_2F_2), LOD ≥ 3,0

Marker	Chr.	Genotype	Allele	F	P	R^2 (%)	DPE
RM3586	3	OM5930, N22	4.150 ± 0.44	10.60	0.000	36.23	B
RM3735	4	OM5930, N22	5.40 ± 0.44	9.16	0.000	32.60	B

DPE: (Direction of Phenotypic Effect); A: OM5930 , B: N22; Chrm: chromosome

Table 6. Single marker analysis on 310 lines of AS996 / Dular (BC_2F_2), LOD ≥ 3,0

Marker	Chr.	Genotype	Allele	F	P	R^2 (%)	DPE
RM3586	3	AS996 Dular	7.40 ± 0.44	7.80	0.000	6.23	B
RM3735	4	AS996 Dular	6.40 ± 0.44	4.16	0.000	32.60	B
RM349	4	AS996 Dular	5.12 ± 0.44	3.15	0.016	3.15	A
RM323	3	AS996 Dular	4.40 ± 0.44	7.80	0.011	4.78	B

DPE: (Direction of Phenotypic Effect); A: AS996, B: Dular; Chrm: chromosome

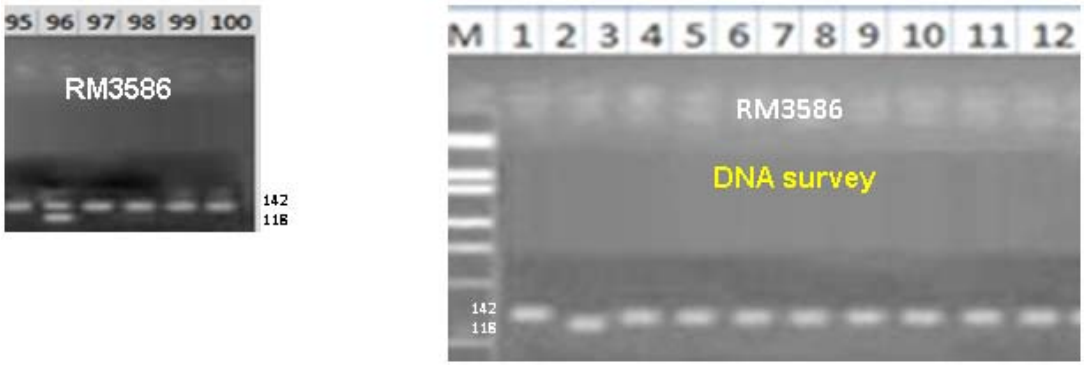

Figure 5. PCR products at locus RM3586 on chromosome 3

Our study will be continued on chromosome 4 in coming year to perform the putative QTLs, which relate to heat tolerance. The proportion of the total phenotypic variation is explained by each QTL was calculated as "R²" value. Amount of phenotypic variation explained together by all the putative QTLs was estimated by fitting a multiple QTL model under Mapmaker/QTL program for the trait. The two genetic loci, especially for RM3586 and RM3735 on chromosome 3 and 4, respectively, can be used in marker-assisted selection approach in heat tolerance rice breeding.

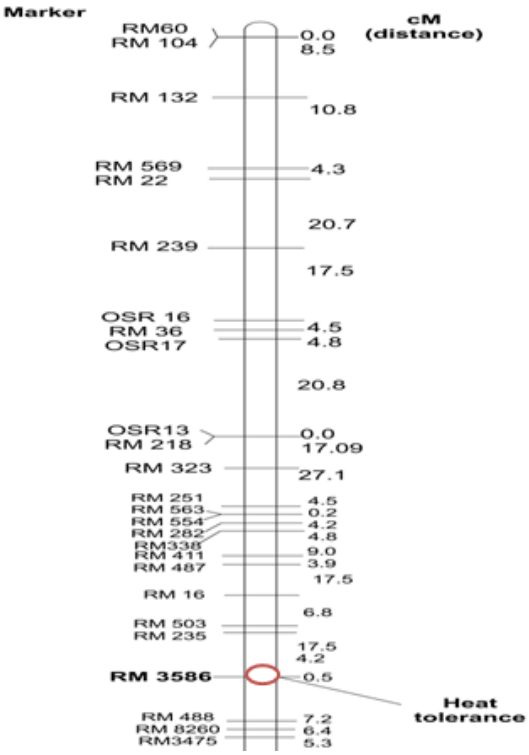

Figure 6. QTL map relating to heat tolerance linked to RM3586 on chromosome 3, LOD ≥ 3.0

Yield Trials:

Yield trials were conducted at 6 sites with 16 genotypes in 2012 dry season. They will be continued in 2013. The different sites are listed as:

1. Qui Nhon: coastal sandy soil
2. Ninh Thuan (two sites): semi arid area
3. Tay Ninh: irrigated upland
4. Dong Nai: irrigated upland
5. Long An: irrigated lowland
6. Can Tho: irrigated lowland

These breeding materials were selected in previous breeding program of CLRRI for climate change adaptation. Sixteen promising genotypes were laid out in RBD, three replications. Their flowering time must be in the critical temperature (36-40^{o}C). Unfilled grain percentage and grain yield were especially noticed. The G x E interaction was analyzed.

Some promising varieties were mentioned as: TLR397, OM8108, OM6707, TLR392, TLR391 due to low unfilled grains and high yield under heat stress.

Table 7. Multilocational Yield Trial (t/ ha) in summer 2012 with critical temperature 36-42^{O}C at heading stage

Designation	Ninh Thuan 1	Tay Ninh	Dong Nai	Ninh Thuan 2	Can Tho	Dong Thap Muoi	Mean	RANK
OM8108	5.76	3.85	1.73	6.28	8.76	5.59	5.3372	3
OM6707	5.58	3.87	3.63	5.68	9.13	6.15	5.6765	1
OM10040	4.18	3.57	2.70	4.77	9.10	5.52	4.9755	7
OM10375	3.12	2.75	0.50	2.50	7.73	3.57	3.3662	15
TLR390	3.66	2.64	0.93	4.87	7.36	4.39	3.9775	14
TLR391	4.56	3.53	2.60	4.74	9.96	5.77	5.1978	5
OM10030	3.95	3.85	0.90	4.63	9.76	5.10	4.7035	10
TLR392	3.59	3.86	3.03	4.67	8.76	5.49	4.9034	8
OM10037-3	3.85	2.88	4.13	4.54	8.76	5.81	4.9998	6
OM10029	4.25	3.77	1.66	4.37	8.16	4.73	4.4966	12
TLR393	3.45	3.63	2.56	3.67	9.40	5.21	4.6566	11
TLR394	4.03	4.03	1.93	5.51	7.93	5.12	4.7623	9
TLR395	4.08	4.23	3.70	4.84	8.86	5.80	5.2562	4
TLR396	3.36	3.38	2.63	3.81	8.56	5.00	4.4599	13
TLR397	4.52	3.96	3.80	5.76	9.10	6.22	5.5633	2

Table 8. The GxE analysis by Eberhard – Russel Model

Designation	b_i	S^2di	Designation	b_i	S^2di
OM8108	1.0078	1.0220	OM10037-3	0.8844	0.7961
OM6707	0.8957	0.1781	OM10029	0.9550	0.162
OM10040	1.0334	-0.0030	TLR393	1.1145	0.3558
OM10375	1.0730	0.3541	TLR394	0.8845	0.3455
TLR390	0.9630	0.4407	TLR395	0.8660	0.1654
TLR391	1.1852	0.0400	TLR396	0.9788	0.1834
OM10030	1.3127	0.2597	TLR397	0.9005	0.1561
TLR392	0.9455	0.1413	***SE***	***0.0603***	***0.0220***

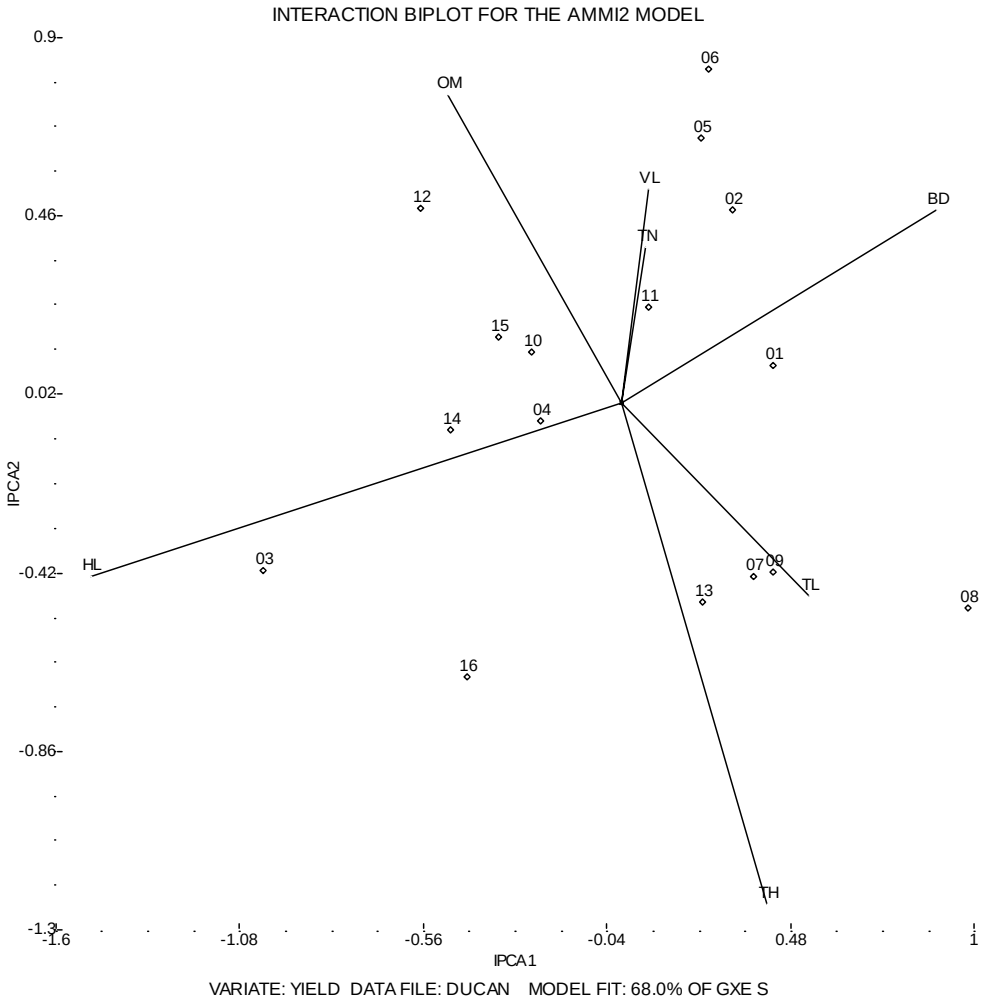

Figure 7. The GxE analysis by BSTAT – AMMI model with 68% of fit model.
Legend: 01: OM10029; 02: OM10030; 03: OM10037-3; 04:OM10040; 05: OM10375; 06: OM10383; 07: OM6707; 08: OM8108; 09: TLR390; 10: TLR391; 11: TLR392; 12: TLR393; 13: TLR394; 14: TLR395; 15: TLR396; 16: TLR397

Further looks

- Perform QTL map for heat tolerance with the total length of 1,600-2,000 cM; ∅ between two marker as ≤ 15 - 20 cM.
- Fine mapping on chromosomes 3 and 4; identify the candidate genes and reliable markers for rice breeding.
- More crosses should be made, marker assisted selection and marker assisted backcrossing should be applied to develop BC_4F_2 and recombinant inbred F_6, F_7 lines
- Conducting the GxE interaction analysis to identify the promising genotypes, which will be released into large scale areas?

Proposal

The heat tolerance rice breeding project supported by Vietnam Government and Korea must go on in phase two because of its effectiveness via the international partnership.

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CHỌN TẠO GIỐNG LÚA CHỐNG CHỊU NÓNG NHỜ CHỈ THỊ PHÂN TỬ (GĐ 1)

Lúa rất nhạy cảm với nhiệt độ nóng nhất là giai đoạn trổ bông đến chín, ảnh hưởng nghiêm trọng đến quá trình vào chắc của hạt. Mục tiêu nghiên cứu: (1) xác định kiểu gen và kiểu hình của các dòng vật liệu ở điều kiện nhiệt độ 37-40^{0}C vào lúc lúa trổ; (2) xác định được các dòng triển vọng đã được chọn nhờ marker, thông qua so sánh năng suất nhiều điểm ở vùng cực trọng vào mùa hè. Hai mươi hai tổ hợp lai được thực hiện bởi IAS và CLRRI. F_4, F_5 và BC_2 được phát triển để có quần thể bản đồ QTL với các dòng RIL và BC. Hồi giao được thực hiện với giống cho gen kháng là N22 và Dular, giống tái tục là OM5930, AS996. Nhiệt độ lúc lúa trổ được theo dõi trên đồng ruộng và trong phytotron với mức cực trọng là 36-40^{o}C. Điều tra đa hình DNA với 45 SSRs trong 218 mẫu giống lúa bản địa và giống lúa cao sản. Năm tính trạng được theo dõi trên quần thể BC_2F_2 của AS996 x Dular (1080 dòng) và OM5930 x N22 (310 dòng) là X1: điểm chống chịu nóng, X2: chiều cao cây, X3: tỷ lệ hạt lép, X4: khối lượng 1000 hạt, X5: năng suất. Phần trăm hạt lép được khẳng định là tính trạng quan trọng nhất, nó có tương quan thuận, chặt chẽ với điểm chống chịu nóng. RM3586 định vị trên nhiễm sắc thể số 3 có thể được sử dụng làm chỉ thị đánh dấu để thực biện MAB nhanh chóng phát triển quần thể BC_4.