G ×E Interaction and QTL Analysis for Agronomic Traits under Different Environments in Rice

HUANG XING

General abstract

Heat stress is one of the major environmental stresses in rice cultivation that can occur both in vegetative and reproductive phases. Temperature beyond critical thresholds can reduce the growth duration of the rice, and more severely it can cause an increase at spikelet sterility, reduce grain-filling duration, and enhance respiratory loss, resulting in yield loss. Understanding how crops interact with their environments is increasingly important in breeding programs, especially in light of highly anticipated climate changes. Development of heat tolerant cultivars is one of the most effective methods to maintain rice production under climate changes in temperate regions. This study was conducted to investigate the genotype x environment interactions of rice cultivars and RILs under different environments, three different latitude regions accounting for seven environments. In addition, QTL mapping analysis for high temperature using single environment analysis and QTL x environment interaction using multi-environment analysis were also undertaken in present study to detect genomic regions linked to important phenotypic traits for adaptation. A total of 105 rice cultivars and a set of recombinant inbred lines (RILS) derived from Dasanbyeo (*indica*) / TR22183 (*japonica*) crosses were used as plant materials to study the genotype X environment interactions

and QTL analysis for high temperature in rice across several environments. Those plant materials were grown in the three locations which showed seven diverse environments including Suwon (Korea) during 2010 and 2011 rice growing seasons, Shanghai (China) during 2010 and 2011 growing seasons, and IRRI (Philippines) during 2010 growing season, 2011 wet season and 2011 dry season. A randomized block design with two replications was used. The materials were cultivated with conventional methods in each location. Eight important agronomical traits including days to heading (DTH), culm length (CL), panicle length (PL), panicle number per plant (PN), spikelet number per panicle (SN), spikelet fertility (SF), 100-grain weight (GW), and grain yield (GY) were measured across seven environments for 105 rice cultivars, 150 RILs population, and their parental lines. The collected agronomic traits were subjected to AMMI (additive main effects and multiplicative interactions) model analysis using CropStat 2.3 software. In regards to QTL mapping analysis, leaves of rice seedlings at the three-leaf stage were harvested and subjected to genomic DNA extraction according to the CTAB method. A 384-plex GoldenGate oligo pool assay (OPA) set and distributed evenly through the 12 rice chromosomes was employed for genotyping 150 RILs population and the parents using VeraCode technology on an Illumina BeadXpress Reader. The results showed that most of rice cultivars exhibited yield stability across all environments and only a few genotypes were unstable. For grain yield, the environment effect of environment at IRRIWS2010 was the highest one, followed by Shanghai2010, IRRIWS2011, IRRIDS2010, Shanghai2011, Suwon2011, and Suwon2010. It is suggested that the yield of tested cultivars was affected by genotypes, environments and G × E interaction, simultaneously. In the meantime, the largest environment effect on spikelet fertility was detected in Shanghai2010 compared to that of in IRRIDS2011 and the other environments which showed less environment effects. Among the evaluated traits, SF and GY showed a high G × E interaction with the mean value of 57% and 39%, respectively suggesting the genotype stability of these two traits were unstable in different trails. The data also revealed that the lowest environment effect was detected in Suwon, followed by in Shanghai,and IRRI. The effect in IRRI were stable compared

to those in Shanghai indicating that Suwon is suitable to obtain a stable cultivars with low G × E interaction environment and can be suitable for control of trials. IRRI was also suitable for investigating stable cultivars with high G × E interaction for high temperature screening. In addition, based on AMMI results, environment B (Shanghai 2010), F (IRRI dry season), C (IRRI wet season) were highly related to the GY revealing these environment suitable for heat tolerant screening. In year replications, environment E (Shanghai2010) did not show highly related to the GY, indicating that heat stresses in Shanghai were not stable. Therefore, it can be concluded that IRRI is more suitable for screening heat stress compared to the other two locations used in present study.

The results come from regional trial data analysis have reference values for crop breeders, and multi-locational screening is a good strategy for developing heat tolerant varieties in rice. For the traits measured in multiple environments, 37 QTLs were detected in which some of the QTLs were detected in at least two environments. Of these, six QTLs were detected for days to heading, six for culm length, four for panicle length, three for panicle number, four for spikelet number, seven for grain weight, three for grain yield and four for spikelet fertility.

The results obtained in the present study provide a scientific basis of stability and adaptability of rice genotypes against high temperature stress and would be helpful in developing rice varieties for high temperature tolerance.

Key words : environments, G × E interaction, rice, AMMI, QTLsStudent Number: 2009-31275

Contents

General Introduction

High temperature have seriously harmed rice production which cause severe damages to both grain quality and yield. Rice is sensitive to high temperature stress at almost its growth stages causing poor grain filling. An increase in temperature 5 ℃ beyond threshold level at rice flowering stage caused floret sterility and resulted in high yield losses (Osada et al. 1973; Stake and Yoshida 1978). When the spikelets were exposed to temperature above 35℃ for 5 days during anthesis, pollen shedding would be disturbed and pollen germination would be impaired resulting in highly spikelet sterility (Yoshida (6). In accordance, after plants exposed to high temperature during anthesis the number of pollen grains shed on stigma was reduced (Tsutomu Matsui *et al.*. 1997, 2001). Heat stress reduces anther dehiscence and pollen fertility rate significantly in heat sensitive variety, while in the heat tolerant variety this effects were smaller. However, spikelet number per panicle, spikelet fertility, grain weight were significantly decreased in both tolerant and sensitive varieties (Cao *et al.* 2008). In addition, a high nighttime temperature decreased pollen germination rate and spikelet fertility (Mohammed et al., 2009). On the other hand, when the temperature raised during grain filling stage, the chalkiness of rice would be increased (Asaoka *et al.*. 1984), polished rice rate would be reduced and lead to reduction of grain yield. Overall, rice is the most sensitive to high temperature at flowering time which cause highly sterile and lead to reduce of yield.

Development of heat tolerant cultivars is one of the most effective methods to

maintain rice production under climate changes in temperate regions (Horie *et al*.. 1996). Breeders and farmers also tried to find other strategies to reduce high temperature effects on grain yield, for example by adjusting seeding time to avoid a high temperature occurs during anthesis episode, known as breed early morning flowering (EMF) varieties on avoiding high temperature (Ishimaru *et al*.. 2010).

Improving tolerance to heat-induced damage is crucial effort to deal with adverse effects of heat stress in rice breeding program. Through the multi-environment method, it is expected to identity stable rice varieties adapted in high temperature environments as well as to identify the heat tolerance genes and/or QTL x environment interaction express in heat condition. A total of 150 rice cultivars and a set of recombinant inbreed lines population of rice derived from a cross between Dasanbyeo (Indica) and TR22183 (Japonica) were developed as plant genetic materials used in present study. Those plant materials were cultivated in seven environment at three different regions, such as in the temperate zone (Suwon), subtropical zone (Shanghai) and tropical zone (IRRI).

The general objectives of present study were to 1) identify rice genotypes tolerance to heat in a specific environment and to evaluate cultivars that have both high mean yield and stable yield performance across different environments, 2) to examine heat tolerant related traits in RILs under different environmental conditions and to investigate the G×E interaction patterns in multi-locational screening of heat tolerance, and 3) to identify the effect of chromosomal segments (QTLs) expressed under different temperatures in several environments and to identify single nucleotide

polymorphism (SNP) markers x environment interaction in order to provide explanations of variable QTL genotypic differences across environments.

Chapter I . Genotype x environment interaction for agronomic traits in rice cultivars under different environments based on AMMI model

Abstract

Rice is sensitive to high temperature stress at almost its growth stages causing poor grain filling. Genotype by environment interaction effect on rice grain yield is usually significant due to large variation in both weather and soil conditions at growing sites. Therefore, a multi-environment yield trial is essential in estimating genotype by environment interaction and identification of superior cultivars. This study were aimed to determine grain yield stability and the response pattern of genotypes across seven environments and to identify high yielding cultivars that could be used as parental lines in breeding program. A total 105 rice cultivars originated from several countries were cultivated in seven different environments and eight agronomic traits were evaluated and subjected to additive main effects and multiplicative interactions (AMMI) model. The results showed that among the agronomic traits, spikelet fertility (SF) and grain yield (GY) showed a high GxE interaction with the mean value of 57% and 39%, respectively. 'Nampung' a Korean's rice cultivar showed the lowest yield stability index (YSI) with high yield mean. While 'IR' serials showed low value of YSI and high yield mean indicating their stability over in all environments. 'Taichung178', 'Hangangchal', and 'Chengcheong' had a high value of yield mean but their AMMI stability value (ASV) rank was very high compared to others suggesting these varieties had a large environmental variation and more suitable for specific environments. Most of varieties

which have low YSI value detected in present study belong to indica subspecies, while those with high YSI value are japonica type. The results come from regional trial data analysis have reference values for crop breeder.
Key words: rice, heat stress, cultivar, AMMI, stability

Introduction

Rice is a staple food for over half of the world's population and the rice consumption tends to increase in every country from year to year. In conjunction with the world's rising consumption level, the world's rice production should be also expanded in order to meet the demand. However, the increasing threat of climate change is already having a substantial impact on agricultural production worldwide as heat waves caused significantly yield losses with great risks for future global food security (Christensen and Christensen, 2007). A temperature increase of 3–4^{o}C could cause crop yields to fall by 15–35% in Africa and Asia and by 25–35% in the Middle East (Ortiz et al 2008).

In rice, high temperature have seriously harmed rice production which cause severe damages to both grain quality and yield. Previous studies revealed that the temperature increases 5 ℃ beyond threshold level at rice flowering stage caused floret sterility and resulted in high yield losses (Osada et al. 1973; Stake and Yoshida 19782). Improving tolerance to heat-induced damage is considered as one of the best ways to deal with adverse effects of heat stress in rice breeding program. Therefore, it is necessary to develop new varieties with improved heat tolerance in rice production.

Screening for heat tolerance in the field presents a challenge due to interactions

with other environmental factors but a wide variety of screen-able traits is available that allows successful selection in the field (Hall, 2011). The use of multi-environments method allows us to identity rice genotypes tolerance to heat in different temperature environments as tolerance to high temperature is a multigenic characters. Through this method stable varieties under high temperature condition can be easily selected.

In the multi-environment trials, increasing attention has been recently paid to the interaction between the genotype and environment (GxE). The GXE interaction structure is a valuable aspect of plant breeding programmes and the introductions of new crop cultivars. Hence, it is necessary to understand the causes of GxE interaction in order to establish breeding objectives, identify ideal test condition and to formulate recommendations for areas of optimal cultivar adaptation (Sabaghnia et al. 2008).

A variety of statistical procedures are in fact available to analyze and determine the results of multi-location trials and GxE interaction data. However, in order to provide a better description of static and dynamic concepts of stability for interpreting GxE interaction, the additive main effects and multiplicative interaction (AMMI) model is considered as an effective approach to explore the interaction. AMMI model proved to be a powerful tool in diagnosing GxE interaction patterns (Crossa 1990). The AMMI analysis is useful in informing important decisions in breeding program, such as which genotypes exhibit specific adaptation and the selection of testing environments (Edbon and Gauch 2002). It can also be used to determine stability of the genotypes across locations using the PCA (principal component axis) scores and ASV (AMMI stability value).

In addition, AMMI analysis provides a graphical representation (biplot) to summarize information on main effects and interactions of both genotypes and environments simultaneously. The biplot facilitates a very powerful graphical tool to display the results of AMMI model. Guach (1988) has firstly used the AMMI model in multi-environment trials. Increasing experiences in the theory and application research proved that the AMMI analysis can separate errors from residual and improve the accuracy of estimation and by using the biplot, genotype environment interactions can be described.

Purchase (2000) developed the AMMI stability value (ASV) based on the IPCA1 and IPCA2 value for each genotype. ASV is defined as the distance from zero in AMMI2 result which IPCA1 scores against IPCA2 scores. Farshadfar et al. (2011) recommended to use another approach known as genotype selection index (GSI) in evaluating AMMI stability value and mean yield for yield stability. In present study, a number of rice genotypes were cultivated in different temperature condition regions to evaluate the stability of each genotypes.

The objectives of the study were to identify rice genotypes tolerance to heat in a specific environment and to evaluate cultivars that have both high mean yield and stable yield performance across different environments. Therefore, to achieve our goals, a number of rice cultivars in present study were cultivated in different temperature condition regions in order to evaluate the stability of each genotype.

Materials and methods

Plant materials and experimental design

A total of 105 rice cultivars from Crop Molecular Breeding Laboratory collection, Department of Plant Science, Seoul National University were used as plant materials in this study. These plant materials were cultivated in three countries which showed seven diverse environments including Suwon (Korea) during 2010 and 2011 rice growing seasons, Shanghai (China) during 2010 and 2011 growing seasons, and IRRI (Philippines) during 2010 growing season, 2011 wet season and 2011 dry season. In details, the experimental conditions during 2010–2011 at the three locations are listed in Table 1.

Rice cultivars were seeded in plastic-tunnel seeding nursery and the seedlings were transplanted in the field in a complete block design with two replications. The seedlings were then transplanted to irrigated field condition at one seedling per hill with a density of 15cm between plants and 30cm between rows. Fertilizers were properly applied at the rate of 100 kg N ha^{-1}, 80 kg P ha^{-1}, and 80 kg K ha^{-1} (100-80-80 kg/ha N-P-K).

Phenotypic evaluation

Eight traits comprising culm length (CL), panicle length (PL), panicle number per plant (PN), spikelet number per panicle (SN), spikelet fertility (SF), 100-grain weight (GW), and grain yield (GY) were measured for all cultivars across three locations and two years experiment.

Statistical analysis

A combined analysis of variance and AMMI analysis were performed using CropStat7.2 software obtained from IRRI website. Analysis of variance is used to partition variance into three components: genotype deviations from the grand mean, environment deviations from the grand mean, and GE deviations from the grand mean. Subsequently, multiplication effect analysis is used to partition GE deviations into different interaction principal component axes (IPCA), which can be tested for statistical

significance through ANOVA. Combined ANOVA across the seven environments was performed on each measured trait. The AMMI analysis based on the first two interaction principal component axes (IPCAs) was used to categorize the experimental environments into mega-environment groups and to detect the homogenous sub-regions having similar G× E interaction patterns. A full AMMI model was conducted for each trait. Biplots with the first two multiplicative terms were used to summarize the G× E interaction patterns. The AMMI stability value (ASV) as described by Purchase (2000) was calculated as follows:

$$ASV = \sqrt{\left[SS_{IPCA1}/SS_{IPCA2}\,(IPCA1_{score})\right]^2 + (IPCA2_{score})^2}$$

where SS referred as sum of square. IPCA1 is interaction principal component axis 1 value and IPCA2 is interaction principal component axis 2 value. The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller ASV scores indicate a more stable genotype across environments.

The value of yield stability, known as yield stability index was calculated as follows:

$$YSI = YASV_{rank} + Yield_{rank}$$

where $YASV_{rank}$ is indicated the rank of yield AMMI stability value and $Yield_{rank}$ is the rank of mean yield of genotypes across environments. YSI incorporate both mean yield and stability in a single criterion. Low value of this parameter shows desirable genotypes with high mean yield and stability.

Fertility stability index (FSI) was measured as:

$$FSI = FASV_{rank} + Fertility_{rank}$$

where $FASV_{rank}$ is the rank of fertility AMMI stability value and $Fertility_{rank}$ is mean fertility of genotypes across environments.

Results

Meteorological environments

The daily maximum, minimum, average temperatures of the experimental locations during the rice-growing periods in the years 2010 and 2011 are presented in Fig.1. Among the seven environments, the highest value of daily maximum temperatures occurs during anthesis period was recorded in Shanghai2010 (August), followed by that of in Suwon 2010 (late-July to August). In 2011, the daily maximum temperature value during anthesis were also higher in both Shanghai and Suwon in comparison to that of observed in IRRI. In overall, a high daily average air temperature was observed in Shanghai followed by that of in IRRI and Suwon. While, the low daily average temperature observed in IRRI was higher than those in Shanghai and Suwon.

Phenotypic analysis

Phenotypic analysis of some importance agronomic traits observed from varieties are summarized in Table 2. Detailed performances of each trait in the seven environments are described below.

Culm length and Panicle length

In general, the highest mean value of culm length observed in seven environments of growing season was recorded in Suwon year 2010 (78.9 cm, ranged from 58.7 to 132.7 cm) followed by that of in Shanghai 2011 (78.3 cm, ranged from 55.4 to 161.9 cm) and Suwon 2011 (75.1 cm, ranged from 58.9 to 136.4 cm), while the lowest one was in IRRI at dry season 2011 with mean value of 45.8 cm ranging from 25.9 to 85.6 cm (Table 2). In comparison to the two locations (Shanghai and Suwon) in both years 2010 and 2011, the culm length observed at IRRI tends to be lower in the three growing seasons (2010 wet season, 2011 wet season and 2011 dry season). This phenomenon might be caused by air temperature effect in which a slightly differences value between the daily maximum and low air temperature was observed in IRRI compared to that of in Shanghai and Suwon (Fig 1). The highest mean value of panicle length (PL) was

observed in cultivars grown in Suwon in both year 2010 and 2011. However, mean value of PL recorded in Suwon showed not significantly difference with that of observed in Shanghai 2010 and 2011. On the other hand, the mean value of PL recorded from IRRI showed the shortest one and it is significantly different with the mean values observed in Suwon and Shanghai (Table 1).

Table 1 Environment location and coordinates

Code	Replications	Environments
Su2010	2	Experimental farm of Seoul National University . Suwon, Korea, 126°59' E, 37°16' N, 28.5m a.s.l. May-October, 2010
Sh2010	2	Experimental farm of Shanghai Academy of Agricultural Science. Shanghai, China, 121°24' E, 30°54' N, 5.6m a.s.l. May-October, 2010
IRRI2010	2	Experimental farm of International Rice Research Institute. Laguna, Philippines, 121°15' E, 14°10 ' N, 16.3m a.s.l. June-September 2010
Su2011	2	Experimental farm of Seoul National University . Suwon, Korea, 126°59' E, 37°16' N, 28.5m a.s.l. May-October, 2011
Sh2011	2	Experimental farm of Shanghai Academy of Agricultural Science. Shanghai, China, 121°24' E, 30°54' N, 5.6m a.s.l. May-October, 2011
IRRIDS2011	2	Experimental farm of International Rice Research Institute. Laguna, Philippines, 121°15' E, 14°10 ' N, 16.3m a.s.l. Jauary-May, 2011.
IRRIWS2011	2	Experimental farm of International Rice Research Institute. Laguna, Philippines, 121°15' E, 14°10 ' N, 16.3m a.s.l. June-September 2011

Yield and yield component traits

Yield component traits such as panicle number (PN) observed in Suwon and Shanghai showed a lower mean values compared to those observed in IRRI. The lowest PN mean value of 6.6 was observed in Suwon 2011, while the highest one was recorded in IRRI 2010 wet season (17.4). Overall, mean value of PN observed in IRRI exhibited almost double than that in Suwon and Shanghai (Table 1). On the other hand, spikelet number per panicle (SN) in Suwon and Shanghai exhibited a higher mean values compared to those observed in IRRI. The SN mean values observed in Shanghai and Suwon showed opposite pattern with those observed in IRRI in which PN Mean values observed in Suwon and Shanghai were almost double than IRRI.

The highest mean values of 1000-grain weight was observed in Suwon and IRRI wet growing season of the year 2010 and 2011. In the two growing seasons, GW showed a no significant differences in the mean values between Suwon 2010 (2.58 g, ranged from 1.98 to 3.12 g) and that of in Suwon 2011 growing season (2.75, ranged from 1.99 to 3.58 g). Similarly, the mean values of GW observed in Suwon were also not significantly different with those in both IRRI 2010 wet season (2.56 g, ranging from 2.14 to 3.14 g) and IRRI 2011 wet season (2.69 g, ranging from 2.17 to 3.16 g). In contrast, the mean value of GW recorded in Shanghai 2010 and 2011, and IRRI 2011 dry season showed significantly smaller than other locations (Table 1) indicating environmental conditions affected to 100-grain yield

Another yield component trait, spikelet fertility (SF) observed from seven environments showed a different manner. The lowest SF mean value was significantly recorded in Shanghai 2010 growing season (69.3%, ranged from 24.4 to 96%) followed by IRRI 2011 dry season (82.3%, ranging from 34.7 to 96.9%) and IRRI 2011 wet season (81.5%, ranged from 51.8 to 93.2%). On the other hand, higher mean value of SF in Suwon during the year 2010 (88.9%) and 2011 (92.0%) growing seasons was recorded. This value was not significantly different with SF mean value observed in Shanghai 2011 (89%) and IRRI 2010 wet season (84%). These findings indicated that a

high in daily air temperature at 2010 in Shanghai affected in reducing spikelet fertility of rice cultivars. Moreover, in Shanghai 2010 growing season, the lowest value of 24.4% was observed (Table 1). Despite the lowest mean value of SF observed in Shanghai, since the range of SF value was ranged to 96%, indicating that at least one cultivar showed tolerance to high temperature which promisingly adapted in certain condition.

Response of GY in the seven environments showed similar pattern with SF. The mean value of GY in 2010 Suwon showed the highest one compared to GY recorded in other locations. In accordance to GY in 2010, the mean of GY in Suwon 2011 growing season was also the highest value, followed by GY in Shanghai 2011 growing season. On the contrary, the GY observed in IRRI 2011 wet and dry seasons was the lowest one.

Table 2 Means and phenotypic variations in varieties for agronomic traits

Traits	Location	Mean	Range	Location	Mean	Range
CL	su10	78.9A	58.7-132.7	su11	75.1A	58.9-136.4
	sh10	70.6B	51.4-134.9	sh11	78.3A	55.4-161.9
	phws10	56.6C	34.8-95	phws11	58.8B	34.8-116.6
				phds1	45.8C	25.9-85.6
PL	su10	21.7A	13.6-30.1	su11	21.6A	15-35.1
	sh10	20.8A	15.8-33.3	sh11	21.4A	14.3-28.1
	phws10	18.4B	12.1-31.1	phws11	17.9B	11.4-26.7
				phds11	17.8B	11.3-28.5
PN	su10	8.5B	4.9-14.4	su11	6.6C	2.5-10.8
	sh10	9B	5.2-13.7	sh11	8.3B	5-18.3
	phws10	17.4A	9.7-26.8	phws11	16.9A	9.3-26.7
				phds11	16A	6.4-28.1
SN	su10	158A	74-278	su11	164A	97-302
	sh10	152A	82-316	sh11	162A	95-293
	phws10	86B	27-260	phws11	97B	27-193
				phds11	74C	27-253
GW	su10	2.58A	1.98-3.12	su11	2.75A	1.99-3.58
	sh10	2.41B	1.86-2.93	sh11	2.4B	1.68-2.99
	phws10	2.56A	2.14-3.14	phws11	2.69A	2.17-3.16
				phds11	2.4B	1.61-3.18
GY	su10	228.7A	80.7-324.7	su11	230.5A	99.9-325.6
	sh10	155B	64.8-309.5	sh11	181.6B	97.1-332.2
	phws10	170.5B	43.7-445.1	phws11	151.6C	43.2-367
				phds11	154.6C	79-259.1
SF	su10	88.9A	61.5-97.3	su11	92A	73.3-98
	sh10	69.3C	24.2-96	sh11	89B	74.2-98.1
	phws10	84.1B	55.9-96	phws11	81.5C	51.8-93.2
				phds11	82.3C	34.7-96.9

Behind letters indicated 5% level in LSD test between environments.

Analysis of variance and G×E interactions on GY and SF

Analysis of variance

Three factors including genotype, environment, and interaction were involved in the analysis of variance (Table 3). The partitioning in pooled analysis of variance showed that genotypes x environments interaction was significant (Table 3). The ANOVA also revealed a highly significant differences among other factors such as genotypes and environments for all traits. Based on the ANOVA analysis, the largest effect of genotypic was observed for PL indicated by the sum square (SS) value of 59%, followed by CL (45%). While and the following traits including PN, SN, and CL showed larger effect of the locations indicated by SS value of 70%, 43%, and 43%, respectively. Present study showed that the highest valued of G×E effects were detected on SF (57%), followed by GxE effects of GY (39%) and GW (32%). On the other hand, the lowest GxE effects was observed in PL (SS value of 20%) and CL (SS value of 12%) (Table 3).

Table 3 Combined analysis of variance and AMMI analysis for agronomic traits

	DF	CL		PL		PN		SN	
		MS	SS %	MS	SS %	MS	SS %	MS	SS %
TOTAL	670		100		100		100		100
Genotypes	104	904	45	51	59	24.7634	14	7610.48	39
Environment	6	15137	43	304	21	2127.41	70	148264	43
G X E	560	46	12	3	20	5.34489	16	676.651	18
G X E									
AMMI 1	109	95**	40	8.1**	49	12.0**	44	1247**	37
AMMI 2	107	63**	26	2.6**	15	6.9**	25	816**	23
AMMI 3	105	34**	14	2.3**	14	5.1**	18	556**	15
AMMI 4	103	24**	10	1.5**	9	1.7**	6	415**	11
G X E RESIDUAL	136		10		13		7		14

	DF	GW		GY		SF	
		MS	SS %	MS	SS %	MS	SS %
TOTAL	670		100		100		100
Genotypes	104	0.18	41	9541.81	36	0.01	13
Environment	6	2.01	27	113178	25	0.56	30
G X E	560	0.03	32	1879.86	39	0.01	57
G X E							
AMMI 1	109	0.060**	42	4351**	46	0.030**	60
AMMI 2	107	0.030**	22	1716**	17	0.010**	18
AMMI 3	105	0.020**	13	1307**	13	0.000**	7
AMMI 4	103	0.010**	10	1247**	12	0.000**	7
G X E RESIDUAL	136		13		12		8

** Significant at P<0.01.

AMMI analysis on GY and SF

AMMI uses ANOVA to test the main effects of genotypes and environments. The ANOVA further divided SS into genotype, environment and G×E interaction. In the G×E interaction, AMMI-4 was used for all traits (Table 3) since the AMMI-4 model was significant in the analysis. However, in present study to test the GxE interaction effects, we focused on the two traits, including GY and SF as they are the most important traits targeting for tolerance to high temperature. The result showed that G×E effects on GY explained the SS value of 39% SS and that of on SF was 57%. While, in other traits the genotypes x environments were also significant different explained by the SS.

Furthermore, in the AMMI analysis, the first two IPCAs (AMMI1 and AMMI2) explained a total of 63% of interaction variation on GY and a total of 78% explained the interaction variation on SF. In order to summarize information on main effects and interaction of genotyped and environments, a graphical representation (biplot) in the AMMI analysis was conducted. The AMMI biplot composed of IPCA1 scores and the means of genotypes or provided the pattern of G×E interaction for each trait. The biplot results of the G×E interactions and main effects in GY and SF are presented in figure 2.

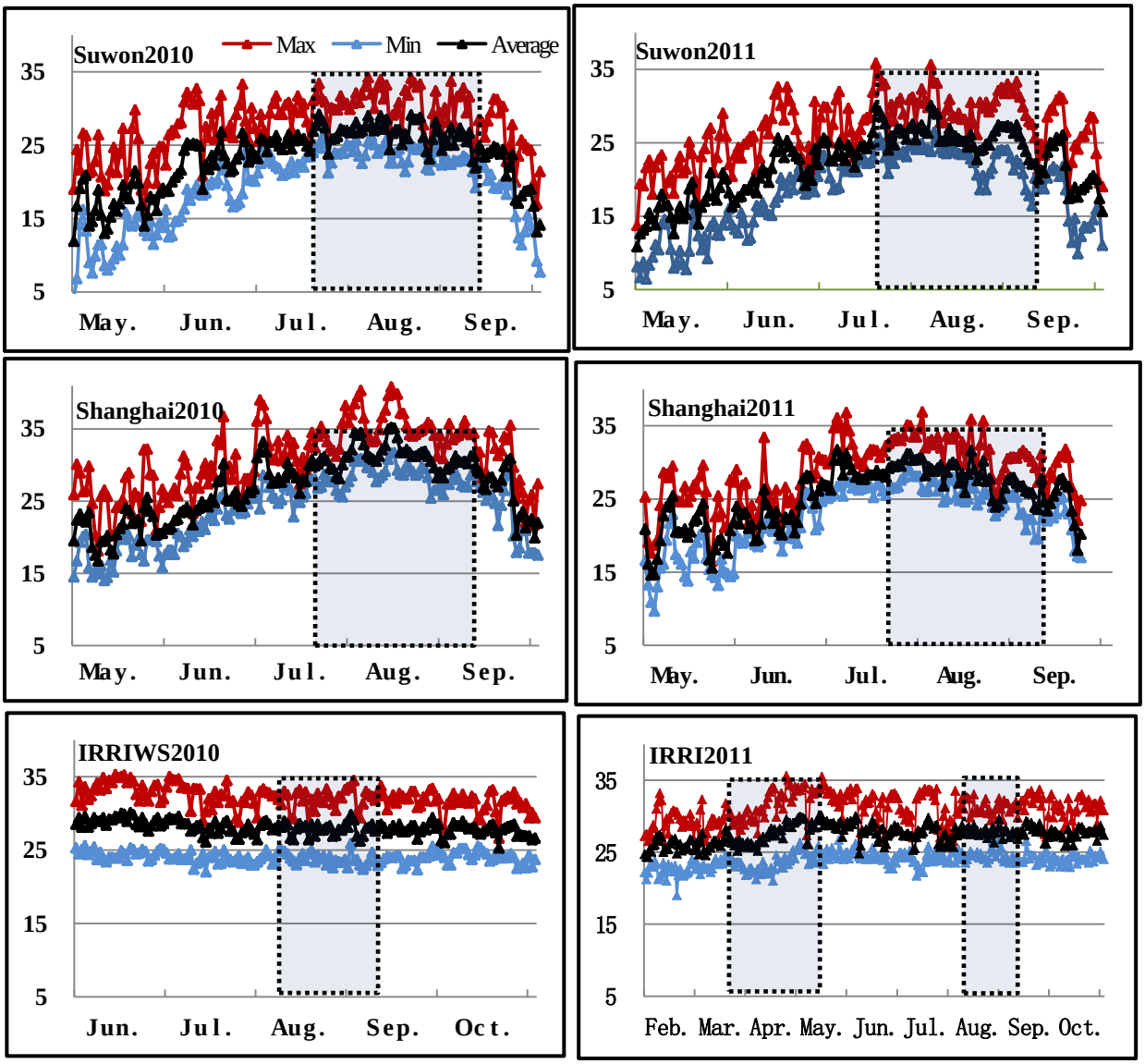
Variation obtained among genotypes (cultivars) was higher compared to those in the environments. Most of cultivars were concentrated except for some varieties which showed high mean yield among the environments (Fig. 2). Based on the IPCA1 value, we found that cultivars were divided into two groups. The first group indicates a positive interaction across the environments A, D, E, and F, while the other groups showed a positive interaction over the environment B, C, and G. The cultivars with high mean yield showed positive interaction in environment B, C, G.

In addition, the AMMI2 explained a total of 63% of the G×E interaction. Most of varieties which located near to the origin of the coordinate showed the most stable ones across all environments. On the other hand, the varieties which located near to the environments of B, C, and G reflected their suitability and stability across these environments.

Regarding biplot results presented in Figure 3, SF traits for most of cultivars were

stable across environments with low variation value indicated by high environmental means values compared to the grand mean. However, in those cultivars was not stable for SF in the environment B, as indicated by lower value of environmental means in comparison to the grand means. Based on the biplots presented in Figure 4, it showed that most varieties were located near to the origin of coordinate reflecting a high stability of cultivars across all environments. However, these varieties showed low positive or negative interactions with the environments and for some varieties were found to locate farther from the origin of the coordinate in comparison with other varieties. Those varieties showed a high positive or negative interaction with environments especially in environment B and F indicating that they are not stable across all environments.

Fig. 1 Daily maximum, average (upper), average (middle), and minimum (lower) air temperature at three locations during the cultivation period in 2010 and 2011.



AMMI stability value (ASV), yield stability index (YSI) and fertility stability index (FSI)

The AMMI model does not provide quantitative stability measure. Therefore, ASV value was proposed to solve this problem. ASV value is defined as distance from zero in a two dimensional scattergram of IPCA1 against IPCA2. ASV was calculated in order to rank genotypes in terms of stability. The ASV rank is the rank of ASV value in all varieties. Lower ASV value indicates greater stability of the varieties in different environments.
Furthermore, yield mean rank the highest yield mean takes the rank one. While, the yield stability index (YSI) is the sum of yield mean rank and ASV rank. The least YSI is considered as the most stable with high grain yield. Likewise, FSI is sum of fertility mean rank and ASV rank.
Based on the analysis presented in Table 5, Nampung cultivar showed the lowest YSI with high yield mean. While IR serials showed low value of YSI and high yield mean indicating their stability over in all environments. Furthermore, the cultivars Yan504, Chenga exhibited low value in both YSI mean yield suggesting that these varieties were stable but low in yield. Another phenomenon showed by cultivars, such as Taichung178, Hangangchal, Chengcheong had a high value of yield mean but their ASV rankwas very high compared to others. This indicated that these varieties had a large environmental variation and more suitable for specific environments. According to the Table 5, surprisingly, most of varieties which have low YSI value belong to indica subspecies, while those with high YSI value are japonica type.
Due to most varieties showed a high value of mean fertility, ASV rank is more effective than FSI value in selection of stable fertility varieties in all environments. According to Fig. 4, varieties which located in the zone A showed a high mean value with a high ASV rank means suggesting that these varieties were affected by G×E interaction and noticed as non-stable varieties across environments. Conversely, varieties that located in the zone B showing a high mean value with low value of ASV rank means revealed that these varieties were stable varieties across all environments.

Fig 2 Biplot of G × E interaction for GY of rice (AMMI 1 and AMMI 2)

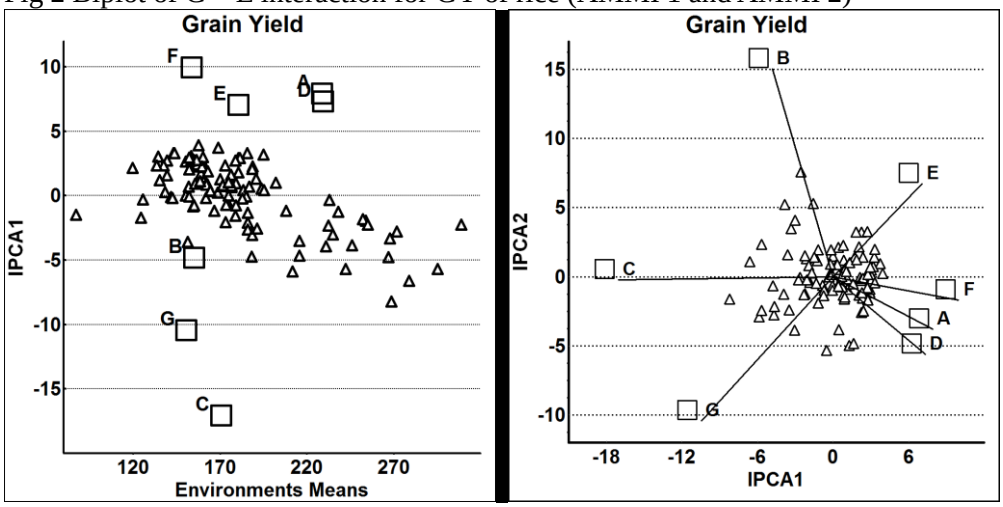


Fig 3 Biplot of G × E interaction for SF of rice (AMMI 1 and AMMI 2)

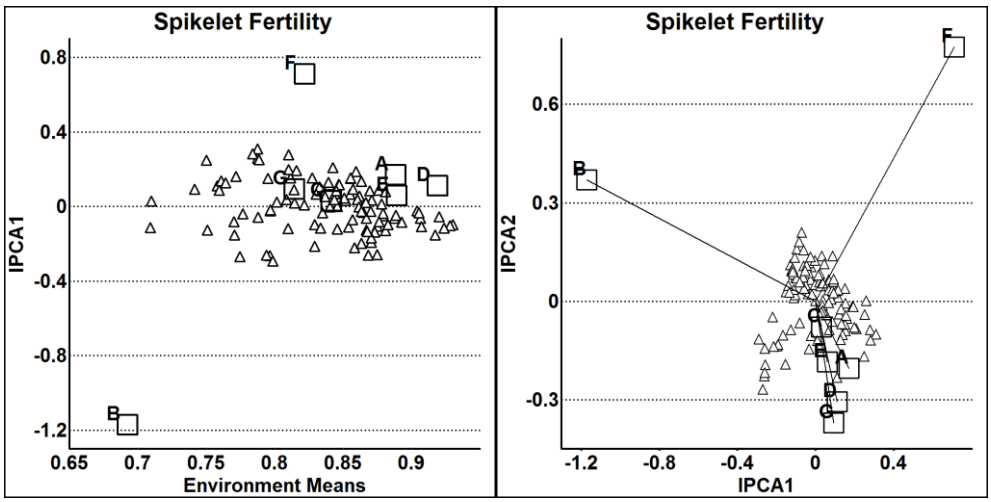


Fig 4 ASV rank and grain yield and spikelet fertility

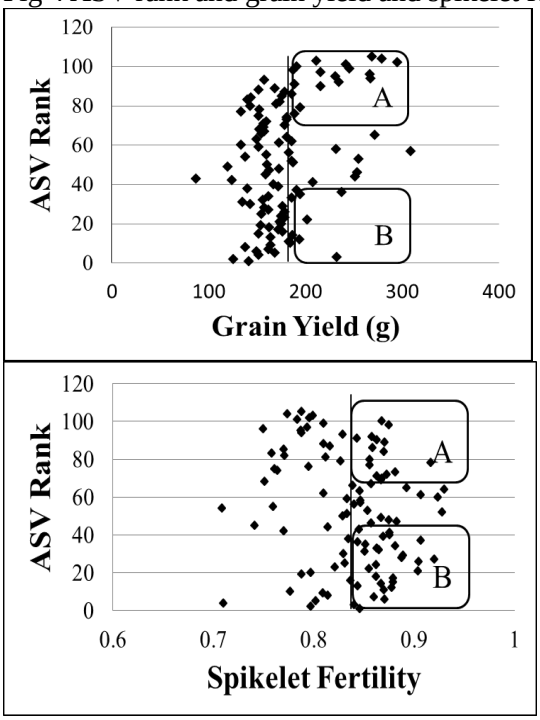


Table 4 ASV rank and YSI of each varieties

Culitvar	Mean	rank	ASV	rank	YSI
Nampung	233	15	0.514	3	18
Junamjosaeng	194	25	0.983	12	37
08JD9	202	22	1.659	22	44
Woonmi	187	32	1.136	14	46
Shingeumo	185	37	0.969	10	47
IR 8	238	13	2.312	36	49
Tong 88-7	183	39	0.974	11	50
IR 36	252	10	2.973	44	54
IR 24	254	9	3.19	46	55
Nongan	195	23	2.256	35	58
Gumei 4	309	1	3.895	57	58
IR 56	255	8	3.801	53	61
Hosun 19	208	21	2.704	41	62
08JD6	177	47	1.348	16	63
Taichung 65	191	27	2.329	37	64
Hwanggeumbora	169	60	0.67	5	65
Taeseong	186	33	2.167	33	66
Ilpum	179	44	1.681	23	67
Odae1	179	43	1.823	26	69
IR 72	272	4	4.439	65	69
Hoban	175	50	1.487	20	70
Yan 504	164	62	0.969	9	71
Malgeumi	172	56	1.385	17	73
Cheonga	162	67	0.954	7	74
La Crosse	174	53	1.502	21	74
Yunjingyou 1	232	16	3.921	58	74
Yangang 19	175	51	1.727	24	75
Simnong 27	164	63	1.052	13	76
Koshihikari	177	48	1.849	29	77
Hwashin 1	188	30	3.595	51	81
Pyeongwon	163	65	1.422	18	83
Della	186	34	3.612	52	86
Hwanggeumnuri	152	85	0.65	4	89
Reimei	162	66	1.827	27	93
Chilbo	183	38	3.89	56	94
TR22183	142	94	0.218	1	95
Hapgang 21	150	90	0.689	6	96

Odae	172	57	2.623	39	96
Handeul	186	35	4.209	62	97
Goun	154	81	1.442	19	100
IR 64	268	6	6.373	94	100
Samnambyeo	158	73	1.848	28	101
Shinunbong	167	61	2.677	40	101
Dongjin 2	152	87	1.212	15	102
Jogwang	162	68	2.187	34	102
Zenith	173	54	3.32	48	102
Saenuri	195	24	5.209	79	103
Dasan	267	7	7.676	96	103
Borami	126	102	0.492	2	104
Chucheong	189	28	4.878	76	104
Keunseom	295	2	9.44	102	104
Kunmingxiaob	138	97	0.966	8	105
Hitomebore	155	80	1.78	25	105
Culitvar	Mean	rank	ASV	rank	YSI
Sandeuljinmi	181	42	4.363	64	106
IR7452-29-4	235	14	6.323	92	106
Hangangchal	279	3	10.669	104	107
Yunjing 21	216	18	6.096	90	108
Poongmi	156	78	2.108	32	110
Mil 23	246	11	8.08	99	110
Taichung 178	269	5	13.296	105	110
Ungwang	163	64	3.269	47	111
Dianjingyou	231	17	6.447	95	112
Hanmaeum	181	40	4.819	73	113
Chengcheong	242	12	9.406	101	113
Dami	181	41	4.829	74	115
Poongmi 1	173	55	4.067	61	116
Hopyeong	179	46	4.657	70	116
Dianxi 4	216	19	7.759	97	116
Hwaseong	159	72	3.108	45	117
Juan 1	161	69	3.595	50	119
Jinmi	189	29	6.238	91	120
Zhendao 88	186	36	5.695	86	122
Yan 308	143	93	1.951	30	123
Hexi 41	212	20	9.861	103	123
Pyeongan	160	71	3.853	55	126

Nongho 6	191	26	8.611	100	126
Chujing 27	188	31	8.054	98	129
Gangbaek	135	99	2.107	31	130
Shuangfeng 1	179	45	5.865	87	132
Sobaek	140	95	2.6	38	133
Cheongan	174	52	5.41	82	134
Seoan 1	176	49	5.517	85	134
Hopum	170	58	5.389	81	139
Cheongdam	158	75	4.477	67	142
Hwanggeumnodel	160	70	4.815	72	142
Short labell	124	103	2.736	42	145
Wuyujing3	152	86	3.948	59	145
Nipponbare	155	79	4.462	66	145
Donghaejinmi	157	76	4.6	69	145
Yan 304	87	105	2.765	43	148
Samgwang	157	77	4.75	71	148
Cheongho	169	59	6.076	89	148
Jinbu	153	82	4.479	68	150
Manna	138	98	3.812	54	152
Hwagwang	150	89	4.273	63	152
Gopum	120	104	3.466	49	153
Sangok	152	84	4.845	75	159
Onnoori	134	101	4.059	60	161
Hanam	153	83	4.948	78	161
Hwacheong	158	74	6.338	93	167
Nampyeong	143	92	5.353	80	172
Seopyeong	144	91	5.473	84	175
Blue bonnet	152	88	5.973	88	176
Haechanmoolg	134	100	4.938	77	177
Lijiangxintu	140	96	5.47	83	179

Table 5 ASV rank and FSI of each varieties

Culitvar	Mean	rank	ASV	rank	FSI
Hanmaeum	0.871	24	0.066	6	30
TR22183	0.878	18	0.084	12	30
Hoban	0.904	9	0.113	21	30
Zhendao 88	0.92	4	0.136	27	31
Hosun 19	0.879	17	0.105	15	32
Hwacheong	0.879	16	0.109	17	33
Junamjosaeng	0.905	8	0.131	26	34
Handeul	0.87	27	0.081	11	38
Sandeuljinmi	0.888	12	0.137	28	40
Yan 308	0.889	11	0.139	29	40
Goun	0.907	7	0.157	37	44
Hanam	0.86	40	0.067	7	47
Odae1	0.867	33	0.092	14	47
Koshihikari	0.881	15	0.145	34	49
Hapgang 21	0.846	53	0.001	1	54
Gumei 4	0.928	2	0.201	52	54
Gangbaek	0.862	38	0.11	18	56
Simnong 27	0.876	19	0.162	40	59
Samnambyeo	0.883	13	0.191	47	60
Cheonga	0.876	20	0.164	41	61
Hwaseong	0.84	59	0.051	3	62
Woonmi	0.862	39	0.124	24	63
Yunjingyou 1	0.924	3	0.231	60	63
08JD6	0.93	1	0.239	64	65
Ilpum	0.865	34	0.141	32	66
Hitomebore	0.869	28	0.161	39	67
Wuyujing3	0.907	6	0.237	61	67
Manna	0.855	47	0.122	22	69
Onnoori	0.844	57	0.086	13	70
Yan 504	0.863	37	0.142	33	70
Dianjingyou	0.875	22	0.192	48	70
Nongho 6	0.893	10	0.239	65	75
Borami	0.837	62	0.108	16	78
Keunseom	0.814	73	0.069	8	81
Taichung 65	0.851	50	0.141	31	81
Seopyeong	0.867	32	0.193	49	81
IR7452-29-4	0.917	5	0.282	78	83

Jogwang	0.852	49	0.149	35	84
Sangok	0.797	83	0.038	2	85
Nipponbare	0.802	80	0.065	5	85
08JD9	0.881	14	0.261	73	87
Hwanggeumnuri	0.809	79	0.073	9	88
Yangang 19	0.857	43	0.183	46	89
Dongjin 2	0.831	66	0.13	25	91
Chucheong	0.844	56	0.155	36	92
Hwagwang	0.821	71	0.122	23	94
Short labell	0.873	23	0.258	72	95
Odae	0.83	67	0.139	30	97
Tong 88-7	0.845	55	0.167	43	98
Mil 23	0.867	31	0.248	69	100
Yunjing 21	0.868	30	0.25	70	100
Jinmi	0.835	63	0.159	38	101
Chengcheong	0.854	48	0.205	53	101

Culitvar	Mean	rank	ASV	rank	FSI
Gopum	0.777	92	0.076	10	102
Haechanmoolguer	0.797	82	0.113	20	102
Taeseong	0.863	35	0.255	71	106
IR 24	0.788	88	0.113	19	107
Sobaek	0.71	104	0.064	4	108
Reimei	0.847	51	0.215	57	108
Hopyeong	0.847	52	0.222	58	110
Taichung 178	0.87	26	0.325	84	110
Nongan	0.857	44	0.246	67	111
IR 56	0.871	25	0.379	89	114
Hwanggeumbora	0.833	65	0.198	51	116
Dami	0.84	60	0.214	56	116
Nampung	0.846	54	0.239	63	117
Ungwang	0.814	74	0.17	44	118
Della	0.829	68	0.194	50	118
Hangangchal	0.875	21	0.511	98	119
Saenuri	0.856	45	0.282	77	122
IR 8	0.833	64	0.227	59	123
Juan 1	0.856	46	0.288	80	126
IR 36	0.863	36	0.388	90	126
Samgwang	0.839	61	0.242	66	127
Hwashin 1	0.859	41	0.348	86	127

Hexi 41	0.868	29	0.527	100	129
Dianxi 4	0.858	42	0.409	92	134
La Crosse	0.77	96	0.164	42	138
Yan 304	0.81	78	0.237	62	140
Pyeongwon	0.741	103	0.18	45	148
Shingeumo	0.827	70	0.287	79	149
Chilbo	0.843	58	0.393	91	149
Lijiangxintu	0.76	99	0.211	55	154
Cheongan	0.812	75	0.295	81	156
Kunmingxiaob	0.709	105	0.21	54	159
Shinunbong	0.816	72	0.358	87	159
Cheongho	0.795	85	0.278	76	161
IR 64	0.829	69	0.416	93	162
Donghaejinmi	0.81	77	0.374	88	165
Blue bonnet	0.751	101	0.247	68	169
Nampyeong	0.764	97	0.262	74	171
Malgeumi	0.761	98	0.274	75	173
Hwanggeumnodel	0.81	76	0.518	99	175
Jinbu	0.771	94	0.301	82	176
Dasan	0.77	95	0.342	85	180
Seoan 1	0.788	87	0.461	94	181
Hopum	0.758	100	0.308	83	183
IR 72	0.794	86	0.5	97	183
Shuangfeng 1	0.799	81	0.548	103	184
Poongmi	0.787	90	0.474	95	185
Chujing 27	0.796	84	0.536	102	186
Pyeongan	0.784	91	0.531	101	192
Poongmi 1	0.788	89	0.575	105	194
Zenith	0.774	93	0.563	104	197
Cheongdam	0.75	102	0.487	96	198

Correlation and path coefficient analysis for agronomic traits

Correlation coefficients among the eight agronomic traits in Varieties are presented in Suppl. Table 2 and path coefficient among yield component traits were in Suppl. Table2. Significant correlations ($p<0.05$, $p<0.01$) were observed among traits.

The results showed that most of correlations among all environments showed a similar pattern. Correlation among yield component traits showed some differences across environments. In Suwon, GY showed a significantly positive correlations with PN but those in IRRI and Shanghai 2010 growing season, they showed a low or non-significant correlations. SN had a significantly correlation with GY for all location except that of in Suwon 2010 growing season. SF was significantly correlated with GY in all environment except with those in Shanghai 2011 growing season. To investigate direct and indirect effects via yield component traits on GY, a path analysis between yield component traits was conducted. Present study exhibited that in Suwon, path analysis revealed that all agronomic traits showed a high positive direct effect on GY. While those in Shanghai and IRRI showed same pattern. Of these, only in Shanghai2011 SF showed negative direct effects on GY.

Discussion

High yield is the most important purpose in rice breeding. Stability analysis provides a general solution for the response of the genotypes to environmental change. In stability analysis models, AMMI model was reported as the best model (Kandus et al. 2010). AMMI is a combination of ANOVA for the main effects of the genotypes and the environment together with principal components analysis (PCA) of the genotype-environment interaction (Zobel et al., 1998; Gauch, 1988).

In our trial, the largest genotype effect obtained from AMMI analysis was observed on traits of CL, PL and GW, while the largest environment effect was observed in PN and SN. Meanwhile, the largest GxE interaction was recorded in GY and SF. In overall, present study revealed that most of genotypes showed yield stability across all environments and only a few genotypes was unstable one. For grain yield, the environment effect of environment at IRRI wet season 2010 was the highest one, followed by Shanghai 2010, IRRI wet season 2011, IRRI dry season 2010, Shanghai 2011, Suwon 2011, and Suwon 2010. It is suggested that the yield of tested cultivars was affected by genotypes, environments and GxE interaction, simultaneously.

For the spikelet fertility, the largest environment effect were detected in Shanghai 2010 in comparison to that of in IRRI dry season 2011 and the other environments which showed less environment effects. In our trails, most genotypes showed stable traits or high environmental effects. SF and GY showed a high GxE interaction with the mean value of 57% and 39%, respectively. This result indicated that genotype stability of the two traits were unstable in different trails. The lowest environment effect was detected in Suwon, followed by that of in Shanghai, and IRRI. Based on our results, it can be concluded that Suwon is suitable to obtain a stable cultivars with low GxE interaction environment and can be suitable for control of trials. According to ASV value for GY, the most stable cultivars was observed in Suwon 2010 growing season which showed the lowest ASV score (8.8) among tested environments, while the lowest ASV score for SF was observed in IRRI 2010 (Table 4). Therefore, trial carried out at

IRRI is suitable for investigating stable cultivars with high GxE interaction for high temperature screening.

The effect of high temperature on different growing stage, meiosis (stage 14) and flowering (stage 15) stages of rice has been studied (Jagadish et al. 2007; Jagadish et al. 2008; Shrivastava et al. 2012). In our study, during flowering stage, the highest maximum daily air temperature was observed in in Shanghai 2010, while during all stages, IRRI showed higher mean temperature (Fig. 1). According to AMMI analysis Shanghai 2010 and IRRI showed high environmental effects and genotype by environment effects. It can be summarized that in our trail temperature may the biggest environmental effect on the tested traits. The use of cultivars which promisingly adapted in specific location found in present study and or those showing a stable across all environments could help to reduce the anticipated yield losses caused by spikelet sterility at anthesis as a result of expected global warming.

Chapter II. Genotype × environment interaction for agronomic traits in rice RILs under different environments based on AMMI model

Abstract

Heat stress is one of the major environmental stresses in rice cultivation during meiophase and anthesis. In this experiment, a set of recombinant inbred lines (RILS) derived from Dasanbyeobyeo (indica) / TR22183 (japonica) crosses was grown in three different environments including Shanghai (subtropical zone), IRRI (tropical zone) and Suwon (temperate zone) to evaluate the genotype × environment interactions (GEI) for heat tolerance. The materials were the most damaged in Shanghai 2010 and dry season in IRRI. Significant G × E interactions in all measured agronomic traits were detected and the additive main effects and multiplicative interaction (AMMI) statistical model was applied to dissect the G × E interactions. The biplots of grand mean and IPCA1 (interaction principal component axes) of heat related traits accounted for most of the total treatment sums of squares. The IPCA scores of spikelet fertility was relatively smaller in Suwon and wet season IRRI than in Shanghai2010 and dry season IRRI, implying that heat tolerance by high temperature during anthesis was more sensitive to G×E interaction in dry season IRRI and 2010 Shanghai than other places. These results demonstrate that multi-locational screening is a good strategy for developing heat tolerant varieties in rice.

Key words: genotype, environment, biplot, RIL, heat tolerance

Introduction

Under the future climates, a greater need of protection against environmental stress will be required for maintaining crops productivity. According to previous research of the UK's Hadley Center, the increase in global air temperature will cause a reduction in the main crop yield up to 37% in the next 20-80 years (Erda *et al.*, 2005). The impact of high temperature can cause severe damage to the yield of crops, particularly when high temperature episodes coincide with flowering (Wheeler et al., 2000). Further studies proved that brief periods of high temperature which occur near flowering can severely reduce the crop yields (Challinor et al., 2005). Besides, the change of winter temperature will change reproductive mode of crop pest in which more aphids could survive in warm winter and they can migrate great distance across large areas of monotonous environments (Gilabert *et al.* 2009).

Another previous study related to the impact of temperature on crops yield revealed that yields increase with temperature up to 29 °C for corn, 30 °C for soybeans, and 32 °C for cotton but that temperatures above these thresholds were very harmful (Wolfram Schlenker *et al.*, 2009). In rice, the temperature above the optimum temperature for the normal development of rice which ranges from 27 to 32^{o}C (Yin et al. 1996) affects almost all the growth stages of rice, especially during the flowering phase, fertilization and seed production resulting in reduced yield (Porter, 2005). Of these, the flowering and booting stages of rice are most susceptible to high temperatures (Shah et al. 2011).

Several studies reported that the warmer climate resulted in loss of the yield of rice annually which mainly caused by the several factors, such as high temperature during anthesis (Jagadish *et al.* 2007), heat stress during meiosis stage (Lin *et al.*.2008), high night temperature (Peng *et al.*, 2004), heat stress during grain filling stage and so on. When the spikelets were exposed to temperature above 35℃ for 5 days during anthesis, pollen shedding would be disturbed and pollen germination would be impaired resulting in highly spikelet sterility. In accordance, after plants exposed to high temperature during anthesis, the number of pollen grains shed on stigma was reduced (Matsui *et al.*.

1997, 2001). Heat stress reduces anther dehiscence and pollen fertility rate significantly in heat sensitive variety, while in the heat tolerant variety this effects were smaller. However, spikelet number per panicle, spikelet fertility, grain weight were significantly decreased in both tolerant and sensitive varieties (Cao *et al*. 2008). In addition, a high nighttime temperature decreased pollen germination rate and spikelet fertility (Mohammed et al., 2009). While when the temperature raised during grain filling stage, the chalkiness of rice would be increased (Asaoka *et al*.. 1984), polished rice rate would be reduced and lead to reduction of grain yield. Overall, rice is the most sensitive to high temperature at flowering time which cause highly sterile and lead to reduce of yield.

Development of heat tolerant cultivars is one of the most effective methods to maintain rice production under climate changes in temperate regions (Horie *et al*.. 1996). Besides, breeders and farmers tried to find another strategy to reduce high temperature effects on grain yield, for example by adjusting seeding time to avoid a high temperature occurs during anthesis episode, known as breed early morning flowering (EMF) varieties on avoiding high temperature (Ishimaru *et al*. 2010). In regards to the improvement of cultivars tolerance in high temperature, we have developed a set of recombinant inbreed lines population of rice derived from a cross between Dasanbyeo (Indica) and TR22183 (Japonica) and they were cultivated in three different regions, such as in the temperate zone (Suwon), subtropical zone (Shanghai) and tropical zone (IRRI).

To screen heat tolerant rice effectively, the temperature of environments must stable and high in order to make the treating environments were reliable. Therefore, daily maximum, minimum, average temperature in the locations were recorded. In present study, the genotype × environment interactions (GEI) of the RILs materials for heat tolerance was evaluated in order to select promisingly lines with stable rice genotypes, especially high grain yield either in a particular environment or adapted in a broader range of environment. The relative performances of genotypes may vary in different environments which is known as genotype × environment (G × E) interaction. When the G × E interactions present, there is an interaction between a particular genotype and a

particular environment. Therefore, such a genotype can be suggested to be grown in a specific environment, whereas the lack of G×E interactions allow a genotype to be adopted in a broader range of environments (Cooper *et al.*. 1996). In present study, the AMMI (additive main effects and multiplicative interaction) and GGE (genotype + genotype × environment) were used for the analysis of principle component of G × E interaction (Kumar, 2012). AMMI model used in this study displays incorporates more of genotype main effects and captures more of the G × E interaction than the genotype main effects and genotype × environment interaction effects (GGE) (Gauch et al. 2008). A studies on rice G×E interactions for heat tolerant through two environments have been conducted (Sierra *et al.*. 2009). Up to now, a few studies on the analysis of G×E interactions for heat tolerance related traits was reported.

The objectives of this study were to examine heat tolerant related traits in RILs under different environmental conditions and to investigate the G×E interaction patterns in multi-locational screening of heat tolerance.

Materials and methods

Development of plant materials

A hundred-sixty recombinant inbred lines (RILs) in the F_{12} generation, derived from the cross Dasanbyeo (Korean indica type) X TR22183 (a temperate japonica type), were developed by single seed descent (SSD). Out of 160 lines, only 154 lines which showed spikelet fertility > 60% (observed in Suwon) were used in the analysis.

Experimental locations and growth conditions

The RILs and parental lines were cultivated in seven environments during the years 2010 and 2011 crop season across three locations including Suwon, Shanghai, and IRRI (Table 1). A randomized block design with two replications was used. The materials were seeded in plastic-tunnel seeding nursery and thirty-day-old seedlings were transplanted to irrigated field condition at one seedling per hill with a 30 x 15 cm of spacing between seedlings. Field management was carried out following normal agronomic practices. Fertilizers were properly applied at the rate of 100 kg N ha^{-1}, 80 kg P ha^{-1}, and 80 kg K ha^{-1} (100-80-80 kg/ha N-P-K).

Phenotypic data collection

Eight important agronomical traits comprising days to heading (DTH), culm length (CL), panicle length (PL), panicle number per plant (PN), spikelet number per panicle (SN), spikelet fertility (SF), 100-grain weight (GW), and grain yield (GY) were measured across seven environments for both RILs population and parental lines. DTH, CL, PL, PN as well as the fully filled grains used for measuring SN, SF, GW and GY were collected according to the Standard Evaluation System (SES) (IRRI 1998).

Statistical analysis

A combined analysis of variance and the AMMI analysis across 7 environments were performed on each trait using the CropStat 7.2. The AMMI analysis based on the first two interaction principal component axes (IPCAs) was used to categorize the experimental environments into mega-environment groups and to detect the homogenous sub-regions having similar G × E interaction patterns. Biplots with the first two multiplicative terms were used to summarize the G × E interaction patterns.

Results

Observation of experimental sites condition

The monthly maximum, minimum, and average temperature during reproductive stage in the three environments are presented in Fig.1 and Table2. Air temperature condition during anthesis as a critically episode of high temperature effect in rice development stage was observed in each environment. In the two locations, Suwon and Shanghai, the anthesis period was mostly occurred in August. In the IRRI 2010 wet season and IRRI 2011 wet season, the period of plant's anthesis occured in September and April, respectively. Maximum daily temperature observed during rice's anthesis stage in Shanghai 2010 crop season was the highest one across locations which exhibited maximum day temperature of at three period, period 1, 2, and 3 was36.1, 37.6, and 34.0^{o}C, respectively (Table 2). The daily maximum temperature during anthesis periode observed in Suwon during the year 2010 and 2011, and in IRRI both during wet season of the years 2010 and 2011 showed a stable temperature. It is indicated by recorded data of the daily maximum temperatures at three period of the anthesis stage as well as the average of daily maximum, minimum, and average temperature during the whole anthesis particular location.

The daily maximum temperature observed during three periods of anthesis in Suwon 2010 were 32.2, 30.9, and 29.1^{o}C for period 1, period 2, and period 3, respectively, while those in Suwon 2011 were 31, 28.3, 31.1^{o}C, for the period 1, period 2, and period 3, respectively (Table 2). The average of daily maximum, minimum, and average temperature during the whole anthesis between two years growing season recorded in Suwon were almost similar.

A similar trend was also observed for the average of the daily maximum, minimum, and average temperature during three periods in anthesis stage between two years wet season observed in IRRI (Table 2). In IRRI 2010 wet season, the daily maximum temperature during anthesis were 32.2, 32.1, 31.7, and 31.5^{o}C, respectively and in 2011

those were 31.2, 30.8, and 31.1^{o}C, respectively. In IRRI 2011 dry season, the the daily maximum temperature during anthesis were 32.1, 33.1, 32.6, and 33.8^{o}C respectively. In details, the average of daily maximum, minimum, average temperature during the whole anthesis of each environment are presented in Table 4.

On the contrary, the daily maximum temperature during anthesis between two years in Shanghai, 2010 and 2011 growing season showed a higher one in Shanghai 2011 crop season. In comparison, the highest maximum daily temperatures was recorded in Shanghai 2011, followed by those in IRRI 2011 dry season across other environments.

Table 1 The tested environments in which varieties evaluated

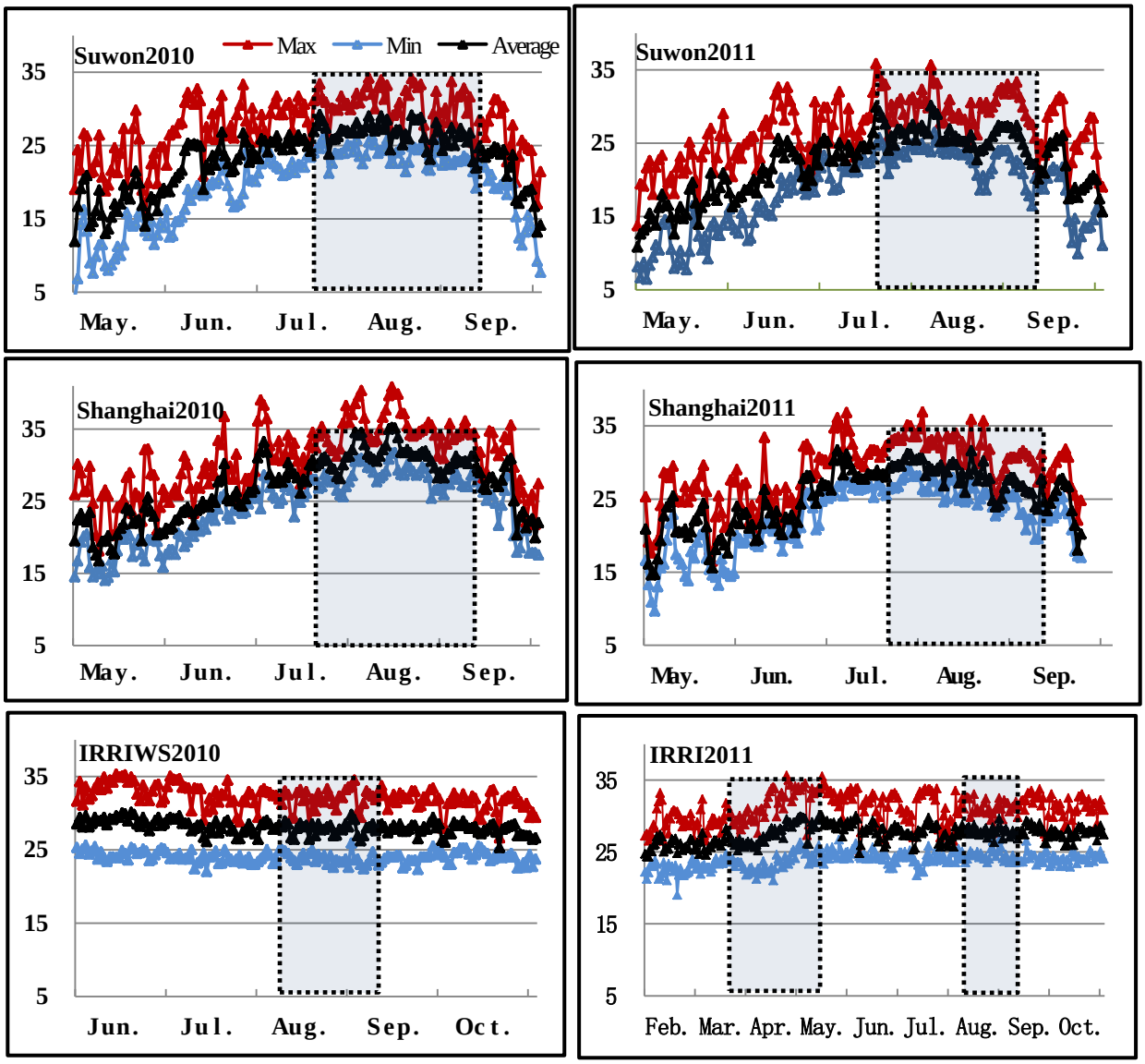
Code	Environments
Su2010	Experimental farm of Seoul National University. Suwon, Korea, 126°59' May-October, 2010
Sh2010	Experimental farm of Shanghai Academy of Agricultural Science. May-October, 2010
IRRI2010	Experimental farm of International Rice Research Institute. Laguna, June-September 2010
Su2011	Experimental farm of Seoul National University . Suwon, Korea, May-October, 2011
Sh2011	Experimental farm of Shanghai Academy of Agricultural Science. May-October, 2011
IRRIDS2011	Experimental farm of International Rice Research Institute. Laguna, January-May, 2011
IRRIWS2011	Experimental farm of International Rice Research Institute. Laguna, June-September 2011

Table. 2 Monthly mean maximum, minimum, and average temperature recorded at reproductive stage in 3 locations

		Suwon2010				Shanghai2010					IRRIWS2010			
		Period 1*	Period 2	Period 3	Mean	Period 1	Period 2	Period 3	Mean		Period 1	Period 2	Period 3	Mean
Max	Jul.	29.4	29.8	30.1	29.8	33.8	31.7	29.6	33.3	Aug.	32.5	31.2	32.3	32
Min		22.4	23.1	24.1	23.2	26.3	26.3	26.3	26.7		24.3	24	23.7	24
Average		25.4	26.1	26.6	26	29.5	28.9	30.2	29.6		28.4	27.6	28	28
Max	Aug.	32.2	30.9	29.1	30.7	36.1	37.6	34	35.8	Sep.	32.2	32.1	31.7	32
Min		24.5	23.6	23.5	23.8	29.6	29.9	27.6	29		23.3	23.7	24.2	23.7
Average		27.7	26.9	26.1	26.9	32.3	33	30.2	31.8		27.8	27.9	27.9	27.9
Max	Sep.	29.7	28.2	22.9	26.9	34.3	32.1	26.7	31	Oct.	31.5	30.7	31.2	31.1
Min		22.4	20.2	13.2	18.6	27.9	25.6	19.8	24.4		24.3	24.4	23.5	24
Average		25.4	23.6	17.7	22.2	30.5	28.2	23	27.2		27.9	27.5	27.3	27.6

		Suwon2011				Shanghai2011				IRRIWS2011					IRRIDS2011			
		Period 1	Period 2	Period 3	Mean	Period 1	Period 2	Period 3	Mean	Period 1	Period 2	Period 3	Mean		Period 1	Period 2	Period 3	Mean
Max	Jul.	28.3	29.6	29.5	29.1	33.3	31.2	34.1	32.9	30.2	32.7	30.3	31	March	28.5	29.1	29.6	29.1
Min		20.8	23.1	23.4	22.4	26.4	26.4	27.6	26.8	24.6	23.4	24.1	24		23	23.3	23.7	23.4
Average		23.9	25.7	26.1	25.3	29.3	28.3	30.2	29.3	27.4	28	27.2	27.5		25.7	26.2	26.6	26.2
Max	Aug.	31	28.3	31.1	30.2	32.6	32.3	29.3	31.3	30.7	31.2	30.8	30.9	Apr.	29.7	32.1	33.1	31.6
Min		24.4	23.2	22	23.2	26.5	25.4	24.7	25.5	24.3	24.6	25	24.6		22.3	22.6	23.9	22.9
Average		27.2	25.2	26	26.1	29	28.6	26.4	27.9	27.5	27.9	27.9	27.8		26	27.4	28.5	27.3
Max	Sep.	28.2	27.2	24.6	26.7	29.5	28.1	-	28.8	31.1	32.3	31.3	31.6	May	32.6	33.8	32.3	32.9
Min		19.8	19	13.5	17.4	21.9	21.5	-	21.8	23.9	24.4	23.9	24.1		24.9	24.6	24.8	24.8
Average		23.6	22.8	18.7	21.7	25.5	24.4	-	24.9	27.5	28.3	27.6	27.8		28.8	29.2	28.6	28.8

Fig 1 Daily maximum, average, minimum day temperature at three locations during cultivated periods



Agronomic traits in RILs

Eight importance agronomic traits of the RILs and their parents are summarized in Table 5. Detailed performances of each trait in the three locations are described belows.
Days to heading

Present study showed that the TR22183 parent headed earlier than the Dasanbyeo parent in all locations. In general, Dasanbyeo and TR22183 revealed early heading in high temperature locations and low latitude locations. Correlations analysis of DTH among the environments showed a highly significant with ranging from p=0.207** (Sh2010 and Ph2010) to 0.935** (Su2010 and Su2011) implying that the photoperiod affected on the DTH of RILs from different latitudes might not be significant (Suppl. Table 2).

The earliest heading date of the parental lines, Dasanbyeo and TR22183 were observed in IRRI followed by that in Shanghai and Suwon. During flowering period, it is also observed that the temperature in Shanghai 2010 crop season was extremely high (Fig 1 and Table 3). In Comparison, the flowering time of the RILs was shortened in response to the high temperature occurs between two years, 2010 and 2011. It is suggested that the high temperature during heading date might be the reason why the heading date was shorted in RILs population (Fig. 1 and Table3 and Table 5).

Table 3 Culture time of the RILs

Location	Seeding date	Transplanting date	Flowering date			Total(d)
			Dasan	TR22183	RILs	
Suwon2010	5/5	6/5	8/16	7/31	7/28~8/29	33
Shanghai2010	5/23	6/18	8/25	8/6	8/1~8/31	30
IRRI2010	7/8	7/29	9/10	9/2	9/4~10/7	33
Suwon2011	4/29	6/4	8/23	8/2	7/29~9/15	49
Shanghai2011	5/20	6/15	8/23	8/2	7/29~9/3	36
IRRIDS2011	2/4	2/25	4/21	4/4	4/7~5/20	44
IRRIWS2011	6/10	6/30	9/6	8/14	8/11~9/5	25

Table 4 Average daily maximum, minimum and average temperature recorded during anthesis in 3 environments

	Suwon2010	Shanghai2010	IRRIWS2010	Suwon2011	Shanghai2011	IRRIWS2011	IRRIDS2011
Max	30.7	35.8	31.9	29.6	31.6	31.0	32.6
Min	24.0	29.0	23.9	22.3	25.4	24.6	23.8
Average	26.9	31.8	27.9	25.4	28.0	27.8	28.2

Table 5 Means and phenotypic variations in parents and RILs for 8 traits in the years 2010, 2011

Traits	Location	2010					Location	2011				
		parents			RILs			Parents			RILs	
		Dasan	TR22183	Diff	MEAN	Range		Dasan	TR22183	Dif	MEAN	Range
DTH	Suwon	103A	87A	*	96A	84-116	Suwon	116A	95A	*	103A	91-139
	Shanghai	100A	81B	*	83B	70-100	Shanghai	95B	77B	*	84B	70-106
	IRRIWS	87B	66C	*	72C	58-91	IRRIDS	84C	67B	*	79C	62-105
							IRRIWS	81D	66B	*	74D	62-87
CL	Suwon	80.1B	68A	*	74.4A	26-123	Suwon	67.6B	62.7B	*	67.6B	40-120
	Shanghai	88.9A	66AB	*	70.2B	42.4-125.5	Shanghai	91.4A	72.3A	*	75.7A	46-126
	IRRIWS	63.7C	62B	NS	60.2C	40-116	IRRIDS	52.6C	45.3D	*	46.4D	22-110
							IRRIWS	70.5B	52.9C	*	62.4C	39-112
PL	Suwon	23.4A	24.5A	NS	24.2A	16-34.4	Suwon	24.2A	26.8A	*	23.8A	14.5-37
	Shanghai	23.7A	26.8A	NS	23.5B	15.4-29.8	Shanghai	24.2A	27.9A	*	23.5B	16.5-33.9
	IRRIWS	21.3A	21.8A	NS	20.8C	10-33	IRRIDS	20.5B	22.3B	*	20.4D	9-31
							IRRIWS	21.8B	21.4B	NS	20.9C	10-32
PN	Suwon	9B	7B	*	15A	3-28	Suwon	8B	6B	*	6D	2-16
	Shanghai	10B	7B	NS	8B	4-16	Shanghai	9B	5B	*	8C	3-27
	IRRIWS	14A	11A	NS	7C	3-31	IRRIDS	10B	7B	*	11B	1-35
							IRRIWS	16A	10A	*	13A	5-29
SN	Suwon	194A	204A	NS	197A	64-344	Suwon	177B	204A	NS	185A	82-338
	Shanghai	187A	172AB	NS	183B	80-381	Shanghai	256A	220A	NS	185A	66-368
	IRRIWS	133A	146B	NS	125C	43-211	IRRIDS	95C	107B	NS	103C	36-324
							IRRIWS	170B	133AB	NS	125B	35-222
GW	Suwon	2.79A	2.48B	*	2.56A	1.78-3.31	Suwon	2.75A	2.75A	NS	2.72A	1.81-3.84
	Shanghai	2.60A	2.50B	*	2.55A	1.66-3.20	Shanghai	2.73A	2.19B	NS	2.39B	1.68-3.22
	IRRIWS	2.81A	2.74A	NS	2.37B	1.75-3.71	IRRIDS	2.30B	2.32B	*	2.52B	1.33-3.29
							IRRIWS	2.85A	2.85A	NS	2.71A	1.99-3.49
GY	Suwon	317.3A	182.9A	*	208A	54.6-365.4	Suwon	337.4A	207.7A	*	215.9A	23.9-373.2
	Shanghai	246.2A	114.8A	*	173.4B	29.4-317	Shanghai	245.5AB	118.5C	*	177.4B	60.8-355.8
	IRRIWS	271.8A	150.2A	*	146C	54.6-373.6	IRRIDS	128.6B	164.6B	NS	98.9C	15.1-400
							IRRIWS	276.6A	97C	*	134.8C	41.3-345.2
SF	Suwon	85.1A	90.6A	NS	78.4A	60-100	Suwon	94.1A	90.3A	NS	81.9A	60-100
	Shanghai	73.5A	74.5B	NS	64.1C	7.2-98.1	Shanghai	75.2AB	90.4A	*	80.1B	60-99.2
	IRRIWS	83.1A	88.3A	*	77.1B	28.3-95.7	IRRIDS	49.4B	93.7A	*	71.5D	20.6-95
							IRRIWS	78.6A	87.3A	NS	77.9C	31.8-95.6

* (Signify difference between parents). NS(none significant).
Behind letters indicated 5% level in LSD test between environments.
Culm length and Panicle length

A large difference in culm length (CL) between the two parents were also observed in all seven environments. The CL of Dasanbyeo was longer than that ofTR22183in all locations. The CL of Dasanbyeo observed in Suwon and Shanghai was similar, but in IRRI it was severely reduced. On the other hand, TR22183 showed similar response with CL by locations (Table 5). Effect of high temperature was also observed on CL of RILs which showed different performance across environments.

The highest mean values of CL in RILs were significantly recorded in Shanghai 2011 (75.7 cm) and Suwon 2010 (74.4 cm). Out of three locations, culm length of RIL observed in IRRI exhibited a lower one, especially those observed during IRR 2011 dry season (46.4 cm) (Table 5). Taken together, the mean values of culm length both in the parents and RILs grown at IRRI were shortened. Panicle length (PL) between the parents in all environments did not different (Table 5). However, mean values of PL in Dasanbyeo observed during IRRI 2011 dry season (20.5 cm) and IRRI 2011 wet season (21.8 cm) were reduced in comparison to those in other environments. Similar pattern was also observed in TR22183 which the lowest mean values of PL observed in IRRI 2011 dry season (22.3 cm) and IRRI 2011 wet season (21.4 cm).

Performances of PL in RILs population, a variation of PL mean value was observed. The mean value of PL in RILs observed in Suwon 2010 and 2010 crop season was the highest one and significantly different with that detected in Shanghai 2010 and 2011 crop season as well as that in IRRI 2010 wet season and IRRI 2011 dry and wet seasons. Among three locations, panicle length of RILs recorded in IRRI across three crop seasons was the lowest one (Table 5).

Yield component traits

A similar response of Dasanbyeo and TR22183on PN was observed in present study. In IRRI, performance of Dasanbyeo and TR22183 showed a higher PN compare to that in Suwon and Shanghai over the years crop seasons. However, mean value of PN in Dasanbyeo and TR22183 between Suwon and Shanghai was not significantly different (Table 5). In RILs progeny, mean value of PN showed a variation across environments.

The highest mean value of PN in RILs was observed in Suwon 2010 crop season (15 panicles) followed by PN mean values observed in IRRI 2011 wet season (13 panicles) and IRRI 2011 dry season (11 panicles).

On the other hand, the lowest mean value of PN in RILs was observed in Suwon 2011 crop season (6 panicles). Since both the highest and lowest PN values existed in Suwon, the differences in temperature condition in this location over two years might be a strong reason explaining why a big difference occurs in the number of panicles between two seasons.

In term of spikelet number per panicle (SN), out of seven environments, SN in Suwon 2010, Shanghai 2010, and Shanghai 2011 crop seasons did not show the differences between the parents. However, SN of Dasanbyeo and TR22183 showed a significantly different in the remaining environments. Of these, TR22183 showed a higher significantly values for SN in IRRI 2010 wet season and IRRI 2011 dry season, while in IRRI 2011 wet season Dasanbyeo showed a higher significantly value for SN (Table 5).

Regardless of SN found in RIL population, the highest mean value of SN was observed in Suwon 2010 crop season (197 spikelets/panicle) and followed by that of in Suwon 2011 (185 spikelets) and in Shanghai 2011 (185 spikelets). The lowest mean value of SN in RILs population was observed in IRRI 2011 dry season (103 spikelets) (Table 5). . For GW, Dasanbyeo exhibited a higher GW than TR22183 in Suwon 2010, Shanghai 2010 and Shanghai 2011 crop seasons. While, in another four environments, including IRRI 2010 wet season, Suwon 2010, IRRI 2011 dry and wet seasons, the GW between Dasanbyeo and TR22183 were not significantly different.

A higher mean values of GW in the RILs population was observed in the four environments, including Suwon 2010 crop season (2.56 g), Suwon 2011 crop season (2.72 g), Shanghai 2010 crop season (2.55 g), and in IRRI 2011 wet season (2.71 g). These values were significantly different with those recorded in IRRI 2010 wet season (2.37 g), Shanghai 2011 crop season (2.39 g), and IRRI 2011 dry season (2.52 g) (Table 5).

In comparison to seven environments, spikelet fertility (SF) between Dasanbyeo and TR22183 was not significantly different in 5 out of seven environments, including in Suwon 2010 crop season, Shanghai 2010 crop season, Suwon 2011 crop season, IRRI 2010 wet season and IRRI 2011 wet season. However, the level of SF for both Dasanbyeo (73.5%) and TR22183 (74.5%) observed in Shanghai 2010 was declined (Table 5).

In other environments, such as in Shanghai 2011 crop season and IRRI 2011 dry season, a significantly different of SF between TR22183 and Dasanbyeo was observed (Table 5). TR22183 exhibited SF mean value of 90.4% and 93.7% in Shanghai 2011 and IRRI 2011 wet season, respectively, which are significantly different with Dasanbyeo's SF mean value of 75.2% and 49.4% in Shanghai 2011 and IRRI dry season, respectively. These results suggests a high temperature occurs during dry season in IRRI and Shanghai affected Dasanbyeo parent (indica type) with severe reduction of spikelet fertility.

In general, the highest SF value of Dasanbyeo was observed in Suwon, followed by that in Shanghai and the lowest one was observed in IRRI dry season. TR22183 showed the lowest SF value recorded in Shanghai 2010 crop season and a similar SF value of TR22183 was recorded in other locations. In Shanghai 2010, both Dasanbyeo and TR22183 showed a lower SF value. In RILs population, the mean SF value showed a similar response with Dasanbyeo to the environments in which the lowest SF mean value of the RILs was observed in Shanghai 2010 crop season.

Dasanbyeo showed a higher grain yield (GY) than TR22183 in all locations. GY obtained from Dasanbyeo was higher than that in Suwon, Shanghai, and IRRI (Table 5). TR22183 showed a similar GY response with Dasanbyeo in all locations. In RILs population, the reduction of GY was very significant. The most severely reduction of GY in Dasanbyeo was observed in IRRI 2011 dry season which showed a similar response in SF found in the same location (Table 5).

Correlation and path coefficient analysis for agronomic traits in RILs

Correlation and path coefficients analysis among the eight agronomic traits in RILs were shown in Suppl. Table 3. Significant correlations ($p<0.05$, $p<0.01$) were observed among traits. In RILs Suwon GY showed significant positive correlations with all other traits. Among yield component traits, SN showed the highest positive correlation with GY in 2010. In 2011 SF showed highest correlation with GY and SN showed higher correlation with GY. In Shanghai2010 grain yield showed significant positive correlations with all other traits except PN.

Among yield component traits SF showed the highest positive correlation with GY than other traits. In Shanghai2011 among yield component traits PN, GW and SF showed significant correlation with GY. In IRRI among yield component traits SF, SN showed significant positive correlations with GY in 2010 wet season. In 2011 dry season, between yield components traits SF showed the highest significant positive correlations with GY. In 2011 wet season, SN showed highest significant positive correlations with GY.

To investigate direct and indirect effects via yield component traits on GY, path coefficient analysis was conducted between yield component traits (Suppl. Table 4). In RILs in Suwon the path analysis revealed that SN had the highest positive direct effect on GY followed by PN and SF, and GW had no significant indirect effects via two traits with GY. In Shanghai, SF, GW had higher positive direct effects on GY than PN SN. In IRRI, SN and SF had high positive direct effect on GY than GW and PN. In all three locations yield component had low indirect effects via GY.

Analysis of variance and G×E interactions

Analysis of variance

Genotype, location, interaction were involved in this analysis of variance (Table 6). The ANOVA revealed G×E interactions as well as differences among genotype, location and year for all traits. Genotypic effects were larger on the CL (42.4%), PL (47.3%) and GW (56.3%) than those on the locational (40.9%, 29%, and 17.4% respectively), G×E effects. Locational effects were larger on DTH, PN, SN, and GY (74.9%, 67.4%, 51.6% and 41.5% respectively) than the genotypic and G×E effects. The highest G×E effects were observed on SF (50.1%) in comparison to other traits which ranged between 8.9%~27.3% and G×E effects were higher on GY(27.3%) .

AMMI analysis

For the AMMI analysis of traits, AMMI-4 was used for all traits (Table 6) in which the AMMI-4 model was significant. In AMMI analysis, the first two IPCAs explained most of the variance in the G×E interactions ranging from 49.1% for GY and 77.1% for SF. The AMMI biplot composed of IPCA1 scores and the means of genotypes or provided the pattern of G×E interaction for each trait (Fig. 2). The components of the biplot (Environments+ G×E) for each trait attributed most of the total treatment SS on traits which provided values of 83.8%, 55.4%, 52.1%, 86.4%, 73.6%, 43.9%, 68.8%, 75,4% for DTH, CL, PL, PN, SN, GW, GY and SF, respectively. Thus, the biplot were suitable to interpret the G×E interactions and main effects except GW, especially for DTH, PN, SN, GY and SF.

According to the biplots (PN, SN, GY, and SF), based on the first two IPCAs the spots of the most RILs were located near the centers of both axes, while the environment spots were widely dispersed (Fig. 2). It is suggested that G×E interaction effects were smaller than the environmental effects.

Furthermore, in the biplots based on the first two IPCAs in SF, the spots of the RILs were located near the centers of IPCA1 axes. The environmental spots were dispersed especially in Shanghai 2010 and IRRI dry season 2011 have a wide IPCA value. Present

study also sought that of the seven environments, SF in the three locations for two years experiments, the environments could be distinguished in three groups. The first group is known as Group A, C, D, E, G had low negative IPCA1 scores and environmental means were close to grand mean. The second group is F with high negative IPCA1 scores and environmental means lower than the grand mean. The third group is B which had highest positive IPCA1 scores and environmental means was also the lowest in these locations, which means that in these locations G× E interactions were B>F>A,C,D,E,G.

For GY, in biplots based on the first two IPCAs, the environmental spots were dispersed especially in Shanghai 2010, IRRI dry season 2011, and IRRI wet season 2010 which have a wide IPCA value. The seven environments could be distinguished in 4 groups. The first group is G and E which had a low IPCA1 scores and environmental means were close to grand mean. The second group is A and D with a higher positive IPCA1, IPCA2 scores. While the third group is B which had a high positive IPCA1 scores. The last group is C, F which had the lowest IPCA1 scores. Therefore, in GY, denotation for G × E interactions were C, F>B>A,D,G,E.

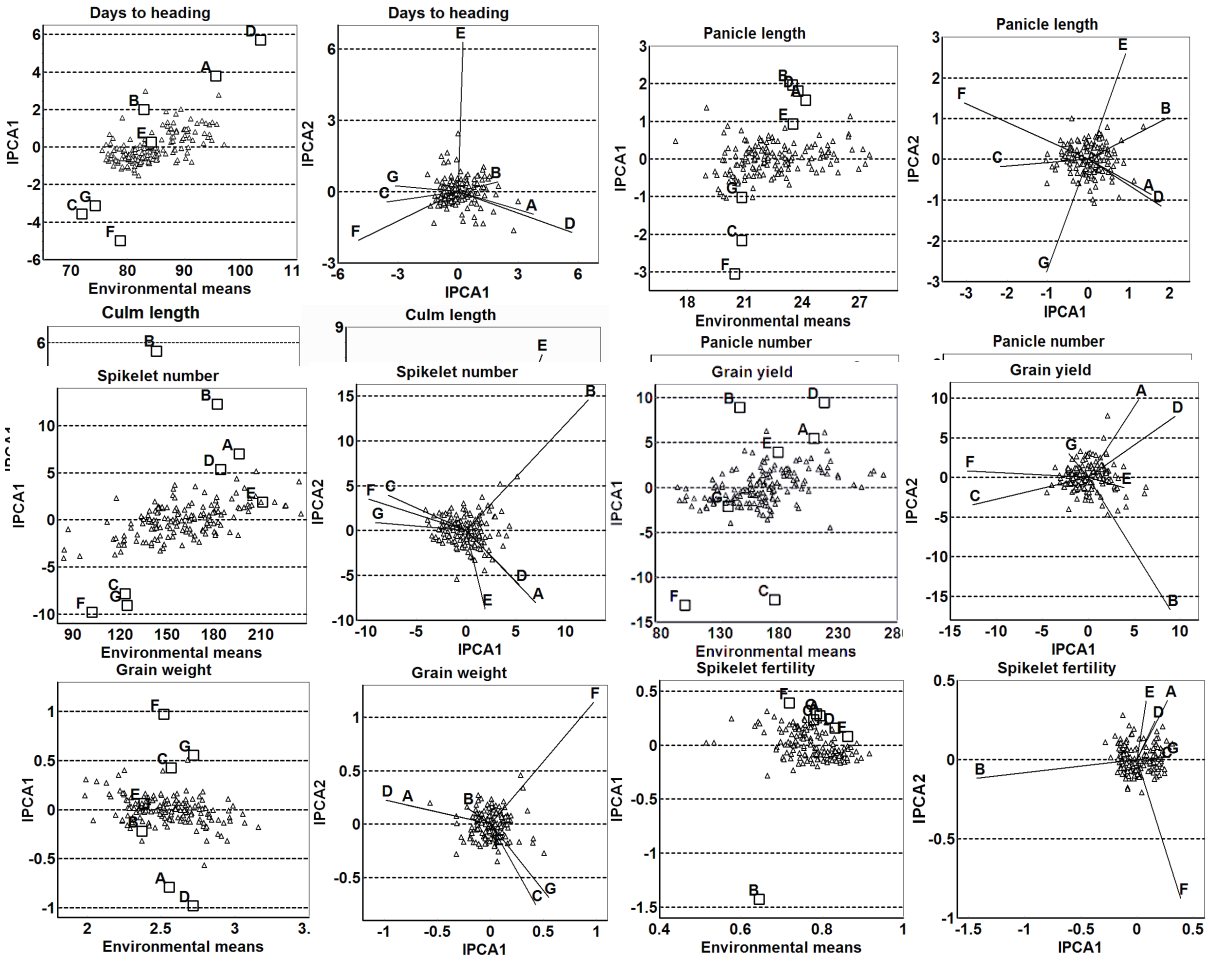
In PN, the group consisted of group C, group F, and group A, B, E, D. While in SN the group consisted of group B, group A, D, and group C, F, G. Taken together, present study suggested that grouping for GY and SF resulting in three divided groups of the environments. The first group was group B (Shanghai 2010), the second group was C (IRRI 2010), F (IRRIDS2011), and the third group was A (Suwon2010), D (Suwon2011), E (Shanghai2011), G (IRRIWS2011).

Table 6 Combined analysis of variance from GLM and AMMI analysis of variance for evaluated traits in the experimental environments and the proportion of the total variance attributable to the sources of variation of RIL

Source	Days to heading			Culm length			Panicle length			Panicle number		
	DF	M.S.	S.S. %	DF	M.S.	S.S. %	DF	M.S.	S.S. %	DF	M.S.	S.S. %
Total	1063		100.0	1056		100.0	1056		100.0	1056		100.0
Genotype	153	173.94	15.4	153	594.50	42.4	153	25.76	47.3	153	12.33	13.5
Environment	6	21626.20	74.9	6	14604.20	40.9	6	402.55	29.0	6	1567.22	67.2
G x E interaction	904	17.09	8.9	897	34.71	14.5	897	2.14	23.1	897	2.99	19.2
CV%		3.70	0.0		7.20	0.0		8.40	0.0		26.80	0.0
AMMI analysis			0.0			0.0			0.0			0.0
G x E interaction	904	17.09	100.0	897		100.0	897	2.14	100.0	897	2.99	100.0
AMMI 1	158	61.32 **	62.7	158	61.32**	41.2	158	4.10 **	33.7	158	5.99 **	35.3
AMMI 2	156	14.77 **	14.9	156	14.77 **	19.2	156	2.41 **	19.6	156	4.50 **	26.1
AMMI 3	154	8.85**	8.8	154	8.85 **	16.0	154	2.30 **	18.4	154	2.90 **	16.6
AMMI 4	152	7.04 **	6.9	152	7.04 **	12.4	152	1.66 **	13.1	152	2.05 **	11.6
Error	284		6.6	277		11.2	277		15.2	277		10.3

Source	Spikelet number			Grain weight			Grain yield			Spikelet fertility		
	DF	M.S.	S.S. %	DF	M.S.	S.S. %	DF	M.S.	S.S. %	DF	M.S.	S.S. %
Total	1062		100.0	1058		100.0	1060		100.0	1062		100.0
Genotype	153	5466.87	26.4	153	0.37	56.3	153	7714.66	30.9	153	0.03	24.6
Environment	6	272199.00	51.6	6	2.96	17.4	6	263738.00	41.5	6	0.79	25.3
G x E interaction	903	772.82	22.0	899	0.03	26.5	901	1154.97	27.3	903	0.01	50.1
CV%		13.90	0.0		6.50	0.0		20.30	0.0		8.50	0.0
AMMI analysis			0.0			0.0			0.0			0.0
G x E interaction	903	772.82	100.0	899	0.03	100.0	901	1154.97	100.0	903	0.01	100.0
AMMI 1	158	1411.91 **	32.0	158	0.06 **	35.0	158	1909.83**	29.0	158	0.04 **	63.8
AMMI 2	156	1122.85 **	25.1	156	0.04 **	23.3	156	1343.61 **	20.1	156	0.01 **	13.3
AMMI 3	154	969.63 **	21.4	154	0.03 **	16.3	154	1250.71 **	18.5	154	0.01 **	8.7
AMMI 4	152	428.64 **	9.3	152	0.02 **	11.4	152	954.77 **	13.9	152	0.00 **	6.9
Error	283		12.2	279		14.0	281		18.4	283		7.3

Fig 2 Biplot of G × E interaction for each trait of rice RILs grown in 2010 and 2011 at three locations. The left plot is that between the mean value of each trait and the first interactive principal components, and the right plot is that between the first and second interactive principal components. The square means the grand means of each RIL, respectively.
(A:Suwon2010, B:Shanghai2010, C:IRRIWS2010, D:Suwon2011, E:Shanghai2011, F:IRRIDS2011, G: IRRIWS2011)



Discussion

This research aimed to evaluate the response of RILs population of rice and the parents under heat stress condition and to obtain the Genotype × Environments interaction pattern of RILs in several environments. Screening RILs population tolerance to high temperature was conducted in various environments including Shanghai 2010 (extremely high day maximum temperature during anthesis), IRRIWS (stable temperature with high mean temperature and minimum temperature during the whole life of rice), and IRRIDS (stable temperature with drought environment), and the experiment was conducted within two years (except for IRRIDS, one year). In present study, we were able to simulate heat damage occurred in certain environments as well as to study the responses of the RILs against different high temperature environments. The G × E interaction results showed that GY and SF were correlated with the maximum daily temperature, how long the stress preserving, and with stage the stress implementing. We found that the damages occurred in several environments and even at the same environment and different year were caused by high temperature stress.

The daily average and daily maximum temperatures during anthesis period were lower in Suwon than those in IRRI and Shanghai in 2010, as well as those recorded in the year 2011 of Suwon, IRRIWS, Shanghai and IRRIDS (Table 4). Compared to other environments, Dasanbyeo and RILs showed the reduction of GY more severely in IRRI where the daily average and minimum temperature was higher. The results agreed with Peng shaobing's research. In their study, it is reported that during the period 1979-2003 grain yield declined by 10% for each 1℃ growing season minimum temperature in the dry season in the Philippines which annual mean maximum and minimum temperature have increased by 0.35 ℃ and 1.13 ℃, respectively (Peng *et al.*, 2004).

In addition, Mohammed et al. (2009) and Wei (2010) suggested that, high nighttime temperature decreased yield. In IRRIDS, the reduction of GY was the most severe one which had similar climate condition with IRRIWS but it combined drought and other abiotic stress during the whole life of rice. Heat stress is main reason to cause sterility

while the heat stress and water stress simultaneously can decrease spikelet fertility (Rang et al. 2011). Present study also found that TR22183 showed more stable fertility than Dasanbyeo in all environments. However it showed the lowest GY s in IRRI. Similarly, DTH and yield components trait of SN also reduced in IRRI. The phenomenon of low GY might be caused by a severe reduction of SN observed in such a location.

According Table 3 and Fig 1, TR22183 avoided high day maximum temperature in Shanghai2010 and IRRIDS. While DTH and SN in TR22183 showed highly positive correlation in all environments at IRRI (Supl Table 3). According Naokuni's (25), combination of *Hd1* and *Ehd1* can reduce the number of primary branches in panicle, resulting in smaller spikelet number per panicle. In present study, TR22183 and some RILs showed a similar pattern. Some studies reported that when water stress occurred during early reproductive stages, decrease in spikelet number per plant would occur (Kato et al., 2006, 2007). TR22183 and some RILs showed a short DTH in IRRI compared to DTH in other regions. Overall, It is supposed that shortened of DTH or other abiotic stress might cause decrease of SN. The decrease in SN resulting in severe loss of the grain yield.

Among the various agronomic traits evaluated in this study, the lowest SF value in RILs was observed in Shanghai 2010 which appeared to be an extremely maximum day temperature during anthesis stage. Dasanbyeo showed a not significantly effect in this environment because it avoided the extremely high temperature during anthesis. In IRRI dry season, the maximum daily temperature was higher during anthesis and SF showed a lower value in all environments, meanwhile, Dasanbyeo exposed to high day temperature during anthesis. This result showed that the maximum temperature during anthesis affected GY by low fertility as reported by other previous studies (Satake *et al.*).

The ANOVA is an additive model describing main effects effectively. It also determine whether or not the G × E interaction is significant as source of variation (Samonte et al., 2005). AMMI analysis supporting in the selection of environments or test site locations

and helpful in making decision in breeding program (Gauch et al., 1997). In present study, AMMI results showed that environment B (Shanghai 2010), F (IRRI dry season), C (IRRI wet season) were highly related to the GY revealing these environment suitable for heat tolerant screening. In year replications, environment E (Shanghai2010) did not show highly related to the GY, revealing heat stresses in Shanghai were not stable. Therefore, it can be concluded that based on the results, IRRI is more suitable for screening heat stress compared to the other two locations used in present study.

Some anomalous results were exist and hard to explain. These results also showed high relations between G × E interactions such as IRRIDS. It can be summarized that abiotic stresses are hard to divide into a single stress. These results demonstrate that multi-locational screening should be a good strategy for developing or screening widely adaptable heat tolerant rice varieties. However, this methods still can be improved.

Correlation analysis among the environments for the evaluated traits

1) Days to heading

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.841**					
Ph2010	0.285**	0.207**				
Su2011	0.935**	0.840**	0.234**			
Sh2011	0.573**	0.572**	0.279**	0.542**		
Phds2011	0.280**	0.384**	0.258**	0.274**	0.347**	
Phws2011	0.425**	0.517**	0.323**	0.446**	0.480**	0.513**

2) Culm length

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.824**					
Ph2010	0.216**	0.248**				
Su2011	0.864**	0.767**	0.242**			
Sh2011	0.687**	0.713**	0.276**	0.683**		
Phds2011	0.367**	0.386**	0.124**	0.345**	0.355**	
Phws2011	0.598**	0.595**	0.181**	0.594**	0.519**	0.312**

3) Panicle length

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.615**					
Ph2010	0.127**	0.243**				
Su2011	0.616**	0.564**	0.151**			
Sh2011	0.411**	0.325**	0.16**	0.385**		
Phds2011	0.3**	0.388**	0.111**	0.272**	0.222**	
Phws2011	0.464**	0.435**	0.115**	0.407**	0.253**	0.283**

4) Panicle number

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.336**					
Ph2010	0.066*	0.104				
Su2011	0.342**	0.22**	0.084**			
Sh2011	0.111**	0.179*	0.021	0.101**		
Phds2011	0.187**	0.177*	0.061*	0.146**	0.037	
Phws2011	0.146**	0.126*	0.026	0.131**	0.065*	0.122**

5) Spikelet number

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.523**					
Ph2010	0.136*	0.179**				
Su2011	0.66**	0.503**	0.163**			
Sh2011	0.36**	0.252**	0.254**	0.263**		
Phds2011	0.335**	0.276**	0.145*	0.254**	0.231**	
Phws2011	0.431**	0.301**	0.091	0.402**	0.139*	0.305**

6) Grain weight

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.796**					
Ph2010	0.284**	0.31**				
Su2011	0.744**	0.722**	0.197**			
Sh2011	0.659**	0.72**	0.318**	0.587**		
Phds2011	0.26**	0.394**	0.183**	0.241**	0.279**	
Phws2011	0.524**	0.644**	0.34**	0.509**	0.6**	0.299**

7) Grain yield

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.462**					
Ph2010	0.092	0.15**				
Su2011	0.657**	0.488**	0.096			
Sh2011	0.438**	0.500**	0.064	0.387**		
Phds2011	0.146*	0.182**	0.050	0.161**	0.224**	
Phws2011	0.423**	0.364**	-0.003	0.411**	0.403**	0.187**

8) Spikelet fertility

	Su2010	Sh2010	Ph2010	Su2011	Sh2011	Phds2011
Sh2010	0.108					
Ph2010	0.137*	0.051				
Su2011	0.545**	0.228**	0.084			
Sh2011	0.435**	0.288**	0.186**	0.365**		
Phds2011	0.184**	0.014	0.144*	0.157**	0.158**	
Phws2011	0.395**	0.060	0.232**	0.36**	0.41**	0.295**

Correlation coefficients among eight traits of DT RILs

Traits	Environment	DTH	CL	PL	PN	SN	GW	GY
	Suwon2010	0.389**						
	Suwon2011	0.294**						
	Shanghai2010	0.576**						
CL	Shanghai2011	0.483**						
	IRRIWS2010	0.254**						
	IRRI DS2011	0.405**						
	IRRI WS2011	0.070						
	Suwon2010	0.90**	0.408**					
	Suwon2011	0.217**	0.367**					
	Shanghai2010	0.218**	0.467**					
PL	Shanghai2011	0.060	0.215**					
	IRRIWS2010	0.255**	0.350**					
	IRRI DS2011	0.435**	0.511**					
	IRRI WS2011	0.114*	0.561**					
	Suwon2010	0.117*	0.022	-0.186**				
	Suwon2011	0.276**	-0.004	-0.127**				
	Shanghai2010	0.073	-0.069	-0.307**				
PN	Shanghai2011	0.063	-0.079**	-0.095**				
	IRRIWS2010	0.023	-0.148**	-0.155**				
	IRRI DS2011	0.119*	-0.041	-0.124**				
	IRRI WS2011	0.013	-0.060*	-0.141**				
	Suwon2010	0.049	0.258**	0.479**	-0.299**			
	Suwon2011	0.132*	0.233**	0.522**	-0.182**			
	Shanghai2010	0.122*	0.198**	0.369**	-0.223**			
SN	Shanghai2011	-0.312	0.084*	0.306**	-0.130**			
	IRRIWS2010	0.69**	0.177**	0.341**	-0.262**			
	IRRI DS2011	0.281**	0.264**	0.397**	-0.169**			
	IRRI WS2011	0.171**	0.360**	0.536**	-0.071			
	Suwon2010	-0.075	0.208**	0.095	-0.056	-0.037		
	Suwon2011	-0.126*	0.200**	0.078	-0.200**	-0.086		
	Shanghai2010	-0.058	0.273**	0.161**	-0.171**	-0.041		
GW	Shanghai2011	0.122*	0.360**	0.106**	-0.041	0.072*		
	IRRIWS2010	0.040	0.184**	0.088	-0.031	-0.233**		
	IRRI DS2011	-0.200**	0.101	-0.105	-0.041	-0.129*		
	IRRI WS2011	0.024	0.096	-0.010	-0.107	-0.122*		
	Suwon2010	0.314**	0.334**	0.239**	0.177**	0.420**	0.252**	
	Suwon2011	0.370**	0.308**	0.234**	0.127*	0.372**	0.249**	
	Shanghai2010	0.468**	0.442**	0.202**	0.066	0.227**	0.312**	
GY	Shanghai2011	0.413**	0.329**	0.175**	0.146*	0.069	0.420**	
	IRRIWS2010	0.157**	0.225**	0.100	0.033	0.306**	0.089	
	IRRI DS2011	-0.065	0.118*	0.021	0.177**	0.158**	0.131*	
	IRRI WS2011	0.327**	0.246**	0.260**	0.226**	0.440**	0.114*	
	Suwon2010	-0.029	0.038	-0.091	-0.085	-0.082	0.093	0.208**
SF	Suwon2011	0.204**	0.239**	0.092	0.056	0.122*	0.143*	0.440**
	Shanghai2010	0.508**	0.370**	0.173**	-0.072	-0.014	0.072	0.535**
	Shanghai2011	0.183*	0.196**	-0.081*	0.0003	-0.121**	0.113**	0.336**

IRRIWS2010	-0.077	0.061	-0.116*	-0.029	-0.240**	0.062	0.342**
IRRI DS2011	-0.342**	-0.105	-0.233**	0.008	-0.140*	0.073	0.307**
IRRI WS2011	-0.052	-0.160**	-0.271**	0.022	-0.308**	0.051	0.030

Path coefficient analysis of direct and indirect effect of traits on yield in DT populations

Suwon		PN	SN	GW	SF	Corr
2010	PN	0.384	-0.169	-0.015	-0.022	0.177
2011		0.237	-0.073	-0.056	0.019	0.127
2010	SN	-0.115	0.566	-0.010	-0.021	0.420
2011		-0.043	0.399	-0.024	0.041	0.372
2010	GW	-0.022	-0.021	0.270	0.024	0.252
2011		-0.047	-0.034	0.282	0.048	0.249
2010	SF	-0.033	-0.046	0.025	0.262	0.208
2011		0.013	0.048	0.040	0.337	0.440
Shanghai		PN	SN	GW	SF	Corr
2010	PN	0.226	-0.067	-0.056	-0.038	0.066
2011		0.175	-0.013	-0.016	0.000	0.146
2010	SN	-0.050	0.298	-0.013	-0.007	0.227
2011		-0.023	0.101	0.028	-0.037	0.069
2010	GW	-0.039	-0.012	0.325	0.038	0.312
2011		-0.007	0.007	0.386	0.035	0.420
2010	SF	-0.016	-0.004	0.023	0.532	0.535
2011		0.000	-0.012	0.044	0.305	0.336
IRRI		PN	SN	GW	SF	Corr
2010	PN	0.185	-0.133	-0.006	-0.013	0.033
2011DS		0.224	-0.044	-0.006	0.003	0.177
2011WS		0.282	-0.039	-0.021	0.004	0.226
2010	SN	-0.048	0.507	-0.043	-0.110	0.306
2011DS		-0.038	0.262	-0.019	-0.046	0.158
2011WS		-0.020	0.540	-0.024	-0.056	0.440
2010	GW	-0.006	-0.118	0.185	0.028	0.089
2011DS		-0.009	-0.034	0.150	0.024	0.131
2011WS		-0.030	-0.066	0.201	0.009	0.114
2010	SF	-0.005	-0.122	0.012	0.457	0.342
2011DS		0.002	-0.037	0.011	0.331	0.307
2011WS		0.006	-0.166	0.010	0.180	0.030

Chapter III. QTL analysis for agronomic traits under different environments in an RIL population

Abstract

Understanding how crops interact with their environments is increasingly important in breeding program, especially in light of highly anticipated climate changes. One method for understanding this relationship is to use genetic maps and quantitative trait loci (QTL) mapping to detect genomic regions linked to important phenotypic traits for adaptation. Detection of quantitative trait loci (QTLs) by environment interaction (QEI), therefore, is an importance strategy in order to dissect consistency of genomic regions associated with quantitative traits. A total of 150 recombinant inbred lines (RILs) of F_{12} generation derived from a cross between two parents, cv. Dasanbyeo (Korean indica type) x TR22183 (temperate japonica type) develop through a single seed descend method from an F_2 population were evaluated at Suwon 2010, Shanghai 2010, IRRI 2010 wet season, Suwon 2011, Shanghai 2011, IRRI 2011 dry season, and IRR 2011 wet season for a total of seven diverse environments. Traits evaluation across seven environments for both RILs population and parental lines include eight important agronomical traits comprising days to heading (DTH), culm length (CL), panicle length

(PL), panicle number per plant (PN), spikelet number per panicle (SN), spikelet fertility (SF), 100-grain weight (GW), and grain yield (GY). A 384-plex GoldenGate oligo pool assay (OPA) set (RiceOPA3.1) distributed evenly through the 12 rice chromosomes was employed for genotyping 150 RILs population and the parents using VeraCode technology on an Illumina BeadXpress Reader. The linkage map constructed in current study covered a total of 926.53 cM with an average two loci interval of 4.01 Cm and the linkage map for 235 SNPs markers used in present study enabled us to identify the location of SNPs markers associated with heat tolerance. In present study, QTLs for heat tolerance were identified for all eight traits of which a total of 44 main-effect QTLs and 35 QTLs x Environment interaction were detected using single environment and multi-environments analyses, respectively in the RIL F_8 population cultivated at seven different environments. Of these, fourteen putative QTLs for DTH, CL, PN, SN, GW and GY found in single environment analysis had the similar position to the QTL x environment interaction for those traits suggesting that these same QTLs from both single-and multi-environments are major and stable QTLs for certain traits. To the best of our knowledge, 12 QTLs consisted of four QTLs for CL (*qCL2*, *qCL8.1*, *qCL8.2*, and *qCL8.3*), six QTLs for GW (*qGW3.1*, *qGW3.2*, *qGW7*, *qGW8*, *qGW10.1*, and *qGW10.2*), one QTL for GY (*qGY3*) and one for SF (*qSF4*) out of 44 QTLs obtained from single environment analysis were novel since no overlapping QTL reported from previous studies. In addition, 12 out of 35 QTLs obtained from multi-environment analysis were also novel as no any QTL regions reported in previous studies overlapped with our study.

Key words: QTLs x environment interaction, single nucleotide polymorphism, environment, RIL

Introduction

High temperature stress is one of the major environmental constraint in world rice production and it has a wide range effects on plants resulting in yield losses. Temperature beyond critical thresholds can reduce the growth duration of the rice, and more severely it can cause an increase at spikelet fertility, reduce grain-filling duration, and enhance respiratory loss, resulting in lower yield and lower quality rice grain (Fitzgerald and Resurreccion, 2009; Kim et al., 2011). Heat stress can occur both in vegetative and reproductive phases, which causes yield loss. In comparison to the high temperatures occurs during the vegetative phase, rice is more tolerant to heat than that occurs during the reproductive phase, particularly at the flowering stage (Jagadish et al., 2010). Understanding how crops interact with their environments is increasingly important in breeding program, especially in light of highly anticipated climate changes. One method for understanding this relationship is to use genetic maps and quantitative trait loci (QTL) mapping to detect genomic regions linked to important phenotypic traits for adaptation.

The majority of studies investigating QTLs for phenotypic traits indicate inconsistence QTL detection across different experiments, environments, and populations (Beavis and Keim, 1996; Dudley, 1993; Paterson 1991, and Tanskley, 1993) due mainly to the association of molecular markers with QTL have collected data in a single environment. Despite, a genotype x environment interactions study involving multiple environments has been reported by Piepho (2002), but since the study has

focused only on a single measurement of a phenotype, a comprehensive picture of how a QTL affects the dynamic process of growth under a range of environmental condition was not obtained. Detection of quantitative trait loci (QTLs) by environment interaction (QEI), therefore, is an importance strategy in order to dissect consistency of genomic regions associated with quantitative traits. However, the genetic factors underlying the genotype x environment interaction (GEI) in line-based phenotypes has also resulted in an inconsistent QTL detection across environments (Beavis and Keim, 1996). Useful methods in detecting GEI and QEI is very importance in order to determine genetic factors associated with GEI, but provide no explanation of environment factors involved.

The availability of climatic data can be helpful in modelling particular aspects of the environment contributing to the differential performance of genotype across environments and it will lead to better explanations of specific environmental factors responsible for GEI and QEI (Campbell et al. 2004). The QEI effect was firstly reported by Cross et al (1999) revealed that temperature differences across environments accounted for a large portion of the QEI detected in a tropical maize (*Zea mays* L.) mapping population. In present study, understanding the genetic basis of genotype x environment (GxE) interaction for quantitative trait loci (QTLs) involved in grain yield and yield-related components is a major issue to achieve our goals.

The availability of a wide range of molecular markers and powerful statistical methods has significantly facilitated QTL mapping. Among the molecular markers, single-nucleotide polymorphisms (SNPs) represent the most abundant type of variation

present in DNA. SNPs are mostly bi-allelic (Krawczak, 1999), co-dominantly inherited, sequence tagged and occur at high density within genomes (Xing et al 2005). SNP based genotyping is preferred because it is highly accurate, quick and automated using limited human intervention. Increasing the marker density on constructing the linkage map proved a powerful in locating the yield-related QTLs (Wu et al 2014). In present study, SNP genotyping was undertaken using a 384-plex marker set for QTL mapping on the BeadXpress platform which was successfully developed by Thompson et al (2012). This marker set has demonstrated a robust and efficient method for marker genotyping in rice.

The objectives of this study were to identify the effect of chromosomal segments (QTLs) expressed under different temperatures in several environments and to identify single nucleotide polymorphism (SNP) markers x environment interaction in order to provide explanations of variable QTL genotypic differences across environments.

Materials and methods

Plant materials and traits evaluation

A total of 150 recombinant inbred lines (RILs) of F_{12} generation derived from a cross between two parents, cv. Dasanbyeo (Korean indica type) x TR22183 (temperate japonica type) develop through a single seed descend method from an F_2 population were evaluated at Suwon 2010, Shanghai 2010, IRRI 2010 wet season, Suwon 2011, Shanghai 2011, IRRI 2011 dry season, and IRR 2011 wet season for a total of seven diverse environments. The environments consists of three locations, Suwon, Shanghai, and IRRI represented diversed agroecological zones of rice production. Suwon experimental site was located in farm of Seoul National university, Korea and carried out during May to October 2010 and 2011 (126°59′E, 37 °16′N, 28m a.s.l), the experiment in Shanghai (China) was also done during May to October in 2010 and 2011 (121°24′E, 30 °54′N, 5.6m a.s.l), while the experiments at IRRI (Philippines) were carried out during June to September in the years 2010 and 2011 wet season and during January to May in the year 2010 dry season (121°15′E, 14 °10′N, 16.3m a.s.l).

Traits evaluation across seven environments for both RILs population and parental lines include eight important agronomical traits comprising days to heading (DTH), culm length (CL), panicle length (PL), panicle number per plant (PN), spikelet number per panicle (SN), spikelet fertility (SF), 100-grain weight (GW), and grain yield (GY). DTH, CL, PL, PN as well as the fully filled grains used for measuring SN, SF,

GW and GY were collected according to the Standard Evaluation System (SES) (IRRI 1998).

DNA Extraction and Genotyping Analysis

Leaves of rice seedlings at the three-leaf stage were harvested and subjected to genomic DNA extraction according to the CTAB method (Murray and Thompson, 1980) with minor modification. DNA concentrations were measured using ND-1000 Spectrophotometer (NanoDrop) and diluted to the required concentration of 50 ηg/μl. a 384-plex GoldenGate oligo pool assay (OPA) set (RiceOPA3.1) designed by Thompson et al (2012) and distributed evenly through the 12 rice chromosomes was employed for genotyping 150 RILs population and the parents using VeraCode technology on an Illumina BeadXpress Reader. All genotype calling results were manually checked and any obvious errors in calling the homozygous or heterozygous clusters were corrected. Prior to SNP analysis, the markers genotype calling results were filtered where individuals or markers presenting 5% or more missing data were excluded from the construction of linkage map analysis.

Statistical analysis

The mean evaluated traits data of two replicates (blocks) in different trials (environments) from all 150 lines (genotypes) were analyzed for frequency distributions,

standard errors, pearsons correlation coefficients and ANOVA using SAS Statistics package (Schlotzhauer et al 1997).

The SNP genotype data were then subjected to JoinMap4 software to construct the linkage map (Kyazma, Wageningen, Netherlands). Linkage groups were determined using a minimum LOD value of 3.0. The Kosambi mapping function was used to translate recombination frequencies into map distances. The linkage map was graphically visualized with MapChart 2.2 (Voorrips 2002).

QTLs were detected for each of the seven traits using the MCIM method implemented in QTLNetwork 2.0 (Yang et al 2005). In addition, QTL Network was also used to identify QTL epistasis and QTL-environment (QE) interactions of one trait in several trials with three replicates together, which employed the genome scan parameters of a 10 cM testing window, 1 cM walk speed and 10 cM filtration window. Two-dimensional (2D) genome scans were carried out to search for multiple interacting QTLs. A genome-wide threshold value of the *F*-statistic ($\alpha = 0.01$) for declaring the presence of a QTL was estimated by 1,000 random permutations. A Monte Carlo Markov Chain method with Gibbs sample size of 20,000 was used to estimate QTL effects (Jiang et al 1997). The sum of individual phenotypic variance explained by each QTL was calculated as the total phenotypic variance explained by all QTL for each trait.

Results

Linkage map construction

In present study, a 384-plex GodenGate OPA set (RiceOPA3.1) was subjected to genotyping the parents and the 150 RIL population derived from a cross between Dasanbyeo and TR22183. A linkage map based on the RIL population was then constructed. A linkage map is pre-requisite for the mapping of quantitative trait loci (QTL) and a high-resolution linkage map facilitates fine mapping of quantitative trait loci (QTLs) and can be produced because of the abundance of SNPs within the genome (Davey et al 2011). The linkage map constructed in current study covered a total of 926.53 cM with an average two loci interval of 4.01 cM (Table 1). Figure 1 shows the linkage map for 235 SNPs markers used in present study which showed the location of SNPs markers associated with heat tolerance.

Table 1. Genetic map for heat tolerance on 12 chromosomes of RIL F8 derived from Dasanbyeo/TR22183

Chromosome	Marker number	Length	Average (cM)
1	30	112.92	3.76
2	29	114.77	3.96
3	22	156.73	7.12
4	23	91.25	3.97
5	18	79.76	4.41
6	6	21.74	3.62
7	9	59.93	4.81
8	20	78.54	3.93
9	20	37.06	1.85
10	10	47.90	4.79
11	22	65.88	2.99
12	24	67.51	2.81
Total	235	926.53	4.01

Identification of main-effect QTLs for heat tolerance using single environment analysis

In order to understand tolerance mechanisms under environmental stresses, the detection of adaptive QTLs for high temperature tolerance is required. Several studies have already identified genetic markers related to different environmental stresses, including heat (Roy et al., 2011). QTL mapping studies for heat tolerance have been conducted on various rice populations at flowering stages. However, confirmation and fine mapping of the identified QTLs for heat tolerance have not been reported yet (Ye et al., 2012). In other crops, several loci for heat tolerance have been identified, such as in wheat (Paliwal et al., 2012) and maize (Bai, 2011).

In present study, QTLs for heat tolerance were identified for all eight traits (Fig. 1). All QTLs were detected above an empirically determined experiment-wise significance threshold equivalent to $P < 0.01$. An experiment-wise significance threshold of $P < 0.05$ was used to declare a QTL significant for supporting environments if a QTL was already established at the same position in at least one environment at $P < 0.01$. QTLs for heat tolerance were identified and summarized in Table 2, Table3 and Figure 1. Based on composite interval mapping analysis employed by QTL Network software, a total of 44 main-effect QTLs were detected for eight traits in the RIL F8 population grown at seven different environments. These main-effect QTLs were mapped to nine chromosomes including chromosomes 1, 2, 3, 4, 5, 7, 8, 9 and 10.

Eight QTLs for days to heading (DTH) were mapped on chromosomes 1, 3, 8, and 10. In comparison to other detected QTLs for days to heading (*qDTH)*, the *qDTH8*

(*qDTH8.1, qDTH8.2,* and *qDTH8.3*) located on chromosome 8 showed the highest effect on phenotypic variation (Table 2). Of these, the *qDTH8.1* and *qDTH8.3* were identified only at one environment of Suwon2010 and Sanghai2011, respectively and each QTL showed 29.6% and 24.7% phenotypic variation, respectively. The *qDTH8.2* was detected at two environments of Sanghai2010 and Suwon2011 explained 27.6% and 30.5% phenotypic variation, respectively. Dasanbyeo allele increased the days to heading at all QTLs loci (Table 2).

Five QTLs for culm length (CL) were mapped on chromosome 1, 2 and 8. A QTL on chromosome 1, *qCL1* was detected in RIL progenies grown during the two growing seasons, wet and dry season year 2011 at the Philippines. The *qCL1* explained about 10% of phenotypic variation in the two environments. QTL for CL identified on chromosome 2 (*qCL2*), showed additive effect for increasing CL at three environments, Suwon2010, Suwon2011, and Philippines wet season 2011 explaining 11.2, 9.8 and 9.9% of phenotypic variation, respectively. The remaining three QTL loci for CL were identified on chromosomes 8, namely *qCL8.1*, *qCL8.2*, and *qCL8.3*. The *qCL8.1* was detected at four environments, including Suwon2010, sanghai2010, Philippines2010, and Philippines wet season 2011 explaining a phenotypic variation of 156, 23.1, 10 and 11.5%, respectively. The *qCL8.2* was only identified at one environment, Philippines2010 explaining 10.9% of phenotypic variation. While the *qCL8.3* was detected at two environments, explaining the phenotypic variation of 11.9 at Suwon2011, and 19.3% that of at Sanghai2011 (Table 2). Additive effect to increase the value of culm length provided by the QTL loci of the *qCL1, qCL2* and *qCL8.2* were derived from

TR22183 allele which is reflected by negative additive value. On the other hand, Dasanbyeo allele increased the culm length at the *qCL8.1* and *qCL8.3*.

Five QTLs for panicle length (PL) were mapped on chromosome 1, 3 and 4. Of these, none of QTLs, was detected in more than one environments. Those QTLs consisted of the *qPL1.1* (Philippines dry season 2011), *qPL1.2* (Philippines dry season 2011), *qPL3* (Suwon 2011), *qPL4.1* (Suwon 2010) and *qPL4.2* (Suwon 2011) which explained the phenotypic variation of 7.3, 11.5, 8.8, 6.7, and 10.9%, respectively. Of these, Dasanbyeo allele increased panicle length in Suwon2011, while TR22183 increased PL on the remaining four loci (Table 2).

Four QTLs responsible for panicle number (PN) were mapped to chromosomes 1, 4, and 8 (Table 2). The *qPN1* was observed in RIL population grown in the Philippines dry season 2011, which showed a 12.5% of phenotypic variation explained value. The increase of panicle number was derived from Dasanbyeo allele effect. The *qPN4.1* was detected at two environments, including Suwon2010 and Suwon2011 explaining 13.3 and 12% of phenotypic variation, respectively. Dasanbyeo allele increased the panicle number at this locus. Another QTL locus detected on chromosome 4, *qPN4.2* was only identified at environment of Suwon2010. This locus explained 12.6% of phenotypic variation and an increased in panicle number was contributed by Dasanbyeo allele. While, the qPN8 detected on chromosome 8 was only detected in the environment of Suwon2011 which explained 11.9% of phenotypic variation. The Dasanbyeo allele also increased panicle number at this locus.

A total of eight QTLs related to spikelet number (SN) were detected in present

study. These QTLs were mapped to chromosomes 2, 4, 5, and 9. Of these, four QTLs were mapped to chromosomes 2, namely *qSN2.1*, *qSN2.2*, *qSN2.3*, and *qSN2.4*. The *qSN2.1* was detected at growing season of Philippines2010, explaining a 13.8% of the phenotypic variation. The *qSN2.2* existed at the Shanghai2011 which the value of PVE of 5%. The *qSN2.3* which explained 12.8% of the phenotypic variance was found at the environment of philippines2010. While the *qSN2.4* was detected in RIL population at the environment of Philippines dry season 2011 and this QTL has PVE value of 13%. The additive effect on the increase of spikelet number at these loci were derived from Dasanbyeo alleles. QTL regions related to SN on chromosome 4 were named as *qSN4.1* and *qSN4.2*. The *qSN4.1* was found in RIL population grown at Suwon year 2010 and explained 12.6% of the phenotypic variation. While, the *qSN4.2* was detected at Sanghai year 2011 and explaining 14.4% of the phenotypic variation. These two QTLs had additive effect derived from TR22183 alleles in increasing the spikelet number. In addition, in present study, we also found another two loci for spikelet number including, *qSN5* on chromosome 5 and qSN9 on chromosome 9. The *qSN5* which explained 3.4% of PVE was detected at Suwon 2011 and *qSN9* which explained 9.2% of PVE was detected at environment of the Philippine dry season 2011.

Seven QTLs for grain weight (GW) were mapped to chromosomes 1, 3, 7, 8, and 10. The *qGW1*, located on chromosome 1 was detected at two environments of the Sanghai2010 and Philippines2010. Phenotypic variation explained by this locus was 5.1 and 8.6% in Sanghai 2010 and Philippines 2010, respectively. The TR22183 allele at this loci increased grain weight at the two environments. Two QTLs for GW on

chromosome 3 were also observed, namely qGW3.1 and qGW3.2. The *qGW3.1* in the RIL population was detected at four environments including Suwon2010, Sanghai2010, Philippines2010, and Philippines wet season 2011 which explained a 9.2, 12.7, 16.5, and 16.1% of phenotypic variation, respectively. The allele of Dasanbyeo increased grain weight at this locus. While *qGW3.2* was only detected at one environment of Sanghai2010 explaining 7.2% of phenotypic variation and the additive effect on grain weight was derived from TR22183 allele. The *qGW7* and *qGW8* were the QTLs for GW detected at Philippines2011 and Suwon2011, respectively. The former had PVE value of 11.4%, the later was 9.3%. Dasanbyeo allele increased the grain weight at this locus. Meanwhile, *qGW10.1* and *qGW10.2* were the QTLs for GW detected on chromosome 10. The *qGW10.1* was identified from RIL population grown at Sanghai2010, while the *qGW10.2* was from Sanghai2011. The *qGW10.1* showed PVE value of 14.2% and the *qGW10.2* was 8.7%. These two QTLs showed that the positive additive effect in increasing the grain weight was derived from Dasanbyeo allele.

A total of three QTLs for grain yield were mapped to chromosome 3, 9, and 10. The *qGY3*, a QTL on chromosome 3 showed 12% of the phenotypic variation explained, while the *qGY9*, a QTL for GY on chromosome 9 explained 14.6% of the phenotype variation. In addition to QTL for GY, the *qGY10* was detected on chromosome 10 exhibited a 14.2% of PVE. The *qGY3* was identified in RIL population grown at environment of Suwon2010, while *qGY9* was detected at Philippines dry season 2011. The *qGY10* was detected on the environment of Suwon2010. Dasanbyeo allele increased the grain yield at all the three QTLs (Table 2).

Spikelet fertility is one of the most important characters on QTL mapping for heat tolerance. In present study, we detected four QTL loci for spikelet fertility, including *qSF1* (located on chromosome 1), *qSF2* on chromosome 2, *qSF4* on chromosome 4, and *SF8* on chromosome 8. The *qSF1* was detected from RIL population grown at the environment of Sanghai2011 and explaining 10.6% of the phenotypic variation. Dasanbyeo allele increased the spikelet fertility at this locus. The *qSF2* was found at the environment of the Philippines wet season 2011 and explaining a 10.4% of the phenotypic variation. The TR22183 allele increase spikelet fertility at this locus. The *qSF4* and *qSF8* were observed from RIL population at the environment of Sanghai2011 and the Philippines wet season 2011, respectively. The *qSF4* showed 6.6% of the phenotypic variation explained value, while the PVE value showed by the *qSF8* was 9.8%. Dasanbyeo allele increased the SF at the *qSF4*, whereas the TR22183 allele increased the SF at the *qSF*

Table 2. Putative QTLs for heat tolerance in RILs population at different environments using single environment analysis

[1]Significance levels of $P<0.001$ is denoted by **, negative value means allele from TR22183 had positive additive value (the TR22183 allele increases the value of the traits), whereas positive one means the Dasanbyeo allele increases the value of the traits.

Traits	Chr.	QTL	Interval	Suwon2010		Sanghai2010		Philippines2010		Suwon2011		Sanghai2011		Philippines DS2011		Philippines WS2011	
				Additive effect[1]	h^2(a)	Additive effect	h^2(a)	Additive effect	h^2(a)	Additive effect	h^2(a)	Additive effect	h^2(a)	Additive effect	h^2(a)	Additive effect	h^2(a)
DTH	1	*qDTH1*	id1003056-id1003344													-0.95**	0.051
	3	*qDTH3.1*	id3000695-id3001422	2.06**	0.068												
	3	*qDTH3.2*	fd10-id3015453			1.87**	0.11										
	3	*qDTH3.3*	id3015533-id3016090							2.54**	0.079						
	8	*qDTH8.1*	id8002187-ud8000441	3.92**	0.296												
	8	*qDTH8.2*	id8002025-id8002187			3.77**	0.276			4.87**	0.305						
	8	*qDTH8.3*	id8001299-id8002025									3.81**	0.247				
	10	*qDTH10*	id10000185-ud10000265	2.31**	0.09	1.17**	0.062										
CL	1	*qCL1*	id1023174-id1025455											-1.44**	0.101	-1.91**	0.107
	2	*qCL2*	id2000007-id2001468	-3.36**	0.112					-1.61**	0.098					-2.23**	0.099
	8	*qCL8.1*	id8002025-id8002187	4**	0.156	6.35**	0.231	2.31**	0.1							2.19**	0.115
	8	*qCL8.2*	id8007093-id8007595					-2.6**	0.109								
	8	*qCL8.3*	id8001299-id8002025							2.05**	0.119	5.68**	0.193				
PL	1	*qPL1.1*	id1001764-id1003056											-0.61**	0.073		
	1	*qPL1.2*	id1023174-id1025455											-0.72**	0.115		
	3	*qPL3*	id3014361-fd10							0.71**	0.088						
	4	*qPL4.1*	id4005704-ud4001552	-0.63**	0.067												
	4	*qPL4.2*	id4006867-id4007698							-0.77**	0.109						
PN	1	*qPN1*	id1022407-id1023174											1.08**	0.125		
	4	*qPN4.1*	id4008442-id4008981	0.4**	0.133					0.4**	0.12						
	4	*qPN4.2*	id4010200-id4011666	0.41**	0.126												
	8	*qPN8*	id8004221-id8005235							0.41**	0.119						
SN	2	*qSN2.1*	id2002293-id2003067					9.44**	0.138								
	2	*qSN2.2*	id2007679-ud2001198									12.29**	0.05				
	2	*qSN2.3*	wd2000539-id2005345					7.32**	0.128								

	2	*qSN2.4*	id2004617-wd2000539										8.99**	0.13		
	4	*qSN4.1*	id4006867-id4007698	-18.29**	0.126											
	4	*qSN4.2*	id4010200-id4011666									-17.53**	0.144				
	5	*qSN5*	id5003662-id5004086							7.68**	0.034						
	9	*qSN9*	id9007180-id9007328											7**	0.092		
GW	1	*qGW1*	id1026052-dd1002041			-0.07**	0.051	-0.08**	0.086								
	3	*qGW3.1*	id3000695-id3001422	0.09**	0.092	0.08**	0.127	0.13**	0.165							0.11**	0.161
	3	*qGW3.2*	id3014361-fd10			-0.07**	0.072										
	7	*qGW7*	id7004065-id7004210													0.04*	0.114
	8	*qGW8*	id8007595-id8007764							0.08**	0.093						
	10	*qGW10.1*	id10004500-ud10000989			0.12**	0.142										
	10	*qGW10.2*	ud10000989-id10005049									0.08**	0.087				
GY	3	*qGY3*	id3015533-id3016090	9.94**	0.12												
	9	*qGY9*	id9004148-id9004978											13.23**	0.146		
	10	*qGY10*	id10003891-id10004500	17.9**	0.142												
SF	1	*qSF1*	id1023174-id1025455									0.02**	0.106				
	2	*qSF2*	wd2000377-id2004617													-0.03**	0.104
	4	*qSF4*	id4002032-id4002348											0.03**	0.066		
	8	*qSF8*	wd8001250-id8002968													-0.02**	0.098

Table 3. QTL analysis for heat tolerance across seven environments

	Chrom	QTL	Marker interval	A	AE1	AE2	AE3	AE4	AE5	AE6	AE7	h^2(a)
DTH	1	*qEDTH1*	id1026052-dd1002041	0.84**	0.26NS	0.24NS	-0.51NS	0.51NS	0.03NS	-0.6NS	0.07NS	0.003
	3	*qEDTH3.1*	id3000695-id3001422	0.67**	0.68NS	0.16NS	-0.56NS	0.49NS	0.05NS	-0.49NS	-0.33NS	0.01
	3	*qEDTH3.2*	fd10-id3015453	1.18**	0.02NS	0.43NS	-0.18NS	0.27NS	-0.34NS	-0.14NS	-0.06NS	0.047
	8	*qEDTH8*	id8002025-id8002187	2.66**	1.25**	1.03*	-1.42**	2.26**	0.83*	-2.37**	-1.43**	0.162
	10	*qEDTH10*	ud10000265-id10003260	0.91**	0.56NS	0.23NS	-0.17NS	0.56NS	-0.07NS	-0.6NS	-0.5NS	0.039
	12	*qEDTH12*	id12005212-id12005547	1.64**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.05
CL	1	*qECL1.1*	id1015984-id1016790	4.28**	0.2NS	0.8NS	-0.55NS	0.22NS	0.85NS	-0.97NS	-0.54NS	0.009
	1	*qECL1.2*	id1023174-id1025455	-2.99**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.056
	1	*qECL1.3*	id1018870-id1020384	2.14**	0NS	0NS						0.017
	2	*qECL2*	id2000007-id2001468	-2.29**	-0.5NS	-0.58NS	0.82NS	-0.21NS	-0.49NS	1.01NS	-0.04NS	0.069
	5	*qECL5*	id5007205-id5008218	2.02**	0.01NS	0.06NS	-0.03NS	-0.04NS	-0.02NS	0.03NS	-0.01NS	0
	8	*qECL8*	id8001299-id8002025	4.62**	0.32NS	2.77**	-0.91NS	-0.36NS	1.93**	-2.9**	-0.81NS	0.122
PL	2	*qEPL2*	id2003067-id2003244	0.53**	0.02NS	0.01NS	-0.01NS	0.02NS	-0.01NS	-0.01NS	-0.02NS	0.031
	4	*qEPL4*	id4004185-id4005704	-0.58**	-0.06NS	-0.08NS	0.09NS	-0.11NS	0.1NS	0.08NS	-0.02NS	0.023
	5	*qEPL5*	id5003662-id5004086	0.35**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.037
	11	*qEPL11*	id11001601-id11002336	-0.63**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.033
PN	4	*qEPN4*	id4008442-id4008981	0.33**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.033
	5	*qEPN5*	id5005882-id5006365	-0.31**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.026
	10	*qEPN10*	id10005049-wd10003790	-0.19**	0.07NS	-0.04NS	0.05NS	0.03NS	0.02NS	-0.09NS	-0.04NS	0.009
SN	2	*qESN2*	id2002293-id2003067	14.08**	1.13NS	2.35NS	0.02NS	-0.19NS	-1.33NS	-1.14NS	-0.81NS	0.072
	4	*qESN4*	id4008981-id4009413	-10.86**	-3.75NS	-0.42NS	2.23NS	-2.04NS	-2.54NS	3.53NS	3NS	0.05
	5	*qESN5*	id5013798-id5014669	-8.91**	-1.63NS	-1.47NS	1.18NS	-0.88NS	0.31NS	1.4NS	1.06NS	0.017
	10	*qESN10*	id10004500-ud10000989	6.19**	1.57NS	0.53NS	-2.71NS	0.74NS	0.83NS	-0.17NS	-0.76NS	0.018
GW	1	*qEGW1*	id1026052-dd1002041	-0.09**	0NS	0NS	-0.01NS	0NS	0NS	0.02NS	0NS	0.038
	2	*qEGW2*	ud2001198-id2008501	-0.06**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.048
	3	*qEGW3.1*	id3000695-id3001422	0.06**	0NS	0NS	0.02NS	0NS	0NS	-0.04*	0.01NS	0.092
	3	*qEGW3.2*	id3014361-fd10	-0.04**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.013
	7	*qEGW7*	id7003591-id7004065	0.04**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.06

	8	*qEGW8*	ud8001618-id8007093	0.03**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.03
	10	*qEGW10*	id10004500-ud10000989	0.07**	0NS	0NS	0NS	0NS	0NS	0NS	0NS	0.077
GY	3	*qEGY3*	id3015533-id3016090	8.73**	2.38NS	0.87NS	-3.35NS	3.32NS	1.08NS	-3.07NS	-1.25NS	0.044
	8	*qEGY8*	id8001299-id8002025	-3.45**	-1.16NS	3.7NS	-2.84NS	1.89NS	4.05NS	-4.09NS	-1.55NS	0.017
	10	*qEGY10*	id10003891-id10004500	9.92**	1.17NS	1.31NS	0.24NS	2.77NS	-0.63NS	-3.65NS	-1.24NS	0.088
SF	1	*qESF1.1*	id1018870-id1020384	0.03**	-0.01NS	0.01NS	0NS	0NS	0NS	0NS	0NS	0.033
	5	*qESF5*	id5006456-id5007205	0.03**	0NS	0.01NS	0.01NS	0NS	0.01NS	-0.02*	0NS	0.023
	8	*qESF8*	id8002025-id8002187	0**	-0.01NS	0.04**	-0.01NS	0NS	0.01NS	-0.01NS	-0.02NS	0.002

Identification of QTLs using multi-environment analysis

For the traits measured in multiple environments, 37 QTLs denoted as E-QTLs were detected in which some of the QTLs were detected in at least two environments. Of these, six QTLs were detected for days to heading, six for culm length, four for panicle length, three for panicle number, four for spikelet number, seven for grain weight, three for grain yield and four for spikelet fertility. The additive effect, marker intervals, and interaction of additive and environment effects on the QTLs were summarized in Table 3. Among six E-QTLs detected for days to heading, *qEDTH8* was a QTL with strong QTL x environment interaction (QEI) for days to heading, while the others did not show significant QEI. For this QTL the allele from parent *A* (Dasanbyeo) had significantly higher days to heading in Suwon 2010, Shanghai 2010, Suwon 2011, and Shanghai 2011 than the allele from parent *B* (TR22183) in IRRIWS 2010, IRRIDS 2011, and IRRIWS 2011 (Table 3). This result indicated that *qEDTH8* had the large environmental effects on heading date explained of 16.2% phenotypic variation and the effects of remaining QTL for DTH had the same sign in each environment.

In general, contributions of Dasan alleles on DTH showed significantly higher in Suwon 2010, Shanghai 2010, and Suwon 2011 for all E-QTLs for days to heading, while alleles from TR22183 had significantly higher days to heading in IRRIWS 2010 and IRRIDS 2011 for all DTH related QTLs, except *qEDTH12*. In Shanghai 2011, the positive additive effect in increasing the days to heading for *qEDTH3.2* and *qEDTH10* was derived from TR22183 allele, while that for other QTLs was derived from

Dasanbyeo allele (Table 3). In environment IRRIWS 2011, the positive effect observed in *qEDTH3.1*, *qEDTH3.2*, *qEDTH8*, and *qDTH10* in increasing days to heading come from TR22183 while those in *qEDTH1* and *qEDTH12* come from Dasanbyeo allele. The results revealed that gene(s) controlling days to heading in rice had a significant QTL x environment interaction (QEI).

Of the total six detected E-QTLs for CL, the *qECL8* on chromosome 8 was a QTL with showed QTL with strong QTL x environment interaction mainly in Shanghai 2010, Shanghai 2011, and IRRIDS 2011 explaining a total of 12.2% the phenotypic variation. The positive effect in increasing CL by the *qECL8* was derived from Dasanbyeo in environment E2 (Shanghai 2010) and E5 (Shanghai 2011). The allele effect of Dasanbyeo on *qECL1.1* found in chromosome 1 was identified in E1 (Suwon 2010), E2 (Shanghai 2010), E4 (Suwon 2011), and E5 (Shanghai 2011), whereas the effect of TR22183 on this QTL was observed in E3 (IRRIWS2010), E6 (IRRIDS 2011), and E7 (IRRIWS 2011). This QTL explained only 0.9% of phenotypic variation (Table 3). The positive allele in increasing culm length in *qECL1.2* was derived from Dasanbyeo across environments which explained 5.6% of the phenotypic variation. For the remaining two QTLs for CL, *qECL2* and *qECL5* had low PVE values compared to other QTLs for CL. The positive allele in increasing CL in the *qECL2* at IRRIWS 2010 and IRRIDS 2011 was derived from Dasanbyeo allele, while that at other environments was derived from TR22183 allele. In the *qECL5*, Dasanbyeo allele contributed in increasing CL at the environments of Suwon 2010, Shanghai 2010, and IRRIDS 2011, while in the remaining four environments, the positive effect was derived from TR22183

allele (Table3).

Present study failed to find QTLs with strong QTL x environment interaction (QEI) for yield (GY) and yield related traits, such as panicle length (PL), panicle number (PN), and spikelet number per panicle (SN). Among the tested yield related traits, significant QEI were detected only in traits 100-grain weight (GW) and spikelet fertility (SF) with a low PVE value (Table 3). In the GW, *qEGW3* on chromosome 3 was a QTL showed a significant QTL x environment interaction mainly in IRRIDS 2011 explaining a total of 9.2% the phenotypic variation. A weak significant QEI was also observed in QTL for spikelet fertility which identified on chromosomes 5 and 8 in IRRIWS 2011 (PVE, 2.3%) and Shanghai 2010 (PVE, 0.2%), respectively (Table 3).

Among four QEI responsible for PL detected in present study, Dasanbyeo allele in the *qEPL5* and *qEPL11* had the positive effect in increasing the length of panicle across seven environments explaining 3.7 and 3.3% phenotypic variation, respectively. For the *qEPL2*, positive effect in increasing PL in Suwon 2010, Shanghai 2010, and Suwon 2011 was derived from Dasanbyeo allele, while that in IRRIWS 2010, Shanghai 2011, IRRIDS 2011, and IRRIWS 2011 was derived from TR22183. PVE value observed in this QTL was 3.1%. The *qEPL4* had explained 2.3% phenotypic variation and the positive effect in increasing PL in IRRIWS 2010, Shanghai 2011, and IRRIWS 2011 was derived from Dasanbyeo allele, whereas that in Suwon 2010, Shanghai 2010, Suwon 2011, and IRRIWS 2011 was derived from TR22183 allele.

The QEI for panicle length, the allele from parent *A* (Dasanbyeo) in the *qEPN4* located on chromosome 4 and *qEPN5* on chromosome 5 had a positive effect in seven

environments, explaining a 3.3% and 2.6% of the phenotypic variation, respectively. Phenotypic variance explained (PVE) value for the *qEPN10* was the lowest one (0.9%) among three QTL x Environment observed in present study and the positive effect in increasing the number of panicle in this QTL was derived from Dasanbyeo allele in Suwon 2010, IRRIWS 2010, Suwon 2011, and Shanghai 2011. While in the remained environments, Shanghai 2010, IRRIDS 2011, and IRRIWS 2011, the allele from TR22183 increased the number of panicle.

The QTLs x environment interaction in seven environments for the number of spikelet per panicle were identified in four chromosomal regions, namely *qESN2*, *qESN4*, *qESN5*, and *qESN10* which detected on chromosomes 2, 4, 5, and 10, respectively. The positive effect in increasing SN in the *qESN2* detected in Suwon 2010, Shanghai 2010, and IRRIWS 2010 was derived from Dasanbyeo allele, while that in other environments was from TR22183. For the *qESN4*, Dasanbyeo allele increased the SN at IRRIWS 2010, IRRIDS 2011, and IRRIWS 2011, while TR22183 allele increased the SN at Suwon 2010, Shanghai 2010, Suwon 2011 and Shanghai 2011. The positive effect in increasing SN in the *qESN5* detected in IRRIWS 2010, Shanghai 2011, IRRIDS 2011 and IRRIWS 2011 was derived from Dasanbyeo allele, while that in another three environments was from TR22183. For the *qESN10*, Dasanbyeo allele increased the SN at Suwon 2010, Shanghai 2010, Suwon 2011 and Shanghai 2011, while TR22183 allele increased the SN at IRRIWS 2010, IRRIDS 2011, and IRRIWS 2011.

The QTLs x environment interaction in seven environments for the 100-grain weight (GW) were identified in seven chromosomal regions, namely *qEGW1*, *qEGW2*,

qEGW3.1, *qEGW3.2*, *qEGW7*, *qEGW8* and *qEGW10*. Of these, except for *qEGW1* and *qEGW3.1* the positive effect in increasing grain weight was derived from Dasanbyeo allele throughout seven environments. Among these QTLs, the highest value of phenotypic variation was explained by the *qEGW3.1* (9.2%). The positive effect of TR22183 in increasing grain weight in this locus was only observed in IRRIDS 2011, while in the remaining environments was from Dasanbyeo allele. On the other hand, in the *qEGW1*, TR22183 allele had a positive effect on grain weight at IRRIWS 2010 and in the remaining environments was contributed by Dasanbyeo allele (Table 3).

The QTLs x environment interaction in seven environments for the grain yield were identified in four chromosomal regions, namely *qEGY3*, *qEGY8*, and *qEGY10* which detected on chromosomes 3, 8, and 10, respectively. Of these, *qEGY10* provided a high PVE value of 8.8% in which Dasanbyeo allele had a positive effect in increasing grain yield at Suwon 2010, Shanghai 2010, IRRIWS 2010, and Suwon 2011. While TR22183 allele had a positive effect on grain yield at the rest of environments (Table 3).

The QTLs x environment interaction in seven environments for the spikelet fertility were identified in three chromosomal regions, namely *qESF1*, *qESF5*, and *qESF8* which detected on chromosomes 1, 5, and 8, respectively. The *qESF1* provided a high PVE value of 3.3% in which Dasanbyeo allele had a positive effect in increasing spikelet fertility at all environments except in Suwon 2010. While in the *qESF5*, the positive effect in increasing spikelet fertility observed in all environments, except in IRRIDS 2011 was derived from Dasanbyeo allele. In the *qESF8*, Dasanbyeo allele had a positive effect on spikelet fertility was observed in Shanghai 2010, Suwon 2011, and

Shanghai 2011, whereas in the four remaining environments, increasing in spikelet fertility was derived from TR22183 allele (Table 3).

Discussion

A wide range of molecular markers availability such as single-nucleotide polymorphisms (SNPs) and powerful statistical methods has significantly facilitated QTL mapping. The use of SNPs on QTL mapping is preferred because it is highly accurate, quick and automated using limited human intervention. Therefore, by increasing the marker density on constructing the linkage map would prove a powerful in locating the yield-related QTLs (Wu et al 2014).

A large number of loci or QTLs affecting importance agronomic traits under high temperature across several environments in rice have been identified. However, most previous studies used common molecular markers, such as SSR, STS, RFLP, etc. on identifying the chromosomal regions associated with target traits. The use of SNPs markers in locating a QTL may provide a precise location of gene (s) controlling importance traits in a chromosomal region. In present study, a total of 44 main-effect QTLs and 35 QTLs x Environment interaction were detected using single environment and multi-environments analyses, respectively, for eight traits in the RIL F_8 population cultivated at seven different environments. Of these, fourteen putative QTLs for DTH, CL, PN, SN, GW and GY found in single environment analysis had the similar position to the QTL x environment interaction for those traits (Table 3 and Table 4) suggesting that these same QTLs from both single-and multi-environments are major and stable QTLs for certain traits. In details, those putative QTLs identified from single environment were consisted of three QTLs for DTH including *qDTH3.1*, *qDTH3.2*, and

qDTH8.2 showing a similar position with *qEDTH3.1*, *qEDTH3.2*, and *qEDTH8*, respectively analyzed from multi-environments, three putative QTLs for CL including *qCL1*, *qCL2*, and *qCL8.3* showed similar position with *qECL1.2*, *qECL2*, and *qECL8*, respectively, one QTL for PN, *qPN4.1* was same position with *qEPN4*, one QTL for SN, *qSN2.1* was same position with *qESN2*.

In addition, three putative QTLs controlling GW including *qGW3.1*, *qGW3.2*, and *qGW10.1* detected from single-environment analysis had a same position to the *qEGW3.1*, *qEGW3.2*, and *qEGW10*, respectively identified from multi-environment analysis. For GY trait, two putative QTLs including *qGY3* and *qGY10* were found to have a similar position to the *qEGY3* and *qEGY10*, respectively, while for SF trait, one QTL on chromosome 8, *qSF8* identified from single environment analysis had a same position with *qESF8* detected from multi-environment analysis. Present study showed that the phenotypic variation value explained by QTLs which observed from single environment analysis was commonly higher than those obtained by multi-environment analysis (Table 3 and Table 4). This results suggested that the QTLs with low effect may not be detected by single analysis, but it may be detected by multi-environment analysis. Conversely, QTLs detected by the single analysis but not by the environment analysis should be linked to a gene whose expression varies in different environments.

Several QTLs detected in our study were also identified in previous studies for the same or other traits. The QTLs found in this study compared with already reported QTLs are shown in Table 4 and Table 5 for those analyzed using single-and multi-environments, respectively. To the best of our knowledge, no overlapping QTL

reported from previous studies with the 12 out of 44 QTLs obtained from single environment analysis, suggesting that the 12 QTLs were novel (Table 4). These QTLs consisted of four QTLs for CL (*qCL2*, *qCL8.1*, *qCL8.2*, and *qCL8.3*), six QTLs for GW (*qGW3.1*, *qGW3.2*, *qGW7*, *qGW8*, *qGW10.1*, and *qGW10.2*), one QTL for GY (*qGY3*) and one for SF (*qSF4*). Furthermore, 12 out of 35 QTLs obtained from multi-environment analysis were also novel since no any QTL regions reported in previous studies overlapped with our study (Table 5). These QTLs x Environment interaction comprising of two QTLs for CL, *qECL2* (same with *qCL2*) and *qECL8* (same as *qCL8.3*), one for PL (*qEPL11*), one for PN (*qEPN5*), one for SN (*qESN5*), five QTLs for GW, including *qEGW2*, *qEGW3.1* (same as *qGW3.1*), *qEGW3.2* (same as *qGW3.2*), *qEGW7*, and *qEGW10*, and two QTLs for GY, including *qEGY3* (same as *qGY3*), and *qEGY10*.

The remaining 32 QTLs detected in single environment and 23 QTLs x environment interaction obtained from the multi-environments analysis correspond to those reported by previous studies. Comparison of QTL obtained from single environment for the days to heading trait with QTL for DTH in previous study showed that the *qDTH1* locus located at 711,264-4,004,917 bp of the Nipponbare Pseudomolecule annotated by IRGSP Build5, in chromosome 1 is overlapped with QTL related to days to heading, QHd1a (Li et al. 2003) and dth1.1 (Thomson et al. 2003). The *qDTH3.1* at the location of 1,086,244-2,572,805 of chromosome 3 is overlapped with QHd3a (Li et al. 2003) and QHd3 (Mei et al. 2003). The *qDTH3.2* locus located at 32,363,157-32,934,112 bp on chromosome 3 is overlapped with QTL for DTH (no

published QTL symbol) reported by Li et al. (2003). The *qDTH3.3* locus located at 33,021,181-34,128,235 bp on chromosome 3 is overlapped with qHD-3-2 (Takeuchi et al. 2001). While the QTLs on chromosome 8, including *qDTH8.1* located at 6,227,953-7,650,506 bp is overlapped with qHD-8 (Li et al. 1999), dth8 (Zhao et al. 1996), and QHd8a (Price et al. 1996), the *qDTH8.2* located at 5,846,154-6,227,953 bp is overlapped with qHD-8 (Li et al. 1999), qDTH-8 (Yamamoto et al. 2001), dth8 (Xiao et al. 1996), and dth8.1 (Xiao et al. 1998), and the *qDTH8.3* locus at 4045340-5846154 bp is overlapped with qHD-8 (Li et al. 1999), qDTH-8 (Yamamoto et al. 2001), dth8 (Xiao et al. 1996), dth8.1 (Xiao et al. 1998), and the QTL for DTH (no published symbol) reported by Wang et al. (2002) (Table 4). For the QTLs x environment interaction related to days to heading, we found that the *qEDTH1* locus located at 42,675,857-44,337,823 bp on chromosome 1 is overlapped with the QTL for DTH (no published symbol) reported by Mei et al. (2003). The *qEDTH10* locus at 3,962,918-12,370,697 bp chromosome 10 is overlapped with QTL for DTH reported by Doi et al. (1998). In addition, the *qEDTH12* locus located at 14,638,463- 15,926,299 bp on chromosome 12 is overlapped with qHD-12 (He et al. 2001) (Table 5).

Comparison of the QTL regions related to the culm length in previous studies showed that the *qCL1* (same as *qECL1.2*) located at 38,241,825-39,984,340 bp on chromosome 1 is overlapped with qCL-1-2 (Yamamoto et al. 2001) and qCL-1 (Takeuchi et al. 2001). The *qECL1.1* locus located at 29,381,516-30,349,382 bp on chromosome 1 is overlapped with qCL-1-3 and qCL-1-2 (Yamamoto et al. 2001). The *qECL5* locus located at 17,936,082-19,968,541 bp on chromosome 5 is overlapped with

cl5a for culm length (Mu et al. 2004) (Table 4 and Table 5).

QTL regions related to the panicle length detected in present study was also overlapped with some QTL region for PL in previous studies. In single environment analysis, the *qPL1.1* locus for panicle length which located at 258,237-3,711,264 bp on chromosome 1 is overlapped with QPl1b (Mei et al. 2003), and the *qPL1.2* locus at 38,241,825-39,984,340 on chromosome 1 is overlapped with QTLs for PL, pl1.1 (Thomson et al. 2003), qPL-1 (Hittalmani et al. 2002), pl1.1 (Septiningsih et al. 2003), and QTL for PL (no symbol) reported by Hittalmani et al. (2002). The *qPL3* locus at 31,085,342-32,363,157 bp on chromosome 3 is overlapped with qPL-3-2 (Yamamoto et al. 2001). While QTL for PL identified on chromosome 4, the *qPL4.1* locus at 19,624,577-20,617,915 bp is overlapped with qPL-4 (Yamamoto et al. 2001) and pl4 (Zhuang et al. 1997), and the *qPL4.2* locus at 21,364,314-22,964,670 bp is overlapped with qPL-4 (Yamamoto et al. 2001) (Table 4). The QTLs x environment interaction for PL obtained from multi-environment analysis showed that the *qEPL2* locus located at 5,837,031-6,286,548 bp on chromosome 2 is overlapped with the QTL for PL (no symbol) reported by Mei et al. (2003). Another two overlapping QTL regions for PL, the *qEPL4* locus located at 14,187,948-15,944,694 bp is overlapped with pl4 (Zhuang et al. 1997), and the *qEPL5* locus at 7,257,028- 8,010,690 bp on chromosome 5 is overlapped with qPL5-1 (Cui et al. 2002) and pl5 (Xing et al. 2001) (Table 5).

QTL regions for PN detected in present study showed overlapping with the QTL region for PN in previous studies. In single environment analysis, the *qPN1*, located in 37,298,025-38,241,825 bp on chromosome 1 is overlapped with qpn1.3 (Lanceras et

al. 2004). The *qPN4.1* (same as *qEPN4*) locus at 25,714,022-27,854,801 bp with ppp4 (Xiao et al. 1996), qPN-4 (Cho et al. 2003), qpn4.2 and tp4 (Lanceras et al. 2004). The *qPN4.2* locus, at 30,669,315-31,195,061 bp on chromosome 4 is overlapped ppp4 (Xiao et al. 1996), qNOP-4 (Hittalmani et al. 2002), qpn4.8 and qpn4.2 (Lanceras et al. 2004), and np4 (Zhuang et al. 1997). The *qPN8* locus located at 15,702,199-19,699,897 bp on chromosome 8 is overlapped with QTL for PN (no symbol) reported by xiao et al. (1996) and Liao et al. (2001) (Table 4). While in multi-environments analysis, one QTL x environment interaction for PN, the *qEPN10* locus located at 18,072,931-19,875,588 bp is overlapped with QTL for PN (no symbol) studied by Xiao et al. (1996) and tp10 (Yu et al. 1997) (Table 5).

Most of QTL regions for SN detected in present study showed overlapping with the QTL region for SN in previous studies. In single environment analysis, the QTL for SN in chromosome 2 showed overlapping with those in previous studies. The *qSN2.1* (*qESN2*) located at 4,361,466-5,837,031 bp is overlapped with QTL for SN (no symbol) reported by Mei et al. (2003). The *qSN2.2* locus located at 20,564,464-21,953,870 bp is overlapped with tns2 (Zhuang et al. 1997) and QTL for SN (no symbol) reported by Obara et al. (2001) and Yamaya et al. (2002). The *qSN2.3* locus, located at 10,301,049-11,445,702 bp and the *qSN2.4* locus, located at 9,582,164-10,301,049 bp is also overlapped with tns2 (Zhuang et al. 1997). In chromosome 4, the *qSN4.1* locus which located at 21,364,314-22,964,670 bp is overlapped with qSPN-4b (Teng et al. 2002), while *qSN4.2* locus at 30,669,315-31,195,061 bp is overlapped with QTL for SN (no symbol) reported by Brondani et al. (2002) and qtsn4.2 (Lanceras et al. 2004). The

qSN5 locus, located at 7,257,028-8,010,690 bp on chromosome 5 is overlapped with qtsn5.1 and snp5.2 (Marri et al. 2005), and with QTL for SN (no symbol) reported by Xiao et al. (1996). The *qSN9* locus, at 21,666,419-22,296,947 bp on chromosome 9 is overlapped with sppl9 (Xiao et al. 1996) (Table 4). The QTL x environment interaction for SN obtained from multi-environment analysis showed that the *qESN4* locus located at 27,854,801-28,961,502 bp on chromosome 4 is overlapped with qNOS-4-2 (Hittalmani et al. 2003), while the *qESN10* locus at region of 16,570,436-17,407,900 bp on chromosome 10 is overlapped with QTL for SN (no published symbol) reported by Xiao et al. (1996).

Among detected QTLs for GW found in present study, only one QTL was overlapped with that in previous studies. The *qGW1* (same as *qEGW1*) locus located at 42,675,857-44,337,823 bp on chromosome 1 is overlapped with gw1.1 (Thomson et al. 2003) (Table 4 and Table 5). For GY, two identified QTLs were co-located with those identified in previous studies. The *qGY9* locus found from single environment analysis which located at 15,517,207-16,979,246 bp on chromosome 9 is overlapped with QTL for GY (no symbol) reported by Cai and Morishima (2009) and the *qEGY8* locus identified from multi-environment analysis which located at 4,045,340- 5,846,154 bp is overlapped with yld8.1 (Suh et al. 2005) (Table 4 and Table 5).

For the spikelet fertility trait, the *qSF1* locus, located at 38,241,825-39,984,340 bp on chromosome 1 is overlapped with QTL responsible for SF (no published symbol) reported by Mei et al. (2003). The *qSF2* locus located at 8,004,029-9,582,164 bp on chromosome 2 is overlapped with sf2 (Zhuang et al. 1997) and QSf2 (Mei et al. 2003).

The *qSF8* (*qESF8*) locus located at 8,424,668-9,201,609 bp on chromosome 8 is overlapped with QTL for SF (no symbol) reported by Mei et al. (2003) (Table 4). The *qESF1* locus located at 33,063,495-34,654,331 bp on chromosome 1 is overlapped with sf1.1 (Marri et al. 2005) and sf1 (Lin et al. 1996; Zhuang et al. 1997). The *qESF5* locus located at 16,163,117-17,936,082 on chromosome 5 is overlapped with the SF QTL region (no symbol) reported by Mei et al. (2005) (Table 5).

Based on the results, in overall we can conclude that present study enabled to detect some new QTLs related to important agronomic traits under high temperature, several stable QTLs across different environments were also found, and QTLs corresponding to previously reported QTLs suggesting a functional conservation of QTLs across other rice cultivar in different environmental conditions. Despite the estimated QTL additive effects of the same trait measured in different environments were not comparable, the phenotypic variation value explained by QTLs which observed from single environment analysis was commonly higher than those obtained by multi-environment analysis suggesting that the QTLs with low effect may not be detected by single analysis, but it may be detected by multi-environment analysis. On the other hands, QTLs detected by the single analysis but not by the environment analysis must be related to a gene whose expression varies in different environments. The information of QTLs found in present study can be taken into consideration that the QTL region for more environment-specific indicated by precisely SNP markers would be useful in applying marker-aided selection

Table 4. Comparison of QTLs for agronomic traits detected under high temperature condition with previous studies in rice

Traits	Chr.	QTL	Interval	Physical distance IRGSV4 position (bp)	Previous studies that identified common regions
DTH	1	*qDTH1*	id1003056-id1003344	3711264-4004917	QHd1a (Li et al. 2003); dth1.1 (Thomson et al. 2003)
	3	*qDTH3.1*	id3000695-id3001422	1086244-2572805	QHd3a (Li et al. 2003); QHd3 (Mei et al. 2003)
	3	*qDTH3.2*	fd10-id3015453	32363157-32934112	# (Li et al. 2003)
	3	*qDTH3.3*	id3015533-id3016090	33021181-34128235	qHD-3-2 (Takeuchi et al. 2001)
	8	*qDTH8.1*	id8002187-ud8000441	6227953-7650506	qHD-8 (Li et al. 1999); dth8 (Zhao et al. 1996); QHd8a (Price et al. 1996)
	8	*qDTH8.2*	id8002025-id8002187	5846154-6227953	qHD-8 (Li et al. 1999); qDTH-8 (Yamamoto et al. 2001); dth8 (Xiao et al. 1996); dt h8.1 (Xiao et al. 1998)
	8	*qDTH8.3*	id8001299-id8002025	4045340-5846154	qHD-8 (Li et al. 1999); qDTH-8 (Yamamoto et al. 2001); dth8 (Xiao et al. 1996); dt h8.1 (Xiao et al. 1998); # (Wang et al. 2002)
	10	*qDTH10*	id10000185-ud10000265	907516-1008240	# (Doi et al. 1998)
CL	1	*qCL1*	id1023174-id1025455	38241825-39984340	qCL-1-2 (Yamamoto et al. 2001); qCL-1 (Takeuchi et al. 2001)
	2	*qCL2*	id2000007-id2001468	9619- 2410918	New
	8	*qCL8.1*	id8002025-id8002187	5846154-6227953	New
	8	*qCL8.2*	id8007093-id8007595	26059709- 27732718	New
	8	*qCL8.3*	id8001299-id8002025	4045340-5846154	New
PL	1	*qPL1.1*	id1001764-id1003056	2258237-3711264	QPl1b (Mei et al. 2003)
	1	*qPL1.2*	id1023174-id1025455	38241825-39984340	pl1.1 (Thomson et al. 2003); qPL-1 (Hittalmani et al. 2002); pl1.1 (Septiningsih et al. 2003); # (Hittalmani et al. 2002)
	3	*qPL3*	id3014361-fd10	31085342-32363157	qPL-3-2 (Yamamoto et al. 2001)
	4	*qPL4.1*	id4005704-ud4001552	19624577- 20617915	qPL-4 (Yamamoto et al. 2001); pl4 (Zhuang et al. 1997)
	4	*qPL4.2*	id4006867-id4007698	21364314- 22964670	qPL-4 (Yamamoto et al. 2001)
PN	1	*qPN1*	id1022407-id1023174	37298025- 38241825	qpn1.3 (Lanceras et al. 2004)
	4	*qPN4.1*	id4008442-id4008981	25714022- 27854801	ppp4 (Xiao et al. 1996); qPN-4 (Cho et al. 2003); qpn4.2 (Lanceras et al. 2004); tp4 (Lanceras et al. 2004)
	4	*qPN4.2*	id4010200-id4011666	30669315- 31195061	ppp4 (Xiao et al. 1996); qNOP-4 (Hittalmani et al. 2002); qpn4.8 (Lanceras et al. 2004); np4 (Zhuang et al. 1997); qpn4.2 (Lanceras et al. 2004)

	8	*qPN8*	id8004221-id8005235	15702199-19699897	# (xiao et al. 1996; Liao et al. 2001)
SN	2	*qSN2.1*	id2002293-id2003067	4361466-5837031	# (Mei et al. 2003)
	2	*qSN2.2*	id2007679-ud2001198	20564464-21953870	tns2 (Zhuang et al. 1997); # (Obara et al. 2001; Yamaya et al. 2002)
	2	*qSN2.3*	wd2000539-id2005345	10301049- 11445702	tns2 (Zhuang et al. 1997)
	2	*qSN2.4*	id2004617-wd2000539	9582164-10301049	tns2 (Zhuang et al. 1997)
	4	*qSN4.1*	id4006867-id4007698	21364314-22964670	qSPN-4b (Teng et al. 2002)
	4	*qSN4.2*	id4010200-id4011666	30669315- 31195061	# (Brondani et al. 2002); qtsn4.2 (Lanceras et al. 2004)
	5	*qSN5*	id5003662-id5004086	7257028-8010690	qtsn5.1 and snp5.2 (Marri et al. 2005); # (Xiao et al. 1996)
	9	*qSN9*	id9007180-id9007328	21666419- 22296947	sppl9 (Xiao et al. 1996)
GW	1	*qGW1*	id1026052-dd1002041	42675857- 44337823	gw1.1 (Thomson et al. 2003)
	3	*qGW3.1*	id3000695-id3001422	1086244-2572805	NEW
	3	*qGW3.2*	id3014361-fd10	31085342-32363157	NEW
	7	*qGW7*	id7004065-id7004210	23807673- 24174224	NEW
	8	*qGW8*	id8007595-id8007764	27732718- 27921569	NEW
	10	*qGW10.1*	id10004500-ud10000989	16570436- 17407900	NEW
	10	*qGW10.2*	ud10000989-id10005049	17407900-18072931	NEW
GY	3	*qGY3*	id3015533-id3016090	33021181- 34128235	NEW
	9	*qGY9*	id9004148-id9004978	15517207- 16979246	# (Cai and Morishima 2009)
	10	*qGY10*	id10003891-id10004500	15239556- 16570436	NEW
SF	1	*qSF1*	id1023174-id1025455	38241825- 39984340	# (Mei et al. 2003)
	2	*qSF2*	wd2000377-id2004617	8004029- 9582164	sf2 (Zhuang et al. 1997); QSf2 (Mei et al. 2003)
	4	*qSF4*	id4002032-id4002348	4741077- 5518866	NEW
	8	*qSF8*	wd8001250-id8002968	8424668- 9201609	# (Mei et al. 2003)

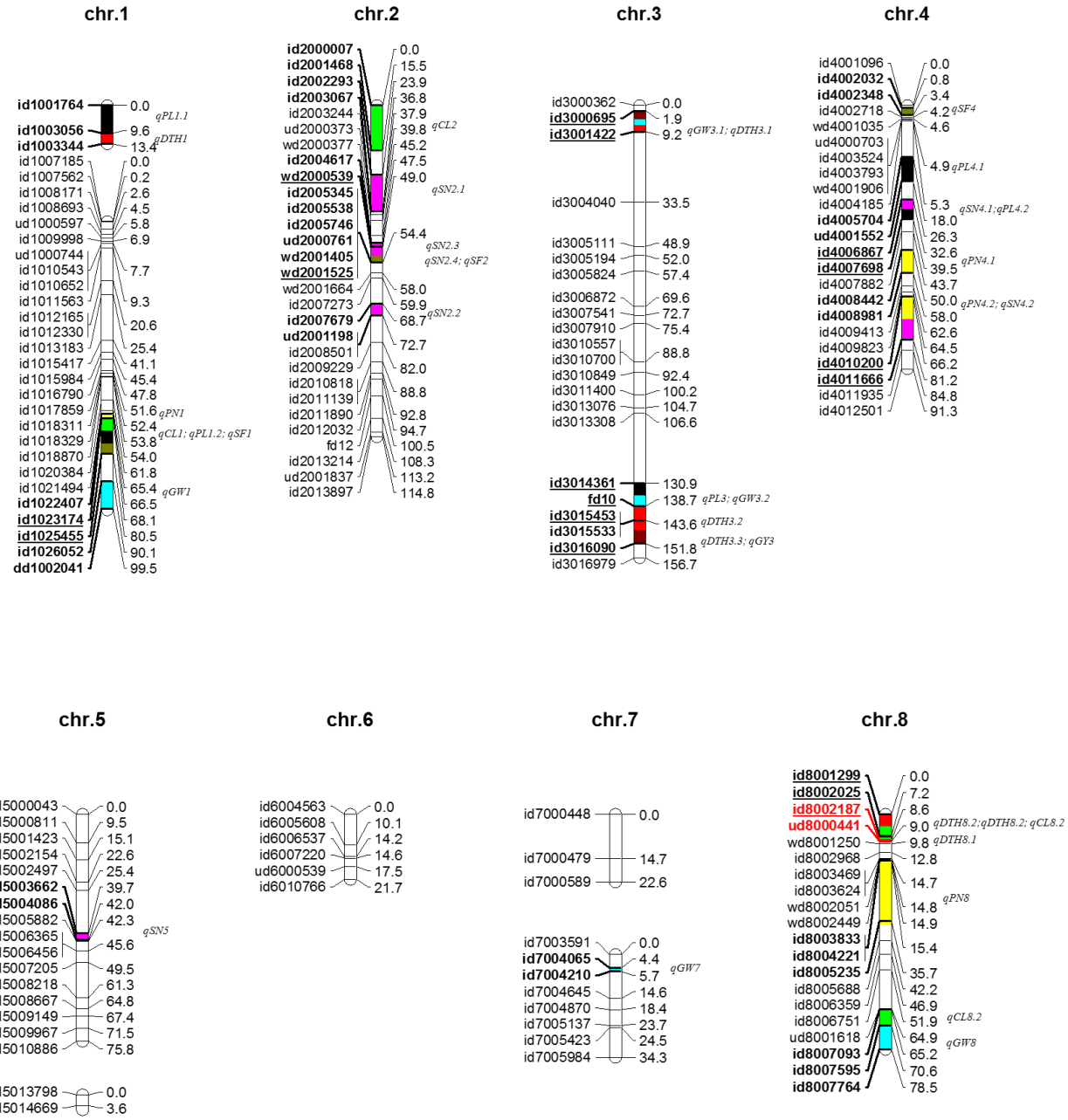
E1=Suwon10, E2=Shanghai10, E3=IRRIWS10, E4=Suwon11, E5=Shanghai11, E6=IRRIDS11, E7=IRRIWS

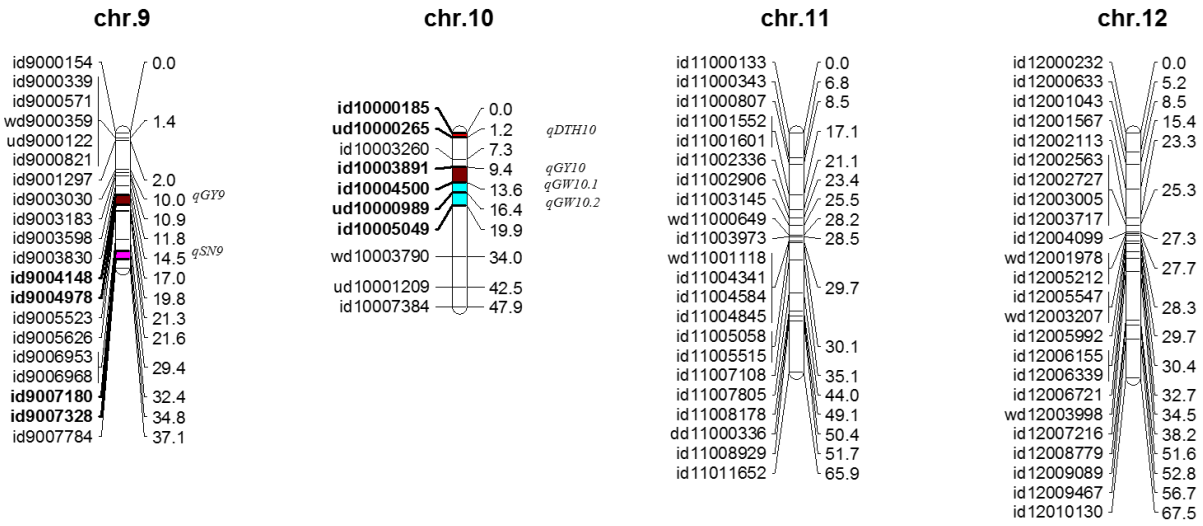
Table 5. Comparison of E-QTLs with previous studies

	Chrom	QTL	Marker interval	Physical distance IRGSV4 position (bp)	Previous studies that identified common regions
DTH	1	*qEDTH1*	id1026052-dd1002041	42675857-44337823	# (Mei et al. 2003)
	3	*qEDTH3.1*	id3000695-id3001422	1086244- 2572805	QHd3a (li et al. 2003); QHd3 (Mei et al. 2003)
	3	*qEDTH3.2*	fd10-id3015453	32363157-32934112	# (Li et al. 2003); qHD-3-2 (Takeuchi et al. 2001)
	8	*qEDTH8*	id8002025-id8002187	5846154- 6227953	qHD-8 (Li et al. 1999); qDTH-8 (Yamamoto et al. 2001); dth8 (Xiao et al. 1996); dth8.1 (Xiao et al. 1998)
	10	*qEDTH10*	ud10000265-id10003260	3962918- 12370697	# (Doi et al. 1998)
	12	*qEDTH12*	id12005212-id12005547	14638463-15926299	qHD-12 (He et al. 2001)
CL	1	*qECL1.1*	id1015984-id1016790	29381516-30349382	qCL-1-3 and qCL-1-2 (Yamamoto et al. 2001)
	1	*qECL1.2*	id1023174-id1025455	38241825-39984340	qCL-1-2 (Yamamoto et al. 2001); qCL-1 (Takeuchi et al. 2001)
	2	*qECL2*	id2000007-id2001468	9619- 1475052	NEW
	5	*qECL5*	id5007205-id5008218	17936082-19968541	cl5a (Mu et al. 2004)
	8	*qECL8*	id8001299-id8002025	4045340- 5846154	NEW
PL	2	*qEPL2*	id2003067-id2003244	5837031- 6286548	# (Mei et al. 2003)
	4	*qEPL4*	id4004185-id4005704	14187948-15944694	pl4 (Zhuang et al. 1997)
	5	*qEPL5*	id5003662-id5004086	7257028- 8010690	qPL5-1 (Cui et al. 2002); pl5 (Xing et al. 2001)
	11	*qEPL11*	id11001601-id11002336	4062854- 5572513	NEW
PN	4	*qEPN4*	id4008442-id4008981	25714022-26441221	ppp4 (Xiao et al. 1996); qPN-4 (Cho et al. 2003); qpn4.2 and tp4 (Lanceras et al. 2004)
	5	*qEPN5*	id5005882-id5006365	14336683-15976102	NEW
	10	*qEPN10*	id10005049-wd10003790	18072931-19875588	# (Xiao et al. 1996); tp10 (Yu et al. 1997)
SN	2	*qESN2*	id2002293-id2003067	4361466- 5837031	# (Mei et al. 2003)
	4	*qESN4*	id4008981-id4009413	27854801-28961502	qNOS-4-2 (Hittalmani et al. 2003)
	5	*qESN5*	id5013798-id5014669	28147882-29179539	NEW
	10	*qESN10*	id10004500-ud10000989	16570436-17407900	# (Xiao et al. 1996)
GW	1	*qEGW1*	id1026052-dd1002041	42675857-44337823	gw1.1 (Thomson et al. 2003)
	2	*qEGW2*	ud2001198-id2008501	21953870-22034318	NEW
	3	*qEGW3.1*	id3000695-id3001422	1086244- 2572805	NEW

	3	*qEGW3.2*	id3014361-fd10	31085342-32363157	NEW
	7	*qEGW7*	id7003591-id7004065	22444742-23807673	NEW
	8	*qEGW8*	ud8001618-id8007093	24849632-26059709	NEW
	10	*qEGW10*	id10004500-ud10000989	16570436-17407900	NEW
GY	3	*qEGY3*	id3015533-id3016090	33021181-34128235	NEW
	8	*qEGY8*	id8001299-id8002025	4045340- 5 846154	yld8.1 (Suh et al. 2005)
	10	*qEGY10*	id10003891-id10004500	15239556-16570436	NEW
SF	1	*qESF1*	id1018870-id1020384	33063495-34654331	sf1.1 (Marri et al. 2005); sf1 (Lin et al. 1996; Zhuang et al. 1997)
	5	*qESF5*	id5006456-id5007205	16163117-17936082	# (Mei et al. 2005)
	8	*qESF8*	id8002025-id8002187	5846154- 6 227953	# (Mei et al. 2005)

Figure 1. Molecular linkage map on 12 chromosomes in RIL F8 population of Dasanbyeo/TR22183




chr.9
id9000154 0.0
id9000339
id9000571
wd9000359 1.4
ud9000122
id9000821
id9001297 2.0
id9003030 10.0 qGY9
id9003183 10.9
id9003598 11.8
id9003830 14.5 qSN9
id9004148 17.0
id9004978 19.8
id9005523 21.3
id9005626 21.6
id9006953
id9006968 29.4
id9007180 32.4
id9007328 34.8
id9007784 37.1
chr.10
id10000185 0.0
ud10000265 1.2 qDTH10
id10003260 7.3
id10003891 9.4 qGY10
id10004500 13.6 qGW10.1
ud10000989 16.4 qGW10.2
id10005049 19.9
wd10003790 34.0
ud10001209 42.5
id10007384 47.9
chr.11
id11000133 0.0
id11000343 6.8
id11000807 8.5
id11001552
id11001601 17.1
id11002336 21.1
id11002906 23.4
id11003145 25.5
wd11000649 28.2
id11003973 28.5
wd11001118
id11004341 29.7
id11004584
id11004845
id11005058
id11005515 30.1
id11007108 35.1
id11007805 44.0
id11008178 49.1
dd11000336 50.4
id11008929 51.7
id11011652 65.9
chr.12
id12000232 0.0
id12000633 5.2
id12001043 8.5
id12001567 15.4
id12002113 23.3
id12002563
id12002727 25.3
id12003005
id12003717
id12004099 27.3
wd12001978 27.7
id12005212
id12005547 28.3
wd12003207
id12005992 29.7
id12006155
id12006339 30.4
id12006721 32.7
wd12003998 34.5
id12007216 38.2
id12008779 51.6
id12009089 52.8
id12009467 56.7
id12010130 67.5

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Abstract in Korean

벼의 서로 다른 지역에서의 유전자형과 환경사이의 상호작용 및 QTL 분석

초록

고온 스트레스는 가장 중요한 환경적 스트레스이다. 온도는 벼의 생육기간을 감소시키고 임실에 영향을 주며 심백율 복백율에도 영향을 준다. 환경과 작물의 상호관계을 해석하는것은 육종의 중요한 과제중의 하나이다. 고온 저항성이 있는 품종을 개발하는것은 온대 지역에서 현재 기후변화에 대응할수 있는 좋은 방법이라고 할수 있다. 본 연구는 유전자-환경의 상호작용을 분석하기 위하여 품종과 RIL 을 서로 다른 위도에서 재배하였다. QTL 유전자지도는 대조지역인 수원의 데이터로 작성하였고 분석은 QTL x 환경의 방법으로 진행하였다. 실험에는 총 105 개의 품종과 다산/TR22183 RIL 을 사용하였다. 환경은 2010 년 수원, 2010 년 상해, 2010 년 IRRI 우기, 2011 년 수원, 2011 년 상해, 2011 년 IRRI 건기와 우기로 모두 7 개 환경으로 진행하였다. 농업형질은 간장, 수장, 수수, 영화수, 임실, 백립중, 수량, 출수기 등을 조하하였다. 실험에서 얻은 데이터는 AMMI 방법을 이용하여 CropStat.2.3 프로그램을 이용하여 분석을 진행하였다. 실험에서

유전자-환경 상호작용이 제일 많이 나타나는 지역은 2010 년 상해였고 그 다음은 IRRI 2011 년 건기였다. 농업형질 가운데서 수량과 임실은 유전자-환경의 영향을 각각 57%와 39% 로 제일 많이 받았다. 수원에서는 유전자-환경의 상호작용 영향을 다른 두 지역에 비하여 제일 적게 받았다.

본 연구의 결과는 육종가들한테 유용한 데이터를 제공할수 있고 참고로 할수 있다. 다 지역적 스크리닝 방법은 고온 저항성 품종을 개발하는데 적합한 방법이라고 볼수 있다. 또 37 의 QTL 이 탐색하였고 이중 여러 QTL 은 여러 지역에서 탐색되였다. 본 연구의 결과는 새로운 고온 저항성 품종을 육종하는데 좋은 방법을 제공할수 있다.

주요어: 벼, 환경, 유전자-환경 상호작용, AMMI, QTL
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