RESEARCH ARTICLE

Development of heat tolerant introgression lines and preliminary quantitative trait loci (QTL) analysis at flowering stage in *Oryza sativa* L.

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Revised: 25 January 2018; Accepted: 23 February 2018

Abstract: The efficacy of modern rice varieties has been challenged by global warming because of their incapability to tolerate heat stress. Breeding for heat tolerance (HT) is therefore essential. Molecular breeding studies on HT in hybrid rice is sparse although HT quantitative trait loci (QTL) have been discovered. China is the leading country for hybrid rice production, however, introgression of HT alleles to hybrids is not practiced in breeding programmes. Therefore, the objectives of this study were to introgress HT alleles from indica donors Bg 90-2 and Mengguandamagu to SH 527, a popular parent in hybrid variety development, and to identify the associated DNA markers with HT to facilitate hybrid rice molecular breeding in China. The BC₂F_{2.4} introgression lines were produced by crossing donor and recurrent parents and their performances were evaluated at two provinces of China in 2011 and 2012 under normal and heat stress conditions. The data were subjected to ANOVA and HT was compared against the standard heat-check variety N22. Out of 600 SSR markers, 61 and 59 markers were polymorphic for the two crosses SH 527/Bg 90-2 and SH 527/Mengguandamagu, respectively and introgression lines were subsequently genotyped. The single-marker analysis was performed to detect marker trait associations. A total of 46 HT introgression lines were obtained with 88.3 % of homozygosity for HT alleles and seven high yielding HT lines were specifically selected for the provinces. Under heat stress, 42 significant marker trait associations were detected. Two of these were previously reported in HT QTLs implying their applicability in rice breeding programmes.

Keywords: Bg 90-2, global warming, heat stress on rice, heat tolerant QTLs, hybrid rice, Shuhui 527.

INTRODUCTION

Global warming has become one of the major environmental threats that exerts serious influence on rice production (Maestri *et al.*, 2002). Over the past 100 years, the mean global temperature has been increased by 0.6 °C and projected to increase at a rapid rate (Root *et al.*, 2003) with another 0.5 – 2.8 °C upsurge by the end of the 21st century (Meehl *et al.*, 2005; Vuuren *et al.*, 2008). Among rice growth stages, flowering stage is the most sensitive phase to high temperature (Sato *et al.*, 1973; Satake & Yoshida, 1978). The prevalence of high temperature is often a constraint for stable rice production in many tropical and subtropical countries such as China, India, Pakistan, Bangladesh, Thailand and many African countries (Osada *et al.*, 1973; Matsushima *et al.*, 1982; Tian *et al.*, 2009). Satake and Yoshida (1978) have shown that spikelets at anthesis exposed to temperatures more than 35 °C for about five days become sterile and set no seeds. The sterility is caused by poor anther dehiscence and low pollen production; hence low numbers of germinating pollen grains are produced (Matsui *et al.*, 2001; Prasad *et al.*, 2006). However, there is a genetic variation in spikelet sterility at high temperatures (Satake & Yoshida, 1978; Matsui *et al.*, 2001; Prasad *et al.*, 2006) causing certain rice genotypes to express tolerance to the heat stress. The rice genotypes N22 (Yoshida *et al.*, 1981; Prasad *et al.*, 2006) and

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Akitakomachi (Matsui *et al.*, 2001) are the two most heat tolerant genotypes found to date in *Oryza sativa indica* and *O. sativa japonica*, respectively.

Breeding for heat tolerance is often hailed as the most viable solution to produce rice varieties that could be grown in the areas affected by heat stress (Jagadish *et al.*, 2010b). Recently, the production of hybrid varieties with improved heat tolerance over commonly produced recombinant inbred lines has been considered as an efficient strategy to improve the rice yields under heat stress conditions. The hybrid rice production in China began far back in 1964 and large-scale hybrid seed production for commercial use was initiated in 1976 (Yuan & Mao, 1991). Ever since new hybrid rice strains with improved traits such as high yield potential, biotic and abiotic stress tolerance and grains with enhanced quality have been developed in China (Cheng *et al.*, 2007). In recent years, high temperature has caused serious yield losses in several rice producing provinces in China, especially in the Yangtze-Huai River valley (Huang *et al.*, 2003; Yang & Chen, 2007). On-farm observations on high temperature effect on hybrid rice genotypes Shanyou 63, Jinyou 448 and Liangyoupeijiu revealed that average seed set is decreased by 8 % as a result of the reduction in pollen vigour, and number of pollen grains per stigma by 6 % in Shanyou 63 and Jinyou 448 and 8 % in Liangyoupeijiu (Tong *et al.*, 2008). Shuhui 527 (SH 527), an indica restorer line, has been widely used as the top elite restorer for many years even before the hybrid seed production began in the south region of China (Wang *et al.*, 2004). Moreover, SH 527, which was released by the Rice Institute in Sichuan Agricultural University, China, is an essential restorer parent in hybrid seed production because of its many improved traits such as higher grain quality, higher nutrient content and resistance to biotic and abiotic stresses (Cui *et al.*, 2008). Therefore, improving the heat tolerance in SH 527 is identified as one of the key breeding priorities to maintain a higher level of hybrid rice productivity in China.

Currently molecular marker assisted selection (MAS) has become one of the widely used influential techniques in uplifting the efficiency of plant breeding (Cheng *et al.*, 2007). In order to perform MAS, quantitative trait loci (QTL) linked to a particular trait of interest has to be discovered. The heat tolerance (HT) in rice especially at the flowering stage has been characterised as a quantitative trait, and the underlying QTLs and the flanking DNA markers have been discovered and available (Zhang *et al.*, 2009) for genetic and breeding applications. The QTL mapping studies for HT have been conducted on

various rice populations at booting (Zhao *et al.*, 2006a) and flowering (Cao *et al.*, 2003; Chen *et al.*, 2008; Zhang *et al.*, 2008; 2009; Jagadish *et al.*, 2010a; Xiao *et al.*, 2011) stages. Ye *et al.* (2012) have summarised a total of 38 published QTLs linked to HT at flowering stage. This list excluded four QTLs discovered by them using single nucleotide polymorphism (SNP) markers, which was the first attempt of using SNP markers to map the QTLs for HT. The QTLs linked to HT published by different research groups are comparable even when different mapping populations were used for the analyses. For example, the QTLs on rice chromosomes 1, 2, 3, 4, 8, 9, 10 and 11 were reported from at least two different studies (Ye *et al.*, 2012) and heat stress was provided in these studies with the temperature range of 38 – 39 °C.

However, none of these DNA markers have been validated and no genetic characterisation attempts have been made for SH 527 to be used in hybrid rice production. Thus, the objectives of the present study were to introgress HT alleles from indica donors Bg 90 – 2 and Mengguandamagu to SH 527 and to identify the significantly associated DNA markers with the heat tolerance in the introgression lines of SH 527 recurrent background to facilitate hybrid rice breeding programmes in China.

METHODOLOGY

Plant material

Parental genotypes

One recurrent parent, SH 527, the male parent of many elite *O. sativa indica* hybrid cultivars (commercially grown in South China) and two donor parents, Bg 90 – 2 (from Sri Lanka) and Mengguandamagu (from China) were used to develop introgression lines through backcross breeding.

Establishment of progenies

The initial crosses were made among the recurrent parent and two donor parents to produce F_1 populations in summer, 2004 at Hainan, China. Then, these F_1 populations were backcrossed with the recurrent parent in winter, 2004 to produce BC_1F_1 populations. A total of 25 randomly chosen plants from each BC_1F_1 population were backcrossed with the recurrent parent in summer, 2005 to produce bulked BC_2F_1 populations. The bulked BC_2F_1 lines were planted and allowed to self-produce BC_2F_2 seeds. Then, they were bulk-harvested as random BC_2F_2 seeds and stored in a cold storage facility at

the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China in winter, 2005. In summer 2008, BC₂F₂ populations were established at Hainan and screened for high temperature stress using 400 plants for each line and the tolerant individuals which showed more than 40 % spikelet fertility were selected. In the same season at Hainan, 47 % of spikelet fertility was observed only for the recurrent parent SH 527. Then seed multiplication was conducted in winter, 2008 at Hainan by growing two rows, each having 12 plants of BC₂F_{2.3} generation. The progeny test was conducted at the same location in summer, 2009 and HT of the lines was verified. At the same time, genotypic evaluation was conducted using 20 – 30 g of BC₂F_{2.4} seeds of each line at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences.

Screening for heat tolerance

The selected lines in each BC₂F_{2.4} population (mapping population) were evaluated for phenotypic traits of HT at two provinces, Hunan and Hangzhou in 2011 and 2012. The plants were grown in the Agricultural Research Station at Liuyang, Hunan Province, China (28° 09' N, 113° 37' E) in summer 2011. The seeds were sown in the nursery prepared in the field and 28 day old seedlings were transplanted in plastic sheds with two replicates. At the same time, seedlings were transplanted in an open field with three replicates having 10 – 12 plants in a row with 20 × 17 cm space with randomised complete block design. The recurrent parent, donor parent and N22, the standard heat-check variety, were planted repeatedly to obtain synchronised flowering with each set of introgression lines produced in the present study. The field was managed according to the standard practices in the area and plants were raised under flooded condition. The ventilation mechanisms in the plastic shed were kept open to minimise heat stress during the vegetative growth and ripening periods. Heat stress was imposed by closing the side walls and top-vent during the onset of flowering period manually and automatically. The maximum temperature inside the shed was maintained within the range of 37 – 40 °C by opening and closing top and side vents. The night temperature (23 – 28 °C) was not controlled during the flowering period. The daily maximum and minimum ambient temperatures and relative humidity at the canopy level were recorded with a digital electronic thermometer (Oregon, Scientific, IN-OUT Thermo-Hygrometer and Clock, Model: THG312). The heading date in days (HD) was recorded when 50 % of the plants in each line was at flowering. The plant height (PH) and number of panicles (NP) were measured in three middle plants of each line at maturity.

These three middle plants of each introgression line were harvested along with the plants of recurrent parent, donor parents and N22. Number of filled grains per panicle (NFG), number of spikelets per panicle (NSP), filled grain percentage (FG) [calculated as (NFG / NSP) × 100], thousand grain weight (TGW) and grain yield per plant (GY) were evaluated under normal (i.e. control) and heat stress conditions. The HT was evaluated using the heat stress index (HSI) as given in the following formula.

$$HSI = \frac{FG \text{ under normal condition} - FG \text{ under heat stress condition}}{FG \text{ under normal condition}}$$

Belding and Hatch (1955)

The experiment was continued with BC₂F_{2.6} populations for the year 2012 in the same field. All the introgression lines were classified into five groups (104 – 100, 99 – 96, 95 – 92, 91 – 88 and 87 – 82 days) based on the HD observed in 2011 and they were sown on five different days. Then, they were transplanted at two instances to ensure that all lines would head at the same time and exposed to the same heat stress condition at the flowering stage. All the other management practices and data collection methods were same as in the previous year.

Simultaneously introgression lines of two populations were evaluated for high temperature in a glasshouse where temperature (40 ± 2 °C) was controlled using an automated system in the National Rice Research Institute at Hangzhou, China in 2011 and 2012. The plants were grown in plastic pots (30 × 30 cm), each filled with 10 kg of natural clay loamy soil and four plants were grown in each pot. Each line was grown in four pots of which two were used for heat stress and two for control. Only the main stem and other four tillers were maintained and all the other tillers were removed to prevent overcrowding and to keep a unique heading time. Moreover, the water level was maintained above the soil surface and all the pots were maintained under normal conditions (normal temperature and natural light) in a net-house. Once the initiation of flowers was observed in reproductive stage, two pots were transferred into the glasshouse where temperature (40 ± 2 °C) and relative humidity (65 % ± 5) were controlled with the automated system from 9:00 a.m. to 3:00 p.m. and kept at 30 °C for the rest of the day. The night temperature was not controlled. The plants were moved back to the net-house after 15 ds of heat stress treatment and were grown to maturity, and HD was recorded at flowering and the yield parameters were recorded after harvesting.

Genotyping with rice microsatellite markers

DNA was extracted from bulked seedlings from BC₂F_{2.4} populations using CTAB method (Doyle, 1991). A total of 600 rice microsatellite markers (Gramene Markers Database, <http://archive.gramene.org/markers/microsat/>) were screened for the parental polymorphism using 6 % denaturing polyacrylamide gel electrophoresis.

Data analysis

The trait data for HD, PH, NP, NFG, NSP, FG, TGW, GY and HSI of two different provinces and years were separately subjected to ANOVA procedure and mean separation in the statistical package SAS v. 9.1. (SAS Institute, 2009). Scatter plots were drawn for better visualisation of the overall mean yield performance and HT of the best selected introgression lines with respect to the two most important traits GY and HSI (Appendix, Figure I). The GY of SH 527 and HSI of N22 were marked as lines of selection thresholds for each province and year. The introgression lines with a higher GY compared to SH 527 and a lower HSI compared to N22 were considered as the best introgression lines with required level of GY and HT. The marker traits associations were detected by using single-marker analysis with GLM procedure of SAS and the significance threshold of $p < 0.05$ was used to avoid the type II error (Zheng *et al.*, 2007). When a significant marker trait association was detected by two or more linked markers, the one with the highest F-value was presented (Xu *et al.*, 2005).

RESULTS AND DISCUSSION

Development of introgression lines with improved HT

Out of the 400 BC₂F₂ plants screened for heat tolerance, 21 lines (plants) from SH 527/Bg 90 – 2 population and 25 plants from SH 527/Mengguandamagu population were selected by subjecting the two populations to the selection intensities of 5.0 % and 6.3 %, respectively (Table 1). The selected plants were advanced till BC₂F₅ and then subjected to screening under normal and heat stress conditions at Hunan and Hangzhou Provinces in 2011. The same screening was repeated at two provinces for BC₂F₆ in 2012 and the best three lines of each population based on FG, GY and HSI were chosen at the selection intensity of 1.5 %. At BC₂F₅ many donor homozygous genotypes were observed among the selected introgression lines compared to the number of donor heterozygous genotypes in which 90.9 % and 85.7 % homozygotes were observed among BC₂F₅ of SH 527/Bg 90 – 2 and SH 527/Mengguandamagu, respectively (Table 1). In summary, at BC₂F₂ nearly 5 % of rice plants exhibited the introgression of heat tolerant alleles and interestingly 88.3 % of homozygotes were obtained at BC₂F₅. If there is a set of DNA markers available for background genome selection (Pongsri *et al.*, 2016), the recurrent genome could be accurately and quickly recovered to find ‘heat tolerant SH 527’ from the segregating populations to be used in rice farming and hybrid rice production.

Table 1: Results of screening BC₂F₂ populations derived from the crosses between recurrent parent SH 527 and two donors (Bg 90 – 2 and Mengguandamagu) for heat tolerance (HT) and the introgression pattern of the resultant HT introgression lines

Population (cross)	Size of BC ₂ F ₂ original population	Number of selected plants from original population with > 40 % spikelet fertility	Selection intensity (%) on original population	*Number of selected promising introgression lines of BC ₂ F ₅	Selection intensity (%) on BC ₂ F ₅	% HT introgression lines selected from BC ₂ F ₅		
						Donor homozygous	Donor heterozygous	Donor introgression
SH 527/Bg 90 – 2	400	21	5.0	6	1.5	10	1	11
SH 527/Mengguandamagu	400	25	6.3	6	1.5	12	2	14

* These introgression lines were collected from both Hunan and Hangzhou for > 40 % spikelet fertility at flowering. This was the number of selected backcross progenies with significantly improved HT confirmed by progeny testing of their derived BC₂F_{4.5} introgression lines under heat stress at flowering stage.

Table 2: Mean performance of the traits evaluated under normal and heat stress conditions of the introgression lines derived from BC₂F₅ of the two crosses

Treatment / treatment comparison	Trait ^x	2011				2012			
		SH 527/ Bg 90-2	SH 527/ Mungguandamagu	SH 527	N22	SH 527/ Bg 90-2	SH 527/ Mungguandamagu	SH 527	N22
Treatment: heat stress condition at Hunan (mean maximum temperature: 38.5 °C ± 4.5, relative humidity: 68 % ± 7.1 in 2011 and mean maximum temperature: 40.3 °C ± 3.5, relative humidity: 70.0 % ± 5.7 in 2012)	HD (days)	96.5a ^y	94.4a	95.4a	87.6b	85.3a	82.8a	84.0a	76.0b
	PH (cm)	119.0c	124.0b	116.4c	157.6a	114.2b	115.6b	107.3b	131.0a
	NP	6.1a	5.7a	5.8a	5.7a	12.4a	9.9a	10.4a	10.3a
	NFG	109.6b	104.6b	99.5c	117.8a	30.7b	32.9b	30.6b	37.5a
	NSP	140.5b	146.9a	133.3c	144.8a	120.9a	120.2a	120.3a	110.6b
	FG (%)	71.6c	71.8c	74.7b	77.4a	24.9b	28.7a	24.9b	33.6a
	TGW (g)	26.5a	29.0a	28.7a	18.6b	24.0a	26.2a	25.2a	17.4b
	GY (g/plant)	16.3a	17.1a	15.7a	12.0b	9.0a	8.9a	7.9a	6.6a
	HSI	0.07a	0.06a	0.06a	0.03b	0.72a	0.67b	0.71a	0.64b
	Deviation of the treatment means (heat stress vs. normal)								
Treatment comparison: heat stress (as in above cell) vs. normal condition (no heat stress) at Hunan (mean maximum temperature: 33.6 °C ± 3.8, relative humidity: 64 % ± 7.5 in 2011 and mean maximum temperature: 33.8 °C ± 1.8, relative humidity: 67 % ± 7.8 in 2012)	HD (days)	- 0.4b	- 0.3b	- 1.5c	1.0a	- 2.8c	- 1.5b	- 1.0b	6.0a
	PH (cm)	5.2c	7.3b	5.3c	18.4a	- 8.7a	- 10.1a	- 15.6b	- 23.8c
	NP	- 2.2a	- 2.3a	- 2.0a	- 3.4b	1.4a	- 1.0c	0.1b	- 0.5b
	NFG	30.2b	27.9b	15.4c	49.3a	- 102.4a	- 103.1a	- 101.0a	- 105.3a
	NSP	38.1c	45.8b	23.1d	62.0a	- 29.8a	- 33.1a	- 30.9a	- 44.0b
	FG (%)	- 6.4c	- 4.4b	- 2.5a	- 5.1b	- 63.5a	- 60.1a	- 62.3a	- 58.6a
	TGW (g)	- 2.0b	- 1.4b	- 0.2a	- 2.3b	- 5.8b	- 5.2b	- 5.2b	- 3.2a
	GY (g/plant)	- 2.5b	- 1.1a	- 2.3b	- 0.9a	- 34.7b	- 37.8b	- 33.4b	- 25.0a
	Treatment means								
	HD (days)	77.2a	78.7a	76.0a	73.5a	78.4a	77.7a	79.5a	76.0a
Treatment: heat stress condition at Hangzhou (mean maximum temperature: 40.0 °C ± 2.0, relative humidity: 65.0 % ± 5.0 in 2011 and mean maximum temperature: 40.0 °C ± 2.0, relative humidity: 65.0% ± 5.0 in 2012)	NFG	122.0a	110.9b	102.4b	117.9b	86.7a	90.6a	84.0a	54.7b
	NSP	222.6a	206.6b	222.1a	176.0c	178.9a	155.5b	183.6a	145.0c
	FG (%)	54.5b	53.5b	46.1c	70.9a	49.1b	50.8b	44.4c	56.5a
	TGW (g)	26.8a	28.6a	27.7a	17.8b	25.7a	26.9a	24.8a	19.2b
	GY (g/plant)	16.3a	15.8a	14.2b	11.1c	11.3a	10.6a	10.1a	7.8b
	HSI	0.29c	0.35b	0.42a	0.26c	0.39a	0.38a	0.42a	0.36a
	Treatment means								

Continued -

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Treatment / treatment comparison	Trait ^x	2011			2012		
		SH 527/ Bg 90-2	SH 527/ Mengguandamagu	SH 527/ Bg 90-2	SH 527/ Mengguandamagu	SH 527	N22
Treatment comparison: heat stress (as in above cell) vs. normal condition (no heat stress) at Hangzhou (mean maximum temperature: 32.8 °C ± 3.1, relative humidity: 74.8 % ± 8.4 in 2011 and mean maximum temperature: 32.8 °C ± 3.1, relative humidity: 74.8 % ± 8.4 in 2012)	HD (days)	- 0.6b	0.6b	- 2.5c	2.0a	0.5a	- 0.5c
	NFG	- 44.9a	- 52.2a	- 78.3c	- 66.6b	- 72.8c	- 74.6c
	NSP	6.3a	6.7a	- 5.5b	- 17.9c	- 19.9c	0.3a
	FG (%)	- 23.1a	- 28.3a	- 33.4b	- 24.3a	- 31.6a	- 32.5a
	TGW (g)	- 2.1c	- 1.0a	- 0.7a	- 1.5b	- 1.8b	1.7a
	GY (g/plant)	- 7.9a	- 8.4b	- 11.4c	- 6.7a	- 10.6a	- 9.9a

^xHeading date (HD); plant height (PH); number of panicles (NP); number of filled grains per panicle (NFG); number of spikelets per panicle (NSP); filled grain percentage (FG); thousand grain weight (TGW); grain yield (GY) and heat stress index (HSI). ^y Means denoted by different letters within rows are significantly different at $p < 0.05$ separately for years 2011 and 2012 and provinces Hunan and Hangzhou. The underlined trait values are significantly higher than that of SH 527 and N22 ($p < 0.05$).

Heat tolerance of BC₂F₅ introgression lines

The mean trait values of all the introgression lines derived from two crosses in 2011 and 2012 under heat stress conditions at the two provinces were obtained in comparison to the recurrent parent SH 527 and the heat-check variety N22 (Table 2). Also, the deviation of each trait within introgression lines and, SH 527 and N22 from heat stress to normal conditions were calculated (Table 2). The heat-check variety N22 generally got the significantly least HD compared to others. A proteomic analysis of heat treated anthers from N22 provides strong evidences for the involvement of cold shock proteins with heat shock proteins, which function together to improve the heat tolerant trait in indica type (Jagadish *et al.*, 2010b). HD is considered as an important trait in rice breeding because farmers prefer to use short-duration rice varieties in the seasons predicted for prolonged drought periods. The reason for longer HD is that temperature at the early season could be comparatively low taking more days to complete growing degree days or thermal days (i.e. minimum number of days with optimum temperature required for growth and development), which determines the transition of the plant from vegetative stage to reproductive stage. However, longer HD could in turn help to accumulate more assimilates contributing to higher yield. On the other hand, a high temperature with a high relative humidity reduces the fertility of spikelets. Weerakoon *et al.* (2008) have shown that the increased relative humidity from 60 % to 85 % at both 36 °C and 34 °C day time temperatures significantly decreases the percentage of anthers with fertile pollen. At Hunan, the mean maximum temperature and relative humidity under the stress condition in 2012 (40.3 °C ± 3.5, 70 % ± 5.7, respectively) were greater than that of the stress condition in 2011 (38.5 °C ± 0.5, 68 % ± 7.1). This change in temperature and relative humidity has led to a significant reduction in mean GY of the introgression lines in 2012 by 34.7 g for SH 527/Bg 90 – 2 and 37.8 g for SH 527/Mengguandamagu, while GY of N22 was reduced by 25.0 g in Hunan ($p < 0.05$). In contrast, the reduction in mean GY of the introgression lines in 2012 was non-significant and calculated as 9.2 g for SH 527/Bg 90 – 2 and 10.4 g for SH 527/Mengguandamagu, while GY of N22 was reduced by 9.9 g in Hangzhou ($p < 0.05$). In 2011, the similar trend was not observed and at least one set of introgression lines out of SH 527/Bg 90 – 2 and SH 527/Mengguandamagu exhibited significantly similar GY to N22 ($p < 0.05$). Similarly, all the deviations of mean trait values of NP, NFG, NSP, FG, TGW and GY for introgression lines SH 527 and N22 from heat stress to normal conditions in the two provinces and two years are shown in Table 2.

The mean HD values were significantly lower in N22 compared to SH 527 and introgression lines under heat stress condition at Hunan for the two years. However, the mean HD was not significantly different under stress conditions in 2011 and 2012 at Hangzhou. The PH data were only available from Hunan and N22 was the significantly tallest compared to others under any condition imposed ($p < 0.05$, Table 2). Similar to PH measurements, the NP data were available only from Hunan. The mean NP was not significantly different among all the genotypes tested. The mean NFG (122.0) was significantly highest in introgression lines derived from SH 527/Bg 90 – 2 compared to other genotypes at Hangzhou in 2011. None of the introgression line

populations exhibited significantly higher yield related mean trait values compared to SH 527 and N22 under heat stress conditions at Hunan in 2011 and 2012. The lower HSI reflects higher heat tolerance (Belding & Hatch, 1955). The introgression lines of both SH 527/Bg 90 – 2 and SH 527/Mengguandamagu showed similar mean HSI values to SH 527 and was significantly higher than N22 at Hunan in 2011. However, at Hunan in 2012, the introgression lines of SH 527/Bg 90 – 2 showed similar mean HSI value to SH 527 and had significantly higher mean HSI than the introgression lines of SH 527/Mengguandamagu and N22. The introgression lines of SH 527/Bg 90 – 2 and N22 exhibited significantly similar HSI of 0.28 at Hangzhou in 2011 ($p < 0.05$, Table 2).

Table 3: The best selected introgression lines for the two crosses SH 527/Bg 90 – 2 and SH 527/Mengguandamagu based on the combined screening results of FG, GY and HSI in 2011 and 2012

Cross	Province	The best introgression lines selected ^x
SH 527/Bg 90 – 2	Hunan	HA180, HA187, HA193
	Hangzhou	HA181, HA190, HA196
SH 527/Mengguandamagu	Hunan	HA222, HA223, HA224
	Hangzhou	HA205, HA207, HA211

^x These best selected introgression lines have been used to evaluate the mean performances of two crosses at two provinces in 2011 and 2012 under normal and heat stress conditions (Appendix, Tables I and II and Figure I).

Table 4: The significant markers associated with the traits heading date (HD) and plant height (PH) under normal and heat stress conditions at Hunan and Hangzhou in 2011 and 2012

Treatment, province, year ^x	Marker/s	Rice chromosome	F value	R ² %	Additive effect	Previously reported in
HD (days)						
Normal, Hunan, 2011	<i>RM82</i>	7	9.4**	29.0	- 1.8	-
Stress, Hunan, 2012	<i>RM126</i>	8	8.1**	27.7	4.5	-
Normal, Hunan, 2012	<i>RM126</i>	8	5.9*	20.5	3.1	-
Stress, Hunan, 2011	<u><i>RM108-RM189</i></u>	9	6.0*	26.0	- 2.1	Chen <i>et al.</i> , 2008 ^y
Normal, Hunan, 2012	<u><i>RM108-RM189</i></u>	9	5.0**	31.1	2.6	Chen <i>et al.</i> , 2008
Stress, Hunan, 2011	<i>RM286</i>	11	4.7*	18.2	- 1.4	-
Normal, Hunan, 2011	<i>RM286</i>	11	8.3**	27.3	- 1.5	-
Normal, Hunan, 2011	<i>RM229</i>	11	9.5**	34.4	1.6	-
PH (cm)						
Stress, Hunan, 2011	<i>RM547-RM483</i>	8	6.6*	28.0	2.4	-
Stress, Hunan, 2012	<i>RM547-RM483</i>	8	5.7*	21.3	4.9	-

^x The treatment (normal/heat stress condition), the province and the year of the experiment are given.

* $p < 0.05$, ** $p < 0.01$ (the level of significance)

The markers underlined have been previously identified^y in heat tolerant QTLs of rice.

The performances of the best selected introgression lines

A total of three best introgression lines from each cross based on their desirable mean trait performances of FG, GY and HSI (lower HSI reflects higher HT) in 2011 and 2012 were selected for Hunan and Hangzhou separately. None of the introgression lines selected were common for both provinces (Table 3). The mean performance of the introgression lines derived from the two crosses exhibited variable patterns at the two provinces in two years indicating that underlying genetic basis for those traits are subjected to the environmental influences. The mean performances of all the traits of these selected introgression lines under normal and stress conditions at the respective province in 2011 and 2012 are given in Appendix (Tables I and II).

The mean TGW was always the significantly highest in all selected introgression lines compared to N22 under normal and heat stress conditions. These introgression lines also showed equal or significantly higher TGW to SH 527 under all the conditions tested. Since there are seven traits available for the selection of the best introgression lines for each province, many selection strategies could be adopted combining all seven traits. However, the broad aim of the present study was to improve the HT of SH 527 through backcross breeding and to have at least the GY of SH 527 in the produced lines. The selection based on independent culling levels of mean GY of SH 527 and HSI of standard heat-check variety N22 could be implemented for each province and year as depicted in the scatter plots (Appendix, Figure I). The employment of independent culling levels for breeding and selection has been reported

Table 5: The significant markers associated with the trait number of spikelets per panicle (NSP) under normal and heat stress conditions at Hunan and Hangzhou in 2011 and 2012

Treatment, province, year ^x	Marker/s	Rice chromosome	F value	R ² %	Additive effect	Previously reported in
NSP						
Normal, Hangzhou, 2011	<i>RM110-RM279</i>	2	4.8*	17.4	10.4	-
Normal, Hangzhou, 2012	<i>RM290-RM341</i>	2	6.8*	30.0	- 18.5	-
Normal, Hangzhou, 2011	<i>RM208</i>	2	5.3*	18.7	- 19.0	-
Normal, Hangzhou, 2012	<i>RM208</i>	2	7.8**	25.4	- 20.0	-
Stress, Hunan, 2011	<i>RM426-RM135</i>	3	9.1*	29.4	18.4	-
Normal, Hunan, 2011	<u><i>RM190</i></u>	6	5.9*	20.3	- 9.2	Zhang <i>et al.</i> , 2009 ^y
Normal, Hangzhou, 2011	<u><i>RM190</i></u>	6	5.6*	19.7	- 19.5	Zhang <i>et al.</i> , 2009
Normal, Hangzhou, 2011	<i>RM444</i>	9	9.1**	28.4	15.9	-
Stress, Hangzhou, 2012	<i>RM444</i>	9	8.4**	26.8	13.6	-
Normal, Hunan, 2011	<i>RM474-RM216</i>	10	8.8**	34.0	13.3	-
Normal, Hangzhou, 2011	<i>RM474-RM216</i>	10	4.9*	21.3	14.4	-
Stress, Hangzhou, 2011	<i>RM333</i>	10	5.9**	34.8	- 17.1	-
Normal, Hangzhou, 2011	<i>RM333</i>	10	3.5*	23.9	- 14.8	-
Normal, Hangzhou, 2011	<i>RM263-RM318</i>	2	8.4**	26.7	- 7.0	-
Normal, Hangzhou, 2012	<i>RM263-RM318</i>	2	5.5*	19.2	- 4.0	-
Normal, Hangzhou, 2011	<i>RM263-RM318</i>	2	4.5*	16.4	- 5.2	-
Stress, Hunan, 2011	<i>RM482</i>	2	9.0**	34.6	- 5.1	-
Normal, Hangzhou, 2011	<u><i>RM282</i></u>	3	8.8**	27.7	- 5.1	Zhang <i>et al.</i> , 2008
Stress, Hunan, 2011	<i>RM401</i>	4	8.7**	28.3	- 4.7	-
Stress, Hangzhou, 2011	<i>RM401</i>	4	6.8*	22.8	- 6.8	-
Stress, Hangzhou, 2011	<i>RM216</i>	10	5.2*	22.5	- 9.8	-
Stress, Hangzhou, 2011	<i>RM286</i>	11	6.9*	23.9	- 9.6	-
Normal, Hangzhou, 2012	<i>RM286</i>	11	6.3*	22.3	- 4.3	-
Normal, Hangzhou, 2012	<i>RM4</i>	12	6.5*	22.1	- 4.2	-

^x The treatment (normal/heat stress condition), the province and the year of the experiment are given.

* $p < 0.05$, ** $p < 0.01$ (the level of significance)

The markers underlined have been previously identified^y in heat tolerant QTLs of rice.

in many crop improvement programmes (Wricke & Weber, 1986; Zhao *et al.*, 2006b; Yan & Frégeau-Reid, 2008). The introgression lines HA193 and HA187 were selected from SH 527/Bg 90 – 2 for Hunan in 2012 (Appendix, Figure IC), and HA181, HA190 and HA196 were selected from SH 527/Bg 90 – 2 for Hangzhou in 2011 (Appendix, Figure IE). However, only HA196 was selected from SH 527/Bg 90 – 2 for Hangzhou in 2012 (Appendix, Figure IG) while only HA205 was selected from SH 527/Mengguandamagu for Hangzhou in 2012 (Appendix, Figure IH). No introgression lines with

better GY and HSI in combination were detected from the crosses SH 527/Bg 90 – 2 and SH 527/Mengguandamagu for Hunan in 2011 (Appendix, Figures IA and IB, respectively), from SH 527/Mengguandamagu for Hunan in 2012 (Appendix, Figure ID) and from SH 527/Mengguandamagu for Hangzhou in 2011 (Appendix, Figure IF). The seven introgression lines selected here have shown a greater improvement of GY and HSI compared to SH 527 and N22 suggesting that backcross breeding followed by selection for key traits can be used to improve the performance of standard rice varieties.

Table 6: The significant markers associated with the traits number of panicles (NP), number of filled grains per panicle (NFG) and thousand grain weight (TGW) under normal and heat stress conditions at Hunan and Hangzhou in 2011 and 2012

Treatment, province, year ^x	Marker/s	Rice chromosome	F value	R ² %	Additive effect	Previously reported in
NP						
Normal, Hunan, 2012	<i>RM39</i>	5	8.7**	27.3	2.4	-
Normal, Hunan, 2012	<i>RM481</i>	7	8.9**	33.0	1.0	-
Stress, Hunan, 2012	<i>RM4</i>	12	9.6**	36.0	2.1	-
NFG						
Normal, Hunan, 2011	<u><i>RM190</i></u>	6	5.5*	19.4	-6.5	Zhang <i>et al.</i> , 2009 ^y
Normal, Hangzhou, 2011	<u><i>RM190</i></u>	6	5.0*	17.9	-14.9	Zhang <i>et al.</i> , 2009
Normal, Hangzhou, 2012	<u><i>RM190</i></u>	6	8.1**	26.1	-20.4	Zhang <i>et al.</i> , 2009
Stress, Hangzhou, 2011	<u><i>RM190</i></u>	6	5.0*	17.7	-24.1	Zhang <i>et al.</i> , 2009
Normal, Hangzhou, 2012	<i>RM541</i>	6	13.8**	63.3	-22.4	-
Stress, Hunan, 2011	<i>RM444</i>	9	8.4**	27.7	10.8	-
Normal, Hangzhou, 2011	<i>RM216</i>	10	8.5**	32.2	-12.6	-
Normal, Hangzhou, 2011	<i>RM333</i>	10	6.0**	35.2	-14.3	-
Normal, Hangzhou, 2012	<i>RM333</i>	10	6.2**	36.1	-17.4	-
Stress, Hangzhou, 2011	<i>RM333</i>	10	6.3**	36.3	-19.8	-
Stress, Hunan, 2011	<i>RM4</i>	12	5.7*	20.7	-11.5	-
Normal, Hangzhou, 2011	<i>RM4</i>	12	5.0*	17.8	-12.4	-
Stress, Hangzhou, 2011	<i>RM4</i>	12	7.4**	24.3	-9.8	-
TGW						
Normal, Hangzhou, 2011	<i>RM208</i>	2	5.6*	19.6	1.2	-
Stress, Hangzhou, 2012	<i>RM208</i>	2	4.6*	16.6	1.6	-
Normal, Hangzhou, 2012	<i>RM208</i>	2	6.1*	20.9	1.1	-
Stress, Hangzhou, 2011	<i>RM114</i>	3	3.7*	25.2	0.0	-
Normal, Hangzhou, 2012	<u><i>RM190</i></u>	6	4.3*	15.9	1.0	Zhang <i>et al.</i> , 2009
Stress, Hangzhou, 2012	<i>RM402-RM541</i>	6	5.7*	41.7	1.8	-
Normal, Hangzhou, 2012	<i>RM402-RM541</i>	6	4.3*	15.9	1.0	-
Normal, Hangzhou, 2012	<u><i>RM271</i></u>	10	8.7**	27.3	-1.7	Kui <i>et al.</i> , 2008

^x The treatment (normal/heat stress condition), the province and the year of the experiment are given.

* $p < 0.05$, ** $p < 0.01$ (the level of significance)

The markers underlined have been previously identified^y in heat tolerant QTLs of rice.

Marker trait associations

Out of 600 rice microsatellite markers screened for recurrent and donor parents, only 61 and 59 were polymorphic for the two crosses SH 527/Bg 90 – 2 and SH 527/Mengguandamagu, respectively. The significant markers associated with the studied traits, the percentage variation explained by each marker in terms of percentage correlation coefficient (R^2) and the estimated additive effects are shown in Tables 4 – 7. No significant markers associated with FG were detected for any of the two crosses under the conditions tested ($p > 0.05$). A total of four markers (RM126, RM108, RM189 and RM286) were found to be significantly associated with HD while the two markers RM547 and RM483 exhibited a significant association with PH ($p < 0.05$, Table 4). A total of eight markers (RM426, RM135, RM444, RM333, RM482, RM401, RM216 and RM286) were found to be significantly associated

with NSP (Table 5). The marker RM4 was significantly associated with NP while the four markers RM190, RM444, RM333 and RM4 were associated with the trait NFG only under heat stress condition. Moreover, the four markers RM208, RM114, RM402 and RM541 showed a significant association with TGW and none of the markers were significantly associated with GY under heat stress condition (Tables 6 and 7). A total of 11 markers namely RM576, RM200, RM14, RM110, RM279, RM290, RM401, RM1376, RM216, RM286 and RM4 were found to be significantly associated with HSI ($p < 0.05$, Table 7). The significant additive effects (a) of all the significant markers are given in Tables 4 – 7 for the traits studied. Only two significant dominant effects (d) were detected out of all the significant marker trait associations and both these effects were found on RM114 of chromosome 3 associated with TGW under stress condition at Hangzhou in 2011 ($d = 2.97$ g) and 2012 ($d = 3.11$ g) indicating the heterosis that favours hybrid rice production.

Table 7: The significant markers associated with the traits grain yield (GY) under normal and heat stress conditions and heat stress index (HSI) under heat stress condition at Hunan and Hangzhou in 2011 and 2012

Treatment, province, year*	Marker/s	Rice chromosome	F value	R^2 %	Additive effect	Previously reported in
GY (g/plant)						
Normal, Hunan, 2011	RM576-RM493	1	4.7*	20.6	1.5	- ^y
Normal, Hunan, 2011	RM231	3	5.2*	18.3	1.6	-
Normal, Hunan, 2011	RM234	7	8.8**	27.6	1.9	-
HSI						
Stress, Hangzhou, 2012	RM576	1	6.3*	26.0	-0.1	-
Stress, Hunan, 2011	RM200	1	4.4*	29.5	0.1	-
Stress, Hunan, 2011	RM14	1	8.8**	28.3	0.1	-
Stress, Hunan, 2011	RM110-RM279	2	6.5*	27.7	0.1	-
Stress, Hunan, 2011	RM290	2	4.8*	32.6	-0.1	-
Stress, Hangzhou, 2012	RM290	2	6.4*	27.3	-0.2	-
Stress, Hunan, 2011	RM401	4	5.4*	19.8	0.1	-
Stress, Hangzhou, 2011	RM401	4	6.6*	22.2	0.1	-
Stress, Hunan, 2011	RM1376	8	9.4**	35.7	0.1	-
Stress, Hunan, 2011	RM216	10	8.6**	28.1	0.1	-
Stress, Hunan, 2011	RM286	11	8.9**	29.7	0.1	-
Stress, Hangzhou, 2011	RM286	11	8.6**	28.1	0.1	-
Stress, Hunan, 2011	RM4	12	6.6*	23.1	0.1	-
Stress, Hangzhou, 2011	RM4	12	9.2**	28.5	0.1	-

*The treatment (normal/heat stress condition), the province and the year of the experiment are given.

* $p < 0.05$, ** $p < 0.01$ (the level of significance)

No markers have been previously identified^y in heat tolerant QTLs of rice unlike in Tables 4 – 6.

Table 8: The overall picture of the significant marker trait associations detected in the introgression lines

Trait ^x	Province, Year	Treatment ^y	Rice chromosome and rice microsatellite (RM) markers																																										
			1				2				3				4				5				6				7				8				9				10				11		12
			RM576	RM493	RM14	RM200	RM208	RM482	RM263	RM318	RM110	RM279	RM290	RM341	RM114	RM135	RM426	RM282	RM231	RM401	RM39	RM190	RM541	RM402	RM82	RM481	RM234	RM1376	RM483	RM547	RM126	RM108	RM189	RM444	RM216	RM474	RM333	RM271	RM286	RM229	RM4				
HD	Hunan, 2011	N																																											
	Hunan, 2012	S																																											
		N																																											
PH	Hunan, 2011	S																																											
	Hunan, 2012	S																																											
NP	Hunan, 2012	N																																											
		S																																											
NFG	Hunan, 2011	N																																											
	Hangzhou, 2011	S																																											
		N																																											
	Hangzhou, 2012	S																																											
N																																													
NSP	Hunan, 2011	N																																											
	Hangzhou, 2011	S																																											
		N																																											
	Hangzhou, 2012	S																																											
N																																													
TGW	Hangzhou, 2011	N																																											
	Hangzhou, 2012	S																																											
		N																																											
	Hangzhou, 2012	S																																											
N																																													
GY	Hunan, 2011	N																																											
HSI	Hunan, 2011	S																																											
	Hangzhou, 2011	S																																											
	Hangzhou, 2012	S																																											

^xHeading date (HD); plant height (PH); number of panicles (NP); number of filled grains per panicle (NFG); number of spikelets per panicle (NSP); thousand grain weight (TGW); grain yield (GY) and heat stress index (HSI).

^yThe treatment levels are normal (N) and heat stress (S) conditions. Background colours are indicated to clearly represent the different traits. Blue shaded cells represent the significant marker trait association for normal condition and the red shaded cells represent the significant marker trait association for heat stress condition ($p < 0.05$). Other cells indicate non-significant marker trait associations ($p > 0.05$).

When the markers associated with multiple traits at two provinces in 2011 and 2012 were considered separately, 47 and 42 significant associations were detected under normal and heat stress conditions, respectively ($p < 0.05$, Table 8). RM208 of chromosome 2 was significantly associated with NSP and TGW at Hangzhou in both years under normal condition. RM190 of chromosome 6 was significantly associated with NFG at Hunan in 2011 and at Hangzhou in 2011 and 2012; and NSP at Hunan and Hangzhou in 2011 under normal condition. RM401 of chromosome 4 was significantly associated with NSP and HSI at Hunan and Hangzhou in 2011 under stress condition. RM401 was not significantly associated with any of the traits measured under normal condition indicating that it could be located in a QTL that is only expressed under heat stress. RM286 of chromosome 11 was significantly associated with HD at Hunan in 2011 and NSP at Hangzhou in 2012 under normal condition. The same marker was significantly associated with HD at Hunan in 2012; NSP at Hangzhou in 2011; and HSI at both Hunan and Hangzhou in 2011 under stress condition. RM4 of chromosome 12 was significantly associated with NFG at Hangzhou in 2011 and NSP at Hangzhou in 2012 under normal condition. The same marker was significantly associated with NP at Hunan in 2012; NFG and HSI at Hunan and Hangzhou in 2011 and HSI under stress condition. Overall RM4 showed the highest number of significant associations detected in the present study (Table 8).

The detection of different markers associated with the traits under normal and stress conditions at two provinces and years supported the differential expression of many quantitative traits, which is influenced by both the environment and the interaction among non-allelic genes. Hence, the results of QTL mapping under different genetic backgrounds and environments might be diverse in terms of their expression (Lu *et al.*, 1996; Xing *et al.*, 2002). A total of 11 significant marker trait associations detected in the present study were previously reported in QTL mapping studies for HT (Kui *et al.*, 2008; Chen *et al.*, 2008; Zhang *et al.*, 2008; 2009). The first attempt of QTL mapping for HT at the flowering stage using SNP markers revealed that the QTL qHTSF1.1 at 150.5 cM of chromosome 1 could explain 12.6 % of fertility of spikelets whereas the present study found that RM200 could explain 29.5 % of HSI suggesting that this might be the same QTL (Ye *et al.*, 2012). Xiao *et al.* (2011) detected another QTL named qPF4 around 14.3 cM of chromosome 4 for pollen fertility whereas the present study detected RM401 of the same locus implying that the same QTL was detected. An important association with RM190 on chromosome 6 was detected in the present study for NFG, NSP and TGW, which had been

previously reported as a QTL linked to pollen fertility by Zhang *et al.* (2009) and again reported by Xiao *et al.* (2011). The significant marker trait associations of RM108 of chromosome 9 with HD and RM271 of chromosome 10 with TGW had been previously reported in QTLs linked to spikelet fertility by Chen *et al.* (2008) and Kui *et al.* (2008), respectively.

CONCLUSION

The backcrossing of standard rice variety SH 527, a parental genotype for hybrid seed production, as a recurrent parent with heat tolerant indica donor parents yielded a total of 46 heat tolerant introgression lines at BC₂F₅ with a spikelet fertility > 40 %, and 88.3 % of these introgression lines display homozygosity for heat tolerant alleles. Overall performances of the introgression lines are better than SH 527 and N22 for NFG and GY in Hangzhou in 2011 under stress condition. A total of seven high yielding and heat tolerant introgression lines could be specifically selected based on GY and HSI as independent culling levels for the respective province and years. In single-marker analysis, 47 and 42 significant marker trait associations were detected under normal and heat stress conditions, respectively for multiple traits measured at Hunan and Hangzhou in 2011 and 2012. Out of these significant marker trait associations, 11 cases were previously reported as heat tolerant QTLs validating the results of the present study. Therefore, the detected DNA markers associated with yield and heat tolerant traits can be used in marker assisted hybrid seed production involving SH 527 as a parental genotype. It is evident that indica donor parents could be readily used in improving HT of standard rice varieties.

Acknowledgement

This study was funded by the “863” programme from the Ministry of Science and Technology of China and the Bill & Melinda Gates Foundation (OPP51587) – Phase II.

Authors wish to acknowledge the valuable contribution from the following scientists: Q. Tang, Hunan Agricultural University, Changsha, China; L. Tao, National Rice Research Institute, Hangzhou, China; X. Chao and Y.M. Gao, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, 12, South Zhong-Guan-Cun Street., Beijing, 100081, China; Z.K. Li, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, 12, South Zhong-Guan-Cun Street., Beijing, 100081, China; and the International Rice Research Institute, DAPO Box 7777, Metro Manila, The Philippines.

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Appendix

Table I: Mean performances of the best selected introgression lines evaluated under normal and heat stress conditions at Hunan, 2011 and 2012

Genotype	Treatment	HD ^x (days)	PH (cm)	NP	NFG	NSP	FG (%)	TGW (g)	GY (g/ plant)	HSI
SH 527/Bg 90 - 2 from Hunan, 2011										
HA180	Normal	94.0b ^y	114.4b	7.7b	83.5c	110a	77.1b	30.1a	18.9a	-
HA187		97.0a	115.6b	8.2b	88.2b	112.5a	78.5b	30.4a	21.9a	-
HA193		95.3a	115.2b	7.4b	93.6a	111.9a	83.3a	28.4a	20.0a	-
SH 527		96.9a	111.1c	7.8b	84.1c	110.2a	77.2b	29.1a	20.4a	-
N22		86.6c	139.2a	9.1a	68.5d	82.8b	82.5a	20.9b	12.9b	-
HA180	Stress	96.5a	115.3c	9.2a	93.6d	141.9a	66.9b	28.5a	24.8a	0.13a
HA187		95.0a	124.7b	6.2b	98.4c	144.1a	68.1b	29.2a	18.0c	0.13a
HA193		97.0a	128.2b	6.5b	111.2b	145.6a	77.5a	29.0a	20.9b	0.07b
SH 527		95.4a	116.4c	5.8c	99.5c	133.3b	68.5b	28.7a	13.1d	0.11a
N22		87.6b	157.6a	5.7c	117.8a	144.8a	77.4a	18.6b	12.0d	0.06b
SH 527/ <i>Mengguandamagu</i> from Hunan, 2011										
HA222	Normal	95.3b	112.8c	8.2b	74.0c	94.7c	78.1b	30.4a	18.7c	-
HA223		98.0a	120.3b	9.7a	79.6b	110.4a	72.1c	30.6a	23.4a	-
HA224		99.7a	118.9b	8.6b	79.6b	101.3b	78.6b	29.7a	20.2b	-
SH 527		96.9a	111.1c	7.8c	84.1a	110.2a	77.2b	29.1a	20.4b	-
N22		86.6c	139.2a	9.1a	68.5b	82.8d	82.5a	20.9b	12.9d	-
HA222	Stress	92.0b	116.0c	6.3b	87.4d	135.3b	64.8d	29.5a	16.4b	0.17a
HA223		97.0a	134.0b	8.3a	90.4c	134.8b	67.0c	30.0a	22.3a	0.07c
HA224		99.0a	124.3b	6.0b	92.9c	127.7c	73.2b	29.3a	16.9b	0.08c
SH 527		95.4a	116.4c	5.8c	99.5b	133.3b	68.5c	28.7a	13.1c	0.11b
N22		87.6c	157.6a	5.7c	117.8a	144.8a	77.4a	18.6b	12.0c	0.06c
SH 527/Bg 90-2 from Hunan, 2012										
HA180	Normal	84.3c	119.8d	12.2a	129.7b	149.3c	87.1b	30.7b	48.4a	-
HA187		93.7a	126.2b	10.2b	143.8a	157.3b	91.1a	32.9a	47.6a	-
HA193		92.0a	125.7b	9.2c	143.4a	163.9a	87.7b	30.8b	40.4b	-
SH 527		89.3b	122.9c	10.3b	131.6b	151.2c	87.2b	30.4b	41.3b	-
N22		70.0d	154.8a	10.8b	142.8a	154.6b	92.2a	20.6c	31.6c	-
HA180	Stress	82.7b	116.3b	17.3a	22.4e	119.3b	18.8d	24.3b	9.5b	0.78a
HA187		91.3a	116.0b	11.7b	48.7b	119.0b	40.9a	25.6a	14.5a	0.54c
HA193		89.3a	107.3c	8.3d	69.7a	171.9a	40.5a	23.8b	13.8a	0.54c
SH 527		87.7a	107.3c	10.4c	30.6d	120.3b	24.9c	25.2a	7.9c	0.71a
N22		76.0c	131.0a	10.3c	37.5c	110.6c	33.6b	17.4c	6.6c	0.63b
SH 527/ <i>Mengguandamagu</i> from Hunan, 2012										
HA222	Normal	79.7b	126.8c	13.0a	132.9d	157.3b	84.5b	32.0a	54.8a	-
HA223		90.0a	130.5b	8.2c	148.9a	173.2a	86.0b	30.9b	37.5d	-
HA224		88.3a	125.8c	10.7b	136.9c	150.4c	91.0a	32.8a	47.8b	-
SH 527		89.3a	122.9d	10.3b	131.6d	151.2c	87.2b	30.4b	41.3c	-
N22		70.0c	154.8a	10.8b	142.8b	154.6b	92.2a	20.6c	31.6e	-

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Genotype	Treatment	HD ^x (days)	PH (cm)	NP	NFG	NSP	FG (%)	TGW (g)	GY (g/ plant)	HSI
SH 527/Mengguandamagu from Hunan, 2012										
HA222	Stress	84.3b	115.4c	11.0a	29.0b	139.1b	21.2c	24.7b	7.8b	0.75b
HA223		86.3a	123.0b	8.8c	35.3a	143.8a	24.8b	28.3a	8.8a	0.71b
HA224		89.0a	115.6c	9.6c	23.5c	134.6c	18.5d	26.7a	6.1c	0.80a
SH 527		87.7a	107.3d	10.4b	30.6b	120.3d	24.9b	25.2b	7.9b	0.71b
N22		76.0c	131.0a	10.3b	37.5a	110.6e	33.6a	17.4c	6.6c	0.63c

^x Heading date (HD), plant height (PH), number of panicles (NP), number of filled grains per panicle (NFG), number of spikelets per panicle (NSP), filled grain percentage (FG), thousand grain weight (TGW), grain yield (GY) and heat stress index (HSI). ^y Means denoted by different letters within cross and within each treatment level are significantly different at $p < 0.05$ separately for years 2011 and 2012 in the Hunan province.

Table II: Mean performances of the best selected introgression lines evaluated under normal and heat stress conditions at Hangzhou, 2011 and 2012

Genotype	Treatment	HD ^a (days)	NFG	NSP	FG (%)	TGW (g)	GY (g/plant)	HSI
SH 527/Bg 90-2 from Hangzhou, 2011								
HA181	Normal	-	153.9c	216.4c	71.2b	28.1a	21.6a	-
HA190		-	162.0b	215.0c	75.8b	27.9a	22.6a	-
HA196		-	153.7c	249.3a	61.6c	26.7a	20.5a	-
SH 527		-	167.5b	222.5b	75.3b	28.0a	23.5a	-
N22		-	184.5a	193.9d	95.2a	19.3b	17.8b	-
HA181	Stress	77.0.a ^y	143.9a	241.2a	59.6c	26.2a	18.9a	0.16b
HA190		77.0a	133.0b	198.8c	67.0b	27.7a	18.4a	0.11c
HA196		79.0a	142.8a	241.4a	59.1c	26.3a	18.8a	0.04d
SH 527		77.0a	132.6b	223.4b	59.3c	27.5a	18.2a	0.21a
N22		72.0b	117.9c	176d	77.5a	17.8b	12.1b	0.18b
SH 527/Mengguandamagu from Hangzhou, 2011								
HA205	Normal	-	181.8b	236.6a	76.8b	29.1a	26.4a	-
HA207		-	171.9c	189.4c	90.7a	30.6a	26.3a	-
HA211		-	198.1a	217.7b	91.1a	28.7a	28.4a	-
SH 527		-	167.5d	222.5b	75.3b	28.0a	23.5b	-
N22		-	184.5b	193.9c	95.2a	19.3b	17.8c	-
HA205	Stress	78.0b	159.7a	252.6a	63.0c	27.8a	22.2a	0.18c
HA207		84.0a	153.5a	215.6c	71.2b	29.4a	22.6a	0.22b
HA211		76.0b	117.2c	196.9d	59.8d	28.5a	16.7b	0.34a
SH 527		77.0b	132.6b	223.4b	59.3d	27.5a	18.2b	0.21b
N22		72.0c	117.9c	176.0a	77.5a	17.8b	12.1c	0.18c
SH 527/Bg 90-2 from Hangzhou, 2012								
HA181	Normal	-	175.7a	213.2a	82.5a	25.8b	22.7a	-
HA190		-	140.7c	179.3d	78.4b	29.6a	20.9a	-
HA196		-	144.0c	182.6c	78.7b	26.2b	18.9b	-
SH 527		-	125.4d	171.8d	74.1c	25.3b	16.2b	-
N22		-	156.9b	203.5b	76.5c	26.6b	20.7a	-

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Genotype	Treatment	HD* (days)	NFG	NSP	FG (%)	TGW (g)	GY (g/plant)	HSI
SH 527/Bg 90-2 from Hangzhou, 2012								
HA181	Stress	77.0b	98.0c	190.1b	51.6c	27.2a	13.3a	0.37a
HA190		78.0b	116.4a	220.7a	53.1b	25.6a	14.9a	0.32a
HA196		81.0a	117.6a	181.8c	64.5a	25.1a	14.8a	0.18c
SH 527		79.0b	105.5b	195.5b	53.2b	25.7a	13.5a	0.26b
N22		76.0b	54.7d	145.0d	56.5b	19.2b	7.9b	0.26b
SH 527/Mengguandamagu from Hangzhou, 2012								
HA205	Normal	-	165.8b	199.5b	83.1c	29.0a	24.0a	-
HA207		-	145.2d	161.2d	90.1a	29.6a	21.5b	-
HA211		-	177.2a	202.5a	87.5b	27.6b	24.5a	-
SH 527		-	125.4e	171.8c	74.1d	25.3b	16.2c	-
N22		-	156.9c	203.5a	76.5d	26.6b	20.7b	-
HA205	Stress	79.0b	118.9a	177.5c	67.2b	25.8b	15.4a	0.19c
HA207		84.0a	92.0d	127.8e	72.5a	28.5a	13.1a	0.20c
HA211		76.0c	113.1b	189.3b	59.7c	26.0b	14.7a	0.32a
SH 527		79.0b	105.5c	195.5a	53.2d	25.7b	13.5a	0.26b
N22		76.0c	54.7e	145.0d	56.5c	19.2c	7.9b	0.26b

^aHeading date (HD), number of filled grains per panicle (NFG), number of spikelets per panicle (NSP), filled grain percentage (FG), thousand grain weight (TGW), grain yield (GY) and heat stress index (HSI). ^y Means denoted by different letters within cross and within each treatment level are significantly different at $p < 0.05$ separately for years 2011 and 2012 in the Hangzhou province.

Figure I: Scatter plots indicating the positions of the best selected introgression lines with respect to the recurrent parent SH 527 and the standard heat-check variety N22. X axis represents the grain yield (GY) in g/plant and Y axis represents the heat stress index (HSI).