

Genome-wide association analysis for heat tolerance of processes of anthesis in rice detected a large set of genes involved in adaptation to thermal stresses



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INTRODUCTION

Sensitivity of rice pollen development to heat is a major issue for climate change scenarios in the tropics. QTL mapping and transcriptome studies have identified stress response pathways involved in this development. The aim of this study was to address the sensitivity of processes of anthesis to heat and to confront detected genes with already published pathways.

MATERIAL AND METHODS

Plant material: The panel used was composed of 165 traditional and improved *indica* and two *Aus* accession including the heat tolerant N22..

Genotyping: Genotyping was done, following a method combining Diversity Arrays Technology (DArT) and next generation sequencing called DArTseq™. It provided a total of 8214 DArT markers and 8018 SNP markers with less than 5% missing data, representing a average density of one marker every 22.9kb. The missing data were estimated using Beagle v3.3.

Association analysis : The adjusted means of sterility rate for each accession was transformed with the 2ASin Square-root function to tend toward a normal distribution. Association analysis was performed under a Mixed Linear Model using the percentages of admixture (Q matrix) and the kinship matrix (K) as random effects. The analysis was performed using Tassel-MLM exact procedure. The threshold for significant association was set at $p < 1e-05$ and $q < 0.05$ (false discovery rates).

RESULTS

Genetic diversity and linkage disequilibrium:

Structure analysis pointed the most likely number of subgroups as 4: G1 (48 accessions) composed of local *indica* varieties of diverse geographic origin; G2 (14) composed of local varieties from the Madagascar highlands; G3 (33) and G4 (13) composed mainly of improved *indica*; admixture (59) that could not be assigned to any of the 4 subgroups for more than 80% of their genotype (Fig. 2).

Linkage disequilibrium was high and decreased slowly: average r^2 was superior to 0.5 for distances below 20 kb and reached half of its initial level only at about 300 kb distance between markers

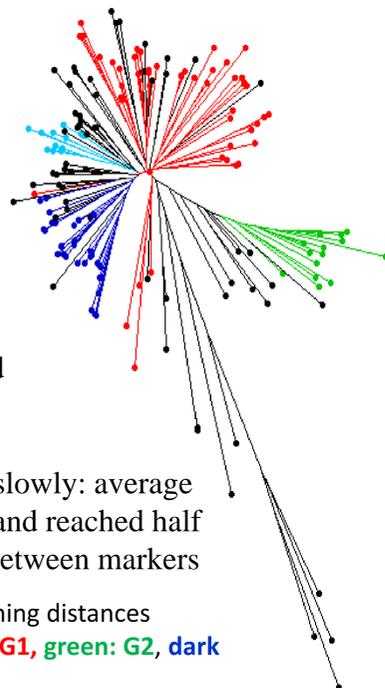


Fig. 2: Unrooted neighbour joining tree of simple matching distances between the 167 accessions of association panel. **Red: G1, green: G2, dark blue: G3, light blue: G4, and black: Admixt.**

Loci involved in heat tolerance of processes of anthesis:

- The semi-dwarfing gene *sd1* was detected at the expected position (Fig. 4A) confirming the effectiveness of our association analysis procedures.
- 91 significant associations ($p < 1e-04$, $q < 0.05$) were detected, representing at least 12 independent loci for heat tolerance on 8 chromosomes (Fig. 4B).
- The 12 independent loci corresponded to gene families involved in temperature stress sensing and signaling, heat shock proteins, DNA damage and replication controls, transcription factors regulating growth and defense responses against abiotic stresses, as well as pollen development (Table 1).

Table 1: Major gene families associated with heat tolerance of processes of anthesis

Chr.	Position (bp)	p-value	q-value	Alleles	Effect	MAF (%)	Predicted gene	Gene function
1	28 116 036	1.00E-05	0.0106	A/G	0.219	28	Os01g48980.1	GPI transamidase component family protein, putative, expressed
1	30 476 158	1.19E-05	0.0106	C/A	0.202	37	Os01g53020.1	heat shock protein DnaJ, putative, expressed
3	12 537 441	3.29E-06	0.0106	C/T	0.581	4	Os03g21870.1	DUF623 domain containing protein, expressed
4	17 668 138	7.04E-06	0.0106	T/A	-0.215	52	Os04g29680.1	OsWAK38 - OsWAK receptor-like protein kinase, expressed
6	3 331 725	3.79E-05	0.0202	T/A	0.503	5	Os06g07040.1	OsIAA20 - Auxin-responsive Aux/IAA gene family member, expressed
6	3 512 853	1.01E-05	0.0106	C/T	0.513	5	Os06g07380.1	OsFBX188 - F-box domain containing protein, expressed
7	9 000 865	1.82E-04	0.0472	C/T	0.304	11	Os06g07220.1	LTPL128 - Protease inhibitor/seed storage/LTP family protein precursor, expressed
7	29 312 153	8.07E-05	0.0481	G/T	-0.247	19	Os07g49010.1	TOPBP1B - Similar to DNA replication protein TOPBP1 from, expressed
10	21 182 537	4.25E-06	0.0106	A/T	0.268	17	Os10g39680.1	CHIT14 - Chitinase family protein precursor, expressed
11	442 456	1.54E-06	0.0100	A/T	-0.239	27	Os11g01780.1	SAG20, putative, expressed
11	1 061 145	1.22E-05	0.0106	T/A	-0.210	51	Os11g02964.1	FAR1 family protein, expressed
12	25 624 918	1.40E-07	0.0018	A/T	0.661	3	Os12g41290.1	Spc97 / Spc98 family protein, putative, expressed



Fig. 1: Heat stress during processes of anthesis

Phenotyping for heat tolerance: during spikelet flowering: four sets of the 167 accessions were grown in a greenhouse under 29/21°C day/night temperature conditions from sowing until few days before anthesis. At start of its anthesis, each single plant from two of the four sets (as two reps of each of the two treatments) was transferred from 8am to 2pm during 6 consecutive days into a 38°C greenhouse compartment, while it was grown within the initial compartment for the remaining time. Spikelet sterility, calculated as the ratio of unfilled spikelets at maturity, was quantified rigorously: it was reported from the top half only (to avoid considering empty spikelets due to lack of carbohydrates) of the sole panicles whose overall anthesis was completed by the end of the 6-days period. Plant height was measured at time of anthesis as the distance from the soil surface up to the bottom of the panicle of the main tiller.

Diversity for heat tolerance of processes of anthesis:

the applied heat stress was severe, leading to high level of sterility within the association panel (Fig. 3A). Groups detected by structure analysis differed significantly ($P < 0.004$) for heat tolerance (Fig. 3B). G2 and G3 had the lowest tolerance. The highest tolerance were observed in N22 a *Aus* variety from India and PEH KUH a G1 from Taiwan.

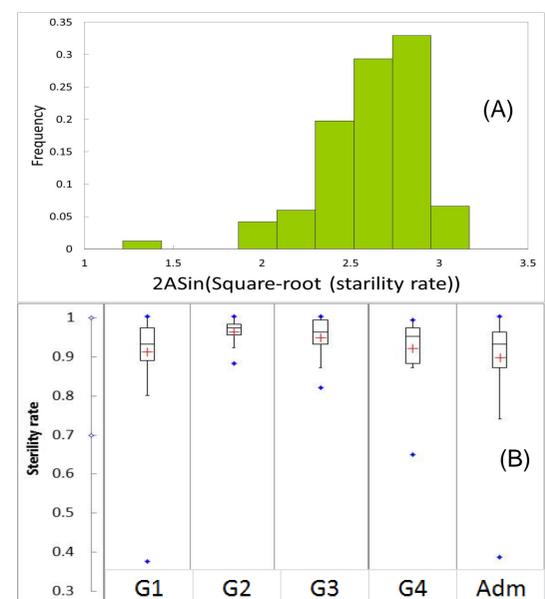


Fig. 3: Distribution of heat tolerance of processes of anthesis among the association panel (A) and within subgroups (B).

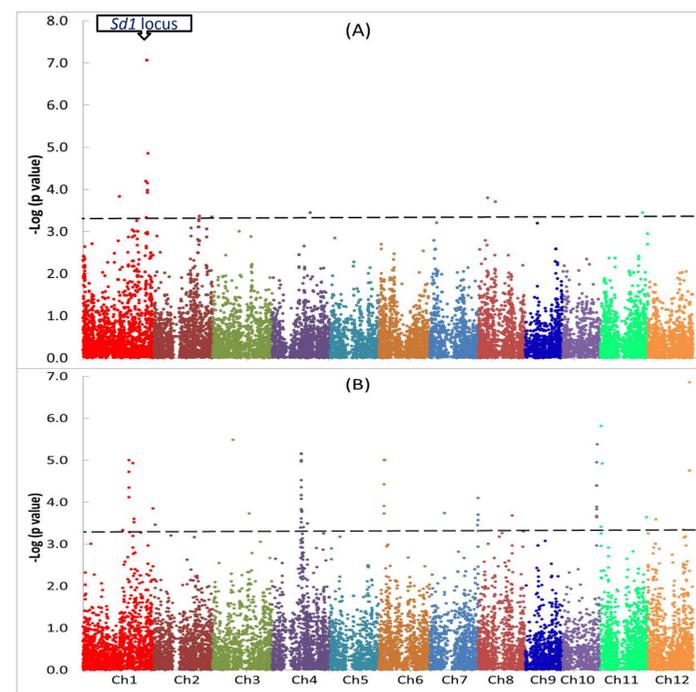


Figure 4: P-values by chromosome for 16232 tests of association between SNP loci and phenotypic traits within the *indica* panel of 167 accessions. A: plant height; B: heat tolerance at flowering time.