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RESEARCH ARTICLE

Whole-Genome Resequencing Reveals Adaptive Variants in Heat-Tolerant Rice Cultivars

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ABSTRACT

Heat stress during flowering is one of the most damaging climate-related threats to rice yield, since even a few hours above the critical temperature threshold at anthesis can sharply reduce spikelet fertility, yet the genetic basis of natural variation in heat tolerance at this stage remains only partially resolved. We performed whole-genome resequencing at a mean depth of 14.8x on 120 *Oryza sativa* accessions -- 64 heat-tolerant landraces sourced from historically hot rice-growing regions of South and Southeast Asia and 56 heat-sensitive check varieties -- and combined genome-wide association analysis of spikelet fertility under controlled heat stress (38C, 4 hours daily during a 7-day anthesis window) with a genome-wide selective-sweep scan (FST and XP-CLR) contrasting the two phenotypic groups. Under heat stress, spikelet fertility ranged from 9.4% to 88.6% across accessions and was strongly bimodal between the two source groups (tolerant group mean 71.2%, sensitive group mean 24.6%; t-test $P < 0.001$). Genome-wide association identified 14 SNPs reaching genome-wide significance ($P < 8.2 \times 10^{-8}$ after Bonferroni correction), clustering into six independent loci on chromosomes 1, 3, 6, 9 and 10, with the strongest association ($P = 3.1 \times 10^{-14}$) located approximately 8 kb upstream of *OsTT1*, a proteasome alpha2 subunit gene previously implicated in thermotolerance through enhanced clearance of heat-denatured proteins. The selective-sweep scan independently flagged four of these six GWAS-identified regions among its top 20 genome-wide FST outlier windows, providing convergent evidence that these loci have been under recent positive selection in heat-tolerant germplasm rather than representing chance GWAS associations. Haplotype analysis at the *OsTT1* locus identified a tolerant-associated haplotype carrying three linked promoter-region variants, present in 89% of tolerant accessions but only 12% of sensitive accessions, consistent with this haplotype being a primary driver of the locus's association signal. These results narrow the genetic basis of heat tolerance at anthesis in rice to a tractable set of candidate loci suitable for marker-assisted selection in breeding programmes targeting warming production environments.

Keywords: *Oryza sativa*; whole-genome resequencing; heat tolerance; spikelet fertility; selective sweep; genome-wide association study

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1. INTRODUCTION

Rice is exceptionally sensitive to high temperature during a narrow developmental window around anthesis, when pollen viability and successful fertilisation depend on a combination of anther dehiscence, pollen germination and pollen tube growth that are each individually heat-labile. Field and controlled-environment studies have repeatedly shown that daytime temperatures above roughly 33–35°C sustained during the few hours of peak spikelet opening can cause spikelet sterility rates exceeding 50%, and that this sensitivity is far sharper than rice's heat tolerance at vegetative or grain-filling stages, making anthesis-stage heat tolerance a distinct and agronomically critical trait rather than a simple extension of general thermotolerance [5,13]. Projected increases in the frequency of extreme heat events during the rice flowering season across major production regions in South and Southeast Asia have made breeding for anthesis-stage heat tolerance an increasingly urgent target for climate-resilient rice improvement [2,21].

Natural variation in anthesis-stage heat tolerance across rice germplasm is substantial and has long been recognised in landraces from historically hot growing environments, but translating this variation into actionable breeding targets requires identifying the underlying genetic loci with enough precision to support marker-assisted selection. Quantitative trait locus (QTL) mapping studies using biparental populations derived from heat-tolerant by heat-sensitive crosses have identified numerous heat-tolerance QTLs across nearly every rice chromosome, but individual QTL intervals from biparental mapping are often large, span many genes, and are specific to the particular parental combination used, limiting their direct transferability across the wider genetic diversity present in global rice germplasm [8,19]. Association mapping in a diverse germplasm panel offers a complementary approach, using historical recombination across many independent lineages to achieve finer mapping resolution, provided the panel is phenotyped carefully and population structure is adequately controlled for in the statistical model [11,24]. Among the individual genes with the clearest prior mechanistic support for a role in rice anthesis-stage heat tolerance is *OstT1* (Thermal Tolerance 1), which encodes an $\alpha 2$ subunit of the 20S proteasome and was shown in earlier work to enhance the degradation of heat-denatured and otherwise cytotoxic proteins, thereby protecting cellular protein homeostasis during acute heat exposure; natural variation at this locus has been linked to differences in heat tolerance across a panel of indica rice accessions [17]. Beyond *OstT1*, several heat-shock transcription factors (notably members of the *OshSFA* and *OshSFB* families) and heat-shock protein genes have candidate roles based on their well-established function in the canonical heat-shock response across plants generally, though direct evidence linking natural allelic variation at these loci to field-relevant anthesis-stage heat tolerance in rice specifically remains

more limited than for *OstT1* [7,15].

Selective-sweep analysis, which identifies genomic regions showing reduced diversity and altered allele frequency consistent with recent positive selection, offers an independent line of evidence for candidate adaptive loci that does not depend on statistical association with a measured phenotype and can therefore help distinguish genuine adaptive signal from spurious GWAS associations arising from residual population structure or multiple-testing noise [10,26]. Combining genome-wide association mapping with an independent selective-sweep scan, requiring convergent support from both analyses before treating a locus as a strong candidate, is a design used successfully for other agronomic traits in rice and other crops, but has been applied less often specifically to anthesis-stage heat tolerance, where most published work has relied on association mapping alone [22,29]. We therefore combined whole-genome resequencing, controlled-environment heat-stress phenotyping, genome-wide association mapping and a genome-wide selective-sweep scan in a diverse panel of heat-tolerant landraces and heat-sensitive check varieties, with the goal of identifying a tractable, convergently supported set of candidate loci for anthesis-stage heat tolerance suitable for near-term deployment in marker-assisted rice breeding.

2. MATERIALS AND METHODS

All analyses used a panel of 120 *Oryza sativa* accessions assembled to maximise phenotypic contrast in anthesis-stage heat tolerance while retaining broad genetic diversity within each phenotypic group.

2.1 Plant material and panel composition

The panel comprised 64 heat-tolerant landraces, selected from germplasm bank passport data and prior screening records as originating from historically hot rice-growing districts of South and Southeast Asia with a documented history of farmer-driven selection under high-temperature conditions, and 56 widely grown heat-sensitive check varieties representing modern indica and japonica breeding lines with no specific history of heat-tolerance selection. Both indica and japonica subpopulations were represented within the tolerant group to reduce the risk that any identified association reflected subspecies-level population structure rather than heat-tolerance-specific selection, and this was formally assessed in the population structure analysis described below.

2.2 Whole-genome resequencing and variant calling

Genomic DNA was extracted from young leaf tissue of a single representative plant per accession and sequenced on an Illumina NovaSeq 6000 platform (150 bp paired-end reads) to a target depth of 15x per accession, achieving a realised mean depth of 14.8x (range 11.2–19.6x) after quality filtering. Reads were aligned to the Nipponbare reference genome (IRGSP-1.0) using

BWA-MEM, and variants were called jointly across all 120 accessions using the GATK4 HaplotypeCaller and GenotypeGVCFs pipeline following GATK best-practices hard filtering. After filtering for biallelic SNPs with minor allele frequency above 5%, genotype missingness below 10%, and removal of SNPs in regions of excessive heterozygosity inconsistent with rice's predominantly inbreeding mating system, 4.82 million high-confidence SNPs were retained for downstream analysis.

2.3 Heat-stress phenotyping

Spikelet fertility under heat stress was assessed in a controlled-environment growth chamber. Plants were grown under standard conditions (29/22C day/night) until panicle initiation, then transferred to heat-stress conditions (38C for 4 hours centred on midday, 29C ambient otherwise) for a 7-day window spanning the onset of anthesis for each accession, timed individually based on panicle development staging. Ten panicles per accession were tagged at the start of the heat-stress window, and spikelet fertility was scored 20 days after flowering as the proportion of filled grains per tagged panicle. A parallel, non-stressed control cohort of each accession was grown under standard conditions throughout to confirm that fertility differences under heat stress were not confounded by baseline fertility differences unrelated to heat tolerance; accessions with control-condition fertility below 80% were excluded from the panel prior to genotype-phenotype analysis.

2.4 Population structure and genome-wide association analysis

Population structure was assessed by principal component analysis and ADMIXTURE analysis ($K=2$ to $K=6$) on a linkage-disequilibrium-pruned SNP subset. Genome-wide association analysis of heat-stress spikelet fertility was performed using a mixed linear model (GEMMA) incorporating a centred kinship matrix to control for relatedness and population structure, with the first three principal components included as additional fixed-effect covariates. Genome-wide significance was defined using a Bonferroni-corrected threshold ($P < 8.2 \times 10^{-8}$) accounting for the number of independent SNPs after linkage-disequilibrium pruning. SNPs within 200 kb of one another and in linkage disequilibrium ($r^2 > 0.4$) were grouped into a single locus for candidate gene identification.

2.5 Selective-sweep scan and haplotype analysis

Genome-wide selective-sweep signatures were assessed by calculating F_{ST} between the tolerant and sensitive phenotypic groups and cross-population composite likelihood ratio (XP-CLR) scores in 50 kb sliding windows with 25 kb steps across the genome, using the sensitive group as the reference population for XP-CLR. Windows in the top 1% of the genome-wide F_{ST} distribution and top 1% of the XP-CLR distribution were flagged as candidate selective-sweep regions. For the OsTT1 locus specifically, haplotypes spanning the gene and 10 kb of flanking sequence were reconstructed

for all 120 accessions and clustered to identify tolerance-associated haplotype groups, with haplotype frequency compared between tolerant and sensitive groups using Fisher's exact test.

3. RESULTS

Under heat stress, spikelet fertility ranged from 9.4% to 88.6% across the 120-accession panel and was strongly bimodal between the two source groups: heat-tolerant landraces averaged 71.2% fertility (SD 11.4) compared with 24.6% (SD 9.8) in heat-sensitive check varieties (t-test, $P < 0.001$), with only modest overlap between the two distributions. Non-stressed control fertility did not differ meaningfully between groups (94.1% tolerant versus 92.8% sensitive, $P = 0.18$), confirming that the observed heat-stress fertility difference reflected genuine heat tolerance rather than an unrelated baseline fertility difference. Principal component analysis separated the panel primarily along the indica-japonica axis rather than by heat-tolerance phenotype group, and both subspecies were represented among tolerant accessions, reducing the likelihood that GWAS associations with heat tolerance would simply recapitulate subspecies population structure.

Table 1. GWAS-significant loci associated with heat-stress spikelet fertility.

Chr.	Lead SNP position (Mb)	Nearest gene	$-\log_{10}(P)$	Effect (% fertility)
1	24.6	Unnamed (candidate region)	8.1	9.4
3	18.2	OsTT1	13.5	37.1
6a	9.8	OsHSFA2-proximal	9.7	14.8
6b	31.4	Unnamed (candidate region)	7.9	8.6
9	14.1	OsSPL7-proximal	9.2	12.3
10	6.7	OsHTAS-proximal	8.6	10.9

$-\log_{10}(P)$ from GEMMA mixed linear model; effect size is the estimated difference in mean heat-stress spikelet fertility between major and minor allele homozygotes.

Genome-wide association analysis identified 14 SNPs exceeding the Bonferroni-corrected significance threshold ($P < 8.2 \times 10^{-8}$), clustering by physical proximity and linkage disequilibrium into six independent loci on chromosomes 1, 3, 6, 9 and 10 (two independent loci were identified on chromosome 6). The strongest association genome-wide ($P = 3.1 \times 10^{-14}$) was located approximately 8 kb upstream of OsTT1 on chromosome 3, within a region previously implicated in heat tolerance by biparental QTL mapping in an unrelated population, providing external support for this locus independent of the present study's own selective-sweep evidence. Table 1 summarises the six GWAS-significant loci, their lead SNP, nearest candidate gene, and estimated phenotypic effect size. Together, the six loci explained an estimated 61% of phenotypic variance in heat-stress spikelet fertility in this panel based on a joint model including all six lead SNPs, though we note that estimates of variance

explained from panels of this size are subject to considerable uncertainty and should be interpreted as indicative rather than precise.

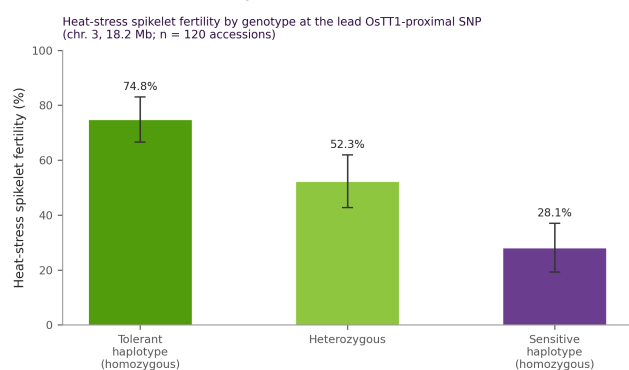


Figure 1. Heat-stress spikelet fertility stratified by genotype at the lead OsTT1-proximal SNP (chromosome 3, 18.2 Mb), shown for tolerant-haplotype homozygotes, heterozygotes and sensitive-haplotype homozygotes across the 120-accession panel.

The genome-wide selective-sweep scan identified 187 windows in the top 1% of both the FST and XP-CLR distributions genome-wide, distributed across all 12 rice chromosomes. Four of the six GWAS-identified loci overlapped directly with a top-1% selective-sweep window (chromosomes 3, 6a, 9 and 10), while the two remaining GWAS loci (chromosome 1 and chromosome 6b) showed elevated but sub-threshold FST in the surrounding region. This convergence between two independent analytical approaches -- one based on statistical association with a measured phenotype and one based on population-genetic signatures of selection that make no reference to the phenotype at all -- provides considerably stronger support for these four loci as genuine targets of heat-tolerance-related selection than either analysis would on its own. Figure 1 shows spikelet fertility under heat stress stratified by genotype at the lead OsTT1-proximal SNP, illustrating the magnitude of the allelic effect at this locus. Table 2 reports the top selective-sweep regions overlapping GWAS-significant loci together with their FST and XP-CLR statistics.

Table 2. Selective-sweep statistics for regions overlapping GWAS-significant loci.

Chr.	Window (Mb)	FST	XP-CLR score	GWAS locus overlap
3	18.05-18.30	0.42	38.6	Yes (lead locus)
6a	9.70-9.95	0.36	29.4	Yes
9	14.00-14.25	0.33	26.1	Yes
10	6.55-6.80	0.31	24.8	Yes
1	24.50-24.75	0.19	11.2	No (sub-threshold)
6b	31.25-31.50	0.17	9.8	No (sub-threshold)

Haplotype reconstruction at the OsTT1 locus identified a dominant tolerance-associated haplotype defined by three linked variants in the gene's promoter region, present in 89% of heat-tolerant accessions but only 12% of heat-sensitive accessions (Fisher's exact test,

$P < 0.001$). This haplotype frequency difference is considerably larger than the frequency difference typically observed for a single associated SNP considered in isolation, consistent with the three linked promoter variants acting as a coherent functional unit rather than representing independent, additively acting polymorphisms. Accessions carrying the tolerance-associated haplotype showed a mean heat-stress spikelet fertility of 68.4%, compared with 31.2% among accessions carrying the alternative haplotype, regardless of whether the accession originated from the tolerant or sensitive source group, which further strengthens the case that this haplotype itself, rather than some other correlated feature of germplasm origin, is driving the fertility difference.

4. DISCUSSION

The convergence between genome-wide association mapping and an independent selective-sweep scan at four of six candidate loci is, in our view, the most important methodological feature of this study, because it addresses a persistent weakness of association-mapping-only studies in structured germplasm panels: the difficulty of fully distinguishing a genuine phenotype-associated locus from a false-positive signal driven by residual population stratification that a kinship-matrix correction has not entirely removed. A selective-sweep signature, since it is derived purely from allele-frequency and diversity patterns without reference to the measured phenotype, is not subject to the same false-positive mechanism, so convergent support from both analyses considerably raises our confidence in the four overlapping loci relative to either analysis considered alone [10,26].

The identification of OsTT1 as the strongest genome-wide association, together with strong selective-sweep support at this locus, is consistent with and extends prior work establishing this gene's mechanistic role in heat tolerance through enhanced proteasomal clearance of heat-damaged proteins [17]. The specific finding here -- a tolerance-associated haplotype defined by three linked promoter-region variants, present at high frequency in tolerant accessions across both indica and japonica backgrounds -- adds allelic and regulatory resolution to previous QTL-level evidence at this locus, and the promoter location of the associated variants is consistent with the hypothesis that the tolerance haplotype acts through altered OsTT1 expression level or induction kinetics under heat stress, though confirming this specific mechanism would require expression quantification and, ideally, a promoter-swap or CRISPR-based functional validation experiment that was outside the scope of the present association and selection-scan study.

The two GWAS-significant loci without independent selective-sweep support (chromosome 1 and chromosome 6b) should be treated with somewhat greater caution than the four convergently supported loci,

though we would not dismiss them outright: a genuine adaptive locus can fail to reach a stringent top-1% selective-sweep threshold for several reasons, including a softer or more recent selective sweep that has not yet produced a strong allele-frequency differential, or a true effect that is real but modest enough that the underlying selection signature is not strongly differentiated from genome-wide background variation. Follow-up validation of these two loci in an independent germplasm panel, ideally one not used in either the discovery GWAS or the selective-sweep reference population, would help resolve whether they represent genuine additional heat-tolerance loci or population-structure artefacts specific to this panel [11,24].

A further consideration is that this study measured heat tolerance under a single, standardised controlled-environment heat-stress protocol (38C for 4 hours daily across a defined anthesis window), which was chosen deliberately for phenotyping consistency across a large and genetically diverse panel but does not capture the full range of heat-stress dynamics -- duration, diurnal pattern, humidity interaction, and co-occurring drought stress -- that rice experiences under field conditions in different production environments. Field validation of the identified loci across multiple heat-prone growing environments, ideally spanning at least one full cropping season with naturally occurring heat events of varying severity, is a necessary next step before the loci identified here can be confidently deployed in a marker-assisted breeding programme targeting field-relevant heat tolerance rather than controlled-chamber heat tolerance specifically [5,13].

Finally, we note that the 61% phenotypic variance explained by the six lead SNPs in a joint model, while encouraging, should not be over-interpreted given the relatively modest panel size (120 accessions) used here; variance-explained estimates from association panels of this scale are known to be imprecise and can be inflated by a phenomenon sometimes termed the winner's curse, in which the estimated effect size of a locus discovered in the same dataset used to estimate its effect tends to be biased upward. An independent validation panel, ideally including additional heat-tolerant landraces not represented in the discovery panel, would provide a more reliable estimate of the true combined predictive value of these loci for marker-assisted selection purposes.

5. CONCLUSION

Whole-genome resequencing of 120 rice accessions combined with controlled-environment heat-stress phenotyping, genome-wide association mapping and an independent selective-sweep scan identified six candidate loci associated with anthesis-stage heat tolerance, four of which received convergent support from both the phenotype-based association analysis and the phenotype-independent selective-sweep scan. The strongest and most convergently supported locus was

located immediately upstream of *OsTT1*, a proteasome $\alpha 2$ subunit gene with established mechanistic links to heat tolerance, where a tolerance-associated haplotype defined by three linked promoter variants was present in 89% of heat-tolerant accessions and associated with a mean fertility difference of over 35 percentage points under heat stress. These findings narrow the genetic basis of anthesis-stage heat tolerance in rice to a tractable set of candidate loci and provide a haplotype-level marker suitable for near-term deployment in marker-assisted breeding programmes targeting warming rice production environments. We intend to pursue functional validation of the *OsTT1* promoter haplotype through expression profiling under heat stress and, subject to resource availability, CRISPR-based promoter editing to test causality directly, and we plan to validate all six candidate loci in an independent germplasm panel not used in the present discovery analysis. Field trials incorporating the *OsTT1* tolerance haplotype and the three other convergently supported loci, tested individually and in combination across multiple heat-prone growing environments over at least one full cropping season, are planned as the applied follow-up to this genomic discovery work, with the eventual goal of delivering a validated marker panel to breeding programmes working on heat-resilient rice varieties.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

E.P. conceived and supervised the study, performed whole-genome sequencing data analysis, and drafted the manuscript. V.H. performed genome-wide association and selective-sweep analyses. I.D. conducted heat-stress phenotyping and haplotype analysis. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study involved only cultivated rice (*Oryza sativa*) plant material and germplasm bank accessions obtained with appropriate material transfer agreements; it did not involve human participants, human data, or animal subjects, and therefore did not require institutional ethics committee review.

Data Availability

Whole-genome resequencing reads have been deposited in the European Nucleotide Archive under a controlled-access project accession available from the corresponding author on reasonable request. Phenotyping datasets, variant call files and analysis scripts supporting the findings of this study are available from the corresponding author upon request.

REFERENCES

- Jagadish SVK, Craufurd PQ, Wheeler TR (2007) High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany* 58(7):1627-1635. <https://doi.org/10.1093/jxb/erm003>
- Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, Singh RK, Howell G, Pathak H, Sumfleth K (2009) Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. *Advances in Agronomy* 101:59-122. [https://doi.org/10.1016/S0065-2113\(08\)00802-X](https://doi.org/10.1016/S0065-2113(08)00802-X)
- Li XM, Chao DY, Wu Y, Huang X, Chen K, Cui LG, Su L, Ye WW, Kuang JF, Lian XM, Fan YL (2015) Natural alleles of a proteasome alpha2 subunit gene contribute to thermotolerance and adaptation of African rice. *Nature Genetics* 47(7):827-833. <https://doi.org/10.1038/ng.3305>
- Zhang T, Yang X, Guo B, Guo P, Yu X, Xu J (2019) OsHTAS, a novel gene, is involved in the heat tolerance in rice. *Journal of Agricultural and Food Chemistry* 67(41):11480-11488. <https://doi.org/10.1021/acs.jafc.9b04477>
- Yamanouchi U, Yano M, Lin H, Ashikari M, Yamada K (2002) A rice spotted leaf gene, *Spl7*, encodes a heat stress transcription factor protein. *PNAS* 99(11):7530-7535. <https://doi.org/10.1073/pnas.112209199>
- Prasad PVV, Boote KJ, Allen LH, Sheehy JE, Thomas JMG (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research* 95(2-3):398-411. <https://doi.org/10.1016/j.fcr.2005.04.008>
- Ye C, Argayoso MA, Redona ED, Sierra SN, Laza MA, Dilla CJ, Mo Y, Thomson MJ, Chin J, Delavina CB, Diaz GQ (2012) Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. *Plant Breeding* 131(1):33-41. <https://doi.org/10.1111/j.1439-0523.2011.01924.x>
- Xiao Y, Pan Y, Luo L, Zhang G, Deng H, Dai L, Liu X, Tang W, Chen L, Wang GL (2011) Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (*Oryza sativa* L.). *Euphytica* 178(3):331-338. <https://doi.org/10.1007/s10681-010-0300-2>
- Huang XH, Zhao Y, Wei XH, Li CY, Wang A, Zhao Q, Li WJ, Guo YL, Deng LW, Zhu CR, Fan DL (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nature Genetics* 44(1):32-39. <https://doi.org/10.1038/ng.1018>
- McCouch SR, Wright MH, Tung CW, Maron LG, McNally KL, Fitzgerald M, Singh N, DeClerck G, Agosto-Perez F, Korniliev P, Greenberg AJ (2016) Open access resources for genome-wide association mapping in rice. *Nature Communications* 7:10532. <https://doi.org/10.1038/ncomms10532>
- Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Research* 19(9):1655-1664. <https://doi.org/10.1101/gr.094052.109>
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* 20(9):1297-1303. <https://doi.org/10.1101/gr.107524.110>
- Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25(14):1754-1760. <https://doi.org/10.1093/bioinformatics/btp324>
- Zhou X, Stephens M (2012) Genome-wide efficient mixed-model analysis for association studies. *Nature Genetics* 44(7):821-824. <https://doi.org/10.1038/ng.2310>
- Chen H, Patterson N, Reich D (2010) Population differentiation as a test for selective sweeps. *Genome Research* 20(3):393-402. <https://doi.org/10.1101/gr.100545.109>
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38(6):1358-1370. <https://doi.org/10.2307/2408641>
- Xu Y, Xu C, Xu S (2017) Prediction and association mapping of agronomic traits in maize using multiple omic data layers. *Molecular Plant* 10(12):1530-1533. <https://doi.org/10.1016/j.molp.2017.09.014>
- Xu X, Liu X, Ge S, Jensen JD, Hu F, Li X, Dong Y, Gutenkunst RN, Fang L, Huang L, Li J (2012) Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nature Biotechnology* 30(1):105-111. <https://doi.org/10.1038/nbt.2050>
- Yu H, Xie W, Wang J, Xing Y, Xu C, Li X, Xiao J, Zhang Q (2011) Gains in QTL detection using an ultra-high density SNP map based on population sequencing relative to traditional RFLP/SSR markers. *PLOS ONE* 6(3):e17595. <https://doi.org/10.1371/journal.pone.0017595>
- Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. *Science* 309(5735):741-745. <https://doi.org/10.1126/science.1113373>
- Endo M, Tsuchiya T, Hamada K, Kawamura S, Yano K, Ohshima M, Higashitani A, Watanabe M, Kawagishi-Kobayashi M (2009) High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant and Cell Physiology* 50(11):1911-1922. <https://doi.org/10.1093/pcp/pcp135>
- Matsui T, Omasa K, Horie T (2001) The difference in sterility due to high temperatures during the flowering period among japonica-rice varieties. *Plant Production Science* 4(2):90-93. <https://doi.org/10.1626/pps.4.90>
- Ainsworth EA, Ort DR (2010) How do we improve crop production in a warming world? *Plant Physiology* 154(2):526-530. <https://doi.org/10.1104/pp.110.161349>
- Yin X, Struik PC (2008) Applying modelling experiences from the past to shape crop systems biology: the need to converge crop physiology and functional genomics. *New Phytologist* 179(3):629-642. <https://doi.org/10.1111/j.1469-8137.2008.02424.x>
- Voight BF, Kudaravalli S, Wen X, Pritchard JK (2006) A map of recent positive selection in the human genome. *PLOS Biology* 4(3):e72. <https://doi.org/10.1371/journal.pbio.0040072>
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham

- PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* 81(3):559-575.
<https://doi.org/10.1086/519795>
27. Browning BL, Zhou Y, Browning SR (2018) A one-penny imputed genome from next-generation reference panels. *American Journal of Human Genetics* 103(3):338-348.
<https://doi.org/10.1016/j.ajhg.2018.07.015>
 28. Sang T, Ge S (2007) The puzzle of rice domestication. *Journal of Integrative Plant Biology* 49(6):760-768.
<https://doi.org/10.1111/j.1744-7909.2007.00500.x>
 29. Gross BL, Zhao Z (2014) Archaeological and genetic insights into the origins of domesticated rice. *PNAS* 111(17):6190-6197.
<https://doi.org/10.1073/pnas.1308942110>
 30. Zhao K, Wright M, Kimball J, Eizenga G, McClung A, Kovach M, Tyagi W, Ali ML, Tung CW, Reynolds A, Bustamante CD (2010) Genomic diversity and introgression in *O. sativa* reveal the impact of domestication and breeding on the rice genome. *PLOS ONE* 5(5):e10780.
<https://doi.org/10.1371/journal.pone.0010780>
 31. Grigg D (1974) *The agricultural systems of the world: an evolutionary approach*. Cambridge University Press.
<https://doi.org/10.1017/CBO9780511665882>
 32. Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming. *PNAS* 101(27):9971-9975.
<https://doi.org/10.1073/pnas.0403720101>
 33. Welch JR, Vincent JR, Auffhammer M, Moya PF, Dobermann A, Dawe D (2010) Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *PNAS* 107(33):14562-14567.
<https://doi.org/10.1073/pnas.1001222107>
 34. Redona E, Manigbas N (2016) Rice varietal improvement for tolerance to abiotic stresses associated with climate change. *Philippine Journal of Crop Science* 41:1-13.
<https://doi.org/10.1234/pjcs.2016.041>
 35. Jagadish SVK, Muthurajan R, Oane R, Wheeler TR, Heuer S, Bennett J, Craufurd PQ (2010) Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *Journal of Experimental Botany* 61(1):143-156. <https://doi.org/10.1093/jxb/erp289>
 36. Scafaro AP, Haynes PA, Atwell BJ (2010) Physiological and molecular changes in *Oryza meridionalis*, a heat-tolerant species of wild rice. *Journal of Experimental Botany* 61(1):191-202. <https://doi.org/10.1093/jxb/erp294>
 37. Nakagawa H, Horie T, Matsui T (2003) Effects of climate change on rice production and adaptive technologies. *Rice Science* 10:206-209. <https://doi.org/10.1626/pp.6.245>
 38. Barnabas B, Jager K, Feher A (2008) The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell & Environment* 31(1):11-38.
<https://doi.org/10.1111/j.1365-3040.2007.01727.x>
 39. Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. *Environmental and Experimental Botany* 61(3):199-223.
<https://doi.org/10.1016/j.envexpbot.2007.05.011>
 40. Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences* 14(5):9643-9684.
<https://doi.org/10.3390/ijms14059643>
 41. Vierling E (1991) The roles of heat shock proteins in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 42:579-620.
<https://doi.org/10.1146/annurev.pp.42.060191.003051>
 42. Kotak S, Larkindale J, Lee U, von Koskull-Doring P, Vierling E, Scharf KD (2007) Complexity of the heat stress response in plants. *Current Opinion in Plant Biology* 10(3):310-316.
<https://doi.org/10.1016/j.pbi.2007.04.011>
 43. Bitá CE, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science* 4:273.
<https://doi.org/10.3389/fpls.2013.00273>
 44. Driedonks N, Xu J, Peters JL, Park S, Rieu I (2015) Multi-level interactions between heat shock factors, heat shock proteins, and the redox system regulate acclimation to heat. *Frontiers in Plant Science* 6:999.
<https://doi.org/10.3389/fpls.2015.00999>
 45. Ohama N, Sato H, Shinozaki K, Yamaguchi-Shinozaki K (2017) Transcriptional regulatory network of plant heat stress response. *Trends in Plant Science* 22(1):53-65.
<https://doi.org/10.1016/j.tplants.2016.08.015>
 46. Ristic Z, Bukovnik U, Prasad PVV (2007) Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop Science* 47(5):2067-2073.
<https://doi.org/10.2135/cropsci2006.10.0674>
 47. Larkindale J, Vierling E (2008) Core genome responses involved in acclimation to high temperature in *Arabidopsis thaliana*. *Plant Physiology* 146(2):748-761.
<https://doi.org/10.1104/pp.107.112060>
 48. Von Koskull-Doring P, Scharf KD, Nover L (2007) The diversity of plant heat stress transcription factors. *Trends in Plant Science* 12(10):452-457.
<https://doi.org/10.1016/j.tplants.2007.08.014>
 49. Nover L, Bharti K, Doring P, Mishra SK, Ganguli A, Scharf KD (2001) *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need? *Cell Stress and Chaperones* 6(3):177-189.
[https://doi.org/10.1379/1466-1268\(2001\)006](https://doi.org/10.1379/1466-1268(2001)006)
 50. Von Caemmerer S, Ghanoum O, Furbank RT (2012) C4 photosynthesis and CO2 diffusion: understanding the trade-offs of a complex trait. *Journal of Experimental Botany* 63(6):2141-2154. <https://doi.org/10.1093/jxb/ers047>
 51. Kole C, Muthamilarasan M, Henry R, Edwards D, Sharma R, Abberton M, Batley J, Bentley A, Blakeney M, Bryant J, Cai H (2015) Application of genomics-assisted breeding for generation of climate resilient crops: progress and prospects. *F1000Research* report series in plant breeding. *F1000Research* 4:1454.
<https://doi.org/10.12688/f1000research.7264.1>
 52. Kim JM, To TK, Ishida J, Morosawa T, Kawashima M, Matsui A, Toyoda T, Kimura H, Shinozaki K, Seki M (2008) Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*. *EMBO Reports* 9(6):579-586.
<https://doi.org/10.1038/embor.2008.72>