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Author/s:

Harrop, TWR; Mantegazza, O; Luong, AM; Béthune, K; Lorieux, M; Jouannic, S; Adam, H; Wellmer, F

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RESEARCH PAPER

# A set of *AP2*-like genes is associated with inflorescence branching and architecture in domesticated rice

Thomas W. R. Harrop<sup>1</sup>, Otho Mantegazza<sup>2</sup>, Ai My Luong<sup>2</sup>, Kevin Béthune<sup>2</sup>, Mathias Lorieux<sup>3</sup>, Stefan Jouannic<sup>2</sup> and Hélène Adam<sup>2,\*</sup> 

<sup>1</sup> Laboratory for Evolution and Development, Department of Biochemistry, University of Otago, PO Box 56, Dunedin 9054, Aotearoa New Zealand

<sup>2</sup> University of Montpellier, DIADE, IRD, France

<sup>3</sup> Rice genetics and Genomics Laboratory, International Center for Tropical Agriculture, Cali 6713, Colombia

\* Correspondence: [helene.adam@ird.fr](mailto:helene.adam@ird.fr)

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## Abstract

Rice yield is influenced by inflorescence size and architecture, and inflorescences from domesticated rice accessions produce more branches and grains. Neither the molecular control of branching nor the developmental differences between wild and domesticated rice accessions are fully understood. We surveyed phenotypes related to branching, size, and grain yield across 91 wild and domesticated African and Asian accessions. Characteristics related to axillary meristem identity were the main phenotypic differences between inflorescences from wild and domesticated accessions. We used whole transcriptome sequencing in developing inflorescences to measure gene expression before and after the transition from branching axillary meristems to determinate spikelet meristems. We identified a core set of genes associated with axillary meristem identity in Asian and African rice, and another set associated with phenotypic variability between wild and domesticated accessions. *AP2/EREBP*-like genes were enriched in both sets, suggesting that they are key factors in inflorescence branching and rice domestication. Our work has identified new candidates in the molecular control of inflorescence development and grain yield, and provides a detailed description of the effects of domestication on phenotype and gene expression.

**Keywords:** *AP2/EREBP*-like genes, development, domestication, inflorescence, rice.

## Introduction

Rice produces grains on complex raceme inflorescences called panicles, which consist of a series of branches of different orders. The rachis is the main axis, and primary, secondary, and tertiary branches form higher-order axes (see [Supplementary Fig. S1](#) at *JXB* online). Grain yield in rice is linked to inflorescence branching, because the number of spikelets produced on the higher-order branches determines the number of grains per panicle ([Doebley et al., 2006](#); [Xing and Zhang, 2010](#); [Olsen](#)

and [Wendel, 2013](#)). Inflorescence size and architecture are key targets in selective breeding for improved rice grain yield and quality ([Doust, 2007](#); [Wang and Li, 2011](#)), and these characteristics are different between domesticated and wild accessions.

Inflorescence branching and the number of grains per inflorescence vary between clades, species, and accessions of rice in the genus *Oryza* ([Yamaki et al., 2010](#); [Taguchi-Shiobara et al., 2011](#)). Asian and African clades diverged around 2 million years

ago and were domesticated independently (Zhu and Ge, 2005). Domesticated Asian rice, *Oryza sativa*, diverged 10 000 years ago via a complex domestication process from the wild Asian rice species, *Oryza rufipogon*. The *O. sativa* species complex involves a network of subspecies, including *O. sativa* ssp. *indica* and *O. sativa* ssp. *japonica* (Huang *et al.*, 2012; Choi *et al.*, 2017; Stein *et al.*, 2018). Domesticated African rice, *Oryza glaberrima*, has a simpler history. It was domesticated from its wild relative, *Oryza barthii*, 3000 years ago, without subsequent introgression (Vaughan *et al.*, 2008; Cubry *et al.*, 2018). In both African and Asian clades, domesticated species produce inflorescences that are more complex and ramified than those of their wild relatives (Linares, 2002; Yamaki *et al.*, 2010; Ta *et al.*, 2017).

Panicle structure is established early after floral transition. The vegetative shoot meristem develops into a reproductive rachis meristem, which produces axillary meristems until its abortion. The axillary meristems on the rachis all develop as primary branches, which themselves produce a variable number of axillary meristems. Axillary meristems on the primary branches can differentiate into secondary branches, some of which may also produce tertiary branch meristems. Axillary meristems on the primary branches can also differentiate directly into spikelet meristems. Finally, all axillary meristems and terminal meristems differentiate gradually from the top to the base of the panicle into spikelet meristems and then florets. Each rice grain is produced from a single spikelet (reviewed by Itoh *et al.*, 2005).

The complexity of panicle branching and the number of grains are determined by two basic developmental outcomes: the number of axillary meristems produced along all panicle axes; and the rate of meristem fate transition, which determines whether an axillary meristem grows into a higher-order branch or differentiates into a spikelet and gives rise to a grain. The maturation rate and identity of reproductive and axillary meristems are controlled by transcriptional regulators, some of which have been identified in *O. sativa* (Xing and Zhang, 2010; Wang and Li, 2011). Genes including *G1 LIKE PROTEIN 5* (*TAWAWA1/TAW1*), *LAX PANICLE 1* (*LAX1*), and *LAX PANICLE 2* (*LAX2*) control the branching phase via establishment and activity of indeterminate meristems (Komatsu *et al.*, 2001; Tabuchi *et al.*, 2011; Yoshida *et al.*, 2013). Genes such as *SUPERNUMERARY BRACT GENE* (*SNB*), *FRIZZY PANICLE* (*FZP*), and *LEAFY HULL STERILE 1* (*LHS1*) regulate the transition from indeterminate meristems to determinate spikelet meristems and the subsequent transition from spikelet to floret meristem (Jeon *et al.*, 2000; Komatsu *et al.*, 2001; Agrawal *et al.*, 2005; Chen *et al.*, 2006; Lee *et al.*, 2007; Khanday *et al.*, 2013). Regulatory changes in some genes, including *FZP*, *WEALTHY FARMER'S PANICLE* (*IDEAL PLANT ARCHITECTURE 1*, *WFP/IPA1/SPL14*), *GRAIN SIZE 2* (*G22/GRF4*), and *GRF6*, are also associated with modified panicle phenotype (Jiao *et al.*, 2010; Miura *et al.*, 2010; Bai *et al.*, 2016; Duan *et al.*, 2015; Huang *et al.*, 2018).

Despite advances in understanding molecular mechanisms that regulate panicle branching in rice (Furutani *et al.*, 2006; Harrop *et al.*, 2016), and characterization of individual genes associated with variation in panicle branching (Bai *et al.*, 2012; Ikeda *et al.*, 2013), the transcriptional control of

axillary meristem identity that underlies the phenotypic diversity of branching across rice species is not fully understood. The independent domestications of African and Asian rice in the genus *Oryza* provide a comparative context to study the evolution of agronomic traits such as panicle architecture, the mechanisms underlying parallel evolution of phenotype, and the basic molecular control of inflorescence branching. Here, we phenotyped panicles from domesticated and wild accessions of Asian and African rice, and used whole-transcriptome sequencing (RNAseq) to reveal gene expression patterns associated with the diversity of inflorescence architecture. Our analysis reveals an association between expression of *AP2/EREBP*-like genes, panicle architecture and domestication.

## Materials and methods

### Plant material and growth conditions

Panicle morphological traits were measured in 91 accessions of *O. rufipogon*, *O. sativa*, *O. glaberrima*, and *O. barthii*, grown in Cali, Colombia and Montpellier, France (Supplementary Table S1). At panicle maturity, we collected the three main panicles from three plants per accession, per replicate (i.e. 18 panicles per accession). We used four accessions for expression analysis: *O. sativa* ssp. *indica* IR64, *O. rufipogon* W1654, *O. glaberrima* Tog5681, and *O. barthii* B88. These accessions were grown in a greenhouse in Montpellier, France, in June 2014, under long day conditions (14 h light–10 h dark). After 6–8 weeks they were transferred to short day conditions (11 h light–13 h dark) to induce flowering. To confirm panicle phenotypes in the growth conditions used for RNAseq, we evaluated panicle traits for nine panicles from each accession, which were grown in the greenhouse under the same growth conditions. The *arl5* and *smos1-3* mutants (Kitomi *et al.*, 2011; Aya *et al.*, 2014) were grown in a greenhouse in Montpellier, France, in October 2017 under short day conditions (11 h light–13 h dark). At least 18 panicles were used for panicle phenotyping. All greenhouse plants were grown at 28 °C with 80% relative humidity. For phenotyping analyses, each panicle was spread out and fixed on white paper using adhesive tape. Panicles were photographed and the images were used for panicle structure and seed number analysis with P-TRAP software (AL-Tam *et al.*, 2013).

### Tissue collection and RNA sequencing

For expression analysis, we collected 15 immature panicles each from at least 10 plants per accession, per stage, collected from 4 to 15 d after floral induction (i.e. transfer to short day conditions) for each biological replicate. For sample collection, leaves surrounding the young panicle were removed by hand and the reproductive tissue was cut with a sharp blade under a Stemi 508 (Zeiss, Germany) stereo microscope to identify developmental stage. The reproductive tissues were immediately frozen in liquid nitrogen, and total RNA including small RNA was extracted using the RNeasy Plant Mini kit with RLT and RWT buffers (Qiagen, Germany). DNase treatments were performed using the RNase-free DNase set (Qiagen, Germany). RNA integrity numbers of the extracted RNA, measured using a 2100 Bioanalyzer (Agilent, USA), were between 8.6 and 10. Stage specificity was validated with quantitative real-time RT-PCR (qPCR) using stage-specific marker genes (Supplementary Table S2); 400 ng of total RNA was used for each sample for RNAseq library preparation with the TruSeq Stranded Total RNA with Ribozero Plant kit (Illumina, USA). After quantification with a 2100 Bioanalyzer, 125-base paired-end reads were generated on a HiSeq 2500 (Illumina) by the GeT platform (Toulouse, France).

### qPCR

cDNA was synthesized from 1 µg of DNase-treated total RNA using the SuperScript III First-Strand Synthesis System (Invitrogen, USA).

A Biomark HD Microfluidic Dynamic Array (Fluidigm, USA) was used for large-scale qPCR. A 96×96 Dynamic Array Integrated Fluidic Circuit (Fluidigm, USA) was loaded with cDNA and primer combinations after 15 cycles of specific target amplification and exonuclease I treatment. A fast cycling protocol with EvaGreen dye (Bio-Rad Laboratories, USA) was used for amplification. Three biological replicates were performed for each sample. Data were normalized using four genes (*LOC\_Os06g11170*, *LOC\_Os06g48970*, *LOC\_Os01g16970*, and *LOC\_Os03g61680*). Gene expression relative to the normalization factors was estimated using the  $2^{-\Delta\Delta CT}$  method without a calibrator sample (Livak and Schmittgen, 2001). Primer sequences are listed in Supplementary Table S2.

### Data analysis

We trimmed reads and removed adaptors with cutadapt (Martin, 2011), before mapping to the MSU v7 annotation of the *Oryza sativa* ssp. *japonica* cv. Nipponbare reference genome (Ouyang et al., 2007) using STAR in two-pass mode (Dobin et al., 2013). To generate per-library gene expression cutoffs, we used the 95th percentile of reads that mapped to intergenic regions of the genome, as described previously (Harrop et al., 2016). We used DESeq2 (Love et al., 2014) for differential expression analysis of genes that passed the cutoff. We used annotations from PlnTFDB v3.0 (Pérez-Rodríguez et al., 2010) and PlantTFDB v4.0 (Jin et al., 2017) to analyse expression of transcription factors. Soft clustering of transcription factor genes was performed with Mfuzz (Kumar and Futschik, 2007), and enrichment of transcription factor family genes was tested with the GSEA method using the FGSEA package (Subramanian et al., 2005; Sergushichev, 2016, Preprint).

### Reproducibility and data availability

Raw sequence data are hosted at the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) under accession PRJNA518559. The code we used to analyse the RNAseq data and panicle phenotype data is hosted at <https://github.com/tomharrop/5acc>, and the code for qPCR analysis is at <https://github.com/othomantegazza/5acc-qpcr>. We used snakemake (Köster and Rahmann, 2012) to arrange analysis steps into workflows and monitor dependencies, and Singularity (Kurtzer et al., 2017) to capture the computing environment. The final results and all intermediate steps can be exactly reproduced from the raw data with a single command using snakemake and Singularity. The source for this manuscript is hosted at <https://github.com/tomharrop/ird-5acc-paper>.

## Results

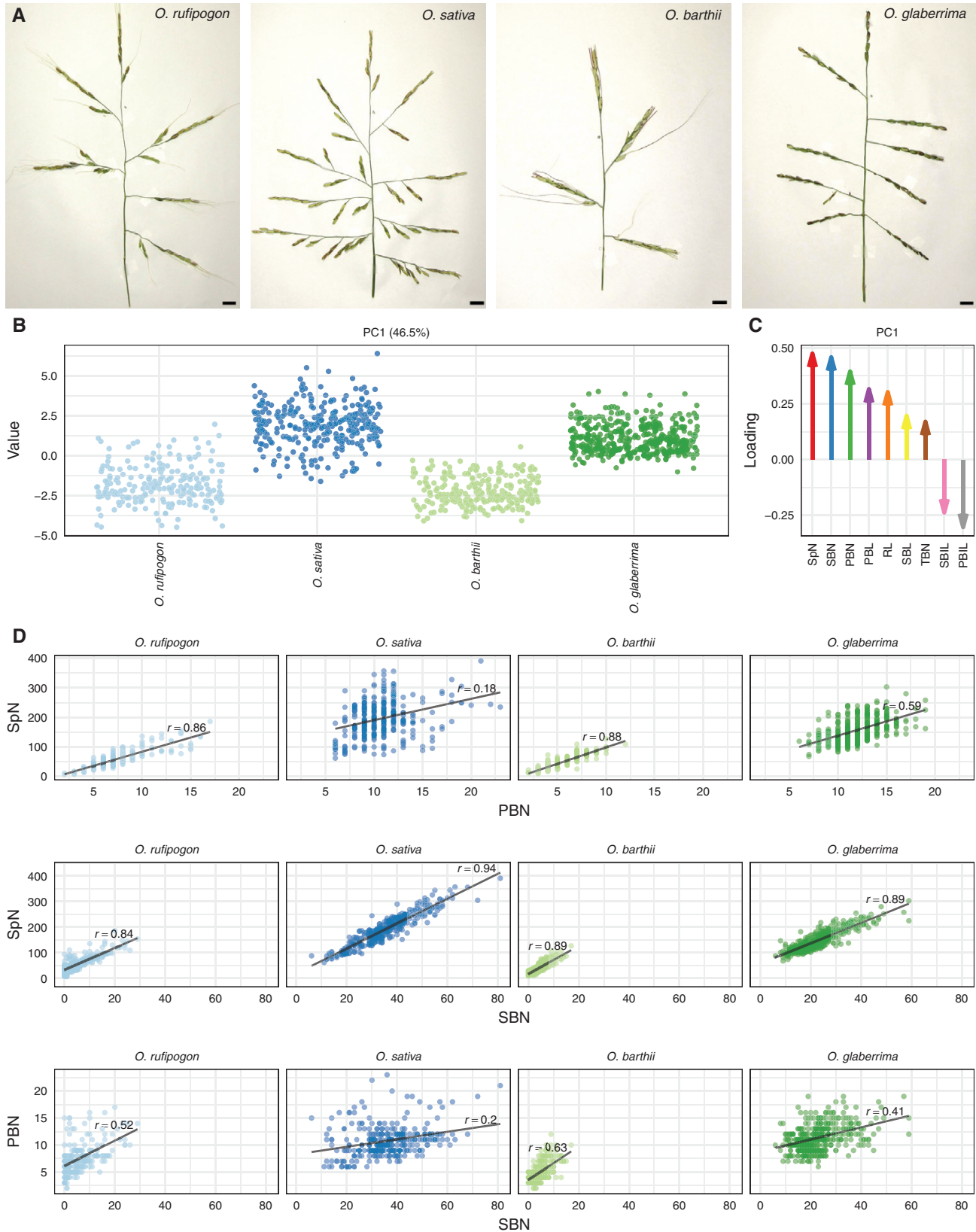
### Parallel changes in panicle architecture between wild and domesticated accessions

To measure the diversity of panicle architecture, we phenotyped 91 rice accessions (Supplementary Table S1), including wild Asian rice (*Oryza rufipogon*), domesticated Asian rice (*Oryza sativa*), wild African rice (*Oryza barthii*), and domesticated African rice (*Oryza glaberrima*), using P-TRAP image analysis software for automated measurement of traits (Fig. 1A; Supplementary Fig. S1; Supplementary Tables S1, S3; AL-Tam et al., 2013). Principal components analysis (PCA) of the phenotyping data identified a major coordinate (PC1) that accounts for 46.5% of variability (Fig. 1B). PC1 separates domesticated and wild accessions, but not Asian and African accessions, and is the only component that separates panicles from different accessions (Supplementary Fig. S2). Spikelet number, secondary branch number, and primary branch number have the highest loadings on PC1, whereas length traits have lower absolute loading on PC1 (Fig. 1C). For all species, spikelet number correlates more with secondary branch number than

it does with primary branch number. Primary branch number correlates with spikelet number more in wild than in domesticated species, but this correlation is weaker in Asian species than in African species. Primary and secondary branch numbers do not correlate, suggesting they are controlled by different genetic mechanisms (Fig. 1D). Our phenotypic analysis indicates similar changes in panicle architecture between wild and domesticated accessions in the independent African and Asian domestication processes, which presumably result from parallel, artificial selection on panicle architecture. Spikelet number, secondary branch number and primary branch number are the main contributors to these differences in panicle architecture, and these phenotypes are all related to axillary meristem formation and fate transition (Teo et al., 2014; Zhang and Yuan, 2014).

### Measurement of gene expression in developing panicles

We investigated gene expression changes associated with the diversity of panicle architecture and differences between the Asian and African domestication processes via RNAseq. We used a single accession each of domesticated Asian rice (*O. sativa* ssp. *indica* IR64) and its wild relative (*O. rufipogon* W1654), and domesticated African rice (*O. glaberrima* Tog5681) and its wild relative (*O. barthii* B88). Based on the extensive phenotyping described above, the chosen accessions are consistent with species-wide patterns of panicle architecture (Supplementary Fig. S3). To measure whole-transcriptome gene expression in these accessions, we collected immature panicles from each accession at four developmental stages: rachis meristem (RM); indeterminate meristem (IM), including panicles displaying primary branch initiation, elongation of primary branches, and axillary meristem initiation; determinate meristem (DM), including panicles wherein axillary meristems had transitioned into early spikelet differentiation; and floret meristem (FM), with early differentiation of floral organs (Supplementary Fig. S4A). We first confirmed staging of the panicles by extracting RNA from pooled immature panicles at each stage and measuring expression of markers of panicle development by quantitative real-time RT-PCR (qPCR) (Supplementary Fig. S4B). Because branching complexity is related to branch meristem establishment and meristem fate transition (Kyoizuka et al., 2014), and secondary branch number and spikelet number contribute to differences between wild and domesticated accessions (Fig. 1), we chose the IM and DM stages for RNAseq. cDNA libraries for sequencing were constructed from rRNA-depleted RNA samples from three biological replicates at both stages for all four accessions. Using the *O. sativa* ssp. *japonica* cv. Nipponbare reference genome to map reads (Ouyang et al., 2007), we obtained an average of more than 20 million uniquely mapped reads within exons for each accession, including African rice species (Supplementary Table S4). Our analysis was limited to transcripts from the four studied accessions that have homologs in the reference annotation that are similar enough for reads to map unambiguously. To allow for differences in mapping between accessions, we compared genes using between-stage read count differences within accessions, rather than read count



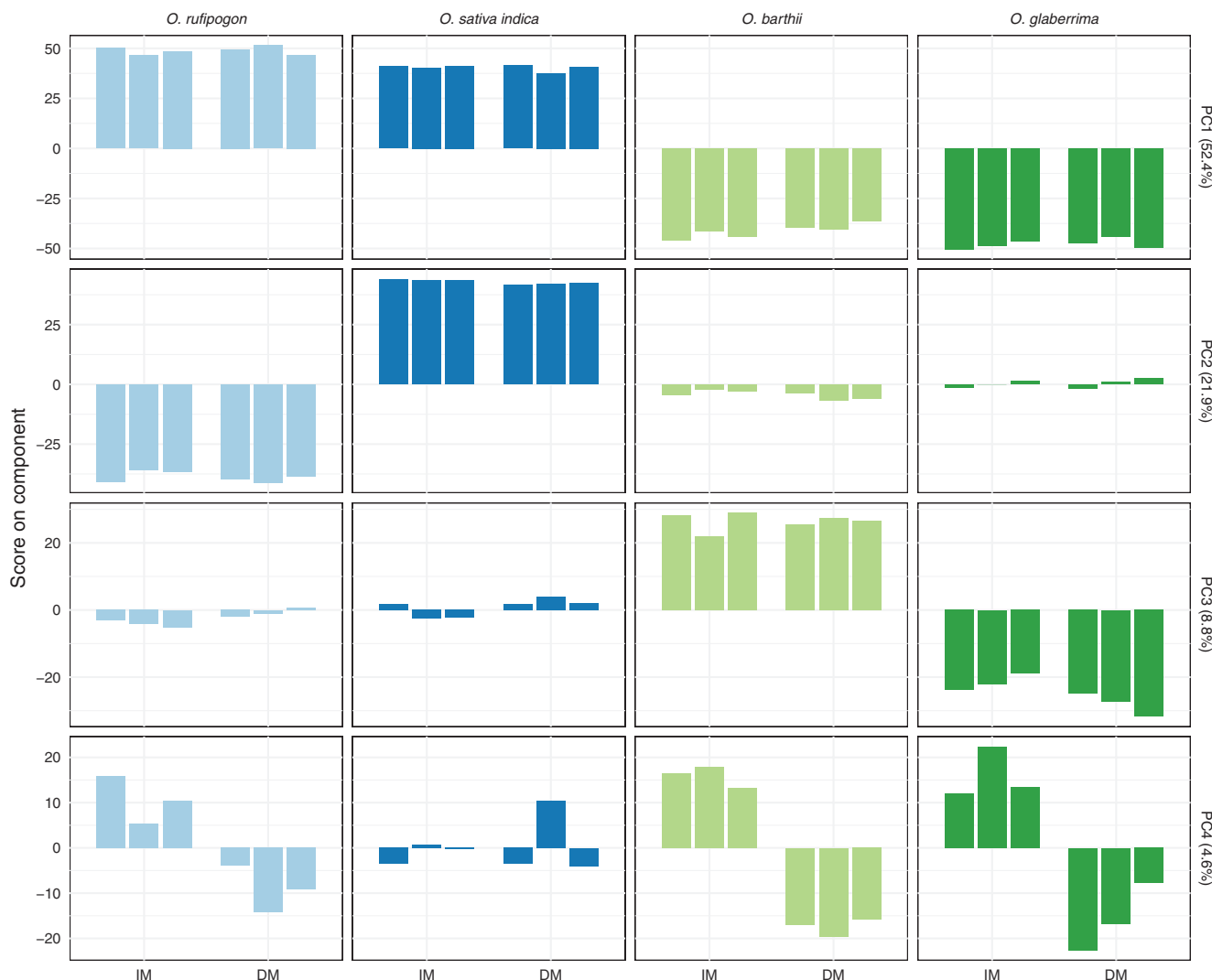
**Fig. 1.** Panicle complexity of 91 rice accessions. The main component of variability in panicle phenotypes splits accessions by domestication status, and is related to spikelet number, secondary branch number, and primary branch number. (A) We measured traits using spread panicles from *O. rufipogon*, *O. sativa*, *O. barthii*, and *O. glaberrima*. (B) The first principal component (PC1) in the panicle phenotype data accounts for 46.5% of variability and separates wild and domesticated accessions independently of continent. (C) Spikelet number (SpN), secondary branch number (SBN), and primary branch number (PBN) have the highest loadings on PC1. (D) Correlation between the main panicle traits that contribute to panicle architecture diversity. Primary branch number and spikelet number correlate in wild species. Secondary branch number and spikelet number correlate more in cultivated species than in wild species. Primary and secondary branch numbers do not correlate. PBIL, primary branch internode length; PBL, primary branch length; RL, rachis length; SBIL, secondary branch internode length; SBL, secondary branch length; TBN, tertiary branch number.

differences between accessions. Pairwise distances between samples, calculated on the number of reads per gene from all detected genes, grouped samples first by stage, then by accession, and then by continent (Supplementary Fig. S5). We did not observe grouping by domestication status, suggesting that transcriptome-wide changes during domestication are subtle compared with differences between species.

We used PCA on transformed raw counts to investigate general patterns of variation in the transcriptomes (Fig. 2). PC1 separates African and Asian accessions, and PC2 and PC3 separate wild and domesticated accessions in Asian and African samples, respectively. PC1–PC3 may relate to species-specific differences unrelated to panicle architecture, or mapping biases introduced by mapping all libraries against the *O. sativa* ssp. *japonica* reference. While the first three PCs separate different combinations of accessions, PC4 separates developmental stages across all four accessions, although separation is weaker in *O. sativa* ssp. *indica*. This suggests a conserved mechanism that controls the transition from indeterminate to determinate phase of axillary meristem development in all accessions.

### AP2/EREBP-like transcription factors are core regulators of panicle branching

To identify the core set of genes involved in axillary meristem determination in all four accessions, we used differential expression (DE) tests to find genes that were up- or down-regulated between stages across all accessions. Positive  $\log_2$ -fold change values ( $L_2FCs$ ) indicate higher expression in DM than in IM. There were 153 genes that had at least 1.5-fold DE between stages in all species at a false-discovery rate of 0.1, including 88 genes up-regulated in DM samples and 65 genes down-regulated in DM samples (Supplementary Table S5). There was an enrichment of transcription factor (TF) genes in the list of 153 differentially expressed genes (37 TF genes;  $P=6.0\times 10^{-12}$ , hypergeometric test), including *LHS1*, *LAX1*, *PANICLE PHYTOMER2 (PAP2)*, and *MOSAIC FLORAL ORGANS 1 (MFO1)*, which regulate inflorescence architecture or meristem fate transition in rice (Komatsu *et al.*, 2001; Ohmori *et al.*, 2009; Kobayashi *et al.*, 2010, 2012; Khanday *et al.*, 2013). This indicates that RNAseq of developing panicles at the IM and



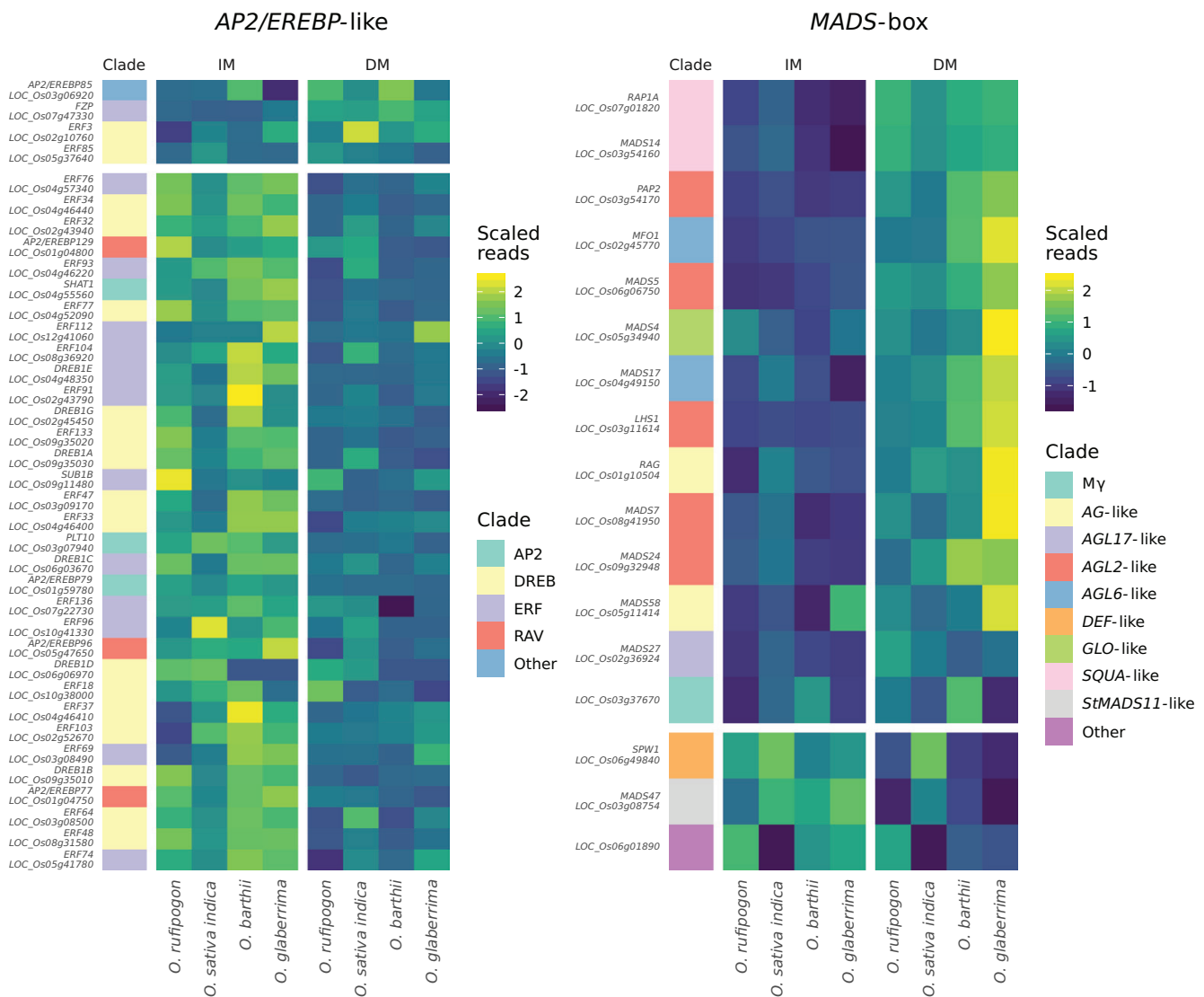
**Fig. 2.** Principal components analysis of transformed read counts for each library. Principal component 4 (PC4) separates RNAseq samples by developmental stage, and explains 4.6% of total variability. The first three components explain 83.1% of variability, and separate RNAseq samples by species. Bars show single samples (three replicates per accession per stage).

DM stage identifies genes that control branching, and suggests that transcription factors are prominent among these genes.

We used gene set enrichment analysis (GSEA; [Subramanian \*et al.\*, 2005](#); [Sergushichev, 2016](#), Preprint) on genes ranked by  $L_2FC$  to test for family-level enrichment among transcription factors. *AP2/EREBP*-like genes and MADS-box genes were both enriched ( $P_{adj}=3.5\times 10^{-5}$  and  $P_{adj}=4.0\times 10^{-4}$ , respectively, GSEA permutation *t*-test; [Supplementary Table S6](#)). Correspondingly, the list of 153 differentially expressed genes included 10 MADS-box genes and seven *AP2/EREBP*-like genes ([Supplementary Table S5](#)). Most differentially expressed MADS-box genes are more highly expressed at the DM stage, whereas most differentially expressed *AP2/EREBP*-like genes are more highly expressed at the IM stage. The majority of *AP2/EREBP*-like genes that have higher expression at the IM stage are from the ERF and DREB clades ([Fig. 3](#)). The DE

results are consistent with the role of transcriptional regulation in panicle branching, and highlight a set of candidate core regulators of axillary meristem determination and branching that are conserved between rice species. The pattern of expression of *AP2/EREBP*-like genes may indicate a role in the promotion of indeterminate axillary meristem identity or suppression of the transition from axillary meristem to spikelet meristem. MADS-box genes may have an inverse role as promoters of determinate meristem. Co-regulation of members of TF families, sometimes at the clade level, highlights the redundant or overlapping functions of TF families in meristem establishment and fate transition.

To test the role of *AP2/EREBP*-like genes in the control of panicle architecture, we phenotyped panicles from two loss-of-function mutants. The *crowns rootless5 (crl5)* mutant of the *AP2*-like gene *PLETHORA 8 (PLT8)*; [Kitomi \*et al.\*, 2011](#)) produced



**Fig. 3.** *AP2/EREBP*-like and MADS-box transcription factors change expression between IM and DM. For each family, we plotted genes that were in the top 10% of all genes by absolute  $L_2FC$  between IM and DM, without filtering on adjusted *P*-value. Genes in the upper panels had higher expression at the DM stage, whilst genes in the lower panels had higher expression at the IM stage. Most *AP2/EREBP*-like genes that pass the 10% cutoff were more highly expressed in the IM. *AP2/EREBP*-like genes that were more highly expressed in IM mainly belong to the ERF and DREB clades. In contrast, most MADS-box genes that pass the cutoff were more highly expressed at the DM stage. Clades for *AP2/EREBP*-like genes are from the Plant Transcription Factor Database v4.0 ([Jin \*et al.\*, 2017](#)) and [Sharoni \*et al.\* \(2011\)](#). MADS-box clades were manually tabulated from [Arora \*et al.\* \(2007\)](#).

panicles with a shorter rachis with fewer primary branches (Fig. 4; Supplementary Table S7), consistent with a peak of *PLT8* expression in rachis meristem tissues from *O. sativa* ssp. *japonica* (Supplementary Fig. S6; Harrop *et al.*, 2016). Panicles from the *small organ size1* (*smos1*) mutant of *ERF142* (Aya *et al.*, 2014) have a reduced number of primary and secondary branches, and fewer spikelets (Fig. 4; Supplementary Table S7). *ERF142* expression is highest in primary branch and elongating primary branch meristem tissues in *O. sativa* ssp. *japonica* (Supplementary Fig. S6; Harrop *et al.*, 2016). Although neither gene was differentially expressed in our RNAseq dataset, these phenotypes support the involvement of AP2/EREBP-like genes in control of panicle architecture.

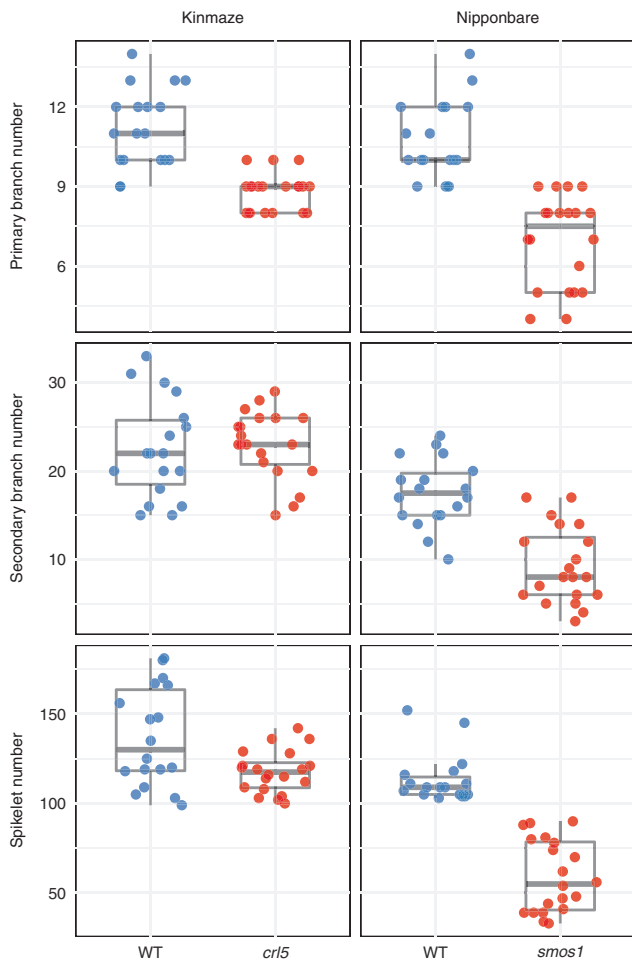
#### AP2/EREBP-like gene expression is associated with panicle diversity and domestication

To identify common patterns of expression of transcriptional regulators related to variation in panicle phenotypes, we used soft clustering of scaled L<sub>2</sub>FCs between IM and DM on the subset of annotated TF genes that were detected in our

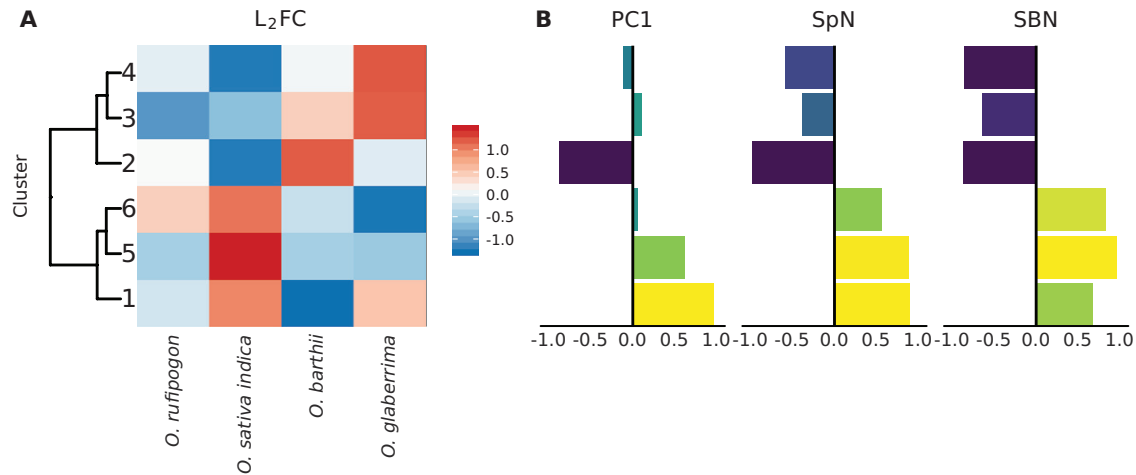
RNAseq dataset. We recovered six clusters comprising a total of 119 genes (Fig. 5; Supplementary Table S8). To determine which clusters were related to domestication, we calculated correlations between the mean L<sub>2</sub>FC value of genes in each cluster and PC1 in the phenotyping data, and between mean L<sub>2</sub>FC and the number of secondary branches and spikelets from repeat panicle phenotyping for the accessions used for RNAseq (Figs 1, 5B; Supplementary Fig. S7).

Clusters 3, 4, and 6 correlated with spikelet number (SpN) and secondary branch number (SBN), but not with PC1, meaning that the L<sub>2</sub>FC of genes in those clusters does not correlate with the phenotypic differences between wild and domesticated accessions. Clusters of genes with high L<sub>2</sub>FC in *O. sativa* ssp. *indica* have a positive correlation with SBN and SpN, whereas clusters of genes with low L<sub>2</sub>FC in *O. sativa* ssp. *indica* have a negative correlation with SBN and SpN. Cluster 4 had an enrichment of HB genes (9 out of 31 genes;  $P_{adj}=2.5\times 10^{-4}$ ). It also contained three MIKC<sup>C</sup>-type MADS-box genes (*LHS1*, *MFO1*, and *MADS14*), which promote spikelet meristem termination (Jeon *et al.*, 2000; Ohmori *et al.*, 2009; Kobayashi *et al.*, 2012), and three AP2/EREBP-like genes including *OsINDETERMINATE SPIKELET 1* (*OsIDS1*), which also controls inflorescence architecture (Chuck *et al.*, 2008; Lee and An, 2012). L<sub>2</sub>FCs of genes in this cluster are low in *O. sativa* ssp. *indica*, high in *O. glaberrima* and intermediate in the two wild accessions. Although these genes may be involved in regulation of panicle complexity, their expression did not appear to have changed in parallel in the two domestications. L<sub>2</sub>FCs of genes in clusters 3 and 6 change between accessions from the two continents. In cluster 3, L<sub>2</sub>FCs are higher in African species than in Asian species, meaning that the genes are more highly expressed in DM stages in African species. Genes in cluster 6 have the opposite pattern, with higher L<sub>2</sub>FCs in Asian species compared with African species. Cluster 3 contained *LAX1* and *ABERRANT PANICLE ORGANIZATION 2* (*FLO-LFY HOMOLOG OF RICE*, *AP02/RFL*), which are involved in axillary meristem establishment and outgrowth and promotion of indeterminate meristematic activity in rice, respectively (Komatsu *et al.*, 2001; Ikeda-Kawakatsu *et al.*, 2012). Their higher expression at the DM stage in panicles from both wild and domesticated African accessions could be associated with a reduced number of spikelets.

Clusters 1, 2, and 5 correlated with the main principal component (PC1) in the phenotyping data, which separates wild and domesticated species independently of continent. Clusters 1 and 5 are also positively correlated with spikelet number and secondary branch number, whereas cluster 2 is negatively correlated. The correlation of L<sub>2</sub>FC patterns with PC1 suggests that genes in these clusters may be associated with changes in panicle architecture between wild and domesticated species. Cluster 1 and cluster 5 both had a positive correlation with PC1. L<sub>2</sub>FCs are higher in domesticated accessions for genes in cluster 1, meaning that they are more highly expressed at the DM stage in domesticated accessions. Genes in cluster 2 have lower L<sub>2</sub>FCs in domesticated accessions, meaning that they are more highly expressed at the DM stage in wild accessions. Cluster 2 also had the strongest negative correlation with PC1, and low L<sub>2</sub>FCs in *O. sativa* ssp.



**Fig. 4.** Mutants in two AP2/EREBP-like genes, *PLT8* and *ERF142*, have defects in panicle architecture compared with their background accessions. The *crl5* mutant of *PLT8* (*LOC\_Os07g03250*) produced fewer primary branches and spikelets, and the *smos1-3* mutant of *ERF142* (*LOC\_Os05g32270*) produced fewer primary branches, secondary branches, and spikelets.



**Fig. 5.** Gene expression clusters correlate with the main component of diversity of panicle architecture (PC1) and the number of secondary branches (SBN) and spikelets (SpN). Clusters contained 19–31 genes each (Supplementary Table S8). (A) Mean, scaled log<sub>2</sub>-fold change (L<sub>2</sub>FC) of genes by cluster and accession. (B) Pearson correlation with PC1, SBN, and SpN. PC1 is the main principal component that separates panicles from domesticated and wild accessions of rice (Fig. 1). Correlations with SBN and SpN are based on repeat panicle phenotyping for the accessions used for RNAseq in greenhouse conditions (Supplementary Fig. S8; Supplementary Table S10).

*indica*. The lower L<sub>2</sub>FCs in domesticated accessions could implicate cluster 2 genes in promotion of determinate meristem fate, because their lower expression at the DM stage may result in more activity of indeterminate axillary meristems. Eight *GROWTH-REGULATING FACTOR1* (GRF) family genes, which are involved in the regulation of cell proliferation (Kim and Tsukaya, 2015), were detected in our dataset, and three of them were present in cluster 2 ( $P_{\text{adj}}=7.6\times 10^{-3}$ , hypergeometric test). In contrast to cluster 2, most of the genes in cluster 5 have L<sub>2</sub>FCs close to zero in *O. sativa* ssp. *indica*, and negative L<sub>2</sub>FCs in the other accessions (Supplementary Fig. S8), meaning that the expression of these genes decreases at the DM stage in all accessions except *O. sativa* ssp. *indica*. The lack of repression of cluster 5 genes and to a lesser extent cluster 1 genes at the DM stage in *O. sativa* ssp. *indica* could result in more branching via the promotion of indeterminate axillary meristem identity. Cluster 5, which had the highest mean L<sub>2</sub>FC in *O. sativa* ssp. *indica*, had an enrichment of *AP2/EREBP*-like genes (6 out of 17 genes;  $P_{\text{adj}}=7.6\times 10^{-3}$ , hypergeometric test), and cluster 1 also contains 3 *AP2/EREBP*-like genes (Supplementary Table S8). We used qPCR to confirm these patterns in all four stages of each accession for all *AP2/EREBP*-like genes in cluster 5 (Supplementary Fig. S9). The enrichment of *AP2/EREBP*-like genes in cluster 5 and the presence of three *AP2/EREBP*-like genes in cluster 1 suggest that their pattern of expression is associated with differences in panicle architecture across wild and domesticated accessions.

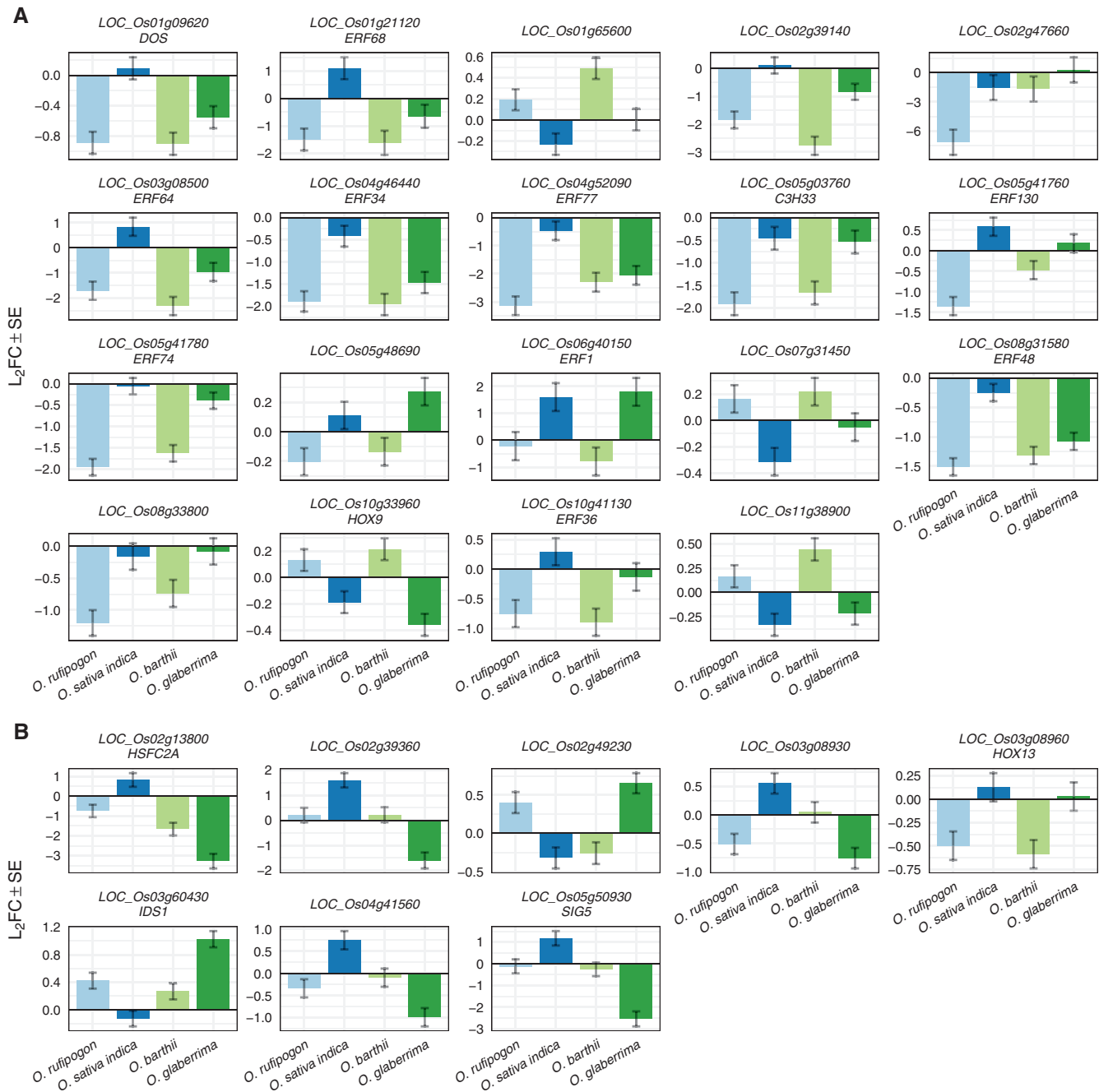
To find TF genes associated with parallel changes in panicle architecture during domestication, we tested the stage $\times$ domestication interaction for *O. rufipogon*, *O. sativa* ssp. *indica*, *O. barthii* and *O. glaberrima* at a false discovery rate of 0.1 (Supplementary Table S9). We detected 19 genes with a stage $\times$ domestication interaction, including nine *AP2/EREBP*-like genes ( $P=4.4\times 10^{-7}$ , hypergeometric test; Fig. 6A). These genes are putative targets of parallel selection on panicle architecture that occurred during domestication. *AP2/EREBP*-like genes were also prominent when we tested the stage $\times$ accession

interaction separately for each domestication (12 out of 85 genes in Asian accessions; 8 out of 50 genes in African accessions; Supplementary Table S9). Consistent with its presence in cluster 4, *OsIDS1* was also differentially expressed in both Asian and African domestications, although the direction of change was different (Fig. 6B). Genes with this pattern of expression in the four accessions may have also been targets of selection on panicle architecture, but evolved divergently.

The prominence of *AP2/EREBP*-like genes among putative core regulators of branching in all four *Oryza* species, and among genes associated with differences between wild and cultivated accessions, suggest that they were key targets of artificial selection for improvement in panicle architecture, and were involved in changes to the regulatory network controlling branching that occurred during domestication.

## Discussion

The purpose of this study was to identify genetic factors underlying the diversity of panicle architecture, which influences grain number in rice. Our transcriptomic comparison of panicles at indeterminate and determinate stages of axillary meristem development revealed a core set of transcription factors associated with axillary meristem phase transition in wild and domesticated African and Asian rice (Fig. 2). Some of these transcription factors, including *AP2/EREBP*-like and MIKCC-type MADS-box genes, appear to be co-regulated at the family or clade level (Fig. 3). Our phenotypic survey of 91 accessions showed that characteristics related to axillary meristem formation and fate transition are the main factors separating wild and domesticated rice (Fig. 1). Three clusters of gene expression correlated with the major component of phenotypic variability between wild and domesticated accessions, containing enrichments of *GRF* and *AP2/EREBP*-like transcription factor genes (Fig. 5). We observed a correlation between expression of *AP2/EREBP*-like transcription factors, domestication status, and derived phenotypes, suggesting



**Fig. 6.** Parallel and divergent evolution of gene expression associated with domestication. (A) Expression of genes with a stage $\times$ domestication interaction when all four accessions were tested together. We used this test to identify genes where the change in  $L_2FC$  between indeterminate (IM) and determinate (DM) stages changed in the same direction in both African and Asian domesticated accessions. (B) Expression of genes with a stage $\times$ domestication interaction when tested separately for African and Asian accessions. These genes have divergent changes between wild and domesticated accessions. The genes plotted in (B) had an interaction in both of the separate tests, but not in the single test used to identify the genes in (A).

that expression of these genes has changed as a result of artificial selection during domestication. As well as basic insights into molecular control of branching in rice, this work provides an overview of the outcome of the domestication process at phenotypic and whole-transcriptome levels.

A set of AP2/EREBP-like genes decrease in expression over the course of wheat spike development (Li *et al.*, 2018), and the molecular function of some individual AP2/EREBP-like genes has been reported in relation to inflorescence or root development. *FZP* represses the formation of axillary

meristem and induces transition from spikelet to floral meristem (Komatsu *et al.*, 2001). Along with the AP2/EREBP-like gene *FZP*, AP2/EREBP85, *ERF3*, and *ERF85* were more highly expressed at the DM stage across all four accessions, consistent with a role in the regulation of axillary meristem identity (Fig. 3). Conversely, most of the AP2/EREBP-like genes that change expression between stages had lower expression in DM than in IM, including mainly ERF and DREB clade genes and three RAV-like genes. In Arabidopsis, RAV orthologs repress flowering genes (Matías-Hernández *et al.*,

2014), and *ERF3* interacts with the HB gene *WOX11* to promote crown root development (Zhao *et al.*, 2015). Our results suggest additional roles for these genes in promoting reproductive axillary meristem initiation. This family- and clade-level view of their expression suggests that *AP2/EREBP*-like play a central role in the regulation of phase transition from indeterminate to determinate state.

*AP2/EREBP*-like genes are also associated with domestication. The *AP2*-like wheat domestication gene *Q* regulates inflorescence and glume shape and spike length, and has variation in binding sites for microRNA miR172 between wild and domesticated species (Simons *et al.*, 2006; Debernardi *et al.*, 2017; Greenwood *et al.*, 2017). Recently, characterization of a quantitative trait locus associated with grain yield and panicle branching and variation in the promoter region showed that *FZP* regulates panicle architecture and is associated with rice domestication (Bai *et al.*, 2017; Fujishiro *et al.*, 2018; Huang *et al.*, 2018). Our analysis indicates a modified expression pattern of some *AP2/EREBP*-like genes between wild and domesticated rice accessions. One of the clusters of genes with expression patterns that correlate to the main phenotypic differences between wild and domesticated accessions contained an enrichment of *AP2/EREBP*-like genes. In addition, nine out of the 19 genes with a stage $\times$ domestication interaction across the entire dataset were *AP2/EREBP*-like genes (Fig. 6A). Eight of these nine genes have lower expression in panicles at the DM stage, and may be involved in promotion of indeterminate axillary meristem and/or suppression of the transition from axillary meristem to spikelet meristem. Among these genes, *ERF130* (*MULTI-FLORET SPIKELET1*, *MFS1*) regulates the timing of the transition of spikelet meristems to terminal spikelets and positively regulates the expression of *OsIDS1* and *SNB* (Ren *et al.*, 2013). Taken with the roles of *AP2* mutants in modification of rice panicle architecture (this study; Komatsu *et al.*, 2001; Lee and An, 2012), our results implicate several *AP2/EREBP*-like genes as putative targets of artificial selection during rice domestication resulting in parallel evolution of expression.

*OsIDS1* controls panicle branching in rice, and positively regulates branch meristem identity by repressing genes that specify spikelet identity (Lee and An, 2012). We found a stage $\times$ domestication interaction in expression of *OsIDS1*, but the change was divergent between African and Asian accessions (Fig. 6B). This suggests that even if genes are involved in similar regulatory networks, their expression may diverge during selection. Parallel morphological evolution during Asian and African rice domestication is sometimes associated with different genomic modifications (Furuta *et al.*, 2015; Cubry *et al.*, 2018; Hu *et al.*, 2018; Lv *et al.*, 2018), illustrating that the interaction between genetic variation and the diverse selection pressures associated with domestication can result in diverse genomic outcomes.

Our interspecific analysis showed that parallel domestication of African and Asian rice resulted in similar modifications in panicle architecture, with changes in branch number contributing to derived phenotypes more than branch length traits (Fig. 1B, C). Secondary branch number correlated with spikelet number in all species, but primary branch number

only correlates with spikelet number in wild species (Fig. 1D). Yield improvements seem to have occurred mainly through increased ramification of branching, rather than, for example, elongation of the primary axis. This suggests that increased production of secondary branches from axillary meristems rather than direct differentiation into spikelets was important in both domestications.

The molecular control of phase transition from indeterminate to determinate axillary meristems is not fully understood in rice panicle development. Microarray analysis of developing panicles has identified a small set of differentially expressed genes, enriched for TFs (Furutani *et al.*, 2006). In microdissected meristem tissues, gene expression changes gradually during transition in axillary meristem identity (Harrop *et al.*, 2016), similar to the gradual meristem maturation during tomato inflorescence development (Park *et al.*, 2012). We also observed a small set of genes that were differentially expressed between IM and DM consistently in all accessions (153 of 25 229 tested), with an enrichment of TFs (37 of 153 genes). More genes were upregulated in DM than down-regulated, as in wheat spike development (Wang *et al.*, 2017). These results suggest that the gene regulatory network controlling phase transition from IM to DM is controlled by a small subset of core, conserved genes in Asian and African rice.

Because of redundant and overlapping activities of TFs, gene regulatory networks are robust to perturbations in single genes, allowing them to produce a stable transcriptional output in variable cellular and environmental conditions (Gitter *et al.*, 2009; Dai *et al.*, 2009; Wu and Lai, 2015). *AP2/EREBP*-like and MADS-box genes were enriched among differentially expressed genes. Expression of *AGL6*-like, *AP1/FUL*-like, and four of the five *SEPALLATA*-like MADS-box genes all increased between indeterminate and determinate stage, as expected given their roles in spikelet determination and inflorescence development (Yoshida and Nagato, 2011; Zhang and Yuan, 2014). This suggests family-level or clade-level co-regulation of TFs in rice panicle development. Our observation that some genes appear to be co-expressed at the family or clade level may indicate partial functional redundancy between groups of homologous TFs in the rice genome that act in the gene regulatory network controlling axillary meristem determination.

This work has revealed the core set of genes that are associated with the determination of axillary meristem identity across Asian and African rice accessions. We have also discovered candidate targets of artificial selection on panicle phenotype during domestication. In particular, the interspecific expression pattern of *AP2/EREBP*-like genes suggests that they regulate axillary meristem determination and have evolved during domestication. Along with efforts to characterize the molecular function of the candidate genes we have identified and to measure their expression in other accessions, further investigation of the gene regulatory network controlling meristem identity in developing panicles would continue to drive an understanding of the process of inflorescence development in rice and its relationship to grain yield.

## Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Spread mature rice panicle.

Fig. S2. Principal component analysis (PCA) of panicle phenotyping data showing components 1–4.

Fig. S3. The accessions used for RNAseq are consistent with species-wide patterns of panicle architecture.

Fig. S4. Early stages of rice panicle development used for gene expression analysis.

Fig. S5. Heatmap of pairwise distances between RNAseq samples.

Fig. S6. Expression of *AP2/EREBP*-like genes in *O. sativa* spp. *japonica* cv. Nipponbare meristems (data from Harrop *et al.*, 2016).

Fig. S7. Phenotyping of the five *Oryza* accessions used for RNAseq.

Fig. S8. Most genes in cluster 5 have negative  $L_2FC$ s between IM and DM in *O. rufipogon*, *O. barthii*, and *O. glaberrima*, but  $L_2FC$ s in *O. sativa* spp. *indica* are closer to zero.

Fig. S9. Expression analysis along early panicle development of *AP2/EREBP*-like genes present in cluster 5.

Table S1. Rice accessions used in this study.

Table S2. Sequences of primers used.

Table S3. Quantification of panicle traits in 91 accessions from wild and domesticated Asian and African rice species.

Table S4. Read and mapping statistics for all RNAseq samples.

Table S5. Differential expression test results between stages across all species; we used an arbitrary differential expression threshold of 1.5-fold change in expression and adjusted *P*-value (false discovery rate) less than 0.1.

Table S6. Transcription factor family enrichment by  $L_2FC$ .

Table S7. Quantification of panicle traits from *crl5* and *smos1* mutants.

Table S8. Clustered genes.

Table S9. Differential expression test results for the stage $\times$ accession interaction in Asian and African accessions.

Table S10. Detailed quantification of panicle traits from rice accessions used for sequencing analysis.

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## Author contributions

HA and SJ designed the research with input from TWRH and OM; HA, TWRH, OM, KB, and AML performed the research; TWRH, OM, HA, and ML performed data analysis, collection, and interpretation; TWRH, SJ, and HA wrote the manuscript.

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