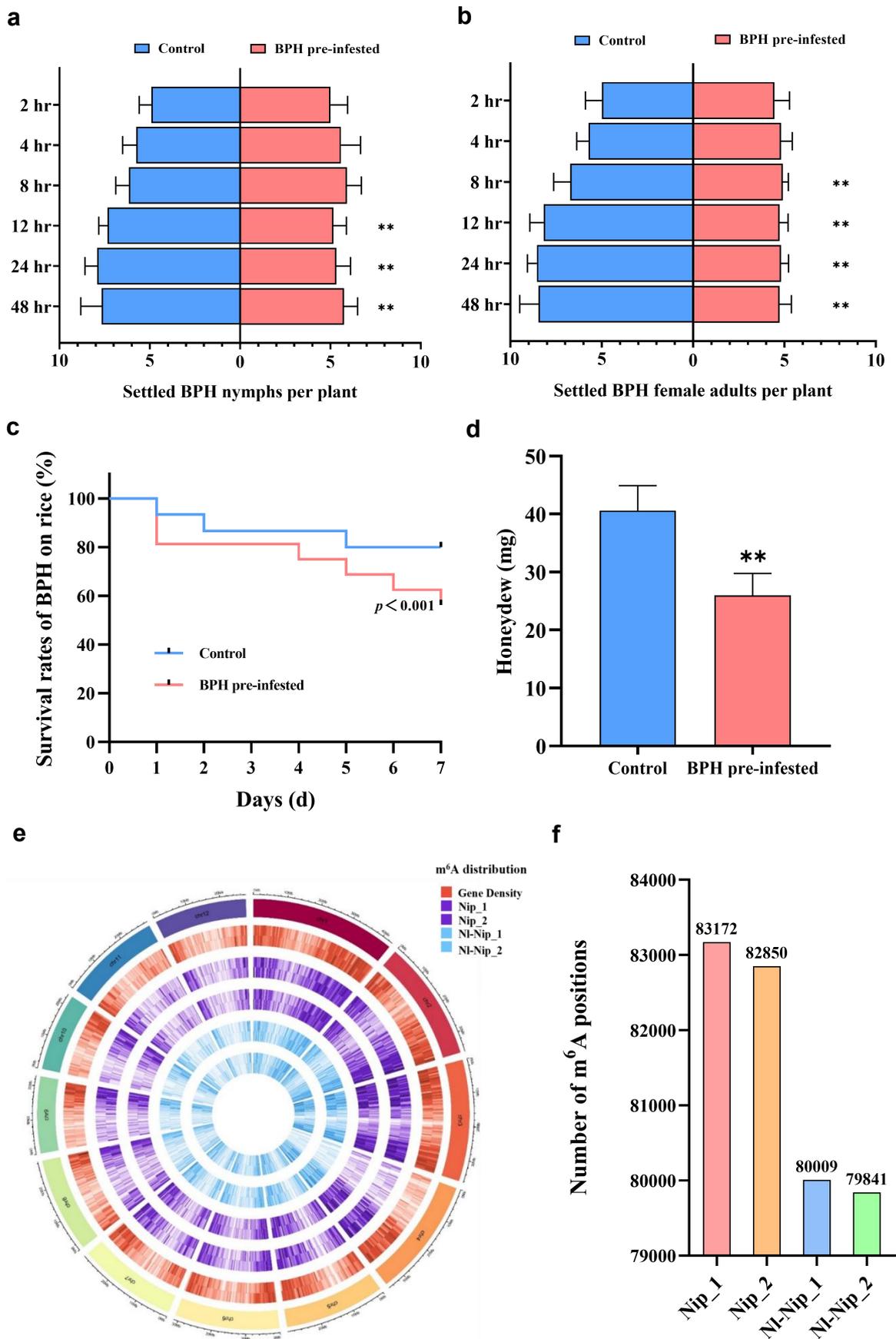
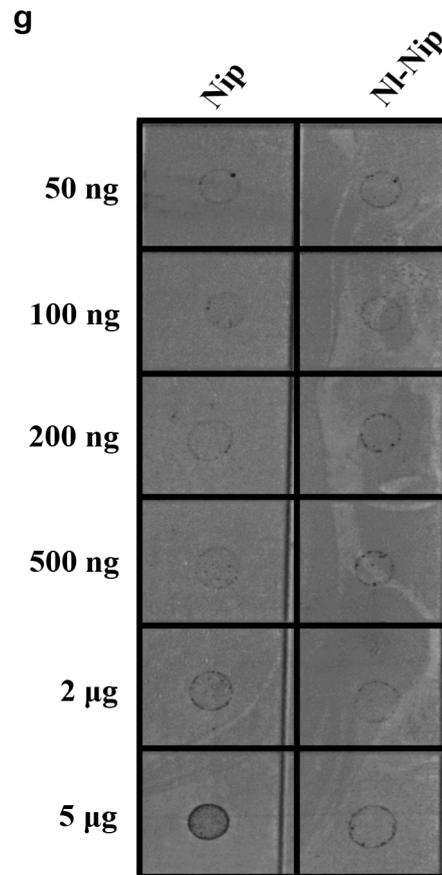


# 1 Figure Legends





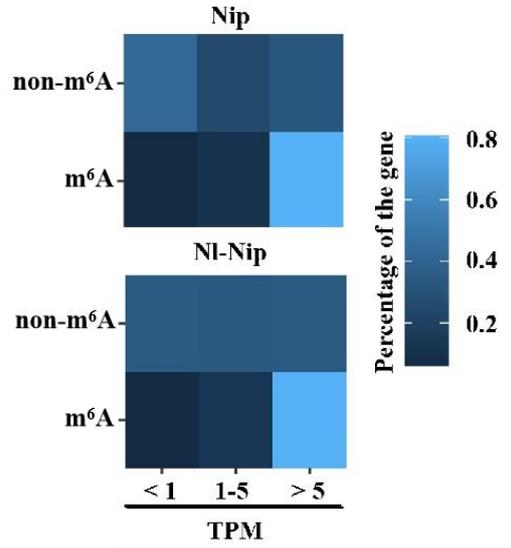
3

4 **FIGURE 1** The increase of rice resistance infested by BPH gravid females and its  
5 overall m<sup>6</sup>A modification

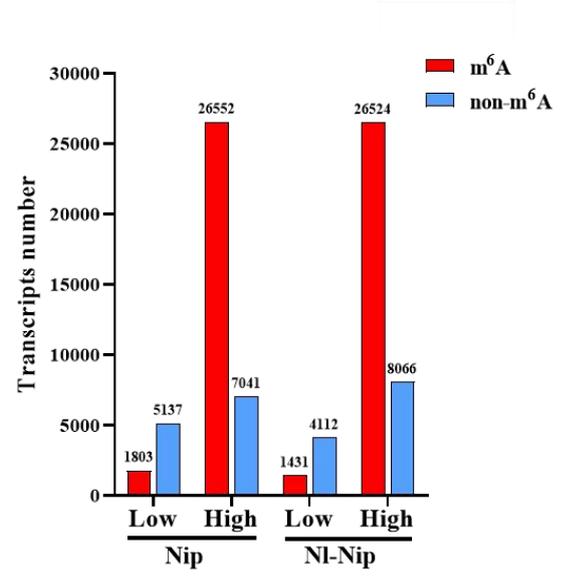
6 (a, b) Mean number of BPH nymphs (a) and female adults (b) per plant for plant pairs  
7 ( $n = 12$ ; BPH pre-infested rice vs control rice). Fifteen fourth instar nymphs and  
8 gravid female adults were released for each replicate. (c) Mean survival rates of  
9 nymphs across time ( $n = 8$ ). (d) Mean amount of honeydew excreted by a female  
10 adult 24 h after the start of the experiment ( $n = 18$ ). All choice and no-choice assays  
11 were performed after 24 h of continuous gravid females infestation on rice leaf  
12 sheaths (BPH pre-infested), whereas untreated rice plants were used as the controls. (e)  
13 Circo plots of m<sup>6</sup>A methylome in rice plants. The six rings from outside to inside  
14 show the genomic positions (1<sup>st</sup> ring), gene density (2<sup>nd</sup> ring), m<sup>6</sup>A methylome density  
15 of control rice plants (3<sup>rd</sup> and 4<sup>th</sup> rings), m<sup>6</sup>A methylome density of BPH-infested  
16 plants (5<sup>th</sup> and 6<sup>th</sup> rings). The outer loop of each ring (3<sup>rd</sup>–6<sup>th</sup> rings) represents the

17 plus-strand of the genome and the inner loop represents the minus-strand. (f)  
18 Histograms showing the number of m<sup>6</sup>A methylation positions in control (Nip\_1,  
19 Nip\_2) and BPH-infested Nipponbare plants (NI-Nip\_1, NI-Nip\_2). The Y-axis  
20 represents the total position number and X-axis represents the two treatments. Two  
21 biological replicates were used for each treatment. (g) Dot-blot analysis of m<sup>6</sup>A levels  
22 in total RNA extracted from rice samples using the specific anti-m<sup>6</sup>A antibodies. Error  
23 bars represent standard errors. Asterisks indicate significant differences (\*\*  $p < 0.01$ ;  
24 ns, no significant difference; Student's *t*-test). m<sup>6</sup>A, N<sup>6</sup>-methyladenosine; BPH, brown  
25 planthopper; Nip, control Nipponbare rice; NI-Nip, BPH-infested Nipponbare rice.

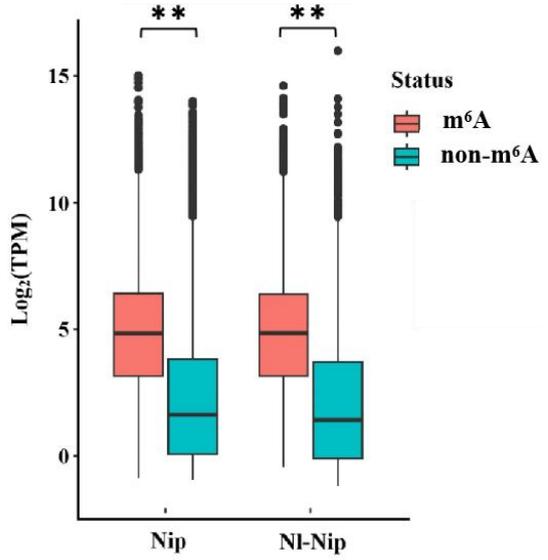
**a**



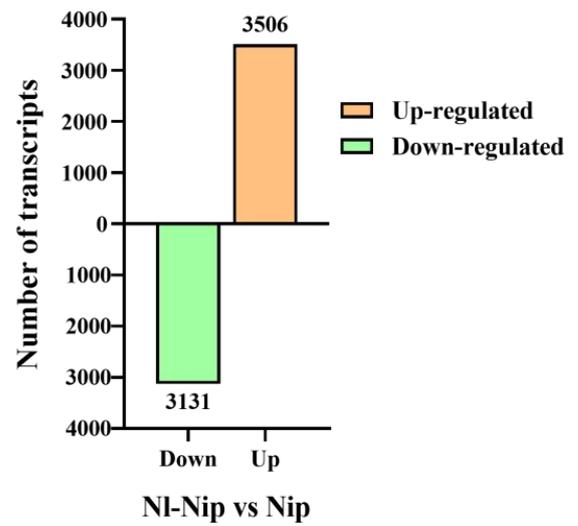
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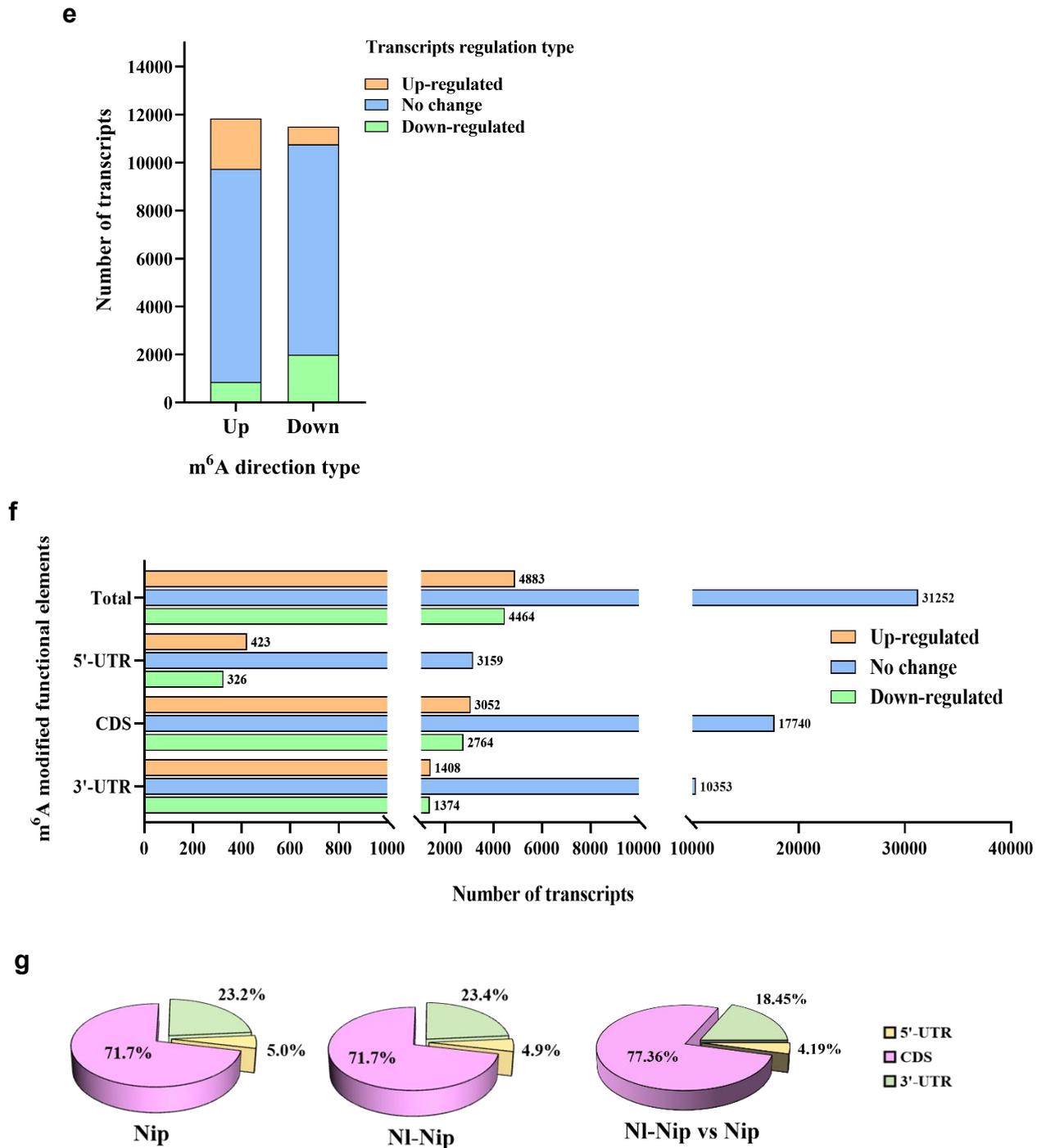


**c**



**d**

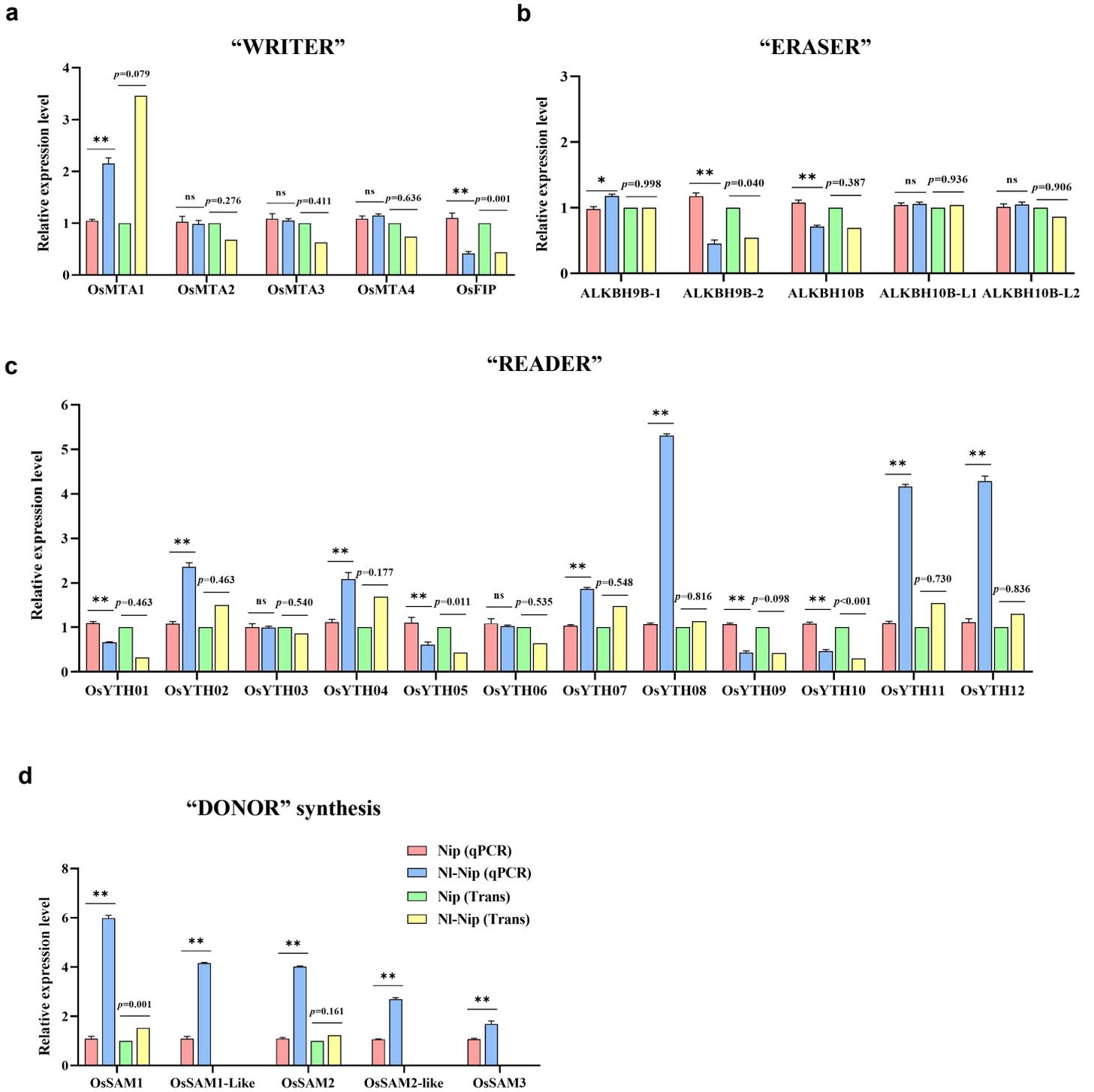




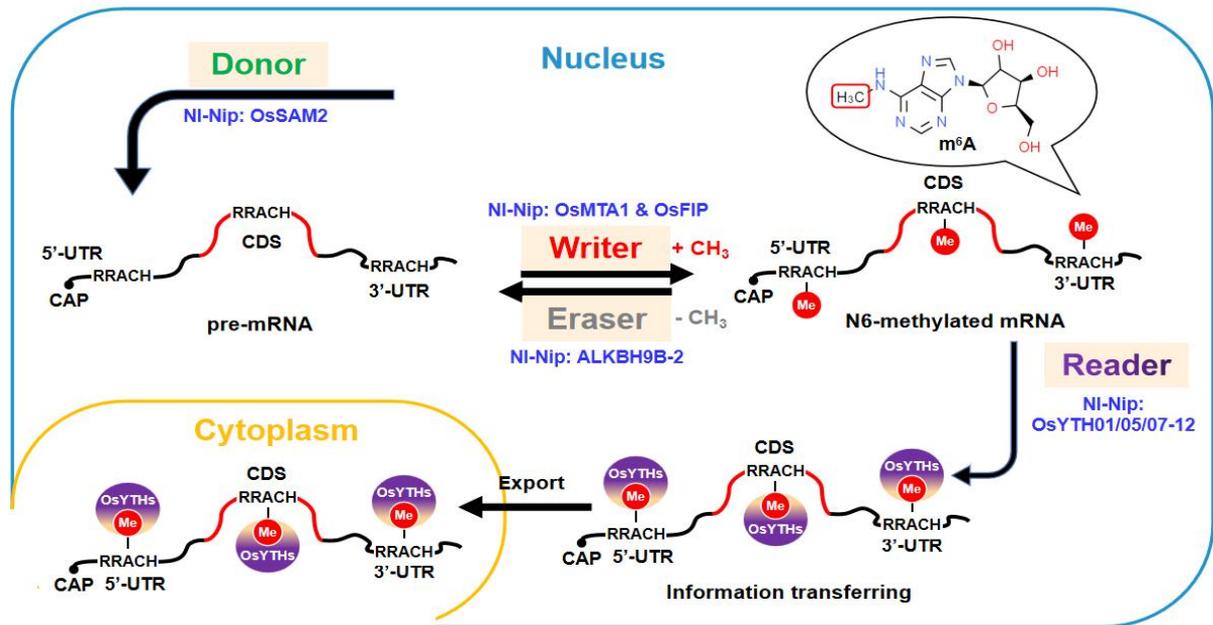
27 **FIGURE 2** Integrated analyses of the relationship between transcripts regulation and  
 28 m<sup>6</sup>A modification  
 29 (a) The percentage of rice m<sup>6</sup>A methylated and un-methylated genes at defined TPM  
 30 (Transcripts Per kilobase of exon model per Million mapped reads) levels (< 1, 1–5,  
 31 and > 5). Color densities indicate different percentages of genes in a category. (b)

32 Comparisons of the number of non-m<sup>6</sup>A methylated genes and m<sup>6</sup>A methylated genes  
33 in their gene bodies with high (High: TPM > 1) and lower (Low: TPM < 1) expression  
34 levels in each treatment. (c) Box plot comparing TPM expression levels between  
35 non-m<sup>6</sup>A and m<sup>6</sup>A methylated genes in each treatment. Asterisks indicate significant  
36 differences (\*\*  $p < 0.01$ ; Student's  $t$ -test). (d) Histograms showing the number of  
37 significantly up- and down-regulated transcripts that contain m<sup>6</sup>A modifications in  
38 NI-Nip vs. Nip comparison group. (e) Stack diagram of the relationship between the  
39 m<sup>6</sup>A methylation differential types and the corresponding transcripts differential types  
40 in NI-Nip vs. Nip comparison group. The Y-axis represents transcripts of different  
41 regulation types as well as their numbers, and X-axis shows the up- and  
42 down-directed m<sup>6</sup>A methylated transcripts in NI-Nip vs. Nip group. (f) Widely  
43 integrated analyses of the relationship between the transcript expression levels and  
44 m<sup>6</sup>A methylated functional elements using the m<sup>6</sup>A modified transcripts in NI-Nip vs.  
45 Nip group. The Y-axis represents the different gene structure of m<sup>6</sup>A modification  
46 regions; X-axis shows the number of transcripts with different regulatory types. (g)  
47 Percentages of each gene body in differentially m<sup>6</sup>A-methylated genes in control Nip,  
48 NI-Nip, as well as in NI-Nip vs. Nip group. m<sup>6</sup>A, N<sup>6</sup>-methyladenosine; BPH, brown  
49 planthopper; Nip, control Nipponbare rice sample; NI-Nip, BPH-infested Nipponbare  
50 rice sample.

51  
52



e



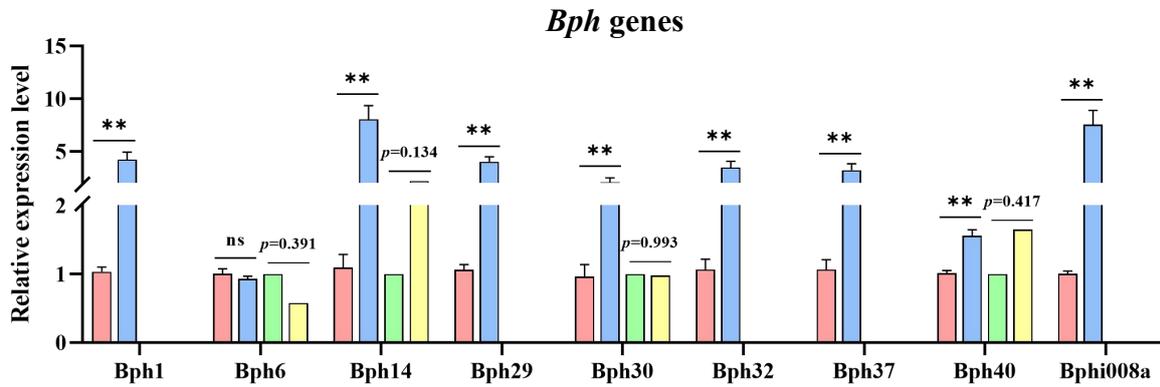
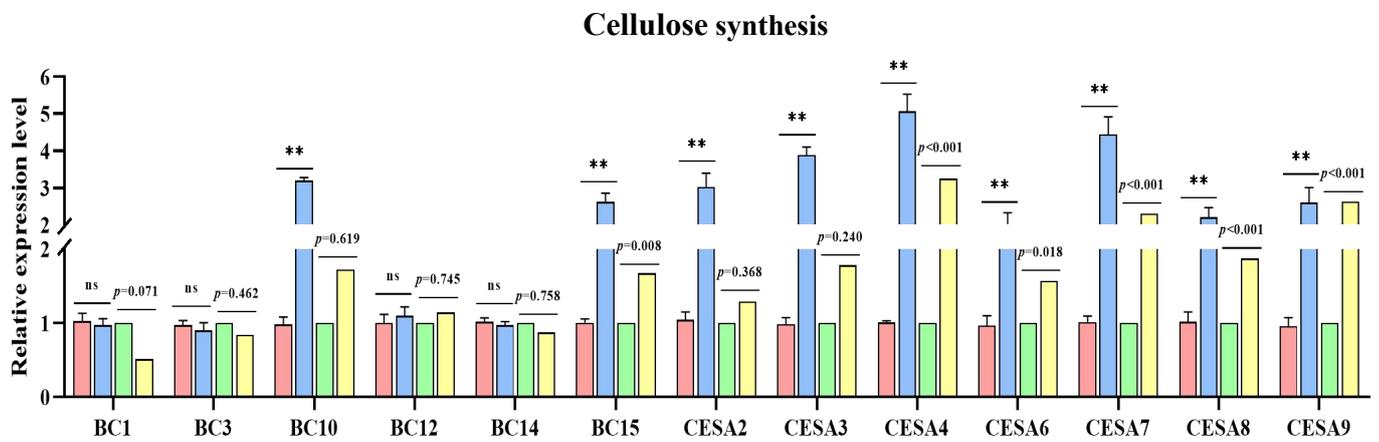
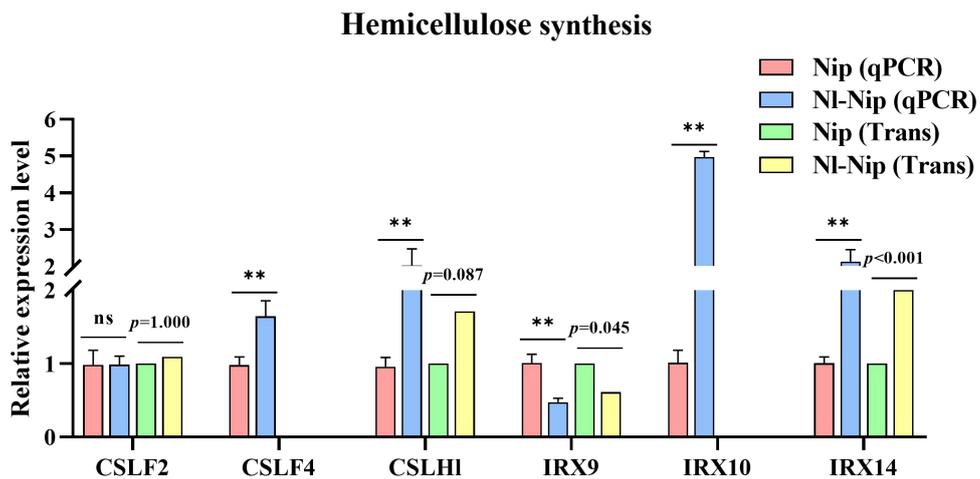
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54

55 **FIGURE 3** Relative expression levels of rice genes involved in m<sup>6</sup>A methylation  
 56 machinery under BPH infestation

57 (a–d) RT-qPCR and transcriptome analysis of the relative expression of 5 “WRITER”  
 58 genes (a), 5 “ERASER” genes (b), 12 “READER” genes (c), and 5 methyl “DONOR”  
 59 synthesis genes (d) in Nip and NI-Nip plants. Some undetected transcriptome data  
 60 indicated the absence of m<sup>6</sup>A methylation sites in the transcript. Error bars represent  
 61 standard errors. Asterisks indicate significant differences (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns,  
 62 no significant difference; Student’s  $t$ -test). (e) Rice m<sup>6</sup>A methylation pathways and  
 63 related genes upon BPH infestation. Blue letters indicate differentially expressed  
 64 transcripts containing differentially directed m<sup>6</sup>A methylation with the same  
 65 regulatory trend. *OsSAM2* means that *OsSAM2* was up-regulated and showed  
 66 up-directed m<sup>6</sup>A methylation in NI-Nip compared with in Nip plants. *OsFIP* indicates  
 67 that *OsFIP* was down-regulated and also showed down-directed m<sup>6</sup>A methylation in  
 68 NI-Nip compared with in Nip plants. The differentially expressed transcripts showed a  
 69 transcriptional expression fold change of  $< 0.5$  or  $> 2$  ( $p < 0.05$ ), along with a  
 70 significant m<sup>6</sup>A methylation direction ( $p < 0.05$ ), and  $|\text{meth diff}| > 10$ . m<sup>6</sup>A,

- 71  $N^6$ -methyladenosine; BPH, brown planthopper; Nip, control Nipponbare rice; NI-Nip,  
72 BPH-infested Nipponbare rice.

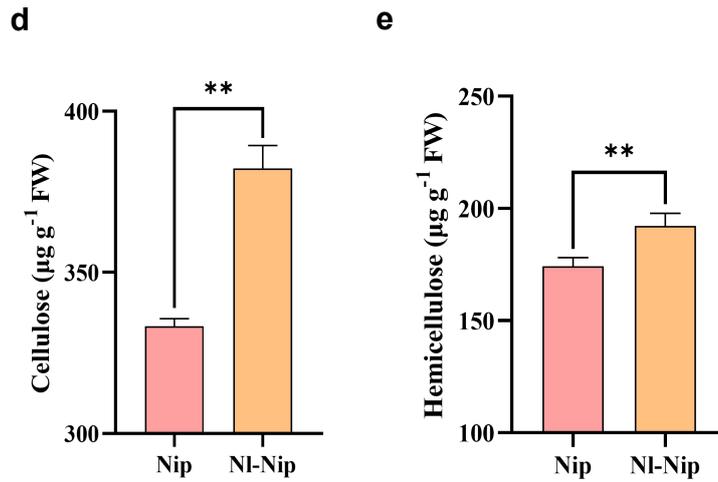
**a****b****c**

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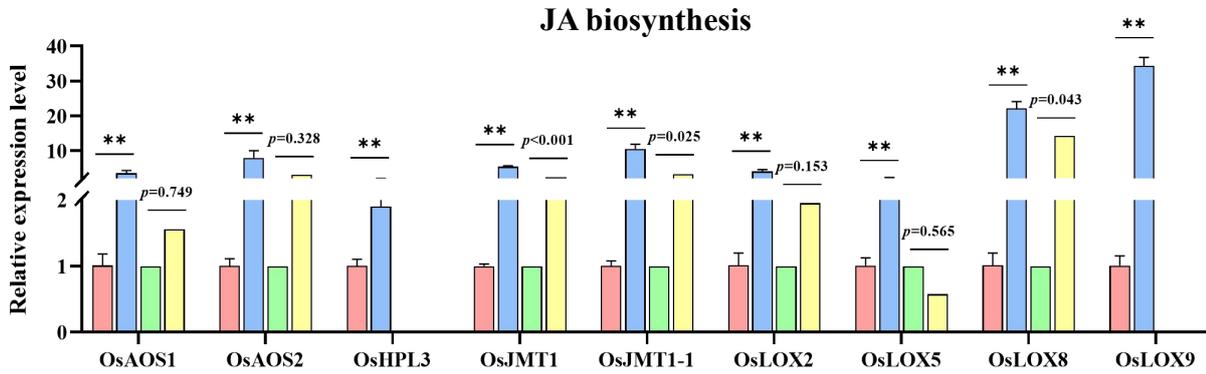
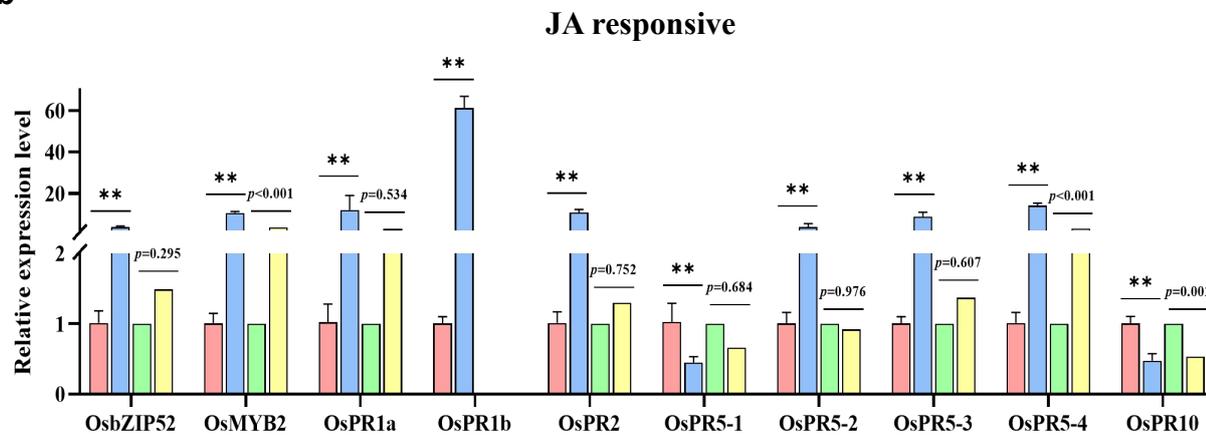
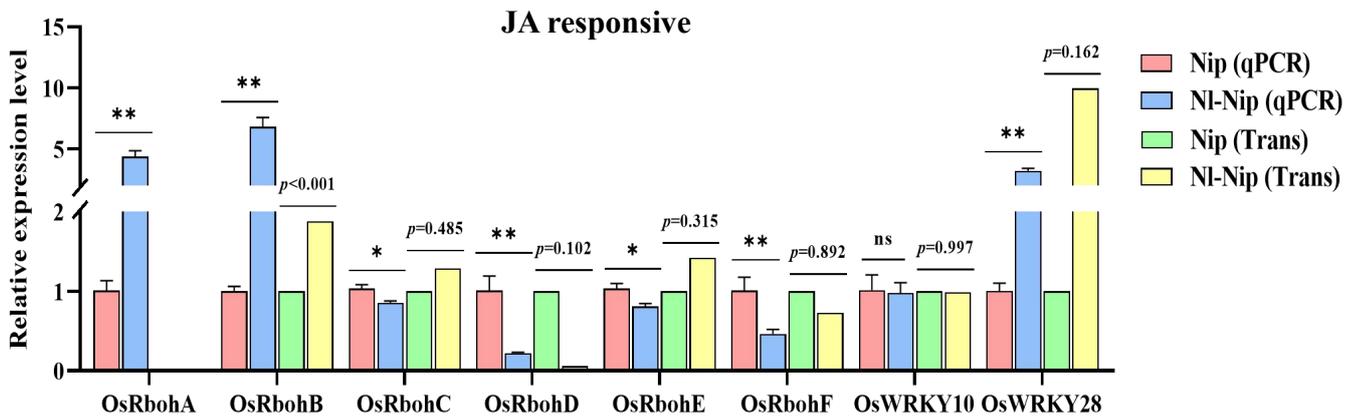
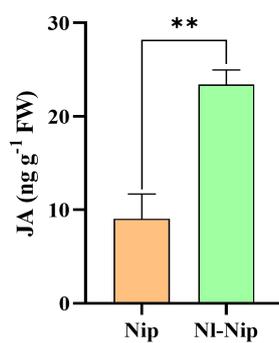
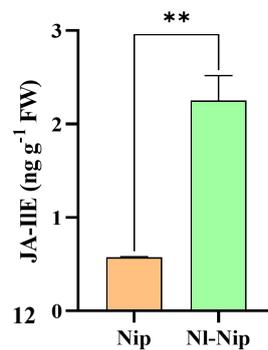
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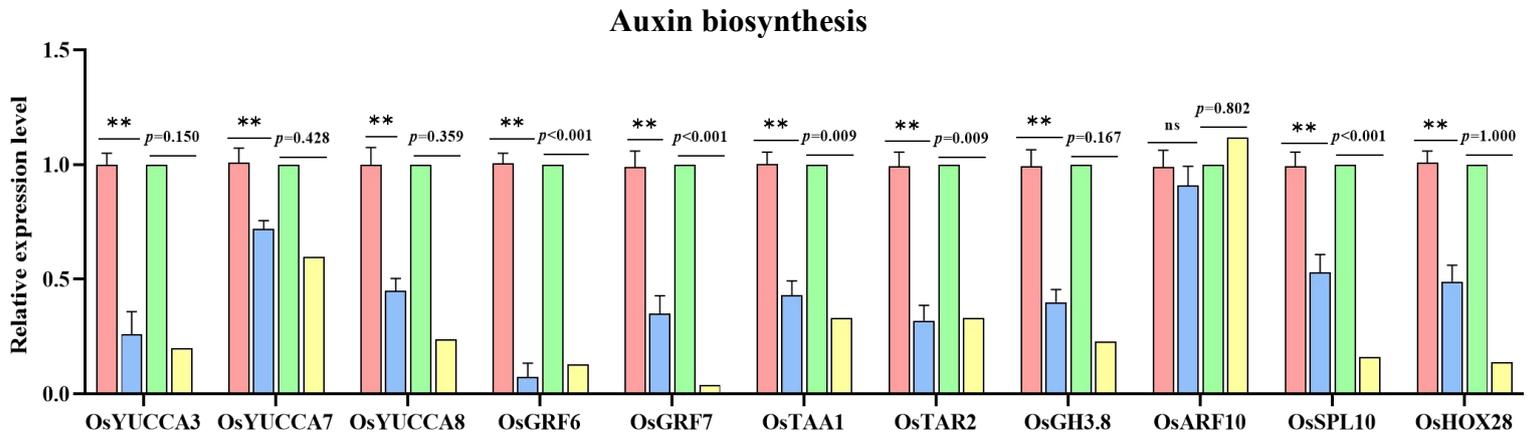
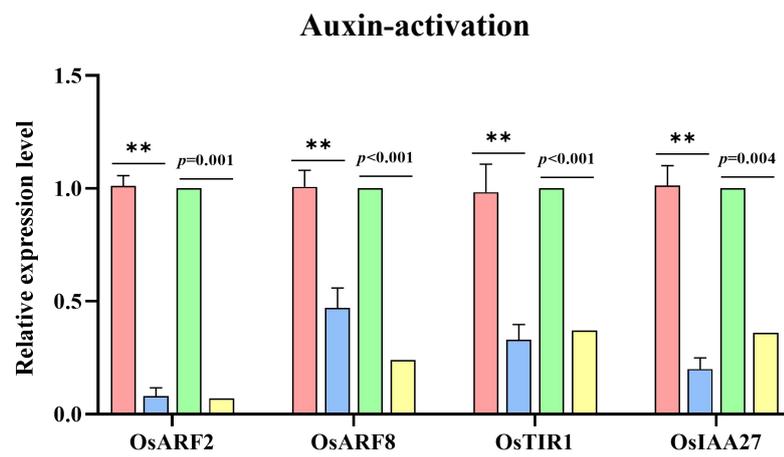
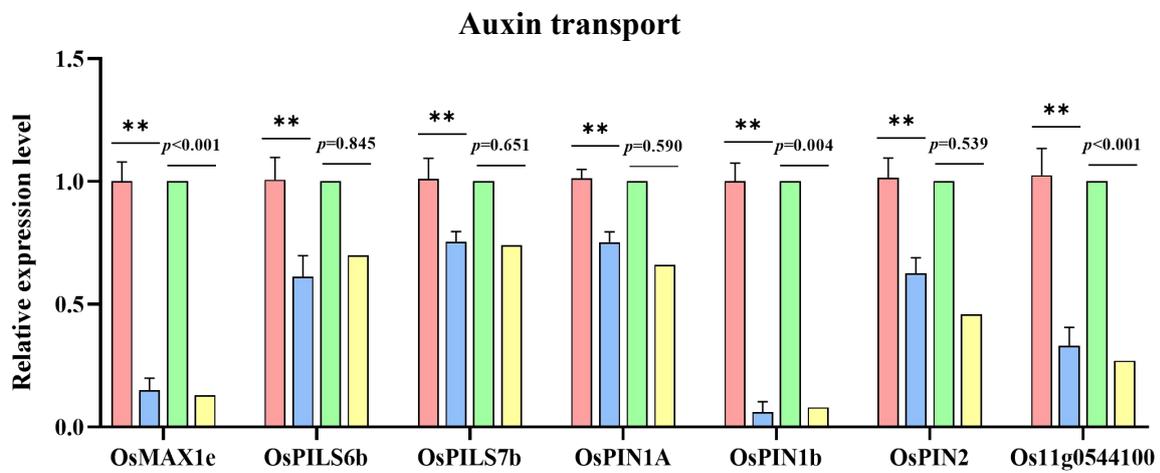
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79 **FIGURE 4** The BPH infestation activated cellulose- and hemicellulose-synthesis  
80 pathways

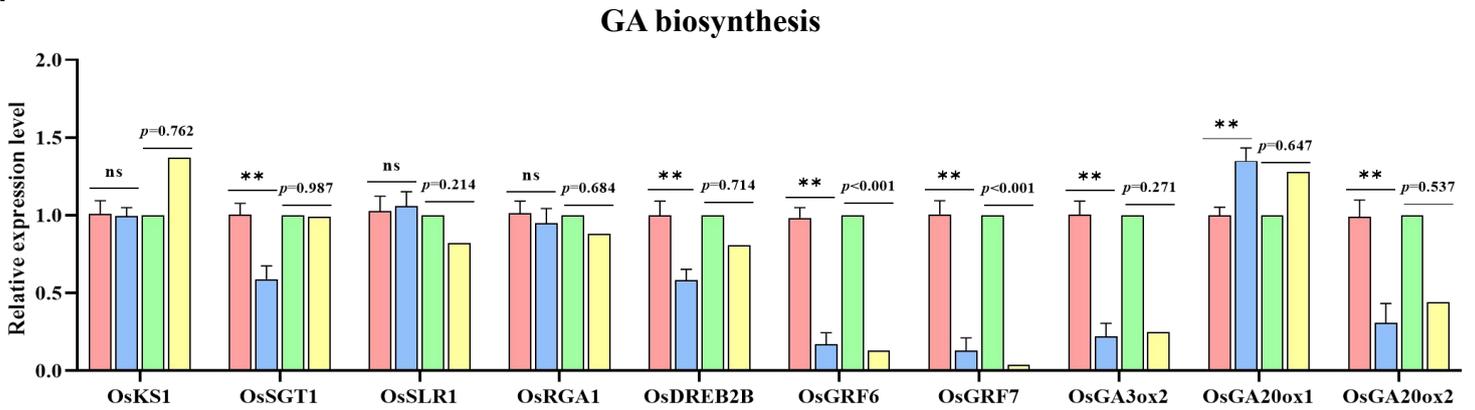
81 (a–c) RT-qPCR and transcriptome analysis of the relative expression of 9 *Bph* genes  
82 (a), 13 cellulose synthesis genes (b), and 6 hemicellulose synthesis genes (c) in Nip  
83 and NI-Nip plants. Some undetected transcriptome data indicated the absence of m<sup>6</sup>A  
84 methylation sites in the transcript. (d, e) Mean levels ( $n = 6$ ) of cellulose (d) and  
85 hemicellulose (e) in Nip and NI-Nip plants. Error bars represent standard errors.  
86 Asterisks indicate significant differences (\*\*  $p < 0.01$ ; ns, no significant difference;  
87 Student's *t*-test). Nip, control Nipponbare rice; NI-Nip, BPH-infested Nipponbare rice;  
88 FW, fresh weight.

**a****b****c****d****e**

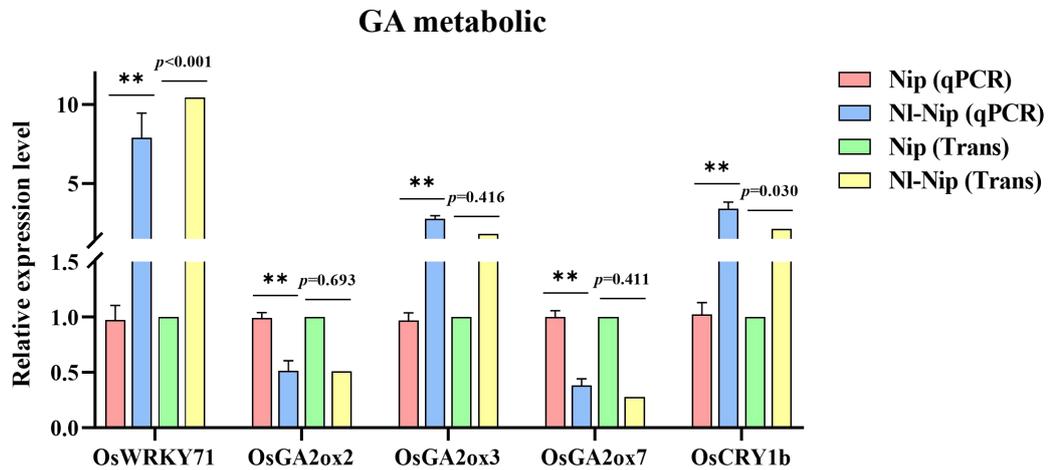
91 **FIGURE 5** Jasmonic acid biosynthesis and responsive pathways were activated in  
92 BPH-infested rice plants  
93 (a–c) RT-qPCR and transcriptome analysis of the relative expression of 9 JA  
94 biosynthesis-related genes (a), and 18 JA responsive genes (b, c) in Nip and NI-Nip  
95 plants. Some undetected transcriptome data indicated the absence of m<sup>6</sup>A methylation  
96 sites in the transcript. (d, e) Mean levels ( $n = 6$ ) of JA (d), and jasmonoyl-isoleucine  
97 (JA-ILE) (e) in Nip and NI-Nip plants. Error bars represent standard errors. Asterisks  
98 indicate significant differences (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns, no significant difference;  
99 Student's  $t$ -test). Nip, control Nipponbare rice; NI-Nip, BPH-infested Nipponbare rice;  
100 FW, fresh weight.

**a****b****c**

d



e



103

104 **FIGURE 6** Auxin pathways and gibberellic acid biosynthesis process were activated

105 in BPH-infested rice plants

106 (a–e) RT-qPCR and transcriptome analysis of the relative expression of Auxin

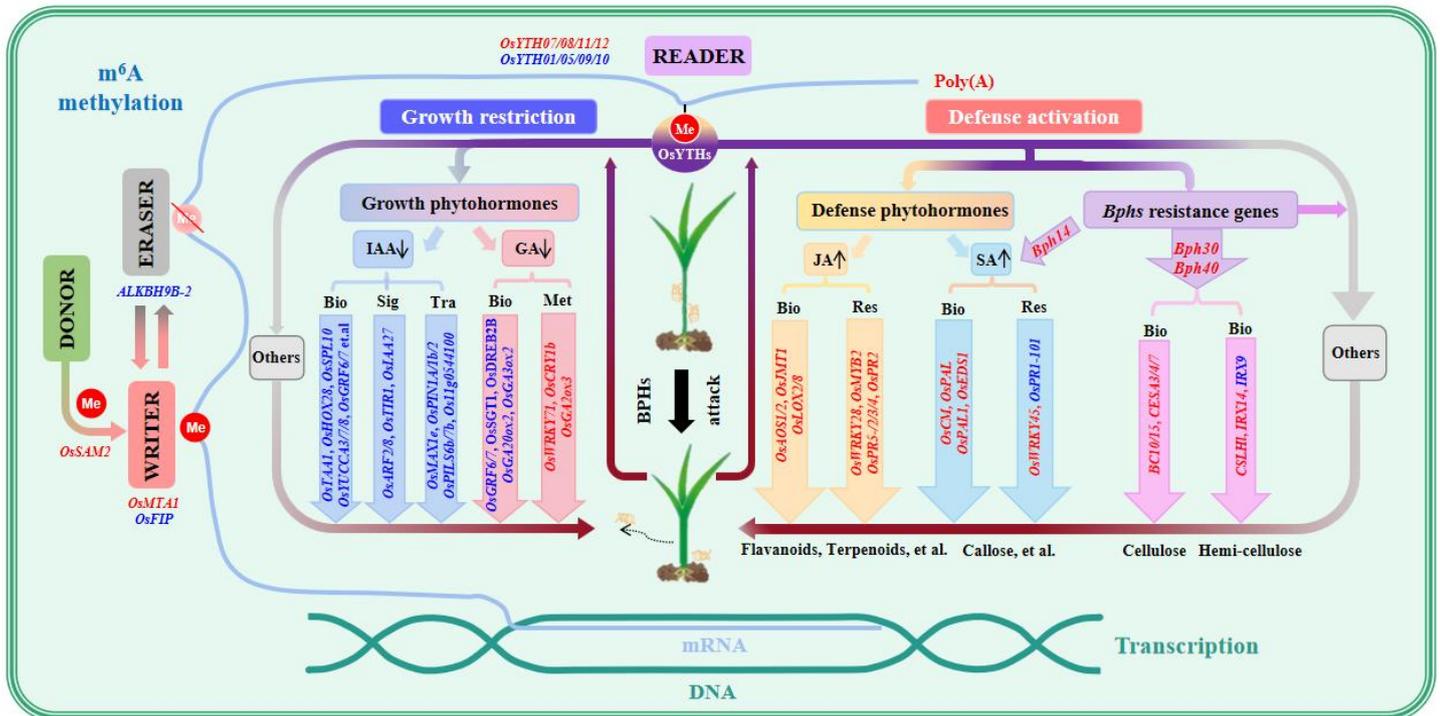
107 biosynthetic process (a), Auxin-activated signaling pathway (b), Auxin

108 transport-related (c), gibberellic acid (GA) biosynthetic (d), and GA metabolic (e)

109 genes in Nip and NI-Nip plants. Error bars represent standard errors. Asterisks

110 indicate significant differences (\*\*  $p < 0.01$ ; ns, no significant difference; Student's111  $t$ -test). BPH, brown planthopper; Nip, control Nipponbare rice; NI-Nip, BPH-infested

112 Nipponbare rice.



**FIGURE 7** Proposed model of the rice m<sup>6</sup>A RNA methylation modulation in key defense and growth pathways regulated by BPH infestation

The genes highlighted in red denote both transcriptional up-regulated ( $p < 0.05$ ) and up-directed m<sup>6</sup>A modification ( $p < 0.05$  and  $|\text{meth diff}| > 10$ ). The number of up-directed m<sup>6</sup>A sites occurring on the target pathway in NI-Nip vs. Nip group was less than that of down-directed m<sup>6</sup>A sites. Genes highlighted in blue represent both transcriptional down-regulated ( $p < 0.05$ ) and down-directed m<sup>6</sup>A modification; the number of down-directed m<sup>6</sup>A sites occurring on the target pathway in NI-Nip vs. Nip group was less than that of up-directed m<sup>6</sup>A sites ( $p < 0.05$  and  $|\text{meth diff}| > 10$ ). The upward arrows indicate increased phytohormones content, and downward arrows indicate decreased phytohormones content. Bio, biosynthetic process; Sig, activated signaling process; Tra, transport process; Met, metabolic process; Res, responsive process; m<sup>6</sup>A, N<sup>6</sup>-methyladenosine; BPH, brown planthopper; Nip, control Nipponbare rice; NI-Nip, BPH-infested Nipponbare rice.