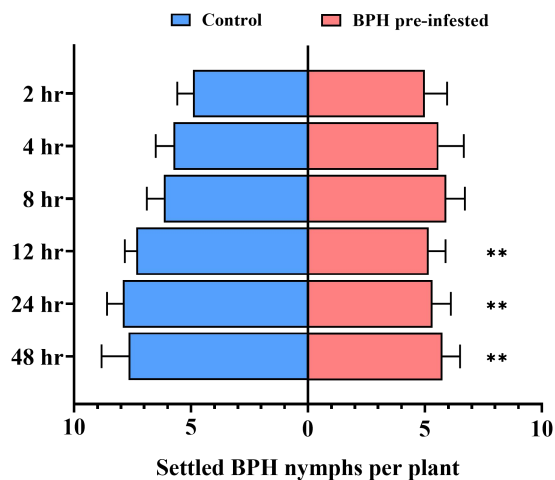
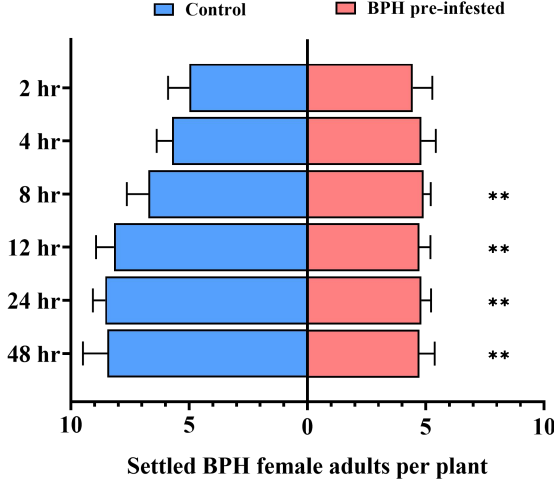


1 **Figure Legends**

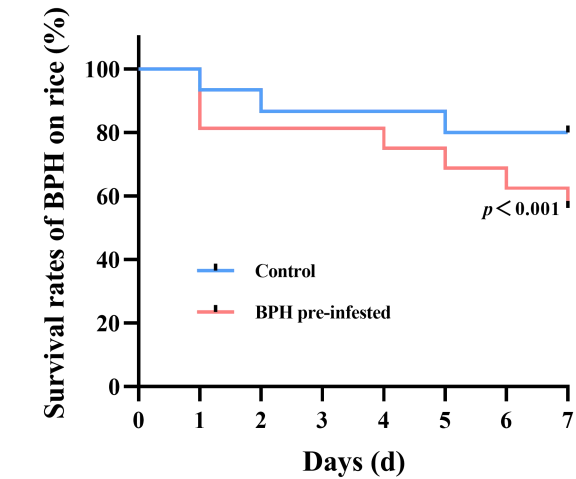
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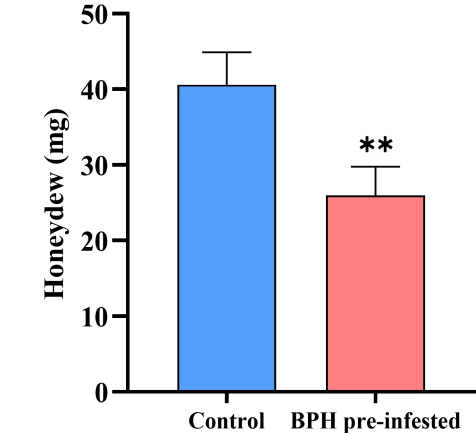
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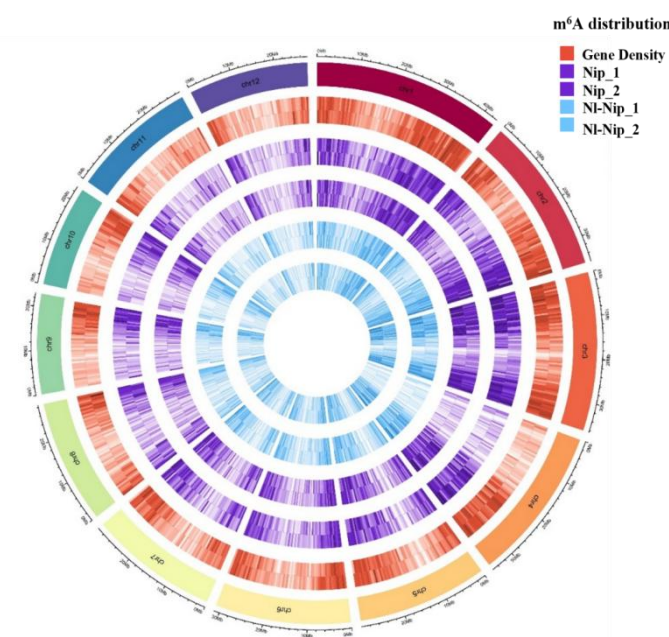
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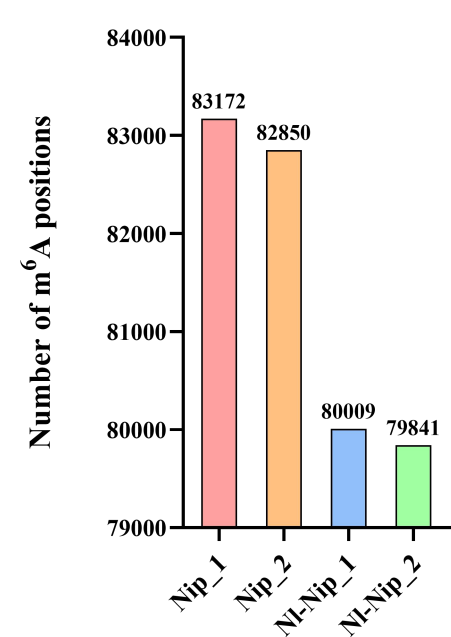
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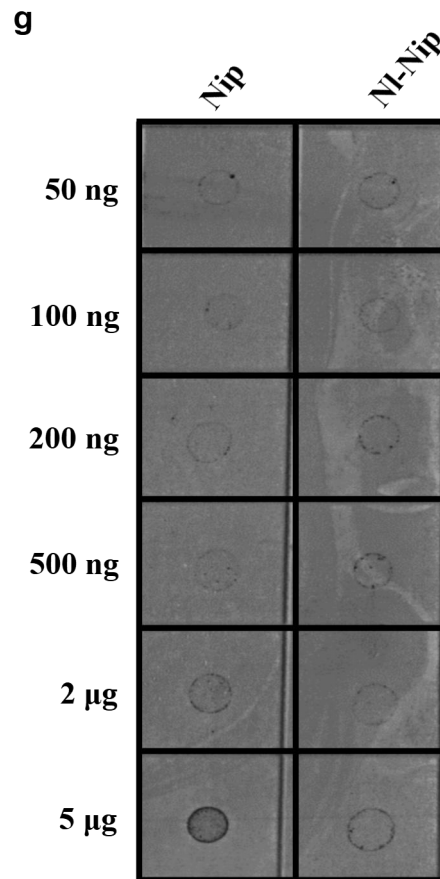


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f





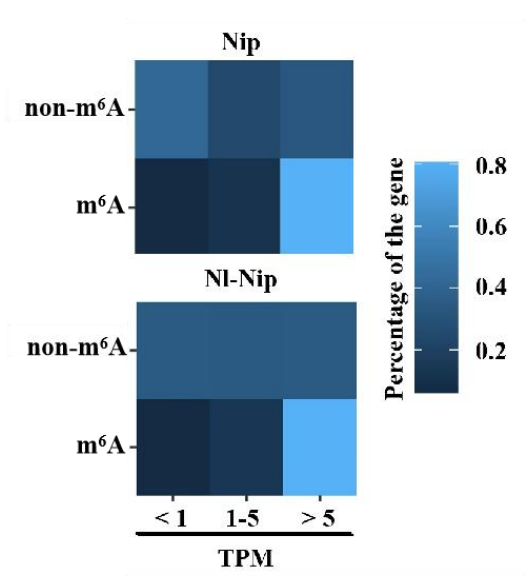
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4 **FIGURE 1** The increase of rice resistance infested by BPH gravid females and its
 5 overall m⁶A modification

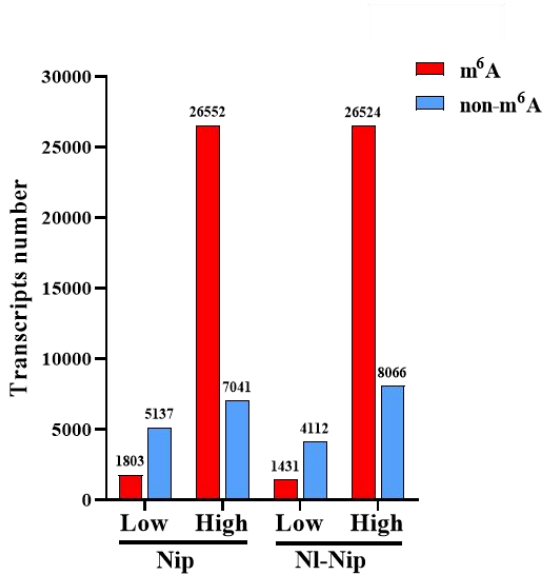
6 (a, b) Mean number of BPH nymphs (a) and female adults (b) per plant for plant pairs
 7 ($n = 12$; BPH pre-infested rice vs control rice). Fifteen fourth instar nymphs and
 8 gravid female adults were released for each replicate. (c) Mean survival rates of
 9 nymphs across time ($n = 8$). (d) Mean 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 m⁶A methylome in rice plants. The six rings from outside to inside
 14 show the genomic positions (1st ring), gene density (2nd ring), m⁶A methylome density
 15 of control rice plants (3rd and 4th rings), m⁶A methylome density of BPH-infested
 16 plants (5th and 6th rings). The outer loop of each ring (3rd–6th rings) represents the

17 plus-strand of the genome and the inner loop represents the minus-strand. (f)
18 Histograms showing the number of m⁶A methylation positions in control (Nip_1,
19 Nip_2) and BPH-infested Nipponbare plants (Nl-Nip_1, Nl-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 m⁶A levels
22 in total RNA extracted from rice samples using the specific anti-m⁶A antibodies. Error
23 bars represent standard errors. Asterisks indicate significant differences (** $p < 0.01$;
24 ns, no significant difference; Student's *t*-test). m⁶A, N⁶-methyladenosine; BPH, brown
25 planthopper; Nip, control Nipponbare rice; Nl-Nip, BPH-infested Nipponbare rice.

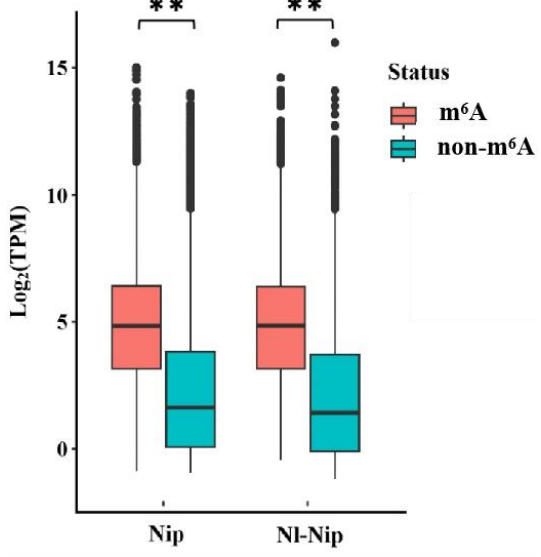
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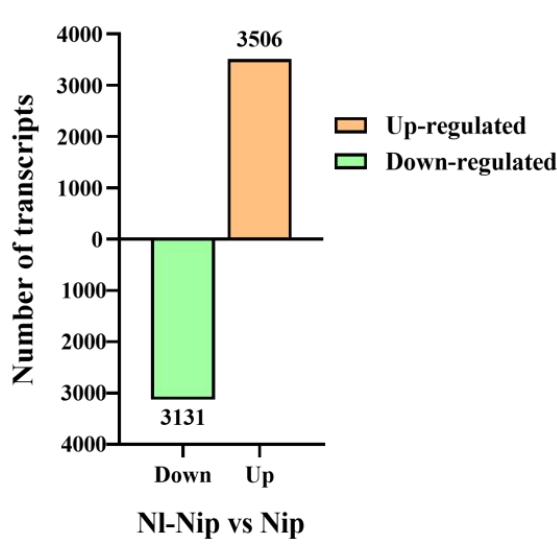
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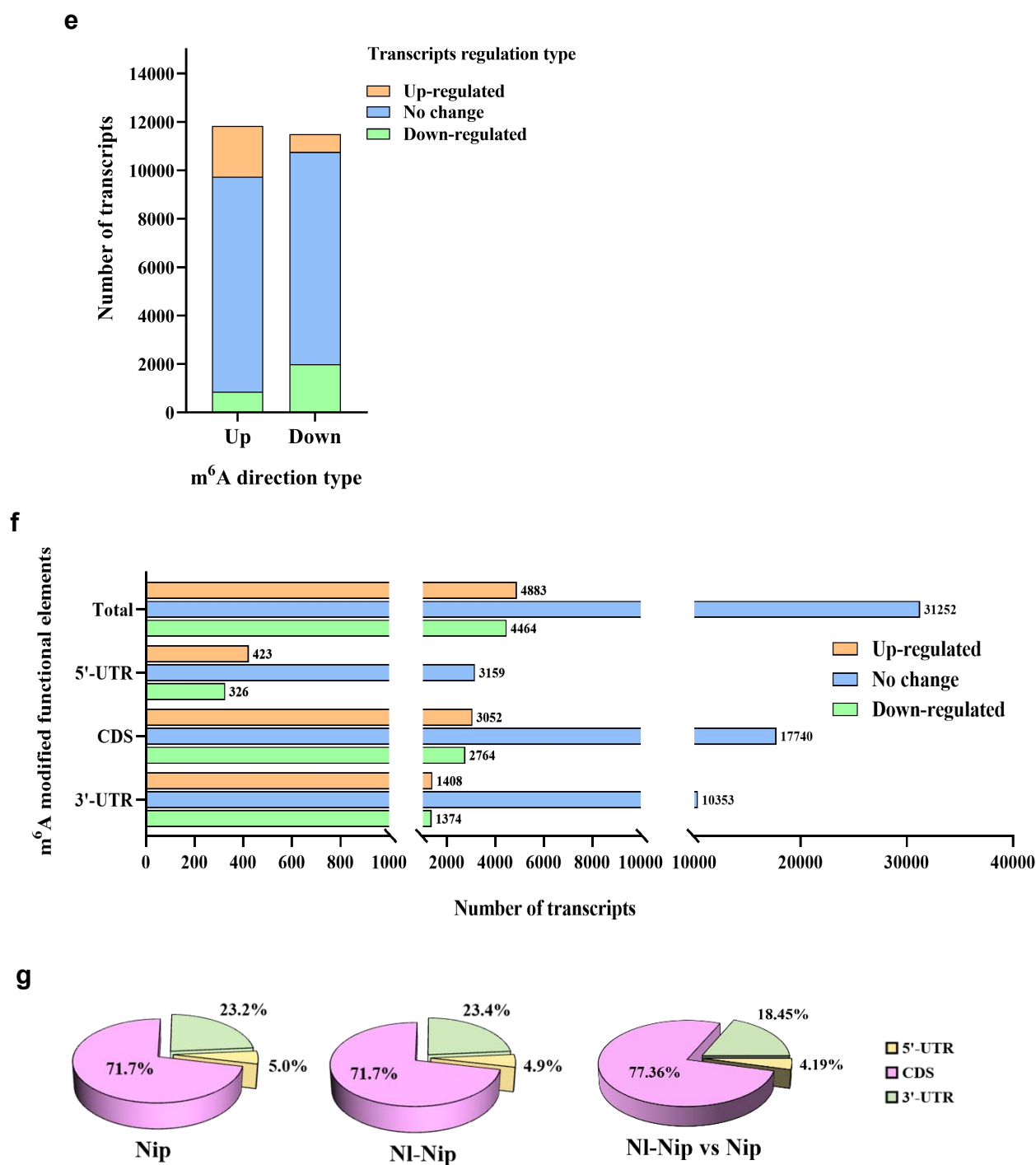


FIGURE 2 Integrated analyses of the relationship between transcripts regulation and m⁶A modification

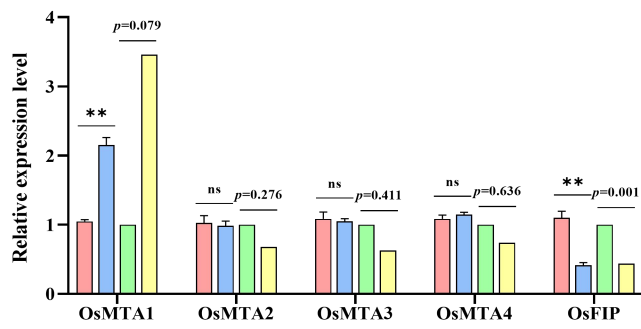
(a) The percentage of rice m⁶A methylated and un-methylated genes at defined TPM (Transcripts Per kilobase of exon model per Million mapped reads) levels (< 1, 1–5, and > 5). Color densities indicate different percentages of genes in a category. (b)

Comparisons of the number of non-m⁶A methylated genes and m⁶A methylated genes in their gene bodies with high (High: TPM > 1) and lower (Low: TPM < 1) expression levels in each treatment. (c) Box plot comparing TPM expression levels between non-m⁶A and m⁶A methylated genes in each treatment. Asterisks indicate significant differences (** $p < 0.01$; Student's t -test). (d) Histograms showing the number of significantly up- and down-regulated transcripts that contain m⁶A modifications in NI-Nip vs. Nip comparison group. (e) Stack diagram of the relationship between the m⁶A methylation differential types and the corresponding transcripts differential types in NI-Nip vs. Nip comparison group. The Y-axis represents transcripts of different regulation types as well as their numbers, and X-axis shows the up- and down-directed m⁶A methylated transcripts in NI-Nip vs. Nip group. (f) Widely integrated analyses of the relationship between the transcript expression levels and m⁶A methylated functional elements using the m⁶A modified transcripts in NI-Nip vs. Nip group. The Y-axis represents the different gene structure of m⁶A modification regions; X-axis shows the number of transcripts with different regulatory types. (g) Percentages of each gene body in differentially m⁶A-methylated genes in control Nip, NI-Nip, as well as in NI-Nip vs. Nip group. m⁶A, N⁶-methyladenosine; BPH, brown planthopper; Nip, control Nipponbare rice sample; NI-Nip, BPH-infested Nipponbare rice sample.

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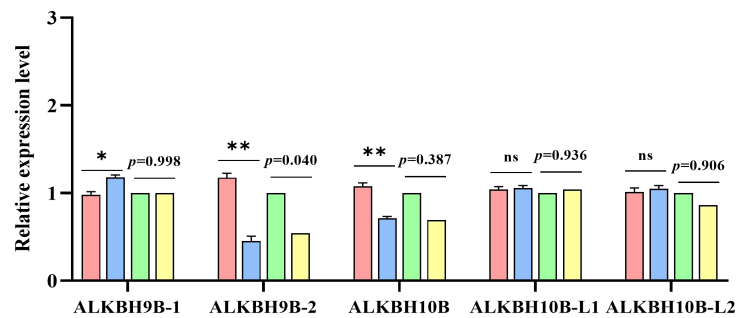
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“WRITER”



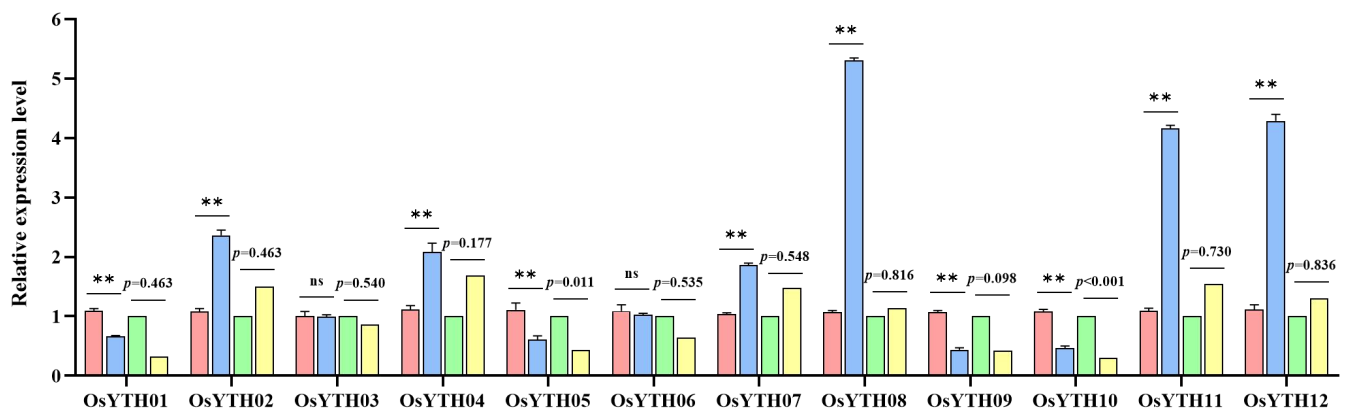
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“ERASER”



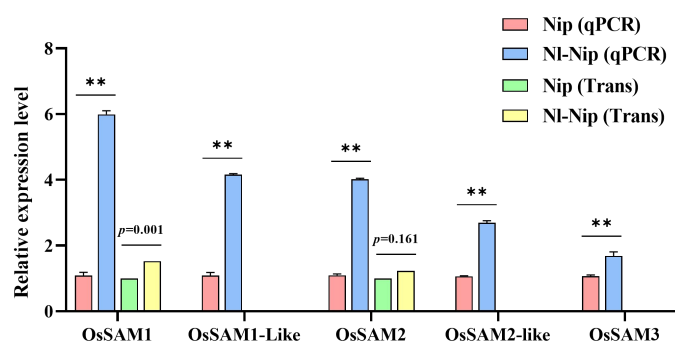
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“READER”



d

“DONOR” synthesis



e

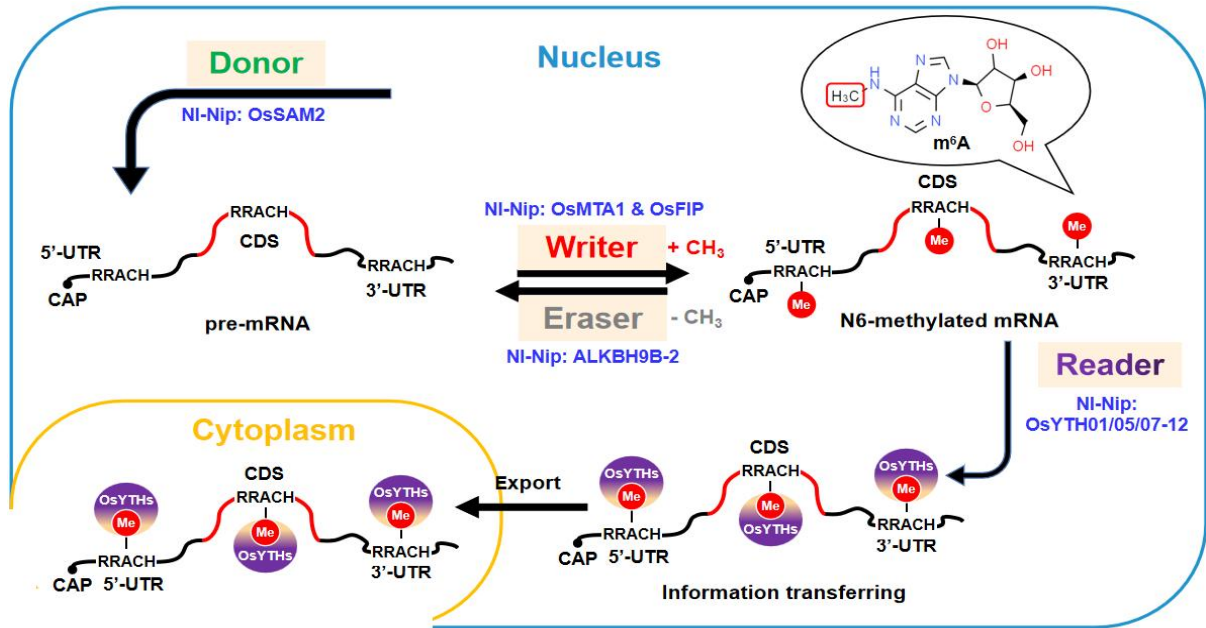
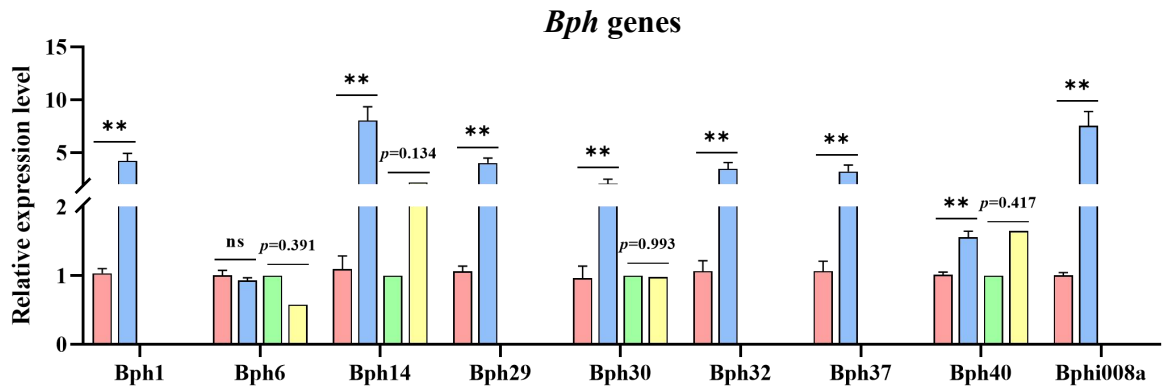


FIGURE 3 Relative expression levels of rice genes involved in m⁶A methylation machinery under BPH infestation

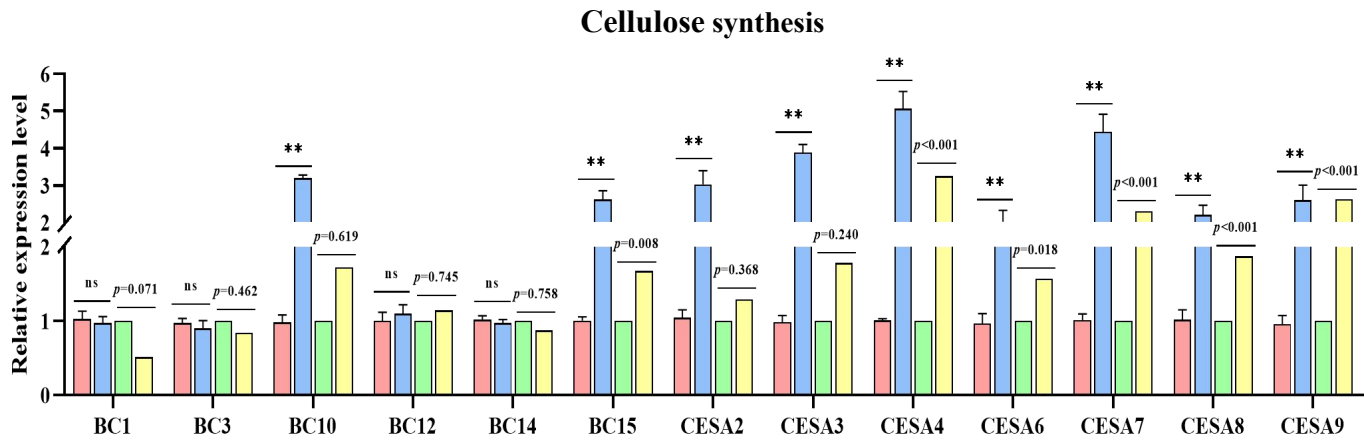
(a–d) RT-qPCR and transcriptome analysis of the relative expression of 5 “WRITER” genes (a), 5 “ERASER” genes (b), 12 “READER” genes (c), and 5 methyl “DONOR” synthesis genes (d) in Nip and NI-Nip plants. Some undetected transcriptome data indicated the absence of m⁶A methylation sites in the transcript. Error bars represent standard errors. Asterisks indicate significant differences (* $p < 0.05$; ** $p < 0.01$; ns, no significant difference; Student’s t -test). (e) Rice m⁶A methylation pathways and related genes upon BPH infestation. Blue letters indicate differentially expressed transcripts containing differentially directed m⁶A methylation with the same regulatory trend. *OsSAM2* means that *OsSAM2* was up-regulated and showed up-directed m⁶A methylation in NI-Nip compared with in Nip plants. *OsFIP* indicates that *OsFIP* was down-regulated and also showed down-directed m⁶A methylation in NI-Nip compared with in Nip plants. The differentially expressed transcripts showed a transcriptional expression fold change of < 0.5 or > 2 ($p < 0.05$), along with a significant m⁶A methylation direction ($p < 0.05$), and |meth diff| > 10. m⁶A,

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

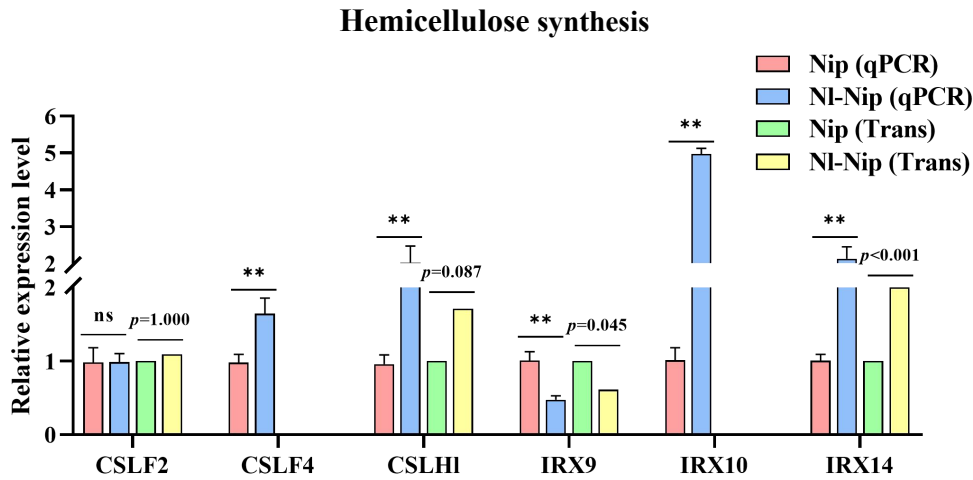
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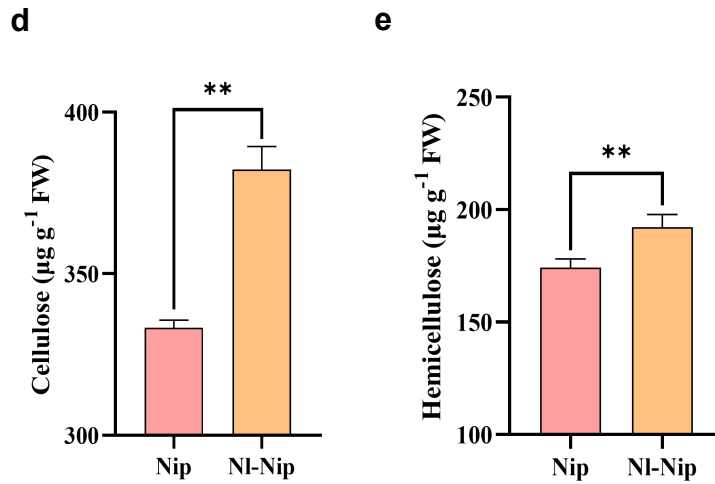


FIGURE 4 The BPH infestation activated cellulose- and hemicellulose-synthesis pathways (a–c) RT-qPCR and transcriptome analysis of the relative expression of 9 *Bph* genes (a), 13 cellulose synthesis genes (b), and 6 hemicellulose synthesis genes (c) in Nip and NI-Nip plants. Some undetected transcriptome data indicated the absence of m⁶A methylation sites in the transcript. (d, e) Mean levels ($n = 6$) of cellulose (d) and hemicellulose (e) in Nip and NI-Nip plants. Error bars represent standard errors. Asterisks indicate significant differences (** $p < 0.01$; ns, no significant difference; Student's t -test). Nip, control Nipponbare rice; NI-Nip, BPH-infested Nipponbare rice; FW, fresh weight.

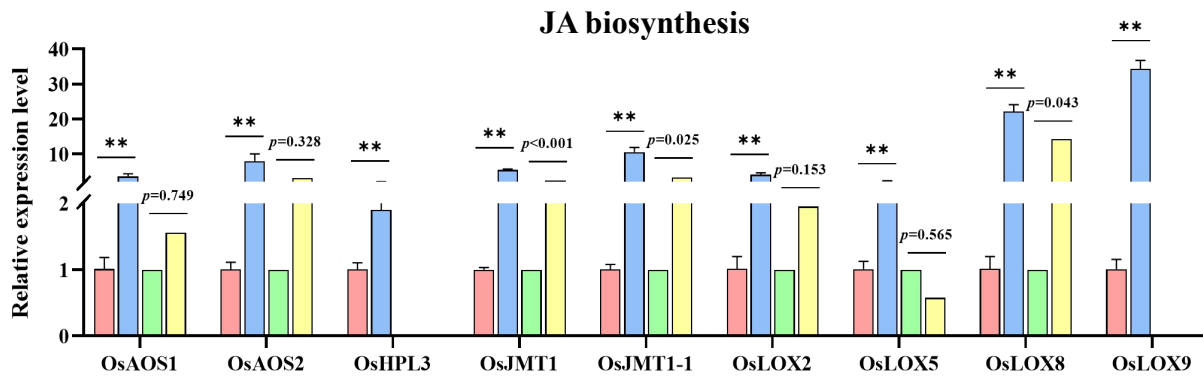
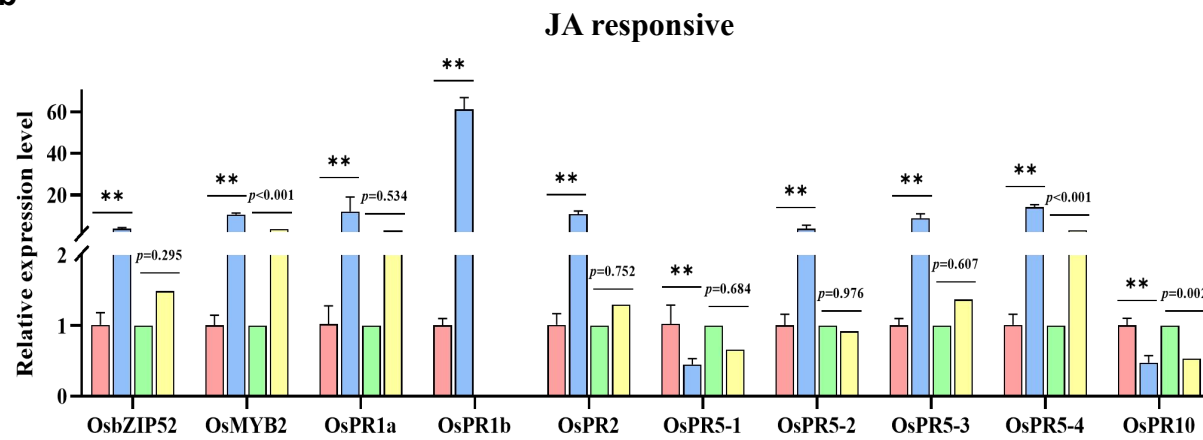
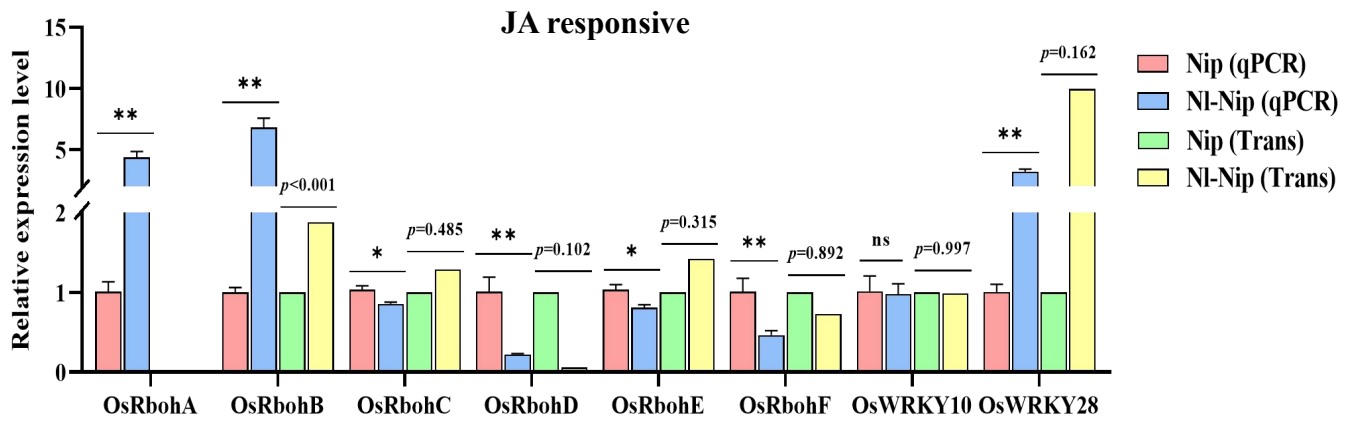
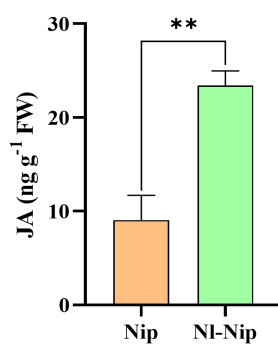
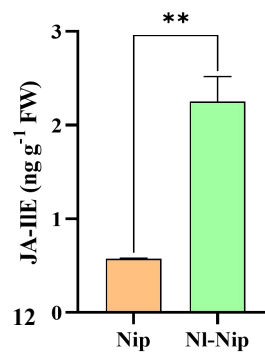
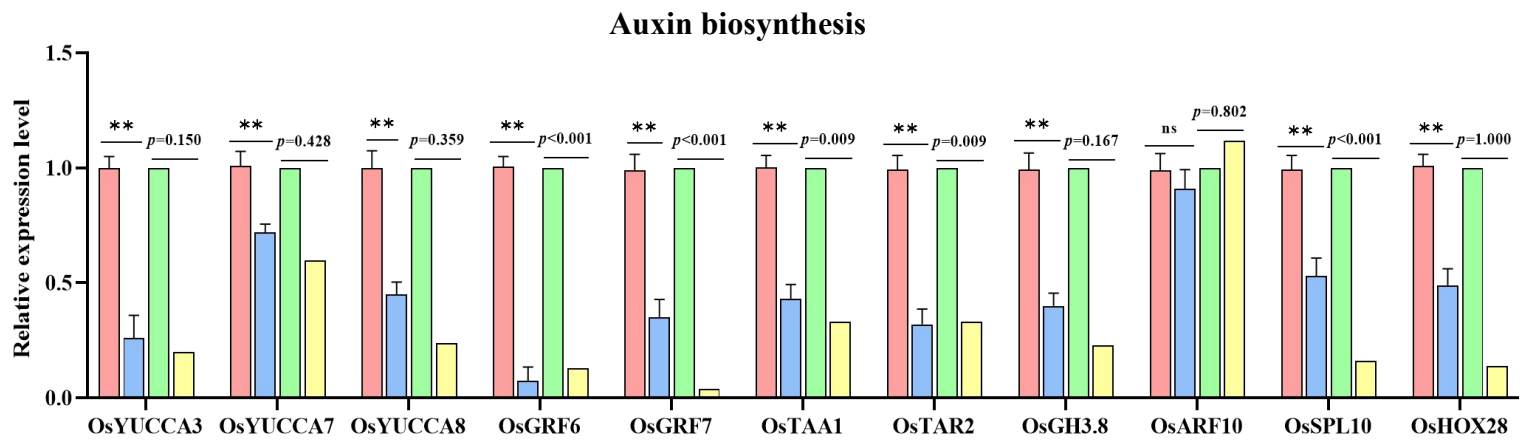
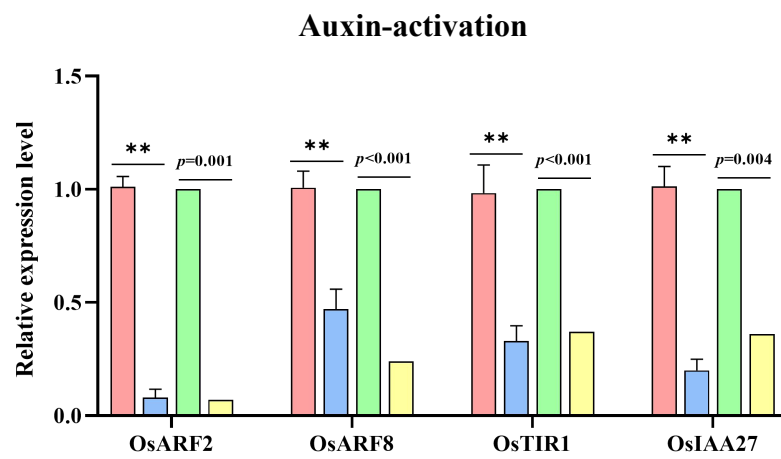
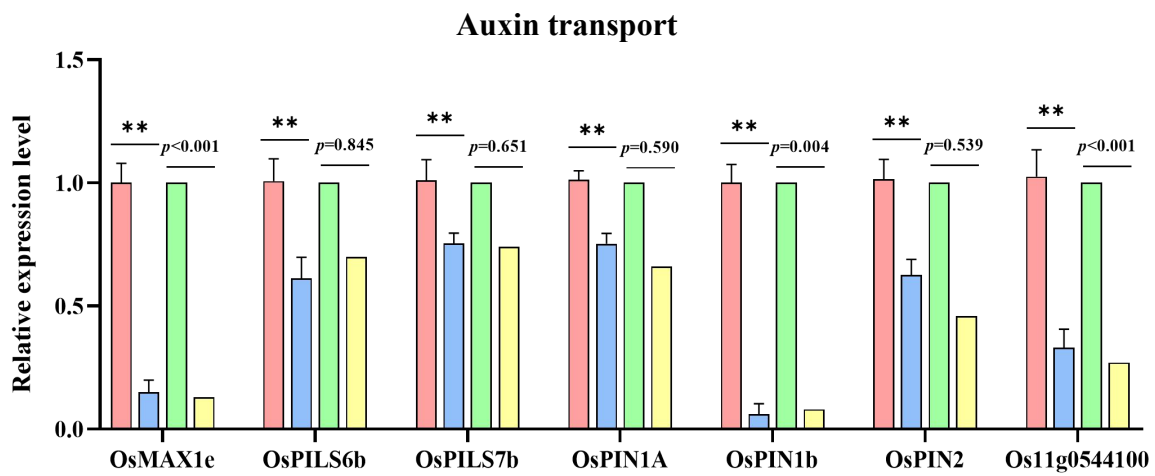
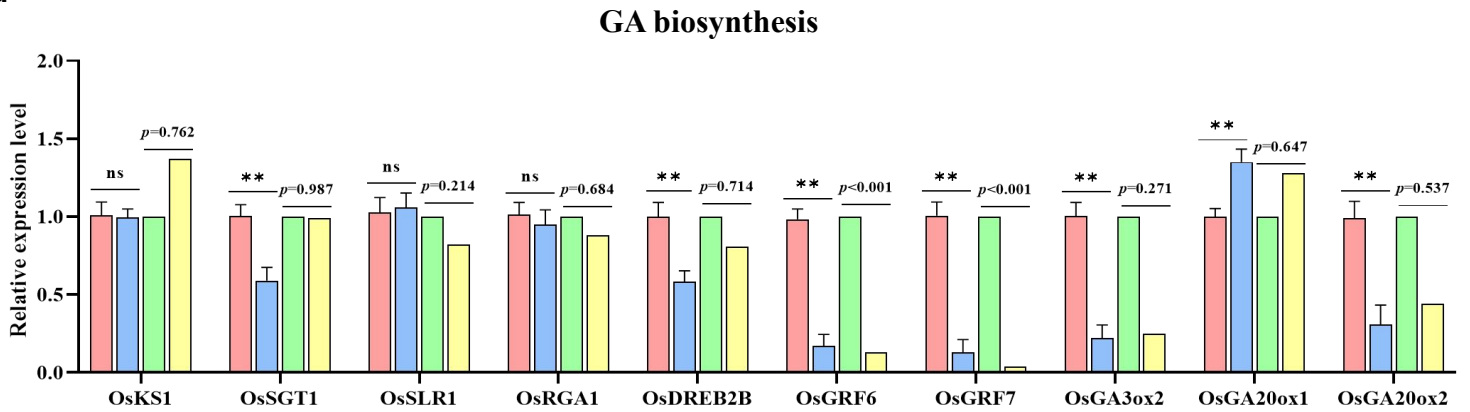
a**b****c****d****e**

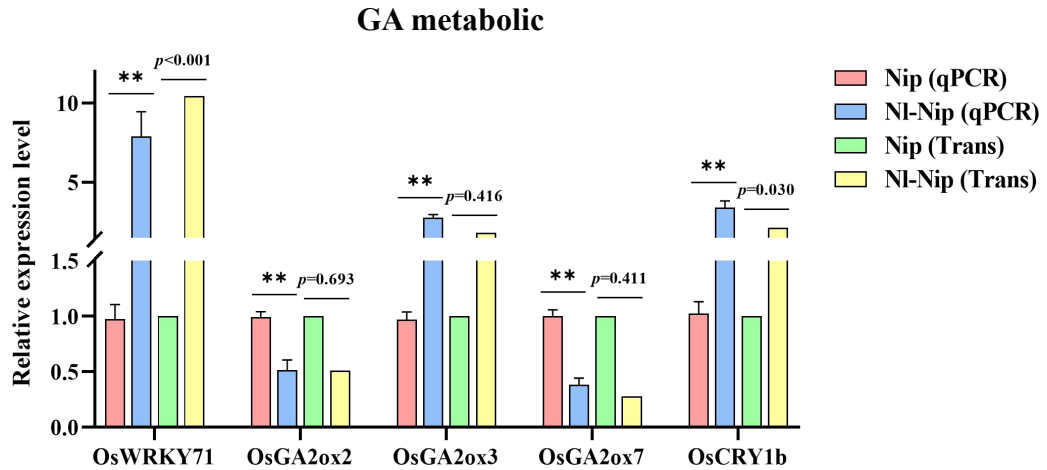
FIGURE 5 Jasmonic acid biosynthesis and responsive pathways were activated in BPH-infested rice plants (a–c) RT-qPCR and transcriptome analysis of the relative expression of 9 JA biosynthesis-related genes (a), and 18 JA responsive genes (b, c) in Nip and NI-Nip plants. Some undetected transcriptome data indicated the absence of m⁶A methylation sites in the transcript. (d, e) Mean levels ($n = 6$) of JA (d), and jasmonoyl-isoleucine (JA-ILE) (e) in Nip and NI-Nip plants. Error bars represent standard errors. Asterisks indicate significant differences (* $p < 0.05$; ** $p < 0.01$; ns, no significant difference; Student's t -test). Nip, control Nipponbare rice; NI-Nip, BPH-infested Nipponbare rice; 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.

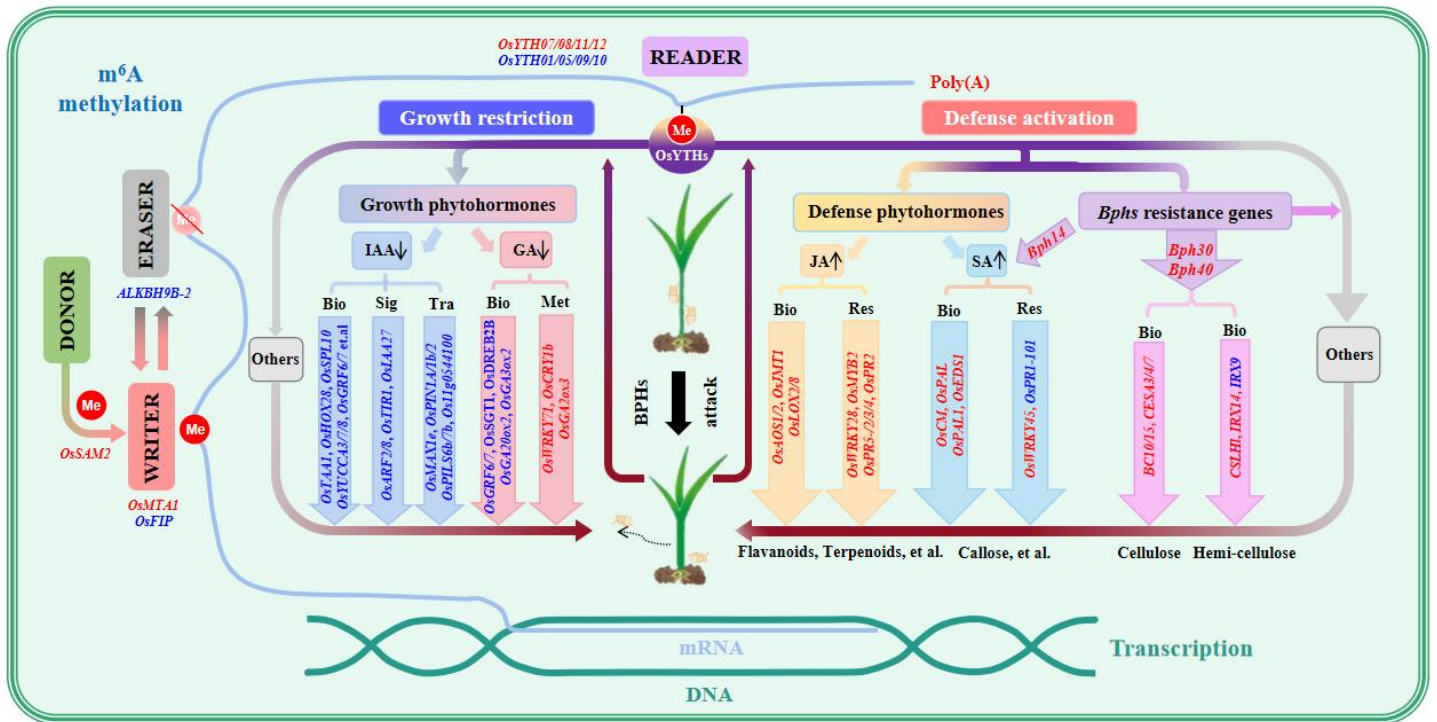


FIGURE 7 Proposed model of the rice m⁶A RNA methylation modulation in key defense and growth pathways regulated by BPH infestation

The genes highlighted in red denote both transcriptional up-regulated ($p < 0.05$) and up-directed m⁶A modification ($p < 0.05$ and $|\text{meth diff}| > 10$). The number of up-directed m⁶A sites occurring on the target pathway in NI-Nip vs. Nip group was less than that of down-directed m⁶A sites. Genes highlighted in blue represent both transcriptional down-regulated ($p < 0.05$) and down-directed m⁶A modification; the number of down-directed m⁶A sites occurring on the target pathway in NI-Nip vs. Nip group was less than that of up-directed m⁶A 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; m⁶A, N⁶-methyladenosine; BPH, brown planthopper; Nip, control Nipponbare rice; NI-Nip, BPH-infested Nipponbare rice.