Widespread DNA N6-methyladenine plays a crucial role in parasitic nematodes

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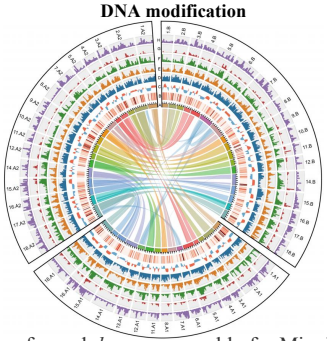
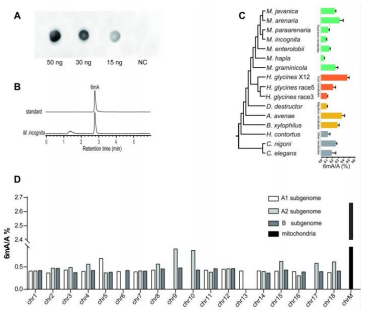
DNA *N*6-methyladenine (6mA) is a noncanonical DNA modification that is present at low levels in different eukaryotes. Previous studies have verified the presence of 6mA methylation in *Caenorhabditis elegans* and the mutant demethylase NMAD-1 alone still has high viability in the 20 generation, but its prevalence and importance associate with genomic function in nematodes remains poorly understood. Here we report that 6mA is widespread in parasitic nematode, and regulation the lifecycle in parthenogenetic *Meloidogyne incognita* (Mi). Through our newly assemblied Mi genome, three putative 6mA demethylase have been idendified. Host-induced silencing (HIS) of demethylase gene reduce the infectivity of Mi, compared with the GFP control, effector Mi-XYl1, Mi-CRT, Mi8D05, MiPFN3, 16D10, MiSGCR1 and MiIDL1 down-regulated 22, 126, 10, 28, 27, 5 and 13-fold in female, respectively. RNA-seq of nematodes after demethylase gene RNAi found that the effector expression pattern of nematodes was disordered, which cause the decrease of the ability to infection.

Improved Mi reference genome assist the identification of DNA modification



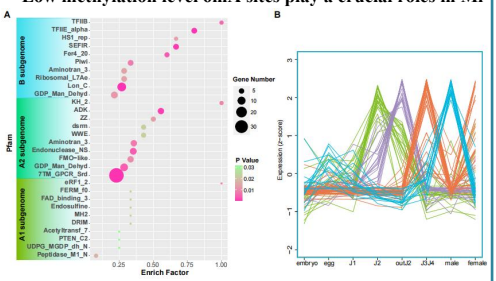
We performed *de novo* assembly for Mi with PacBio data, and correction with Illumina data. The initial contigs were further corrected and scaffolding by 120G Hi-C data. The final assembled Mi reference genome size was 213 Mb, distinguished 3 sets of subgenome.

6mA is widespeard in nematode



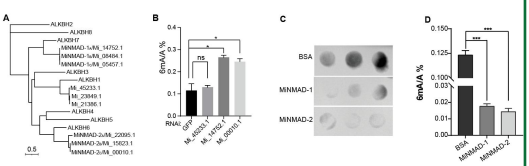
A, 6mA Dot blot of Mi genomic DNA. **B,** LC-MS/MS for 6mA standard and 6mA levels in genomic DNA purified from Mi egg. **C,** 6mA levels identified by LC-MS/MS in different nematode. **D,** the 6mA abundance of each haplotype chromosome, suggest the highest level in mitochondria.

Low methylation level 6mA sites play a crucial roles in Mi



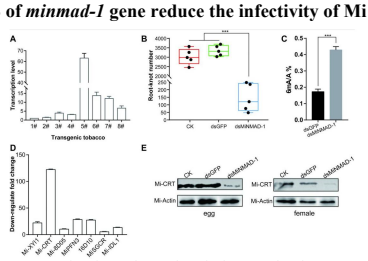
A, Pfam enrichment of low methlation level 6mA site assosiate gene display a more important function. **B,** the effectors located in A2 subgenome low methylation regions displayed a dynamic expression module.

MiNMAD-1/2 is involved in 6mA demethylase



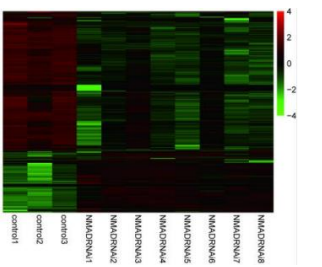
A, putative demethylase of 6mA in Mi. **B,** the 6mA level of Mi after putative demethylase gene RNAi. **C,** in vitro enzyme activity verification of putative demethylase. **D,** the Mi egg genomic DNA 6mA level identified by LC-MS/MS of putative demethylase treatment in vitro.

HIS of *minmad-1* gene reduce the infectivity of Mi



A, Transcription level of demethylase gene in tobacco. **B,** root-knot number of HIS tobacco and control. **C,** the 6mA level of Mi in HIS tobacco. **D,** the effector thranscription level of Mi in HIS tobacco. **E,** western blot of effector Mi-CRT for Mi egg and female.

6mA regulation the expression module of effector



RNA-seq of Mi female after *minmad-1* RNAi found that the effector expression pattern of Mi was disordered, which cause the decrease of the ability to infection.

In summary, DNA N6-methyladenine is widely exist in parasitic nematodes and it is an essential function gene in RKN. Additionally, compare with the C. elegans NMAD-1 mutants, Mi24650 is crucially for lifecycle in *Meloidogyne* spp. We speculate that epigenetics may play a more important role in parthenogenetic reproduction than sexual reproduction.