

Abstract

RNA *N*₆-methyladenosine (m₆A) modifications are essential in plants. Here, we show that transgenic expression of the human RNA demethylase FTO in rice caused a more than threefold increase in grain yield under greenhouse conditions. In field trials, transgenic expression of FTO in rice and potato caused ~50% increases in yield and biomass. We demonstrate that the presence of FTO stimulates root meristem cell proliferation and tiller bud formation and promotes photosynthetic efficiency and drought tolerance but has no effect on mature cell size, shoot meristem cell proliferation, root diameter, plant height or ploidy. FTO mediates substantial m₆A demethylation (around 7% of demethylation in poly(A) RNA and around 35% decrease of m₆A in non-ribosomal nuclear RNA) in plant RNA, inducing chromatin openness and transcriptional activation. Therefore, modulation of plant RNA m₆A methylation is a promising strategy to dramatically improve plant growth and crop yield.

Main

To meet the increasing food demand caused by population growth, numerous strategies have to be attempted to increase plant production¹. More than 30 years of transgenic research have led to notable advances in engineering crops for higher yield through transformation of exogenous and endogenous genes², for example, by introduction of insect resistance with bacterial toxins³, herbicide tolerance with bacterial enzymes degrading herbicides⁴ and engineering disease resistance with endogenous genes⁵. Multiple endogenous genes have also been manipulated to directly increase yields by engineering plant architecture, nitrogen use and other pathways^{6,7,8}. Modulation of photorespiratory pathways to enhance C₃ crop photosynthetic efficiency is another modern strategy to increase yield via engineering of plant glycolate metabolism with bacterial genes⁹. The emerging field of epitranscriptomics has revealed extensive post-transcriptional regulation of RNA metabolism affecting cell differentiation and development¹⁰, but whether the epitranscriptome can be engineered to stimulate crop production remains unexplored.

m₆A is the most abundant mRNA modification in higher eukaryotes; it can be dynamically written, read and erased to regulate RNA processing and metabolism^{11,12}. In mammalian systems, the m₆A methylation mark is essential: knockout of the gene encoding METTL3 m₆A methyltransferase is embryonic lethal in mice¹³. Human FTO, originally identified as a fat mass- and obesity-associated protein¹⁴, mediates RNA

m₆A demethylation¹⁵. Different RNA modifications can be demethylated by FTO in human cells, including m₆A in poly(A) RNA and U6 small nuclear RNA (snRNA), N₆,2'-O-dimethyladenosine (m₆A_m) at the cap +1 position in poly(A) RNA, cap and internal m₆A_m in U1 and U2 snRNA and N₁-methyladenosine (m₁A) in tRNA^{15,16,17,18}. FTO-mediated m₆A demethylation was shown to affect cell growth and proliferation¹⁹. In plants, m₆A is also required for normal development: early works showed that disruption of an m₆A writer subunit leads to embryonic lethality in *Arabidopsis*^{20,21,22,23} and early degeneration of microspores in rice²⁴; that plant RNA m₆A demethylases ALKBH10B and ALKBH9B, homologs of the human m₆A demethylase ALKBH5 (ref. 25), affect floral transition²⁶ and viral infection²⁷; and that plant m₆A reader proteins appear to affect multiple physiological properties in *Arabidopsis*^{28,29,30,31,32,33,34}. As these results all indicate that m₆A does affect plant growth and physiology, we speculated that manipulation of plant m₆A levels via introduction of an m₆A demethylase might offer a new means to affect plant growth.

Because plants do not have an FTO homolog, we pursued this idea about potential growth-altering effects of manipulating the plant epitranscriptome by introducing human FTO. We envisioned that, as a 'foreign' protein, FTO may not be recognized or controlled by plant components. Its demethylation activity may affect multiple potential targets to yield unexpected effects. Here we report that transgenic expression of FTO increases rice and potato yields and biomass by ~50% in field trials. Expression of FTO enhances root growth, tiller bud formation, photosynthetic efficiency and drought tolerance. FTO specifically promotes root meristem cell proliferation, leading to larger root numbers and length. All these phenotypes require the demethylation activity of FTO. We further demonstrated that FTO-mediated m₆A demethylation promotes chromatin openness and induces transcriptional activation. Our study reveals that RNA m₆A modification is critical to control plant growth, and its modulation provides a new, promising approach to substantially elevate crop production.

Results

Active *FTO* transgene increases crop yield and biomass

We generated transgenic rice plants expressing FTO in two genetic backgrounds, Nipponbare (Nipp) and Zhonghua11 (ZH11), as well as transgenic Nipp plants expressing a demethylation activity-dead mutant variant of FTO (FTO_{R316Q/R322Q}, designated *FTO*_{mut} rice) (Supplementary Fig. 1a,b). After confirming expression of FTO in plants of *FTO-1*, *FTO-2* (Nipp background) and *FTO-3* (ZH11 background)

transgenic lines and expression of $FTO_{R316Q/R322Q}$ in FTO_{mut} transgenic rice plants (Nipp background) (Supplementary Fig. 1c,d and Supplementary Tables 1 and 2), we characterized agronomic traits in the greenhouse (Supplementary Fig. 2a–d). To our surprise, the expression of FTO led to a more than threefold increase in grain yield and a more than twofold increase in aerial biomass (that is, above-ground matter, excluding grain) compared with those of wild-type (WT) control rice under greenhouse conditions (Supplementary Fig. 2d). We subsequently conducted field trials with homozygous transgenic and WT plants at two different locations in China (Jiangxi and Beijing). Consistently, rice expressing catalytically active FTO showed significantly increased grain yield and biomass compared to both WT and inactive FTO_{mut} plants in the field (Fig. 1a,b).

Fig. 1: Transgenic expression of FTO increases yield and biomass of rice and potato plants in the field.

a, Photos of mature WT (Nipp), *FTO* and *FTO*_{mut} (a demethylation activity-dead mutant variant of *FTO*) rice plants grown in the field. Scale bar, 10 cm. **b**, Total grains per plant in WT (Nipp), *FTO*_{mut}- and *FTO*-transgenic rice plants in the field. Scale bars, 10 cm. **c–e**, Grain yield (**c**), dry biomass (**d**) and productive tiller number per plant (**e**) in the indicated rice lines grown in the field. Data are mean ± s.e.m. (**c**, $n = 15$ Nipp, $n = 22$ *FTO*_{mut}, $n = 15$ *FTO*-1, $n = 27$ *FTO*-2, $n = 20$ ZH11, $n = 15$ *FTO*-3; **d**, $n = 12$ Nipp, $n = 23$ *FTO*_{mut}, $n = 15$ *FTO*-1, $n = 25$ *FTO*-2, $n = 19$ ZH11, $n = 15$ *FTO*-3; **e**, $n = 20$ Nipp, $n = 25$ *FTO*_{mut}, $n = 16$ *FTO*-1, $n = 26$ *FTO*-2, $n = 27$ ZH11, $n = 24$ *FTO*-3). **f**, Photos of tubers harvested from 20 potato plants of WT (EM3) and *FTO*-transgenic backgrounds grown in the field. **g,i**, Tuber yield (**g**) and tuber number (**i**) in the

indicated potato plants grown in the field. Data are mean \pm s.e.m. of n plots (**g**, $n = 7$ EM3, $n = 17$ *FTO*; **i**, $n = 5$ EM3, $n = 20$ *FTO*; each plot contained over 50 plants). **h**, Dry biomass per plant of the indicated potato plants grown in the greenhouse. Data are mean \pm s.e.m. ($n = 6$ EM3, $n = 36$ *FTO*). P values are from unpaired, two-tailed Student's t -tests.

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Specifically, all three transgenic rice lines expressing catalytically active *FTO* showed over 47% higher grain yield and grain number and over 40% higher biomass in the field (Fig. 1c,d and Supplementary Fig. 3a). Moreover, examination of plants in the field before the heading stage showed that *FTO*-transgenic rice exhibited a more than 42% increase in the number of total tillers as well as total productive tillers per plant (that is, tillers that ultimately produce a grain-bearing panicle) compared with WT or *FTO*_{mut} rice (Fig. 1e and Supplementary Fig. 3b); almost all of the increased tillers in *FTO* rice plants eventually developed into productive tillers. Further evaluation of the various phenotypes in this field study revealed no significant differences in grain size, thousand-grain weight, main panicle grain number, major spike length, mature plant height or heading time (Supplementary Fig. 3c–h), suggesting that the observed higher grain yield and aerial biomass in *FTO*-transgenic rice result primarily from the substantial increase in the productive tiller number. Thus, the RNA demethylation activity of *FTO* can confer massive increases in multiple economically impactful agronomic traits in the globally essential monocot cereal crop rice. These growth phenotypes are dependent on the demethylation activity of *FTO*, as *FTO*_{mut} plants expressing catalytically inactive *FTO* did not exhibit these traits.

The growth promotion effect was not limited to monocot rice. When we transgenically expressed *FTO* in the eudicot tuber crop potato (*Solanum tuberosum*, Emalingshu3 (EM3) background) (Supplementary Fig. 1c,d and Supplementary Tables 3 and 4), we observed similarly massive increases in both yield and aerial biomass (Fig. 1f–h). We found that, although *FTO* did not cause potato plants to produce significantly more tubers (Fig. 1i), it led to ~50% increases in yield (assessed as total tuber weight) and aerial biomass (Fig. 1g,h). Thus, transgenic introduction of *FTO* can confer drastic yield and biomass increases in dicotyledonous plants.

We further investigated whether the *FTO* expression level and/or inheritance of the *FTO* transgene across generations may affect the high yield and biomass agronomic traits of rice plants. After testing the field phenotypes of four *FTO*-transgenic rice lines (Nipp background) with varied *FTO* expression levels and three generations (T_2 to T_4) of the *FTO-1* lines, we found that all these plants

exhibited the aforementioned phenotypes, including more than a 40% increase in grain yield (Supplementary Fig. 4a,b). More discussion is provided in the [Supplementary Results](#). We also evaluated protein and amylose contents in *FTO*-transgenic rice grains and carbohydrate, starch, vitamin C and overall protein contents in *FTO*-transgenic potato tubers and found that expression of *FTO* did not alter these nutrition-related traits in rice grains or potato tubers as compared to those in the corresponding WT or *FTO*_{mut} plants (Supplementary Fig. 5a–f).

Active *FTO* transgene promotes root growth

Seeking to further characterize the physiological impacts of introducing *FTO*, we examined 15-d-old seedlings and 2-month-old plants at the tillering stage of WT (Nipp), *FTO*_{mut} and *FTO*-transgenic rice. We found that fresh weights of whole plants, aerial tissues and roots were all markedly increased at tillering and seedling stages of *FTO*-transgenic rice (Fig. 2a and Supplementary Figs. 6 and 7). Detailed evaluation of root morphological parameters at both stages using WinRHIZO software (Regent Instruments) revealed that roots offered the most visually striking difference for *FTO*-transgenic rice at the tillering stage (Supplementary Figs. 8 and 9 and [Methods](#)). Compared to WT seedlings, *FTO* rice seedlings showed around 33% and 45% increases in the total root number and the total length of lateral roots (Supplementary Fig. 8b,g). We also noted that *FTO* rice plants exhibited more than a 3.3-fold increase in both number and length of primary roots (4.4-fold and 3.3-fold increases in number and length, respectively) at the tillering stage when compared with WT plants (Supplementary Fig. 9b,e). Similar increases were also detected for lateral roots (3.7-fold increase in both number and length) (Supplementary Fig. 9c,f). There was no obvious differences in root diameter between *FTO* and WT plants at either the seedling (Supplementary Fig. 8h,i) or the tillering stage (Supplementary Fig. 9g,h). Note that we also compared WT and *FTO*_{mut} plants in these analyses and found no phenotypic differences.

Fig. 2: *FTO* increases root meristem cell proliferation, root growth, tiller formation and photosynthesis efficiency.

a, Root photos of 2-month-old Nipp, *FTO*_{mut-} and *FTO*-transgenic rice at the tillering stage grown in the greenhouse. Scale bars, 2 cm. **b**, PI staining of root tips in 10-d-old Nipp, *FTO*_{mut-} and *FTO*-transgenic rice and quantitative analysis of root meristem length, cell length and cell number within the same length of the root meristem (as indicated in white boxes and magnified images at the bottom). Arrowheads indicate ends of root meristems. The cell length was calculated from the same length of the root meristem as indicated by the yellow line. Data are mean \pm s.e.m. ($n = 4$ (left); $n = 9$ Nipp, $n = 8$ *FTO*_{mut}, $n = 12$ *FTO* (middle); $n = 8$ Nipp, $n = 8$ *FTO*_{mut}, $n = 10$ *FTO* (right)). Scale bars, 20 μ m. **c**, Longitudinal view of EdU-labeled cells in root meristems of 4-d-old Nipp, *FTO*_{mut-} and *FTO*-transgenic rice seedlings. Scale bars, 20 μ m. Numbers of EdU-labeled cells in root tips were counted. Data are mean \pm s.e.m. ($n = 6$ Nipp, $n = 10$ *FTO*_{mut}, $n = 12$ *FTO*). **d**, Shoot basal regions of four-leaf stage Nipp, *FTO*_{mut-} and *FTO*-transgenic rice seedlings. Arrowheads indicate tiller buds. Scale bars, 1 mm. Data are mean \pm s.e.m. ($n = 10$ Nipp, $n = 10$ *FTO*_{mut}, $n = 9$ *FTO*). **e**, Longitudinal sections of shoot apices and tiller buds of 10-d-old Nipp, *FTO*_{mut-} and *FTO*-transgenic rice. Scale bars, 100 μ m. The experiment was repeated three times independently with similar results. **f–h**, Photosynthetic rate (**f**), stomatal conductance (**g**) and transpiration rate (**h**) of Nipp, *FTO*_{mut-} and *FTO*-transgenic rice at the filling stage grown in a field in Beijing. Data are mean \pm s.e.m. (**f**, $n = 16$ Nipp, $n = 10$ *FTO*_{mut}, $n = 11$ *FTO*; **g**, $n = 16$ Nipp, $n = 10$ *FTO*_{mut}, $n = 9$ *FTO*; **h**, $n = 17$ Nipp, $n = 9$ *FTO*_{mut}, $n = 11$ *FTO*). *P* values are from unpaired, two-tailed *t*-tests.

Active *FTO* transgene enhances root cell proliferation

To characterize phenotypic differences in roots at the histological level and to seek clues about altered plant development programs, we examined cell size in root zones and mature leaves. Staining of longitudinal tissue sections of rice and potato plants showed that cell sizes in both root zones and mature leaves of *FTO* rice and potato plants did not differ from those of the corresponding WT or *FTO*_{mut} plants (Supplementary Fig. 10a,b). These observations suggest that increases in root growth are likely caused by cell division, not cell elongation. Subsequently, we used confocal microscopy to examine meristem cells in propidium iodide (PI)-stained root tips. The size of *FTO* rice root apical meristem zones did not differ from that of WT or *FTO*_{mut} roots; however, meristem cells of *FTO* roots had lesser longitudinal length and had higher overall meristem cell numbers (Fig. 2b), indicating that *FTO* expression in rice increases root apical meristem cell proliferation.

We also examined the shoot apical meristem and observed that shoot apical meristem size and numbers of the first layer of meristem cells were not altered among WT, *FTO* and *FTO*_{mut} rice (Supplementary Fig. 11), consistent with our findings that *FTO*-transgenic rice did not differ in rice leaf size or plant height. To further confirm that *FTO* does increase root cell proliferation, we labeled newly replicated DNA using 5-ethynyl-2'-deoxyuridine (EdU) and found that the EdU signal was significantly increased in *FTO* rice roots compared with those of WT and *FTO*_{mut} plants (Fig. 2c and Methods), supporting the notion that root cell division rate was increased by *FTO*. Collectively, these results reveal that expression of *FTO* in plants enhances root development by promoting cell division in the root meristem.

Active *FTO* transgene promotes tiller bud formation

Yields in cereal crops are strongly impacted by the number and type of tillers produced by individual plants³⁵. We found that *FTO*-transgenic rice had a greater than 40% increase in the number of productive tillers (Fig. 1e). Given that the development of productive tillers is controlled in a two-stage process including the formation of an axillary bud at the unelongated basal internode and the subsequent outgrowth of such buds³⁵, we next characterized the role of *FTO* in rice tillering ability at the anatomical level. *FTO*-transgenic rice as four-leaf-stage seedlings had significantly more axillary buds on the unelongated basal internode than WT and *FTO*_{mut} plants at the same growth stage (Fig. 2d). Longitudinal sections further

confirmed that 10-d-old *FTO*-transgenic rice had already formed a normal tiller bud but WT and *FTO*_{mut} rice had not (Fig. 2e). Note that *FTO*-transgenic, WT and *FTO*_{mut} plants did not have tillers on elongated upper internodes during field trials.

FTO increases photosynthesis and drought tolerance

Our observations of notable increases in diverse plant organs upon *FTO* expression suggest that this RNA-demethylase enzyme may affect multiple physiological processes in plants. Any increase in plant growth must be accompanied by increased carbon acquisition. Indeed, we found that *FTO*-expressing plants exhibited ~36% higher net photosynthetic efficiency than WT or *FTO*_{mut} plants in the field (Fig. 2f). Consistently, *FTO*-transgenic plants also showed a ~34% increase in stomata conductance and a ~78% increase in transpiration rates (Fig. 2g,h). Considering that stomatal opening might induce hypersensitivity in droughts³⁶, we tested whether *FTO* expression in rice affects drought resistance. Results showed that both 3- and 6-week-old *FTO* rice plants exhibited significantly higher survival rates under two drought stress treatment conditions compared to WT and *FTO*_{mut} plants (Supplementary Fig. 12 and [Supplementary Methods](#)). Thus, these results show that *FTO*-transgenic plants possess enhanced photosynthetic efficiency and drought tolerance. More discussion on photosynthesis and drought tolerance is provided in the [Supplementary Results](#).

FTO mediates substantial nuclear m₆A demethylation

To explore the potential molecular pathways through which *FTO* confers the observed phenotypes, we next investigated effects on RNA caused by expression of *FTO*. Both stably transformed *35S::FTO-eGFP* transgenic *Arabidopsis* root and tobacco leaves transformed with the *35S::FTO-eGFP* construct for transient expression showed that *FTO* is primarily localized in the nucleus (Supplementary Fig. 13), consistent with the main subcellular localization of *FTO* observed in mammals¹⁵. We examined how *FTO* affects m₆A levels in diverse organs of transgenic rice and potato plants. We isolated poly(A) RNA, non-ribosomal RNA and non-ribosomal nuclear RNA from different organs of WT, *FTO*_{mut} and *FTO* plants and quantified the ratio of m₆A/A (a relative m₆A level) by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Consistent with the known m₆A-demethylation function of *FTO*, samples from 15-d-old *FTO*-transgenic rice displayed noticeably reduced m₆A levels (reduction of around 7%) in poly(A) RNA isolated from both shoots and roots compared to those from WT and *FTO*_{mut} plants.

Notably, the non-ribosomal nuclear RNA portion showed a decrease of around 35% in m₆A level in *FTO*-transgenic rice seedlings compared to that from WT plants (Fig. 3a). *FTO* expression clearly caused more m₆A reduction in rice non-ribosomal nuclear RNA than that in rice total poly(A) RNA, which is consistent with the nuclear localization of overexpressed *FTO* in plants (Supplementary Fig. 13). This trend was evident for all tested organs at different stages and for all poly(A) RNA and non-ribosomal RNA (Supplementary Fig. 14a–d). Consistently, m₆A levels in poly(A) RNA were also notably reduced in *FTO*-transgenic potato plants (Supplementary Fig. 14e). We further determined m₆A levels in U6 snRNA isolated from shoots and roots of WT, *FTO*_{mut} and *FTO* rice and found that *FTO* expression did not mediate demethylation of m₆A in rice U6 snRNA (Supplementary Fig. 14f).

Fig. 3: Transcriptome-wide identification and analysis of *FTO*-mediated m₆A-demethylation sites in rice.

a, Quantification of the m⁶A/A ratio in non-ribosomal nuclear RNA (left) and poly(A) RNA (right) isolated from 15-d-old WT (Nipp), *FTO*_{mut}⁻ and *FTO*-transgenic rice shoots, roots and whole seedlings by LC-MS/MS. Data are mean ± s.e.m. (*n* = 12 Nipp, *n* = 4 *FTO*_{mut}, *n* = 12 *FTO* (left); *n* = 4 Nipp, *n* = 5 *FTO*_{mut}, *n* = 6 *FTO* in shoots; *n* = 6

Nipp, $n = 4$ *FTO*_{mut}, $n = 5$ *FTO* in roots (right)). **b**, Quantification of the ratio of poly(A) RNA to a synthesized poly(A) RNA spike-in control in a total RNA Pico Chip analysis using an Agilent 2100 Bioanalyzer. Poly(A) RNA along with the spike-in control were isolated from the same mass of shoots and roots of 15-d-old WT (Nipp), *FTO*_{mut}- and *FTO*-transgenic rice. Data are mean \pm s.e.m. ($n = 6$ Nipp, $n = 5$ *FTO*_{mut}, $n = 4$ *FTO* in shoots; $n = 3$ Nipp and *FTO*_{mut}, $n = 5$ *FTO* in roots). *P* values are from unpaired, two-tailed *t*-tests. nt, nucleotides. **c**, Cumulative frequency plots and box plots showing the distribution of transcript expression in WT (Nipp), *FTO*_{mut}- and *FTO*-transgenic rice shoot (top) or root (bottom) tissues. In box plots, lower and upper hinges represent first and third quartiles, the center line represents the median, and whiskers represent $\pm 1.5\times$ the interquartile range. *P* values were determined using one-tailed Mann–Whitney *U*-tests. TPM, transcripts per million. **d**, Violin-and-box plots displaying the distribution of significant m₆A hypomethylation levels ($\log_2(\text{FC}) < -0.5850$) in mRNA and repeat RNA in shoots and roots of *FTO*-transgenic rice plants. In box plots, lower and upper hinges represent first and third quartiles, the center line represents the median, and whiskers represent $\pm 1.5\times$ the interquartile range. *P* values were determined using two-tailed Mann–Whitney *U*-tests.

[Full size image](#)

Considering that FTO can demethylate other RNA modifications (m₆A_m and m₁A in different RNA types) in mammalian cells^{16,17,18} and N₆-methyldeoxyadenosine (6mA) in single-stranded DNA in vitro¹⁵, we subsequently investigated whether RNA modifications m₆A_m and m₁A and DNA 6mA could be demethylated by FTO in *FTO*-expressing plants. We found that plants contained neither the homolog of human cap m₆A_m methyltransferase PCIF1 (ref. 37) (Supplementary Fig. 15a) nor cap m₆A_m in rice poly(A) RNA (Supplementary Fig. 15b). Cap and internal m₆A_m in U1 and U2 snRNA, m₁A in tRNA and DNA 6mA are not substrates of expressed FTO in *FTO*-transgenic plants (Supplementary Fig. 16a–h and Methods). More discussion on the demethylation substrates of FTO is provided in the [Supplementary Results](#). Collectively, these results indicate that FTO notably demethylates m₆A in poly(A) RNA, non-ribosomal RNA and non-ribosomal nuclear RNA in *FTO*-transgenic plants.

FTO mediates m₆A demethylation of mRNA and repeat RNA

We subsequently investigated potential molecular mechanisms through which FTO-mediated m₆A demethylation may confer the observed profound increases in multiple economically impactful agronomic traits. We performed m₆A methylated RNA immunoprecipitation (MeRIP) sequencing, calibrated using synthesized

m₆A-modified poly(A) RNA spike-in controls in equal masses of shoot and root materials from 15-d-old WT, *FTO*_{mut} and *FTO* rice plants. Calibrated m₆A sequencing analysis in shoots and roots of WT, *FTO*_{mut}- and *FTO*-transgenic rice identified ~12,000 m₆A peaks in each genotype, with good reproducibility across replicates (Supplementary Fig. 17a and Methods). The m₆A motif and the distribution of detected m₆A sites along transcripts were consistent with previous m₆A sequencing results in plants^{24,26,29} (Supplementary Fig. 17b). We then analyzed differentially methylated m₆A sites in mRNA. Compared to those of WT rice, both shoots and roots of *FTO*-transgenic rice showed more hypomethylated m₆A peaks (hypo-m₆A, 222 in shoots and 331 in roots) than hypermethylated m₆A peaks (hyper-m₆A, 63 in shoots and 127 in roots) in mRNA (Supplementary Fig. 17c and Supplementary Data 1). The increased hypo-m₆A peak number in mRNA in both shoots and roots of *FTO*-transgenic rice is consistent with the m₆A-demethylation function of FTO. Hypo-m₆A peaks in mRNA were highly enriched within coding sequences and 3' untranslated regions (Supplementary Fig. 18a and Supplementary Data 1). Gene ontology (GO) analysis of these hypo-m₆A-modified transcripts in shoots (222 genes) and roots (330 genes) of *FTO*-transgenic rice revealed enrichments for functional annotations related to 'cellular homeostatic process', 'one-carbon and small-molecule metabolic process' and 'gene expression' (Supplementary Fig. 18b and Supplementary Data 1). By contrast, no pathway enrichment was detected in our GO analyses of hypo-m₆A-containing genes from *FTO*_{mut}-transgenic shoots (31 genes) or roots (337 genes) (Supplementary Fig. 17c and Supplementary Data 1 and 2), suggesting that variant m₆A peaks in *FTO*_{mut} plants are apparently random and non-functional.

The RNA modification m₆A was shown to be deposited onto chromatin-associated regulatory RNA (carRNA), including promoter-associated RNA, enhancer RNA and RNA transcribed from transposable elements (repeat RNA); further, m₆A was shown to promote the degradation of a subset of these repeat RNA species through nuclear exosome-targeting-mediated nuclear degradation³⁸. Mammalian repeat RNA species, such as those in the long interspersed element 1 (LINE1) family, are known to affect chromatin remodeling and gene transcription^{39,40}; removal of m₆A from LINE1 protects it from nuclear degradation and subsequently enhances both chromatin openness and downstream transcription in mouse embryonic stem cells³⁸. Considering that repeat RNA such as mammalian LINE1 contains a poly(A) tail, we asked whether FTO expressed in rice could demethylate m₆A in repeat RNA.

Compared to WT, 547 and 415 hypo-m₆A peaks, representing 408 and 323 repeat RNA species, were respectively identified in shoots and roots of *FTO* rice (Supplementary Fig. 19a and Supplementary Data 1). We found that the extent of m₆A hypomethylation was more pronounced for repeat RNA than that for mRNA (Fig. 3d and Supplementary Data 1). We ranked repeat classes or families according

to their m₆A hypomethylation levels, which showed that MuDR and En-Spm are the most strongly affected repeat RNA classes or families in *FTO* shoots and roots, respectively (Supplementary Fig. 19b and Supplementary Data 1).

FTO induces transcriptional activation and more open chromatin

Over the course of poly(A) RNA-purification steps during sample preparation for m₆A detection by LC–MS/MS, we repeatedly noted that, even though we started with equal masses of *FTO*-transgenic and WT samples, *FTO*-transgenic plants consistently yielded higher amounts of poly(A) RNA (Supplementary Fig. 20a). To verify this, we isolated poly(A) RNA from samples of equal plant mass along with a synthesized poly(A) RNA spike-in control and quantified the ratio of poly(A) RNA to spike-in RNA by total RNA Pico Chip analysis using an Agilent 2100 Bioanalyzer. *FTO*-transgenic plants accumulated higher levels of poly(A) RNA than WT and *FTO*_{mut} plants (Fig. 3b, Supplementary Fig. 20b–e and Methods). We next asked whether *FTO* affects plant ploidy, which could potentially explain the enhanced accumulation of transcripts; polyploidy has been a major force in plant evolution and crop domestication and has been harnessed for crop improvement⁴¹. Measurement of DNA content per cell nucleus by flow cytometry showed that *FTO*-transgenic rice was diploid, the same as WT and *FTO*_{mut} plants (Supplementary Fig. 21).

Seeking biological insights about the regulation network(s) through which the *FTO*-mediated m₆A demethylation enhances the accumulation of transcripts and affects yield and biomass phenotypes, we sampled equal masses of shoot and root materials from 15-d-old WT, *FTO*_{mut} and *FTO* rice plants and performed quantitative RNA sequencing (RNA-seq) with an External RNA Controls Consortium (ERCC) RNA spike-in control. Quantitative RNA-seq analysis confirmed that *FTO*-transgenic plants did indeed accumulate higher overall levels of poly(A) RNA compared to WT and *FTO*_{mut} plants (Fig. 3c, Supplementary Fig. 22, Supplementary Data 3 and Methods), further supporting our earlier observations (from RNA extractions) of increased poly(A) RNA expression in *FTO* plants.

Although m₆A is known to increase transcript degradation^{22,26,42}, the relatively modest levels of hypo-m₆A-modified mRNA in *FTO*-transgenic rice shoots (222 genes) and roots (330 genes) may only contribute to a limited extent to overall increased mRNA levels in *FTO*-transgenic rice shoots and roots. We instead observed 11,342 and 6,933 significantly upregulated mRNA species (fold change (FC) > 1.5) in shoots and roots of *FTO* plants compared to those in WT control rice, respectively, from our quantitative RNA-seq experiment (Supplementary Fig. 23a and Supplementary

Data 3). Increased overall expression levels of poly(A) RNA observed in *FTO*-transgenic rice shoots and roots are most likely derived from *FTO*-induced transcriptional activation.

Analysis of our quantitative RNA-seq data, focusing on differential expression of mRNA and repeat RNA, revealed that, compared to those of WT rice, shoots of *FTO* rice had 11,342 significantly upregulated mRNA species and 7,432 upregulated repeat RNA species ($FC > 1.5$) but had 539 significantly downregulated mRNA species and 918 downregulated repeat RNA species ($FC < 2/3$) (Supplementary Fig. 23a and Supplementary Data 3). Similar trends were also observed in *FTO* root samples: 6,933 significantly upregulated mRNA species and 7,068 upregulated repeat RNA species versus 2,699 significantly downregulated mRNA species and 2,009 downregulated repeat RNA species (Supplementary Fig. 23a and Supplementary Data 3). Overlap of these *FTO*-regulated mRNA or repeat RNA species between shoots and roots uncovered around 50% of overlapped transcripts, and their *FTO*-induced differential expression levels were organ specific (Supplementary Fig. 23b,c and Supplementary Data 3). These findings collectively suggest that, similar to the known biological impacts of mammalian m₆A in modifying transcriptional activation³⁸, *FTO*-mediated m₆A demethylation of plant repeat RNA may change the chromatin state. This can help to explain the global transcriptional upregulation that we observed in *FTO*-transgenic plants.

To validate whether the demethylation activity of *FTO* can affect chromatin state in plants, we performed DNase I-treated terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays on shoot apices and found a notable increase in chromatin openness in *FTO*-transgenic rice compared to that in WT and *FTO*_{mut} plants (Fig. 4a and Methods). Moreover, both *FTO*-transgenic rice and potato plants showed markedly decreased levels of histone 3 lysine 9 dimethyl (H3K9me2) and histone 3 lysine 27 trimethyl (H3K27me3) (both known transcription-repressing marks), suggesting that a more open chromatin state was indeed induced in *FTO* plants (Fig. 4b and Supplementary Fig. 24). These results confirm that *FTO*-mediated m₆A-demethylation promotes a more open chromatin state and induces transcriptional activation. This process could be through m₆A-modified repeat RNA or other carRNA species that affect chromatin state as recently shown in mammals^{38,43,44,45}.

Fig. 4: *FTO* increases plant chromatin accessibility and affects various metabolic pathways.

a, DNase I-treated TUNEL assays in shoot apices showing chromatin accessibility in 15-d-old *FTO*-transgenic rice compared with WT (Nipp) and *FTO*_{mut}-transgenic rice plants. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Scar bars, 20 μ m. Data are mean \pm s.e.m., $n = 10$ biological replicates. *P* values are from unpaired, two-tailed *t*-tests. **b**, Immunoblot assays of H3K9me2 and H3K27me3 levels in 15-d-old *FTO*-transgenic rice compared with those in WT (Nipp) and *FTO*_{mut}-transgenic rice plants. The histone H3 level was quantified relative to the β -actin level; H3K9me2 and H3K27me3 levels were quantified relative to the histone H3 level. Data are mean \pm s.e.m., $n = 3$. *P* values are from unpaired, two-tailed *t*-tests. **c**, GSEA network analysis of upregulated (normalized enrichment score (NES) < 0) and downregulated (NES > 0) pathways in shoots and roots of *FTO*-transgenic rice. Each pie chart represents a pathway; colors in pies indicate NES of pathways; positive and negative NESs indicate gene set enrichment in pathways of Nipp and *FTO*-transgenic rice, respectively; insignificantly enriched pathways (false discovery rate (FDR) > 0.1) are colored in gray; and left and right halves of each pie, respectively, represent pathways enriched in roots or shoots. Pie size indicates the number of genes in the pathway. Edges represent overlap between pathways, and the width represents the number of genes that overlap. To simplify the network, only edges meeting the cutoff (similarity coefficient > 0.1) are shown.

[Source data](#)

We also investigated potential involvement of transcriptional activation programs in the observed high-yield and biomass phenotypes of *FTO*-transgenic rice. Gene set enrichment analysis (GSEA) between WT and *FTO*-transgenic rice shoots and roots revealed that many plant functional pathways were upregulated in *FTO*-transgenic rice, including pathways related to photosynthesis, ATP synthesis-coupled electron transport and regulation of nitrogen use (Fig. 4c and Supplementary Data 4). GSEA analysis also unveiled that most *FTO*-induced pathways were shoot- or root-specific (Fig. 4c). Thus, *FTO*-mediated m⁶A-demethylation upregulates organ-specific transcripts associated with these pathways and transduces their downstream regulatory effects in our observed specific plant development and physiology, which coordinately increase crop yield and biomass.

Discussion

In summary, we achieved dramatic induction of both crop yield and plant biomass for both monocot and eudicot plants under agricultural field conditions by expressing the human *FTO* protein in plants. Mechanistically, we detected no cap m⁶A_m in rice poly(A) RNA, consistent with our previous report on *Arabidopsis*²⁶. Instead, we found that *FTO* mediates m⁶A demethylation of both mRNA and repeat RNA in plants. The introduction of *FTO* caused elevated overall poly(A) RNA production and more open chromatin in plant cells. Elevated tissue-specific mRNA species encoding proteins play functional roles in root cell proliferation, tiller formation and photosynthetic efficiency, contributing to elevating crop yield and biomass.

We observed notably elevated repeat RNA species upon transgenic expression of *FTO* in plants. We propose that *FTO*-mediated demethylation of some of these repeat RNA species and other carRNA species could drive chromatin opening, as chromatin regulation by repeat RNA methylation has recently been reported in mammals^{38,43,44,45}. Thus, beyond its empirical demonstration that expressing an RNA demethylase in plants dramatically boosts arguably the most important agronomic trait (yield) to increase agricultural productivity, our study represents a starting point for scientific investigations about the mechanisms by which RNA m⁶A dynamics may regulate chromatin and gene transcription in plants.

Methods

Plasmid construction and plant transformation

Native human *FTO* cDNA (GenBank accession no. [NP_001073901.1](#)) and optimized *FTO* cDNA with codon optimization in rice were subcloned into the binary vector pCAMBIA1307 carrying the CaMV 35S promoter between HindIII and KpnI restriction sites with sequence for C-terminal 1×Flag, generating the plasmids *35S::FTO-Flag* and *35S::optFTO-Flag*, respectively. Double mutation of *FTO* (resulting in R316Q and R322Q) was performed using the Agilent QuikChange II XL Site-Directed Mutagenesis kit (Agilent, 200521) to obtain the plasmid *35S::FTO_{mut}-Flag*. For rice transformation, two plasmids, *35S::FTO-Flag* and *35S::FTO_{mut}-Flag*, and one plasmid, *35S::optFTO-Flag*, were respectively transformed into Nipp and ZH11 plants using *Agrobacterium tumefaciens* strain EHA105 according to a published method⁴⁶. Medium supplemented with hygromycin was used to select transformants. Twenty independent T₀ transformations were generated to produce T₁ progeny. T-DNA copy number (CN) was determined in T₁ plants by quantitative PCR with reverse transcription analysis using forward and reverse primers for *HPT* (hygromycin phosphotransferase) and *SPS* (sucrose phosphate synthase) according to published methods^{47,48}. At least three independent transformation lines with one CN of the target gene were selected and produced T₂ progeny (Supplementary Tables 1 and 2). Non-single insert lines were not further characterized. Homozygous lines were confirmed in T₂ offspring by genomic PCR, testing for the presence of both *HPT* and *FTO* using the primers *HPT*-gDNA PCR forward and reverse and *FTO*-gDNA PCR forward and reverse, respectively.

For potato transformation, the plasmid *35S::FTO-Flag* was transformed into EM3 using *A. tumefaciens* GV3101 according to a published method⁴⁹. Medium supplemented with hygromycin was used to select transformants. Transgenic plants were tested by genomic PCR in the T₁ generation for the presence of both *HPT* and *FTO* using the same primers described above. T-DNA CN was determined in T₁ plants by quantitative PCR with reverse transcription analysis using the primers *HPT*-CN forward and reverse and *Urease*-CN forward and reverse according to published methods^{47,50}. Transgenic lines with one CN insertion were used for further study (Supplementary Tables 3 and 4). All primers used for plasmid construction and transgenic line verification are listed in Supplementary Table 5.

Field experiments

Single-copy insert and homozygous *FTO*-transgenic rice plants (*FTO-1*, *FTO-2*, *FTO-4*, *FTO-5* and *FTO_{mut}* in the Nipp background; *FTO-3* in the ZH11 background) along with WT Nipp and ZH11 plants were grown in standard paddy conditions during normal rice planting seasons in Beijing (N 40° 06' 13.07", E 116° 25' 6.34") and Jiangxi (N 28° 22', E 115° 55'), China. The planting density was 15 cm in a row and 17 cm between rows with one plant per hill. Normal field management including irrigation, fertilization and disease control was carried out following normal agricultural practices in rice cultivation. Harvested grains were air dried and stored at room temperature. Yield per plant, total grain number per plant, biomass per plant, effective tiller number per plant, plant height, spike length, total grain number per major panicle and 1,000-grain weight were determined after harvesting. Values are mean \pm s.e.m.

WT (EM3) and *FTO*-transgenic potato plants were grown in the field in the normal potato-planting season in Hebei (N 39° 27', E 115° 51'), China. The planting density was 30 \times 30 cm with one bud per hill in a ridging planting mode. The ridge height was about 30 cm, and the ridge width was about 60 cm. Normal field management including irrigation, fertilization and disease control was carried out following normal agricultural practices in potato cultivation. Tuber weight and tuber number per plot were determined after harvesting.

Measurement of photosynthesis parameters

Photosynthesis parameters were measured using a LI-6800 system (LI-COR) in flag leaves of Nipp, *FTO_{mut}*- and *FTO*-transgenic rice at the heading stage grown in the field from 9:00 to 11:30 and from 14:00 to 16:30 under a daily photoperiod of 12 h of light and 12 h of dark with 55–60% humidity⁵¹.

Root morphological analysis

Plants were harvested at the tillering stage (3 months after planting in soil) and the seedling stage (15 d after growth under hydroponic conditions). All visible roots of one plant were carefully collected. After washing to be free of soil, cleaned roots were dispersed in water in a transparent tray (30 \times 20 \times 3 cm) and scanned using an

Epson scanner (Epson Expression, 1600 Pro) to acquire root images. Root images were then analyzed with WinRHIZO software (Regent Instruments) to determine root morphological parameters such as root length and root number⁵².

Histological analysis

Rice lateral root tips were stained with PI for 1 min and then imaged using a Zeiss LSM 700 laser scanning confocal microscope. Root apical meristem size and meristem cell length and number were determined according to the file of cortex cells from confocal microscopy images⁵³. Shoot apical meristem size and L1 cell number were calculated according to a previous report⁵⁴. Paraffin sections of rice as well as potato tissues were immersed in xylene to remove paraffin and rehydrated with a series of ethanol solutions. After washing with water, sections were stained with Safranin O (Coolaber) and Fast Green (Coolaber)⁵⁵ and imaged under bright field through a microscope (Olympus, IX73). Tiller buds at shoot basal regions of four-leaf-stage seedlings were imaged under bright field through a microscope (Nikon, SMZ18).

EdU staining

EdU staining was performed as previously reported⁵⁶ with modifications. Four-day-old seedlings were immersed in a 100 μ M EdU solution for 6 h. After fixation for 30 min in 4% formaldehyde, the EdU-detection cocktail was made according to the protocol from the Click-iT EdU Alexa Fluor 488 Imaging kit (C10083, Invitrogen). Images were captured using a Zeiss LSM 700 laser scanning confocal microscope, and numbers of EdU-stained cells in rice root meristems were quantified using ImageJ software.

Isolation of poly(A) RNA, non-ribosomal RNA, non-ribosomal nuclear RNA, tRNA and U1, U2 and U6 snRNA

Total RNA was isolated with the TRIzol reagent (Invitrogen). Poly(A) RNA was isolated with Dynabeads oligo(dT)₂₅ (Thermo Fisher Scientific) following the manufacturer's protocol. Non-ribosomal RNA was extracted from total RNA via removal of rRNA using the Ribo-Zero rRNA Removal kit (Illumina) following the manufacturer's

protocol. Non-ribosomal nuclear RNA was isolated from total nuclear RNA via removal of rRNA using the Ribo-Zero rRNA Removal kit (Illumina) after total nuclear RNA extraction from plant nuclei with the TRIzol reagent (Invitrogen). For isolation of total tRNA, the small RNA fraction (<200 nucleotides) was isolated from total RNA using the RNA Clean & Concentrator-5 kit (Zymo Research, R1016) and separated on a 15% TBE–urea gel, and total tRNA bands were sliced and recovered from the gel¹⁷. For isolation of U1, U2 and U6 snRNA, the small RNA fraction (<200 nucleotides) was separated on a 6% TBE–urea gel, and bands corresponding to U1, U2 and U6 snRNA^{17,18} were sliced and recovered from the gel. RNA concentration was measured with the Qubit 2.0 (Thermo Fisher Scientific) and the Equalbit RNA HS Assay kit (Vazyme, EQ211–02). The quality of all isolated types of RNA was confirmed using an Agilent 2100 Bioanalyzer instrument (Agilent).

LC–MS/MS quantification of m₆A, m₁A, m₆A_m and 6mA

For quantification of m₆A, 100 ng RNA (including poly(A) RNA, non-ribosomal RNA, non-ribosomal nuclear RNA and U6 snRNA) was digested with 1 U Nuclease P1 (Wako, 145-08221) in 20 µl buffer containing 10 mM ammonium acetate (pH 5.3) at 42 °C for 4 h, followed by the addition of 1 U Shrimp Alkaline Phosphatase (NEB, M0371L) and 100 mM MES (pH 6.5) at 37 °C for 4 h. After centrifuging at 15,000 r.p.m. for 10 min, supernatants of digested samples were injected into an LC–MS/MS system for analysis. For quantification of m₁A in tRNA¹⁷, 200 ng tRNA was digested with 1 U Nuclease P1 (Wako, 145-08221) in 40 µl buffer containing 10 mM ammonium acetate (pH 5.3) at 60 °C for 2 h, followed by the addition of 1 U Shrimp Alkaline Phosphatase (NEB, M0371L) and 100 mM MES (pH 6.5) at 37 °C for 3 h. After centrifuging at 15,000 r.p.m. for 10 min, supernatants of digested samples were injected into an LC–MS/MS system for analysis. For quantification of total m₆A_m (cap and internal) and internal m₆A_m in poly(A) RNA and snRNA, according to previous reports^{17,18}, 100 ng poly(A) RNA and U1 and U2 snRNA was decapped with 10 U RppH (NEB, M0356S) in NEB ThermoPol buffer (NEB, B9004S) for 3 h at 37 °C. RNA after decapping (for quantification of total m₆A_m) and RNA without decapping (for quantification of internal m₆A_m) were subsequently digested with 1 U Nuclease P1 (Wako, 145-08221) in 20 µl buffer containing 10 mM ammonium acetate (pH 5.3) at 42 °C for 4 h, followed by the addition of 1 U Shrimp Alkaline Phosphatase (NEB, M0371L) and 100 mM MES (pH 6.5) at 37 °C for 4 h. After centrifuging at 15,000 r.p.m. for 10 min, supernatants of digested samples were injected into an LC–MS/MS system for analysis. For quantification of cap m₆A_m in snRNA according to a previous report¹⁸, 100 ng U1 and U2 snRNA was decapped with 25 U RppH (NEB, M0356S) in NEB ThermoPol buffer (NEB, B9004S) for 3 h at 37 °C. The 5' phosphates of the exposed cap-adjacent base were removed by the addition of 5 U Shrimp Alkaline Phosphatase (NEB, M0371L) in 100 mM MES (pH 6.5) at 37 °C for 1 h. After phenol–chloroform extraction and ethanol precipitation, RNA samples were then

digested with 1 U Nuclease P1 (Wako, 145-08221) in 20 μ l buffer containing 10 mM ammonium acetate (pH 5.3) at 42 °C for 3 h. After centrifuging at 15,000 r.p.m. for 10 min, supernatants of digested samples were injected into an LC–MS/MS system for analysis.

For quantification of 6mA in genomic DNA⁵⁷, genomic DNA was purified from the chromatin of 15-d-old *FTO*-transgenic rice plants to avoid bacterial contamination. Chromatin was extracted according to a previously described protocol⁵⁸. In total, 500 ng DNA was digested with 5 U DNA Degradase Plus (Zymo Research, E2021) in 40 μ l 1 \times DNA Degradase Reaction Buffer at 37 °C overnight. After centrifuging at 15,000 r.p.m. for 10 min, supernatants of digested samples were injected into an LC–MS/MS system for analysis.

Nucleosides were separated by ultra-performance liquid chromatography (Shimadzu) on a Zorbax SB-Aq column (Shimadzu) with mass spectrometry detection using a Triple Quad 5500 mass spectrometer (AB SCIEX) in positive ion mode by multiple-reaction monitoring. MS parameters were optimized for m_6A , m_1A , m_6A_m and 6mA detection. Nucleosides were quantified using nucleoside-to-base ion mass transitions of m/z 268.0 to 136.0 (A), m/z 282.0 to 150.1 (m_6A), m/z 282.0 to 150.1 (m_1A), m/z 296.0 to 150.0 (m_6A_m), m/z 266.1 to 150.1 (6mA) and m/z 252.1 to 136.1 (dA). m_6A/A , m_6A_m/A , m_1A/A and 6mA/dA ratios were calculated by fitting signal intensities to standard curves obtained from a concentration series of pure nucleoside standards (Sigma-Aldrich) run with the same batch of samples.

Quantitative analysis of poly(A) RNA isolation by Bioanalyzer

A 908-nucleotide synthesized poly(A) RNA spike-in control was transcribed in vitro using the MEGAscript kit (AM1333, Thermo Fisher Scientific) (Supplementary Table 6). Total RNA was extracted from equal masses of 15-d-old rice shoots and roots using the TRIzol reagent (Invitrogen) and was added with the spike-in control (the same amount of spike-in for equal mass of tissue sample). Poly(A) RNA, along with the spike-in, was isolated using Dynabeads oligo(dT)₂₅ (Thermo Fisher Scientific) following the manufacturer's protocol and subsequently loaded in a total RNA Pico Chip (RNA 6000 Pico kit, 5067-1513, Agilent) using an Agilent 2100 Bioanalyzer Instrument (Agilent). The mimic gel and chromatograms of the isolated poly(A) RNA and the spike-in were obtained from the Agilent 2100 Bioanalyzer. The ratio of poly(A) RNA to spike-in was determined by calculating the peak area ratio of poly(A) RNA versus spike-in.

Ploidy measurement using flow cytometry

To examine the ploidy level of *FTO* plants in this analysis, fresh leaves were chopped with a sharp razor in precooled extraction buffer (0.1 M citric acid, 0.5% Tween-20, pH 2.3). After filtered through a 30- μ m cell strainer, the DNA content per nucleus stained with DAPI was measured using the BD LSRFortessa cell analyzer according to a previous report⁵⁹.

DNase I-treated TUNEL assay

Paraffin sections of shoot apices were immersed in xylene to remove paraffin and rehydrated with a series of ethanol solutions. After washing with water, sections were treated with 0.2 U ml⁻¹ DNase I digestion (NEB, M0303L) and then post-fixed with 4% formaldehyde. TUNEL assays were subsequently performed on fixed tissues using the DeadEnd Fluorometric TUNEL System (Promega) according to the manufacturer's instructions³⁸. The nuclear area was stained with DAPI. Images were captured using a Zeiss LSM 700 laser scanning confocal microscope, and intensity of nuclear TUNEL signal was quantified using ImageJ software.

Calibrated m⁶A MeRIP sequencing

Poly(A) RNA along with spike-in controls (5 μ g) was isolated from equal masses (1 g) of 15-d-old shoots and roots of WT (Nipp), *FTO*_{mut} and *FTO* plants from hydroponic experiments and subjected to m⁶A MeRIP sequencing^{11,12}. The mixture of spike-in controls contained three 20% m⁶A-modified RNA species with different sequences and three non-m⁶A-modified RNA species with different sequences (Supplementary Table 6), which were transcribed in vitro using a kit (AM1333, Thermo Fisher Scientific). Poly(A) RNA with spike-in controls was fragmented into molecules of ~100 nucleotides in length using RNA Fragmentation Reagents (Ambion) and subjected to m⁶A immunoprecipitation using the EpiMark N⁶-Methyladenosine Enrichment kit (NEB) following the manufacturer's protocol. Library preparation was performed using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB) according to the manufacturer's protocol. Sequencing was performed on an Illumina HiSeq 4000 machine in paired-end mode with 150 bp per read (Genewiz).

Quantitative RNA sequencing

Equal masses of 15-d-old shoots and roots of WT (Nipp), *FTO*_{mut} and *FTO* plants from hydroponic experiments were used for quantitative RNA-seq. After total RNA isolation, ERCC RNA spike-in control (Ambion) was added to each isolated total RNA sample (0.1 μ l per 5 mg of plant mass). Total RNA with spike-in controls was subjected to non-ribosomal RNA purification using the Ribo-Zero rRNA Removal kit (Illumina, MRZPL1224). After rRNA removal, purified non-ribosomal RNA was fragmented into molecules \sim 100 nucleotides in length using RNA Fragmentation Reagents (Ambion) according to the manufacturer's protocol. Library preparation was performed using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB) according to the manufacturer's protocols. Sequencing was performed on an Illumina HiSeq 4000 machine in paired-end mode with 150 bp per read (Genewiz).

Calibrated m⁶A-seq data analysis

Adaptor sequences of raw reads were trimmed by Cutadapt version 1.15 (ref. [60](#)) Reads longer than 15 nucleotides after trimming were mapped to the rice genome (MSU 7.0) using HISAT2 version 2.1.0 (ref. [61](#)) with the following parameters: '-p 24-time-reorder-dta-no-unal-pen-noncansplice 12-rna-strandness RF -k 5'. mRNA and repeat RNA annotations were downloaded from the Rice Genome Annotation Project and the Rice Annotation Project Database, respectively. Mapped reads were separated by strands, and then MeTPeak₆₂ was used to detect m⁶A peaks with mRNA and repeat RNA annotations ('FRAGMENT_LENGTH=200, READ_LENGTH=150, PEAK_CUTOFF_FDR=0.05, WINDOW_WIDTH=50, SLIDING_STEP=10, FOLD_ENRICHMENT=2'). Peaks overlapping between two replicates were merged and considered for further analysis. Counts of reads in union peaks from pairwise groups (Nipp versus *FTO*, Nipp versus *FTO*_{mut}) were calculated by featureCounts ('-t exon -g gene_id -C -M -O-fraction -T 24 -s 2 -p-readShiftType downstream-readShiftSize 100-read2pos 5') from Subread version 1.6.4 (ref. [63](#)). The number of reads mapped to all union peaks divided by the number of reads mapped to m⁶A-modified spike-in controls represented the whole m⁶A level. Next, fold enrichment of individual union peaks was normalized to RPKM_{IP/input} based on the normalized whole m⁶A level. Differential peaks were defined as those meeting the fold-change cutoff (\log_2 (FC) > 0.5850 or \log_2 (FC) < -0.5850) and read-density cutoff (RPKM_{input} > 0.5) in input samples. Motif enrichment from peaks was performed by HOMER version 4.9 (ref. [64](#)).

Quantitative RNA-seq data analysis

Adaptor sequences of raw reads were trimmed by Cutadapt version 1.15 (ref. [60](#)). Trimmed reads shorter than 16 nucleotides were discarded, and remaining reads were mapped to the rice genome (MSU 7.0) using HISAT2 version 2.1.0 (ref. [61](#)) with the above parameters. mRNA and repeat RNA annotations were downloaded from the Rice Genome Annotation Project and the Rice Annotation Project Database, respectively. Reads for mRNA and repeat RNA were counted by featureCounts ('-t exon -g gene_id -C -M -O -fraction -T 24 -s 2 -p') from Subread version 1.6.4 (ref. [63](#)) and then normalized to TPM values. TPM values were further normalized using RNA ERCC spike-in controls using the function 'normalize.loess' from the 'affy' package⁶⁵ according to a previous study⁶⁶. Normalized TPM values were used for downstream analysis.

Gene ontology and gene set enrichment analysis

GO analysis on hypomethylated transcripts from *FTO*-transgenic rice shoot and root tissues compared to those of WT was performed using agriGO version 2.0 (ref. [67](#)). GSEA software⁶⁸ downloaded from the Broad Institute was employed for GSEA of RNA-seq data with the GO annotation set (http://ge-lab.org/gskb/7-plant-results-database/oryza_sativa_asian-rice_gmt2.gmt). Results (FDR < 0.1) were further organized into a network by EnrichmentMap⁶⁹.

Statistical analysis

Statistical analysis was conducted using GraphPad. Data are presented as mean \pm s.e.m. Unpaired Student's *t*-tests were used for comparing agronomic traits of each transgenic line with those of the control.

Reporting Summary

Further information on research design is available in the [Nature Research Reporting Summary](#) linked to this article.

Data availability

m⁶A-seq and quantitative RNA-seq data generated by this study were deposited in the GEO database under the accession number [GSE135549](#). [Source data](#) are provided with this paper.

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Ethics declarations

Competing interests

A patent application has been filed by EpiPlanta Biotech Ltd. for the technology disclosed in this publication. C.H. is a scientific founder and a member of the scientific advisory board of Accent Therapeutics.

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Supplementary information

Supplementary Information

Supplementary Figs. 1–24, Tables 1–6, Results and Methods.

Reporting Summary

Supplementary Data 1

Hypo-m⁶A-containing genes in transgenic rice shoots and roots.

Supplementary Data 2

GO analyses of hypo-m⁶A-containing genes in transgenic rice shoots and roots.

Supplementary Data 3

Analysis of quantitative RNA sequencing data, focusing on differential expression of mRNA and repeat RNA.

Supplementary Data 4

Upregulated pathways revealed by GSEA between WT and *FTO*-transgenic rice shoots and roots.

Supplementary Data 5

Unprocessed gel for Supplementary Fig. 1c.

Supplementary Data 6

Unprocessed western blot for Supplementary Fig. 1d.

Supplementary Data 7

Unprocessed western blot for Supplementary Fig. 24.

Supplementary Data 8

Flow cytometry sequential gating strategy for Supplementary Fig. 21.

Source data

Source Data Fig. 4

Unprocessed western blot for Fig. 4b.