An Acyl-CoA N-Acyltransferase Regulates Meristem Phase Change and Plant Architecture in Barley

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The modification of shoot architecture and increased investment into reproductive structures is key for crop improvement and is achieved through coordinated changes in the development and determinacy of different shoot meristems. A fundamental question is how the development of different shoot meristems is genetically coordinated to optimize the balance between vegetative and reproductive organs. Here we identify the MANY NODED DWARF1 (HvMND1) gene as a major regulator of plant architecture in barley (Hordeum vulgare). The mnd1.a mutant displayed an extended vegetative program with increased phytomer, leaf, and tiller production but a reduction in the number and size of grains. The induction of vegetative structures continued even after the transition to reproductive growth, resulting in a marked increase in longevity. Using mapping by RNA sequencing, we found that the HvMND1 gene encodes an acyl-CoA N-acyltransferase that is predominately expressed in developing axillary meristems and young inflorescences. Exploration of the expression network modulated by HvMND1 revealed differential expression of the developmental microRNAs miR156 and miR172 and several key cell cycle and developmental genes. Our data suggest that HvMND1 plays a significant role in the coordinated regulation of reproductive phase transitions, thereby promoting reproductive growth and whole plant senescence in barley.

Shoot architecture is a major determinant of plant function, diversification, and adaptation and is fundamentally important for the productivity of crop plants. It is largely defined by plant height, the arrangement and shape of leaves, branching (tillering) patterns, and inflorescence morphologies (Wang et al., 2018). These features are determined by the activity and fate of the shoot apical meristem (SAM), axillary meristems (AXMs), leaf meristems, and intercalary meristems (Teichmann and Muhr, 2015; McMee, 2019). During vegetative growth, the SAM initiates leaf primordia on its flanks, which later develop into leaves connected to the stem via a node. The leaf of cereal crops is an elongated structure consisting of the proximal sheath enclosing the meristem and culm, and the distal blade, which projects away from the stem axis to optimize light interception (Smith and Hake, 1992; Johnston et al., 2015; Digel et al., 2016; Conklin et al., 2019). In each leaf axil, typically a single AXM is initiated and forms, together with the leaf, node, and subtending internode, a phytomeric unit (McMaster, 2005; McSteen

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A.W., G.W.v.E., R.S., and M.v.K. conceived and designed the experiments; A.W. and G.W.v.E. performed the field experiments; A.W. conducted the phenotypic analyses; A.W. and G.W.v.E. conducted the RNA sequencing experiment and analyzed the data; G.G. contributed the characterization of the mhd mutant plant; A.W. performed allelism tests, gene rescuing, quantitative PCR experiments, and phylogenetic analysis; G.K.K. conducted in situ hybridization and protein localization experiments; A.B. and I.F. performed protein activity tests; and A.W. wrote the article with help from G.W.v.E. and M.v.K.

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and Leyser, 2005). First, an AXM develops into an axillary bud (AB), which subsequently either remains dormant or grows out to form a primary tiller, including leaves, stem, inflorescence, and a succession of secondary tillers (Schmitz and Theres, 2005). In cereals, such as barley (Hordeum vulgare) and wheat (Triticum aestivum), only the ABs at basal unelongated internodes grow out and form tillers, while aerial ABs remain dormant (Oikawa and Kyoizuka, 2009; Kebrum et al., 2013; Rossini et al., 2018). Meristems adopt different identities during development, where meristem identity refers to the type of primordia produced (Poethig, 2010; Bartlett and Thompson, 2014; Bomert and Whipple, 2018; McKim et al., 2018). The SAM transitions from a vegetative meristem to an inflorescence meristem which then forms floral meristems on its flanks. Meristem determinacy regulates the number of primordia developed by each meristem and when and whether the meristem is eventually consumed in the production of primordia (Bartlett and Thompson, 2014). Developmental transitions typically take place in a highly regulated spatial and temporal context, with clear directionality (Keyte and Smith, 2014). However, meristem reversion may also occur, where, for example, floral meristems revert to vegetative branches (Tooke et al., 2005; Trevaskis et al., 2007; Müller-Xing et al., 2014; Asbe et al., 2015; Li et al., 2019). Consequently, shoot architecture is dependent on the initiation of meristems, the timing of identity transitions, and meristem determinacy, which denotes when a meristem ceases to produce lateral organs and terminally differentiates, i.e. into flowers.

The initiation and development of the different shoot meristems need to be coordinated to optimize the allocation of limited resources to the different plant organs and thus optimize plant performance and fitness (Liller et al., 2015). Generally, plants follow different life strategies. They either invest more in vegetative structures, maintaining undifferentiated meristems for extended growth and survival, or they invest more in seed production, typically followed by whole plant senescence and death (Alban and Coupland, 2010). Perennial plants that live over many seasons versus annual plants that only reproduce once and then die represent extremes of the spectrum. Trade-offs or linkage between the development and fate of different shoot organs also controls productivity in annual crop plants (Sadras, 2007; Guo et al., 2018). In cereal crops, negative correlations between tiller (spike) number, grain number, and grain size have hampered the genetic improvement of overall yield (Liller et al., 2015). Consequently, a fundamental question is how the activity and development of different shoot meristems are coordinately regulated. Elucidating the underlying genes and regulatory networks would offer valuable guidance for plant breeding practices and provide genetic tools for the dissection of linked traits.

The highly regulated progression through developmental phase changes and coordinated development of different plant meristems suggests the presence of master regulators that trigger critical developmental transitions simultaneously in several different tissues. The conserved plant microRNAs miR156 and miR172 have been identified as such key regulators of phase changes in plant meristems. Overexpression of miR156 or miR172 causes strong pleiotropic phenotypes such as increased tillering, aerial branching and defective spike branching in wheat, maize (Zea mays), and rice (Oryza sativa; Xie et al., 2006; Chuck et al., 2007; Zhu et al., 2009; Liu et al., 2017). The identification of further regulators and genetic dissection of their activity and function in different plant meristems of cereal crops is important for precise manipulation of meristem identity and activity during plant development. While the control of phase change and its profound effects on shoot architecture have been studied in the grass crops maize and rice (Poethig, 1988; Kyozuka et al., 2014; Hibara et al., 2016), much less is known about the coordinated control of different shoot meristems, the timing of meristem phase changes, and investment in vegetative versus reproductive plant structures in the temperate cereals wheat and barley.

The discovery of genes and regulatory networks underlying shoot architecture in barley is facilitated by the large collection of developmental mutants, many of which have been backcrossed to cv Bowman to generate near isogenic lines (NILs; Bossinger et al., 1992; Franczowiak et al., 1996). This mutant collection includes many termed “many noded dwarf” (mnd) that have a short plant stature and increased node numbers, tillering, and vegetative biomass (Harlan and Pope, 1922; Gustafsson, 1947). These mnd mutants represent a valuable resource for detecting genes that control shoot branching and vegetative versus reproductive growth. Among the mnd mutants, only the gene underlying the mnd4/6 mutation has been cloned so far, and it encodes a cytochrome p450 protein, an ortholog of the rice PLAS-TOCHRON1 (PLAT1) gene (Mascher et al., 2014).

Here we characterized the pleiotropic phenotype of allelic barley high-tillering mutant plants. We demonstrated that these mutants exhibit a delay in the transition to reproductive growth that is linked to a strong increase in the number of leaves and tillers but an overall decrease in grain yield. Outgrowth of ABs at aerial nodes, floral reversion, and bract development indicated reduced apical dominance in the mnd1 mutant plants. Mapping by RNA sequencing revealed that the HvMND1 locus encodes an acyl-CoA N-acyltransferase. Expression analysis demonstrated that this gene is specifically transcribed in the AXMs and SAMs and controls the expression of key developmental genes and genes of the cell cycle machinery. Our data suggest that HvMND1 plays a significant role in the coordinated regulation of phase transition, thereby promoting reproductive versus vegetative growth.

**RESULTS**

**MND1 Regulates Shoot Branching and Inflorescence Development**

We investigated the macro- and microscopic phenotypes of the mnd1.a mutant, which was originally...
identified as a high-tillering mutant in a mixed field of wheat and barley (Harlan and Pope, 1922). We scored plant height, flowering time, and yield in the original mutant line in cv Mesa, where the mnd1.a mutation had occurred spontaneously, and its backcross-derived NILs in cv Bowman as well as in the parental lines in outdoor experiments over two consecutive years. For simplicity, we will hereafter refer to the mutants as mnd1.a (M) for the original mutant line and mnd1.a for the backcross-derived NIL in cv Bowman. The mnd1.a mutants in both backgrounds were stunted and exhibited a high-tillering phenotype (Fig. 1, A–C) in agreement with previous reports (Harlan and Pope, 1922; Bregitzer et al., 2014). Moreover, both mnd1.a mutant lines flowered significantly later than the corresponding wild-type plants, with wild-type cultivars and mutant lines flowering ~73 d and 86 d or more, respectively, after seedling emergence (Fig. 1D). Although the mutants were 40% shorter than the corresponding wild types, they nevertheless produced significantly more vegetative biomass (Fig. 1E). In contrast, the generative biomass per plant and the thousand grain weight (TGW) were lower in both mutants (Fig. 1, F and G) due to 20% smaller kernels caused by a decrease in grain width and length (Supplemental Fig. S1, B–D). Additionally, the spike length and number of grains per spike were significantly lower in the mutants compared to the corresponding wild-type plants (Fig. 1H; Supplemental Fig. S1A). Consequently, the mnd1.a mutation increased the vegetative biomass but reduced the generative biomass given the smaller grain size and grain number.

Since the mnd1.a mutants exhibited a delay in flowering time, we further investigated the development of the main SAM in cv Bowman and the introgression line under inductive long-day (LD) conditions according to the Waddington scale (Fig. 2A). The Waddington scale is a quantitative scale for barley and wheat development based on the morphogenesis of the shoot apex and the carpel of its most advanced flower (Waddington et al., 1983). The emergence of the first spikelet primordia on the shoot apex at the double ridge stage (W2.0) specifies a reproductive SAM. The first floral organ primordia differentiate and stem elongation starts at the stamen primordium stage (W3.5). Anthesis and pollination of the most advanced floret occur at the last stage of the Waddington scale (W10.0). In the mnd1.a mutant, spikelet initiation and inflorescence development were delayed compared to those of cv Bowman (Fig. 2A). This delay in spikelet initiation was linked to an increase in the number of leaves on the main shoot of the mutant (Fig. 2B). Five weeks after emergence (WAE), no further leaves appeared on the main shoot in cv Bowman, and the plants flowered with approximately six leaves. In contrast, the mnd1.a mutant showed a steady increase in leaf number throughout development until 9 WAE, when the experiment was terminated. The production of supernumerary leaves on the main shoot was associated with an increase in the number of elongated internodes (Supplemental Fig. S2A). Although mnd1.a mutant plants initiated leaf primordia for a longer period, the plastochron, which is the leaf initiation rate, was unchanged between cv Bowman and mnd1.a (Supplemental Table S1). However, the appearance of successive leaves on the main culm was faster in the mutant (Fig. 2B). The time between the visual appearance of two successive leaf tips was 5.6 d in cv Bowman plants, whereas mnd1.a mutant plants developed two successive leaves in only 2.2 d (Supplemental Table S1). This demonstrates that the mnd1.a mutation caused a shorter phyllochron and an increase in phytomers due to the prolonged vegetative development. In addition, lengths and widths of the leaf blades and lengths of the leaf sheaths were significantly reduced in the mutant, as shown for the first three fully developed leaves (Fig. 2, C and D; Supplemental Fig. S2C). The short leaf phenotype coincided with an earlier termination of leaf growth of the first leaf (L1) in the mutant compared to L1 in cv Bowman (Supplemental Fig. S2B). To determine whether the shorter leaves in the mutant were caused by a change in cell elongation or proliferation, we examined the adaxial epidermal cells of L1 in both genotypes. No differences were found for average cell length (Fig. 2E; Supplemental Fig. S2, D and E), but cells located over veins next to sclerenchyma and lateral cells were enlarged in the mutant (Supplemental Fig. S2, D and E). Consequently, the shorter leaves in the mutant were not caused by a reduction in cell size, but resulted from a lower cell number.

To determine whether the higher tiller number was linked to the development of additional leaves (Fig. 2, B and G), we dissected tiller development at defined developmental stages in the wild-type and mutant plants. Significantly more tillers were already observed on the main shoot in the mnd1.a mutant compared to cv Bowman at the glume primordium stage (W3.0; Fig. 2F). At the awn primordium stage (W5.0), cv Bowman had developed two to three tillers, whereas the main shoots of the mnd1.a mutant had established >40. We then analyzed the pattern of AB formation at the main stem at three different stages of early development (Fig. 3A). For this purpose, the leaf axil of each leaf >0.3 mm on the main culm was examined for the presence or absence of an AB or tiller. We classified ABs as young when they were surrounded by only the first leaf or mature when they were enclosed in more than one leaf and secondary ABs had potentially formed. As bud outgrowth progressed, we further classified tillers into young tillers with only one visible shoot and mature tillers if secondary side shoots were apparent. At 1 WAE, one to two more leaves were produced in the mnd1.a mutant compared to cv Bowman. However, mnd1.a and wild-type plants produced the same relative number of ABs per leaf axil (0.6 and 0.6 at 1 WAE, 0.4 and 0.5 at 3 WAE, and 0.5 and 0.5 at 5 WAE for cv Bowman and mnd1.a, respectively), but buds were further developed in mnd1.a than in cv Bowman. At 3 WAE, cv Bowman plants did not form further leaves on the main culm due to the initiation of spikelet primordia
Figure 1. Phenotypic characteristics of adult mnd1.a mutants grown under outdoor conditions. A, Morphology and plant architecture of the spontaneous mnd1.a mutant in cv Mesa [mnd1.a (M)] and its backcross-derived NIL in cv Bowman (mnd1.a). Scale bars = 25 cm. B and C, Comparison of plant height (B) and tiller number (C) between the mnd1.a mutants and the corresponding parents representing each genetic background. D, Flowering time in days until the appearance of the first awns from the flag leaves. E and F, Vegetative (E) and reproductive (F) biomass per plant after senescence and an additional drying period. G and H, TGW (G) and grains per spike (H) for each genotype. Data were obtained from outdoor trials in the consecutive years 2014 and 2015 (n = 10–40). Box plots show the median and interquartile range, error bars show the 10th and 90th percentiles, and dots are outliers. Statistical significance was assessed for each mutant and the corresponding genetic background cultivar by a two-tailed unpaired Student’s t test (*P < 0.05, **P < 0.01, and ***P < 0.001).
and termination of the vegetative program. In contrast, the mnd1.a mutant continued to form leaves or leaf-like structures until 5 WAE, when spikelets were initiated (Fig. 3A). However, the ratio of AB to leaf number remained the same at 3 WAE for the mutant and wild type. This indicated that the high number of ABs in the mnd1.a mutant was a consequence of the increased leaf number. Moreover, AB formation continued during inflorescence development and was observed at aerial nodes in the mnd1.a mutant, whereas leaf axils at elongated internodes remained without AB formation in cv Bowman (Fig. 3, A and B). In mnd1.a plants, ABs formed at all elongated internodes, with the exception of a restricted region approximately between nodes 12 and 22 (Fig. 3, A and B). In addition, young ABs formed below and at the base of the inflorescence. The development of such aerial ABs was observed starting at early reproductive stages of inflorescence development, after the reproductive transition (W3.0–W3.5; Fig. 3C). The morphology of the young aerial ABs was identical to that of ABs at the plant base, with leaf primordia enclosing a vegetative shoot meristem. Aerial ABs initiated leaves, underwent stem elongation, developed inflorescences, and eventually set kernels (Fig. 3, C and D). We further observed the formation of nodal root buds at nodes where aerial ABs had formed (Supplemental Fig. S4D). In a separate experiment we determined the duration of tillering of the mnd1.a mutant under controlled conditions. While Bowman plants underwent whole-plant senescence within 3 months, the mnd1.a mutant plants continued to generate new leaves and tillers at the base of senescent tillers for >12 months, when the experiment was stopped (Supplemental Fig. S4, A–C). Consequently, the mnd1.a locus affected not only tiller number but also duration of tillering and therefore longevity and whole-plant senescence in barley.

To determine the origin of the ABs at the base of inflorescences in the mnd1.a mutant, we compared the morphology of developing inflorescences in wild-type and mutant plants at the glume primordium stage (W3.0), the stamen primordium stage (W3.5), and the awn primordium stage (W5.0) by scanning electron microscopy. We found that the aerial ABs at the base of the inflorescence developed from leaf axils at elongated internodes, which is consistent with the observation that AB formation continued during inflorescence development in the mnd1.a mutant.
inflorrence base were present at all analyzed stages in the mnd1.a mutant (Fig. 4A). These branch meristem-like structures were still vegetative at the stamen primordium stage but initiated spikelet primordia when the inflorescence had transitioned to the awn primordium stage. In particular, the development of the branch meristem-like organs coincided with a disturbed bract suppression in mnd1.a inflorescences. In cv Bowman, bract growth at the collar and rachis nodes was suppressed. However, in the mnd1.a mutant, leaflike structures termed floral bracts and known as third outer glumes (Houston et al., 2012) were subtending the triple spikelet primordia at the rachis nodes and coincided with the reversion of the triple spikelet meristem to a branch meristem (Fig. 4A). Eventually, bracts at the basal rachis nodes expanded and were able to enclose the spike, whereas bracts at the upper rachis nodes were not visible in maturing mnd1.a spikes (Fig. 4B). Moreover, the rachis internodes were elongated at the location of bract outgrowth, causing the mnd1.a spikes to adopt an accordion-shaped morphology (Fig. 4B). Cv Bowman and cv Mesa are two-rowed barley cultivars where only the central spikelet meristem develops a flower and grain, while the floral organ development of lateral spikelets is impaired, resulting in infertility. However, in the mnd1.a mutant, the
development of these lateral spikelet structures was impaired. Some lateral floral meristems failed to initiate after the differentiation of the corresponding lateral glume primordia, especially at the basal region of the inflorescence where bracts grew out (Fig. 4C). In addition to branches at the base of the mnd1.a inflorescences, we also observed the occasional formation of ABs in the axils of flag leaves (Fig. 4B). Consequently, reduced bract suppression at lower rachis nodes, reversion of spikelet meristems to branch meristem-like organs, and initiation of ABs in flag leaf axils contributed to a highly branched shoot architecture in the mnd1.a mutant. Moreover, we observed differences in inflorescence length and spikelet numbers between the mnd1.a
mutant and cv Bowman (Supplemental Fig. S3, A and B). The length of mnd1.a inflorescences was comparable to that of wild-type inflorescences at spikelet initiation (W2.0). During further development, inflorescence growth (W3.5–W6.0) and rate of spikelet initiation were reduced in mnd1.a compared to the wild type. At W6.0, cv Bowman plants had initiated an average of 34 spikelets, compared to 24 in mnd1.a mutant plants. The lower number of spikelets in the mnd1.a mutant was also caused by the reversion to branch meristem-like organs at the basis of inflorescences, as described above (Supplemental Fig. S1A).

Taken together, the mnd1.a mutant produced more leaves and tillers than did wild-type plants due to (1) a longer vegetative growth period, (2) faster leaf outgrowth, (3) formation of ABs at aerial nodes, and (4) reduced bract suppression and floral reversion. Consequently, the mutation underlying the mnd1.a locus coordinates the timing of developmental programs and thus is a major pleiotropic modifier of barley shoot and inflorescence architecture.

Identification of the Gene Underlying the mnd1.a Locus

To map the gene, determine the polymorphism underlying the mnd1.a mutation, and identify potential molecular targets of the gene in developing inflorescences, we performed a RNA sequencing experiment as previously described (van Esse et al., 2017). We sequenced total RNA from main inflorescences of the backcross-derived mnd1.a mutant and cv Bowman at three developmental stages: spikelet initiation (W2.0), the stamen primordium stage (W3.5), and after awn primordia had emerged and started to elongate (W5.0). Furthermore, RNA was extracted and sequenced from leaf-enriched SAM samples at W1.0 of the original parent cv Mesa for comparison of sequence variation originating from this line. To identify polymorphisms in the sequenced genotypes, all reads from one genotype were pooled regardless of the developmental stage of the SAM, enabling better coverage of expressed transcripts and ultimately more support for variant identification. We compared the variants identified for cv Bowman and the mnd1.a mutant and found 261 polymorphic alleles. By mapping the 261 alleles to the ordered sequence of the barley reference genome (Mascher et al., 2017), we identified two introgression regions on chromosomes 2H and 7H in the backcross-derived mnd1.a mutant (Fig. 5, A and B), corroborating previous reports (Druka et al., 2011). To narrow down the list of 261 candidate genes, polymorphisms shared between the mnd1.a introgression line, the original parent cv Mesa, and reference cv Morex were excluded as candidates for the mnd1.a phenotype, revealing a total of 56 genes with unique mutations to mnd1.a (Fig. 5B). Among these, 32 alleles carried nonsynonymous mutations, 10 mutations were located in a conserved domain, and only two were predicted to be functionally important according to the PROVEAN score (cutoff of −2.5; Supplemental Table S2; Choi and Chan, 2015). The candidate gene located within the introgression region on chromosome 2H encodes a ribosomal L12 family protein (HORVU2HR1G029240), whereas the candidate gene located within the introgression on chromosome 7H encodes an acyl-CoA N-acyltransferase superfamily protein (HORVU7HR1G113480.3). The ribosomal L12 family protein carried an amino acid substitution in its conserved ribosomal protein L7/L12 domain (Supplemental Table S2), and the acyl-CoA N-acyltransferase superfamily protein carried a frameshift mutation due to an 8-bp insertion in the mnd1.a mutant allele (Fig. 5C; Supplemental Table S2). This insertion was located in the second of three exons at the beginning of the conserved N-acyltransferase domain, leading to a mistranslation and a premature stop codon. To confirm the mutations obtained from the RNA sequencing reads, we sequenced both candidate genes in cv Bowman, cv Mesa, and the backcross-derived and original mnd1.a mutant lines (Supplemental Table S3). The 8-bp insertion in the acyl-CoA N-acyltransferase was detected in both mnd1.a mutant lines but was absent from the parental cultivars. However, we detected the candidate polymorphism in the ribosomal L12 family protein only in the backcross-derived mnd1.a line and not in the original mnd1.a mutant or the parental cultivars. Because the ribosomal L12 family protein polymorphism was not shared between both mnd1.a mutant lines, we selected the acyl-CoA N-acyltransferase as the candidate gene underlying the mnd1.a locus. To confirm the candidate gene, we made use of two independent mutant lines, many branched (mbd) in the background of cv ZOH (Zhang, 1997) and MHOR198 in the background of cv HOR3069. Both mutants were late flowering and produced aerial branches like the mnd1.a mutant (Supplemental Figs. S5 and S6; Supplemental Tables S4 and S5). We performed complementation experiments by crossing mnd1.a that carried a recessive mutation with either the mbd or MHOR198 mutant (Harlan and Pope, 1922; Hor, 1924). Compared to the parental wild-type cultivars, the resulting F1 progeny were late flowering and characterized by prolific tiller production, more nodes, and the presence of aerial branches (Supplemental Figs. S5 and S6; Supplemental Tables S4 and S5). Consequently, the mbd and MHOR198 mutants were identified as allelic to mnd1.a. Sequencing the two candidate genes in the mbd and the MHOR198 lines revealed no mutation in the L12 family protein, while the candidate acyl-CoA N-acyltransferase gene was deleted in mbd and carried a 2-bp insertion in the second exon, causing a frame-shift mutation in MHOR198 (Supplemental Table S3). We therefore concluded that the mnd1.a phenotype is caused by a loss-of-function mutation in the acyl-CoA N-acyltransferase in the three allelic mnd1.a mutant lines.

The maximum-likelihood tree of HvMND1 homologs defined a MND1-family clade comprising the barley MND1 gene along with 57 MND1-like proteins from...
nine monocots, nine dicots, and the spikemoss *Selaginella moellendorffii*. The MND-like proteins from the angiosperms fell into the two monophyletic clades of dicots and monocots. The tree topology indicated a series of independent duplications of the MND1-like genes within the Poaceae clade (Supplemental Fig. S7). In barley, a recent duplication event was observed resulting in HvMND1 and its closest paralog HORVU5Hr1G071620.1 located on chromosome 5H. The previously identified GCN5-related N-acetyltransferase-like protein OsglHAT1 (LOC_Os06g44100.1; Song et al., 2015) is the closest homolog of HvMND1 in rice (74.81% amino acid identity), and the encoding gene is located in a syntenic region on chromosome 6 (Mayer et al., 2011). In the Arabidopsis (*Arabidopsis thaliana*) genus, we identified three homologs of HvMND1, all acyl-CoA N-acyltransferase (NAT) superfamily proteins, including the ethylene response gene *HOOK-LESS1* (HLS1), which is essential for seedling growth (Lehman et al., 1996). We further investigated the natural diversity of *HvMND1* using resequencing data of 91 wild barley, 137 landrace, and 91 research and elite breeding lines (Russell et al., 2016). Only polymorphisms in the coding sequence (CDS) of *HvMND1* were extracted and aligned together with corresponding sequences from cv Mesa and the *mnd1.a* allele (Supplemental Dataset 1). The subsequent haplotype analysis resulted in 11 haplotypes (excluding cv Mesa and the *mnd1.a* allele; Supplemental Fig. S8). The majority of analyzed accessions carried one of two main haplotypes, whereas the remaining genotypes belonged to nine minor haplotypes (Supplemental Fig. S8, Supplemental Table S6). Among the polymorphic sites underlying the haplotype network, only six were nonsynonymous changes. Variant-effect analysis using PROVEAN (Choi and Chan, 2015) predicted neutral effects on protein function for all nonsynonymous changes except one (single amino acid substitution V92A). However, when we grew plants that fell into this haplotype, we did not observe any phenotype resembling the *mnd1.a* mutant. The *mnd1.a* allele itself and the related Mesa allele were derived from one of the major haplotypes. Overall, we observed a high sequence conservation for *HvMND1*, in agreement with its function as a major regulator of barley development.

**HvMND1 Transcript Localization**

We investigated the localization of *HvMND1* transcripts in cv Bowman SAMs, inflorescences, and crown tissue by mRNA in situ hybridization. We observed distinct *HvMND1* expression foci at the abaxial base of

![Figure 5](https://example.com)
young developing leaves, and this persisted through all investigated stages of vegetative (W1.0–W1.5) and reproductive (W2.0, W3.5, and W5) SAM development (Fig. 6, A–D). This expression pattern was also observed in young AFs, where HvMND1 transcripts localized to the abaxial base of young leaves enclosing the AXM (Fig. 6B). In addition, we detected HvMND1 expression in SAMs undergoing the transition from vegetative to reproductive development (W2.0), with HvMND1 transcripts localizing broadly to the vasculature of the developing inflorescence, but not in vegetative SAMs or in later stages of inflorescence development. We verified the localization of HvMND1 expression in cv ZOH and tested for expression in the derived deletion mutant mbd, as well as in the cv Bowman-derived mnd1.a mutant line at spikelet initiation (W2.0). The localization of HvMND1 mRNA was comparable between the mbd parental background cv ZOH and cv Bowman (Supplemental Fig. S9). HvMND1 expression was not detected in inflorescences of the mnd1.a and mbd mutants at W2.0 (Supplemental Fig. S9, G and H). Furthermore, cellular localization of HvMND1 was investigated using infiltrated Nicotiana benthamiana leaves transiently expressing HvMND1 fused to GFP. We found that HvMND1 localized in the nucleus and colocolated with the plasma membrane of the N. benthamiana cells (Supplemental Fig. S9I). To assess whether HvMND1 is a functional acetyltransferase, we compared proteome-wide Lys acetylation in young developing in cv Bowman and the loss-of-function mutant mnd1.a by immunoblot analysis (Supplemental Fig. S10). We did not detect any differences in the amount or abundance of Lys-acetylated proteins, including histones, indicating that the putative acetyltransferase activity of HvMND1 might be very specific to a subset of cells and/or target proteins, as suggested by the very localized expression of HvMND1 in the inflorescence. The spatial and temporal expression of HvMND1 was further investigated by reverse transcription quantitative PCR (RT-qPCR) in different plant tissues of cv Bowman and in a developmental series of SAMs in cv ZOH. In cv Bowman, HvMND1 expression was detected in the crown tissue of 3-d-old seedlings and in the nodes of the uppermost elongated internode segment of plants at W3.5 (Supplemental Fig. S11A). In the roots of 3-d-old seedlings, HvMND1 expression levels were close to the detection limit, whereas HvMND1 was not expressed in fully expanded leaves. Moreover, HvMND1 expression was detected in the leaf-enriched SAM samples of cv ZOH at all sampled stages, from vegetative SAMs to young developing inflorescences (Supplemental Fig. S11B). In summary, HvMND1 was expressed in leaf axils at AXM initiation zones close to the SAM and in the vasculature of SAMs undergoing floral transition but was absent or showed low expression in other plant organs.

Transcriptional Profiling by RNA Sequencing

To determine the molecular function and potential target genes of HvMND1, we investigated transcriptional changes in developing inflorescences in cv Bowman and the backcross-derived mnd1.a mutant. For this purpose, the whole transcriptome data set used for candidate gene identification was screened for transcripts that were differentially expressed between the genotypes in leaf-enriched inflorescence tissues at early reproductive developmental stages (W2.0, W3.5, and W5.0). Principal component analysis of all expressed transcripts in cv Bowman and the mnd1.a mutant revealed that the developmental stage explained most of the variance (principal component [PC] 1; 34% of the total variance) followed by the genotype (PC2; 11.73% of the total variance; Supplemental Fig. S12A). A hierarchical cluster analysis of differentially expressed transcripts (DETs) showed that DETs at W2.0 grouped separately from those at W3.5 and W5.0, which were more similar in terms of their expression profile (Supplemental Fig. S12B). Furthermore, the number of DETs increased with developmental stage, and the largest number was observed at the awn primordium stage (W5.0), where we also observed the strongest phenotypic differentiation between the genotypes, including floral reversion and bract outgrowth in the mnd1.a mutant (Supplemental Fig. S12C).

We discovered a core set of DETs for all three developmental stages in the mnd1.a mutant that consisted of 43 transcripts (Supplemental Fig. S12C). Of the 24 upregulated core DETs, 20 showed no or very low expression levels in the inflorescence of cv Bowman and were clearly expressed in the mnd1.a mutant. Conversely, 12 of the 19 DETs downregulated at all stages exhibited no or very low expression levels in mnd1.a but were strongly expressed in the wild-type inflorescences (Fig. 6E). Genes expressed only in the inflorescence of the mnd1.a mutant had roles in cell cycle control, cell division, differentiation, and morphogenesis and included, for example, two ribonucleoside-diphosphate reductase large subunit genes (RNRs; HORVU2Hr1G065760.1 and HORVU3Hr1G089710.1), a RING/U-box superfamily protein (HORVU7Hr1G107430.1), two F-box superfamily proteins (HORVU1Hr1G088190.2 and HORVU6Hr1G082250.1), and two receptor kinase genes (HORVU2Hr1G037740.1 and HORVU7Hr1G115940.2; Fig. 6E; Deshaies and Joazeiro, 2009; De Smet et al., 2009; Vierstra, 2009). Moreover, transcripts with roles in light signal transduction were also primarily upregulated in the mnd1.a mutant and included, for example, two FAR-RED IMPAIRED1 (FAR1) genes (HORVU0Hr1G038960.25 and HORVU4Hr1G014170.1; Fig. 6E), which have multiple roles in plant development, including meristem as well as floral development (Wang and Wang, 2015; Liu et al., 2016). Interestingly, transcripts of Lys-specific demethylase 5D (HORVU2Hr1G045000.1) were detected only in Bowman, and not in apices of the mnd1.a mutant (Fig. 6E). Functions of Lys-specific demethylases include demethylation of Lys residues on histones that regulate chromatin structure and, thereby, gene expression including that of the central floral homeotic genes (Jiang et al., 2007; Jeong et al., 2009).
Figure 6. HvMND1 expression and transcriptome analysis in developing inflorescences. A to D, Longitudinal sections of cv Bowman SAMs before floral transition (W1.0–W1.5; A), at the spikelet primordium initiation stage (W2.0; B), at the glume stage (W3.0, stitched and fused to assemble a single image; C), and at the awn primordium stage (W5.0; D), hybridized with an HvMND1 antisense probe. Expression of HvMND1 is restricted to the base at the abaxial site of developing leaves and in the inflorescence at W2.0 (marked by arrowheads). IFM, Inflorescence meristem; L, leaf. E, Heatmap of core DETs showing a significant regulation (LogFC $\geq$−1.5 or $\leq$1.5; false discovery rate $\leq$0.05) at all three investigated developmental stages of mnd1.a inflorescence (W2.0, W3.5, and W5.0). The number of raw counts for each gene in each biological replicate is color coded.
Furthermore, several transcripts involved in biotic and abiotic defense were partly or completely downregulated in mnd1.a inflorescences, including three putative nucleotide-binding site Leu-rich repeat resistance genes (HORVU7Hr1G111650.1, HORVU7Hr1G117570.5, and HORVU7Hr1G120020.16; Fig. 6E). These results suggest that the mnd1.a mutation caused changes in the tissue-specific expression of genes that have functions in cell cycle control, development, and defense across all three developmental stages.

We further investigated transcriptional changes for each developmental stage separately to identify transcripts linked to the stage-specific phenotypic differences between wild-type and mutant plants (Supplemental Dataset 2). We found an enrichment of photosynthesis-related DETs in mnd1.a inflorescences at the spikelet initiation stage (W2.0). For example, several genes encoding for chlorophyll a/b-binding proteins, which are part of the light-harvesting complex, and PSI and PSII subunits were upregulated in the mnd1.a mutant compared to cv Bowman. Notably, these expression changes in photosynthesis-related genes coincide with the derepression of inflorescence bract development followed by the outgrowth of bracts into leaf-like structures in the mutant. In mnd1.a inflorescences at the stamen primordium (W3.5) and awn primordium (W5.0) stages, we observed changed expression of several genes that are implicated in developmental control. For example, four LIGHT-DEPENDENT SHORT HYPOCOTYLS (LSH) genes (HORVU2Hr1G089190.3, HORVU3Hr1G088000.1, HORVU6Hr1G058340.3 and HORVU6Hr1G088790.1) were upregulated. Members of the LSH protein family are known suppressors of organ differentiation in boundary regions in Arabidopsis and rice (Takeda et al., 2011; Yoshida et al., 2013). Furthermore, seven barley MADS-box (BM) genes were downregulated in mnd1.a inflorescences at W3.5 and W5.0. MADS-box genes encode floral homeotic transcription factors that are involved in flower development and vegetative organogenesis (Davies and Schwarz-Sommer, 1994; Trevaskis et al., 2007). These BM genes included barley homologs of floral patterning genes, such as APETALA1 (API)-like (HvBM8; HORVU2Hr1G063800) and a SEPALATA-like gene (SEP1; HORVU5Hr1G095710), that were differentially regulated at the stamen primordium stage. In addition, transcripts of LEAFY (LFY; HORVU2Hr1G102590.2), which is involved in the initiation of floral meristems as well as tillering and particle branching, were reduced in mnd1.a (Weigel et al., 1992, Rao et al., 2008). Moreover, we observed a strong upregulation of two TERMINAL FLOWER1 (TFL1)-like genes in mnd1.a shoot apices (HORVU2Hr1G072750.4 and HORVU4Hr1G078770.1). TFL1-like genes are key repressors of flowering time and regulators of shoot architecture (Shannon and Meeks-Wagner, 1991; Bradley et al., 1996; Pnueli et al., 1998; Nakagawa et al., 2002). Among the identified TFL1-like DETs was the barley homolog of snapdragon (Antirrhinum majus) CENTRORADIALIS (HvCen), an important regulator of seasonal flowering and growth habit that has been targeted during barley improvement (Comadran et al., 2012; Bi et al., 2019). The upregulation of floral repressors (TFL1-like and LSH) and downregulation of floral inducers (BM) was associated with the delayed floral development in the mnd1.a mutant. In addition, several homebox-Leu zipper protein family genes (Hox) were differentially regulated in developing mnd1.a inflorescences at W3.5 and W5.0. Among them, two WUSCHEL-related HOX (WOX) transcription factor genes (HORVU2Hr1G113820.5 and HORVU4Hr1G051530.8) showed higher transcript levels in the mutant. WOX genes confer several regulatory roles in plants, including stem cell maintenance, cell proliferation, floral transition, and lateral organ formation (Wu et al., 2007; Vandenbussche et al., 2009; Dolzblasz et al., 2016). We also observed changes in transcript levels of several genes controlling shoot and spike architecture. For example, transcript levels of four TEOSINTE-LIKE1, CYCLOIDEA, and PROLIFERATING CELL FACTOR1 (TCP) transcription factor family genes were increased in mnd1.a inflorescences at W5.0 (HORVU7Hr1G038130.1, HORVU6Hr1G075650.1, HORVU3Hr1G073830.1, and HORVU5Hr1G000490.1), of which HORVU5Hr1G000490.1 encodes for a TEOSINTE BRANCHER1 (TBM)-like TCP transcription factor. This gene showed expression in mnd1.a developing inflorescences, but transcripts were absent in cv Bowman inflorescences at W3.5 and W5.0. However, barley TBM (HORVU4Hr1G007040) and GRASSY TILLERS1 (GT1; HORVU4Hr1G070610), which acts downstream of TBM to control lateral bud outgrowth (Whipple et al., 2011), were not differentially regulated in the mnd1.a mutant. HvMND1 itself showed overall lower transcript levels in mutant apices than in cv Bowman at all three investigated stages.

Taken together, our transcriptome analysis revealed a core set of DETs consistently regulated at all investigated stages of inflorescence development in the mnd1.a mutant. A high proportion of the core DETs showed expression in mutant inflorescences, but transcripts were absent in cv Bowman, and vice versa. Most of these transcripts are associated with regulatory functions in cell cycle control and development. Furthermore, genes controlling phase-change transitions, floral meristem identity, and floral development were particularly differentially regulated during inflorescence development (W3.5 and W5.0) in mnd1.a. We therefore hypothesize that HvMND1 is a regulator of organ-specific expression of genes involved in cell proliferation and meristem transitions.

The mnd1.a Mutant Exhibits Altered Expression Levels of the Developmental miRNAs miR156 and miR172

The pleiotropic mnd1.a phenotype and differential regulation of floral homeotic genes were reminiscent of the effects caused by misregulation of the developmental
miRNAs miR156 and miR172. The sequential expression of miR156 and miR172 regulates phase change and plant architecture in different plant species (Schwab et al., 2005; Xie et al., 2006; Wu et al., 2009). We therefore tested whether the mnd1.a phenotype was linked to changes in the levels of miR156 and miR172 in the developing apices of Bowman and the mnd1.a mutant. We collected main SAMs and surrounding leaves from mutant and wild-type plants over a time period of 1 to 32 d after emergence (DAE) and determined the Waddington stages at each sampling time point. The analysis revealed significant differences in the transcript levels of both miRNAs. When miRNA abundance was compared between the two genotypes for several days after plant emergence, miR156 levels decreased in both lines but were significantly higher in the mnd1.a mutant than in cv Bowman until 18 DAE (Fig. 7A). In contrast, levels of miR172ac and miR172b increased and were significantly lower in the mutant than in the wild type between 14 and 32 DAE and between 18 and 25 DAE, respectively (Fig. 7, B and C). The delays in vegetative phase change and floral development in mnd1.a were therefore linked to higher levels of miR156 and reduced levels of miR172 in the apex. When differences in the miRNA abundance were compared between genotypes across the same stages, miR156 levels were significantly higher in mnd1.a compared to cv Bowman only during the early vegetative stage (Fig. 7D). The mature miR172ac and miR172b transcripts were significantly higher in the mutant than in cv Bowman at spikelet initiation (W2.0) and the glume primordium stage (W3.0; Fig. 7, E and F). We further investigated the expression of seven SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes in barley, which carry putative miR156 target sites (Tripathi et al., 2018). The expression of SPL3, SPL11, SPL13, SPL16, and SPL18 showed a gradual increase in expression over time in cv Bowman (Supplemental Fig. S13). Expression of SPL17 and SPL23 could not be detected in our study. In the mnd1.a mutant, all SPL-like genes showed significantly lower expression, and the gradual increase in expression over time was less pronounced, as observed in cv Bowman, with the exception of SPL16, which did not display significant expression differences between cv Bowman and the mutant. When comparing the expression of the SPL-like genes between genotypes at the same developmental stages, all SPL-like genes, except for SPL3, showed increased expression in the mutant similar to the changes in miR172 transcript abundance at the same developmental stages.

Taken together, the delay in vegetative phase transition and reproductive development in mnd1.a was associated with a delay in the downregulation of miR156 and upregulation of miR172 over time. However, comparison of miR172 levels across genotypes at the same stage revealed that mnd1.a mutants exhibited significantly higher levels of miR172 levels at the reproductive stages compared to cv Bowman.

**DISCUSSION**

We identified HvMND1, an acyl-CoA N-acyltransferase, as a regulator of developmental phase change in barley. HvMND1 had pleiotropic effects on many shoot and inflorescence architecture traits. The SAM of mnd1.a mutant plants remained vegetative for a longer period and generated more leaves on the main shoot. Leaves in the mnd1.a mutant were smaller, possibly due to a
shorter cell proliferation phase and faster leaf maturation linked to a shortened phyllochron, while the leaf initiation rate was not altered. The higher number of fast-outgrowing leaves was associated with an increased number of initiated ABs and tillers, suggesting that the increase in leaf number leads to the high-tillering phenotype in the mnd1.a mutant. As such, the mnd1.a phenotype resembles barley mnd6 and rice pla1 mutants characterized by increased leaf production and short plant stature (Itoh et al., 1998; Miyoshi et al., 2004; Mascher et al., 2014). However, MND6 and PLA1 encode for cytochrome P450 proteins, which are neither related to the MND1 gene nor differentially regulated in the mnd1.a mutant plants. In contrast to mnd6 and pla1, mnd1.a mutants showed a strong delay in the transition from vegetative to reproductive growth. Furthermore, not only was the transition to reproductive growth delayed in the mnd1.a mutant, but the successive progression of vegetative to reproductive growth was impaired. The induction of vegetative structures continued even after the transition to reproductive growth so that the mutants exhibited an overlap of vegetative and reproductive development. These were the development of tillers at aerial nodes and the derepression of bracts coinciding with the reversion of triple-spikelet meristems to vegetative branch meristems. Bracts have been proposed to act as signals of triple-spikelet meristems to vegetative branch meristems (Whipple, 2017). Insufficient bract suppression might therefore have affected the determinant fate of triple-spikelet meristems and subsequent reversion to branch meristem-like organs in the mnd1.a inflorescences.

Floral reversion, bract development, and AB growth at aerial nodes in the mnd1.a mutant indicated reduced apical dominance (Cline, 1997; Cline, 2000). Apical dominance is established by the phytohormone auxin that is produced in the growing shoot tip and transported downward to suppress bud outgrowth (Müller and Leyser, 2011; Gallavotti, 2013). Reduced apical dominance in the mnd1.a mutants therefore indicated reduced auxin production or transport. The release of bract outgrowth, spikelet meristem reversion, and reduced lateral spikelet development in the mnd1.a mutant were only observed at basal inflorescence nodes, and these phenotypes gradually diminished toward the apical inflorescence. Basal-distal gradients in spike morphology have been observed before and are associated with gradients in gene expression (Debernardi et al., 2017) and in cytokinin, gibberellin, and auxin levels along the spike (Youssef et al., 2017), suggesting that the mnd1.a mutation interacted with gene expression or hormone level differences across the inflorescence. In addition, the outgrowth of branches at aerial nodes subtending the inflorescence, as well as the floral reversion and strong vegetative program of the mnd1.a mutant, indicated reduced apical dominance. Interestingly, the mnd1.a mutant plants continued to generate tillers much longer than those of wild-type plants, suggesting that HvMND1 controls longevity and whole-plant senescence in barley. These profound changes in the developmental program resulted in mutant plants with increased vegetative and decreased reproductive biomass. Similar phenotypes have been described for the maize mutant Corngrass1 (Cg1) which is characterized by overproduction of leaves and inflorescences with changed floral architecture and abolished bract suppression (Poethig, 1988; Chuck et al., 2007). Similarly, the rice mutant pla1 develops ectopic shoots at primary rachis branches that are subtended by outgrown and enlarged bracts (Miyoshi et al., 2004). These aberrations in the timing of reproductive and vegetative transitions, including changes in the strict temporal and spatial progression of developmental events, have been termed heterochrony. In plants, heterochronic changes have been identified in gametophyte development, embryogenesis, vegetative development, shoot maturation, and floral morphogenesis and were presumably responsible for many morphological innovations during land plant evolution (Li and Johnston, 2000; Buendía-Monreal and Gillmor, 2018; Pabón-Mora et al., 2019). The collective phenotypes of the mnd1.a mutant resemble that of a heterochronic mutant. We therefore propose that HvMND1 is a heterochronic gene that controls phase change in barley.

We demonstrated that HvMND1 codes for an acyl-CoA N-acyltransferase. Acylation transferases are widespread among plants, yeasts, and other organisms and show a range of targets for amino group acetylation. These targets include small molecules such as auxin-related metabolites (Epstein and Ludwig-Müller, 1993), N termini of larger proteins that are blocked from degradation when acetylated (Driessen et al., 1985), and the Lys residues of histones. The latter are posttranslational modifications of histone tails that result in either permissive or repressive chromatin states modulating gene expression (Chen and Tian, 2007). Our phylogenetic analysis demonstrated that HvMND1 has three homologs in Arabidopsis, one of which is the functionally characterized HLS1 gene. HLS1 is a major regulator of seedling growth that integrates environmental and endogenous hormonal signals to control the formation of the apical hook (Lehman et al., 1996). The molecular function of HLS1 was recently demonstrated, revealing its interaction with the Mediator complex in histone acetylation (Liao et al., 2016). Similarly, the closest HvMND1 homolog in rice (OsgiHAT1) acetylates histone H4, and associated proteins specify its Lys acetylation spectrum (Song et al., 2015). In our protein localization experiment, we detected HvMND1 proteins not only colocalizing with the plasma membrane but also in nuclei, and we thus propose that HvMND1 might act as a regulator of gene expression through the acetylation of histones in the chromatin of specific target genes. However, we did not detect differences in the Lys acetylation of proteins in young inflorescences, probably because HvMND1 is only expressed in a subset of cells in the SAM or might acetylate very specific proteins or target promoter sequences, as has been
observed for its rice and Arabidopsis homologs (Song et al., 2015; Liao et al., 2016). Furthermore, the spatial and temporal expression profile of OsglHAT1 is similar to that of HvMND1 (Song et al., 2015). In both barley and rice, expression was detected on the abaxial side of young developing leaves and only at the onset of reproductive development in developing inflorescences. Additionally, OsglHAT1 was expressed in bracts but only during the primary and secondary branch differentiation stages of inflorescence development, suggesting a potential function in bract outgrowth repression. Transgenic knockdown OsglHAT1 plants, like the mnd1.a mutants, are stunted and have smaller grains (Song et al., 2015). However, the authors do not report higher leaf and tiller numbers, bract outgrowth, or a flowering time phenotype. Our data suggest that HvMND1 and its paralog HORVU5Hr1G071620 arose from a recent duplication event in barley, so the regulation of inflorescence development and vegetative growth by HvMND1 may be due to subsequent neo-functionalization. We did not detect expression of HORVU5Hr1G071620 in our RNA sequencing data, whereas HvMND1 expression was clearly detected (Supplemental Dataset 2). Diversity analyses using a panel of wild and cultivated barley revealed two major haplotypes and only a few genotypes with non-synonymous nucleotide substitutions. This strong sequence conservation indicates a functionally important protein that acts as a hub regulator of phase change and meristem development of different shoot meristems in barley.

Homology to OsglHAT1 and HLS1 suggested that HvMND1 might control gene expression through histone modifications. Indeed, the mutation in mnd1.a caused differential expression of 43 genes that were exclusively expressed in either the wild-type or mutant inflorescences. These transcripts showed enrichment in cell cycle-associated transcripts that were specifically expressed in the mnd1.a inflorescences but not in cv Bowman. Previous studies have highlighted the role of the cell cycle machinery in shaping plant architecture (Xu et al., 2012; Yang et al., 2018). It is well known that histone acetyltransferases play a crucial role in controlling cell fate and influence cell cycle progression through either histone modification of promoter regions or direct deacetylation of target nonhistone proteins or both (Telles and Seto, 2012). Consequently, HvMND1 might directly control cell cycle and proliferation. However, the enrichment in cell cycle genes might also be a consequence of the highly proliferating and morphologically different SAM and inflorescence tissue in mnd1.a compared to cv Bowman. Moreover, an ortholog of the Lys-specific demethylase 5D RELATIVE OF EARLY FLOWERING 6 (REF6) was not expressed in mnd1.a inflorescences but was present at all stages in cv Bowman. In Arabidopsis, REF6 is a histone demethylase and promotes flowering through transcriptional regulation of floral integrators such as FLOWERING LOCUS T (FT) and SUPPRESSOR OF CONSTANS OVEREXPRESSION1 (SOC1; Noh et al., 2004; Lu et al., 2011). Such methylation marks located at histones, including marks at Lys residues, maintain repression of their target genes and are important for correct expression of plant developmental genes (Zhang et al., 2007). Consequently, HvMND1 likely acts as an epigenetic modifier to turn on or off the expression of a core set of genes that are putatively involved in regulating meristem identity and development. In addition, mnd1.a was characterized by altered expression levels of the sequentially expressed miRNAs miR156 and miRNA172. Typically, the sequential transcript levels of miR156 and miR172 are negatively correlated. The mnd1.a mutant, however, displayed high levels of both miR156 in the early vegetative phase and miR172 in the reproductive inflorescence. Overexpression of miR156 in the dominant Cg1 mutant in maize caused a delay in juvenile-to-adult phase change, an increase in the number of leaves and tillers, a delay in flowering, and development of small ears with large bracts (Chuck et al., 2007). Similarly, a semidominant rice mutant, Bushy dwarf tiller1 (Bdt1), with increased expression of miR156, displayed high tillering and small panicles with overgrown bracts and leaf-like structures (Hayashi-Tsugane et al., 2015). In addition, overexpression of miR172b in rice delayed the transition from spikelet meristem to floral meristem. This resulted in defects in floral and grain development, including changes in the number and identity of floral organs and reduced fertility and grain weight (Zhu et al., 2009). miRNAs bind to complementary sequences within mRNA molecules and cause silencing of these mRNA molecules by cleavage, destabilization, or reduced protein translation (Bartel, 2009). miR156 targets the SPL gene family encoding plant-specific transcription factors that control leaf, floral, and fruit development in different plants (Rhoades et al., 2002; Bonnet et al., 2004; Xie et al., 2006; Tripathi et al., 2018). In mnd1.a, high expression levels of miR156 in the early vegetative phase were associated with a reduction in miR156-targeted SPL transcript levels, such as those of HvSPL3 and HvSPL13. In rice, increased expression levels of miR156f in the Oryza sativa multi-tillering and dwarf1 (osmtd1) mutant reduced the transcript levels of its targets OsSPL3, OsSPL12, and OsSPL14, resulting in a pleiotropically changed shoot architecture characterized by high tillering, aerial tillering, dwarfism, and reduced grain yield (Liu et al., 2015). Furthermore, the rice homolog OsSPL13, a target of miR156 in rice, controls grain size by altering grain thickness and length, as well as panicle length and branch number (Si et al., 2016). Consequently, altered expression levels of these miRNAs and miR156-targeted SPL genes may have caused some of the pleiotropic phenotypes in the mnd1.a mutant. Histone acetylation by epigenetic modifiers is associated with the gradual down-regulation of miR156 in Arabidopsis (Kim et al., 2015; Picó et al., 2015; Li et al., 2017; Xu et al., 2018). HvMND1 may therefore act as a positive regulator of the vegetative-to-reproductive phase transition by epigenetic control of miRNA156 and/or miRNA172 expression.
However, further biochemical studies of acetylation targets of HvMND1 should be conducted to determine its molecular function.

The delay in spikelet initiation observed for the mnd1.a mutant was associated with the downregulation of the floral homeotic LFY gene and the upregulation of floral repressor TERMINAL FLOWER1 (TFL1/CEN) at the stamen primordium stage. LFY and TFL1 act antagonistically in the meristem for the acquisition of a floral meristeme fate in Arabidopsis and rice, and changes in the dosage of either of these genes have strong effects on timing of the reproductive phase transition (Ratcliffe et al., 1998; Rao et al., 2008; Nakagawa et al., 2002). We also observed the down-regulation of MADS-box transcription factors involved in floral development and patterning, such as barley homologs of PI, SEPALLATA, and API/FUL. Interestingly, rice double mutants for the API/FUL-like genes OsMADS14 and OsMADS15 exhibited AB formation at stem nodes, delayed flowering, derepression of bracts, and floral reversion in the inflorescence comparable to the inflorescence phenotypes we observed for the mnd1.a mutant (Kobayashi et al., 2012; Wu et al., 2017). Similarly, inflorescences of Arabidopsis soc1-3 ful-2 double mutants show floral reversion, the generation of many small leaves with AXMs, and bract formation (Melzer et al., 2008). In addition, the mutant plants were characterized by secondary growth and survival of stems and apical rosettes, while the wild-type plants senesced and died after seed maturation. We also observed a significant increase in the longevity and ability to generate new tillers even after the transition to flowering in the mnd1.a mutant, while cv Bowman plants underwent whole-plant senescence after flowering. Furthermore, the SHORT VEGETATIVE PHASE (SVP)-like gene BMI was upregulated in the mnd1.a apices. Overexpression of BMI causes reversion of spikelet meristems to branch meristems at the inflorescence base and late flowering due to an extended reproductive phase in barley (Trevaskis et al., 2007). In rice, the tawawa1-d (taw1) mutant, carrying a gain-of-function mutation in an Arabidopsis LSH1 and Oryza G1 (ALOG) family protein, also showed increased inflorescence branching. Lateral meristems acquired a branch-meristem rather than a spikelet-meristem identity through upregulation of the SVP family genes OsMADS22, OsMADS47, and OsMADS55 (Yoshida et al., 2013). Similarly, the upregulation of LSH-encoding transcripts and SVP-like genes in the mnd1.a mutant was linked to the formation of branch meristems instead of spikelet meristems at the base of the inflorescences. Consequently, reversion of spikelet meristems to branch meristems and longevity in mnd1.a were likely the consequence of changes in the expression dosage of floral homeotic genes.

The phenotype of the mnd1.a mutant is interesting also from an evolutionary perspective. It shares several features with the wild progenitor of barley and Hordeum sp. from the secondary and tertiary gene pool, such as a more pronounced vegetative program, prolonged tillering, and a reduced ratio of reproductive to vegetative biomass (Kernich et al., 1995). Selection and breeding of modern barley may have caused a progressive reduction in the vegetative program or the relative duration of the juvenile and adult phases of shoot development. Since spike size is generally related to the duration of the reproductive phase, human selection for large spikes may have resulted in the suppression of juvenile traits. However, sequencing of HvMND1 in diverse accessions did not reveal strong differences between cultivated and wild barley. Future studies need to analyze differences between the expression domains or levels of HvMND1 and relevant target genes in wild and cultivated barley.

Taken together, we propose that HvMND1, identified as an acyl-CoA N-acyltransferase, is a positive regulator of the vegetative-to-reproductive phase change, possibly by coordinating expression of the genes involved in phase transitions and floral development in barley.

**MATERIALS AND METHODS**

**Plant Material and Cultivation**

Barley (Hordeum vulgare) seeds of the original mnd1.a mutant line in the background of cv Mesa, the derived backcross line in the background of cv Bowman, as well as the parental cultivars Bowman and Mesa, were obtained from the U.S. Department of Agriculture (Supplemental Table S7). Seeds of the mnd mutant, the parental cv ZOH, MHOR198, and HOR3069 were obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research. Previously identified mutations of both mutant lines are summarized in Supplemental Table S7.

**Outdoor Plant Cultivation and Phenotyping**

The original and backcrossed mnd1.a mutant, as well as the respective parental cultivars, were sown in 96-well trays in February of 2014 and 2015. Plants germinated in the greenhouse and were transferred to 12-L pots with one plant per pot and at least 16 replicates per genotype. The soil was composed of peat and a clay-soil mixture (Einheitserde, Einheitserdewerke Werkverband) and was supplemented with long-term fertilizer. Plants were cultivated outdoors in Cologne, Germany, irrigated with a sprinkling robot, and fertilized or treated with pesticides, as described previously (Liller et al., 2015). The pots were randomized and arranged in 22 rows, with 34 pots per row placed at a distance of 10 cm. The plots were surrounded by one row of pots with cv Morex plants to ensure an even planting density for every experimental plant. At 89 DAE, plant pictures were taken of one representative plant per genotype. Flowering time was measured in days from plant emergence until the appearance of the first awns from the flag leaf, called tipping. At plant maturity, ~2 weeks before harvest, tiller numbers were counted at the base of each plant and plant height was measured (from soil surface to the base of the uppermost spike). After harvest in the year 2015, whole plants were dried for 2 weeks, spikes were removed, and the vegetative dry weight of each plant was determined. Exemplary spikes for each genotype were scanned. Grain measurements were performed on three to five spikes of one plant, and five plants per genotype, of the 2014 and 2015 outdoor trial. Grain cleaning was performed by hand for each spike individually and measurements were performed using the MARVIN Seed Analyzer (GTA Sensorik) to assess the number of grains per spike, the TGW, and grain length, width, and area.

**Greenhouse Cultivation and Phenotyping**

For detailed macroscopic phenotyping of the investigated mnd1.a mutant, cv Bowman and the mnd1.a NIL were sown in 96-well trays (Einheitserde, Einheitserdewerke Werkverband). After stratification for 3 d at 4°C, plants were
cultivated in controlled LD greenhouse chambers (Bronson Climate) with 16 h light (22°C; photosynthetic photon flux density [PPFD] 270 μmol m⁻² s⁻¹) and 8 h dark (18°C) day/night cycles. After emergence of the second leaf, plants were transferred to 20-Well growing trays. At 3 DAE and subsequently every week after emergence, five plants per genotype were dissected until the end of the experiment at 9 WAE. At each time point, the developmental stage of the SAM of the main stem was determined according to the quantitative scale introduced by Wallingford et al. (1983) using the stereomicroscope Nikon SMZ18 and NIS-Elements BR software (version 3.4; Nikon Instruments Europe). In addition, the number of visible leaves, tillers, and elongated internodes (distance to previous node >5 mm) were counted at each time point. To study phyllochron in wild-type cv Bowman and mnd1.a mutant plants, leaf emergence rates during the first 5 WAE were compared. Phyllochron was calculated as the leaf emergence rate ¹ from the slope of the linear regressions in R (R version 3.3.2; R Development Core Team, 2011). We used the tidy function of the “broom” package (Robinson and Hayes, 2018) to extract the significance of the fitted linear regression, the slope (phyllochron), and the se. The number of spikelet primordia was counted for main shoot apices from the stage of pistil primordium initiation (W4.0) until the stage of style formation (W6.0), and length of dark (18°C) day/night cycles. After emergence of the second leaf, plants were repotted to 1-L pots when the two-leaf stage was reached to investigate the genotype of plants at 3 WAE grown under controlled LD conditions as described in van Esse et al., 2015). Further, candidate variants were evaluated by PROVEAN (for protein variation effect analyzer; http://provean.jcvi.org), a software that computationally predicts the influence of single amino acid substitutions on protein biological function. The pairwise sequence alignment-based score (PROVEAN score) measures the change in sequence similarity of a query sequence to a protein sequence homolog before and after introducing an amino acid variation to the query sequence. The chosen candidate genes were confirmed by Sanger sequencing using the primers listed in Supplemental Table S8.

RNA Sample Preparation and RNA Sequencing

Inflorescences of main culms from cv Bowman, cv Mesa, and the backcross-derived mnd1.a mutant grown in controlled LD greenhouse chambers (Bronson Climate) with 16 h light (22°C, PPFD 270 μmol m⁻² s⁻¹) and 8 h dark (18°C) day/night cycles were used for RNA isolation. The following stages of inflorescence development were selected: spikelet initiation (W2.0), stamen primordium stage (W3.5), and anther primordium (W5.0). In the case of cv Mesa plants, only vegetative SAM tissue (W1.0) was harvested. Collected tissues included the SAM, its base, and small surrounding leaves and were sampled in the middle of the day, 6 to 7 h before the onset of the night period (Supplemental Fig. S14). To assure that all samples were collected at the correct stages, three plants per genotype were dissected before sampling. At least three separate biological replicates, each consisting of a pool of at least 10 meristems, were collected. RNA sample preparation, RNA sequencing, quality control, and adaptor trimming were performed as described in van Esse et al. (2017). Additionally, vegetative SAMs (W1.0–W1.5) from cv Mesa plants grown at the same conditions were harvested and total RNA was extracted. Two biological replicates of cv Mesa SAMs with at least 10 pooled meristems were subjected to RNA sequencing.

Single-Nucleotide Polymorphism Calling and Candidate Gene Selection

Reads obtained from sequencing the total mRNA of SAMs were mapped to a combined set of high-confidence and low-confidence CDSs of the recently described genome of barley ‘Morex’ (Mascher et al., 2017). For single-nucleotide polymorphism detection, all reads obtained from one genotype were pooled regardless of developmental stage to enable better coverage of expressed transcripts and ultimately more support for called variants. The alignments to the reference CDSs were performed using BWAMEM (version 0.7.15; Li, 2013). Mapped reads were filtered and processed as described in van Esse et al. (2017). Subsequent variant calling was performed using the UnifiedGenotyper of GATK (version 3.6; McKenna et al., 2010). Although barley is a diploid plant species, we treated the reads as originating from a haploid species to preclude heterozygous calls and adjust the variant calling to inbreeding plant features. In addition, a minimum Phred-scaled confidence threshold for calling and calling of single-nucleotide polymorphisms was set to 50 (W1.0) and 30, respectively. Variants were filtered in a custom R script (R version 3.3.2; R Development Core Team, 2011) that only allowed for variants with support of at least 30 in read depth, at least 98 for genotype quality, and a Phred quality score of >2,000. Introggression regions were defined by comparing filtered variants of mnd1.a and cv Bowman. Candidate genes were selected when a variant observed for mnd1.a was not detected in either cv Bowman, cv Mesa, or the reference of cv Morex. Pairwise alignments of translated candidate protein sequences were performed using MUSCLE (Edgar, 2004) to reveal nonsynonymous variants. Location of conserved domains were assigned using the National Center for Biotechnology Information Conserved Domain Database (Marchler-Bauer et al., 2015). Further, candidate variants were evaluated by PROVEAN (for Protein Variation Effect Analyzer; http://provean.jcvi.org), a software that computationally predicts the influence of single amino acid substitutions on protein biological function. The pairwise sequence alignment-based score (PROVEAN score) measures the change in sequence similarity of a query sequence to a protein sequence homolog before and after introducing an amino acid variation to the query sequence (Choi and Chan, 2015). The mutations in the chosen candidate genes were confirmed by Sanger sequencing using the primers listed in Supplemental Table S8.

Complementation Tests

Allism tests were performed through crosses between the backcross-derived mnd1.a mutant line in cv Bowman and the shoot architecture mutants mhd and MHOR198 in the cv ZOH and cv HOR3069 background, respectively. Plants used for crosses were grown in LD greenhouses, emascu- lated, and pollinated with pollen from a single plant. Backcross-derived mnd1.a mutants were used as pollen donors in these studies. The F1 progeny of the two crossing combinations mhd × mnd1.a and MHOR198 × mnd1.a was genotyped using PCR primer sequencing sanger sequencing of the mnd1.a-specific marker and the derived gene HORV7HrG113480.3 with the primers listed in Supplemental Table S8. True heterozygous crosses were grown in greenhouse LD conditions along with the parental mutant lines and the respective genetic background parents. Parental lines and F1 progeny of MHOR198 × mnd1.a crosses were
vernalized for 8 weeks at 4°C in a plant growth chamber. Flowering time was assessed in days after emergence until tipping; height, tiller number, node number, and aerial branching were assessed when plants senesced. Plant pictures were taken when most of the analyzed genotypes were flowering.

Phylogenetic Analysis

To identify homologs of HoMND1, a BLASTP search was performed using the HoMND1 protein sequence as query against peptide sequences from the following species: Aegilops tauschii, Arabidopsis thaliana, Brachypodium distachyon, papaya (Carica papaya), cucumber (Cucumis sativus), barley, Medicago truncatula, rice (Oryza sativa), poplar (Populus trichocarpa), common bean (Phaseolus vulgaris), castor bean (Ricinus communis), sorghum (Sorghum bicolor), rye (Secale cereale), foxtail millet (Setaria italica), tomato (Solanum lycopersicum), Selaginella moellendorfii, wheat (Triticum aestivum), grapevine (Vitis vinifera), and maize (Zea mays). For barley, the current database of predicted peptide sequences was used (Mascher et al., 2017). For all other species the Phytozome 12.1.6 database (Goodstein et al., 2012) was used to retrieve homologous proteins. BLAST results were filtered with an E-value cutoff of 1E−10. The nucleotide CDSs of the homologous proteins were then extracted, one representative isoform was selected in case of alternative splicing variants, and sequences were aligned using MUSCLE (Edgar, 2004) in MEGA version 5 (Tamura et al., 2011) based on translated codons. Visually unaligned leading or trailing nucleotide sequences were removed manually from the multiple sequence alignment. The conversion to phylip format was performed using a stream editor (sed) script. The maximum-likelihood tree was generated using RAxML 8.2.10 (fixed-base frequencies, GAMMA model, and “autoMRE” for an optimal number of bootstrap repeats; Stamatakis et al., 2008). Dendroscope 3 (Huson and Scornavacca, 2012) was used for visualization of the tree, which was rooted using S. moellendorfii as the outgroup.

Diversity Analysis of HoMND1

A haplotype analysis of HoMND1 was performed based on exome resequencing data from 39 research/breeding lines, 137 landraces, and 91 wild barley accessions published in Russell et al. (2016). Polymorphisms of the CDS of HoMND1, which is located on morgex_contig1619442 and morgex_contig362150, were extracted and further used to create alternate fasta sequences with the implemented function (e = 0).

Analysis of DETs in the Transcriptomic Data Set of Developing Inflorescences

Whole transcriptome expression analysis was performed on the RNA sequencing reads obtained from main SAM tissues, including the base and small surrounding leaves, of cv Bowman and mndl.a. The reads were aligned to the barley high-confidence transcript sequences (Mascher et al., 2017) using the quasi-mapping based mode in Salmon with default settings (Patro et al., 2015). Further processing and read quantification were performed as described in van Ee et al. (2017). DETs in mndl.a mutants were selected based on adjusted P ≤ 0.05 and log2 fold change ≤ −1.5 or ≥ 1.5. Hierarchical clustering of DETs was performed using Pearson correlation coefficients in R. The number of DETs per stage was visualized using the R package “eulerr”, which generates area-proportional Euler diagrams. Gene ontology (GO) overrepresentation analysis was performed using Blast2GO (version 5.0; Götz et al., 2008) with the Fisher’s exact test for significantly enriched GO terms (P ≤ 0.05). The GO terms obtained were reduced to the most specific terms with the implemented function in Blast2GO.

RNA In Situ Hybridization and Localization in Nicotiana benthamiana Leaves

RNA in situ hybridizations were performed on SAMs at stages W1.0 to W1.5, W2.0, W3.5, and W5.0 as described in Kirschner et al. (2017). Plant cultivation and sampling time were according to the above-described RNA sequencing experiment (“RNA Sample Preparation and RNA Sequencing” section), although more leaves and plant base remained on the collected SAM samples. Probe for detecting the HoMND1 mRNA were prepared from genomic DNA of barley cv Mores from the HoMND1 start to stop codon (1,690 bp), or 861 bp upstream of the stop codon (EcoX3). The DNA was cloned into the pGCG000 entry vector of the GreenGate cloning system (Lampropoulos et al., 2013) and then amplified, including the T7 and SP6 promoter sites, by PCR. RNA probes were built as described in Hejatko et al. (2006). The long RNA probes (1,609 bp) were hydrolyzed by adding 50 μL carbonate buffer (0.08 μM NaHCO3 and 0.12 μM Na2CO3) to 50 μL RNA probe and incubating at 60°C for 54 min. On ice, 10 μL 10% (v/v) acetic acid, 12 μL sodium acetate, and 312 μL ethanol were added, and the RNA was precipitated and dissolved in RNA-free distilled water. Polyvinyl alcohol was added to the nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate staining buffer to a final concentration of 10%. Permanent specimens were created by washing the slides in 50% ethanol, 70% ethanol, 95% ethanol, and 100% ethanol for 2 min each, and in xylo for 10 s, and, after drying, adding a few drops of Entellan (Merck) and a coverslip. The construction of estradiol-inducible expression vectors with GFP as translational fusions at the C terminus for transient expression in N. benthamiana leaves and the transformation of N. benthamiana leaves was described before (Bleckmann et al., 2010). The open reading frame sequence of HoMND1 (from genomic DNA) was inserted upstream of the fluorophore, without the stop codon and in frame with GFP. Nuclei were stained with 4,6-diamino-phenylinole (1 μg mL−1), and plasma membranes were stained with FM1-43FX (20 μM; Thermo Scientific). Pictures of the RNA in situ hybridization samples were taken using a plan-neofluor 10× objective with a NA of 0.30 using the Zeiss Axioskop light microscope, and image processing, i.e. stitching, was performed with the Stitching Plugin in Fiji (Preibisch et al., 2009; Schindelin et al., 2012). For imaging of the N. benthamiana leaves, the confocal laser scanning microscope Zeiss LSM780 with a C-Apochromat 40×/1.2 W Kol M27 was used. GFP was excited at 488 nm and emission was detected at 490 to 508 nm, 4,6-diamino-phenylinole was excited at 405 nm and detected at 410 to 483 nm, and FM1-43FX was excited at 488 nm and detected at 545 to 668 nm.

Immunoblot Analysis of Lys-Acetylated Proteins in Inflorescence Extracts

Frozen young inflorescences in the stamen primordium stage (W3.5) were ground to a fine powder, and proteins were extracted with hot extraction buffer (0.1 μM HCl [pH 7.6], 4% [w/v] SDS, and 0.1% dithiothreitol) for 5 min at 95°C. DNA was sheared by sonication and cell debris was removed by centrifugation twice for 30 min at 18,000g. The supernatant was collected, and the protein yield was determined via the Pierce 660 nm Protein Assay Reagent and the compatible Ionic Detergent Compatibility Reagent (Thermo Fisher). For immunoblot analysis, 50 μg protein was separated on a 15% SDS-PAGE and transferred onto nitrocellulose membrane. Immunodetection of Lys-acetylated proteins was performed as described previously (Hartl et al., 2015). A secondary anti-rabbit antibody IRDye 800CW was used, and infrared fluorescence signals were detected with an Odyssey FC Imaging System (Li-Cor). The membrane was after- stained with 0.1% (w/v) amido black in 10% (v/v) acetic acid for detection of total proteins blotted.

Expression Analysis Using RT-qPCR

To study the expression of HoMND1 in different plant tissues in cv Bowman, as well as in developing meristems of cv ZOH, RT-qPCR was performed. Wild-type cv Bowman plants were germinated on Whatman cellulose filter paper (Sigma-Aldrich) in petri dishes sealed with parafilm. Enriched seedling crown tissue containing vegetative SAMs surrounded by leaves and whole roots was sampled 3 d after germination. Plants for leaf and node tissues of cv Bowman were grown in 96-well trays, and SAM development was staged every 3 d. At W3.5, leaf material was collected from the youngest fully elongated leaves. Additionally, nodes from the uppermost elongated internode located close to the SAM were harvested. For expression analysis of HoMND1 in cv ZOH, plants were grown in 96-well trays, and leaf-enriched developing inflorescences were collected at W1.0, W2.0, W3.5, and W5.0 as described for the RNA sequencing experiment. To determine expression of miRNAs and putative downstream targets, cv Bowman and mndl.a plants were grown in 96-well trays and stem segments containing the developing SAM were sampled 1, 4, 8, 14, 18, 25, and 32 DAE. All plants were grown in chambers with 16 h light (22°C), PPFD 270 μmol m−2 s−1/8 h dark (18°C) day/night cycles, and samples were taken 6 to 7 h before onset of the night period. Three biological
replicates per tissue and genotype were collected, each consisting of a pool from three or more individual plants. Isolation of total RNA, cDNA synthesis, and RT-qPCR were performed as described in Campoli et al. (2012). RNA isolation, reverse transcription with stem-loop primers, and miRNA quantification were performed as described in Bergonzoni et al. (2013). For each sample, two technical replicates were used and quantification was based on the titration curve using LightCycler 480 software (version 1.5, Roche). Transcript levels of miRNAs were normalized using Hv18S and Hvsnor23 as controls, whereas HvMND1 and SPL expression levels were normalized using HvACTIN and HvGAPDH. All primers used in the RT-qPCR analysis are listed in Supplemental Table S8.

Accession Numbers

Accession numbers of all major genes and proteins mentioned in this study are listed in Supplemental Table S8. Short read Illumina data and raw variant expression data are accessible through Gene Expression Omnibus series accession number GSE149110 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE149110).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Spike and seed shape parameters of mnd1.a mutants in cv Bowman and cv Mesa.

Supplemental Figure S2. Internode and detailed leaf phenotype of the mnd1.a mutant.

Supplemental Figure S3. Impaired inflorescence development in mnd1.a mutant plants.

Supplemental Figure S4. Duration of leaf and tiller outgrowth in mnd1.a mutant plants.

Supplemental Figure S5. Allelism crosses between the backcross-derived mnd1.a line and the putative HvMND1 mutant lines mbd and MHOR198.

Supplemental Figure S6. Aerial shoot branching in mbd, mnd1.a, and MHOR198 mutants and in heterozygous F1 progeny of crosses mbd × mnd1.a and MHOR198 × mnd1.a.

Supplemental Figure S7. Maximum likelihood phylogenetic tree of acyl-CoA N-acyltransferase-like genes in 19 monocot and eudicot plant species.

Supplemental Figure S8. Diversity analysis of HvMND1.

Supplemental Figure S9. Corresponding negative controls of the HvMND1 in situ hybridization experiment and HvMND1 transcript localization in cv ZOH.

Supplemental Figure S10. Lys-acylated proteins in young developing inflorescences (W3.5) in cv Bowman and mnd1.a.

Supplemental Figure S11. HvMND1 expression determined by RT-qPCR.

Supplemental Figure S12. Principal-component analysis of DETs in cv Bowman and mnd1.a.

Supplemental Figure S13. Expression analysis of miR156-targeted SQUAMOSA-promoter binding like (SPL) genes in the mnd1.a mutant.

Supplemental Figure S14. Representation of leaf-enriched inflorescence tissue dissected for RNA extraction.

Supplemental Table S1. Phyllochron and plastochron of cv Bowman and mnd1.a mutant plants.

Supplemental Table S2. Nonsynonymous mutations assigned to a conserved domain of the corresponding protein in mnd1.a backcross-derived mutants identified through mapping by RNA sequencing.

Supplemental Table S3. Resequencing of candidate polymorphisms in the parental lines cv Bowman and cv Mesa, the backcross-derived mnd1.a mutant (mnd1.a), and the original mnd1.a mutant in cv Mesa [mnd1.a (M)].

Supplemental Table S4. Allelism test between mnd1.a and mbd mutants in the cv Bowman and cv ZOH genetic backgrounds, respectively.

Supplemental Table S5. Allelism test between mnd1.a and MHOR198 in the cv Bowman and cv HOR3809 genetic backgrounds, respectively.

Supplemental Table S6. Haplotypes of HvMND1 and assignment of alleles carrying the HvMND1 alleles.

Supplemental Table S7. Mutants used in this study.

Supplemental Table S8. Primer pairs used in this study.

Supplemental Dataset S1. Multiple sequence alignment of the HvMND1 CDS.

Supplemental Dataset S2. Differentially expressed transcripts in developing mnd1.a mutant inflorescences.

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