The *dicer-like1* Homolog *fuzzy tassel* Is Required for the Regulation of Meristem Determinacy in the Inflorescence and Vegetative Growth in Maize

Beth E. Thompson, a, 1 Christine Basham, a Reza Hammond, b, c Queying Ding, a Atul Kakrana, b, c Tzuu-Fen Lee, c Stacey A. Simon, c Robert Meeley, d Blake C. Meyers, c and Sarah Hake, a

a Department of Biology, East Carolina University, Greenville, North Carolina 27858
b Center for Bioinformatics and Computational Biology, University of Delaware, Newark, Delaware 19714
c Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware 19711
d Pioneer, A DuPont Company, Johnston, Iowa 50131

ORCID ID: 0000-0003-3436-6097 (B.C.M.)

Plant architecture is determined by meristems that initiate leaves during vegetative development and flowers during reproductive development. Maize (*Zea mays*) inflorescences are patterned by a series of branching events, culminating in floral meristems that produce sexual organs. The maize *fuzzy tassel (fzt)* mutant has striking inflorescence defects with indeterminate meristems, fascination, and alterations in sex determination. *fzt* plants have dramatically reduced plant height and shorter, narrower leaves with leaf polarity and phase change defects. We positionally cloned *fzt* and discovered that it contains a mutation in a *dicer-like1* homolog, a key enzyme required for microRNA (miRNA) biogenesis. miRNAs are small noncoding RNAs that reduce target mRNA levels and are key regulators of plant development and physiology. Small RNA sequencing analysis showed that most miRNAs are moderately reduced in *fzt* plants and a few miRNAs are dramatically reduced. Some aspects of the *fzt* phenotype can be explained by reduced levels of known miRNAs, including miRNAs that influence meristem determinacy, phase change, and leaf polarity. miRNAs responsible for other aspects of the *fzt* phenotype are unknown and likely to be those miRNAs most severely reduced in *fzt* mutants. The *fzt* mutation provides a tool to link specific miRNAs and targets to discrete phenotypes and developmental roles.

INTRODUCTION

Plant development is dependent on the activity of meristems, groups of indeterminate, self-renewing cells that initiate new organs. Maintenance of the balance between organ initiation at the periphery and self-renewal in the central stem cells is critical for plant growth (Steeves and Sussex, 1989). The shoot apical meristem initiates leaf primordia during vegetative development. As the plant becomes reproductive, leaf primordia become smaller and axillary branch meristems, in the axis of leaves, become more prominent. Ultimately, inflorescence meristems are formed that will produce flowers. Meristems are considered indeterminate if the central stem cells are maintained during the production of meristem or organ primordia, whereas meristems are considered determinate if the central stem cells are consumed, as in a floral meristem.

Maize (*Zea mays*) produces two inflorescences, the tassel and the ear, which produce male and female flowers, respectively. The tassel is the product of the apical inflorescence meristem, while the ear is the product of an axillary meristem. In both the tassel and the ear, the inflorescence meristem initiates secondary and higher order meristems, culminating in the formation of floral meristems. The imposition of determinacy on the higher order meristems determines inflorescence architecture.

MicroRNAs (miRNAs) are key regulators of meristem fate and function in maize and other plants. In maize, *tasselseed4* (*ts4*), which encodes miR172e, is required for meristem determinacy in multiple higher order meristems, in addition to playing a role in sex determination. *ts4/miR172e* represses two AP2-like genes, *ids1* and *sid1* (Chuck et al., 2007b, 2008), a regulatory module that is conserved in *Arabidopsis thaliana* and rice (*Oryza sativa*) (Aukerman and Sakai, 2003; Lee and An, 2012). miR156 is required for leaf suppression in the inflorescence and plays a key role in determining the meristem and leaf boundary (Chuck et al., 2010). The dominant mutant *Congrass1* (*Cg1*), which is caused by the overexpression of miR156, has a fasciated inflorescence meristem, indicating that miR156 also plays a role in stem cell homeostasis (Chuck et al., 2007a).

miRNAs have key roles in vegetative development. The balance between miR156 and miR172 is critical to determine the switch from vegetative to reproductive development, also known as phase change, in maize, *Arabidopsis*, and other plants (Wu and Poethig, 2006; Chuck et al., 2007a, 2011; Poethig, 2013). miR165 and miR166 repress class III homeodomain-leucine zipper transcription factors to regulate abaxial/adaxial leaf polarity (McConnell et al., 2001; Juarez et al., 2004).
miRNAs are small noncoding RNAs of 20 to 22 nucleotides in length that posttranscriptionally repress gene expression in plants and animals (Bushati and Cohen, 2007; Bartel, 2009; Chen, 2009; Voinnet, 2009; Krol et al., 2010). miRNA genes are transcribed by RNA polymerase II as long, primary microRNA (pri-miRNA) transcripts that contain one or more hairpin structures (Xie et al., 2005). The pri-miRNA undergoes two sequential processing events to generate the mature miRNA. The pri-miRNA is cleaved to release a small RNA duplex, consisting of the miRNA and its complement, the pre-miRNA (pre-miRNA). The pre-miRNA is then cleaved to release the mature miRNA.

We isolated a maize mutant, **fuzzy tassel (fzt)**, with a broad range of vegetative and reproductive defects. *fzt* mutants have particularly striking inflorescence defects, including increased indeterminacy of multiple meristems and defects in stem cell homeostasis and sex determination. *fzt* mutants also have vegetative defects, including reduced plant stature, and short narrow leaves with mild polarity defects. Positional cloning showed that *fzt* contains a mutation in DCL1, a key enzyme in the miRNA biogenesis pathway. The levels of most miRNAs are moderately reduced in *fzt* mutants; however, a few miRNAs are more dramatically reduced, suggesting that developmental defects in the *fzt* mutant are caused by reduced levels of a subset of miRNAs and the upregulation of specific miRNA-targeted miRNAs.

**RESULTS**

**fzt Is Required during Vegetative and Reproductive Development**

*fzt* was isolated by screening an M2 population of A619 ethyl methanesulfonate (EMS)-mutagenized plants. The mutant was backcrossed to A619 a minimum of three times prior to analysis; analysis was done in the A619 inbred background unless noted otherwise. *fzt* is recessive, 100% penetrant, and has striking reproductive defects and reduced plant stature (Figure 1; Supplemental Figure 1). We also backcrossed the *fzt* mutation to Mo17 and B73 a minimum of three times for analysis. *fzt* phenotypes are qualitatively similar in all inbred backgrounds examined; however, some defects appear more severe in the Mo17 and B73 inbred backgrounds (see below).

Plant stature is dramatically reduced in *fzt* mutants. *fzt* plants are less than one-third the height of normal sibling plants (Figure 1A; Supplemental Figure 1A). We counted the number of leaves, including the first juvenile leaves, to determine if this loss of stature was due to short or missing internodes. Whereas normal siblings produced on average 15 leaves, *fzt* mutants produced only an average of 12 leaves, suggesting that the short stature was a combination of both missing and shorter internodes (Supplemental Figure 1B). To confirm this finding, we quantified the number and length of internodes at maturity and found that normal plants had an average of 10.5 nodes per plant whereas *fzt* plants had an average of only 8 nodes per plant (Supplemental Figure 1C). We also measured internode length in *fzt* and normal plants. *fzt* plants had significantly shorter internodes, except for the top-most internode of *fzt* plants (internode 8) (Supplemental Figure 1D).

Leaf size was also reduced in *fzt* plants; *fzt* leaves were only about two-thirds the length and less than one-half the width of normal leaves (Supplemental Figures 1E and 1F). To determine if reduced leaf size was due to a decrease in cell size or cell number, we counted total epidermal cell number per unit area as a measure of cell size; increased cell number per unit area would be indicative of decreased cell size in *fzt* plants. Surprisingly, we found that *fzt* plants had a slight decrease in cell number per unit area compared with normal siblings, suggesting that cell size is slightly increased in *fzt* leaves compared with normal siblings (Supplemental Figure 1G). Thus, the decrease in leaf size is likely due to a combination of decreased cell size and increased cell number.
due to a decrease in total cell number rather than decreased cell
size.

The reproductive defects in fzt plants are particularly striking
(Figures 1B to 1I). In normal maize plants, tassels produce sta-
minate flowers and ears produce pistillate flowers, due to the
abortion of pistils in the tassel and stamen arrest in the ear. In
addition to sex organs, maize inflorescences contain grass-speci-
cific organs, including lodicules, palea, and lemma. Lodicules have ho-
mology to petals (Ambrose et al., 2000), and palea and lemma are
bract-like organs. Spikelets are produced in pairs; each spikelet
consists of two florets enclosed by two bracts, called glumes
(Figure 1C). In ears, one of the two florets aborts (Figure 1G). In fzt
plants, both male and female inflorescences exhibit multiple de-
fects resulting in complete sterility. fzt tassels often produce extra
spikelets, and the spikelets contain more than two florets (Figure
1E). fzt tassel florets make an excess of palea/lemma-like organs,
and the stamens are small, undeveloped, and never shed pollen.
fzt spikelets also lack recognizable glumes, resulting in exposed
floral organs and their “fuzzy” appearance (Figure 1D). On average,
fzt tassels produce only one-half the number of tassel branches as
normal siblings (5 branches in fzt versus 9.5 branches in normal
siblings) (Supplemental Figure 1H). fzt tassels occasionally pro-
duce silks, indicating that carpel abortion is defective.

The fzt mutation also severely affects ear development. fzt ear
spikelets are enclosed by bracts that morphologically resemble
tassel glumes and contain extra florets (Figure 1I). Ear florets
also make extra palea-like organs and often contain immature
stamens, indicating that fzt is required for multiple aspects of
sex determination in the ear.

We observed similar phenotypes when fzt was introgressed
into the Mo17 and B73 inbred backgrounds (Supplemental
Figure 2). The fzt mutation had similar effects on plant stature in
all three inbred backgrounds (Supplemental Figures 2A, 2B, 2J,
and 2K); however, the inflorescence defects were more severe in
Mo17 and B73 than in the A619 inbred. fzt[Mo17] and fzt[B73]
tassels were highly branched and formed no recognizable flo-
rets, although tassel “spikelets” produced lemma/palea-like or-
gans and a few undeveloped and abnormal stamens (Supplemental
Figures 2D, 2G, 2M, and 2N). fzt[B73] ears were highly branched
and contained many immature meristems at maturity. Almost no
floral organs were produced, except for rare immature and abnor-
amal stamens (Supplemental Figures 2F, 2H, and 2I). fzt[Mo17]
plants generally lacked ears.

fzt Contains a Mutation in DCL1

To gain insight into the molecular underpinnings of the fzt
phenotype, we positionally cloned the gene. fzt mapped to the
short arm of chromosome 1 between the simple sequence re-
peat markers bnlg1124 and umc1292. We developed new
polymorphic markers to narrow the fzt-containing region to an 
−3.2-centimorgan region, which included 33 predicted genes,
26 of which had functional annotations (gene predictions were
obtained from the filtered gene set of the maize B73 RefGen_v2)
(Figure 2A). One gene in this interval, dcl1, stood out as a par-
icularly strong candidate. DCL1 is a key enzyme required for
miRNA biogenesis in Arabidopsis and other plant species and is
broadly expressed during development (Sekhon et al., 2011).
Given the well-established role of miRNAs in many developmental
processes, including the regulation of meristem determinacy in
maize, a mutation in dcl1 seemed likely to underlie the pleiotropic
phenotypes of fzt.

DCL proteins contain several conserved domains, including
a bipartite helicase domain, a DUF283 domain that was recently
defined as a novel RNA binding motif (Qin et al., 2010), two
RNase III domains (RNase IIIa and RNase IIIb), and two double-
stranded RNA binding domains (Figure 2B). The dcl1 locus
corresponds to gene model GRMZM2G040762. To assemble
the full dcl1 genomic sequence and predict a full-length coding
sequence, we assembled maize BAC sequences and used
similarity with the rice and Arabidopsis DCL1 protein sequences
to predict a full-length maize DCL1 protein (1929 amino acids)
and corresponding coding sequence (5790 nucleotides) (see
Methods). We sequenced the predicted dcl1 coding region from
fzt and A619 plants and found a G-to-A mutation in fzt mutants
corresponding to exon 17 and predicted to cause an S-to-N
substitution in the RNase IIIa domain (Figure 2B; Supplemental
Figure 3).

To generate additional alleles and confirm that we isolated the
correct gene, we conducted a noncomplementation screen in
which Mo17 EMS-mutagenized pollen was crossed onto fzt
heterozygotes and the resulting progeny were scored for fzt
phenotypes. We found one plant with the fzt phenotype that
contained a G-to-A mutation in exon 19, which is predicted to
introduce a premature stop codon and truncate DCL1 by 39
amino acids. The fzt/fzt-EMS plant was sterile, and we were
unable to recover the new allele for further experiments. We
obtained four additional dcl1 alleles (dcl1-mum1 to dcl1-mum4)
through reverse genetics resources (Bensen et al., 1995). Three
alleles (dcl1-mum2 to dcl1-mum4) contain Mu insertions in the

Figure 2. fzt Contains a Mutation in dcl1.
(A) Mapping data for fzt.
(B) Genomic region of dcl1 with mutant alleles indicated. Black triangles indicate insertion sites of Mutator transposon insertions (dcl1-mum1–4). Orange boxes indicate protein-coding exons, and gray boxes indicate 3’ untranslated regions.
(C) Schematic of the DCL1 protein with conserved domains indicated. The predicted effects of the mutant alleles on the DCL1 protein are indicated. a.a., amino acids.
protein-coding region of exon 1 and are likely null alleles; all three alleles fail to complement fzt. Based on dcl1 null phenotypes in Arabidopsis, we hypothesized that null alleles of dcl1 are embryonic lethal. We examined the self-progeny of dcl1-mum3 heterozygotes, one-quarter of which are predicted to be dcl1-mum3 homozygotes. We found that 49 of 172 (28.4%) seeds lacked recognizable embryos, and all seeds with a recognizable embryo contained at least one normal dcl1 allele (Supplemental Figure 4B). Thus, dcl1-mum3 homozygotes are indeed early embryonic lethal. Homozygous dcl1-mum2 and dcl1-mum4 plants were never recovered, suggesting that these alleles are also embryonic lethal. We also asked if plants were never recovered, suggesting that these dcl1-mum3 alleles were phenotypically normal and complemented the fl allele, and we refer to our allele as dcl1-fzt. We also obtained three dcl1 tilling alleles from the Maize Tilling Project (Till et al., 2004) that contained nonsilent point mutations in or near the helicase domain (Figures 2B and 2C). Interestingly, plants homozygous for these alleles were phenotypically normal and complemented the fzt mutation, suggesting that the helicase domain may not be critical for DCL1 function in an otherwise normal genetic background, although it is also possible that these mutations do not impair helicase function.

**dcl1-fzt Mutants Are Defective in miRNA-Regulated Processes**

miRNAs have well-known roles in plant development (Bartel, 2009; Chen, 2009; Rubio-Somoza and Weigel, 2011). Given that dcl1-fzt carries a mutation in a key enzyme required for miRNA biogenesis, we predicted that dcl1-fzt mutants would have reduced miRNA levels. Therefore, we examined dcl1-fzt mutants for defects in several miRNA-regulated processes.

miRNA regulation is required to regulate meristem determinacy in the inflorescence. The dcl1-fzt inflorescence defects resemble those of the ts4 mutant, which is caused by a loss-of-function mutation in miR172e. Reduced miR172e expression leads to the loss of meristem determinacy and sex determination defects (Chuck et al., 2007b, 2008). To more closely examine dcl1-fzt mutants for these defects, we examined early inflorescence development by scanning electron microscopy. In normal plants, the inflorescence meristem (IM) gives rise to ordered rows of spikelet pair meristems (SPMs). Each SPM gives rise to two SMs (asterisks in Figure 3J) and SMs of fzt mutants persisted (arrow in Figure 3L), initiating more than the normal two FMs (asterisks in Figure 3L). We also found that miR172e was substantially reduced in dcl1-fzt mutants compared with normal controls (see below), consistent with the observed meristem determinacy defects.

Normal meristems grow as a single apex by balancing stem cell growth with the rate of primordium initiation. Meristems that lose this homeostasis broaden and become fasciated (Aichinger et al., 2012; Pautler et al., 2013). We consistently noted fasciation in dcl1-fzt IMs (Figure 3H; Supplemental Figure 5). The broadened tip produced many more meristems than normal. We also observed fasciation in the BMs (Figure 3H, arrowhead). Finally, SPMs were not initiated in ordered rows and were irregular in shape and size (Figures 3E and 3G; Supplemental Figure 5). These defects are not observed in the ts4 mutant, suggesting that miRNAs in addition to miR172e are reduced in the dcl1-fzt mutant and have key roles in meristem homeostasis and determinacy.

miRNAs also have well-established roles in vegetative development. Normal maize leaves have a ligule on the adaxial surface and distinct hairs on the adaxial and abaxial surfaces. Mutations in the miR166 binding site of Rolled-leaf1 cause the adaxialization of leaf surfaces, resulting in curled leaves and abaxial ligules (Juarez et al., 2004). Since dcl1-fzt leaves did not exhibit these macroscopic polarity defects, we examined the leaves for subtle polarity defects. In normal leaves, macrohairs are restricted to the adaxial surface of leaf blades and are often used as adaxial markers. We examined the distribution of macrohairs on dcl1-fzt and normal leaf blades and found that dcl1-fzt leaves contained fewer macrohairs on the abaxial blade compared with normal siblings (Figures 4A and 4B). In addition, dcl1-fzt leaves contained macrohairs on the abaxial blade, while normal leaves did not (Figures 4C and 4D). Interestingly, this defect was more severe in the Mo17 inbred background (Figures 4A to 4D) than in the A619 background (Supplemental Figure 6), in which abaxial macrohairs were restricted to the leaf margins and were not present throughout the blade (Supplemental Figure 5D).

Vascular bundles are also polarized, with the xylem positioned at the adaxial pole and phloem positioned at the abaxial pole. We examined the polarity of vascular bundles in dcl1-fzt [Mo17] and normal sibling plants in cross sections. We found that dcl1-fzt [Mo17] mutants had subtle defects in vascular organization. The xylem was more disorganized in dcl1-fzt [Mo17] mutants than in normal siblings and extended farther toward the abaxial pole than normal (Figures 4E and 4F). Together, these results are consistent with mild leaf polarity defects in dcl1-fzt mutants, in which the abaxial surface acquired adaxial characteristics.

Phase change from the juvenile to adult life phase is another well-known miRNA-regulated process in plants and is controlled by the antagonistic activities of miR156 and miR172 (Chuck et al., 2007a; Poethig, 2013). Juvenile and adult leaves make distinct epidermal waxes, which can be distinguished by toluidine blue staining: juvenile cells stain violet in color, while adult cells stain blue. We found that in the A619 inbred background, dcl1-fzt mutants begin making adult waxes about one leaf later than normal siblings (Supplemental Figure 7). Combined with the node number data (Supplemental Figure 1C), this indicates that, on average, dcl1-fzt [A619] plants gain one juvenile internode and lose four adult internodes. By contrast, in the Mo17 inbred background, dcl1-fzt mutants begin making
adult leaf waxes approximately one leaf earlier than normal siblings (Supplemental Figure 7). While initially it seems paradoxical that dcl1-fzt has opposite effects on phase change depending on the inbred background, this is not necessarily unexpected, given that the timing of phase change is regulated by the opposing activities of miR156 and miR172 and their target genes. Slight changes in the relative levels of these genes could shift the balance of downstream target genes and the timing of phase change. An alternative explanation for the apparent background effects on phase change is incomplete introgression. A619 and Mo17 flower at different times, with Mo17 up to 2 weeks later than A619, and thus the early phase change in dcl1-fzt [Mo17] plants could be due to residual A619 alleles that promote early phase change. We think that this hypothesis is unlikely for two reasons. First, we compared dcl1-fzt [Mo17] with normal siblings; both groups should have similar amounts of residual A619 DNA. Second, normal siblings from both the A619 and Mo17 introgressions transition at the same

Figure 3. dcl1-fzt Inflorescences Make Abnormal Meristems.

(A) to (H) Scanning electron micrographs of normal ([A] to [D]) and dcl1-fzt mutant ([E] to [H]) inflorescences. A young normal ear (A) is compared with a young dcl1-fzt mutant ear (E). dcl1-fzt IMs are flattened and broader than normal, indicating mild fasciation. dcl1-fzt SPMs are enlarged and not initiated in ordered rows. An older normal ear (B) is compared with a dcl1-fzt older ear (F). Normal tassels ([C] and [D]) are compared with dcl1-fzt mutant tassels ([G] and [H]). The white arrowhead in (H) indicates fasciated BMs.

(I) Normal spikelet pair contains two SMs. Each SM is subtended by a glume.

(J) fzt mutant spikelet “pair” contains extra SMs, and not all SMs are subtended by glumes.

(K) Normal spikelet pair during floral development. Each spikelet consists of an upper FM and a lower FM. Floral organs are initiated in a stereotypical, ordered manner.

(L) Older fzt mutant spikelet pair. Spikelets initiate extra FMs. Floral development is abnormal, and floral organs are not initiated properly. An indeterminate branch-like meristem persists (black arrow). Black asterisks indicate SMs, and white asterisks indicate FMs/developing florets. Bars in (A) to (H) = 0.5 mm; bars in (I) to (L) = 100 μm.
Together, these results indicate that earlier transition in the presence of the Mo17 versus leaf 7 in A619). Thus, Mo17 alleles promote an increase in meristem determinacy, leaf polarity, and phase change, consistent with decreased miRNA levels in known miRNA-regulated processes in plants, including meristem and vascular polarity. This is supported by the observation that the DCL1-FZT enzyme is defective in dcl1-fzt mutants (Griffiths-Jones et al., 2006). Eight miRNAs were reduced in both seedlings and tassel primordia tissues, consistent with a defect in the processing of specific miRNAs (miRNAs in boldface and underlined in Figure 5). Not all miRNAs are affected to the same extent in dcl1-fzt mutants, suggesting a processing defect in a subset of miRNAs. For example, miR167a-d-5p was reduced 20- to 30-fold in both seedlings and tassel primordia, whereas miR160a-e,g-5p was decreased only 3- to 5-fold, and many miRNAs did not meet the statistical threshold for differential expression. We note, however, that although many miRNAs did not meet the statistical threshold for differential expression, nearly all of these “nonsignificant miRNAs” (38 of 40 in seedlings and 24 of 31 in tassel primordia) appeared to decrease in dcl1-fzt mutants, suggesting a broad, moderate reduction in the levels of miRNAs.

Mature miRNAs are processed from primary miRNA transcripts, and miRNA processing mutants often have increased levels of miRNA precursors (Kurihara and Watanabe, 2004; Kurihara et al., 2006; Yang et al., 2006; Laubinger et al., 2010). Therefore, we also examined the expression of pri-miRNA transcripts in dcl1-fzt and normal controls using RNA sequencing (RNA-seq; see below). In both seedlings and tassel primordia, ~50% of detectable miRNAs were differentially expressed between dcl1-fzt and normal controls (P < 0.05; 16 of 38 in seedlings and 20 of 39 in tassel primordia). Nearly all differentially expressed precursors were increased in dcl1-fzt mutants compared with normal controls (16 of 16 in seedlings and 17 of 20 in tassel primordia) (Supplemental Figure 9 and Supplemental Table 3). For approximately half of the upregulated precursors, the corresponding mature miRNA was decreased in dcl1-fzt mutant plants (pri-miRNAs are in boldface and underlined in Supplemental Figure 9). The molecular analysis of miRNA and pri-miRNA levels, combined with genetic analysis of the dcl1-fzt allele, strongly suggests from which arm of the hairpin precursor the mature miRNA is processed (Griffiths-Jones et al., 2006). Eight miRNAs were reduced in both seedlings and tassel primordia tissues, consistent with a defect in the processing of specific miRNAs (miRNAs in boldface and underlined in Figure 5). Not all miRNAs are affected to the same extent in dcl1-fzt mutants, suggesting a processing defect in a subset of miRNAs. For example, miR167a-d-5p was reduced 20- to 30-fold in both seedlings and tassel primordia, whereas miR160a-e,g-5p was decreased only 3- to 5-fold, and many miRNAs did not meet the statistical threshold for differential expression. We note, however, that although many miRNAs did not meet the statistical threshold for differential expression, nearly all of these “nonsignificant miRNAs” (38 of 40 in seedlings and 24 of 31 in tassel primordia) appeared to decrease in dcl1-fzt mutants, suggesting a broad, moderate reduction in the levels of miRNAs.

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Plant miRNAs primarily regulate gene expression by promoting the cleavage and degradation of target mRNAs (Bartel, 2004). Therefore, we expect mRNAs targeted by miRNAs reduced in dcl1-fzt to be increased in dcl1-fzt plants compared with normal

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**Figure 4.** dcl1-fzt Is Required for Normal Leaf Cell Differentiation.

Macrohairs (arrows) are present on the adaxial surface of a normal leaf blade (A) but absent from the abaxial surface (C). dcl1-fzt [Mo17] contains fewer macrohairs on the adaxial surface (B), but macrohairs are present on the abaxial surface (D). Vascular polarity is also perturbed in dcl1-fzt [Mo17] mutants (E and F). A normal vascular bundle is shown in (E). Xylem cells (red asterisks) are positioned adaxially relative to the phloem cells (P). In dcl1-fzt [Mo17] mutants (F), the xylem cells are disorganized and extend farther toward the abaxial pole than normal. Bars in (A) and (B) = 1 mm; bars in (C) and (D) = 400 μm; bars in (E) and (F) = 50 μm.

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miRNA Levels Are Reduced in fzt Mutants

To determine the effect of the dcl1-fzt mutation on miRNA biogenesis, we analyzed the small RNA populations in 14-d-old seedlings and tassel primordia of dcl1-fzt and normal plants by deep sequencing. In total, small RNA libraries representing three biological replicates of seedlings and tassel primordia from dcl1-fzt and normal controls were generated and sequenced (Supplemental Table 1). The raw small RNA sequences were processed to remove adapter sequences and matched to the maize genome (AGPv2). To compare between libraries, the count/abundance of each small RNA was normalized based on the sequencing depth as reads per 10 million. We found that the small RNA profiles from normal and dcl1-fzt seedlings and tassel primordia are similar (Supplemental Figure 8). To determine if the dcl1-fzt mutation affected the accumulation of a subset of miRNAs, we compared the levels of individual miRNAs in dcl1-fzt seedlings and tassel primordia with normal controls. Approximately one-third of the detectable miRNAs (22 of 63 in seedlings and 14 of 45 in tassel primordia) were differentially expressed in dcl1-fzt mutants compared with normal controls (P < 0.05 and false discovery rate [FDR] < 0.05) (Figure 5; Supplemental Table 2). miRNAs are denoted as -5p or -3p to indicate from which arm of the hairpin precursor the mature miRNA is processed (Griffiths-Jones et al., 2006). Eight miRNAs were reduced in both seedlings and tassel primordia tissues, consistent with a defect in the processing of specific miRNAs (miRNAs in boldface and underlined in Figure 5). Not all miRNAs are affected to the same extent in dcl1-fzt mutants, suggesting a processing defect in a subset of miRNAs. For example, miR167a-d-5p was reduced 20- to 30-fold in both seedlings and tassel primordia, whereas miR160a-e,g-5p was decreased only 3- to 5-fold, and many miRNAs did not meet the statistical threshold for differential expression. We note, however, that although many miRNAs did not meet the statistical threshold for differential expression, nearly all of these “nonsignificant miRNAs” (38 of 40 in seedlings and 24 of 31 in tassel primordia) appeared to decrease in dcl1-fzt mutants, suggesting a broad, moderate reduction in the levels of miRNAs.

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Figure 5. miRNA Levels Are Reduced in dcl1-fzt Mutants.

(A) Comparison of individual miRNA levels in dcl1-fzt and A619 seedlings.

(B) Comparison of individual miRNA levels in dcl1-fzt and normal sibling tassel primordia.
controls. We analyzed the transcriptome of dcl1-fzt and normal control plants in seedlings and tassel primordia using RNA-seq. For this analysis, we analyzed mRNAs that are predicted targets of miRNAs decreased in dcl1-fzt seedlings or tassel primordia (Figure 6; Supplemental Figure 10). We first analyzed the expression of all predicted target mRNAs (having a miRferno [mF] score ≤ 7; see Methods). In the tassel, 314 of 6343 mRNAs predicted as targets of miRNAs were differentially expressed (P < 0.5 and FDR < 0.05); 137 predicted target miRNAs were increased and 177 target miRNAs were decreased in dcl1-fzt tassel primordia compared with normal controls (Figure 6A; Supplemental Data Set 1). In seedlings, 928 of 9420 mRNAs predicted as targets of miRNAs predicted were differentially expressed: 611 predicted target miRNAs were increased and 317 target miRNAs were decreased in dcl1-fzt mutants (Supplemental Figure 10A and Supplemental Data Set 2). Thus, we did not observe a broad increase of miRNA target levels in dcl1-fzt mutants, at least at the level of the tissue used for RNA-seq libraries.

The initial target list may have included many false positives that are not in vivo miRNA targets. Therefore, we also analyzed predicted targets with mF scores ≤ 4, which enrich for higher confidence targets (Fahlgrén et al., 2007). In the tassel, 117 predicted targets had mF scores ≤ 4, of which 14 showed a differential abundance (P < 0.05 and FDR < 0.05) between mutant and normal controls. Of these differentially expressed target miRNAs, 12 of 14 (84%) were increased in dcl1-fzt mutants (Figure 6B; Supplemental Data Set 3). Similarly, in the seedling, 151 predicted target mRNAs had mF scores ≤ 4, of which were differentially expressed (P < 0.05 and FDR < 0.05) between mutant and normal controls. Of the differentially expressed target mRNAs, 19 of 26 (73%) were increased in dcl1-fzt mutants (Supplemental Figure 10B and Supplemental Data Set 4).

Many in vivo miRNA targets have mF scores > 4 due to the loose complementarity in the seed region between the miRNA and target mRNA. Therefore, we also assembled a list of high-confidence targets based on a conserved biological function with miRNA targets in other plant species, which include targets with a range of mF scores (Supplemental Tables 4 and 5). Similar to the mF-enriched targets, a small proportion (11 of 41 in tassel primordia and 17 of 51 in seedlings) of the “biologically defined” targets were differentially expressed between dcl1-fzt and normal controls (P < 0.05 and FDR < 0.05). In the tassel, 10 of 11 of the differentially expressed biologically defined targets were increased in dcl1-fzt mutants, including three MYB-domain transcription factors (miR159 targets), two ARF transcription factors (miR167 targets), three AP2-domain transcription factors (miR172e targets), and two F-box genes (miR394 targets) (Supplemental Table 4). In seedlings, 10 of 17 differentially expressed biologically defined targets were increased in dcl1-fzt mutants. Nearly all of the decreased seedling targets (6 of 7) are targeted by miR397, which targets laccases (Jones-Rhoades and Bartel, 2004; Zhang and Yuan, 2014). Decreased levels of multiple targets corresponding to a single miRNA raise the possibility that miR397 might have a positive role in gene expression, although miR397 downregulates its rice target, OsLAC (Zhang et al., 2013). Alternatively, another miRNA pathway could be epistatic to miR397 regulation, or the predicted miR397 targets might not be bona fide targets. Regardless, the differentially expressed high-confidence miRNA targets (based on mF ≤ 4 or functional conservation) were predominantly increased in dcl1-fzt plants. Although most miRNAs predicted to be miRNA targets were not differentially expressed in dcl1-fzt mutants, miRNA regulation often occurs only in a discrete group of cells at a specific developmental stage, and gene expression analysis at the tissue or whole-plant level is unlikely to uncover this regulation.

To verify our RNA-seq data, we examined the expression of 11 predicted target mRNAs and two pri-miRNA transcripts by quantitative RT-PCR (qRT-PCR) (Figure 6C). The quantitative PCR (qPCR) analysis gave similar results to the RNA-seq data, although in some cases the qRT-PCR analysis indicated a slightly larger fold change than the RNA-seq analysis. Regardless, there was strong agreement between the RNA-seq and qRT-PCR analyses.

**DISCUSSION**

*fzt* Contains a Mutation in *dcl1* and Is Defective in miRNA Processing

We identified a viable maize mutant in *dcl1*, the primary DICER enzyme involved in processing miRNAs in plants. To date, this is the only *dcl1* mutant reported in a plant other than *Arabidopsis*. Several pieces of evidence suggest that the *dcl1-fzt* allele results in decreased DCL1 function and that the *dcl1-fzt* phenotypes are due to decreased levels of a subset of miRNAs. First, *dcl1-fzt* behaves genetically as a partial loss-of-function allele; *dcl1-fzt* is completely recessive, and putative *dcl1* null alleles fail to complement fzt. Second, *dcl1-fzt* contains a mutation predicted to change a conserved serine residue in the RNase III domain of DCL1. The RNase III domains contain the catalytic domains required for RNA cleavage of both the pri-miRNA and pre-miRNA (Kurihara and Watanabe, 2004; Zhang et al., 2004).

Finally, miRNA levels are decreased and pri-miRNA levels are increased in *dcl1-fzt* mutants, consistent with reduced DCL1 function.

miRNAs were not uniformly reduced in *dcl1-fzt* plants. A few miRNAs (miR167a-d-5p and miR394a-b-5p) were dramatically reduced in *dcl1-fzt* mutants, others exhibit a moderate reduction, and another set were not reduced to a statistically significant level. The molecular basis for this differential effect on miRNA...
levels is unclear. One possibility is that the DCL1-FZT protein is defective in processing only a subset of pri-miRNAs with specific structural characteristics. Indeed, pri-miRNA processing seems to be particularly plastic in plants. Most plant pri-miRNAs are processed in a “base-to-loop” fashion by first cleaving the base of the hairpin and then a second cleavage 20 to 24 nucleotides from the initial cleavage site to excise the miRNA/miRNA* duplex (Cuperus et al., 2010; Mateos et al., 2010; Song et al., 2010; Werner et al., 2010). At least two miRNAs in Arabidopsis, miR159 and miR319, are processed in a more complex loop-to-base fashion that requires four cleavage events (Bologna et al., 2009). The processing mechanism of individual miRNAs has not been investigated in maize; thus, it is unclear if fzt mutants are defective in a particular processing pathway.

Alternatively, the differential effect on miRNA levels could be due to molecular redundancy, with DCL2 or DCL4 processing some miRNAs in the absence of fully functional DCL1. In Arabidopsis, DCL4 has been shown to process some miRNAs, including newly evolved miRNAs, with a high degree of complementarity within the pri-miRNA (Rajagopalan et al., 2006; Fahlgren et al., 2007; Ben Amor et al., 2009).

Maize dcl1-fzt mutants phenotypically resemble the Arabidopsis dcl1 alleles dcl1-9 (also known as carpel factor caf1) and dcl1-100, with reduced plant stature and leaf size, increased meristem indeterminacy, and meristem fasciation (Jacobsen et al., 1999; Laubinger et al., 2010). Although Arabidopsis dcl1 alleles have similar effects on plant development, mutations in dcl1 result in dramatically reduced miRNA processing and accumulation. Mature miRNA levels have not been globally examined in dcl1-9/caf1 mutants; however, those miRNAs that have been examined are dramatically reduced or undetectable in the mutant (Park et al., 2002; Reinhart et al., 2002; Kasschau et al., 2003; Papp et al., 2003; Ronemus et al., 2006), and the pri-miRNA of at least some miRNAs accumulate in this mutant (Song et al., 2007). All miRNAs examined in the dcl1-7 mutant allele are also similarly reduced or undetectable. Notably, the phenotype of dcl1-7 is less severe than that of dcl1-fzt, and it produces some pollen (Robinson-Beers et al., 1992). Dcl1-100 mutants also exhibit increased levels of at least some, but not all, pri-miRNA precursors based on tiling array experiments (Laubinger et al., 2010). One unresolved question is why the dcl1-fzt mutation, with a more modest reduction in miRNA levels, exhibits severe phenotypes similar to Arabidopsis dcl1 alleles. There is precedence that at least some single gene mutations have more dramatic consequences in maize than in Arabidopsis, particularly with regard to miRNAs. For example, Cg1 has more severe phenotypes than 35S:miR156 in

Figure 6. Analysis of Predicted miRNA Targets in Tassel Primordia. (A) MA plot showing all predicted miRNA targets (mF ≤ 7) for miRNAs decreased in dcl1-fzt tassel primordia. Red dots indicate miRNA targets differentially expressed in dcl1-fzt mutants (P < 0.05 and FDR < 0.05), and black dots indicate miRNA targets that are not differentially expressed. miRNA targets are not broadly increased in dcl1-fzt mutants. CPM, counts per million; FC, fold change.

(B) MA plot showing predicted miRNA targets with mF ≤ 4. Dot color indicates statistical significance as in (A). Of the differentially expressed targets, the majority (12 of 14) are increased in dcl1-fzt tassel primordia.

(C) qRT-PCR validation of RNA-seq analysis for select miRNA targets and pri-miRNA transcripts. Black bars indicate fold change calculated from qRT-PCR analysis; gray bars indicate fold change calculated from RNA-seq analysis; gray cross-hatched bars indicate transcripts that do not meet the statistical threshold for differential expression in RNA-seq experiments. qRT-PCR data are the result of three biological and three technical replicates. Error bars indicate SE.
miRNAs Are Required Broadly during Inflorescence and Vegetative Development

The wide range of reproductive and vegetative defects observed in dcl1-fzt plants underscores the broad roles of miRNAs during development. Some aspects of the dcl1-fzt phenotype can be explained by known miRNA regulatory networks, such as the regulation of meristem determinacy by ts4/miR172e and the control of phase change by miR156/miR172 and leaf polarity by miR390 and miR166 (Juarez et al., 2004; Chuck et al., 2007a, 2007b). Indeed, miRNA levels in dcl1-fzt correspond well to the severity of specific phenotypes. For example, miR172e is reduced >25-fold in the tassel and dcl1-fzt tassels have severe meristem determinacy defects, whereas miR156a-i-5p, miR156i-5p, and miR166 are not statistically decreased in dcl1-fzt mutants, and dcl1-fzt plants have very mild phase change and leaf polarity defects. Interestingly, dcl1-fzt plants exhibit only relatively modest reductions in most miRNAs and their targets, suggesting that misregulation of a few miRNA target genes underlies most of the dcl1-fzt phenotype. In Arabidopsis, reduced levels of just two miRNA targets, SPL10 and SPL11, largely suppressed the embryonic lethality of dcl1 null mutations, indicating that a large part of the dcl1 embryonic lethality is due to the misregulation of just two targets (Nodine and Bartel, 2010). Thus, even though the levels of most miRNAs are unchanged in dcl1-fzt plants, misregulation of just a few miRNA targets can have large and pleiotropic defects.

The individual miRNAs and/or their miRNA targets that underlie several aspects of the fzt phenotype are still unknown, but the pleiotropic effect is consistent with multiple affected miRNAs and includes pleiotropic effects on stem cell homeostasis, plant height, leaf size, and floral organ and stamen development. miRNAs that exhibit large decreases in dcl1-fzt mutants are good candidates for being responsible for these aspects of the phenotype. Among the miRNAs that are severely reduced in dcl1-fzt tassel primordia are miR159, miR167, and miR319 (Figure 6), which target MYB transcription factors, auxin response factors, and class II TCP transcription factors, respectively, in Arabidopsis and have roles in stamen, ovule, leaf, and petal development (Palatnik et al., 2003; Millar and Gubler, 2005; Wu et al., 2006; Nag et al., 2009; Rubio-Somoza and Weigel, 2013). Importantly, miR159, miR167, and miR319 target these same classes of transcription factors in maize (Zhang et al., 2009), and dcl1-fzt mutants are defective in processes regulated by these miRNAs, including stamen and ovule development. Intriguingly, three MYB transcription factors targeted by miR159 (GRMZM2G423833, GRMZM2G139688, and GRMZM2G050550) as well two ARF transcription factors targeted by miR167 (GRMZM2G081158 and GRMZM2G073735) are statistically increased in dcl1-fzt mutant tassels (Figure 6C; Supplemental Table 4), consistent with three miRNAs contributing to the dcl1-fzt phenotype.

miRNA Regulatory Networks Differ between Inbred Backgrounds

While dcl1-fzt mutants have similar phenotypes in all three inbred backgrounds examined, such as reduced plant stature, reduced leaf size, leaf polarity, phase change, and inflorescence defects, the severity of many of these phenotypes differs depending on inbred background. For example, leaf polarity defects are more severe in the Mo17 inbred background than in A619, and inflorescence defects are more severe in the B73 and Mo17 inbred backgrounds than in A619. Also, phase change occurs approximately one leaf late in the A619 inbred but approximately one leaf early in the Mo17 inbred background. Since the molecular defect in DCL1 is the same in each inbred (all contain the dcl1-fzt mutation, which presumably has the same effect on miRNA processing), these phenotypic differences suggest that genes functioning upstream or downstream of miRNA regulatory networks differ in the three inbreds. The identification of these modifier loci could provide a way to find genes in miRNA regulatory networks not amenable to standard genetic approaches. Most mutant screens isolate strong loss-of-function or null alleles, for which pleiotropy or epistasis might make it difficult to uncover roles in some developmental processes. The natural alleles present at these modifier loci, however, are likely mild alleles that have subtle effects in an otherwise normal genetic background and might provide targets for breeding programs. Similar background differences have not been reported for Arabidopsis dcl1 alleles; thus, work in maize is poised to identify modifiers of miRNA regulatory networks and elucidate how these networks vary in natural populations to regulate plant growth and development.

METHODS

Genetics and Phenotypic Characterization

fzt was generated by EMS mutagenesis in the maize (Zea mays) A619 mutant background. Mapping populations were generated by crossing fzt heterozygotes to the Mo17 and B73 inbreds and self-pollinating. Mutants from segregating populations were tested for linkage to simple sequence repeat markers that spanned the genome. fzt was localized to chromosome 1, bin 1, and flanking markers were defined. The interval was refined
using available markers from the IBM neighbors map and additional single-nucleotide polymorphism markers designed based on maize ESTs in the interval.

The coding region was sequenced from one candidate gene, dcl1 in the fzt allele, which corresponds to gene model GRMZM2G040762. This gene model is incomplete and lacks the conserved DEXD helicase domain found at the N terminus of all DCL proteins. The maize B73 reference genome contains a gap just upstream of gene model GRMZM2G040762; therefore, unordered contigs were assembled from overlapping BACs, AC1S5424, AC191351, and AC191256, and an additional ~53-kb sequence upstream of GRMZM2G040762 was reconstructed. To identify the full-length dcl1 coding sequence, the predicted protein from GRMZM2G040762 was aligned to the predicted rice (Oryza sativa) DCL1 protein (Os03g009220; 1885 amino acids), revealing that GRMZM2G040762 aligns well with amino acids 577 to 1881 (92% identity) (Supplemental Figure 3). To identify the corresponding maize genomic sequences, OsDCL1 amino acids 1 to 576 were BLAST searched in CoGe (Lyns and Freeling, 2008) against the maize genome. This strategy was used iteratively with smaller segments of the N terminus of OsDCL1 to identify maize sequences that align to the entire N terminus. Comparison of the predicted maize DCL1 protein with that from Arabidopsis suggests that a short 114-nucleotide sequence encoding 38 amino acids annotated as an intron in rice is part of an exon. The predicted full-length maize DCL1 protein exhibits 90 and 72% identity to rice and Arabidopsis thaliana proteins, and the N-terminal domain, which contains the conserved DEXD domain, exhibits 85 and 59% identity with rice and Arabidopsis proteins, respectively (Supplemental Figure 3). See Supplemental Figure 11 for the full genomic sequence.

An additional dcl1 allele was generated by crossing EMS-mutagenized Mo17 pollen onto fzt heterozygotes. One individual failed to complement the original fzt mutation in the F1, and the dcl1 coding region in this individual was sequenced. The Mu-insertion sites in the TUSC alleles that failed to complement fzt were also defined. Phenotypic characterization was done using fzt mutant families that had been backcrossed a minimum of three times to A619, Mo17, and B73. Seedling tissue for RNA analysis was genotyped by sequencing the region spanning the fzt mutation.

Vascularure polarity was examined by making hand sections of fresh tissue and staining with 0.5% toluidine blue O stain for 1 min, washing twice with water, and mounting in 100% glycerol for visualization with an Olympus BX41 microscope using a dark field. Epidermal peels were done as described (Gallagher and Smith, 1999).

Scanning Electron Microscopy

Tissue for scanning electron microscopy analysis was dissected and either mounted directly for scanning electron microscopy under low-vacuum conditions on an FEI Quanta 200 Mark 1 scanning electron microscope at an accelerating voltage of 10 to 15 kV or fixed in FAA, critical point dried, sputter coated, and viewed under high vacuum on a Hitachi S-4700 device at an accelerating voltage of 2 kV.

Small RNA and RNA-seq Library Sequencing and Informatics Analyses

A619 and dcl1-fzt mutant seedlings were grown for RNA analysis by germinating seeds on wet paper towels, transplanting seedlings to soil, and growing at 26°C with 12 h of light in a Percival AR-41L3 growth chamber for 14 d. Whole seedlings were removed from soil, washed, and flash-frozen in liquid nitrogen. Tassel primordia (0.5 to 1 cm) from dcl1-fzt and normal siblings were harvested from ~4.5-week-old plants grown in the East Carolina Biology Greenhouse with 16 h of light (two biological replicates) or in the field at the Central Crops Research Station in Clayton, North Carolina (one biological replicate), and flash-frozen in liquid nitrogen.

Total RNA from the materials described above was isolated using Tri Reagent (Molecular Research Center). Small RNA libraries were constructed using the Illumina TruSeq Small RNA sample preparation kit RS-200-0012. RNA-seq libraries were constructed using the Illumina TruSeq RNA sample preparation kit RS-122-2001. Libraries were sequenced on an Illumina HiSeq2000 instrument at the Delaware Biotechnology Institute of the University of Delaware.

The small RNA-seq data were processed as described previously (Nobuta et al., 2010; McCormick et al., 2011). In brief, small RNA libraries were trimmed to remove adapter sequences, low-quality reads were filtered out, and remaining reads (18 to 34 nucleotides) were mapped to the maize genome (Maize Genome Project 5b.60 AGPv2 sequences; http://www.maizesequence.org). The genome-mapped unique reads and their counts were imported to the R statistical environment (R Core Team, 2012). Lowly expressed reads (<1 count per million in less than two samples) were filtered out, library sizes were reset, and normalization was performed using the edgeR (Robinson et al., 2010) package default Trimmed Mean of M values method. Differentially expressed reads (small RNAs) were identified using the Generalized Linear Model approach implemented in the edgeR package. To account for multiple testing, the Benjamini and Hochberg method for controlling FDR was applied with a threshold (q ≤ 0.05) to determine significance (Robinson et al., 2010).

Maize miRNAs annotated in miRBase 21 (mirbase.org) were mapped to this list to identify miRNAs present in each tissue. The seedling A619_3 miRNA abundances appeared elevated compared with its replicates; however, the difference between this nonmutant and mutant sibling pair appeared to be biological, and thus both libraries were included in the analysis.

Raw RNA-seq libraries were processed using an in-house pipeline to trim adapter sequences and filter out low-quality reads. TopHat (Trapnell et al., 2009) was used to map reads to the maize genome (AGPv2 sequences; http://www.maizesequence.org). Genome-mapped reads were assembled, and a “merged” transcriptome assembly was generated using Cufflinks. Quantification of expression levels for genes and transcripts was done using Cuffquant, and count tables were exported using Cuffnorm. These count tables were imported to the R statistical environment (R Core Team, 2012), and reads with low expression were filtered out followed by resetting the library size and normalization by the edgeR default Trimmed Mean of M values method. Mutant versus nonmutant contrasts were used to calculate differential expression using the Generalized Linear Model approach implemented in edgeR. Adjustment for multiple testing was performed by Benjamini and Hochberg’s method (Robinson et al., 2010) to control FDR, and a threshold (q ≤ 0.05) was used to determine significance.

Genome-wide identification of miRNA targets was performed using the sPARTA package (Kakrana et al., 2015). Putative targets were predicted using sPARTA’s built-in target prediction module miFerne with “standard” scoring schema and score cutoff of ≤7. At the cost of sensitivity, targets with scores ≤ 4 were investigated to maintain specificity (Figure 6B; Supplemental Data Sets 3 and 4) (Falahgir et al., 2007). High-confidence target lists based on conserved biological functions (Supplemental Tables 4 and 5) used PFAM identifiers to assign likely biological functions to predicted maize targets. High-confidence targets were selected based on the known functions of conserved miRNAs and their targets in other plant species (Chorostecki et al., 2012).

Gene Expression Analysis

The expression of select target genes was examined using reverse transcription combined with qPCR. Total RNA was extracted from three biological replicates of normal and dcl1-fzt tassel primordia (four tassel primordia per biological replicate) using Trizol according to the manufacturer’s recommendations. RNA was DNase-treated (RNase-free
DNase set; Qiagen), purified using the RNeasy mini elute kit (Qiagen), and reverse transcribed using oligo(dT) primers (SuperScript III first-strand synthesis system; Invitrogen). cDNA equivalent to 25 ng of total RNA was used in a 20-μL PCR with the MyqMix mastermix (Bio-Rad) on a Bio-Rad CFX396 real-time system; data were processed in CFX Manager (Bio-Rad) and Excel. Data represent averages of three biological replicates and three qPCR technical replicates. All primer efficiencies were between 95 and 105%, and data were normalized against gapdh levels. Sequences of the primers used can be found in Supplemental Table 6.

Accession Numbers

The small RNA and RNA sequence data have been submitted to the National Center for Biotechnology Information’s Gene Expression Omnibus with accession number GSE52879.

Supplemental Data

The following materials are available in the online version of this article.

- Supplemental Figure 1. Additional Phenotypic Characterization of fzt Plants.
- Supplemental Figure 2. fzt Phenotypes Introgressed into the B73 and Mo17 Inbreds.
- Supplemental Figure 3. Genomic Structure of Maize dcl1.
- Supplemental Figure 4. dcl1 Alleles Are Transmitted in Mendelian Ratios.
- Supplemental Figure 5. Inflorescence Meristems Are Fasciated in dcl1-fzt Inflorescences.
- Supplemental Figure 6. dcl1-fzt Leaf Polarity Defects in the A619 Inbred Background.
- Supplemental Figure 7. dcl1-fzt Plants Have Phase Change Defects.
- Supplemental Figure 8. Distribution of Small RNAs in dcl1-fzt and Normal Control Plants.
- Supplemental Figure 9. Pri-miRNA Levels Are Increased in dcl1-fzt Mutants.
- Supplemental Figure 10. Analysis of Predicted miRNA Targets in Seedlings.
- Supplemental Figure 11. Maize dcl1 Genomic Sequence.
- Supplemental Table 1. Summary Statistics of Sequence-by-Synthesis (SBS) Small RNA and RNA-seq Libraries Used in This Study.
- Supplemental Table 2. Abundance of miRNAs in Seedling and Tassel Primordium Libraries.
- Supplemental Table 3. Abundance of pri-miRNAs in Seedling and Tassel Primordium Libraries.
- Supplemental Table 4. Summary of Predicted miRNA Targets Based on Conserved Biological Function in dcl1-fzt and Normal Tassel Primordia.
- Supplemental Table 5. Summary of Predicted miRNA Targets Based on Conserved Biological Function in dcl1-fzt and Normal Seedlings.
- Supplemental Table 6. Primers Used in This Study.
- Supplemental Data Set 1. Summary of Predicted miRNA Targets and Fold Difference in dcl1-fzt and Normal Tassel Primordia.
- Supplemental Data Set 2. Summary of Predicted miRNA Targets and Fold Difference in dcl1-fzt and Normal Seedlings.

Supplemental Data Set 3. Summary of Predicted miRNA Targets (mF ≤ 4) in dcl1-fzt and Normal Tassel Primordia.

Supplemental Data Set 4. Summary of Predicted miRNA Targets (mF ≤ 4) in dcl1-fzt and Normal Seedlings.

ACKNOWLEDGMENTS

We thank David Hantz, Julie Calfas, and Julie Marik for care of greenhouse plants and Cathy Herning and Jim Holland for field space and assistance at the Central Crops Research Station. We also thank Amanda Wright for providing technical assistance with epidermal peels. This work was supported by the National Science Foundation (Grant 0604923 to S.H., Grant 1051576 to B.C.M., and Grant 1148971 to B.E.T.) and the National Institutes of Health (National Research Service Award postdoctoral fellowship F32GM082002 to B.E.T.).

AUTHOR CONTRIBUTIONS


Received September 29, 2014; revised October 27, 2014; accepted November 13, 2014; published December 2, 2014.

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The *dicer-like1* Homolog *fuzzy tassel* Is Required for the Regulation of Meristem Determinacy in the Inflorescence and Vegetative Growth in Maize
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*Plant Cell* 2014;26;4702-4717; originally published online December 2, 2014; DOI 10.1105/tpc.114.132670

This information is current as of June 19, 2017

| Supplemental Data | /content/suppl/2015/01/13/tpc.114.132670.DC2.html  
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