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Note

The Interaction of *knotted1* and *thick tassel dwarf1* in Vegetative and Reproductive Meristems of Maize

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ABSTRACT

In Arabidopsis, *SHOOT MERISTEMLESS* (*STM*) and *CLAVATA1* (*CLV1*) competitively regulate meristem homeostasis. Here, we explore the interaction of their maize homologs *knotted1* (*kn1*) and *thick tassel dwarf1* (*td1*). *kn1* mutants form fewer lateral organs and *td1* inflorescences are fasciated with additional floral organs. Double mutants show *kn1* epistatic to *td1* in seedling and ear development but dose-sensitivity exists later to promote leaf initiation. Thus *kn1* and *td1* function in a pathway to maintain meristem homeostasis but their products may interact with different partners during development.

To produce organs predictably, meristems tightly control the opposing processes of meristem maintenance and lateral organ initiation. In Arabidopsis, the *CLAVATA* (*CLV*) genes control balance between these processes. Loss-of-function/hypomorphic *clv* mutants have enlarged shoot and floral meristems, producing more floral organs (Leyser and Furner 1992; Clark *et al.* 1993, 1995; Kayes and Clark 1998). *CLV1* and *CLV2* encode a leucine-rich repeat (LRR) receptor-like kinase protein and an LRR protein lacking a kinase domain, respectively (Clark *et al.* 1997; Jeong *et al.* 1999). *CLV3* encodes a small peptide (Fletcher *et al.* 1999) that physically interacts with CLV1 (Ogawa *et al.* 2008). *BAM1*, *BAM2*, and *BAM3* encode *CLV1*-related receptor kinases. Opposite of *clv* mutants, the double or triple *bam* mutants have smaller meristems (DeYoung *et al.* 2006). Genetic evidence, however, points to a significant role for the *BAM* loci in the *CLV* pathway, as the *bam* mutants ameliorate the *clv3* phenotype but enhance null alleles of *clv1* (DeYoung and Clark 2008).

The homeobox gene *SHOOT MERISTEMLESS* (*STM*), central to meristem maintenance and determinacy (Long *et al.* 1996), functions in a separate pathway (Brand *et al.* 2002; Lenhard *et al.* 2002). Strong *stm* mutants lack vegetative development and produce only cotyledons (Barton and Poethig 1993). Weak *stm* mutants may progress to flowering and demonstrate a function for *STM* in inflorescences (Endrizzi *et al.* 1996; Brand *et al.* 2002; Bhatt *et al.* 2004; Kanrar *et al.* 2006). *clv1* alleles suppress the *stm* phenotype and *stm* alleles suppress the *clv1* phenotype, indicating that these genes play antagonistic roles. This genetic interaction is dose dependent: CLV1 and STM proteins are sensitive to each other, suggesting that their balance is necessary to maintain proper meristem size and shape (Clark *et al.* 1996).

Knotted1 (*kn1*) is a maize homolog of *STM* with similar expression pattern and function (Smith *et al.* 1992; Vollbrecht *et al.* 2000). Expressivity of recessive *kn1* mutations depends upon genetic background and meristem size. In inbred lines with larger meristems, defects are most pronounced during the adult phase: tassels are less branched, ears often absent or with reduced seed set. Ectopic leaves may form above the ear node (Kerstetter *et al.* 1997). In restrictive backgrounds with smaller meristems, a limited shoot phenotype is seen in which zero to two leaves form (Vollbrecht *et al.* 2000).

clv-like mutations affect distinct meristems in the grasses, unlike Arabidopsis, in which all shoot meristems are larger. Mutations in the *CLV3* rice homolog, *FON2*, affect only floral meristems (Suzaki *et al.* 2006), while mutations in another *CLV3* homolog, *FCP1*, affect only vegetative meristems (Suzaki *et al.* 2008). Mutations in the rice *CLV1* homolog, *FON1*, affect only floral meristems (Suzaki *et al.* 2004), suggesting another *CLV1* homolog functions in inflorescence and vegetative meristems. *thick tassel dwarf* (*td1*) encodes the most similar maize CLV1 homolog with 58% amino acid

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TABLE 1

Penetrance of limited shoot phenotype in the B73:Mo17 genetic background

Genotype[a]	*N*	LS[b] (*n*)
kn1/kn1; td1/td1	18	3[c]
kn1/kn1; td1/+	14	4
kn1/kn1; +/+	7	1[c]
kn1/+; td1/td1	11	0
kn1/+; td1/+	13	0
kn1/+; +/+	5	0
Total	68	8

To create a family segregating double mutants in the B73:Mo17 background, *kn1-E1* was backcrossed to Mo17 four times and *td1-glf* was backcrossed to B73 nine times. An F_1 plant with these two parents was self-pollinated to make an F_2 family and assayed by PCR for all possible allelic combinations. Only *kn1-E1* plants showed the limited shoot phenotype. An F_2 individual with the genotype *kn1-E1/+ td1-glf/+* was crossed to a sibling with the genotype *kn1-E1/kn1-E1 td1-glf/+* to enrich for double mutants. Plants were grown under greenhouse conditions.

[a] Not significantly different from expected ratio (X^2, $P > 0.01$).

[b] Limited shoot phenotype.

[c] One plant in each of these classes recovered and grew normally.

identity to CLV1 and 51% identity to BAM1 and BAM2. *td1* mutants have enlarged inflorescence meristems, increased spikelet density and supernumerary floral organs but, in contrast, reduced vegetative growth. Plants are shorter with fewer leaves (Bommert *et al.* 2005). Thus TD1 may promote vegetative meristem growth and restrict inflorescence and floral meristem growth.

To investigate the role of *STM*-and *CLV1*-homologous pathways in the grasses, we have utilized mutations in *kn1* and *td1* to observe double-mutant phenotypes.

***td1-glf* fails to suppress the limited shoot phenotype of *kn1-E1*:** We combined the null *kn1-E1* allele (Vollbrecht *et al.* 2000) with the null *td1-glf* allele (Bommert *et al.* 2005) to determine if *td1-glf* can suppress the limited shoot phenotype caused by *kn1-E1*. The limited shoot phenotype was more penetrant in a mixed B73:Mo17 background than in B73 alone, allowing us to study more individuals. Of 39 *kn1-E1* homozygous plants, 8 (20.5%) exhibited limited shoots (Table 1; Figure 1A). The genotype at the *td1* locus did not affect this phenotype, indicating that *td1-glf* cannot suppress the effects of *kn1-E1* during early vegetative growth and that *kn1-E1* is epistatic to *td1-glf*.

***td1-glf* vegetative meristems are smaller:** In Arabidopsis, *clv1* meristems are larger than wild type during vegetative growth (Clark *et al.* 1993). In contrast, we found that vegetative meristems of *td1-glf* mutants are smaller than those of wild-type siblings (Figure 2). This finding may explain why *td1-glf* does not suppress *kn1-E1*.

***td1-glf* increases the penetrance of the *kn1-E1* ectopic vegetative leaf phenotype:** In permissive backgrounds, plants homozygous for *kn1* loss-of-function alleles develop ectopic leaves in the axils of leaves at a low penetrance (12%) (Kerstetter *et al.* 1997). *td1-glf* enhanced this phenotype dose dependently (Table 2) (Figure 1, B–E). The ectopic leaves are reversed in polarity with their adaxial surface (Figure 1D) facing the adaxial surface of the true leaf. In an F_2 family segregating *td1-glf* and *kn1-E1*, all double mutants had at least one ectopic leaf, while *kn1-E1* homozygotes with one or no copies of *td1-glf* had 40% and 20%, respectively. In a family segregating 50% *kn1-E1* and 50% *kn1-E1/+* in the *td1-glf* mutant background, 90% of double mutants and 40% of *kn1-E1* heterozygotes had ectopic leaves. No *kn1-E1/+ td1-glf/td1-glf* plants had ectopic leaves in the F_2 family, indicating possible epigenetic and/or environmental influence on the phenotype. We also observed fusion of most ectopic leaves of double mutants to the adjacent leaf (Figure 1E) whereas fusion only occurred in a quarter of ectopic leaves of *kn1-E1/+ td1-glf/td1-glf* plants. Thus, we detected a synergistic increase in penetrance of ectopic leaves and an unexpected involvement of *td1* in the regulation of leaf initiation.

***kn1-E1* is epistatic to *td1-glf* in ear development:** *kn1* and *td1* are antagonistic in ear development. In permissive backgrounds, ears of *kn1* mutants are small with reduced spikelet density (Kerstetter *et al.* 1997). Ears of *td1* mutants are fasciated with increased spikelet

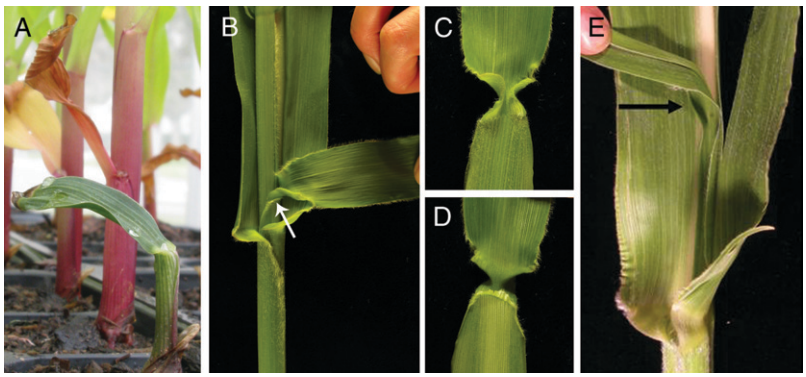
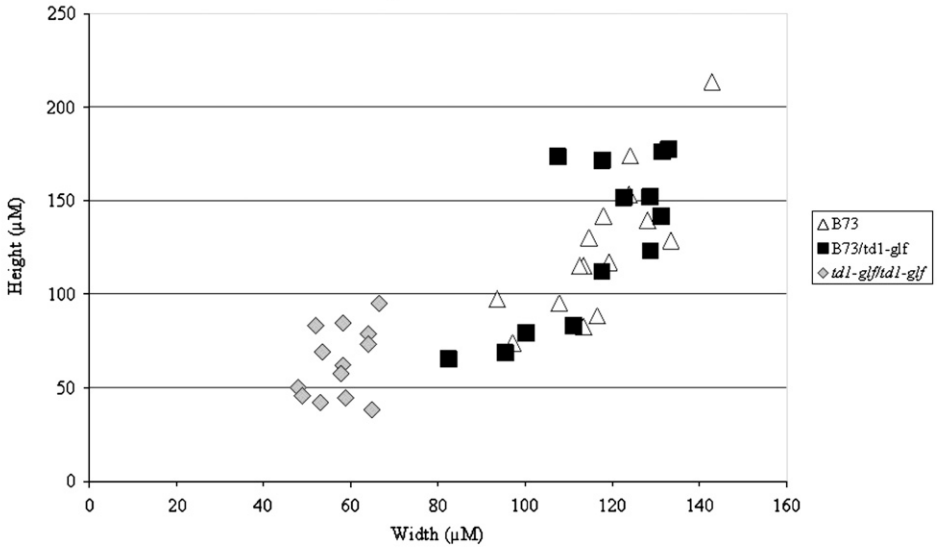


Figure 1.—Vegetative phenotypes of double mutants. (A) A *kn1-E1; td1-glf* seedling showing the limited shoot phenotype of a single leaf. Normal siblings are in the background. Their first leaf has senesced. (B) An ectopic leaf in the axil of a normal leaf. The ligule (arrow) is facing the ligule of the normal leaf (not seen). The ectopic leaf is attached at the meristem. (C–D) Each surface of ectopic leaf from B. (E) A fused ectopic leaf from a double mutant, fused proximal to arrow.

A



B

Means (µm)	height	width	ratio	*P*-values	height	width	ratio
B73 (*n*=16)	124 (36)	117 (12)	1.05 (0.22)	B73 vs. B73/*td1-glf*	0.74	0.86	0.64
B73/*td1-glf* (*n*=13)	129 (43)	116 (16)	1.09 (0.29)	B73 vs. *td1-glf/td1-glf*	0.00	0.00	0.58
td1-glf/td1-glf (*n*=13)	63 (19)	58 (6)	1.10 (0.30)	B73/*td1-glf* vs. *td1-glf/td1-glf*	0.00	0.00	0.94

FIGURE 2.—*td1-glf* vegetative meristems are smaller. (A) Height and width (micrometers) of B73 (open triangles), B73/*td1-glf* (solid squares), and *td1-glf/td1-glf* (shaded diamonds) were measured and plotted. (B) Mean heights, widths, and ratios for each genetic class. Standard deviations are in parentheses. *P*-values for *t*-tests comparing the classes are also shown. The lack of statistical difference in ratios for the three classes indicates they are proportional. B73 is statistically similar to B73/*td1-glf* heterozygotes, indicating that a single *td1-glf* allele has no affect on vegetative meristem size. Plants were grown in a growth chamber with the following conditions: 16 hr of light at 26°; 8 hr of darkness at 22°. Vegetative meristems were measured 14 days after sowing (DAS) after dissection and viewing under a Nikon SMZ800 microscope.

density on a continuous meristematic surface (Figure 3B) (BOMMERT *et al.* 2005). Double-mutant ears (Figure 3D) resembled those of *kn1* plants (Figure 3C), with patches of rachis lacking spikelets and similar spikelet density (Table 3). However, instead of solid rachis, ectopic spikelets formed inside ears of double mutants (Figure 3E) and the rachis fused distally, a phenotype not observed in *kn1* or *td1* single-mutant ears (data not shown).

TABLE 2

***td1-glf* increases the penetrance of the *kn1-E1* ectopic leaf phenotype and promotes fusion of ectopic leaves in the B73 genetic background**

Genotype[a]	Ectopic leaves[b]	Penetrance (%)
F_2 family		
kn1/kn1; td1/td1	4 (2.5; 4)	100
kn1/kn1; td1/+	4 (0.7; 10)	40
kn1/kn1; +/+	1 (0.2; 5)	20
kn1/+; td1/td1	0 (0; 13)	0
kn1/+; td1/+	0 (0; 19)	0
kn1/+; +/+	0 (0; 19)	0
+/+; td1/td1	0 (0; 11)	0
+/+; td1/+	0 (0; 5)	0
+/+; +/+	0 (0; 10)	0
1:1 family		
kn1/kn1; td1/td1	9 (1.90; 10)	90 (12/19)[c]
kn1/+; td1/td1	8 (0.45; 20)	40 (2/9)

td1-glf was backcrossed to B73 nine times and the *kn1-E1* allele was backcrossed to B73 six times. An F_1 plant with these two parents was self-pollinated. F_2 plants were assayed by PCR for all possible allelic combinations and a double mutant was crossed with a *kn1-E1/+ td1-glf/td1-glf* plant to generate a family segregating 1:1 for *kn1-E1* homozygotes in the *td1-glf* mutant background. Plants were grown under greenhouse conditions.

[a] Not significantly different from expected F_2 ratio (X^2, $P > 0.05$).

[b] Number of plants with ectopic leaves followed by mean number of ectopic leaves per plant and sample size in parentheses.

[c] Number of fused ectopic leaves/total number of ectopic leaves.

Summary: *STM* and *CLV* loci competitively regulate the balance of central and peripheral zones of the shoot apical meristem (CLARK *et al.* 1996). To assess whether this relationship exists in maize, we analyzed the interaction of *td1* and *kn1*. *td1-glf* does not suppress the limited shoot phenotype of *kn1-E1*, possibly because *td1* vegetative meristems are smaller. *kn1-E1* suppresses the fasciation of *td1-glf* inflorescences, indicating that the abnormal growth of fasciated meristems requires normal *kn1* function. *td1-glf* fails to suppress the ectopic leaves of *kn1* mutants; rather, it enhances the phenotype. *td1-glf* increases fusion of these ectopic leaves to their subtending leaves, a defect ameliorated by normal *kn1* alleles. In addition, a new phenotype was seen in double mutants: ectopic spikelets inside ears. Thus, KN1 and TD1 function in a linear pathway maintaining homeostasis in the vegetative meristem and ear, during spikelet initiation. Because *td1* mutant ears are fasciated yet their vegetative meristems are smaller, the function

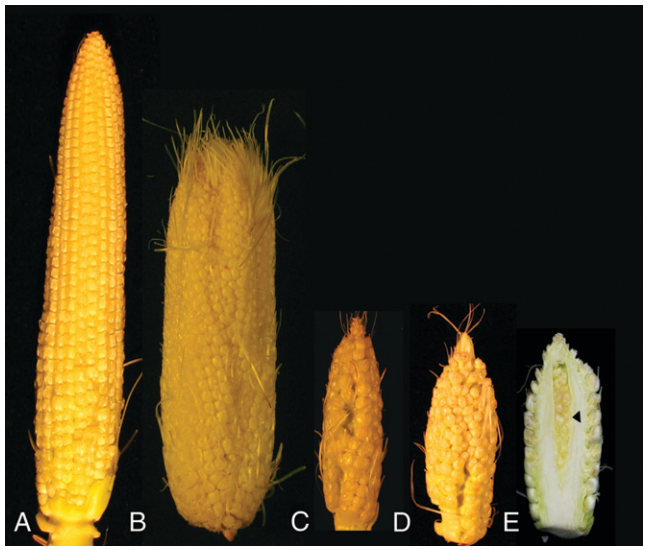


Figure 3.—Reproductive phenotypes. Unpollinated ears of (A) normal sibling, (B), *td1-glf* homozygote, (C) *kn1-E1* homozygote, and (D) a double mutant. Patches of cob have failed to initiate kernel primordia in the double mutant, as seen in *kn1-E1* homozygotes. (E) A longitudinal section of the same ear in D. Arrow in E points to ectopic kernel primordia in the ear core, a phenotype not seen in either single mutant.

of TD1 is similar to CLV1 in the inflorescence and floral meristems, but more similar to BAM (DeYoung *et al.* 2006) in vegetative meristems. The synergism displayed by ectopic leaves and ectopic spikelets suggests that TD1 and KN1 also converge to maintain cob identity and lateral organ initiation. Like the *BAM* genes, *td1* is widely expressed (Bommert *et al.* 2005) and these multiple roles may be dependent upon tissue specificity of other pathway members. We conclude that the relationship between *td1* and *kn1* is not directly comparable to that of *CLV1* and *STM*, as *td1*, like the *BAM* genes, has multiple functions throughout development.

TABLE 3

***kn1-E1* is epistatic to *td1-glf* in ear spikelet formation**

Genotype	Ear spikelets[a] (*n*)
A. Ear spikelet counts	
+/−; +/−	25.39 (12.91; 31)
td1/td1; +/−	30.90 (16.14; 23)
+/−; *kn1/kn1*	10.89 (5.82; 11)
td1/td1; kn1/kn1	11.67 (5.01; 6)
Genotype	***P*-values**
B. Comparisons of ear spikelet counts of the *td1-glf; kn1-E1* F_2 family	
td1/td1; +/− *vs.* +/−; +/−	0.002**
+/−; *kn1/kn1 vs.* +/−; +/−	0.000***
td1/td1; +/− *vs.* +/−; *kn1/kn1*	0.000***
td1/td1; kn1/kn1 vs. +/−; +/−	0.001**
td1/td1; kn1/kn1 vs. td1/td1; +/−	0.000***
td1/td1; kn1/kn1 vs. +/−; *kn1/kn1*	0.975

Plants from the *td1-glf*; *kn1-E1* F_2 family were grown in the greenhouse and observed. (A) Unpollinated ear spikelet counts. Numbers are mean values with standard deviations in parentheses followed by the number of plants measured. (B) *P*-values of a two-tailed Student's *t*-test of ear spikelet counts comparing the genetic classes. **Values are significant at the 1% level; ***values are significant at the 0.1% level.

[a] Numbers of spikelets were counted from a cross section of the ear at its widest point, ~1 cm from the ear tip.

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LITERATURE CITED

Barton, M. K., and R. S. Poethig, 1993 Formation of the shoot apical meristem In *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemless* mutant. Development **119:** 823–831.

Bhatt, A. M., J. P. Etchells, C. Canales, A. Lagodienko and H. D Ickinson, 2004 VAAMANA: a BEL1-like homeodomain protein, interacts with KNOX proteins BP and STM and regulates inflorescence stem growth in *Arabidopsis*. Genes Dev. **328:** 103–111.

Bommert, P. B., C. Lunde, J. Nardmann, E. Vollbrecht, M. P. Running *et al.*, 2005 *thick tassel dwarf1* encodes a putative maize orthologue of the Arabidopsis CLAVATA1 leucine-rich receptor-like kinase. Development **132:** 1235–1245.

Brand, U., M. Grunewald, M. Hobe and R. Simon, 2002 Regulation of *CLV3* expression by two homeobox genes in Arabidopsis. Plant Physiol. **129:** 565–575.

Clark, S. E., M. P. Running and E. M. Meyerowitz, 1993 *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. Development **119:** 397–418.

Clark, S. E., M. P. Running and E. M. Meyerowitz, 1995 *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. Development **121:** 2057–2067.

Clark, S. E., S. E. Jacobsen, J. Z. Levin and E. M. Meyerowitz, 1996 The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in *Arabidopsis*. Development **122:** 1567–1575.

Clark, S. E., R. W. Williams and E. M. Meyerowitz, 1997 The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. Cell **89:** 575–585.

DeYoung, B. J., K. L. Bickle, K. J. Schrage, P. Muskett, K. Patel *et al.*, 2006 The CLAVATA1-related BAM1, BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in Arabidopsis. Plant J. **45:** 1–16.

DeYoung, B. J., and S. E. Clark, 2008 BAM receptors regulate stem cell specification and organ development through complex interactions with CLAVATA signaling. Genetics **180:** 895–904.

Endrizzi, K., B. Moussian, A. Haecker, J. Z. Levin and T. Laux, 1996 The *SHOOT MERISTEMLESS* gene is required for maintenance of undifferentiated cells in *Arabidopsis* shoot and floral meristems and acts at a different regulatory level than the meristem genes *WUSCHEL* and *ZWILLE*. Plant J. **10:** 967–979.

Fletcher, J. C., U. Brand, M. P. Running, R. Simon and E. M. Meyerowitz, 1999 Signaling of cell fate decisions by CLAVATA3 in Arabidopsis meristems. Science **183:** 1911–1914.

Jeong, S., A. E. Trotochaud and S. E. Clark, 1999 The Arabidopsis *CLAVATA2* gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. Plant Cell **11:** 1925–1933.

Kanrar, S., O. Onguka and H. M. Smith, 2006 Arabidopsis inflorescence architecture requires the activities of KNOX-BELL homeodomain heterodimers. Planta **224:** 2263–2273.

Kayes, J. M., and S. E. Clark, 1998 *CLAVATA2*, a regulator of meristem and organ development in Arabidopsis. Development **125:** 3843–3851.

Kerstetter, R. A., D. Laudencia-Chingcuanco, L. G. Smith and S. Hake, 1997 Loss of function mutations in the maize homeobox gene, *knotted1*, are defective in shoot meristem maintenance. Development **124:** 3045–3054.

Lenhard, M., G. Jurgens and T. Laux, 2002 The WUSHEL and SHOOT MERISTEMLESS genes fulfill complementary roles in Arabidopsis shoot meristem regulation. Development **129:** 3195–3206.

Leyser, H. M. O., and I. J. Furner, 1992 Characterisation of three shoot apical meristem mutants of *Arabidopsis thaliana*. Development **116:** 397–403.

Long, J. A., E. I. Moan, J. I. Medford and M. K. Barton, 1996 A member of the KNOTTED class of homeodomain proteins encoded by the *SHOOTMERISTEMLESS* gene of Arabidopsis. Nature **379:** 66–69.

Ogawa, M., H. Shinohara, Y. Sakagami and Y. Matsubayashi, 2008 Arabidopsis CLV3 peptide directly binds CLV1 ectodomain. Science **319:** 294.

Smith, L. G., B. Greene, B. Veit and S. Hake, 1992 A dominant mutation in the maize homeobox gene, *Knotted-1*, causes its ectopic expression in leaf cells with altered fates. Development **116:** 21–30.

Suzaki, T., M. Sato, M. Ashikari, M. Miyoshi, Y. Nagato *et al.*, 2004 The gene FLORAL ORGAN NUMBER1 regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to Arabidopsis CLAVATA1. Development **131:** 5649–5657.

Suzaki, T., T. Toriba, M. Fujimoto, N. Tsutsumi, H. Kitano *et al.*, 2006 Conservation and diversification of meristem maintenance mechanism in *Oryza sativa*: function of the FLORAL ORGAN NUMBER2 gene. Plant Cell Physiol. **47:** 1591–1602.

Suzaki, T., A. Yoshida and H. Hirano, 2008 Functional diversification of CLAVATA3-related CLE proteins in meristem maintenance in rice. Plant Cell **20:** 2049–2058.

Vollbrecht, E., L. Reiser and S. Hake, 2000 Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, *knotted1*. Development **127:** 3161–3172.

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