Development 127, 3161-3172 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV0304

Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, *knotted1*

Erik Vollbrecht[1,*], Leonore Reiser[1] and Sarah Hake[1,2,‡]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA
[2]Plant Gene Expression Center, 800 Buchanan St. Albany, CA 94710, USA
*Present address: Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA
‡Author for correspondence (e-mail: maizesh@nature.berkeley.edu)

Accepted 18 April; published on WWW 22 June 2000

SUMMARY

The *knotted1* (*kn1*) gene of maize is expressed in meristems and is absent from leaves, including the site of leaf initiation within the meristem. Recessive mutations of *kn1* have been described that limit the capacity to make branches and result in extra carpels. Dominant mutations suggest that *kn1* function plays a role in maintaining cells in an undifferentiated state. We took advantage of a *Ds*-induced dominant allele in order to screen for additional recessive alleles resulting from mobilization of the *Ds* element. Analysis of one such allele revealed a novel embryonic shoot phenotype in which the shoot initiated zero to few organs after the cotyledon was made, resulting in plants that arrested as seedlings. We refer to this phenotype as a limited shoot. The limited shoot phenotype reflected loss of *kn1* function, but its penetrance was background dependent. We examined meristem size and found that plants lacking *kn1* function had shorter meristems than non-mutant siblings. Furthermore, meristems of restrictive inbreds were significantly shorter than meristems of permissive inbreds, implying a correlation between meristem height and *kn1* gene function in the embryo. Analysis of limited shoot plants during embryogenesis indicated a role for *kn1* in shoot meristem maintenance. We discuss a model for *kn1* in maintenance of the morphogenetic zone of the shoot apical meristem.

Key words: *knotted1*, Maize, Meristem, Embryo, Homeobox gene

INTRODUCTION

The diversity of plant form relies on events that take place in meristems. Meristems are organized groups of self-renewing cells that produce the organs of the plant. The type of organs produced by a meristem depends on several factors, notably the ontogenetic and developmental context of a particular meristem. The vegetative shoot apical meristem (SAM) of flowering plants produces leaves and stem tissue. Axillary shoot meristems, derived from the SAM, arise in the axil of the leaf. These axillary meristems may reiterate all or part of the main shoot. Most flowering plants have a vegetative phase in which leaves are the predominant organs, followed by a reproductive phase in which the leaves may be highly reduced and flower production becomes dominant.

The shoot apical meristem initiates early in embryogenesis and in most species forms a number of leaves prior to seed dormancy. Cotyledons are the first leaves, but the inception of the SAM and origin of cotyledons has been interpreted in two contrasting ways. Comparative morphological analysis synthesizing information from a broad range of plants suggests that the SAM comprises the apical portion of the globular dicotyledonous embryo, and that cotyledons are the first products of the SAM (Kaplan and Cooke, 1997). Recent studies in *Arabidopsis*, on the other hand, have been interpreted to suggest that the SAM and cotyledons each form independently in the embryo (Barton, 1998). The portion of the shoot above the cotyledon(s), the epicotyl, is unequivocally formed by the activity of the SAM. In dicotyledonous plants, as the SAM enlarges prior to initiating the first leaf of the epicotyl, the meristem becomes prominent between the recently initiated cotyledons. In monocotyledonous plants, the enlarging SAM appears laterally oriented with respect to the single cotyledon (Kaplan, 1973). The cotyledon of maize, a monocot, is termed the scutellum (Randolph, 1936; VanLammeren, 1986). As the scutellum differentiates, the coleoptile is elaborated and surrounds the SAM. The coleoptile has a tubular shape and is considered by some to be the ensheathing base of the scutellum (Weatherwax, 1920; VanLammeren, 1986). The SAM initiates three to five more leaves, each leaf opposite from the previous one, before kernel maturation and the onset of dormancy. These foliage leaf primordia and the meristem constitute the young epicotylar shoot, which is protected by the coleoptile during germination.

The *knotted1* (*kn1*) homeobox gene (Vollbrecht et al., 1991) is expressed in all shoot meristems and not in lateral organs such as leaves or floral organs (Smith et al., 1992; Jackson et al., 1994). *kn1* expression is first detected in the embryo approximately 10 days after pollination (DAP) (Smith et al., 1995) in a region that corresponds to the site where the apical

meristem is first evident histologically (Randolph, 1936). Within the vegetative meristem, *kn1* mRNA disappears in the cells that will form the next leaf. This group of cells is referred to as a plastochron 0 leaf (P_0). Plastochron refers to the time interval between successive leaf initiation events and is also used to identify developing leaves by their positions relative to the apical meristem. A P_0 leaf is not evident as a bump, but a P_1 leaf is a lateral protrusion from the meristem.

The *kn1* gene was first defined by dominant mutations that affect leaf development (Bryan and Sass, 1941; Gelinas et al., 1969; Freeling and Hake, 1985). Most of the dominant *Kn1* alleles result from the insertion of non-autonomous transposable elements into an intron, including a *Dissociation2* (*Ds2*) element and *Mutator* elements. For many of these alleles, the presence of the autonomous element in *trans* enhances expression of the phenotype, presumably due to interference with silencer elements (Hake et al., 1989; Greene et al., 1994). These gain-of-function mutations induce cellular proliferations or knots to form on the leaf blade, and cause alterations in the proximal to distal axis of the leaf. Dominant *Kn1* mutations result from regulatory alterations that confer ectopic *kn1* expression in leaves without affecting the normal pattern of expression in the meristem (Smith et al., 1992). These data and overexpression studies (Sinha et al., 1993; Chuck et al., 1996) point to a role for *kn1* in maintaining cells in an undifferentiated state. Loss of function mutations of *kn1*, including null mutations, were reported to primarily affect meristem function in the inflorescence and have little effect on the function of the vegetative SAM (Kerstetter et al., 1997).

The propensity of *Ds* to transpose to linked sites provides an avenue for obtaining additional alleles once a *Ds* element has transposed into a gene (McClintock, 1947; Greenblatt, 1984; Moreno et al., 1992; Alleman and Kermicle, 1993). We reasoned that mobilization of *Ds2* from the third intron of *Kn1-2F11* could produce a loss-of-function allele. We used such a genetic screen to isolate a new, recessive allele. Some homozygous *kn1-2F11-E1* plants (hereafter referred to as *E1*) had a limited shoot phenotype, in which the apical meristem ceased organ initiation after producing the coleoptile or one to two leaves. The residual cells at the site of the former meristem suggest a role for *kn1* in the morphogenetic zone. Crosses to different inbreds revealed a strong background dependence for the limited shoot phenotype, which correlated with differences in meristem size, and explained why the phenotype had been previously missed.

MATERIALS AND METHODS

Molecular biology

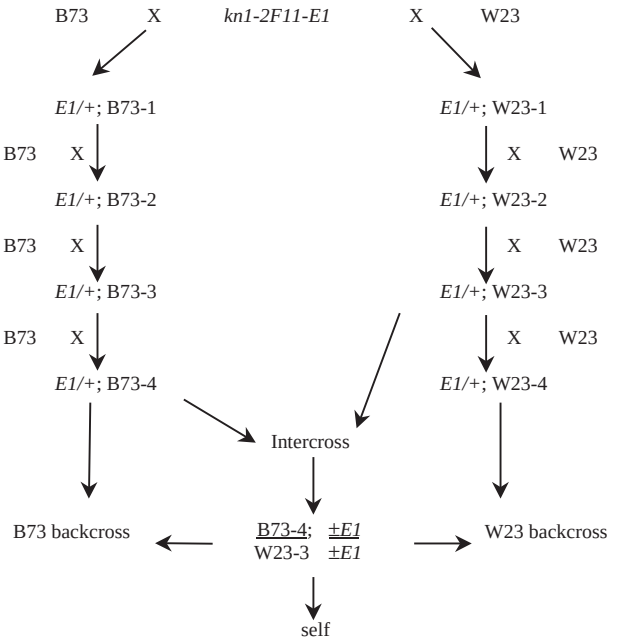
DNA gel blot analysis (Hake et al., 1989), RNA isolation and gel blot protocols (Smith et al., 1992) were as described. The *kn1-2F11-E1* allele (*E1*) was sequenced by PCR amplification of genomic DNA from homozygous *E1* seedlings. Multiple PCR products were sequenced in order to eliminate PCR artifacts. Sequencing reactions were performed according to the manufacturer's instructions (PE Biosystems, Foster City, CA). The *Ac* probe (pAc9) was a generous gift from N. Federoff (University of Pennsylvania, University Park, PA). The *kn1* cDNA probe was described previously (Vollbrecht et al., 1991).

Maize stocks and genetic crosses

Kn1-2F11 contains a *Ds2* at *kn1* (Hake et al., 1989). The *bz2-m::Ds2* allele was used as an *Ac* reporter (Nuffer, 1954). *Ac* stocks were kindly provided by several researchers: *Ac11*, K. Dawe (University of Georgia, Athens, GA); *tr-Ac11* is a transposed, unlinked copy of the *Ac11* element; *r-njm::Ac*, P. Becraft (Iowa State University, Ames, IA); *wx-m7::Ac*, *wx-m9::Ac* and *Ac-st*, P. Chomet (DeKalb Plant Genetics, Mystic, CT); *bz1-m2::Ac*, H. Dooner (Rutgers University, Piscataway, NJ); W22 containing *P-vv::Ac*, *r-njm::Ac* and *Ac-st*, J. Kermicle (University of Wisconsin, Madison, WI); *Ac2* and *Ac2Ac2* (two closely linked copies), Maize Genetics Cooperation Stock Center.

To generate *kn1-2F11-E1*, plants homozygous for *Kn1-2F11* and hemizygous for *Ac11* were pollinated by plants that were homozygous for a normal allele of *kn1* (*kn1+*) and *Ac-st*. Among 15,615 M_1 plants, 593 individuals without the knotted phenotype were self-pollinated. 575 set M_2 seed, and 503 M_2 families were sown in a greenhouse and the phenotypes of the seedlings scored. 63 M_2 families still segregated a knotted phenotype, implying the reversion frequency of *Kn1-2F11* was 3.3%. New phenotypes segregated in many of the remaining 440 revertant families, but only one family segregated the limited shoot phenotype.

The following pedigree diagrams show how introgression, intercross and backcross genotypes (see Table 1) were generated, using the inbred lines B73 (left column) and W23 (right column) as examples. We tracked different *kn1* alleles in introgression pedigrees by *kn1* allele-specific PCR and/or linkage to *adh1* alleles (see below). After introgression, introgressed lines were intercrossed. Intercross genotypes (e.g., B73-4/W23-3) were subsequently self-pollinated or backcrossed to one of the introgression lines (bottom of diagram).



Microscopy and image processing

For embryo analysis, ear sections were removed after pollination, placed in fixative overnight (FAA: 3.7% formalin, 5% acetic acid, 50% ethanol) and then rinsed and stored in 70% ethanol. Dissected, fixed embryos were dehydrated in a graded series of ethanol and optically cleared by passing through a graded series from ethanol into methyl salicylate. Cleared embryos, mounted in methyl salicylate, were observed on a Nikon Axiophot using differential interference contrast (DIC) optics. In some cases, optically cleared embryos were passed back into absolute ethanol, embedded in JB4 plus methacrylate

embedding medium (Polysciences, Warrington, PA), sectioned and stained with toluidine blue (Sigma, St. Louis, Mo). Digital images were captured and measurements taken using NIH Image software (http://rsb.info.nih.gov/nih-image).

Alcohol dehydrogenase assays

kn1 is closely linked (<1 cM) to *alcohol dehydrogenase 1* (*adh1*). Assays for electrophoretic variants of functional *adh1* alleles were performed as described previously (Freeling and Schwartz, 1973). In experiments described here, *Kn1-2F11* was linked to a stable null *adh1* allele, which we derived from the *Ds2*-induced *adh1-2F11* allele (Doring et al., 1984) by imprecise transposon excision. Embryos that were negative for *adh1* activity were considered to be homozygous for the *E1* allele.

RESULTS

Screening for loss of the dominant *Kn1-2F11* phenotype

In the absence of *Ac*, the knotted phenotype of *Kn1-2F11* is barely discernible (Hake et al., 1989). In order to use the *Ds2* element at *Kn1-2F11* to generate recessive alleles, we sought to first increase the penetrance and expressivity of the phenotype. We introgressed different *Ac* elements into the same *Kn1-2F11* line and evaluated the knotted phenotype (Fig. 1C). In each introgression line, plants lacking *Ac* were normal or expressed only a subtle knotted phenotype, while siblings containing an *Ac* element expressed the knotted phenotype more frequently, and often more severely. Among the many *Ac* elements tested in *trans* with *Kn1-2F11*, only McClintock's *Ac-st* (Brutnell et al., 1997) conferred strong expressivity and complete penetrance of the knotted phenotype, regardless of genetic background (Fig. 1A). Modification of *Ac-st* by EMS mutagenesis (P. Chomet, unpublished) eliminated its unique quality to enhance *Kn1-2F11* in *trans*, demonstrating that the interaction was with *Ac-st* (data not shown).

RNA gel blots revealed that the increased severity of the *Kn1-2F11* phenotype in the presence of *Ac-st* correlated with an increase in *kn1* transcript in leaves (Fig. 1B). Higher levels of *kn1* transcript were detected in leaves containing *Ac-st* than in leaves containing standard *Ac*. No increase was apparent in shoot apices. Hybridization with an *Ac* probe revealed the 3.5 kb, transposase-encoding *Ac* transcript in plants containing either standard *Ac* or *Ac-st*. *Ac-st* was expressed at higher levels than standard *Ac* in both young leaves and shoot apices (Fig. 1B). These results demonstrated that *kn1* was ectopically expressed in leaves only when *Ac* was present in *trans*, and that *Ac-st* was expressed at higher levels than *Ac* and resulted in correspondingly higher ectopic *kn1* expression levels.

The enhanced expressivity conferred by *Ac-st* made it feasible to implement a crossing scheme that took advantage

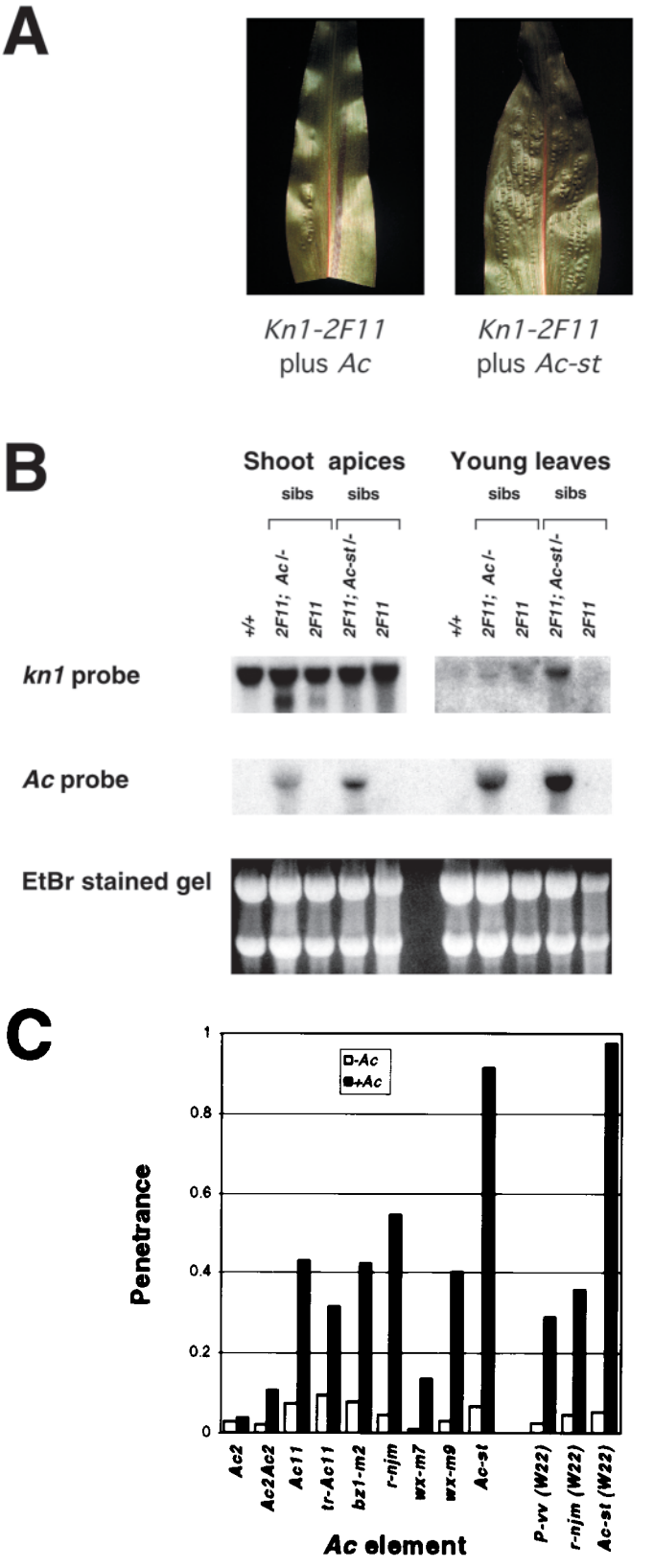


Fig. 1. Penetrance and expressivity of the *Kn1-2F11* phenotype is increased by *Ac-st*. (A) A leaf from a *Kn1-2F11* plant containing one copy of *Ac* (left) has fewer knots than a *Kn1-2F11* leaf containing one *Ac-st* (right). (B) Upper panel, RNA gel blot analysis of plants with the following genotypes: normal (+/+), *Kn1-2F11* lacking *Ac* (*2F11*), *Kn1-2F11* with *Ac* (*2F11; Ac/-*), *Kn1-2F11* with *Ac-st* (*2F11; Ac-st/-*). Middle panel, the same blot probed with an *Ac*-specific probe indicating correlative differences in *Ac* transcript levels. Bottom panel shows equal loading of RNA samples. (C) Several *Ac* elements were individually converged for several generations into an inbred, homozygous *Kn1-2F11* line. Penetrance of the *Kn1-2F11* phenotype is increased in the presence of *Ac* and is highest when *Ac-st* is present. A penetrance of 1 (100%) means that 100% of the plants had knots on the leaves.

of the ability of standard *Ac* to mobilize *Ds2* (see Materials and Methods). One M_2 family showed a new phenotype with genetic linkage to the *kn1* locus. In this family, less than 25% of the seedlings expressed a limited shoot phenotype that showed tight linkage to *kn1* in subsequent generations. The *kn1* allele on this chromosome was dubbed *kn1-2F11-E1* (*E1*). DNA sequencing of *E1* and its progenitor *Kn1-2F11* revealed two sequence polymorphisms. *E1* contained a three base pair deletion in the third intron, at the site of the former *Ds2* insertion, and an eight base pair insertion, in the form of an imperfect direct repeat, in the first exon (Fig. 2A). The three base pair deletion in intron three and the eight base pair insertion in exon one were both consistent with a typical *Ac/Ds* family transposon footprint (Scott et al., 1996).

The eight base pair insertion introduced a coding frameshift into the first exon. The predicted amino acid sequence encoded by the *E1* allele diverged from the progenitor sequence after 23 amino acids, placing the first of several in-frame stop codons after amino acid 42 (Fig. 2B). *kn1* RNA levels were greatly reduced in meristem-enriched tissues from homozygous *E1* individuals, while *kn1* RNA was relatively abundant in tissue from heterozygous *E1*/+ siblings and the *Kn1-2F11* progenitor (Fig. 2C). Based on these results we concluded that *E1* was likely a null mutation of the *kn1* locus.

The *kn1* limited shoot phenotype

Plants homozygous for *E1* could be categorized into two distinct classes. Plants in the first class formed a normal complement of vegetative nodes and flowered in synchrony with their non-mutant siblings. Their primary defect was sparse male (tassel) and female (ear) inflorescences. Ears were small and had reduced seed set and altered kernel orientation (Fig. 3A). Pistillate flowers of the ear had extra carpels (compare Fig. 3B,C). Tassels made fewer long branches and spikelets (data not shown). Occasional individuals (approx. 7% of adult mutant plants) contained a fused, extra leaf, which only occurred in the nodes between the ear shoot and tassel. These phenotypes were all similar to those described previously for recessive mutations induced by EMS (Kerstetter et al., 1997). We confirmed that these defects were due to loss of *kn1* by crosses to the EMS-induced, loss-of-function allele, *kn1-L4* (*L4*), which failed to complement the inflorescence phenotypes. Thus, *E1* was a loss of function allele of *kn1*.

E1 homozygotes in the second class had limited shoots and did not develop to reproductive maturity. These plants always contained a normal coleoptile, but typically lacked visible elements of the epicotylar shoot such as leaves (Fig. 3D). An average of 10% of the limited shoot plants (ranging from 0% to 100% in different families) contained a variably rudimentary epicotyl. The most frequent phenotype for the rudimentary epicotyl was a single leaf (Fig. 3D, center). Single leaves emerged coincident with the first leaves of normal siblings, but were slightly narrower and more upright. Less frequently, we observed plants in which two or three apparently normal leaves emerged before the shoot ceased leaf production. In only a few cases, a short symmetrical leaf emerged from the coleoptile. Limited shoot seedlings were maintained in the greenhouse for several weeks, during which time they initiated no additional structures before they died. *E1* homozygotes that initiated at least four leaves, however, always grew to reproductive maturity and produced sparse tassels and ears.

In the progeny of crosses between *E1* and the recessive alleles *L4* and *R1*, which confer an early splice site defect and a stop codon in the C-terminal homeodomain, respectively (Kerstetter et al., 1997), low frequencies of the limited shoot phenotype were also observed (Table 1, complementation). To

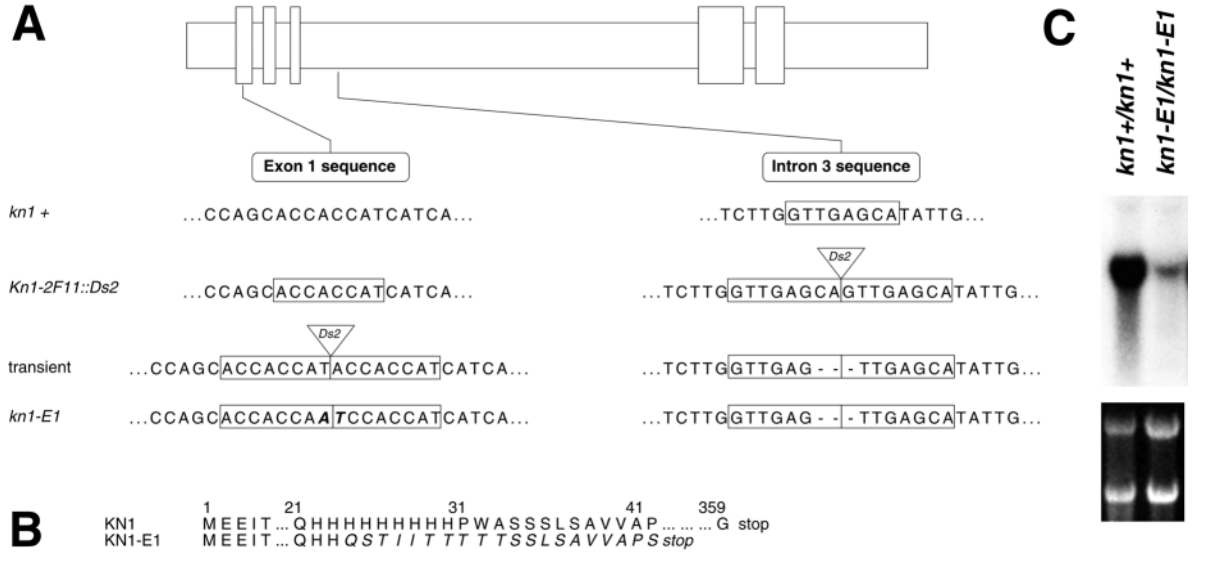


Fig. 2. Isolation of the *kn1-2F11-E1* allele. (A) Structure of the *kn1* locus and a hypothesized mechanism for creation of the *E1* allele. We postulate the transient existence of an intermediate that contained a *Ds2* transposon insertion in exon one, generating an eight base pair direct repeat due to target site duplication. Imprecise transposon excision followed, resulting in an imperfect eight base pair direct repeat with single nucleotide changes flanking the former insertion site. Intron sequences of the normal, *Kn1-2F11*, predicted intermediate and *E1* allele are shown with *Ds* target sites boxed. (B) Comparison of the KN1 protein sequence and predicted KN1-E1 protein. The E1 protein contains novel amino acid sequence (italics) after 23 amino acids and terminates after 42 amino acids. (C) RNA gel blot. Total RNA from normal (*kn1+/kn1+*) and *E1* (*kn1-E1/kn1-E1*) shoot apices was probed with the *kn1* cDNA. Lower panel, EtBr-stained gel.

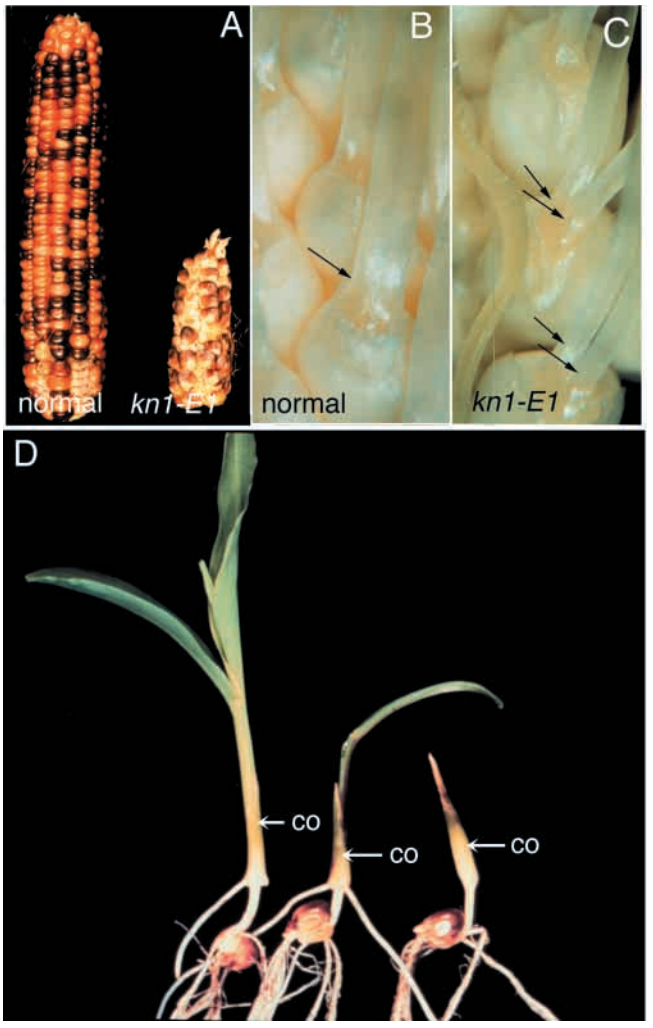


Fig. 3. *E1* mutant phenotypes. (A) Ears of *E1* (right) were smaller and had fewer kernels than normal sibling ears (left). (B) Normal flower of an unpollinated ear showing single silks. (C) *E1* mutant flowers containing two silks (arrow) per flower. (D) Two week old, sibling seedlings from a line expressing the limited shoot phenotype. The normal plant on the left has produced three visible leaves. *E1* plants make a coleoptile (co) and a single leaf (center) or only a coleoptile (right) before ceasing development.

determine whether or not the limited shoot phenotype was specific to *E1* and/or allelic combinations involving *E1*, we examined independent populations segregating *L4* and *R1*. A limited shoot phenotype also showed genetic linkage to those alleles (Table 1, other alleles). Penetrance of the limited shoot phenotype in the *L4* and *R1* populations was relatively low due to genetic background effects, as discussed below, which explained why the phenotype had been previously overlooked. Thus, genetic tests confirmed that the epicotylar shoot defect was due to a loss of *kn1* function.

An early requirement for *kn1* to maintain the shoot apical meristem

Developmental progression of the maize embryo and seedling has been well characterized (Randolph, 1936; Kiesselbach, 1949; VanLammeren, 1986) and the distinct morphological stages identified by Randolph provide a framework for understanding the developmental basis of the limited shoot phenotype. Here, we assign numerals to the stages that Randolph defined by days after pollination (DAP) (Fig. 4), because the rate of development of the embryo depends on genetic background and growth conditions. After fertilization, the zygote divides into an apical cell that gives rise to the proembryo and a larger, basal cell that produces the suspensor. Stage 1 encompasses the development of the globular proembryo, characterized by non-stereotypical cell divisions (Fig. 4). Near the end of stage 1 the shoot apical meristem is evident as a region of densely cytoplasmic cells near the periphery of the embryo apex. These cells coincide with the region of earliest detectable expression of *kn1* gene product (Smith et al., 1995). During stage 2, the root initiates from the base of the compact shoot and the SAM and root apical meristem (RAM) resolve as distinct foci of tissue growth. At stage 3, the coleoptile initiates as a ridge that protrudes from above the apical meristem, and spreads to form a collar around the mound of the SAM. Phyllotaxy in maize is distichous; hence, the first leaf of the epicotyl initiates opposite to the point of origin of the coleoptile, identifying stage 4. Between stages 4 and 5, additional leaves are initiated alternately while the tubular coleoptile continues to expand. At stage 5, kernel maturity, the coleoptile envelops the epicotylar shoot, which contains from three to five leaves surrounding the raised dome of the apical meristem.

To elucidate the developmental basis of the limited shoot phenotype, we first established a scheme to take the variable penetrance into account when examining families that segregated the *E1* mutation. *E1*/+ heterozygotes were self-pollinated to produce ears on which we expected at most 25% limited shoot individuals. At specific time points we cut off the distal half of pollinated ears, and dissected the embryos to

Table 1. Penetrance of the limited shoot phenotype in genetic crosses

Cross type	Female × male	*n*	Penetrance
Complementation	*kn1-L4*(B73) × *kn1-E1* (mixed)	1	3
Other alleles	*kn1-L4* (B73/W32) self	1	3
	kn1-R1 (mixed) self	3	4,14,65
Intercross	*kn1-E1*(4xB73) × *kn1-E1*(3xW23)	2	0,8
Inter→self	*kn1-E1*(3xB73/3xW23) × self	5	0,8,10,11,13
Inter→backcross	*kn1-E1*(4xB73) × *kn1-E1*(3xB73/3xW23)	4	0,0,0,8
	kn1-E1(3xB73/3xW23) × *kn1-E1*(4xB73)	8	0,1,2,3,4,5,6,6
	kn1-E1(3xB73/3xW23) × *kn1-E1*(3xW23)	6	47,52,53,53,55,58

The first column shows the type of cross that was scored. Inter→self and inter→backcross refer to intercross individuals that were subsequently selfed or backcrossed to one of the parental lines, respectively. Parents are shown in the second column; the female precedes the male. *n* refers to the number of families scored for that particular type of cross. The last column gives the penetrance values, as percentages, for each individual family that was examined.

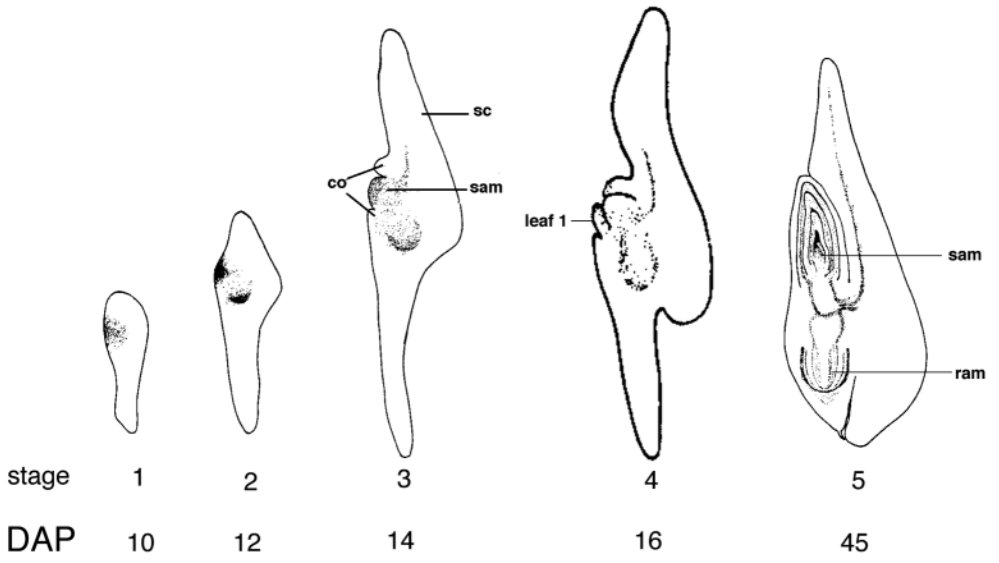


Fig. 4. Schematic illustration of stages of maize embryogenesis. Distinct morphological stages, based on Randolph's (1936) DAP-based stages, are assigned numerals. Stage 5 is not to scale. DAP, days after pollination; co, coleoptile; sam, shoot apical meristem; sc, scutellum; ram, root apical meristem.

process for light microscopy. The lower half of the ear matured on the plant. Mature kernels were then sown in soil to assess penetrance *post facto* by counting the limited shoot individuals. A penetrance value of 0% indicated no limited shoot individuals were found, whereas a penetrance of 100% indicated that one quarter of the individuals had a limited shoot.

We examined optically cleared whole-mount embryos from self-pollinated *E1*/+ ears in which embryos were in stages 3 and 4 of development. Limited shoot plants always contained morphologically normal scutella at the dry kernel stage (stage 5); hence, our analysis targeted SAM function at embryo stages following scutellum initiation. In normal stage 3 embryos the recently initiated coleoptile appeared as a torus, attached to the scutellum along its base and surrounding a smooth, clearly visible meristematic dome (Fig. 5A). By stage 4 the meristem was flanked by an initiating leaf primordium (Fig. 5C). In some sibling stage 3 embryos, the coleoptile sometimes appeared torus-shaped but empty (Fig. 5B). More frequently, we saw the inner coleoptile surfaces appressed along their length (Fig. 5D). Thus, in mutant embryos, the region of coleoptile insertion was similar to that of normal coleoptiles which surrounds the perimeter of the meristem, but we did not detect a SAM in the region circumscribed by the coleoptile base. These SAM defects were observed in five out of 37 embryos from EV97-582-5, which had a penetrance of 52%, and in 10 out of 61 embryos from EV97-582-12, which had a penetrance of 49%. By contrast, embryos from non-mutant ears exhibited only normal morphology as did a total of 83 embryos from the self-pollinated ears. Thus, the frequency of stage 3 and 4 embryos lacking a morphologically distinct SAM correlated with the penetrance of the limited shoot phenotype in each family. We concluded that these meristematic defects were likely to be responsible for the limited shoot phenotype.

To further investigate whether or not defective stage 3 and 4 embryos lacked a SAM, selected optically cleared embryos were rehydrated, embedded and sectioned for histological analysis. In longitudinal section, normal embryos show a meristematic dome, consisting of small, densely staining cells, which is canted relative to the axis of the scutellum. The meristem is surrounded by the coleoptile and, in stage 4 embryos, the first leaf primordium of the epicotyl is present opposite the scutellum (Fig. 5E). By contrast, in mutant embryos, a flattened surface, oriented along the same axis as the SAM in normal embryos (arrows, Fig. 5E-F), stretched across the ring of insertion of the coleoptile (Fig. 5F). Cells within this flattened region were large, vacuolated and histologically similar to surrounding, differentiated cells. Thus, stage 3 and 4 embryos of *kn1* mutants lacked both morphological and histological features characteristic of normal SAMs, suggesting these plants lacked a functioning SAM.

To elucidate further ontogeny of limited shoot embryos and the erstwhile SAM region, we examined shoot apices from plants at later developmental stages, utilizing the close linkage of *kn1* and *adh1* to identify *E1* homozygotes (see Methods). When plants of (*E1*/+) or (+/+) genotype had initiated two leaves of the epicotylar shoot (Fig. 5G), limited shoot individuals had a slightly convex surface between the separated insertion of the coleoptile base (Fig. 5H). Subsequently, stage 5 normal individuals contained an elongated SAM with several flanking leaves surrounded by the coleoptile (Fig. 5I). Homozygous *E1* individuals, on the other hand, frequently lacked a recognizable SAM. Instead, a flattened area or sometimes slightly raised bump occupied the central position within the bounds of the normally expanded coleoptile (Fig. 5J). Occasionally, stage 5 limited shoot plants contained an epicotyl with a leaf opposite the scutellum (Fig. 5K). The extent of development of the single leaf indicated that it had been initiated at a similar time as the first epicotylar leaf of normal siblings. The former SAM region of 1-leaf individuals was morphologically similar to that of individuals lacking an epicotyl entirely (compare Fig. 5J,K), indicating similar meristem dysfunction in the two cases. In general, tissue at the shoot apex retained the tunica-corpus layering characteristic of functioning SAMs (Fig. 5F,H,J,K).

In summary, embryos destined to produce limited shoots always contained a scutellum and properly initiated a coleoptile as a distinct ring encircling the presumptive SAM, consistent with the presence of some component of SAM identity at stage 3. However, most stage 3 embryos lacked morphological or histological evidence of an SAM. Through ontogeny, these plants did not maintain a functional meristem, or did so only briefly, such that zero to a few additional leaves were initiated, and the ring of coleoptile insertion expanded

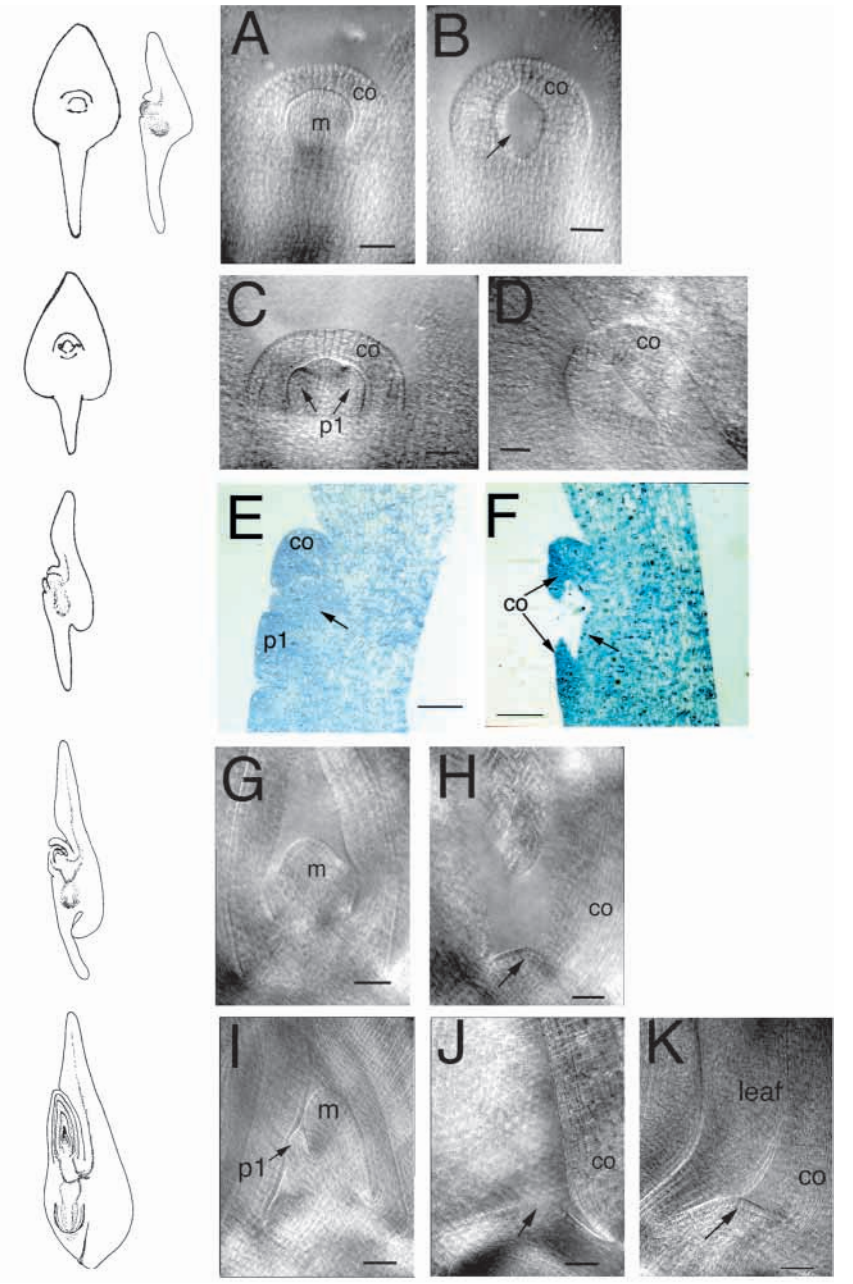


Fig. 5. SAM development in *E1* mutant embryos. Illustrations on the left depict stages of embryo development at which the specimens were obtained. Optically cleared embryos were viewed in either the frontal or longitudinal plane of the embryo using differential interference contrast (DIC) microscopy. (A) Normal stage 3 embryo viewed in frontal plane showing the coleoptile (co) surrounding the bump of the meristem (m). (B) Mutant stage 3 embryo, from same ear as A, with an empty space where the meristem should be (arrow). (C) Normal embryo at stage 4 viewed in frontal plane. The first leaf primordium (p1) has emerged from the flank of the meristem and is inserted 180 degrees from the scutellum. (D) Cleared mutant embryo from the same ear as that in C. The inner surfaces of the coleoptile are appressed, reflecting lack of epicotyl growth. (E) Longitudinal plastic section through normal stage 4 embryo showing the densely cytoplasmic dome of the meristem (arrow) and P1 leaf primordium. The direction of the arrow parallels the embryo root-shoot axis, canted relative to the axis of the scutellum. (F) Longitudinal section through a mutant, stage 4 embryo. The arrow, oriented along the shoot axis, indicates the region that is normally occupied by the meristem. Cells within the region circumscribed by the coleoptile are vacuolated. (G) Normal embryo between stages 4/5. The first leaf of the epicotyl has overgrown the apex and the second leaf has been initiated opposite to the point of insertion of the first leaf. (H) Mutant embryo from the same ear. The arrow points to a slightly raised dome located at the apex of the shoot, within the slightly separated inner surfaces of the coleoptile. There is no epicotyl or SAM. (I) Stage 5 normal embryo that has initiated 5 leaves prior to undergoing dormancy. The dome of the meristem is clearly visible adjacent to the youngest leaf primordium (p1). (J,K) Mutant embryos at stage 5. In J, the arrow points to a slightly raised dome located at the base of and between the inner margins of the coleoptile. The embryo in K has made a single leaf that is inserted opposite to the scutellum. A bump (similar to that seen in J) is indicated by an arrow and lies between the single leaf to the left and the inner margin of the coleoptile on the right. Scale bars, 50 μm.

much less than in a plant with a functioning SAM. Failure to perpetuate a functional meristem had no observed effect on growth of previously initiated leaves. After SAM failure a flattened tissue of vacuolated cells remained at the shoot apex, implying that the SAM was not consumed in the production of organs.

Genetic basis of variable penetrance of the *kn1* limited shoot phenotype

We reasoned that variable penetrance of the limited shoot phenotype might be due to segregation of factors that modified phenotype expression. To investigate this possibility we introgressed *E1* into different inbred backgrounds. Introgression into the W23 background gradually increased and stabilized the penetrance of the limited shoot phenotype. The penetrance ranged from 12% to 67% after one backcross, 31% to 72% after two backcrosses, 58% to 81% after three backcrosses and 77% to 100% after four backcrosses (Fig. 6A). Introgression of *E1* into B73 had an opposite and more abrupt effect. The penetrance was near zero after a single backcross and stayed below 5% after each of four backcrosses (Fig. 6B). After three backcrosses into Mo17 the phenotype showed a range of penetrance from 5% to 26%, whereas after three backcrosses into W22 it showed a penetrance of 46% (Fig. 6B). This distribution of penetrance led us to classify inbreds as being relatively permissive (low penetrance, like B73) or restrictive (high penetrance, like W23). The phenotype of limited shoot individuals was similar in all inbred backgrounds examined. Thus, the penetrance, and not the expressivity, of the phenotype was specifically modified in different inbred backgrounds. Each introgression series approached its characteristic penetrance after only a few backcrosses,

consistent with the presence of discrete modifying loci in each inbred.

Because penetrance of the limited shoot phenotype varied between inbred lines, we tested for dominance relationships (see Methods for crossing schemes). In the F_1 of a cross between *E1*/+ heterozygotes, the permissive modifying activity of the B73 background was dominant to the restrictive backgrounds W23 (Table 1, intercross) and W22 (data not shown), regardless of the B73 parent of origin. Similarly, Mo17 was a dominant, permissive background relative to the restrictive W23 and W22 backgrounds (data not shown).

Additional crosses were then performed with F_1 individuals that arose from crosses between *E1*/+ in B73 and W23 backgrounds. When the B73/W23 F_1 was self-pollinated, we observed low penetrance values (0-13%) in the F_2 (Table 1, Inter→self). For F_2 derived from crosses back to *E1*/+ in the B73 background, we observed similarly low penetrance values (0-8%, Table 1, Inter→backcross). These results affirmed the dominance of permissive B73 modifiers. F_2 derived from crosses back to *E1*/+ in the W23 background, however, showed penetrance values of 47-58% (Table 1, Inter→backcross). In this last cross, half of the progeny would be expected to be homozygous for the W23 allele of a modifying locus and the other half trans-heterozygous for the W23 and B73 alleles. If the restrictive, W23 allele of the modifier were recessive and unlinked, then 50% of the *E1* homozygotes would be expected to show the limited shoot phenotype, as was observed. The data were consistent with the segregation of a single, major modifying locus that was present in a recessive state in W23 and a dominant state in B73.

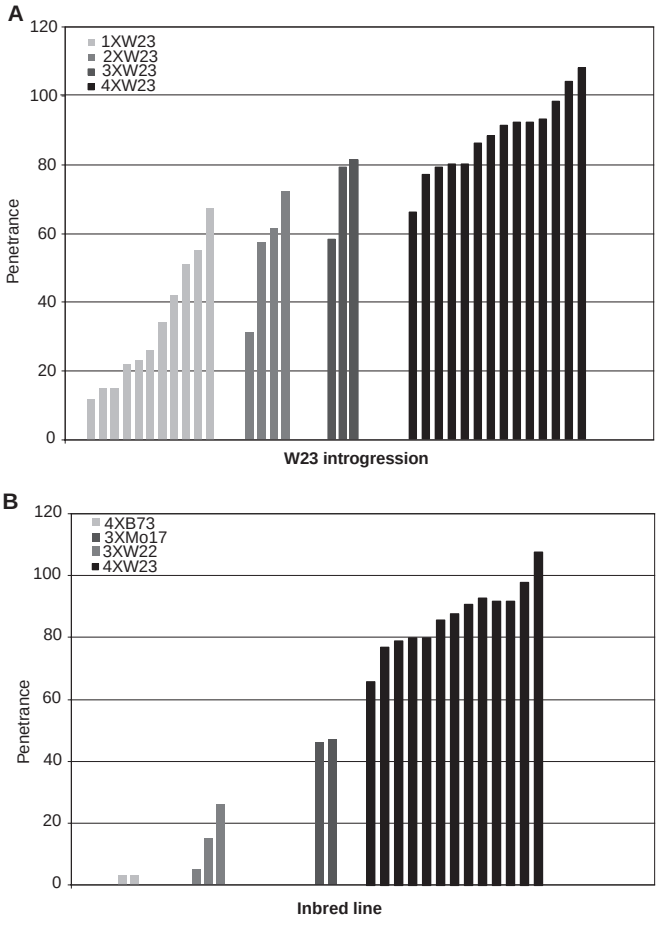


Fig. 6. Effects of background on penetrance of the limited shoot phenotype. Each bar indicates the penetrance of an individual family. Penetrance was calculated as described in the text.
(A) Penetrance of different families after successive backcrosses into W23. (B) Introgression of *E1* into four standard inbred lines (B73, Mo17, W22 and W23). Characteristic inbred effects formed a distribution from strongly restrictive (W23) to strongly permissive (B73) for the limited shoot phenotype.

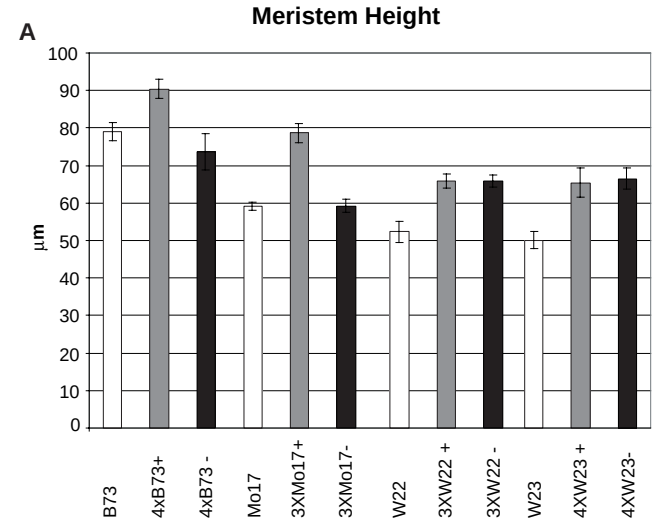
Meristem size is inbred dependent and correlates with penetrance of the limited shoot phenotype

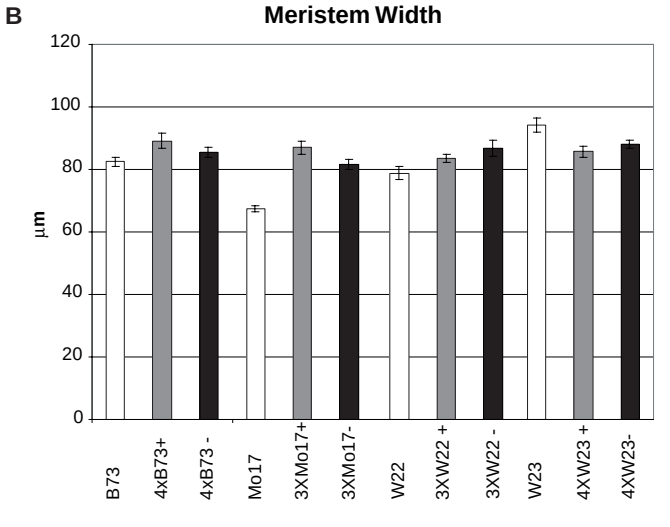
One explanation for the inbred dependence of the limited shoot phenotype was shoot meristem size or geometry differences between inbred lines. We tested this hypothesis by comparing SAM dimensions in embryos from dry kernels (i.e., stage 5, Fig. 4) of B73, W23, W22 and Mo17 inbred lines. For each inbred line examined, the epicotyl contained five leaves, including the most recently initiated primordium (P_1). Thus, embryos from different lines were examined at ontogenetically equivalent stages. Maximal width measurements were taken across the base of the meristem just above the point of most recent leaf insertion. Maximal height was determined by measuring the orthogonal distance from the line of maximal width to the summit of the meristem. We defined aspect ratio of the SAM as the ratio of maximal height to maximal width. Interestingly, relative meristem heights (Fig. 7A, white bars) correlated with relative penetrance of the limited shoot phenotype (Fig. 6B). The B73 inbred, which conferred the lowest penetrance, had the tallest SAMs (highest aspect ratios). In contrast, the restrictive W23 and W22 inbred lines had the shortest (lowest aspect ratios). Mo17 gave intermediate penetrance values and had meristems of intermediate height. Meristem width varied less between inbreds and did not correlate with different penetrance (Fig. 7B, white bars). These data illustrated that SAM geometry varied between inbred lines.

We tested for a relationship between *E1* and shoot meristem geometry by measuring and comparing meristems of homozygous mutants and normal siblings from B73, Mo17, W23 and W22 introgression lines (Fig. 4, stage 5). When tabulating SAM measurements of homozygous *E1* individuals, we only considered SAMs that had developed beyond the 3-leaf stage, and which therefore contained a functioning SAM that was destined to form a complete epicotylar shoot. Mutants and normal siblings contained the same number of leaves as the corresponding inbred, indicating the genotypes contained ontogenetically and developmentally equivalent SAMs. Normal individuals of the four introgression lines displayed the same distribution of relative meristem heights as the inbreds themselves (Fig. 7A, gray columns; compare white columns). Meristem width, on the other hand, differed little between the different introgression lines (Fig. 7B, gray columns), or between mutant and non-mutant classes within lines (Fig. 7B, compare gray and black columns).

SAMs from homozygous *E1* mutants and non-mutant siblings differed in height only if the introgression line was relatively permissive for the limited shoot phenotype. In the permissive B73 and Mo17 lines, meristems of mutants were clearly shorter than meristems of normal sibs (Fig. 7A, gray

versus black columns). By contrast, when *E1* embryos were able to maintain vegetative SAMs in restrictive (W22 and W23) backgrounds, their SAMs were similar in height to those of normal siblings. SAMs of these *E1* and normal embryos were still shorter than the SAMs made by either B73 genotype (Fig. 7A,C). These data demonstrated that lack of *kn1* activity can change meristem geometry, principally by reducing meristem height.





C

Aspect Ratios (Height/Width)

	inbred	*+kn1*	*-kn1*
B73	0.96	1.01	0.87
Mo17	0.87	0.91	0.73
W22	0.66	0.80	0.76
W23	0.53	0.76	0.75

Fig. 7. Meristem geometry in inbred lines and introgressed families segregating *kn1-E1* mutants. Dry kernels were imbibed overnight, and dissected embryos were fixed, cleared and viewed in longitudinal section using Nomarski optics. (A) Height of meristems in stage 5 embryos of B73, Mo17, W22 and W23 inbred lines (white bars), and of normal (+, gray bars) and *kn1* mutant (–, black bars) siblings after B73, Mo17, W22 and W23 introgression. (B) Corresponding width of meristems. (C) Aspect ratios, defined as the ratio of meristem height to width, for inbred lines (first column), and for normal (+*kn1*) and mutant (–*kn1*) siblings in introgression lines. Mutant embryos that did not have meristems are not included. Error bars indicate standard error.

DISCUSSION

We have isolated a new null allele of *kn1*, *kn1-2F11-E1* (*E1*), by screening for revertants of the *Ds*-induced, dominant *Kn1-2F11* allele. Analysis of *E1* mutants revealed a limited shoot phenotype, shown to be a conditional phenotype due to *kn1* loss of function, in which the cotyledon was unaffected and the epicotyl failed to develop. Developmental manifestation of the limited shoot defect suggested a failure to maintain the morphogenetic zone of the SAM. This phenotype showed variable penetrance in different inbred backgrounds, which reflected the action of relatively few modifier loci and correlated with different meristem geometries associated with the inbred lines.

kn1 is required for SAM function in the embryo

Shoot formation requires two conceptually, but not necessarily mechanistically, separable processes. Early in shoot ontogeny, a plant must establish a shoot apical meristem. Once established, SAM function must be maintained. *kn1* limited shoot plants occasionally initiate one to three epicotylar leaves that indicate a normal but transiently functioning SAM. Thus, these plants fail to maintain an already established SAM. Notably, the slightly arched surface of vacuolated cells that occupies the shoot apex of limited shoot plants with an abbreviated epicotyl is also seen at the shoot apex of plants that form only a coleoptile. The similarity of these terminal SAM phenotypes argues that the same SAM dysfunction occurs in both cases. Hence, the data unequivocally illustrate a crucial requirement for *kn1* to maintain SAM function, apart from any role *kn1* may play in establishing the SAM.

What role, if any, does *kn1* play in establishing the SAM during embryogenesis? The answer depends on how embryological events leading to the formation of the SAM and cotyledon are interpreted (Goldberg et al., 1994; Kaplan and Cooke, 1997). In one interpretation, the maize SAM exists at the globular embryo stage and initiates both the cotyledon and epicotyl. Considering the *kn1* phenotype in this context, the normal cotyledon initiation implies that SAM establishment is independent of *kn1* function. Correspondingly, *kn1* expression is not detected when the meristem is first specified, in the apex of the globular embryo. Rather, *kn1* is first detected just prior to coleoptile initiation (Smith et al., 1995), after which point the SAM, evident in section as only a few densely cytoplasmic cells at the embryo periphery, must increase greatly in size in order to initiate the first epicotylar leaf. Thus, in this scenario *kn1* is initially detected just before meristem maintenance functions first become critical. In limited shoot plants, the SAM ceases to maintain meristem function at this stage. Thus, in this context *kn1* would be required to maintain meristem function, but not to form the SAM in the embryo. Loss of an activity required to form the SAM might be expected to result in a globular embryo arrest phenotype rather than the limited

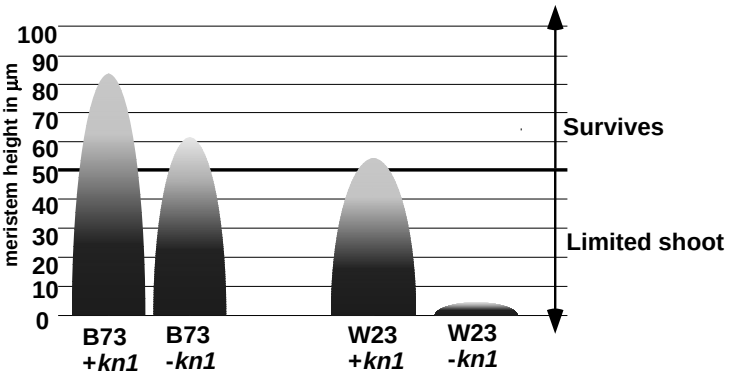


Fig. 8. Model for the effect of *kn1* on meristem size and SAM maintenance in different inbred lines. Meristems of permissive (e.g. B73) backgrounds are taller than meristems of restrictive (e.g. W23) backgrounds, due to the activity of a modifier in permissive lines. A *kn1* mutation makes meristems shorter. When meristem height falls below a critical threshold (50 µm), the meristem cannot be maintained. So, for B73 (a permissive line), in the absence of *kn1* activity meristem height is reduced, but is still above the critical size threshold. In the restrictive W23 line, loss of *kn1* function reduces meristems to below the critical threshold.

shoot phenotype that we have observed. In rice, genes related to *kn1* are appropriately expressed in the apical portion of the embryo to potentially serve as regulators of SAM formation (Sentoku et al., 1999).

An alternative view is that the cotyledon is initiated independently of the meristem. In this context, the *kn1* limited shoot phenotype would be interpreted as a complete lack of SAM function. Therefore, *kn1* would function during meristem initiation, which would be marked by the expression of *kn1* on the flank of the developing embryo. The normally placed coleoptile ring, always observed surrounding the defective SAM in limited shoot plants, would reflect the independence of cotyledon initiation from SAM activity. Furthermore, some process independent of *kn1* and SAM function, perhaps related to cotyledon formation, would specify spatial organization of the apical embryo. *kn1* would thus be required to initiate activity in the otherwise specified SAM tissue. During ensuing shoot development, *kn1* would additionally be required for maintaining the meristem. Therefore, in either scenario *kn1* does not solely define the SAM, it is merely required for SAM function, though the attributes of that function vary. The former interpretation requires only a single function of *kn1*, in meristem maintenance, which our data support unequivocally, and implies a simpler model of embryogenesis in which production of the first leaves (cotyledons) is the first iteration of meristem activity.

Role of *kn1* in meristem maintenance

Once the meristem forms, self-regulatory functions act to maintain organization and organogenic capacity. Two major functional domains have been identified based on extensive histological and molecular studies (Foster, 1938; Ledin, 1954; Nourgarede, 1967; Kaplan and Cooke, 1997; Lenhard and Laux, 1999). The central zone (CZ) is located in the apical central portion of the meristem and consists of slowly dividing, less cytoplasmic cells that ultimately replenish the meristem after organs are initiated. The morphogenetic or peripheral zone (PZ) surrounds the central zone, consists of more densely cytoplasmic cells and is the region where lateral organs are initiated. Replenishment and organogenesis may be viewed as interdependent processes within the meristem, with continued meristem function requiring a balance between these activities. This notion suggests that if either CZ or PZ activity is lost, then meristem maintenance will be lost. But loss of each activity predicts a slightly different shoot apex phenotype, since the shoot apex would initially contain only one complementary activity. If CZ function is lost, then PZ activity without replenishment will consume the shoot apex to produce terminal, differentiated structures representing the consumed meristem, as illustrated by the *pinhead/zwille* mutations in *Arabidopsis* (Moussian et al., 1998; Lynn et al., 1999). If PZ function is lost then CZ cells, now outside the context of an organized SAM, might arrest with CZ or otherwise differentiated identity, but not participate in organogenesis.

We discuss two hypotheses for how *kn1* functions in meristem maintenance. As we suggested previously, *kn1* may prevent differentiation of cells within the CZ that would normally remain indeterminate (Kerstetter et al., 1997). A similar function has been proposed for the *kn1*-like gene *SHOOT MERISTEMLESS* (*STM*) in *Arabidopsis*, where strong *stm* mutants make normal cotyledons that are fused at their bases, but lack all other aspects of shoot development (Barton and Poethig, 1993; Endrizzi et al., 1996). Thus, *kn1* may preserve the CZ, therefore maintaining the SAM by ensuring replenishment of the PZ after organogenesis. On the other hand, the consistent lack of terminal structures at the shoot apex of *kn1* limited shoot plants suggests an alternative function for *kn1* in meristem maintenance. *kn1* is expressed throughout the shoot apex, but is specifically excluded from the PZ cells that are within the site of leaf initiation. We hypothesize that *kn1* function is critical outside the CZ, such as in the PZ where its differential expression defines boundaries of organ initiation. Thus, *kn1* may perpetuate the PZ during meristem ontogeny, perhaps by regulating boundaries that specify the PZ and/or coordinating the contribution of CZ cells to the PZ, or more directly by specifying an indeterminate fate to PZ cells. When *kn1* activity is removed, PZ tissue becomes incorporated into organs, either in a repeating fashion as in the functioning SAM of wild-type plants, or, as in the limited shoot phenotype, in a non-replenished, consuming fashion that leaves the CZ as the only remnant of the SAM. As outlined above, CZ function alone may be insufficient and lead to loss of meristem maintenance. Consistent with this hypothesis, the tissue remaining at the shoot apex of limited shoot plants is histologically similar to CZ cells (Ledin, 1954). Given the small volume of the shoot meristem in the *Arabidopsis* embryo and the corresponding difficulty in resolving the CZ, it is possible that the *stm* mutant phenotype could be interpreted in a similar way.

Inbred-specific modifiers and the effect of *kn1* on shoot meristem size

In lines segregating a *kn1* mutation, the frequency of the limited shoot phenotype depends on the genetic background. Crosses between the permissive B73 and restrictive W23 backgrounds suggested that increased penetrance in W23 could be attributed to the expression state of relatively few genes, which have reduced activity or are non-functional in W23 as

compared to their counterpart B73 alleles. With respect to these two inbreds, our data are consistent with the presence of one major modifying locus segregating independently of *kn1*. Other plausible models, involving different strengths and genetic linkages of modifiers, could also account for the penetrance distributions we have observed. KN1 is a member of a class of related homeodomain (KNOX) proteins in maize that are expressed in shoot meristems and not in leaves (Vollbrecht et al., 1991; Jackson et al., 1994; Kerstetter et al., 1994). A modifier could correspond to a related homeobox gene. Phylogenetic analysis shows that *knox8*, *knox3*, *rs1* and *knox4* are most related to *kn1*(Bharathan et al., 1999; Reiser et al., 2000). *rs1* and *knox4* are likely to be duplicate loci (Schneeberger et al., 1995; Foster et al., 1999); thus, we would expect either both genes or neither gene to be redundant with *kn1*. *knox3* or *knox8* are unlikely to be the modifier given their linkage to *kn1* (Kerstetter et al., 1994). However, the factor that modifies the limited shoot phenotype may well be a global or specific *knox* gene regulator (Timmermans et al., 1999; Tsiantis et al., 1999), or loci that otherwise affect SAM maintenance.

We found that relative SAM size correlated with relative penetrance of the *kn1* limited shoot phenotype. We propose a model wherein there is a critical threshold for meristem size in the embryo, above which the meristem can perpetuate and below which the meristem can not maintain function. 50 μm represents the arbitrary, critical threshold for meristem height in stage 5 embryos (Fig. 8). Relatively permissive inbreds like B73 and Mo17 contain a modifier that increases meristem height. In B73 genotypes containing *kn1* activity, average meristem height is approx. 80 μm. Furthermore, in both B73 and Mo17, lack of *kn1* function decreases average SAM height by approx. 20 μm (Fig. 7). Thus, in B73 the average height of *kn1* mutant SAMs is approx. 60 μm (Fig. 8), which is still greater than the critical threshold. SAMs of W22 and W23 (more restrictive lines) are normally approx. 50 μm tall. Hence, if the *kn1* mutation is converged into W23, then average meristem height decreases by approx. 20 μm and falls below the arbitrary threshold. Mutant meristems that survive in restrictive lines may do so because of stochastic variation in size, and/or because of the sufficient presence of other, minor modifiers. Thus, the absence of either *kn1* or modifier(s) reduces SAM height, explaining why some inbreds have inherently smaller meristems that are more severely affected by loss of *kn1* function. Differences in normal meristem architecture have also been described for *Arabidopsis*; Landsberg (L*er*) ecotype SAMs are shorter and wider than those of the Wassilewskja ecotype (Laufs et al., 1998). Whether or not this ecotype difference affects the phenotype of meristem mutants such as *stm* has not been reported.

kn1 mutations affect specific shoot meristems

Maize appears to have a critical requirement for *kn1* to maintain particular meristems. *kn1* mutants that develop to maturity elaborate fewer first order branches on the main axis of the tassel and ear, while subsequent, higher order branching events are unaffected (Kerstetter et al., 1997). While the unaffected inflorescence meristems may be maintained by inherently different mechanisms than the embryonic SAM and first order branch meristems, the shared requirement for *kn1* may reflect a common quality of affected meristems, perhaps related to their morphology (meristem size) and/or *kn1* expression domains. In both the embryonic SAM and first order branch meristems, the incipient meristem is small and increases in size during development (Abbe et al., 1951; Abbe and Stein, 1954). These small meristems initially express *kn1* in only a few cells, and continue in *kn1* mutants only if they enlarge sufficiently. By contrast, the unaffected inflorescence meristems are each partitioned as a large group of *kn1*-expressing cells (Cheng et al., 1983; Jackson et al., 1994). Small meristems, which may require de novo expression of *kn1*, may be more sensitive to loss of its activity.

We thank Kelly Dawe, Paul Chomet and Brenda Lowe for genetic stocks and advice on transposon genetics, Jennifer Fletcher, David Jackson and members of the Hake lab for critical review of the manuscript, Donald R. Kaplan for advice and discussions, and David Hantz for management of the PGEC greenhouse. The research was supported by NSF grant MCB-9927611 to S. H., an NIH postdoctoral fellowship #18330-03 to L. R. and support from the USDA to S. H.

REFERENCES

Abbe, E. C., Phinney, B. O. and Baer, D. F. (1951). The growth of the shoot apex in maize: internal features. *Am. J. Bot.* **38**, 744-751.

Abbe, E. C. and Stein, O. L. (1954). The growth of the shoot apex in maize: embryogeny. *Am. J. Bot.* **41**, 285-293.

Alleman, M. and Kermicle, J. L. (1993). Somatic variegation and germinal mutability reflect the position of transposable element Dissociation within the maize R gene. *Genetics* **135**, 189-203.

Barton, M. K. and Poethig, R. S. (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.

Barton, M. K. (1998). Cell type specification and self renewal in the vegetative shoot apical meristem. *Curr. Opin. Plant Biol.* **1**, 37-42.

Bharathan, G., Janssen, B. J., Kellog, E. and Sinha, N. (1999). Phylogenetic relationships and evolution of the KNOTTED class of plant homeodomain proteins. *Mol. Biol. Evol.* **16**, 553-563.

Brutnell, T. P., May, B. P. and Dellaporta, S. L. (1997). The *Ac-st2* element of maize exhibits a positive dosage effect and epigenetic regulation. *Genetics* **147**, 823-834.

Bryan, A. A. and Sass, J. E. (1941). Heritable characters in maize. *J. Hered.* **32**, 343-346.

Cheng, P. C., Greyson, R. I. and Walden, D. B. (1983). Organ initiation and the development of unisexual flowers in the tassel and ear of *Zea mays*. *Am. J. Bot.* **70**, 450-462.

Chuck, G., Lincoln, C. and Hake, S. (1996). Knat1 induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis. *Plant Cell* **8**, 1277-1289.

Doring, H. P., Freeling, M., Hake, S., Johns, M. A., Kunze, R., Merckelbach, A., Salamini, F. and Starlinger, P. (1984). A *Ds*– mutation of the *Adh1* gene in *Zea mays*. *Mol. Gen. Genet.* **193**, 199-204.

Endrizzi, K., Moussian, B., Haecker, A., Levin, J. Z. and Laux, T. (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**, 101-113.

Foster, A. (1938). Structure and growth of the shoot apex in *Ginkgo biloba*. *Bull Torrey Bot. Club* **65**, 531-556.

Foster, T., Yamaguchi, J., Wong, B., Veit, B. and Hake, S. (1999). *Gnarley1* is a dominant mutation in the *knox4* homeobox gene that affects cell identity and cell shape. *Plant Cell* **11**, 1239-1252.

Freeling, M. and Hake, S. (1985). Developmental genetics of mutants that specify Knotted leaves in maize. *Genetics* **111**, 617-634.

Freeling, M. and Schwartz, D. (1973). Genetic relationships between the multiple alcohol dehydrogenases of maize. *Genetics* **8**, 27-36.

Gelinas, D., Postlethwaite, S. N. and Nelson, O. E. (1969). Characterization of development in maize through the use of mutants. II. The abnormal growth conditioned by the Knotted mutant. *Am. J. Bot.* **56**, 671-678.

Goldberg, R. B., de Paiva, G. and Yadegari, R. (1994). Plant embryogenesis: zygote to seed. *Science* **266**, 605-614.

Greenblatt, I. M. (1984). A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element Modulator in maize. *Genetics* **108**, 471-485.

Greene, B., Walko, R. and Hake, S. (1994). *Mutator* insertions in an intron of the maize *knotted1* gene result in dominant suppressible mutations. *Genetics* **138**, 1275-1285.

Hake, S., Vollbrecht, E. and Freeling, M. (1989). Cloning *Knotted*, the dominant morphological mutant in maize using *Ds2* as a transposon tag. *EMBO J.* **8**, 15-22.

Jackson, D., Veit, B. and Hake, S. (1994). Expression of maize *KNOTTED1* related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* **120**, 405-413.

Kaplan, D. R. (1973). Comparative developmental analysis of the heteroblastic leaf series of axillary shoots of *Acorus calamus* L. (Araceae). *La Cellule* **69**, 253-290.

Kaplan, D. R. and Cooke, T. J. (1997). Fundamental concepts in the embryogenesis of dicotyledons: a morphological interpretation of embryo mutants. *Plant Cell* **9**, 1903-1919.

Kerstetter, R., Vollbrecht, E., Lowe, B., Veit, B., Yamaguchi, J. and Hake, S. (1994). Sequence analysis and expression patterns divide the maize *knotted1*-like homeobox genes into two classes. *Plant Cell* **6**, 1877-1887.

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

Kiesselbach, T. A. (1949). *The Structure and Reproduction of Corn*, Volume 161. University of Nebraska Agricultural Experiment Station Bulletin.

Laufs, P., Grandjean, O., Jonak, C., Kieu, K. and Traas, J. (1998). Cellular parameters of the shoot apical meristem in Arabidopsis. *Plant Cell* **10**, 1375-1389.

Ledin, R. B. (1954). The vegetative shoot apex of *Zea mays*. *Am. J. Bot.* **41**, 11-17.

Lenhard, M. and Laux, T. (1999). Shoot meristem formation and maintenance. *Curr. Opin. Plant. Biol.* **2**, 44-50.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P. and Barton, M. K. (1999). The PINHEAD/ZWILLE gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the ARGONAUTE1 gene. *Development* **126**, 469-81.

McClintock, B. (1947). Cytogenetic studies of maize and neurospora. *Carnegie Inst. Wash. Year Book* **46**, 146-152.

Moreno, M. A., Chen, J., Greenblatt, I. and Dellaporta, S. L. (1992). Reconstitutional mutagenesis of the maize *P* gene by short range *Ac* transpositions. *Genetics* **131**, 939-956.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G. and Laux, T. (1998). Role of the *ZWILLE* gene in the regulation of central shoot meristem cell fate during *Arabidopsis* embryogenesis. *EMBO J.* **17**, 1799-1809.

Nuffer, G. (1954). Activator of *bz2-m* mutability. *Maize Genetics Coop. Newsletter* **28**, 63-65.

Nourgarede, A. (1967). Experimental cytology of the shoot apical cells during vegetative growth and flowering. *Intern. Rev. Cytol.* **21**, 203-351.

Randolph, L. F. (1936). Developmental morphology of the caryopsis in maize. *J. Ag. Res.* **53**, 881-916.

Reiser, L., Sanchez-Baracaldo, P. and Hake, S. (2000). Knots in the family tree: Evolutionary relationships and functions of *knox* homeobox genes. *Plant Mol. Biol.* **42**,151-66.

Schneeberger, R. G., Becraft, P. W., Hake, S. and Freeling, M. (1995). Ectopic expression of the *knox* homeo box gene *rough sheath1* alters cell fate in the maize leaf. *Genes Dev.* **9**, 2292-2304.

Scott, L., LaFoe, D. and Weil, C. F. (1996). Adjacent sequences influence DNA repair accompanying transposon excision in maize. *Genetics*, **142**, 237-246.

Sentoku, N., Sato, Y., Kurata, N., Ito, Y., Kitano, H. and Matsuoka, M. (1999). Regional expression of the rice KN1-type homeobox gene family during embryo, shoot, and flower development. *Plant Cell* **11**, 1651-1664.

Sinha, N. R., Williams, R. E. and Hake, S. (1993). Overexpression of the maize homeo box gene, *KNOTTED-1*, causes a switch from determinate to indeterminate cell fates. *Genes Dev.* **7**, 787-795.

Smith, L., Greene, B., Veit, B. and Hake, S. (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.

Smith, L. G., Jackson, D. and Hake, S. (1995). The expression of Knotted1 marks shoot meristem formation during maize embryogenesis. *Dev. Gen.* **16**, 344-348.

Timmermans, M. C., Hudson, A., Becraft, P. W. and Nelson, T. (1999). ROUGH SHEATH2: a Myb protein that represses knox homeobox genes in maize lateral organ primordia. *Science* **284**, 151-153.

Tsiantis, M., Schneeberger, R., Golz, J. F., Freeling, M. and Langdale, J. A. (1999). The maize rough sheath2 gene and leaf development programs in monocot and dicot plants. *Science* **284**, 154-156.

VanLammeren, A. A. M. (1986). Developmental morphology and cytology of the young maize embryo (*Zea mays L.*). *Acta Bot. Neerlandia* **35**, 169-188.

Vollbrecht, E., Veit, B., Sinha, N. and Hake, S. (1991). The developmental gene *Knotted-1* is a member of a maize homeobox gene family. *Nature* **350**, 241-243.

Weatherwax, P. (1920). Position of scutellum and homology of the coleoptile in maize. *Bot. Gaz.* **69**, 179-182.