Expression Patterns and Mutant Phenotype of *teosinte branched1* Correlate With Growth Suppression in Maize and Teosinte

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ABSTRACT

The evolution of domesticated maize from its wild ancestor teosinte is a dramatic example of the effect of human selection on agricultural crops. Maize has one dominant axis of growth, whereas teosinte is highly branched. The axillary branches in maize are short and feminized whereas the axillary branches of teosinte are long and end in a male inflorescence under normal growth conditions. Previous QTL and molecular analysis suggested that the *teosinte branched1* (*tb1*) gene of maize contributed to the architectural difference between maize and teosinte. *tb1* mutants of maize resemble teosinte in their overall architecture. We analyzed the *tb1* mutant phenotype in more detail and showed that the highly branched phenotype was due to the presence of secondary and tertiary axillary branching, as well as to an increase in the length of each node, rather than to an increase in the number of nodes. Double-mutant analysis with *anther ear1* and *tassel seed2* revealed that the sex of the axillary inflorescence was not correlated with its length. RNA *in situ* hybridization showed that *tb1* was expressed in maize axillary meristems and in stamens of ear primordia, consistent with a function of suppressing growth of these tissues. Expression in teosinte inflorescence development suggests a role in pedicellate spikelet suppression. Our results provide support for a role for *tb1* in growth suppression and reveal the specific tissues where suppression may occur.

PLANT architecture results from the action of shoot meristems, groups of indeterminate cells whose coordinate activities produce the organs of the shoot. The shoot apical meristem initiates during embryogenesis and produces leaves in a predictable pattern. Axillary meristems initiate from the morphogenetic zone on the periphery of the shoot apical meristem in the axil of a subtending leaf (Steeves and Sussex 1989). The developmental fate of an axillary meristem can be developmentally and environmentally regulated (McDaniel and Hsu 1976; Napoli and Ruehle 1996; Sussex and Kerk 2001). An axillary meristem may develop as a vegetative branch, resulting in additional foliage, or as an inflorescence, resulting in flowers and seeds. An axillary meristem may also remain dormant throughout the life of the plant. The potential for differential fate of axillary meristems provides plants with the necessary plasticity with which to respond to environmental challenges and also provides a mechanism with which to separate functions within the shoot.

The shoot apical meristem often suppresses development of axillary branch growth. In most species, removal of the shoot apex allows axillary buds to grow out due to a phenomenon called apical dominance. The severity of apical dominance is often dependent upon growth conditions such as light quality and plant density. Poor growth conditions are generally associated with an increase in apical dominance (Iltis 1986; Schmitt and Dudley 1996; Pigliucci and Schmitt 1999). Various plant hormones are implicated in the process (Chatfield *et al.* 2000).

Normal maize plants show strong apical dominance with one main axis of growth. They occasionally produce one or two elongated lateral branches, called tillers, from their base and always produce one to two shorter axillary branches midway along the main stem that bear the female inflorescence or ear (Figure 1, A and B). After a defined number of leaves are initiated, the shoot apical meristem undergoes a transition to form an inflorescence meristem, which ultimately produces the male inflorescence or tassel at the apex of the plant (Russell and Stuber 1983; Irish and Nelson 1991).

Teosinte is considered to be the ancestor to maize (Bennetzen *et al.* 2001). A teosinte plant is typically highly tillered with long axillary branches developing from the majority of its nodes. These long branches bear a male inflorescence at their apex (Figure 1, C and D) and a female inflorescence in secondary axillary branch positions. QTL analysis revealed that five gene regions control the major morphological differences between maize and teosinte (Doebley and Stec 1993). One of these regions controls lateral branch length and is also involved in regulating certain aspects of floral development (Doebley *et al.* 1995a). Complementation testing revealed that this QTL corresponds to the maize locus, *teosinte branched1* (*tb1*). Like teosinte, maize *tb1*

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mutants show a reduction in apical dominance with a proliferation of basal tillers (Figure 1E). Lateral branches that would normally give rise to ear shoots are much longer than in normal plants and, similar to tillers, are tipped by a tassel (Figure 1F).

Northern blot analysis of *tb1* revealed expression of a 1.5-kb mRNA in the ears of maize plants carrying a maize allele of *tb1*. A message of similar size but reduced abundance was detected in ears of maize plants that carry a teosinte allele of *tb1*. Furthermore, analysis of nucleotide polymorphisms in a number of maize and teosinte alleles revealed that selection had acted specifically on the 5′ nontranscribed region. Together, the sequence and expression analyses suggest that, during the evolution of maize, regulatory changes had occurred at the *tb1* locus (Doebley *et al.* 1997; Wang *et al.* 1999).

In this article we examined how and when the *tb1* mutation affects development in maize. We used RNA *in situ* hybridization to investigate *tb1* gene expression in vegetative and floral development of maize and teosinte. We also constructed double mutants with *tassel seed2* (*ts2*) and gibberellic acid (GA)-deficient dwarfs to examine the correlation of branch length with inflorescence sex. Our results support a role for *tb1* in growth suppression in distinct tissues where it is expressed.

MATERIALS AND METHODS

Plant materials: All genetic stocks were obtained from the Maize Genetics Coop Stock Center. The B73 inbred line was a gift from Pioneer Hi-Bred International. We used *Zea mays* ssp. *parviglumis* as our teosinte source since this subspecies is considered the ancestor of maize. The stock of teosinte that carries the chromosomal region encompassing the maize allele of *tb1* is described in Doebley *et al.* (1995a).

Morphological analysis: The *tb1-r* allele was introgressed five times into the B73 line of maize. From this introgressed material, a plant heterozygous for *tb1-r* was self-pollinated. Unless otherwise indicated, the progeny of that cross were subjected to restriction fragment length polymorphism (RFLP) analysis and grown in 13-liter pots in the greenhouse. Field-grown plants of the same family were also grown in Brentwood, California. Genotypic categories consisted of homozygous normal (*Tb1-N/Tb1-N*), heterozygous (*Tb1-N/tb1-r*), and homozygous mutant (*tb1-r/tb1-r*) plants. All classes were grown to maturity; morphological traits were scored throughout development depending upon the trait of interest. For some traits, plants from the B73 line were also scored. To determine the total number of culms on greenhouse-grown plants, 10 individuals were scored in each genotypic class and 6 B73 individuals were scored. The number of culms was determined 18 cm from the base of the plant. In the field-grown classes, 15 *tb1-r/tb1-r*, 22 *Tb1-N/tb1-r*, and 10 *Tb1-N/Tb1-N* individuals were scored. To determine the number of nodes on the uppermost lateral branch, 10 *tb1-r/tb1-r*, 11 *Tb1-N/tb1-r*, 10 *Tb1-N/Tb1-N*, and 6 B73 individuals were scored.

Double mutants were identified from their phenotype and segregation ratios. *dwarf1* (*d1*); *tb1* double mutants had as many culms as *tb1* single mutants and were the same stature as *d1* mutants. *anther ear1* (*an1*) mutants were not much shorter than normal plants, but they had a reduced tassel branching phenotype that was easily scored. The *an1*; *tb1* double mutants had as many culms as *tb1* single mutants and the reduced tassel branching phenotype. *ts2*; *tb1* double mutants had a feminized tassel typical of *ts2* mutants but many culms typical of *tb1* mutants.

RFLP analysis: Maize genomic DNA was isolated as described (Chen and Dellaporta 1994). DNA was digested with *Hin*dIII restriction endonuclease, separated on a 0.8% agarose gel, and transferred to Magna nylon membrane (Micron Separations, Westboro, MA). The membranes were hybridized with a 3.4-kb *Hin*dIII *Tb1* genomic clone (Doebley *et al.* 1997). Genotype was also determined by linkage to the *alcohol dehydrogenase 1* (*adh1*) locus. Scutellum slices were subjected to an isozyme analysis as described (Freeling and Schwartz 1973). *tb1-r* is linked to the *Adh1-S* allele and B73 carries the *Adh1-F* allele. Seeds were planted according to the following *Adh1* classes: S/S, S/F, or F/F. Five seedlings were examined from each class for the histological analysis.

Histology: Vegetative tissue was fixed in 45% ethanol, 5% glacial acetic acid, and 10% formaldehyde solution and inflorescence tissue was fixed in 4% paraformaldehyde in 100 mm $NaPO_4$, pH 7. For the histological analysis, sections were stained in a 1:200 dilution of Toluidine Blue O. Unless otherwise noted in text, B73 was used for all *in situ* hybridization experiments**.** Riboprobe preparation and *in situ* hybridizations were performed as described (Jackson 1992). The *knotted1* (*kn1*) gene probe is described by Jackson *et al.* (1994). Subclones of the *tb1* cDNA (0.5-kb *Xba*I and 0.6-kb *Sal*I) were used for antisense and sense hybridization experiments. Signal was not detected from any sense control probes except occasional stem expression as mentioned in text. *kn1* antisense probe was included in all experiments as a positive control to confirm that RNA was detectable and that the procedure was successful.

RESULTS

***tb1* regulates the number and length of axillary branches:** Maize plants in the B73 inbred line lack elongated axillary branches whereas homozygous *tb1* mutant plants have many tillers, resulting in a "bushy" profile (Figure 1, A and E). To quantify the difference between *tb1* mutants and normal siblings, we backcrossed *tb1-r* into B73 for five generations and then carried out measurements on progeny of self-pollinated heterozygotes. The *tb1* gene was used as an RFLP probe to assign genotypes (materials and methods). In greenhouse-grown material, homozygous mutant plants had an average of 13.7 ± 0.32 culms (jointed stems that include the main stem and branches), heterozygous plants had an average of 1.4 ± 0.09 culms, and normal plants always had one culm. Under field conditions, the homozygous mutants had an average of 17 ± 0.49 culms, heterozygous plants had an average of 1.7 ± 0.19 culms, and normal plants again always had one. Thus, heterozygotes were slightly more tillered than wild type (Figure 2A) and field-grown conditions produced slightly more tillers.

The overall length of axillary branches was also affected in homozygous mutants (Table 1A). Homozygous mutants had longer branches along the entire length of the plant with the most dramatic increases in the basal nodes. We asked whether the increase in length was due to an increased number of nodes or an increase

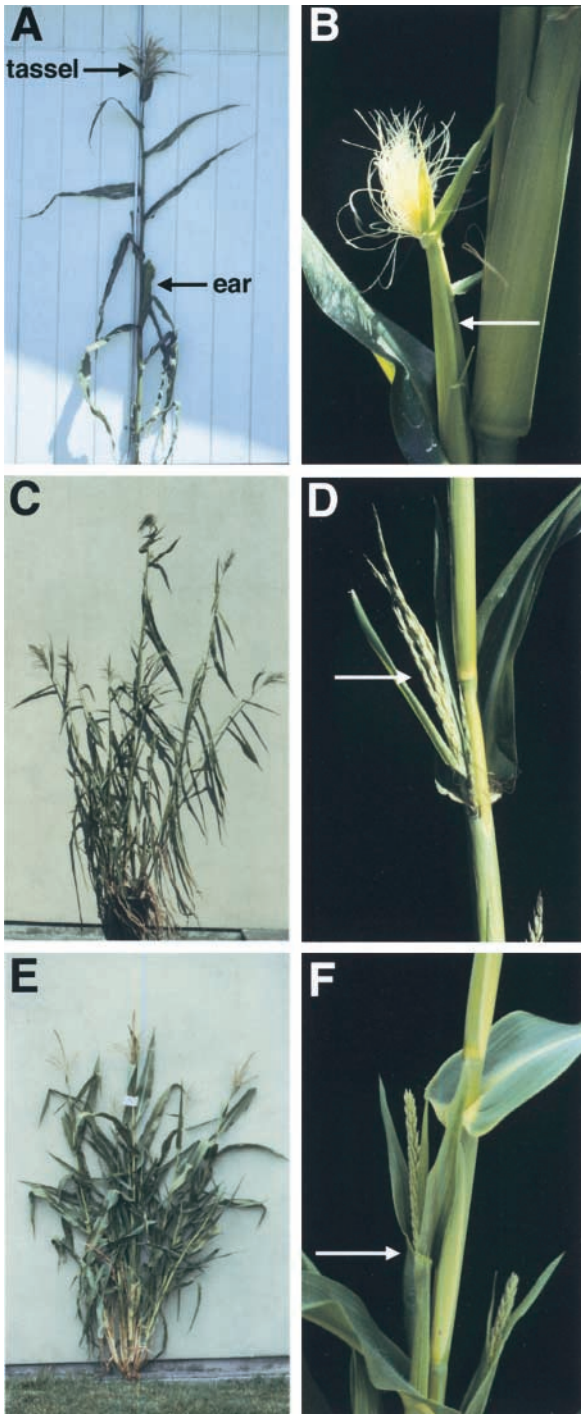

FIGURE 1.—Morphology of mature normal maize, teosinte, and homozygous *tb1* mutant plants. (A) Normal maize plant showing one major axis of growth. (B) Upper axillary branch of a normal maize plant. (C) Teosinte plant. (D) Upper axillary branch of a teosinte plant. (E) Homozygous *tb1* mutant maize plant. (F) Upper axillary branches of a *tb1* homozygous mutant plant.

in length of each internode. The number of nodes on the most apical branch, which is an ear in normal plants, was counted. Homozygous mutants had fewer nodes than normal plants, while heterozygous plants showed no difference in the number of nodes relative to normal plants (Table 1B). This result indicates that mutant branches attain their length through an increase in internode elongation rather than by producing extra nodes. The total number of nodes on the main culm and length of the main culm were also measured to determine if the *tb1* mutation has an effect on either of these aspects of development. No significant differences were seen in homozygous, heterozygous, or normal plants for these traits (data not shown); thus differences in the number of nodes produced and the length of a culm are restricted to axillary branches.

Maize leaves have two distinct parts, a flat blade at the distal end and a proximal sheath that wraps tightly around the culm. The leaves that wrap around an ear are referred to as husk leaves and are often modified such that they have a very short blade portion and are mostly sheath. *tb1* mutants have very long husk leaf blades in comparison to their normal siblings when introgressed into a B73 background (data not shown). Thus, *tb1* appears to play a role in suppression of husk leaf blade growth in addition to its role in branch suppression.

Origin of axillary branches in *tb1* mutants: We investigated the origin of the extra axillary branches in *tb1* mutants by following plants through development. Multiple axillary branches could result from the presence of more than one axillary meristem in a leaf axil, elongation of normally suppressed axillary branches, or reiteration of axillary meristems in the axils of branches. We found no evidence for multiple axillary meristems in one axil; instead we found reiteration of axillary branches due to the formation of secondary and tertiary branches (Figure 2B). In heterozygous plants, the increase in tillering observed was due to elongation of primary branches and not to secondary branching (data not shown).

We observed that 2-week-old homozygous mutants had elongating branches in the axil of their first true (noncotyledon) leaf, whereas axillary branches were never observed in normal seedlings of this age (Figure 2C). To determine when *tb1-r* homozygous mutant plants differed in development from normal plants, we sectioned plants from segregating families at 4, 7, and 13 days after germination. Up to 7 days after germination no difference in development between normal and mutant seedlings was detected (Figure 3, A and B). By 13 days after germination the most basal axillary branch in homozygous mutant seedlings had undergone substantial elongation while the corresponding branch in the homozygous normal plants remained small (Figure 3, C and D).

***tb1* mRNA expression in maize:** To determine the tissue in which *tb1* was expressed and the earliest stage of expression, we carried out RNA *in situ* hybridization. We used the *kn1* cDNA, which is expressed in all shoot meristems, as a control (JACKSON *et al.* 1994). In a serial

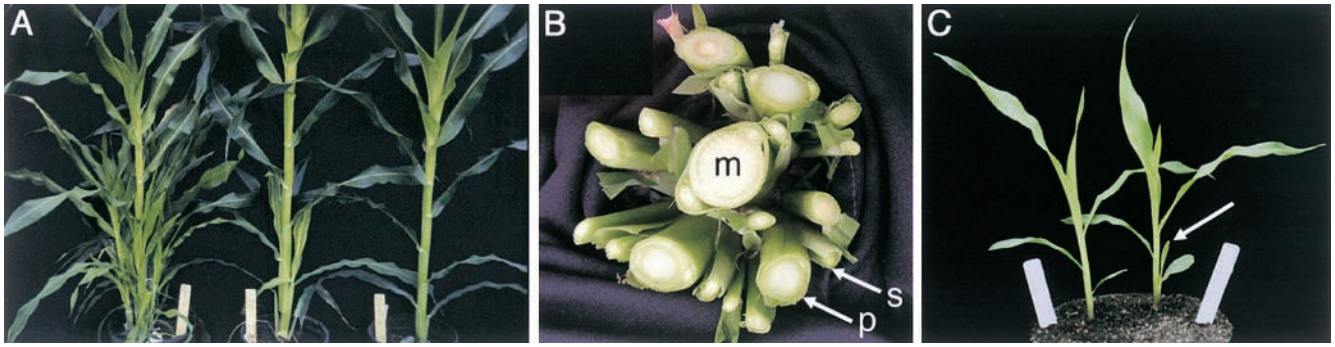

FIGURE 2.—Phenotype of *tb1* mutants. (A) *tb1-r/tb1-r*, *tb1-r/Tb1-N*, and *Tb1-N/Tb1-N* plants from left to right. The heterozygous plant is mildly tillered but will develop a normal female inflorescence at the ear node. (B) A *tb1-r/tb1-r* plant was chopped near the ground to reveal primary (p) and secondary (s) axillary branching. m, main culm. (C) *tb1-r/tb1-r* mutant plants exhibit early basal branching. A normal seedling (left) and a *tb1-r* homozygous mutant seedling (right) at 2 weeks. At this stage of development, a tiller is apparent in the axil of the first true leaf in the *tb1-r* homozygous mutant plant.

section at 5 days after germination, *kn1* expression was very strong in the shoot apical meristem with weaker expression in the stem (Figure 4B). At this stage, axillary buds are just beginning to develop as a bulge in the axil of the fifth leaf from the apex, referred to as a plastochron 5 (P_5) leaf. *kn1* expression was apparent in the axillary region of P_5 and P_6 leaves. *kn1* expression was not detected in the axils of younger leaf primordia, suggesting that the axillary meristems do not form prior to P_5 leaves.

Using the *tb1* cDNA clone, we detected a signal in axillary meristems of seedlings 5 days after germination (Figure 4A). We were not able to detect RNA at 20–24 days after pollination nor at 1–4 days after germination by either *in situ* hybridization or Northern analysis (data not shown). *tb1* expression was just detectable in the axil of P_5 and was clearly apparent in the axil of the P_6 leaf. Expression of *tb1* was not observed in the shoot apical meristem nor in ground tissue (Figure 4A). Thus, *tb1* axillary expression was coincident with the formation of axillary meristems.

In more mature plants, when the vegetative shoot apical meristem became an inflorescence meristem, the expression of *tb1* mRNA in axillary meristems was similar to that observed in seedlings. *tb1* expression was confined to axillary meristems and axillary shoots (Figure 4, C and D). The apparent signal detected in the body of the plant as horizontal stripes was also observed in control sections probed with a sense probe (data not shown) and was therefore considered nonspecific. Again, as seen in 5-day-old seedlings, expression was not detected in axils younger than P_5 or P_6. All axillary meristems and axillary branches P_5 and older showed expression of *tb1* mRNA (Figure 4D). Expression was particularly strong in husk leaves (Figure 4F).

Normal maize development is characterized by the production of axillary meristems called branch meristems from the inflorescence. The branches at the base of the tassel elongate considerably, while the branch primordia in more apical regions are determinate and referred to as spikelet pair primordia. The spikelet pair meristems produce sessile and pedicellate spikelet meristems, each of which produce a pair of glumes and two floral meristems. Up until this stage, development of the ear and tassel are similar although no long branches form on the ear. Early stages of floret development are similar in the ear and tassel: each floret is enclosed in a lemma and palea and all florets produce two lodicules, three stamens, and a gynoecium (Figure 5, A and D). The lower floret initiates first but then lags behind the upper floret in development. Following the initiation of all floral organs, the male and female florets differentiate. In the ear, lower florets arrest and stamens of the upper floret arrest, resulting in a single female floret per spikelet, while in the tassel, selective abortion of the gynoecium produces two male florets per spikelet

TABLE 1

Comparison of morphological traits across genotypic classes

tb1-r/tb1-r	*tb1-r/Tb1-N*	*Tb1-N/Tb1-N*	B73
A. Apical-to-basal branch length (inches)			
22.3 ± 3.8	14.9 ± 1.3	12.6 ± 0.8	12.6 ± 0.6
25.1 ± 4.1	9.8 ± 4.2	7.6 ± 0.9	4.6 ± 0.7
19.4 ± 6.0	5.1 ± 0.9	3.4 ± 1.2	1.0 ± 0
18.0 ± 5.2	2.5 ± 1.3	1.8 ± 0.4	0.8 ± 0.1
14.2 ± 5.3	1.9 ± 1.1	1.1 ± 0.4	0.4 ± 0.1
14.6 ± 6.9	1.0 ± 0.8	0.6 ± 0.2	0
16.3 ± 7.7	0.6 ± 0.3	1.0 ± 0	0
26.7 ± 8.2	0	0	0
41.7 ± 8.2	16.0 ± 0	0	0
54.6 ± 11.4	12.6 ± 5.2	0	0
69.3 ± 14.4	0	0	0
71.1 ± 12.6	0	0	0
B. Number of nodes on apical branch			
5.7 ± 0.6	8.7 ± 0.7	8.9 ± 0.7	9.0 ± 0.6

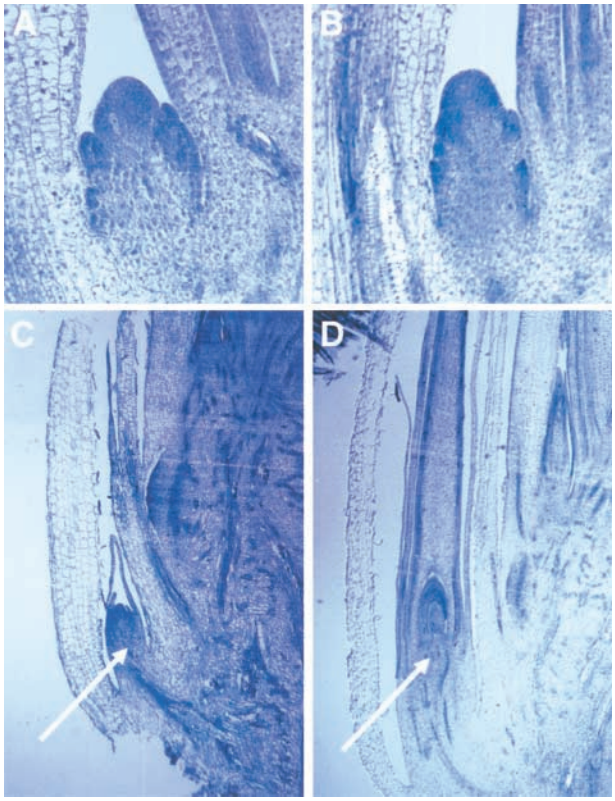

FIGURE 3.—Histological analysis of early developmental differences in normal and mutant plants. Sections showing the most basal axillary branch of normal and *tb1-r* homozygous mutant plants. (A) Seven-day-old normal seedling. (B) Seven-day-old *tb1-r* homozygous mutant. (C) Thirteen-day-old normal seedling. (D) Thirteen-day-old *tb1-r* homozygous mutant.

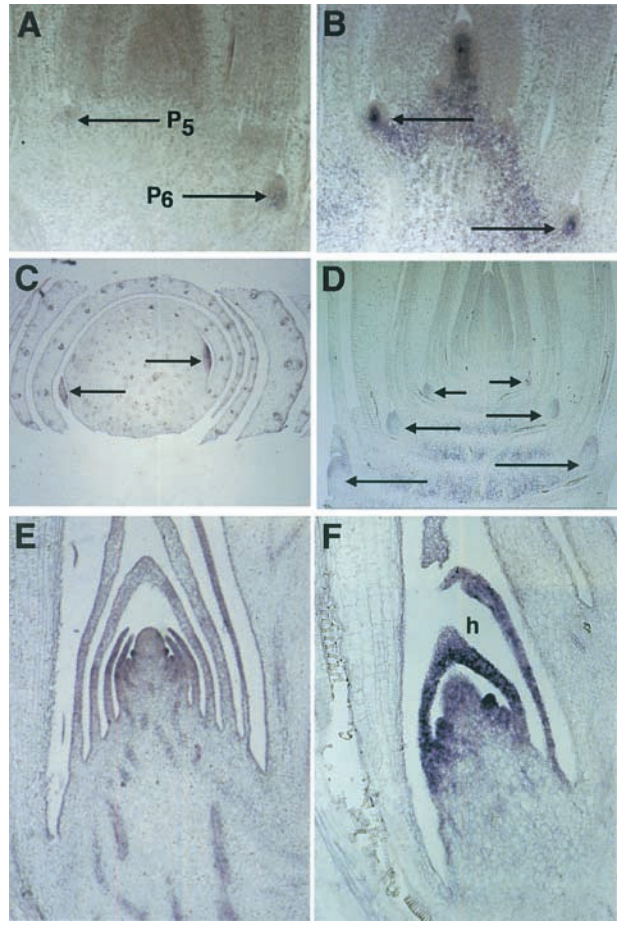

FIGURE 4.—*tb1* expression in axillary meristems. Serial longitudinal sections probed with *tb1* antisense (A, C–F) and with *kn1* (B). In A, *tb1* expression is just detectable in the axil of plastochron 5 (P_5) and is clearly apparent in the axil of P_6 as indicated by arrows in 5-day-old maize seedlings. *kn1* expression in B is coincident with *tb1* expression in the axils of P_5 and P_6 (arrows) and is also strong in the shoot apex and stem. (C) Transverse section reveals *tb1* expression on either side of the shoot in two consecutive axillary meristems (arrows). (D) A longitudinal section through the apex of a plant 42 days after germination. Expression of *tb1* is detected in the axil of P_5 and all older basal axillary meristems and branches. (E) A longitudinal section through the most basal axillary branch of a plant 38 days after germination probed with *tb1* antisense RNA probe. (F) A longitudinal section through the ear shoot 34 days after germination reveals strong expression in husk leaves. h, husk leaves.

(CHENG *et al.* 1983; DELLAPORTA and CALDERON-URREA 1994).

tb1 expression was easily detected in stamen primordia of both upper and lower ear florets at young stages, but was not detected in older stamens shortly after they had undergone selective developmental arrest (Figure 5, B and C). No expression was detected in other lateral organs of the ear florets. Sections through tassel florets also showed expression in stamen primordia; however, the signal was weaker when compared with ear florets. Similar weak expression of *tb1* was also seen in the older stamens of the tassel florets (Figure 5E).

We also examined *tb1* expression in stamen primordia of *an1* mutants. *an1* mutants, like other GA-defective dwarfs, fail to suppress anther development in the ear. If *tb1* was downstream of *an1*, then we might expect to see reduced expression in stamens of female florets on *an1* mutant plants. However, *tb1* expression in *an1* mutants (Figure 5F) was similar to the expression seen in young stamens of normal ear florets. Weak expression persisted in mature stamens similar to what we see in mature male florets. Thus, if an increase in *tb1* expression leads to stamen abortion, *tb1* is upstream of *an1* or in a separate pathway.

At the developmental stage when *tb1* expression was easily detected in the stamens of upper florets of ears, expression was also detected in the lower floral meristem (Figure 5B). This expression was stage specific and not detected in every lower floret. The expression in the lower floral meristem correlates with a suppression in growth of these tissues.

***tb1* expression in teosinte:** We investigated the differences in *tb1* expression between maize and teosinte using *in situ* hybridization. Expression of *tb1* was not detected in a longitudinal section through the shoot apex

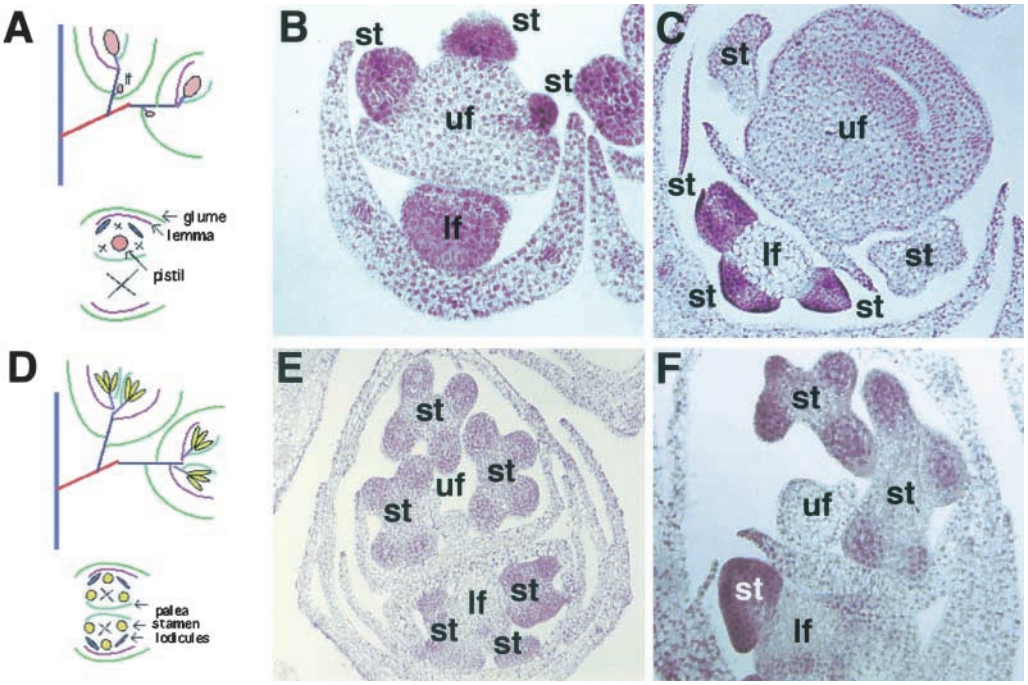

FIGURE 5.—*tb1* expression in floral organs. (A) Cartoon of female spikelet pair in maize. The lower floret and all stamens abort. (B) Young female spikelet showing expression of *tb1* in lower floret and stamens of upper floret. (C) Older female spikelet showing expression in stamens of lower floret. (D) Cartoon of male spikelet pair. The pistils of both upper and lower florets abort. (E) Male spikelet showing weak expression in all stamens. (F) *anther ear1* ear spikelet showing *tb1* expression in young stamens of a developing lower floret. Expression is also detected in the more developed stamens of the upper floret. st, stamen; uf, upper floret; lf, lower floret.

of a 17-day-old teosinte plant, although there were several well-developed primary axillary branches at this stage (Figure 6A). Adjacent sections were probed with the *kn1* antisense probe as a control for the presence of these axillary branches. Expression of *kn1* was detected throughout the stem and most basal axillary branches, showing that axillary branches were present (Figure 6B). Therefore *tb1* expression correlates with axillary branches that are suppressed, as in maize, but not with teosinte axillary branches that elongate.

In teosinte, secondary branches form on the primary (elongated axillary) branches and develop into small female inflorescences. On these "teosinte ears," both the pedicellate and sessile spikelets initiate but the pedicellate spikelet aborts early in development, and the sessile spikelet produces the female florets of teosinte (Figure 7A; DOEBLEY *et al.* 1995b). *tb1* expression was detected in developing female inflorescences of teosinte at the base of the pedicellate spikelet, but not in the sessile spikelet (Figure 7B). The presence of this expression is correlated with suppression of the pedicellate spikelet in teosinte. Similar expression was not detected in maize ears or tassels, which contain both pedicellate and sessile spikelets (data not shown).

We also carried out *in situ* hybridizations with teosinte that carries the maize chromosomal region containing the wild-type allele of *tb1*. The stock, referred to as Teosinte-M1L, was created by backcross breeding with molecular-marker-assisted selection (DOEBLEY *et al.* 1995a). The primary lateral branches of this material are short and feminized, similar to maize, but spikelet arrangement is more similar to teosinte than to maize (DOEBLEY *et al.* 1995a). We asked whether the *tb1* expression pattern was similar to that of maize, teosinte, or a mixture of the two. Expression was seen in the husk leaves, similar to the findings in maize (Figure 7C). Expression was also detected in axillary meristems of the husk leaves, which are suppressed in both maize and Teosinte-M1L, but not in teosinte. Expression in spikelet primordia was similar to that detected in teosinte secondary ears (Figure 7B). Thus, the *tb1* expression pattern in Teosinte-M1L is a mixture of maize and teosinte, largely correlating with the particular aspect of its phenotype.

Genetic analysis of sex determination: Tillers in maize usually end in a tassel, while ears are always female. The elongated branches of *tb1* mutants are tipped with tassels even when they appear in the position of an ear. We asked whether the sexual fate of the axillary branch was dependent on its length, *i.e.*, whether the axillary branches of *tb1* were male because they were elongated. *tb1* mutants were crossed to two different dwarf mutants, *d1* (NEUFFER *et al.* 1997) and *an1* (BENSEN *et al.* 1995). *d1* mutants are ~10% the size of normal siblings whereas *an1* mutants are 60% of normal height. Both *d1* and *an1* mutants are defective in GA biosynthesis. The *d1*; *tb1* double mutants were as short as the single *d1* mutant plants and had as many axillary branches as the *tb1* mutants. The axillary branches were short but were male (data not shown). Similarly, the *an1*; *tb1* double mutant had as many axillary branches as single *tb1* mutants, and the axillary branches at the ear position were short but were completely masculinized (Figure 8A). Therefore, the sexual fate of the axillary branch was not dependent on its length.

We also crossed *tb1* and *ts2* mutants together. Tassels on *ts2* mutant plants have the architecture of normal tassels, but all florets are feminized. Stamen develop-

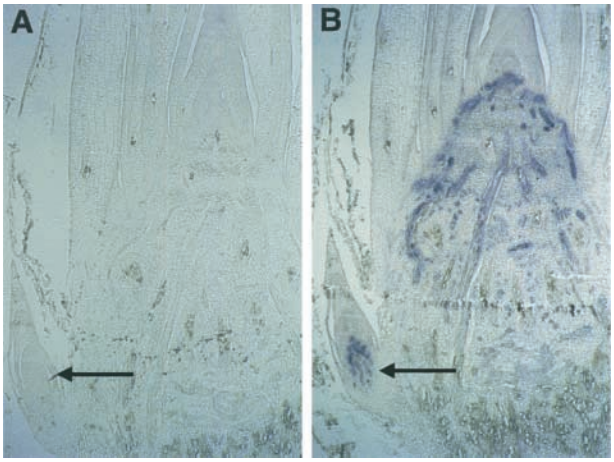

FIGURE 6.—*tb1* expression is not detected in a teosinte shoot apex. Serial longitudinal sections through the shoot apex of a teosinte plant 17 days after germination probed with (A) *tb1* antisense RNA probe and (B) *kn1* antisense RNA probe. This apex includes a well-developed axillary branch (arrows).

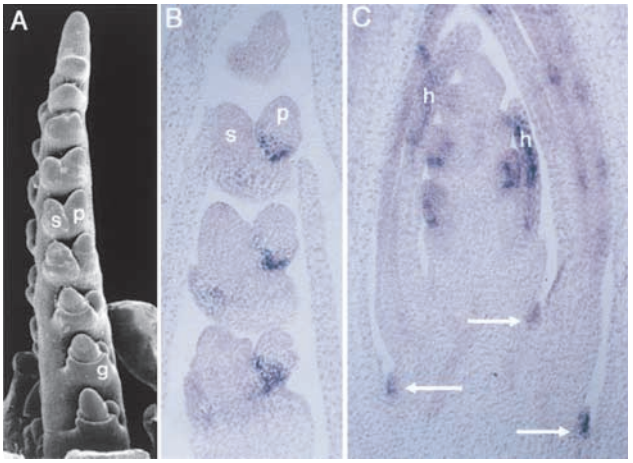

FIGURE 7.—Teosinte inflorescence development. (A) SEM of secondary branch of a teosinte plant. Secondary branches are typically feminized. (B) *In situ* hybridization of *tb1* in a female inflorescence showing strong expression at the base of one of the spikelets. (C) *In situ* hybridization of the primary axillary branch in a Teosinte-M1L plant. s, sessile spikelet; p, pedicellate spikelet; g, glume; h, husk leaf. Arrows point to secondary axillary branches, which are suppressed in Teosinte-M1L.

ment is completely suppressed (DELONG *et al.* 1993). The axillary branches in the ear position of *ts2*; *tb1* double mutants were elongated as in *tb1* single mutants (Figure 8A); however, the inflorescence at the tip of this branch was completely feminized. Therefore, a branch can be long and female. The results of these double-mutant analyses suggest that the length of the branch does not determine whether the inflorescence on that branch is male or female.

We also noted an additional effect of *tb1* in *ts2*; *tb1* double mutants. Normally, in *tassel seed* mutants, the presence of the feminized tassel suppresses development of the ears (IRISH *et al.* 1994 and our observations). However, in *ts2*; *tb1* double mutants, ear development was no longer suppressed (visible as long silks in Figure 8B). Therefore, in the absence of *tb1*, inhibitory signals from the apex no longer have an effect. This finding supports a role for *tb1* in apical dominance as previously proposed (DOEBLEY *et al.* 1997).

DISCUSSION

Differential development of axillary branches can have a dramatic effect on plant architecture. We show that the *tb1* gene of maize is expressed in tissues in maize and teosinte that are suppressed in growth and that this expression correlates with developmental changes that alter the architecture of the plant. These data support previous models that suggest that changes in *tb1* expression were critical to the evolution of maize. In addition, through inferences from expression, phenotype, and QTL analyses, we suggest that *tb1* may be involved in regulating specific steps in maize floral development.

The function of *tb1* in maize: *tb1* regulates the suppression of axillary branches, which in turn regulates formation of secondary axillary branches. We showed that the increased branching nature of *tb1* mutants results from additional axillary branches that form in the axils of elongated branches. The results suggest that a single genetic switch, that of elongation or suppression, may determine whether additional branches form. Interestingly, the long branches actually have fewer nodes, suggesting that mechanisms exist to limit additional nodes from forming when the branches are long.

tb1 was expressed early in development in the axils of P_5 and P_6 leaves in a 5-day-old seedling. The timing of expression was coincident with formation of the first distinct axillary meristem, as detected by *kn1* expression. The last five to six leaves produced on a plant, those corresponding to the region between ear and tassel, are considered sterile (VEIT *et al.* 1993). These "sterile" nodes lack visible axillary buds and do not express *tb1* or *kn1*. A delay of five to six plastochrons between leaf initiation and a visible axillary meristem occurs throughout development of the plant and may reflect a requirement of five plastochrons before completion of bud initiation.

Expression of *tb1* was visible in husk leaves but not in leaves from the main culm. In *tb1* mutants, the leaves that develop on axillary branches (in a similar location to husk leaves) have an enlarged blade region when compared to normal husk leaves in a B73 background. Thus, *tb1* is correlated with suppressing the growth of husk leaf blades in maize. *tb1* was also expressed in the culm of the axillary branch in maize, but not in the main culm, coincident with the lack of elongation seen in maize ears.

Strong expression was detected in young stamen pri-

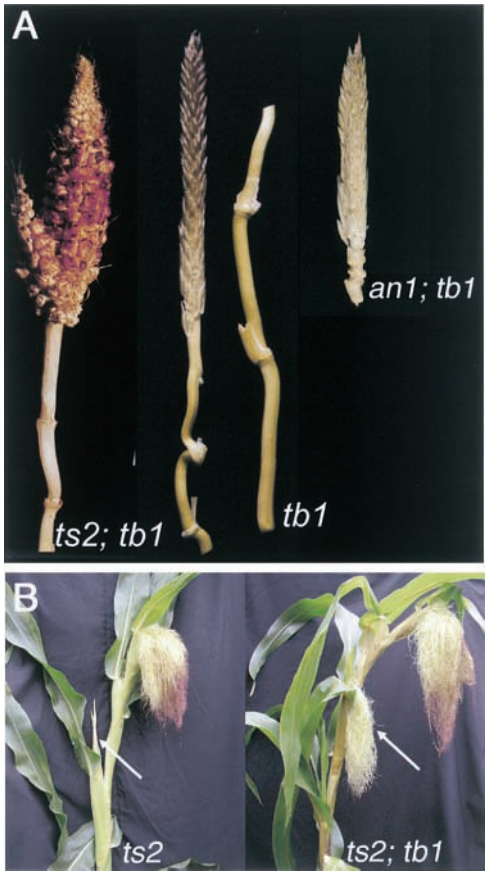

FIGURE 8.—Axillary branch of *tb1* double mutants. (A) The axillary branches shown were in the ear position. The *tb1* branch was cut into two pieces for ease of photographing. (B) Silks grow out precociously in the *ts2*; *tb1* double mutant.

mordia in the ear in both upper and lower florets. Expression persisted in stamens until they underwent developmental arrest. In contrast, weak expression was detected throughout stamen development in the tassel where they do not arrest. These results suggest that *tb1* plays a role in stamen suppression. A QTL for the percentage of staminate spikelets in the primary lateral inflorescence maps very close to *tb1* (DOEBLEY and STEC 1991), supporting such a role for *tb1*.

Function of *tb1* in teosinte: The model proposed for the evolution of maize from teosinte predicts that *tb1* expression would be upregulated in maize relative to teosinte and that this increase in expression would result in the reduction of axillary branches observed in maize (DOEBLEY *et al.* 1995a). In support of this model, Northern blot analysis revealed a reduction in the accumulation of mRNA in axillary branches relative to normal maize, when the teosinte *tb1* allele was introgressed into maize (DOEBLEY *et al.* 1997). We showed by *in situ* hybridization that *tb1* expression was not detected in primary axillary branches of teosinte, which correlates with the elongation of these branches. However, *tb1* was expressed in primary axillary branches of Teosinte-M1L, a stock of teosinte that carries the maize wild-type *tb1* allele. The expression pattern of Teosinte-M1L correlates with its maize-like phenotype (DOEBLEY *et al.* 1995a).

We showed that *tb1* was expressed in specific tissues of the secondary female inflorescences that arise from long axillary branches of teosinte. Expression was localized at the base of the pedicellate spikelet but not the sessile spikelet. This is particularly striking because the pedicellate spikelet undergoes selective developmental arrest, while the sessile spikelet progresses to form the female flowers of teosinte (DOEBLEY *et al.* 1995b). Previous QTL analyses showed that the percentage of cupules lacking the pedicellate spikelet is regulated by a locus that maps very close to *tb1* (DOEBLEY and STEC 1991; DOEBLEY *et al.* 1995a). Therefore, both QTL and expression analyses support a functional role for *tb1* in suppression of the pedicellate spikelet.

Regulation of sexual identity: Our analysis of *tb1* supports the proposed function of *tb1* as a "repressor of organ growth in those tissues in which its messenger RNA accumulates" and supports the model for regulatory changes at the *tb1* locus controlling the evolution of maize from teosinte (WANG *et al.* 1999, p. 236). This model, however, does not account for all aspects of the observed *tb1* mutant phenotype, specifically, conversion of the ear shoot into a male inflorescence. These structures are not simply ears with male florets; the number and length of internodes is altered, the leaf shape is different, and male flowers are converted to female flowers. It is possible that the fate of an axillary branch, that is, whether it is a tiller tipped by a male inflorescence or an ear tipped by a female inflorescence, is a secondary effect of *tb1*. By affecting branch elongation, *tb1* may alter the perception of signaling molecules. For example, the distance between the main stem and apex of the axillary branch could regulate branch identity, feminine *vs.* masculine, or ear *vs.* tiller. This model shares similarities with the catastrophic sexual transmutation theory proposed by ILTIS (1983). Iltis asked whether the polystichous ear of maize had evolved from the distichous ear of teosinte, which is a secondary axillary branch in teosinte, or whether the ear of maize evolved from a shortened, condensed feminized tassel of teosinte in the apical position on primary lateral branches. He proposed that there is an outer male zone of tassels and an inner female zone of ears in teosinte. He further proposed that should the primary branches of teosinte become shortened, they would find themselves within the inner female hormonal zone and produce female instead of male flowers.

We crossed *tb1* to two different gibberellin-deficient mutants to ask whether shortened axillary branches were still masculine. In both double mutants, the axillary branches in the ear position were short and completely male. We also found the converse to be true: a long branch can be female in a *ts2*; *tb1* double mutant. Thus, altering the length of the axillary branch did not change the sexual identity. Given that we can separate the sexual

identity of the axillary inflorescence from its length, it remains possible that *tb1* may play a direct role in inflorescence development in addition to a role in suppressing axillary branch elongation.

Evolutionary considerations: *tb1* is a member of the TCP family of DNA-binding transcriptional regulators (Kosugi and Ohashi 2002) that includes the Antirrhinum gene, *cycloidea* (*cyc*; Cubas *et al.* 1999). A normal Antirrhinum flower has a marked dorsoventral asymmetry due to differential development of the petals and stamens. Flowers of *cyc* mutants have reduced dorsoventral asymmetry resulting in a more radially symmetric flower. RNA *in situ* hybridization shows early expression of *cyc* in the upper, dorsal region of the floral meristem with continued expression in the upper petal and dorsal stamen primordia at later stages. The dorsal stamen does not grow out in wild-type Antirrhinum, but does grow out in *cyc* mutants. Luo *et al.* (1996) suggest that early expression of *cyc* reduces organ number in the dorsal region of the floral meristem and retards the growth rate. Investigations into the expression patterns of a related Arabidopsis gene, *TCP1*, showed a transient, dorsal expression in floral meristems but no expression in floral organs (Cubas *et al.* 2001). Suppression of stamen growth does not occur in Arabidopsis. It is intriguing that members of the TCP family are expressed in axillary meristems in three different species and that their expression in stamen primordia is correlated with suppression.

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