Maize SBP-box transcription factors unbranched2 and unbranched3 affect yield traits by regulating the rate of lateral primordia initiation

George S. Chuck1,2, Patrick J. Brown3, Robert Meeley2, and Sarah Hake1

1Plant Gene Expression Center, Albany, CA 94710; 2Department of Crop Sciences, University of Illinois, Urbana, IL 61801; and 3Pioneer Hi-Bred International, Johnston, IA 50131

The separation of male and female flowers in maize provides the potential for independent regulation of traits that affect crop productivity. For example, tassel branch number controls pollen abundance and length of shedding time, whereas ear row number directly affects kernel yield. Mutations in duplicate SBP-box transcription factor genes unbranched2 (ub2) and ub3 affect both of these yield traits. Double mutants display a decrease in tassel branch number and an increase in ear row number, both of which are enhanced by loss of a related gene called tasselsheath4 (ts4). Furthermore, triple mutants have more tillers and leaves—phenotypes seen in Corngrass1 mutants that result from widespread repression of SBP-box genes. Immunolocalization of UB2 and UB3 proteins revealed accumulation throughout the meristem but absence from the central domain of the meristem where cells regenerate. Thus, ub2, ub3, and ts4h function as redundant factors that limit the rate of cell differentiation to the lateral domains of meristems. When these genes are mutated, cells are allocated to lateral primordia at a higher rate, causing a net loss of cells from the central domain and premature termination of the inflorescence. The ub3 locus is tightly linked to quantitative trait loci (QTL) for ear row number and tassel branch number in both the nested association mapping (NAM) and intermated B73 by Mo17 (IBM) populations of maize recombinant inbreds, indicating that this gene may be agronomically important. Analysis of ear and tassel QTL across biparental families suggests that multiple mutations in ub3 independently regulate male and female inflorescence development.

Significance

Crop yields are dependent on the number of lateral primordia made in the inflorescence. In maize unbranched mutants, excess lateral primordia are made at the expense of the stem cells located in the center of the meristem. Ultimately, the unbranched mutant meristem lacks enough cells to regenerate and thus, terminates prematurely. This study shows that the duplicate transcription factors unbranched2 and unbranched3 function together to decrease the rate of lateral primordia initiation, thus giving the stem cells of the meristem enough time to regenerate. Variants of the unbranched3 gene affect different aspects of lateral primordia initiation that control crop yield.

Author contributions: G.S.C. designed research; G.S.C. and P.J.B. performed research; G.S.C., P.J.B., R.M., and S.H. contributed new reagents/analytic tools; G.S.C., P.J.B., and S.H. analyzed data; and G.S.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

1To whom correspondence should be addressed. Email: georgechuck@berkeley.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1407401112/-/DCSupplemental.
Results

tassel branch number (TBN) and increased ear row number analysis was performed for duplicate within the central domain of the meristem. The restrict initiation of lateral primordia, thus allowing cell renewal not in the central domain, where meristem renewal occurs. These showed expression throughout lateral domains of meristems but not in the central domain, where meristem renewal occurs. These results indicate that ub2 and ub3 function together with tsh4 to restrict initiation of lateral primordia, thus allowing cell renewal within the central domain of the meristem. The ub2 and ub3 mutant phenotypes affect several important agronomic properties, and genome-wide association study analysis implicates two independent natural variants at the ub3 locus with increased tassel branch number (TBN) and increased ear row number (ERN), respectively.

Results

ub2 and ub3 Regulate Primordia Initiation. A reverse genetic analysis was performed for duplicate SBF-box genes that group with tsh4, a gene responsible for repression of bract primordia in maize inflorescences (8). These genes, formerly known as TC305612 and TC282500 (6), were renamed ub2 and ub3 based on their mutant phenotypes. UB2 and UB3 share 79% overall amino acid identity and 86.1% identity within the DNA-binding domains (Fig. S1). Phylogenetic analysis showed that ub2 and ub3 are duplicated loci in maize, with single-copy orthologs present in sorghum, rice, and Brachypodium (Fig. S2). The closest maize homolog to ub2 and ub3 is tsh4. Two independent Mutator (Mu) transposon insertions (12) into ub2 were identified: one in a 99-bp intron near the exon border and one into an exon. In addition, three ub3 insertions into the last exon of ub3 were also identified (Fig. 1C). From RNA gel blots, both ub2 alleles completely disrupt transcription, whereas the ub3-mum1 allele only partially reduces transcription (Fig. 1B).

To test whether these alleles make functional protein, an antibody was raised to full-length UB3 protein. Given the similarity of UB2 and UB3, the serum was predicted to detect both proteins. This result was confirmed by Western blots using nuclei from 3.5-wk-old and 0.5-cm ear primordia, where accumulation was seen in WTs and single mutants but not several double-mutant combinations (Fig. 1C and D). For example, a band was detected in ub3-mum3 single-mutant shoots and ears, although it lacks functional transcript. This band is likely to correspond to UB2 protein, because ub2-mum1/ub3-mum3 double mutants show no detectable protein (Fig. 1C). In ear tissue, however, the ub2-mum1/ub3-mum1 double mutant had a small amount of protein, indicating that ub3-mum1 is only a partial loss of function (Fig. 1D). Thus, the anti-UB3 antibody recognizes both UB2 and UB3 proteins, and the lack of any protein in ub2-mum1/ub3-mum2 and ub2-mum1/ub3-mum3 double-mutant combinations indicates that these alleles are likely loss-of-function null mutants.

The ub2-mum1 and ub3-mum1 alleles were introgressed into the WT W22 background and observed for phenotypic differences relative to W22. Both ub2-mum1 and ub3-mum1 single mutants showed a modest but significant reduction in TBN, with the ub3-mum1 allele being slightly more severe (Fig. 2A and Fig. S3A). This decrease in TBN was greatly enhanced in three different double-mutant combinations (Fig. 2A and Fig. S3A). In addition, thickening appeared near the tip of the central spike of the tassel of the ub2-mum1/ub3-mum3 double mutant (Fig. 2A), indicating the presence of excess spikelets. A similar reduction in TBN was observed in single mutants of tsh4 (8). To determine whether tsh4 may function redundantly with ub2 and ub3, double- and triple-mutant combinations were made. This putative redundancy was confirmed by a reduction in TBN in two different

![Fig. 1. Gene structure and expression of ub2 and ub3. (A) Positions of Mu transposon insertions in ub2 and ub3 genes. (B) RNA gel blot analysis of ub2 and ub3 alleles from 3.5-wk-old dissected shoot apices. The ubiquitin (ubi) gene was used as a loading control. (C) Western blot analysis using 3.5-wk-old nuclear extracts from inbred B73, ub2/ub3 double mutants, and ub3-mum3 single mutants. Ponceau protein staining was done as a loading control. (D) Western blot of nuclear extracts from 0.5-cm ear primordia of B73, Mo17, and ub2 and ub3 single and double mutants. The TSH4 antibody was used as a protein loading control.](Image)

![Fig. 2. Floral phenotypes of ub2 and ub3 mutants. (A) Tassel phenotypes of ub2 and ub3 mutant alleles; ub2-mum1, ub3-mum1, and the double mutant are in the W22 inbred background. Triple-mutant tassel in the W22 background is shown in inset. (B) Ear phenotypes of W22 and double mutants. (C) Razor blade hand sections of the midpoint of the ears in C. Asterisks indicate kernel rows. (D) Fertilized ears of the double mutant compared with WT A619. (E) Mature field-grown plants of W22, ub2, ub3, and the double mutant. (F) Mature field-grown plants of tsh4 and double- and triple-mutant combinations in W22 background showing an increase in tillering.](Image)
Defects in ear length and diameter were observed in single and double mutants (Fig. 2 B–D). The most severe length defect was observed in the triple mutant that was approximately one-half that of WT (Fig. S3B), leading to greatly reduced fertility. Significant increases in ear diameter were seen in ub3 single and ub2 ub3 double mutants (Fig. S3C) that also displayed fasciated tips (Fig. 2 B and D). Sections through the midpoint of the double-mutant ears showed nearly two times the number of kernel rows in the double mutants (Fig. 2C). When fertilized, the tips of these ears were disorganized, and the kernels crowded together (Fig. 2D). Taken together, these phenotypes indicate that meristem function during the floral phase of male and female inflorescences is altered by the simultaneous loss of ub2 and ub3.

**ub2 and ub3 Repress Tiller ing and Leaf Initiation Redundantly with tsh4.** Vegetative phenotypes of ub2 and ub3 single and double mutants were observed in the field. Although ub2 ub3 double mutants do not affect tiller number, tillering was significantly increased in all double-mutant combinations with tsh4 (Fig. 2 E and F), with the strongest increase observed in two different triple-mutant combinations (Fig. S4A).

Leaf number was also altered in the double and triple mutants. Because juvenile leaf number is difficult to assay in the field because of senescence and predation, leaf number above the upper most ear node was counted instead. Apart from ub2 mum1 ub3 mum1, which may not be a complete loss of function, double mutants made significantly more leaves, whereas triple mutants made up to two times as many upper leaves (Fig. S4B), mostly found at the base of the tassel (Fig. 2A). Thus, ub2 ub3 tsh4 functions together redundantly to initiate tassel branches and repress tiller and leaf initiation. Despite the increase in leaf number, the double and triple mutants flowered at the same time as the WT, suggesting that plastochron is, in fact, shortened in SBP-box mutants.

### Lateral Primordia Form at the Expense of the Apical Meristem in Double Mutants.

SEM was performed on the double mutants to determine the origin of the extra primordia in the inflorescence. In normal maize tassels, the main inflorescence meristem (IM) first initiates a limited number of branch meristems (BMs) that determine the origin of the extra primordia in the inflorescence. In the double mutant and determine whether it retained meristem identity. Recessive kn1 mutants are shootless in some backgrounds (16) or have smaller inflorescences with reduced numbers of lateral primordia in others (17). KN1 is found in all meristems, and it is down-regulated in lateral organs (13–15). KN1 immunolocalization experiments showed that the residual meristem of the double mutant has no KN1 expression (Fig. 3 F and G). Moreover, the central cells of the residual meristem appear vacuolated rather than densely cytoplasmic, further indicating that they have lost meristem identity and terminally differentiated (Fig. 3G). These results indicate that meristem maintenance and renewal are compromised in the ub2 ub3 double mutants.
and UB3 proteins continue to be expressed throughout the transition-stage meristem, young leaves, and stem (Fig. 4D), although meristem tip expression is still restricted. In the adjacent section, TSH4 protein was found in a limited domain (Fig. 4E) in leaf primordia and older leaves but not in axillary buds or stems. The expression of TSH4 overlaps completely with UB2 and UB3, consistent with genetic redundancy between all three genes. In female inflorescence ears, UB2 and UB3 were excluded from the tip of the IM as well as the SPMs (Fig. 4F), although high expression was found in bracts subtending the SPM. In early-stage tassels, high UB2 and UB3 expression was found at the base of the tassel, where BMs form, but not in BMs themselves or the inflorescence tips (Fig. 4G). Double labeling with TSH4-specific antibodies showed localization in young bracts subtending the SPM primordia in the upper part of the tassel. At this stage, UB2 and UB3 were expressed in the portion of the tassel that defines the branch zone (BZ) but not the zone that makes SPMs. This complementary expression pattern was confirmed in double-labeling experiments with UB3 and TSH4 or RA2 in radial sections of tassels (Fig. S5 D and E). Thus, UB2 and UB3 are not expressed in the meristems that are affected in the double mutant, including IMs, SPMs, and BMs.

UB3 Maps to Quantitative Trait Loci for TBN and ERN. Quantitative trait loci (QTL) analysis of inflorescence traits was done using the nested association mapping (NAM) population. This population was made by crossing B73 with 25 different inbreds and then selfing to create recombinant inbred lines (18), and detected QTL for TBN, ERN, and BZ at the marker most closely linked to ub3 (19). The ub3-linked ERN QTL has the largest effect of any inflorescence QTL in the NAM population and explains 12% of the variance in ERN across the entire NAM population (Figs. S6 and S7). A larger set of HapMap2 SNPs has now been available for the parents of the NAM population (20). From an ~18-Mb region encompassing the 95% confidence intervals of the ub3-linked ERN and TBN QTL on chromosome 4, we extracted a set of 8,851 polymorphic HapMap2 SNPs that contained no missing data and no heterozygous genotypes among 27 NAM founder lines, including Mo17, and tested each SNP for association with ERN and TBN (Materials and Methods and Fig. 5A). For each trait, we found separate, highly significant nonsynonymous substitutions within the third exon of ub3 downstream of the SBP domain. One variant (Val260Met; 199,457,430 bp) is found in four NAM founder lines (CML103, Mo17, Oh7B, and Tx303) and associated with increased TBN. Another variant (Ser220Asn; 199,457,549 bp) is found in two NAM founders (B73 and Hs301) and associated with increased ERN (Fig. 5B). For both TBN and ERN, genome-wide associations slightly more significant than the nonsynonymous substitution in ub3 were found within the QTL confidence interval (Fig. 5A). These SNPs could represent synthetic associations (21) resulting from either additional alleles at ub3 or closely linked QTL. No correlation was found between QTL effects for TBN and ERN in the ub3 region (Table S1), suggesting that independent mutations, rather than a single pleiotropic mutation, are responsible for the TBN and ERN QTL colocalization.

Another high-resolution mapping resource, intermated B73 by Mo17 (IBM), was made by crossing B73 with Mo17 and intermating the F1 progeny four times before selfing (22). Using a cleaved amplified polymorphic sequence (CAPS) marker that distinguishes the two polymorphisms in ub3, 167 families derived from IBM populations with IBM, Mo17, or B73 parental alleles at the ub3 locus were genotyped. One variant (Val260Met; 199,457,430 bp) is found in four NAM founder lines (CML103, Mo17, Oh7B, and Tx303) and associated with increased TBN. Another variant (Ser220Asn; 199,457,549 bp) is found in two NAM founders (B73 and Hs301) and associated with increased ERN (Fig. 5B). For both TBN and ERN, genome-wide associations slightly more significant than the nonsynonymous substitution in ub3 were found within the QTL confidence interval (Fig. 5A). These SNPs could represent synthetic associations (21) resulting from either additional alleles at ub3 or closely linked QTL. No correlation was found between QTL effects for TBN and ERN in the ub3 region (Table S1), suggesting that independent mutations, rather than a single pleiotropic mutation, are responsible for the TBN and ERN QTL colocalization.

Discussion

The number of lateral primordia made by the male and female inflorescences of maize is a major determinant of yield. In tassels, such as lateral primordia include tassel branches, spikelets, and spikelets, all of which control the length of shedding time and pollen quantity. In ears, the number of spikelet pairs affects kernel yield. Because all lateral primordia are products of meristems, increasing the number of these primordia to improve yield requires alteration of meristem activity. We hypothesize that ub2 and ub3 control the rate at which cells leave the undifferentiated central zone of the meristem and enter the peripheral zone where lateral primordia initiate (Fig. 5D). In ub2 and ub3 double mutants, extra lateral primordia are made too quickly at the expense of the apical meristem, which lacks enough cells to regenerate. Consequently, expression of the MB is reduced (Fig. 3 D and E), leads expression of meristem markers, such as kn1 (Fig. 3 F and G), and terminates prematurely. The roles of these genes in regulating the rate of lateral primordia initiation are similar to the functions of SPL9 and SPL15 in vegetative shoots of Arabidopsis (24, 25), although tsh4, ub2, and ub3 primarily affect the floral phase of development.

UB2 and UB3 accumulate throughout the base of the meristem but are excluded from the central tip of the SAM, where cell renewal occurs. Because these SBP-box transcription factors are targets of miR156 (6), the lack of expression in the central tip of the meristem may be a consequence of microRNA repression. In support of this idea, tsh4, which is also targeted by miR156, is expressed in a pattern complementary to miR156 in the IMs (8). Recent reports show repression of miR156 by sugar (26, 27), which itself is a non-cell-autonomous mobile signal. Because
leaves are carbohydrate sources, perhaps the sugar that they produce travels to the peripheral zone of the meristem to repress microRNA expression and thus, allow $ub2$ and $ub3$ expression. It is possible that these sugars either do not travel far enough to the central tip of the SAM or are actively excluded from the central tip of the SAM, allowing miR156 to remain active and repress $ub2$ and $ub3$.

It is unclear how $SBP$-box transcription factors affect the rate of organ initiation without being expressed in the central zone. Classic experiments performed in potato showed that a diffusible substance travels between leaves to control the rate of leaf growth and initiation (28). More recently, it has been shown that leaf-specific promoters driving $SPL9$ expression in Arabidopsis were able to affect the rate of leaf initiation in the SAM (5). Such results are consistent with the hypothesis that $SBP$-box transcription factors or their target genes may function as nonautonomous signals that move through the shoot apex to regulate plastochron. Because we observed no sign of $UB2$ or $UB3$ protein throughout the IM, SPM, and spikelet meristem or in the apical tips of the vegetative meristem, it is more likely that a downstream factor in the $SBP$-box gene pathway is the noncell-autonomous signal rather than the $SBP$-box proteins themselves.

Loss-of-function mutations in $ub2/ub3$ doubles display seemingly contradictory phenotypes in male vs. female inflorescences with regard to lateral meristems. For example, double mutants initiate several extra rows of SPMs in ears and tassels. In tassels, however, a different type of lateral meristem, the BM, is not made in excess, and the double mutant is nearly unbranched. This difference can be explained by the fact that double mutants make several extra leaves (Fig. S4B). These extra leaves, however, do not simply form in place of tassel branches, because the number of extra leaves does not equal the number of missing tassel branches in the mutants. It is more likely that BM can only form when vegetative growth is suppressed, allowing the tassel to initiate floral structures exclusively. Because both $ub2$ and $ub3$ are expressed exclusively in the basal zone of tassels, where BMs are made (Fig. 4G), it is possible that they function to suppress vegetative leaf initiation in that region. These observations suggest that highly branched inflorescences characteristic of panicoid grasses can only be obtained if vegetative leaf initiation is suppressed during the floral phase by $SBP$-box genes.

Because $ub2$ and $ub3$ affect TBN and ERN, two traits that directly affect yield, polymorphisms in these genes were tested for association with agronomically important inflorescence traits. A previous study using the NAM population showed the presence of $ub3$-linked QTL for TBN and ERN (19). In addition, a previous QTL study in a B73-Mo17 biparental family identified a TBN QTL that mapped close to $ub3$ (29). A more detailed analysis

![Fig. 5. Allelic diversity in $ub3$ and its association with inflorescence variation with a model for function. (A) Physical and genetic positions of $ub3$ relative to marker–trait associations for ERN and TBN in the NAM population. The 95% confidence intervals (95% CIs) for $ub3$-linked ERN and TBN joint linkage QTL are shown in blue. The positions of the most significant markers/5SNPs for joint linkage and genome-wide association study (GWAS) analyses of ERN and TBN are shown as red dots. The position of $ub3$ on each map is shown as green dots. The 95% CI for the $ub3$-linked TBN QTL extends 2.386 Mb and contains 44 predicted genes, whereas the 95% CI for the $ub3$-linked TBN QTL extends 9.695 Mb and contains 191 predicted genes. All physical positions refer to AGPv2.1. (B) Clustal alignments of $UB3$ from teosinte, maize, and other panicoid grasses. Polymorphisms are indicated by asterisks, and amino acid differences are in red. (C) Variation in average BZ length, branch number, and ERN in B73 vs. Mo17 populations of IBM lines. Differences between the two populations are highlighted in red. (D) Model for $UB2/UB3$ function. $UB2/UB3$ is not expressed in undifferentiated cells at the tip of the meristem, where renewal occurs. Red arrows signify $UB2/UB3$ control of cell movement out of the central zone into the peripheral zone, where cell differentiation and lateral organ initiation occur.
revealed the existence of two derived polymorphisms in the 3′-end of UB3 that are not present in teosinte, the progenitor of maize, which possesses two-rowed ears or several other panicle grass orthologs (Fig. 5B). These two polymorphisms in the ub3 gene are associated with distinct effects on male and female inflorescences: the Val260Met mutation associates with higher TBN in inbred lines, such as Mo17, whereas the Ser220Asn mutation associates with higher ERN in inbreds, such as B73 (Fig. 5C). Because both TBN and ERN are yield-related traits in maize, the physical proximity of the candidate SNPs for these traits in ub3 (119 bp apart) could give rise to pseudooverdominance in hybrids that combine two advantageous alleles (e.g., B73 and Mo17). It is unclear at this time how these B73 and Mo17 polymorphisms might affect UB3 function. Western blots comparing B73 and Mo17 ear nuclear extracts appeared similar (Fig. 1D), indicating that the polymorphisms may affect protein function as opposed to protein amount or stability.

The potential for ub3 to be used as a tool to improve the agronomic properties of crop plants is supported by the function of its ortholog in rice, WEALTHY FARMER’S PANICLE (WFP)/IDEAL PLANT ARCHITECTURE (IPA) (Fig. S2). Both WFP and IPA are dominant gain-of-function alleles that improve grain yield in rice by reducing tilling and increasing panicle branching (30, 31). Phenotypes that are the opposite of the ub2/tsh4/SPL14 loss-of-function phenotype. IPA causes overexpression of OsSPL14 through a mutation in the miR156-binding site that releases the gene from negative regulation by the microRNA (30). Extrapolating from the loss-of-function phenotypes of ub2 and ub3, it is possible that overexpression of these SBP-box genes may delay lateral primordia initiation and differentiation, leaving more time for the meristem to renew and regenerate. After the floral transition, more meristem cells would then be available to produce a more complex inflorescence, thus leading to higher yield. Consequently, these SBP-box genes could be important tools with which to alter plant architecture and theoretically, increase yields in a variety of crop plants.

Materials and Methods

Isolation of ub2 and ub3 and Genetic Analysis. All ub2 and ub3 alleles were isolated from pools of Mu transposon-mutagenized populations and screened by PCR with Mu-end oligos and either ub2 or ub3 oligos. The ub2-mum1 and ub3-mum1 alleles were introgressed into the W22 background by backcrossing at least two times and then selfing; ub2 and ub3 were followed by PCR using the Mu-end primer MuS242, the SFI primer (TAAGAA-GAGGCTGCGCAAAACGGCT) for ub2, and the TFI primer (TGCTGTATTCT- CATACCCAGG) for ub3. Triple mutants were made by crossing the single mutants to sb4-M1 in the W22 background, selfing, and scoring by PCR. All tassel, leaf, and tillering phenotypes were scored in the field.

Generation of a UB3 Antibody and Immunolocalization. Full-length UB3 protein coupled to an histidine tag was made in the pET21d expression vector (Novagen). Recombinant protein was isolated under denaturing conditions, dialyzed, and injected into two guinea pigs (Cocalico). The serum was affinity-purified using full-length UB3 protein coupled to a GST tag (Novagen). Immunolocalization was performed as previously described (8).

SEM. Samples were fixed in formaldehyde acetic acid overnight, critical point-dried, sputter-coated, and examined as previously described (8).

Association of ub3 SNPs with ERN and TBN in the NAM and IBM Populations. Association analysis using NAM and IBM populations is described in detail in SI Materials and Methods.

ACKNOWLEDGMENTS. We thank Katsutoshi Tsuda, Michael Lewis, and China Lunde for critical reading of the manuscript. This work was supported by National Science Foundation Grants PGR-1339332 (to G.S.C.) and PGR-1238030 (to P.J.B.) and Binational Agricultural Research and Development Grant IS-4536-12C (to S.H.)

Association of \textit{ub3} SNPs with ERN and TBN in the NAM Population. Linkage and joint linkage analyses of ERN and TBN in the NAM population have been described previously (1). Briefly, in the joint linkage analysis of ERN across all 26 NAM families, the closest marker to \textit{ub3} (m388) was the first QTL to enter the model, explained 12.0\% of the variation in ERN across the entire NAM population, and was the largest-effect ERN QTL across all of the NAM (Fig. S6A) and in 13 of 26 individual families (Fig. S6B). In the joint linkage analysis of TBN across all 26 NAM families, the closest marker to \textit{ub3} (m388) was the sixth QTL to enter the model, explained 1.4\% of the variation in TBN across the entire NAM population, and was not remarkable in terms of effect size (Fig. S7).

Here, we used the newly available set of HapMap2 SNPs (www.panzea.org) to redo a genome-wide association study scan across an \textasciitilde18-Mb interval encompassing the 95\% confidence intervals of the \textit{ub3}-linked joint linkage QTL for ERN and TBN. The phenotypes used for the genome-wide association study consisted of the residuals from the complete joint linkage models excluding QTL on chromosome 4, and confidence intervals were estimated as described previously for leaf architecture traits (2). A set of 575,668 SNPs across this region was filtered to include only those that were polymorphic and contained no missing or heterozygous genotypes across 27 founder lines (8,851). Interval genotypes were imputed as the mean of two flanking markers weighted by physical distance, and phenotypes were regressed on each SNP genotype in turn using family as a covariate.

\textit{ub3} Genotyping and Phenotypic Analysis of IBM Lines. In total, 167 IBM families were scored for either the B73 or Mo17 alleles using a CAPS marker to a fragment amplified using the \textit{ub3} 7F1 primer (TGCTGGATTTCATACCCAAGG) and the 7R2 primer (TGTGCAGCTACGAGATGTGC) and then digested with HpaII, distinguishing the two alleles. These results were corroborated using published \textit{ub3} SNP data (3). These genotype data were correlated with raw measurements for TBN, BZ length, and ERN for these same families across multiple field environments as described previously (1). Best linear unbiased predictors for branch number, BZ, and ERN were compared between 89 RILs that received the B73 \textit{ub3} allele and 79 RILs that received the Mo17 \textit{ub3} allele, and \textit{P} values were calculated by two-sided \textit{t} test.


\textbf{Fig. S1.} Amino acid alignment of UB2 and UB3. Amino acid alignment of (upper rows) UB2 and (lower rows) UB3 full-length proteins. Underlined sequence represents the SBP-box.
Fig. S2. Phylogenetic tree of SBP-box transcription factors from grasses. Neighbor-joining tree of all annotated SBP-box proteins from maize, sorghum, Brachypodium, and rice.
Fig. S3. Tassel and ear measurements of SBP-box mutants. (A) TBN in W22, ub2, ub3, and tsh4 double- and triple-mutant combinations. *Significant differences with W22 (P value < 0.0003 was calculated by t test). (B) Average lengths of inbred W22 and SBP-box mutant ears. Error bar = SD. All mutants are significantly different from W22 based on two-tailed t tests. *P value < 0.0024. (C) Average diameters of W22 and SBP-box mutant ears at midpoint. Only single-, double-, and triple-mutant combinations with ub3 are significantly different from the inbred based on two-tailed t tests. Error bar = SD. *P value < 0.0143.
Fig. S4. Tiller and leaf counts of SBP-box mutants and expression of tillering markers. (A) Tiller counts in field-grown plants of single-, double-, and triple-mutant combinations. *Significant differences from W22 (P value < 1.34E-06 was calculated by t test). (B) Upper leaf number counts above ear nodes of single, double, and triple mutants. *Significant differences from WT W22 (P value < 5.09E-05 was calculated by t test).
Fig. S5. Immunolocalization of early meristem markers in mutant and WT tassels. (A–C) Immunolocalization in adjacent sections of ub2-mum1/ub3-mum3 tassel. (A) KN1 immunolocalization showing expression only in the IM. (B) TSH4 immunolocalization showing expression in stem and early bract primordia (BR). (C) RA2 immunolocalization showing lack of expression. (Scale bars: A–C, 500 μm.) (D) Double labeling of WT tassel with UB3 (blue) and TSH4 (gold) antibodies. The proteins occupy separate domains in the SPM; UB3 is absent from the SPM, whereas TSH4 is present in the BR of the SPM (arrows). (E) Double labeling of the base of WT tassel with UB3 (blue) and RA2 (gold). UB3 is absent from the BM and SPM, whereas RA2 is present in both.
Fig. S6. QTL effects for ERN in the NAM population. (A) QTL effects from joint linkage analysis across the entire NAM population. (B) Joint linkage QTL effects separated by family. The ub3-linked ERN QTL is shown in red.
Fig. S7. QTL effects for TBN in the NAM population. (A) QTL effects from joint linkage analysis across the entire NAM population. (B) Joint linkage QTL effects separated by family. The ub3-linked TBN QTL is shown in red.
Table S1. Comparison of joint linkage and GWAS effects at ub3-linked QTL with genotypes underlying candidate polymorphisms

<table>
<thead>
<tr>
<th>Sample</th>
<th>Joint linkage*</th>
<th>GWAS†</th>
<th>Ser220Asn‡</th>
<th>Joint linkage*</th>
<th>GWAS†</th>
<th>Val260Met§</th>
</tr>
</thead>
<tbody>
<tr>
<td>B97</td>
<td>−0.24</td>
<td>1</td>
<td>1</td>
<td>0.52</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CML103</td>
<td>−0.5</td>
<td>1</td>
<td>1</td>
<td>0.57</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CML228</td>
<td>−0.48</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CML247</td>
<td>−0.52</td>
<td>1</td>
<td>1</td>
<td>0.36</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CML277</td>
<td>−0.32</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CML322</td>
<td>−0.54</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CML333</td>
<td>−0.21</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CML52</td>
<td>−0.62</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CML69</td>
<td>−0.61</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HP301</td>
<td>NS</td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL14H</td>
<td>−0.42</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K11</td>
<td>−0.6</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K13</td>
<td>−0.66</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ky21</td>
<td>NS</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M162W</td>
<td>−0.32</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M37W</td>
<td>−0.35</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mo17</td>
<td>−0.44</td>
<td>1</td>
<td>1</td>
<td>0.56</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mo18W</td>
<td>−0.54</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MS71</td>
<td>NS</td>
<td>0</td>
<td>1</td>
<td>0.28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NC350</td>
<td>−0.83</td>
<td>1</td>
<td>1</td>
<td>−0.39</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NC358</td>
<td>−0.57</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OH43</td>
<td>−0.37</td>
<td>1</td>
<td>1</td>
<td>−0.58</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OH78</td>
<td>−0.23</td>
<td>1</td>
<td>1</td>
<td>0.67</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P39</td>
<td>−0.38</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T303</td>
<td>−0.65</td>
<td>1</td>
<td>1</td>
<td>0.45</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>T218</td>
<td>−0.36</td>
<td>1</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Effects/alleles that differ significantly from B73 are shaded.

*Joint linkage QTL effects. Effects not significant (NS) in an individual family (P ≥ 0.05) are marked.
†Genotypes of the most significant SNP in each genome-wide association study (GWAS) analysis; 0 indicates the reference genome (B73) genotype.
‡Genotypes of the nonsynonymous SNPs in the third exon of ub3 at 199,457,549 and 199,457,430.