

Cloning and Characterization of the Classical Maize Mutant, *Polytypic1*.

By

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Polytypic1 (*Pt1*) is a semi-dominant mutant in maize that affects inflorescence development. To understand how *Pt1* functions in development, I examined the phenotype of *Pt1* mutants in multiple inbred backgrounds. *Pt1* defects are restricted to the inflorescence, but the severity of the phenotype is background dependent. In B73 and Mo17 inbreds, *Pt1*/+ mutants have severe floral defects; floral meristems initiate ectopic floral organs and *Pt1*/+ ears are female sterile. *Pt1*/+ tassels have similar defects as the ear, but are generally less severe. In B73, *Pt1*/+ tassels contain fewer spikelets on the branches, and florets often produce extra floral organs. In Mo17, *Pt1*/+ tassels produce silks. Because *Pt1*/+ ears are sterile in B73 and Mo17, I could not examine the *Pt1* homozygous phenotype. In A619, however, the *Pt1* phenotype is mild and *Pt1*/+ are female fertile. To identify the gene responsible for the *Pt1* mutant phenotype, we pursued a positional cloning approach coupled with RNA-seq. I mapped *Pt1* to a 6.8cM interval (~5Mbp) on chromosome 6 and performed RNA-sequencing to identify RNAs with changes in

expression levels or sequence. The *Pt1*-containing interval contains 111 genes that are expressed in ear primordia, nine of which are differentially expressed. Notably, 13 MADS-box transcription factors, which are known floral regulators, are dramatically downregulated in *Pt1* mutants. The characterization of *Pt1* mutants will give insight into the mechanisms that underlie normal inflorescence development.

Cloning and Characterization of the Classical Maize Mutant, *Polytypic1*.

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TABLE OF CONTENTS

TITLE PAGE.....	i
COPYRIGHT PAGE.....	ii
SIGNATURE PAGE	iii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS	v
LIST OF TABLES.....	vi
LIST OF FIGURES	vii
Introduction	1
Maize is a staple crop and model species for grasses.....	1
Maize inflorescence Development	1
Inflorescence Architecture Depends on Meristem Activity	4
Floral Development in Grasses.....	6
Genetic regulation of Floral Development	6
MADS-box transcription factors regulate floral development in angiosperms.....	6
The ABC Model	7
Classical Maize Mutant <i>Polytypic1</i>	11
Methods	12
Phenotypic Data Collection.....	12
Seed Germination and Plant Growth.....	12
Genotyping	13
Scanning Electron Microscopy (SEM).....	13
RNA Extraction	13
RNA Sequencing and Data Analysis.....	14
Results	18
Characterization of <i>Pt1</i> Phenotype	18
Identification of the gene responsible for the <i>Pt1</i> mutant phenotype.....	29
Discussion and Future Directions.....	38
Phenotypic Characterization.....	38
Identification of the <i>Pt1</i> causative gene	40
<i>Pt1</i> a master regulator in floral development	42
LIST OF REFERENCES.....	43

LIST OF TABLES

Table 1. Summary of Individuals planted and collected	21
Table 2. SNPs predicted within the <i>PtI</i> -containing interval	34
Table 3. Summary of the number of genes expressed and differentially expressed in the <i>PtI</i> mutants.....	35
Table 4. Nine genes are differentially expressed within the <i>PtI</i> -containing interval.....	36
Table 5. MADS-box genes are downregulated in the <i>PtI</i> mutants	37

LIST OF FIGURES

Figure 1. Maize Development	3
Figure 2. Inflorescence Development.....	5
Figure 3. The ABC model	10
Figure 4. Map of <i>Pt1</i>	15
Figure 5. RNA quality	16
Figure 6. MA Plot.....	17
Figure 7. The <i>Pt1</i> Phenotype in B73 inbred	22
Figure 8. The <i>Pt1</i> Phenotype in Mo17 inbred	23
Figure 9. The <i>Pt1</i> Phenotype in A619 inbred.....	24
Figure 10. Spikelet Density Plot.....	25
Figure 11. Silkiness of the <i>Pt1</i> tassels	26
Figure 12. Scanning Electron Micrographs of WT and <i>Pt1</i> tassels in B73.....	27
Figure 13. Scanning Electron Micrographs of <i>Pt1</i> /+ ears in B73	28

Introduction

Maize is a staple crop and model species for grasses

Zea mays (maize) is a key crop to study and is valued because of its high impact in agricultural production and revenue. Maize, along with rice, wheat and barley, belongs to the Poaceae family, commonly known as grasses (Soreng et al., 2015). Maize is a high-yield grass crop and has major economical and agricultural importance, since it is used for human and livestock consumption as well as biofuel production. In order to improve maize yield, it is essential to understand plant and inflorescence architecture, and the mechanisms that underlie grass and floral development (Colasanti & Coneva, 2009).

Zea mays is an important model for plant and floral development due to its great genetic diversity and developmental complexity (Bommert & Whipple, 2018). Maize has numerous genetic tools, including a rich mutant collection and reporter lines that allow us to dissect pathways that control inflorescence architecture. Furthermore, maize serves as an important model for investigating molecular pathways that regulate cell maintenance and cell fate determination and differentiation.

Maize inflorescence Development

Maize is a monoecious plant and male and female flowers (florets) are present on separate inflorescences. The tassel forms florets that contain the male reproductive organs (stamens) and produce pollen, whereas the ear forms florets that contain the female reproductive organs (carpels) and produce kernels after fertilization.

Although inflorescence architecture varies among grasses, all possess a unique structure, the spikelet. Spikelets are short branches that house a variable number of florets depending on the species (Eveland et al., 2014; Kellogg, 2007; B. E. Thompson & Hake, 2009). Maize

spikelets contain two florets, the upper and the lower floret. Each floret consists of a lemma, a palea, two lodicules, three stamens and a central pistil consisting of three fused carpels, surrounding a single ovule. The lemma subtends each floret and the palea surrounds the reproductive organs. Both florets, including the lemma and palea, are enclosed in a pair of glumes. In the ear, the lower floret aborts resulting in the production of spikelets containing a single floret. The sex determination in the two inflorescences occurs when in the tassel floret the central pistil aborts, whereas in the ear spikelets the stamens of the upper floret arrest (Figure 1) (Cheng, Greyson, & Walden, 1983; B. Thompson, 2014).

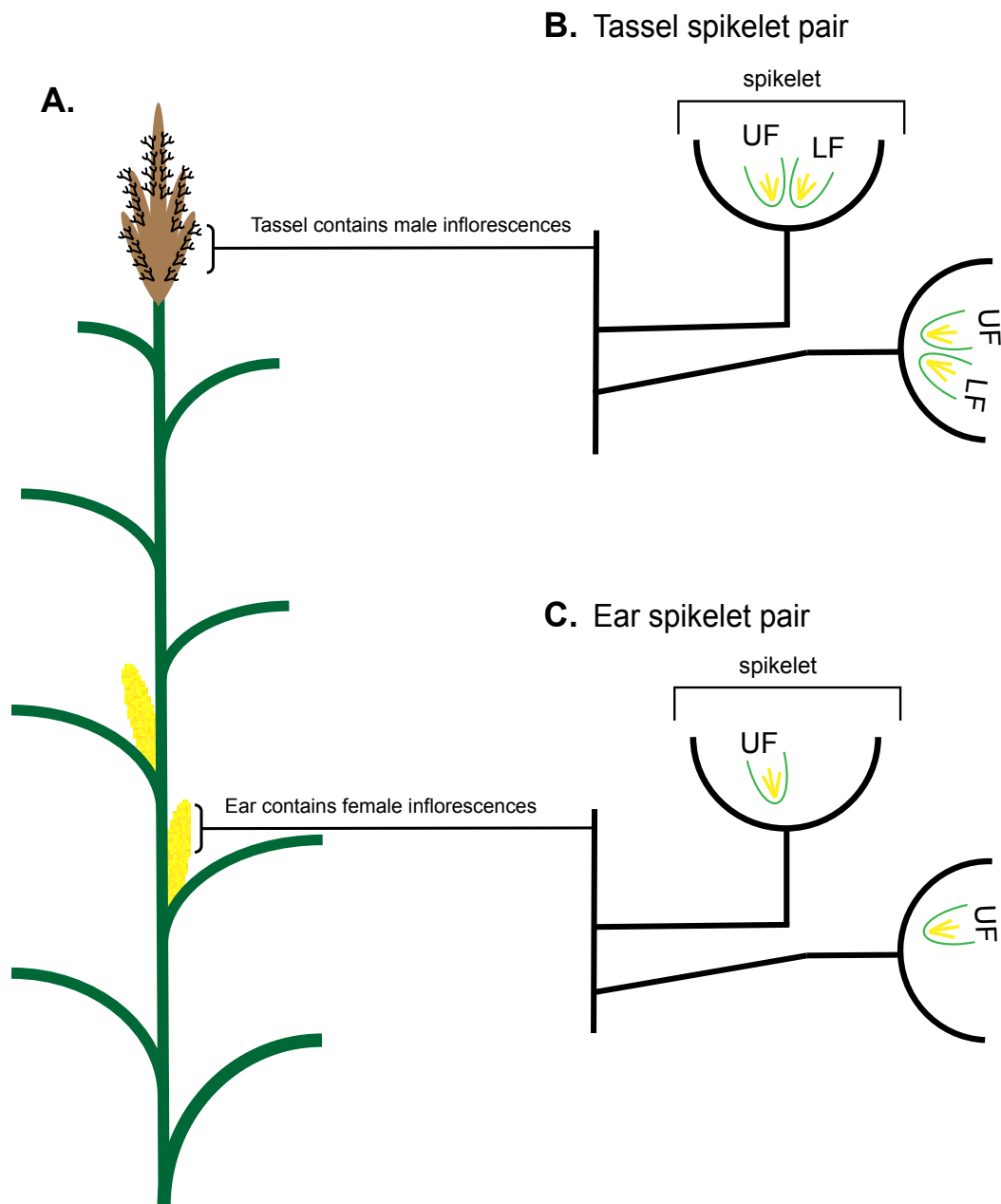


Figure 1. Maize Development. (A) The tassel forms at the apex of the plant and the ears form in the axils of the leaves. Both tassel and ear contain spikelets. (B) Each tassel spikelet contain an upper and lower floret, that house the male floral organs and the non-sexual organs. (C) The ear spikelets only have the upper floret that house the female floral organs and then non-sexual organs, as the lower floret aborts.

Inflorescence Architecture Depends on Meristem Activity

Meristems contain plant stem cells, which self-renew and differentiate to initiate new organs. The shoot apical meristem (SAM) is formed during embryogenesis and maintained throughout the life of a plant by stem cell divisions (Takacs et al., 2012). The SAM is responsible for the organogenesis of all the above ground organs including the inflorescences, the female ears and the male tassel.

Inflorescence development is initiated when the plant transitions from a vegetative to a reproductive stage and the SAM transitions to an inflorescence meristem (IM). Both the tassel and ear arise from a shared program of development involving multiple meristem identities. In the tassel, the IM starts producing lateral meristems, which are called branch meristems (BM) and will form the branches at the base of the tassel. In the ear, the IM does not form BM. The IM also initiates the spikelet pair meristems (SPM) on both the ear and tassel. Each SPM gives rise to two spikelet meristems (SM). Each SM gives rise to two floral meristems (FM), which in turn produce the upper and lower floret (Figure 2) (Bommert & Whipple, 2018; B. E. Thompson et al., 2014).

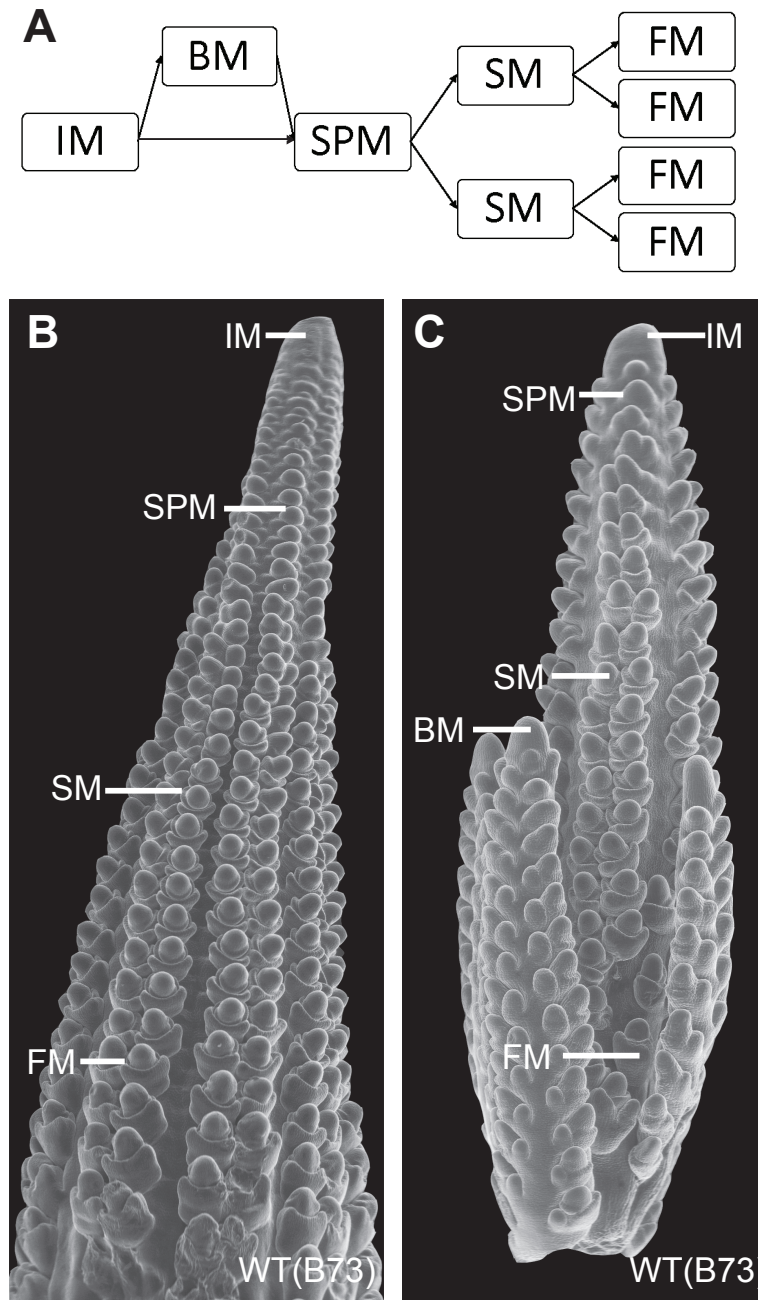


Figure 2. Inflorescence Development. (A) Schematic of maize inflorescence development. (B) The inflorescence meristem (IM) gives rise to spikelet pair meristems (SPM). Each spikelet pair meristem (SPM) then divides into two spikelet meristems (SMs). Each spikelet meristem (SM) will give rise to two floral meristems (FMs) which will develop to floral organs. The inflorescence meristem (IM) of the tassel also produces branch meristems (BMs) (C) whereas the inflorescence meristem (IM) of the ear does not.

Floral Development in Grasses

The angiosperms or flowering plants, such as maize and *Arabidopsis*, have been used and are great models to study flower development. The angiosperm flower is defined by two characteristics: (1) a closed carpel that contains one or more ovules and (2) a nucellus that contains an embryo sac, which after fertilization will develop to form the seed (Judd, Campbell, Kellogg, Stevens, & Donoghue, 2007). The angiosperms exhibit significant variation in floral morphology. Most flowers consist of four whorls of organs. Whorl one (the outer whorl) contains sepals (bract-like organs), whorl two contains petals, whorl 3 contains stamens and whorl 4 (the most inner whorl) contains carpels. These organs are often found in most grasses, however in some species such as maize, some organs are either missing or are modified (Clinton J. Whipple & Schmidt, 2006).

Genetic regulation of Floral Development

MADS-box transcription factors regulate floral development in angiosperms

Plant MADS-box transcription factors are the key regulators that control flowering time, floral organ identity and FM fate (Heijmans, Morel, & Vandenbussche, 2012; Ng & Yanofsky, 2001). The MADS-box family has been defined by the sequence similarity found amongst proteins from different eukaryotic organisms (Kaufmann, Melzer, & Theißen, 2005; Shore & Sharrocks, 1995; Theißen, Melzer, & Rümpler, 2016). MADS-box genes take their name from the four founding members: *MINICHROMOSOME MAINTENANCE FACTOR1* (*MCM1*; from *S.cerevisiae*), *AGAMOUS* (*AG*; from *A.thaliana*), *DEFICIENS* (*DEF*; from *A.majus*) and *SERUM RESPONSE FACTOR* (*SRF*; from *H.sapiens*) (Heijmans et al., 2012; Ng & Yanofsky, 2001; Sommer et al., 1990; Theißen et al., 2016).

MADS-boxes genes can be divided into two major classes, type I and type II genes. Type I genes are present in animals, fungi and plants and are defined by a conserved binding domain, the *SERUM RESPONSE FACTOR* domain (Shore & Sharrocks, 1995). Type II genes are also present in animals, fungi, as well as in plants (Alvarez-Buylla et al., 2000; De Bodt, Raes, Van de Peer, & Theissen, 2003; Zhao et al., 2011). A subset of the MADS-box type II are referred to as MIKC-type and are plant-specific. MIKC-type genes are named for their characteristic domain structure: the MADS-box (M) domain, the Intervening (I) domain, (K) Keratin-like domain and (C) C-terminal domain (Theißen, Kim, & Saedler, 1996). The MADS domain is the most conserved region of the MADS protein sequence and is required for DNA-binding (Theißen et al., 1996, 2016). The I-domain influences the specificity of DNA-binding dimer formation (Riechmann, Krizek, & Meyerowitz, 1996; Theißen et al., 2016). The K-domain is involved in MIKC-type protein function and interactions (Kaufmann et al., 2005; Theißen et al., 2016). The C-domain is the least conserved domain and is involved in stabilizing the interactions facilitated by the K-domain and sometimes in transcriptional activation (Fan, Hu, Tudor, & Ma, 1997; Kaufmann et al., 2005; Pelaz, Gustafson-Brown, Kohalmi, Crosby, & Yanofsky, 2001; Theißen et al., 2016).

The ABC Model

The ABC model emerged from the study of homeotic mutants in *Arabidopsis* and describes how combinatorial function of MADS-box genes from different classes regulates floral organ identity (Coen & Meyerowitz, 1991; Weigel & Meyerowitz, 1994). Nearly all of the MADS-box genes functionally characterized in plants belong to the MIKC class of MADS-box transcription factors and have key roles in floral development. The first two plant homeotic

(organ identity) MADS-box transcription factors identified were the *DEFICIENS (DEF)* gene in *Antirrhinum majus* (Sommer et al., 1990) and *AGAMOUS (AG)* gene in *Arabidopsis*.

MADS-box proteins function in combination with proteins from different classes by forming dimers and then tetramers to regulate the transcription of target genes. The floral organ identity genes are divided into five homeotic functional classes, A, B, C, D and E class (Figure 3) as described in *Arabidopsis* (De Bodt et al., 2003; Theissen, 2001). Class A genes, such as *APETALLA1 (AP1)* regulate sepal identity. Class B genes, including *APETALLA3 (AP3)* and *PISTILLATA (PI)* regulate petals (with class A) and stamens (with class C) (Riechmann et al., 1996; Sommer et al., 1990). Class C genes, such as *AGAMOUS (AG)*, regulate the identity of stamens (with class B) and carpels (Riechmann et al., 1996). Class D genes, including *SEEDSTICK (STK)* and *SHATERPROOF2 (SHP2)*, specify ovule identity (Angenent & Colombo, 1996). Class E genes, *SEPALLATA1/2/3/4 (SEP1/2/3/4)*, function as cofactors for class A,B and C genes and when class E genes are absent all floral organs are converted into leaves (Ditta, Pinyopich, Robles, Pelaz, & Yanofsky, 2004).

Although 75 MADS-box genes have been identified in maize through bioinformatics analysis (Zhao et al., 2011), only a few have been functionally characterized. Some of these mutants include *silky1 (sil)*, *sterile tassel silky ear1 (sts1)*, *zea agamous1 (zag1)* and *bearded-ear1 (bde1)*. *sil* encodes for a gene that is co-orthologous to the class B gene, *ap3* (Ambrose et al., 2000). The phenotype of *sil* mutants includes conversion of lodicules to lemma/palea organs and conversion of stamens to carpels (Ambrose et al., 2000). *sts1*, or other known as *Zmm16* is also a maize class B gene and has the same phenotype as *sil* (Bartlett et al., 2015). The phenotype of *sts1* mutants includes a conversion of lodicules and stamens to lemma/palea organs in the tassel florets (Bartlett et al., 2015). *zag1* is homologous to the class C gene, *ag*. *zag1* mutants

exhibit indetermination of FMs but do not affect identity of the floral organs (Mena et al., 1996). *bde1* encodes for a MADS-box transcription factor and belongs in the AGL6 clade, sister to the SEP clade (Becker & Theissen, 2003; B. E. Thompson et al., 2009). *bde1* mutants have multiple defects in the upper and lower FM (B. E. Thompson et al., 2009).

Floral mutants have provided us with a framework that describes maize floral development and regulation. Analysis of maize mutants such as *sil*, *sts1* and *zag1* indicate that class B and class C functions are conserved in maize (Ambrose et al., 2000; Bartlett et al., 2015; Clinton J. Whipple et al., 2004). However, there is still lot to be learned about MADS-box transcription factors and their interactions as well as their downstream targets.

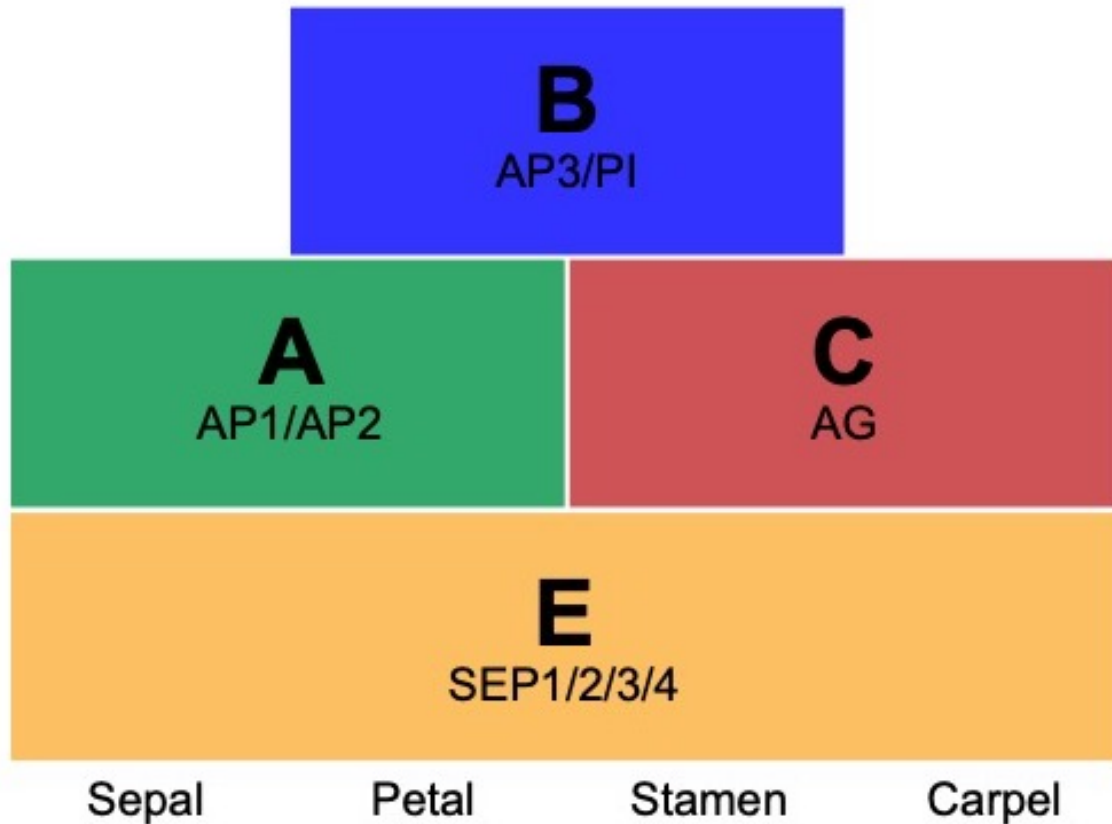


Figure 3. The ABC model. Combinational function of genes from different classes control floral organ identity. Class A genes control sepal identity. Class A in combination with class B genes control petal identity. Class B in combination with class C genes control stamen identity. Class C genes control carpel identity. Class E genes function in combination with all the other class.

Classical Maize Mutant *Polytypic1*

Mutant analysis is a powerful tool to understand gene function. Floral mutant analysis and characterization will lead to better understanding the pathways that regulate meristem and floral organ determinacy and development. *Pt1* was originally identified in a commercial field from an unknown progenitor in the 1950s (Nelson & Postlethwait, 1954). *Pt1* was characterized as a semi-dominant mutant with an excess proliferation of the pistillate spikelet (silks) or an abnormal elongation of the main axis of the ear with partial inhibition of the pistillate spikelet development (Nelson & Postlethwait, 1954). Previous work mapped *Pt1* to chromosome 6, bin 5, however no fine mapping was performed, and the causative gene is unknown. This thesis describes the phenotypic characterization of *Pt1* mutants in three inbred backgrounds as well as the progress towards identifying the causative gene.

Methods

Phenotypic Data Collection

During Summer 2018, I planted families segregating *Pt1* introgressed in A619, B73 and Mo17 (backcrossed a minimum of three times) backgrounds at our summer nursery in Central Crops Research Station (Clayton, NC). Mature inflorescences were scored based on phenotype. Tassels and ears from both mutant and normal plants were harvested from field grown plants and brought into the lab for analysis. Tassels were collected immediately prior to anthesis; ears were collected when silks emerged from normal plants. Branch number in tassels was determined by counting the number of primary branches emerging from the main spike. Spikelet density was determined by counting the number of spikelets in a 10cm region, starting 5cm below the top of the tassel on the main spike. In addition, a qualitative score was assigned to each tassel to indicate the severity of sex determination defects. Also, I recorded any abnormalities found on mutant ears, including sex determination defects.

Seed Germination and Plant Growth

Greenhouse and growth chamber grown plants were germinated by soaking seeds in milli-Q water with gentle shaking for two hours, coating seeds with Captan fungicide and placing them on wet paper towels in the 27°C incubator. Seedlings were transplanted into individual pots once shoots and roots emerge from the seed. Plants were grown in a growth chamber (Percival Intellus Control System) at 26°C, 80% humidity, and 12-hour light and watered once daily. Once the seedlings had initiated at least three leaves they were transplanted into bigger pots in groups of two or three plants per pot and moved to the greenhouse with 12-hour light and 8-hour dark schedule. Plants used for analysis of the mature tassel and ear phenotype were grown in the field at the Central Crops Research Station during Summer 2018.

Genotyping

To definitively identify *Pt1* mutant and normal individuals, all plants were genotyped with the SSR markers *umc1352a* and *bnlg1922*, which flank the *Pt1* locus. At these markers, the *Pt1* mutant chromosome is polymorphic with all inbred backgrounds (B73, A619, Mo17) analyzed in this study. Any individuals with recombinant chromosomes between *umc1352a* and *bnlg1922* were excluded from the analysis and RNA sequencing since their genotype could not be unambiguously determined. SSR markers were amplified using the Taq-Touchdown PCR protocol (Korbie & Mattick, 2008) and PCR products were visualized on a 3.5% metaphor agarose gel. See Table A for primer sequences

Scanning Electron Microscopy (SEM)

Approximately 1cm tassel and ear primordia (~ 5 and 8 weeks after planting) were dissected from both mutant and normal plants and immediately mounted for visualization on a SEM. The tassel and ear primordia were visualized under low vacuum conditions on a FEI Quanta 200 Mark 1 scanning electron microscope at an accelerating voltage of 10 to 20kV.

RNA Extraction

Individual ~1cm *Pt1/+* and normal sibling ear primordia (backcrossed twice to B73), were collected by Dr. Thompson from the field during the Summer 2017 season (Clayton, NC), flash froze in liquid nitrogen and stored at -80°C. RNA was extracted from 32 individual primordia using QIAzol lysis reagent and the QIAGEN miRNeasy Micro kit, including the optional DNase digestion treatment step according to manufacturer instructions. RNA concentration was measured on the NanoDrop Lite Spectrophotometer and visualized on a 1% agarose gel to assess RNA integrity. Total RNA from four mutant or normal siblings was pooled for each biological replicate (500ng/individual, 2µg total RNA/replicate). Integrity of pooled

RNA was also confirmed by analyzing each sampler on a 2100 Agilent Bioanalyzer at the ECU Genomics Core. All samples had an RNA integrity number (RIN) of 10, indicating high quality RNA suitable for sequencing (Figure 5A).

RNA Sequencing and Data Analysis

Illumina directional RNA libraries were constructed by the North Carolina State University Genomic Sciences Laboratory and sequenced on one flow cell of an Illumina NextSeq 500 sequencer (75 bp, paired end sequences).

Trim Galore (FelixKrueger, 2016/2019; Martin, 2011) was used to remove all the adapters from all eight samples. RefGen_v4 (AGPv4) (Jiao et al., 2017) was the reference genome used. HISAT2 (Kim, Langmead, & Salzberg, 2015) was used to align all reads (Figure 5A). samtools (Li et al., 2009) was used to generate a pileup of all reads. Differential Expression analysis of the samples was performed on R version 3.5.0 using DESeq2 (Love, Huber, & Anders, 2014). Principle Component Analysis was performed using pcaExplorer (Marini & Binder, 2017) on R version 3.5.0. Integrative Genomics Viewer (Robinson, Thorvaldsdóttir, Wenger, Zehir, & Mesirov, 2017; Robinson et al., 2011; Thorvaldsdóttir, Robinson, & Mesirov, 2013) was used to visualize all samples and to observe for any aberrant transcripts and if any genes exhibited allele-specific expression. SnpEff (Cingolani et al., 2012) was used to generate all SNPs with their predicted effects.

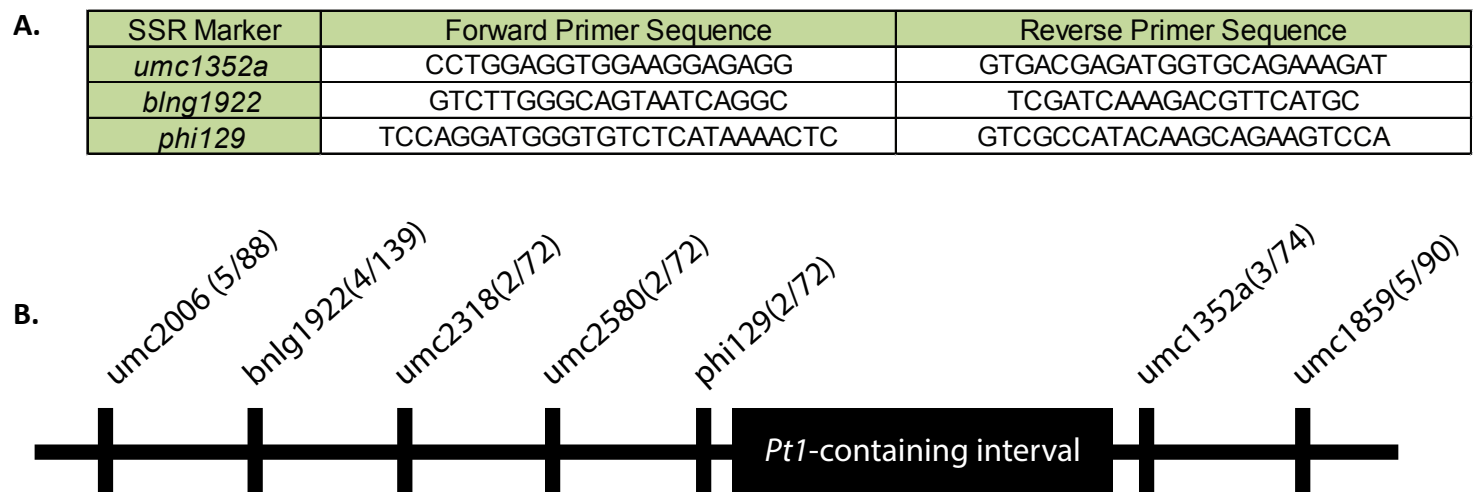


Figure 4. Map of *PtI* (A) The primer sequences for the SSR markers used to genotype all individuals examined. (B) Positional cloning previously mapped *PtI* in a 5.3Mb region between *phi129* and *umc1352a*.

A.

RNA WT pools	RIN	Reads Aligned	RNA <i>Pt1</i> /+ pools	RIN	Reads Aligned
N1	10	95.02%	M1	10	94.92%
N2	10	94.11%	M2	10	94.11%
N3	10	94.37%	M3	10	95.18%
N4	10	94.58%	M4	10	95.34%

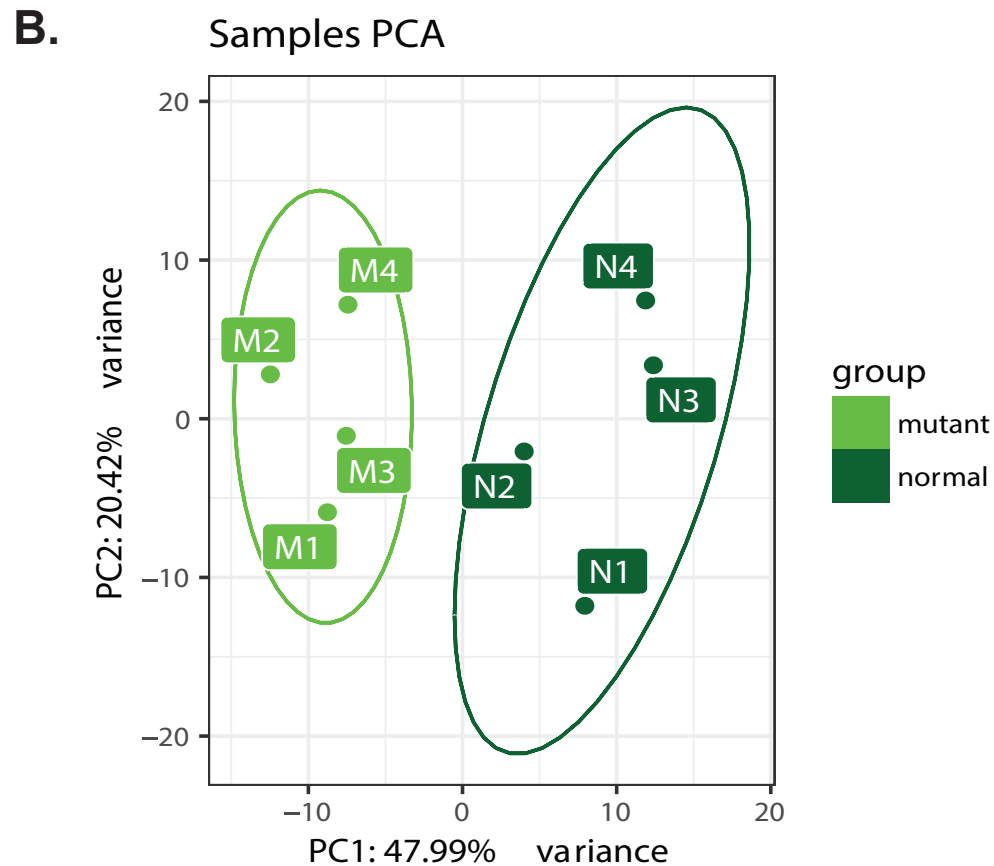


Figure 5. RNA quality (A) Eight RNA pools total were created, four *Pt1*/+ pools and four WT pools. All pools were analyzed through the Bioanalyzer for RNA Integrity Number (RIN). (B) Principle Component Analysis performed on both mutant pools (M1-4) and WT pools (N1-4).

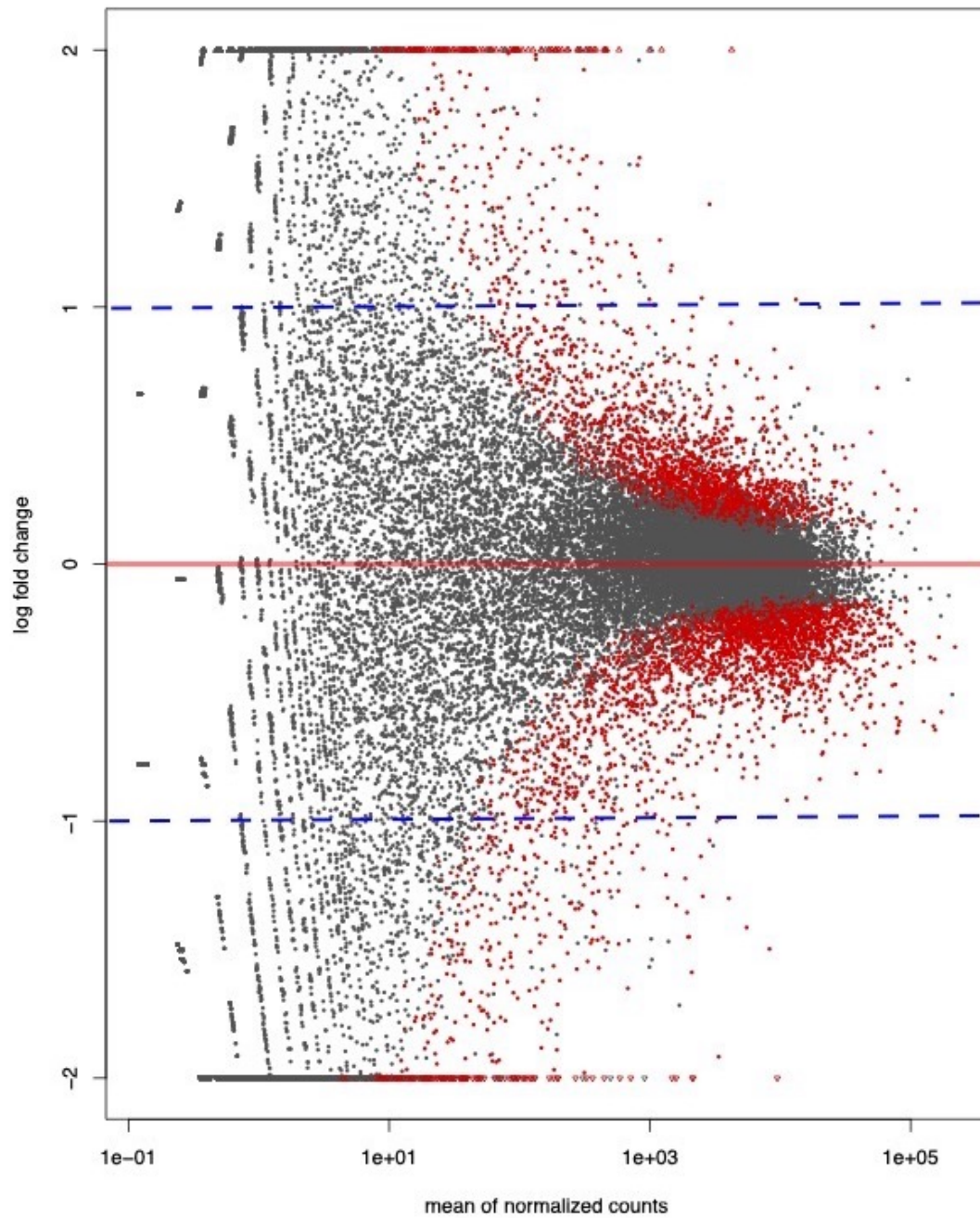


Figure 6. MA Plot. Each dot represents a gene. The red dots represent the differential expressed genes. The blue dashed line indicates the cutoffs for the 2FoldChange.

Results

Characterization of *Pt1* Phenotype

Pt1 is a semi-dominant mutant with severe floral defects in both the tassel and ear (Postlethwait & Nelson, 1964), although the penetrance and expressivity of the mutant phenotype varied significantly depending on the genetic background. Despite being discovered in the 1950s, the *Pt1* mutant phenotype has not been characterized in detail. The initial characterization described *Pt1* to affect ovule differentiation in the ear by producing extra silks (Postlethwait & Nelson, 1964). These analyses only examined the *Pt1* phenotype in mature inflorescences in an unknown genetic background, thus a detailed characterization of the mutant phenotype in a uniform genetic background has not yet been done.

To better understand the effects that *Pt1* expression has on the inflorescences, I examined both young and mature tassels and ears in the B73, A619 and Mo17 inbred backgrounds. To determine the defects on mature inflorescences, I planted individuals and collected mature inflorescences in our summer nursery in Clayton, NC. Overall, the *Pt1* mutant phenotype was penetrant in B73 and Mo17 inbred, while in the A619 inbred the mutant phenotype was variable and difficult to score (Table 1).

In the B73 background, the *Pt1* phenotype is fully penetrant. The *Pt1*/+ tassel has fewer spikelets on the branches (Figure 7B) compared to the wild type sibling (Figure 7A) and has spikelets that often have fewer floral organs and occasionally produce silks (Figure 7D). In the *Pt1*/+ ears the phenotypic defects are more severe than the defects on the *Pt1*/+ tassel. The upper part of the *Pt1*/+ ear lacks spikelets and the spikelets at the base of the ear produce extra silks (Figure 7F). *Pt1*/+ ears also have ovules that produce extra silks and branch like organs (Figure 7H). The *Pt1*/+ ear in B73 is sterile.

In the Mo17 background, the phenotype is fully penetrant similarly to the B73 inbred. The *Pt1/+* tassels have silks and the top of the branches lack spikelets (Figure 8B). The *Pt1/+* tassels often contain spikelets with abnormal organs that produce silks (not shown). The *Pt1/+* ears in Mo17, have the upper part lacking spikelets (Figure 8F) and the spikelets at the base produce extra silks (Figure 7H). The *Pt1/+* ear in Mo17 is sterile.

Unlike B73 and Mo17 inbreds, the *Pt1* phenotype is mild and variable in the A619 background and the *Pt1/+* ear is fertile. The *Pt1/+* tassel (Figure 9B) looks very similar to the normal sibling (Figure 9A). Occasionally, the *Pt1/+* spikelets produce abnormal floral organs and produce silks (Figure 9E). Likewise, the *Pt1/+* ear (Figure 9G) looks very similar to the normal sibling (Figure 9F) and occasionally contains ovules that produce extra silks and protruding nucellus (Figure 9J).

The *Pt1* phenotype is more variable on the tassel than the ear, both within and amongst inbred background lines. In order to detect and compare the defects on the mutant tassel, I determined the spikelet density of both *Pt1/+* and normal tassels. To measure spikelet density, I counted the number of spikelets within a 10cm region on the main spike, starting 5cm below the top of the main spike. Even though there is no significant difference between normal and *Pt1/+* individuals within an inbred line, spikelet density varies a lot in the *Pt1/+* individuals in all inbreds (Figure 10). I also recorded the number of *Pt1/+* mutant tassels that produced silks and the mutant tassels that contained triple spikelets. The silkiness of a tassel was scaled with a score of one being very few silks present to a score of five being many extra silks present (Figure 11). The *Pt1* phenotype in the ear was not as variable as in the tassel. Often times branches would be present at the base of a mutant ear. A common defect across all inbred lines was that *Pt1/+* ear spikelets produced more than one silk.

I looked at tassel and ear primordia to understand the role of *Pt1* in development. Most of characterization was done on *Pt1/+* inflorescences in the B73 background. During early development, *Pt1/+* tassels in B73 have fasciated inflorescences meristems and overall are in a disorganized fashion (Figure 12B) compared to the normal siblings (Figure 12A). In normal tassels, two glumes subtend each spikelet consisting of two florets (Figure 12C and F) . However, in *Pt1/+* tassels, a single enlarged bract subtends two spikelet meristems (Figure 12D). This bract is likely the derepressed bract from the spikelet pair meristems, although may also result from fasciation of the spikelet meristem (Figure 12E). In addition, *Pt1/+* tassels have branches with derepressed bracts (Figure 12G) and clusters of floral meristems (Figure 12H). The *Pt1/+* ears have more severe but less variable defects than the *Pt1/+* tassels. In WT ears, a glume subtends each floral meristem (Figure 13C). In *Pt1/+* ears the spikelet pair meristems arrest (Figure 13B) and often an enlarged bract subtends two floral meristems (Figure 13D). The floral meristems are indeterminate giving rise to branch-like organs and extra silks (Figure 13D).

Inbred	Planted	Collected	<i>Pt1</i>	WT	Penetrance
B73	68	36	21	15	fully
Mo17	57	30	14	16	fully
A619	68	32	24	8	partial

Table 1. Summary of Individuals planted and collected. Each individual was first scored by phenotype. The penetrance of the phenotype for each inbred background was also recorded.

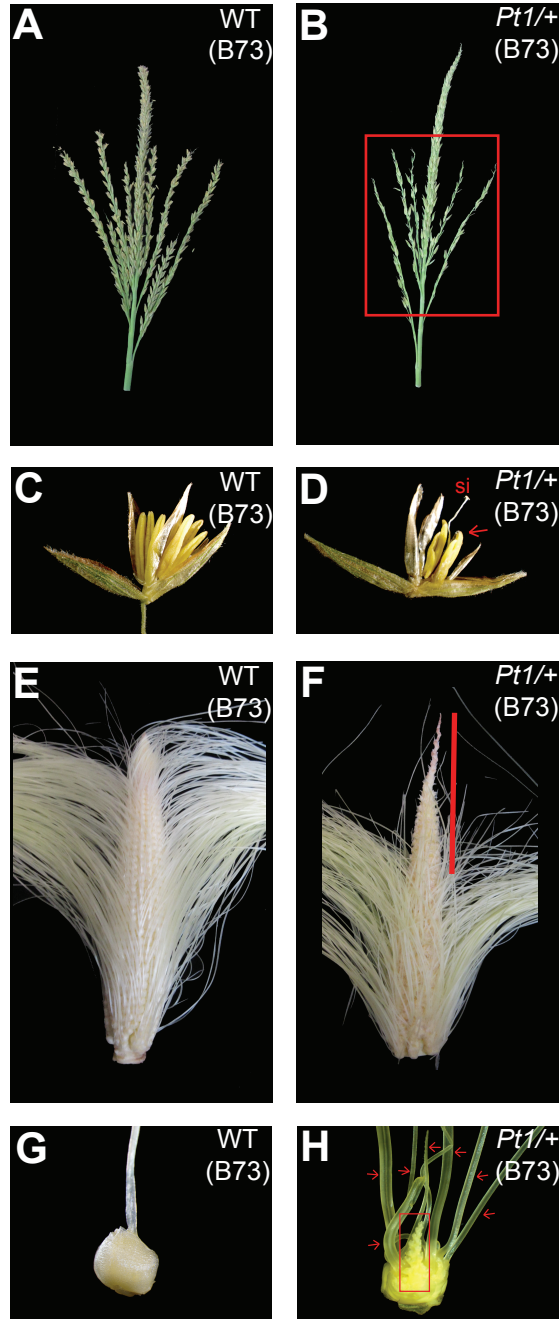


Figure 7. The *Pt1* Phenotype in B73 inbred. The *Pt1/+* tassel (**B**, **box**) has fewer spikelets on the branches compared to the WT sibling (**A**). The *Pt1/+* tassel spikelet (**D**) has abnormal number of floral organs (**D**, **arrow**) and produces silks (**D**, **si**) compared to the WT tassel spikelet (**C**). The *Pt1/+* ear has an upper part barren (**F**, **line**) and is extra silky at the bottom compared to the WT sibling (**E**). The *Pt1/+* ear spikelet produces extra silks (**H**, **arrows**) and branch-like organs (**F**, **box**) compared to the WT ear spikelet (**G**).

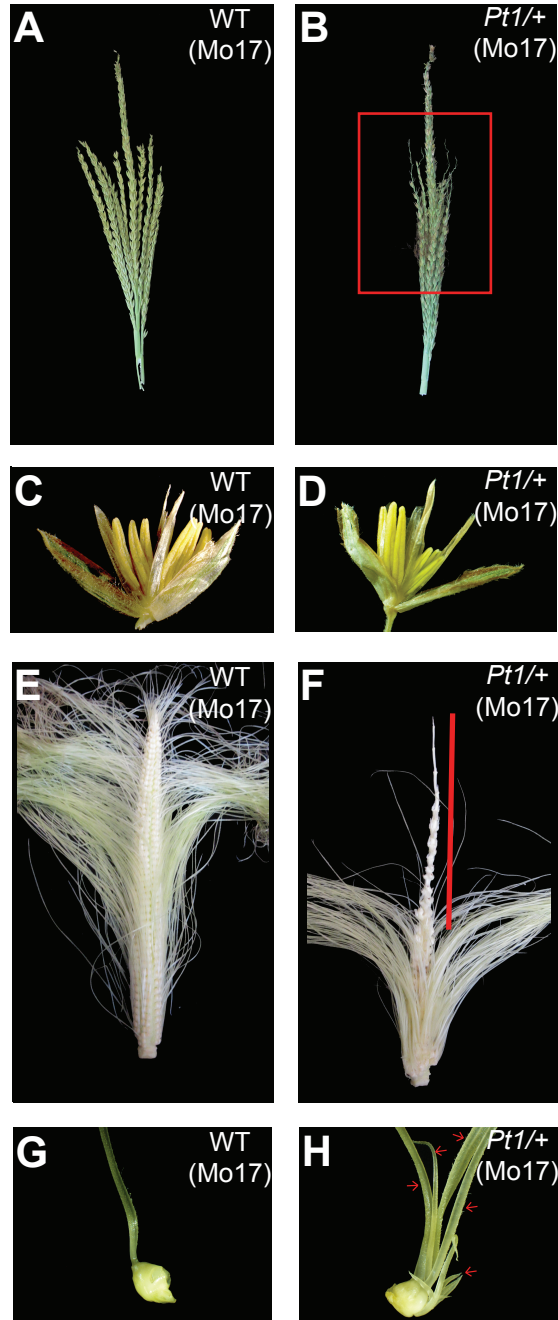


Figure 8. The *Pt1* Phenotype in Mo17 inbred. The *Pt1*/*+* tassel in Mo17 produces silks and has branches with barren tips (**B**, **box**) compared to the WT sibling (**A**). The *Pt1*/*+* tassel spikelet contains abnormal number of floral organs (not shown) compared to the WT tassel spikelet (**C**). The *Pt1*/*+* ear has an upper part that is pin-like (**F**, **line**) and is extra silky compared to the WT sibling (**E**). The *Pt1*/*+* ear spikelet produces extra silks (**H**, **arrows**) compared to the WT ear spikelet (**G**).

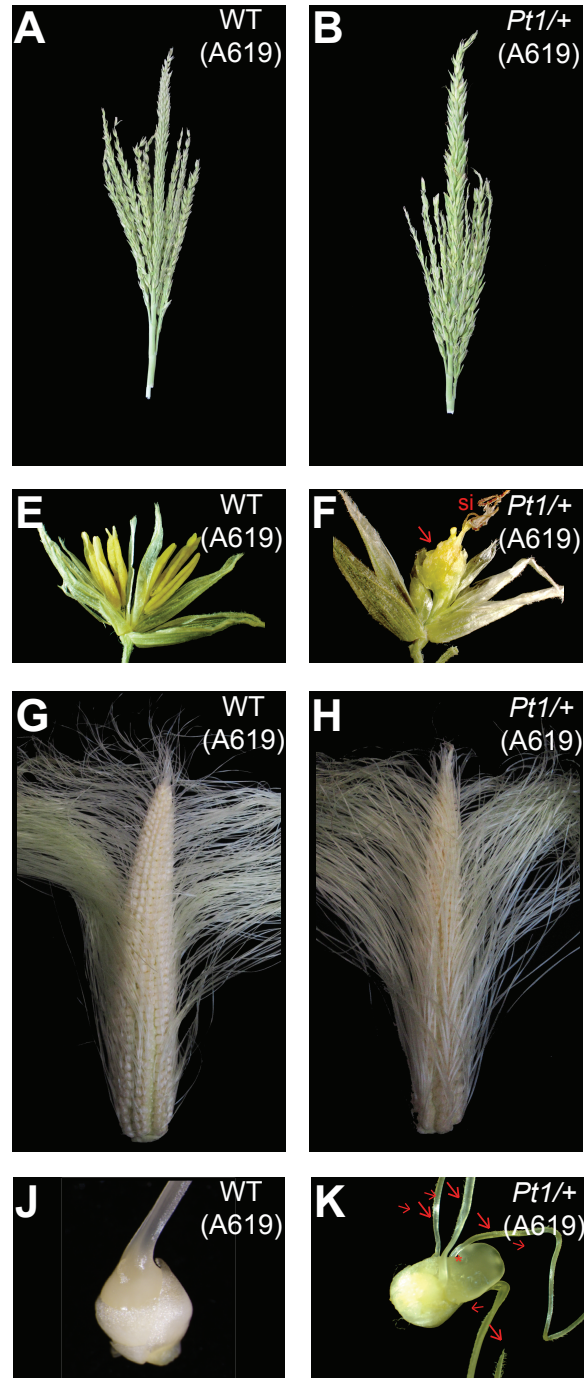


Figure 9. The *Pt1* Phenotype in A619. The *Pt1*/*+* tassel (B) is similar to the WT sibling (A). The *Pt1*/*+* tassel spikelet give rise abnormal floral organs (F, **arrow**) and produce silks (F, **si**) compared to the WT tassel spikelet (E). The *Pt1*/*+* ear (H) is also similar to the WT sibling (G). The *Pt1*/*+* ear spikelet produce extra silks (J, **arrows**) and organs that protrude (J,*) compared to the WT ear spikelet (I).

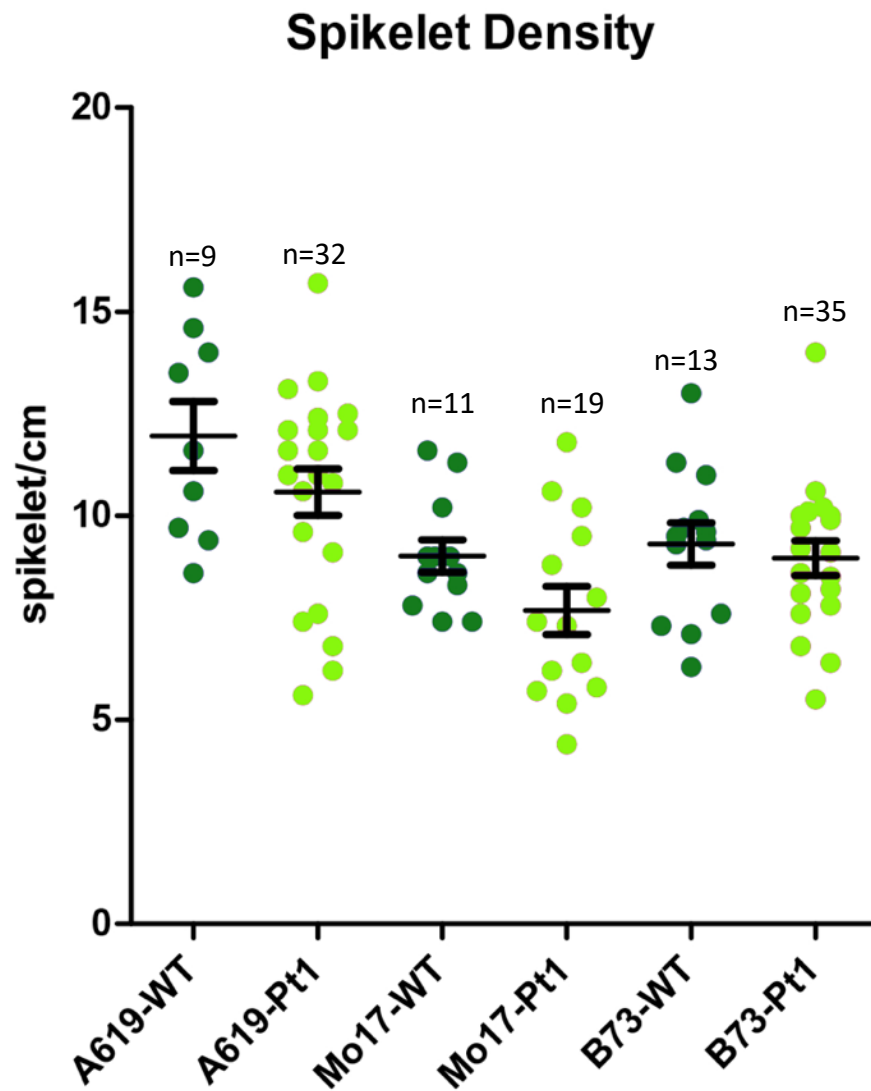


Figure 10. Spikelet Density Plot. Spikelet density measured in all mature tassels collected. Each dot represents one tassel. Statistical t-test was performed for *Pt1* and WT between inbred lines, using the GraphPadPrism v.5.02. There is no statistical significance between mutant and WT individuals in neither A619, Mo17 and B73 inbred backgrounds.

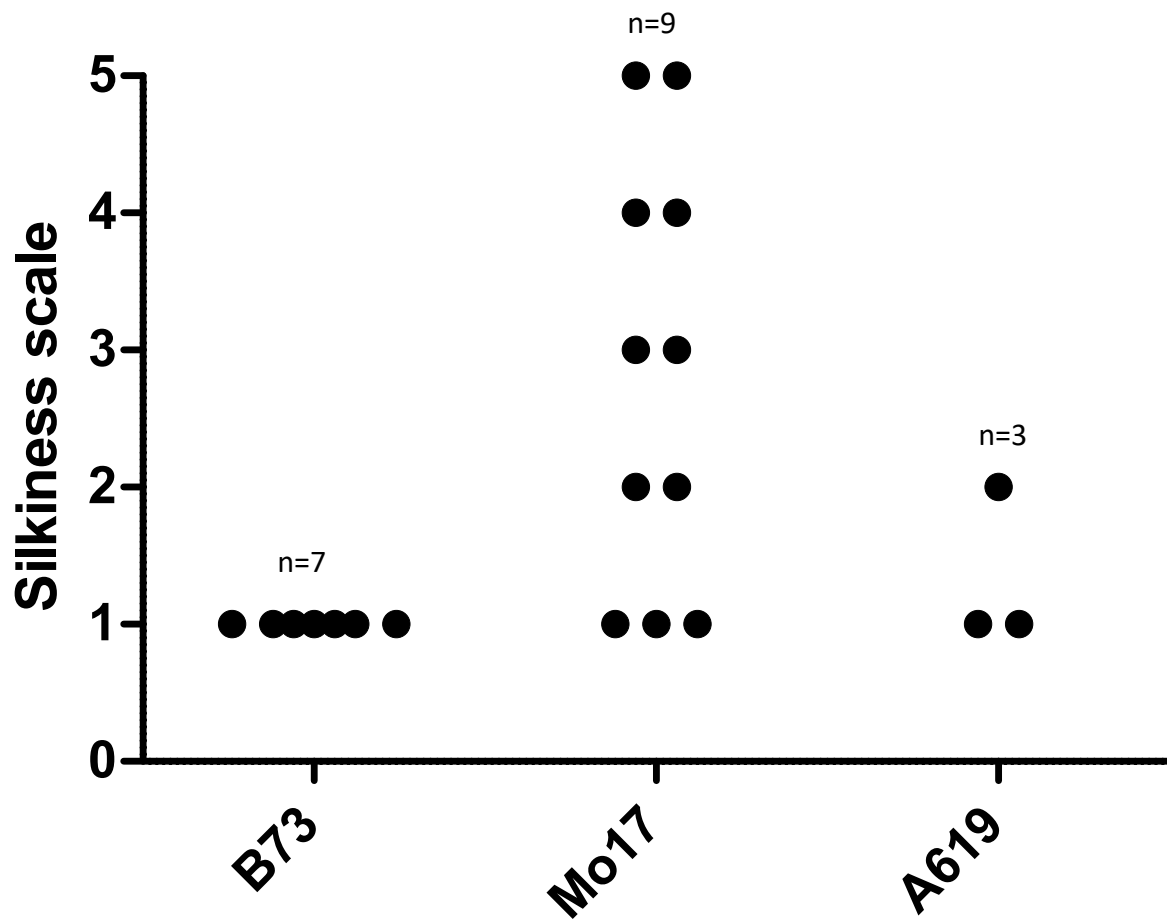


Figure 11. Silkeness of the *Pt1* tassels . The silkeness was recorded of the *Pt1* tassels in all three backgrounds on a scale of 1-5 with 1 being less silky to 5 most silky. The n for each inbred is indicated at the top of each column.

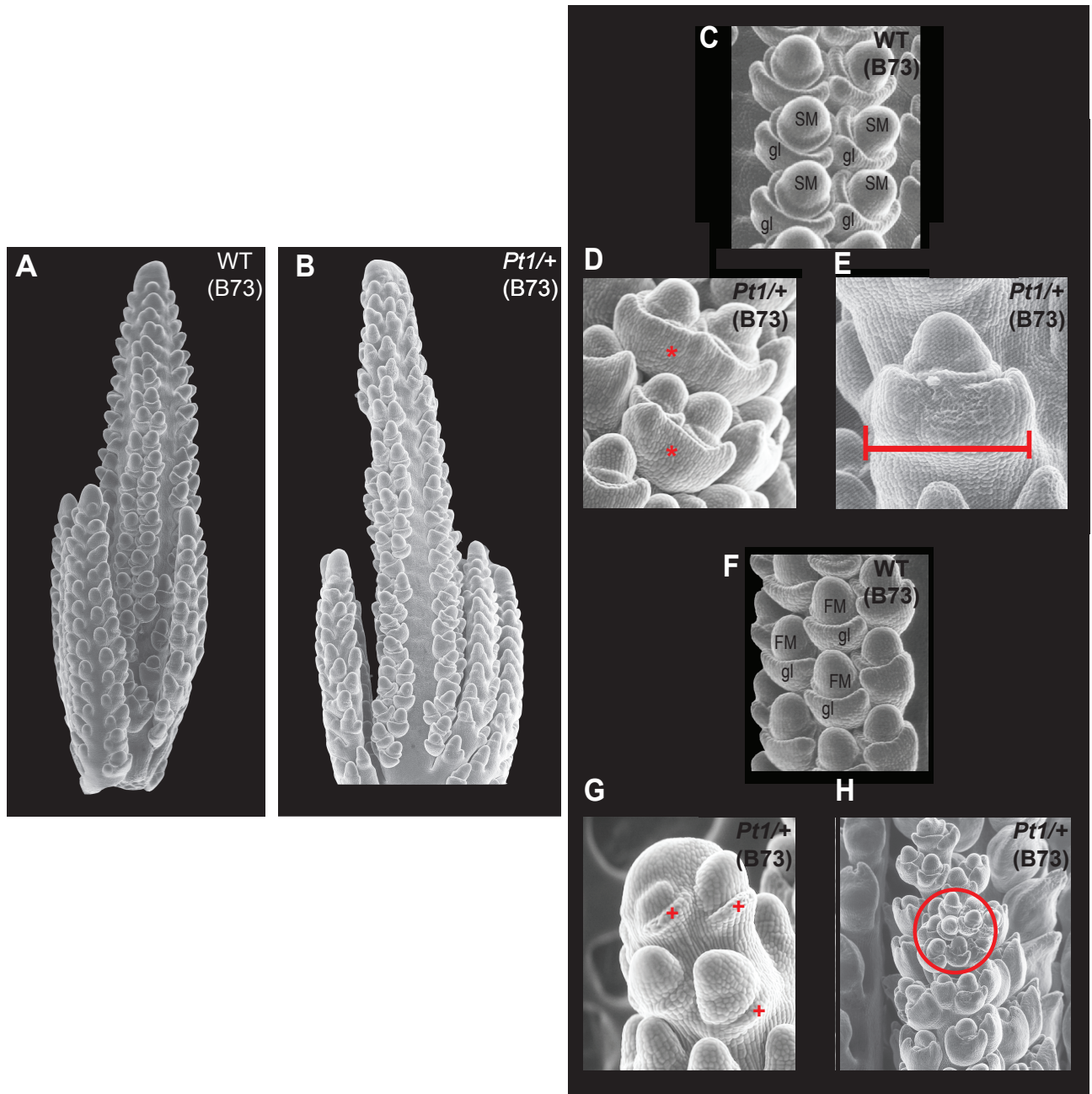


Figure 12. Scanning Electron Micrographs of WT and *Pt1* tassels in B73. *Pt1*/*+* tassels are disorganized with sometimes fasciated inflorescences meristems (**B**) compared to the WT sibling (**A**). (**C**) In the WT tassels a glume (gl) subtends each spikelets meristem (SM). The *Pt1*/*+* tassels often have (**D**) an enlarge glume (gl) subtend two spikelet meristems (SM) or (**E**) a fasciate SM. In the WT tassels (**F**) each glume (gl) also subtends the floral meristems (FM). The *Pt1*/*+* tassels often have derepressed bracts (**G**, +) and have clusters of floral meristems (**H**, circle).

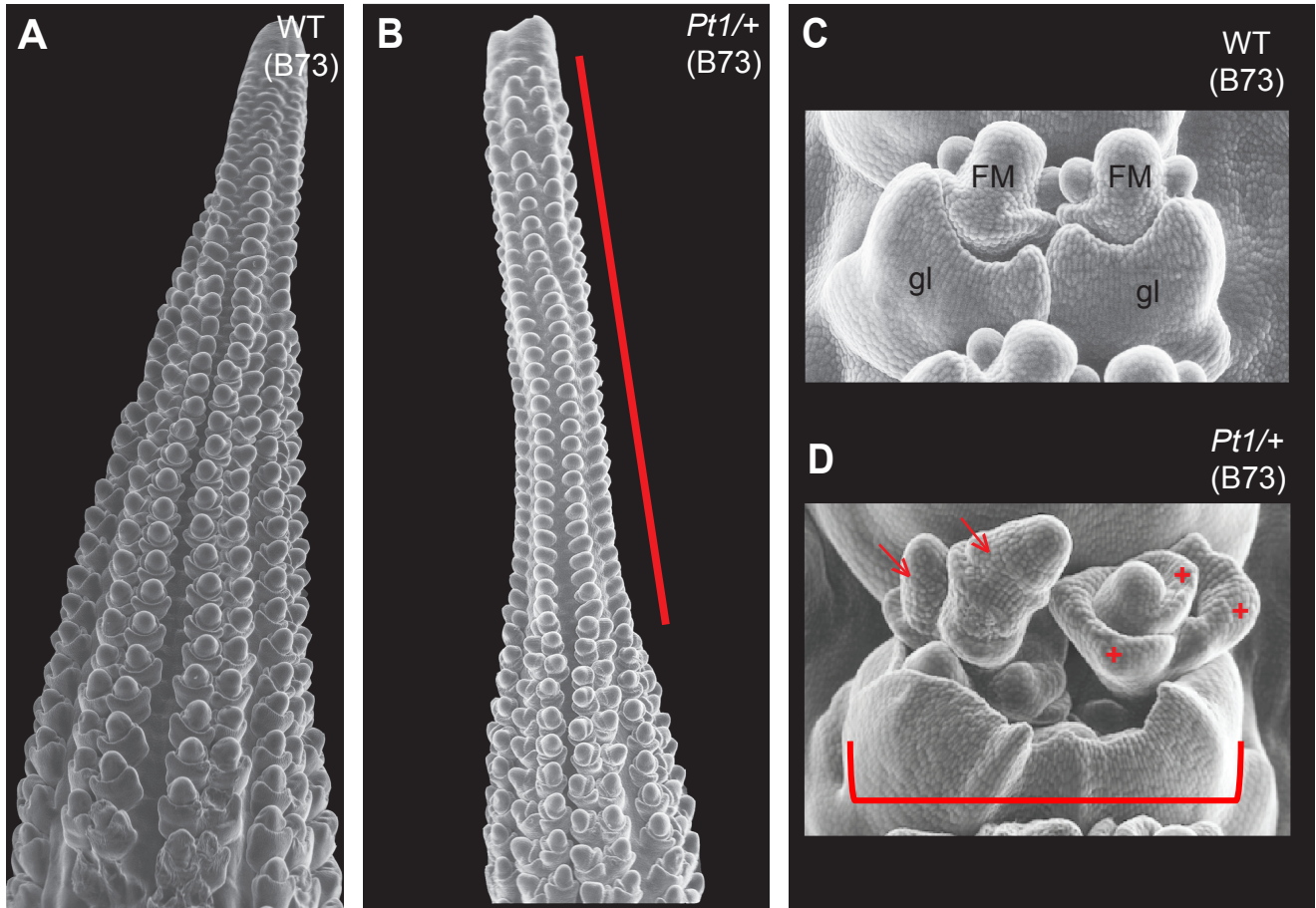


Figure 13. Scanning Electron Micrographs of *Pt1/+* ears in B73. *Pt1/+* ears have fasciated inflorescence meristems (**B**) and spikelet pair meristems that arrest (**B**, line) compared to the WT sibling (**A**). (**C**) The WT ear has floral meristems (FM) that each are subtended by one glume (gl). *Pt1/+* ear has indeterminate floral meristem that produce branch-like organs (**D**, arrows) and extra silks (**D**,+) that are subtended by one enlarged glume (**D**, bracket).

Identification of the gene responsible for the *PtI* mutant phenotype

PtI is a semi dominant mutant that defines a single locus on chromosome 6, bin 5 (Postlethwait & Nelson, 1964). Since *PtI* has not been fine mapped in the past, and to identify the causative gene, I used a map-based approach coupled with RNA sequencing. The *PtI* mutant has severe defects in floral development, suggesting that the underlying gene regulates floral development. To identify the causative gene(s), we initially created a mapping population in A632 and we mapped *PtI* in a 6.8cM (5.3Mb) region between SSR markers *phil29* and *umc1352a* on chromosome 6 (Figure 4B).

I reasoned that the *PtI* mutant phenotype is likely the result of a change in the RNA levels or a change in the RNA sequence of the causative gene(s) (Figure 14), both of which would be detectable by comparing RNA from mutant and normal ear primordia using RNA sequencing. Therefore, I sequenced pooled RNA from ~1cm normal and mutant ear primordia from a segregating *PtI* population backcrossed twice to B73 (the *PtI* phenotype is 100% penetrant and strongly expressed in this genetic background).

To ensure that the individuals used for the normal and mutant pool differ only due to their genotype, I performed Principle Component Analysis (Figure 5B). Principle Component Analysis is a dimension-reduction tool that can be used to reduce a large set of variables to a small set while retaining most of the variation in the data set (Ringnér, 2008). Indeed, the *PtI* mutant pools cluster together and the normal pools cluster as well, indicating that the *PtI* mutant pools differ from the normal pools due to their genotype and not any other environmental effects.

I first asked if *PtI* mutants contained RNAs with a change in the RNA sequence likely to alter protein function (Figure 14B). For this analysis, I only examined single nucleotide polymorphisms within the *PtI*-containing interval. To determine the *PtI*-containing interval I

used the genomic coordinates of *phi129* (127,301,488bp) and *umc1352a* (132,090,730bp). To identify RNAs within the *PtI*-interval that have differences in the RNA sequence, I used SnpEff. SnpEff is a variant annotation and effect prediction tool that predicts the effects of variants on genes. SnpEff categorizes single nucleotide polymorphisms and other variants such as insertions-deletions or multiple nucleotide polymorphisms based on their predicted effects on annotated protein coding genes. Variants defined as high effect are likely to be deleterious on protein function, such as a start or stop gain/loss or frameshift variants. Moderate effect variants are likely to change protein effectiveness and include inframe deletion or missense variants. Low effects variants are unlikely to change protein behavior and include synonymous variants (Cingolani et al., 2012). SnpEff predicted 112 variants with a high or moderate effect in the RNA sequence from genes in the *PtI*-containing interval (Table 2). The genetic background of the *PtI* mutant is unknown and the mutant progenitor is not available for sequencing. Maize is highly polymorphic and thus I expected to identify many polymorphisms between the *PtI* mutant and normal RNA pools. Most of these polymorphisms represent natural variation present within maize and are unlikely to cause the *PtI* mutant phenotype. To evaluate all the variants, I used the Phytozome genome browser (Goodstein et al., 2012) to identify if any of them are novel/unknown. I found that 108 out of the 112 high and moderate effect variants are natural variants in other normal populations and four insertions-deletions variants that were not represented on the genome browser. These insertion-deletion variants would likely result in a strong phenotype loss of function allele. Since *PtI* is a single allele and semi dominant mutant, it is not likely to be the result of a simple loss-of-function mutation.

The *PtI* phenotype could be also caused by changes in the RNA expression levels and thus a mutation in the regulatory DNA (Figure 14A). To look for differences in the expression

levels of the RNA, I performed a differential expression analysis. 632 genes were differentially expressed (DE) between *Pt1* and normal ear primordia (Table 3) ($\log_2\text{FoldChange} >1$ and <-1 and $q\text{value} <0.05$). Of the 111 genes expressed within the *Pt1*-containing interval (Table 3), three genes were upregulated and six genes were downregulated .

If the *Pt1* mutant phenotype is caused by misregulation of one of the nine DE genes (Table 4), this misregulation is likely the result of a change in the regulatory DNA. The mutant pools used for RNA sequencing consist of RNA from *Pt1/+* individuals, and thus I expect that a mutation in the regulatory DNA of the *Pt1* allele will result in misexpression of *Pt1*. I looked for allele specific expression of the DE genes in the *Pt1* heterozygote pools. If the changes in expression levels are due to a cis-regulatory change, we expect to see misexpression of the *Pt1* mutant allele compared to the wild-type (B73) allele. However, if the misexpression of the RNA is due to a trans-acting factor we would expect to see no differences in the expression levels of neither the *Pt1* nor the B73 allele. Therefore, I examined the nine DE genes for allele-specific expression. To detect allele specific expression, I looked for single nucleotide polymorphisms in the DE genes and recorded whether the *Pt1* allele was expressed more than the B73 allele.

From the nine DE genes, six genes contained single nucleotide polymorphisms in my samples and were visualized using Integrative Genomics Viewer (Robinson et al., 2011) to identify allele specific expression (Table 4). Three genes exhibit no allele specific expression suggesting that their misexpression is not caused just by *Pt1* allele. The other three DE genes that contained single nucleotide polymorphisms, Zm00001d037604, Zm00001d037737 and Zm00001d037751 exhibited allele specific expression. Interestingly, the *Pt1* mutant allele was expressed between ~65% to 80% in the SNPs of these three genes over the B73 allele. Zm00001d037604 is homologous to the *Arabidopsis* gene *ethylene response sensor1*, that

negatively regulates the ethylene pathway and exhibited allele specific expression only in the mutant pools. Zm00001d037737, a MADS-box gene *zea agamous1*, and Zm00001d037751, a member of the Late Embryogenesis Abundant hydroxyproline-rich glycoprotein family, they did exhibit allele specific expression but both in the normal and mutant pools.

It is important to understand what other genes might be involved in floral organ development and whether those are acting in combination with *Pt1*. Out of the 632 genes that were differentially expressed in the mutant samples, striking were the MADS-box genes. MADS-box genes are known floral regulators and well-studied in *Arabidopsis*. MADS-box genes are transcription factors that fall into different classes. Combinational function of genes from different classes regulate different organ identity (Figure 3) (Coen & Meyerowitz, 1991; Weigel & Meyerowitz, 1994). Notably, 13 MADS-box genes, all class C genes, and some from class B and class E, are downregulate in *Pt1* mutants (Table 5).

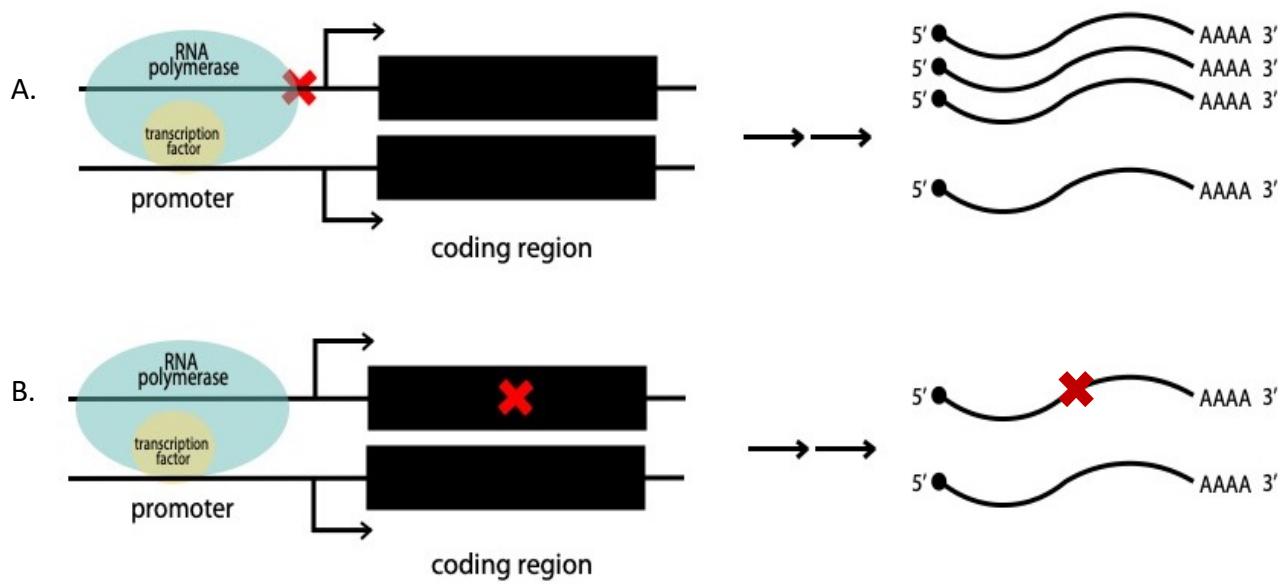


Figure 14. RNA-sequencing rationale. The *Ptl* phenotype could be caused by either **(A)** a mutation in the regulatory DNA resulting in a change in the RNA expression levels or **(B)** a mutation in the coding region resulting in a change in the RNA-sequence.

SNP Effect	SNPs predicted	Natural Variants
High	8	8
Moderate	104	100
Low	290	not examined

Table 2. SNPs predicted within the *Pt1*-containing interval. All high and moderate effects SNPs are natural variants. The low effects SNPs have not been examined yet.

Condition	Genes
Total genes expressed	25895
DE genes with 2FoldChange	632
Genes in the <i>Pt1</i> -containing interval	111
DE genes within the <i>Pt1</i> -containing interval	9

Table 3. Summary of the number of genes expressed and differentially expressed in the *Pt1* mutants. 632 genes are differentially expressed in the *Pt1* mutants. From the 111 genes expressed in the *Pt1*-containing interval, only 9 are differentially expressed.

Maize Gene ID	Gene name/Arabidopsis homologs	log2FoldChange	pvalue	padj	Gene start (bp)	Allele Specific Expression
Zm00001d037565	gibberellin 2-oxidase1	-1.676725845	5.41E-06	0.00014365	129802297	no ASE
Zm00001d037604	Ethylene response sensor 1	1.400008984	2.07E-48	5.37E-45	131348955	~20% ref allele (G) - ~80% alt allele (A) only in mutants
Zm00001d037609	GDSL esterase/lipase	-1.453855935	8.19E-16	1.75E-13	131653518	no ASE
Zm00001d037623	ROTUNDIFOLIA like 12	-1.48436689	0.00233072	0.01948199	132134851	unable to detect - no SNPs
Zm00001d037650	putative disease resistance RPP13-like protein 3	2.775361396	6.67E-12	7.54E-10	132828573	unable to detect - no SNPs
Zm00001d037651	Subtilase family protein	-1.223350729	1.52E-16	3.76E-14	133037545	no ASE
Zm00001d037672	LRR receptor-like serine/threonine-protein kinase	-1.443107521	1.59E-06	5.05E-05	133574540	unable to detect - no SNPs
Zm00001d037737	Zea AGAMOUS homolog1	-3.60333768	6.40E-124	1.66E-119	135893605	~35% ref allele (C) - ~65%alt allele (G) across mutants and normals
Zm00001d037751	LEA hydroxyproline-rich glycoprotein family	1.586739275	3.11E-11	3.14E-09	136355962	~30% ref allele (T) - ~70% alt allele (G) across mutants and normals

Table 4. Nine genes are differentially expressed within the *Pt1*-containing interval. The cutoffs used were (log2FoldChange >1 and <-1 and qvalue <0.05). Out of the nine genes, Zm00001d037604, Zm00001d037650 and Zm00001d037751 are upregulated. The other six genes are downregulated. Allele Specific Expression was recorded by visualizing all samples on IGV.

Gene name	Clade	Chromosome	log2FoldChange	pvalue	padj
Zea AGAMOUS homolog2	AG-like	3	-8.572092093	4.12E-50	1.19E-46
Zea agamous4	AG-like	3	-3.909737894	1.16E-40	2.00E-37
MADS-transcription factor 41	AG-like	3	-6.457407717	1.48E-05	0.000334561
Zea AGAMOUS homolog1	AG-like	6	-3.60333768	6.40E-124	1.66E-119
Zea mays MADS2	AG-like	8	-2.810371461	6.61E-16	1.45E-13
Zea mays MADS1	AG-like	10	-5.374712258	2.77E-29	2.99E-26
silky1	DEF-like	6	-1.451853526	4.13E-23	2.75E-20
Zea mays MADS16	GLO-like	3	-1.300838738	1.48E-51	4.78E-48
Zea mays MADS29	GLO-like	8	-3.744683518	6.32E-19	2.48E-16
Zea mays MADS18	GLO-like	8	-2.543267969	2.44E-30	2.87E-27
Zea mays MADS6	SEP-like	1	-2.320427884	2.89E-42	5.76E-39
Zea mays MADS14	SEP-like	1	-1.414149201	8.23E-63	5.33E-59
Zea mays MADS7	SEP-like	7	-1.052105128	1.98E-17	5.76E-15

Table 5. MADS-box genes are downregulated in the *Ptl* mutants. All class C genes (AG-like), some of class B (DEF-like and GLO-like) and some of class E (SEP-like) are dramatically downregulated. Cutoffs used : log2FoldChange >1 and <-1 and qvalue <0.05.

Discussion and Future Directions

The *Pt1* mutation affects multiple stages of the inflorescence development. Understanding the function of *Pt1* in floral organ identity and development will give significant insight in the floral mechanisms that affect yield. The findings of this thesis suggest that *Pt1* is likely to be involved in multiple determination pathways and further work is required in discovering the causative gene of this mutation.

Phenotypic Characterization

The *Pt1* phenotype is interesting because of its variability within and amongst inbred background lines. Interesting was the fasciation on the inflorescence meristem observed in both inflorescences resulting into enlarged IMs. The mechanism that regulates meristem size is the CLAVATA-WUSCHEL negative pathway and was first characterized in *Arabidopsis thaliana* (Brand, Fletcher, Hobe, Meyerowitz, & Simon, 2000; Schoof et al., 2000). This pathway is conserved amongst grasses, including maize. One of the mutants of the CLAVATA-WUSCHEL pathway in maize is *fasciated ear4 (fea4)*. *fea4* has been genetically characterized to encode for a bZIP transcription factor to regulate the shoot meristem size (Pautler et al., 2015). *fea4* has flat and tapered IMs on both inflorescences (Pautler et al., 2015). Even though, the fasciation observed in the IMs of the *Pt1* mutants was not as severe and less frequent than the *fea4* mutants, it is possible that *Pt1* has an indirect role in meristem size maintenance.

Noteworthy is the barren phenotype found in the upper part of *Pt1/+* ears in B73 and Mo17 inbred lines which lack floral organs. The pin-like phenotype is likely due to failure of the spikelet pair meristems (SPM) to initiate spikelet meristems. SPM determinacy is regulated by the *ramosa (ra)* genes and all *ra* genes have been cloned (Bortiri et al., 2006; Vollbrecht, Springer, Goh, Iv, & Martienssen, 2005). The *ra* mutants are characterized by increased

branching in both tassel and ear (Bortiri et al., 2006; Vollbrecht et al., 2005). However, *Ptl* mutants do not exhibit increased branching, even though secondary branching in the *Ptl* tassel and initiation of branch like organs in the *Ptl* ears have been observed. Thus, *Ptl* might have an indirect role of regulating SPM maintenance, independent of the *ra* genes. To test this hypothesis, I would create a double mutant with *Ptl* and one of the *ra* alleles, to look for epistasis. If the double mutant exhibited the *Ptl* phenotype then the *Ptl* gene would be epistatic to *ra* gene. On the other hand, if the double mutant exhibited a *ra* phenotype then the *ra* gene would be epistatic to the *Ptl* gene.

In addition, a single bract subtends spikelet pairs in *Ptl* mutants. There are two possibilities to explain this phenotype: 1. SMs are fasciated, resulting in enlarged SMs engulfed by large glumes and 2. the bract that subtends the spikelet pair meristems is derepressed. Derepressed bracts are leaf structures subtending a spikelet pair meristem. Derepressed bracts are observed in other floral mutants such as the *tsh1*. *tsh1* mutants fail to suppress bract development resulting in bract growth and encode for a GATA zinc finger protein (C. J. Whipple et al., 2010). The *tsh1* gene is downregulated ($\log_2\text{FoldChange} = -0.52$) in the *Ptl* mutants and the phenotype of the derepressed bract appeared in only few *Ptl* individuals. To further investigate this phenotypic defect, more scanning electron micrographs need to be taken to capture early stages of SM development. However, this indicates that *Ptl* is likely involved in SM determinacy.

The *Ptl* phenotype is also background dependent. In the B73 and Mo17 inbred lines the mutant phenotype is fully penetrant whereas in the A619 background is variable. The defects on the tassel vary more but are less severe than the *Ptl* defects on the ear. A possible explanation of the variation of the *Ptl* phenotype is the presence of a modifier in the A619 inbred line that

suppresses the *Ptl* phenotype. Modifiers are genes that can have small effects in the expression levels of another gene, and thus slightly altering the phenotype (Sang, 2009).

The *Ptl* mutants also have many defects in the floral organs. *Ptl* mutants produce silks on the tassels (in B73 and Mo17) and extra silks on the base of the ears. One of the floral mutants that is characterized by extra silks and is involved in floral organ determinacy is *zagl*. *Zagl* mutants have extra silks because they produce an excess of carpels (Mena et al., 1996). *Zagl* belongs to the C class of MADS-box transcription factors and is involved in floral meristem determinacy (Mena et al., 1996; Schmidt et al., 1993, p. 1). Similarly, *Ptl* mutants have indeterminate floral meristems and produce extra silks in both inflorescences, indicating that *Ptl* is likely involved in FM determinacy at least in part by regulating *zagl*.

Identification of the *Ptl* causative gene

I hypothesized that the *Ptl* mutant phenotype is likely to be caused by either a change in the coding region or a change in the regulatory DNA (Figure 14). If the *Ptl* phenotype is caused by a mutation in the coding region, I expected to find differences in the RNA sequence. On the other hand, if the *Ptl* phenotype is caused by a mutation in the regulatory DNA, I expect to see differences in the RNA expression levels.

I was able to identify 112 variants with a high or moderate predicted effect (Table 2) using SnpEff. However, most of those variants are either known or are naturally occurring variants and thus none of them are likely to cause the *Ptl* phenotype. However, SnpEff also predicted that there are 290 variants with low effect (Table 2). In the future, it is important to examine those low effect variants in more detail because *Ptl* could also be caused by a mutation in a microRNA binding site, in which case would not be predicted as a high/moderate effect by SnpEff.

The DE analysis revealed nine genes as possible candidates for the *Pt1* causative gene. From those 9, three genes are particularly interesting candidates. First interesting gene is the Zm00001d037604, which is homologous to the *Arabidopsis* gene *ers1* (Table 4). *ers1* is an *ethylene response sensor1* that is negatively regulator of the ethylene signaling pathway (Hua & Meyerowitz, 1998). Ethylene is a gaseous hormone that is involved in fruit ripening and in stress and pathogen responses. It is also involved in seed germination, seedling growth, organ development and senescence as well as leaf and petal abscission (Abeles, Morgan, & Jr, 2012; Bleecker & Kende, 2000). In the absence of ethylene, the ethylene receptors ERS1, ERS2, ETR1, ETR2 and EIN4, repress the ethylene responses (Hua & Meyerowitz, 1998). In addition, ethylene also activates the transcription of the ERS1, ERS2 and ETR2. The ethylene pathway has been well studied in *Arabidopsis* and some in tomato and rice, but not much is known about ethylene in maize. To move forward, a GO-term analysis should be performed to possibly identify if there are other ethylene response genes that are affected in *Pt1* mutants.

The second interesting candidate gene is Zm00001d037650, which is homologous to the *Arabidopsis RPP13-like protein3*, which is a putative disease resistant protein. The *RPP13* allele in *Arabidopsis* encodes for a nucleotide binding site – leucine rich repeat protein (NBS-LRR) and is used to recognize a variety of plant pathogens (Bittner-Eddy et al., 1999; Bittner-Eddy, Crute, Holub, & Beynon, 2000). The *RPP13-like protein3* is upregulated in the *Pt1* mutants.

The third interesting candidate gene is Zm00001d037672, which belongs to the Leucine Rich Repeats - Receptor like Kinases (LRRs-RKs). LRR-RKs are receptors involved in the CLAVATA-WUSCHEL pathway (Bommert, Je, Goldshmidt, & Jackson, 2013; Clark, Williams, & Meyerowitz, 1997; Schoof et al., 2000). As mention previously, the CLAVATA-WUSCHEL

negative feedback loop regulates meristem size and maintenance (Bommert et al., 2013; Schoof et al., 2000).

The three genes described above, are all likely candidates for the causative gene of *Ptl* mutation. *Ers1* was the only gene that exhibits allele specific expression. However, *RPP13 – like protein3* and LRR-RKs like genes did not have any single nucleotide polymorphisms that could be used to detect allele specific expression. To differentiate and possibly exclude more genes from the *Ptl*-containing interval, fine mapping could be performed next. The RNA-sequencing performed on *Ptl*/+ mutant pools in the B73 background due to the penetrance of the phenotype. Long read sequencing of a *Ptl* homozygote individual in the A619 inbred would give valuable insight for large genomic rearrangements that could result in the mutant phenotype. Furthermore, in situ hybridization of these differentially expressed within the *Ptl*-containing interval could reveal if any of these genes are expressed specifically in meristems or floral organs that the *Ptl* mutation affects.

Ptl a master regulator in floral development

Striking were the 13 MADS-box genes, all class C genes, and some from class B and class E, are downregulated in *Ptl* mutants (Table 5). Class C are AGAMOUS like and known to regulate stamen (in combination with B and E class genes) and carpel (in combination with E class) identity. Class B genes or APETALLA3/PISTILLATA are known to regulate petal (in combination with A and E class) and as previously mentioned stamen (with class C and E) identity (Alvarez-Buylla et al., 2000; De Bodt et al., 2003; Heijmans et al., 2012; Ng & Yanofsky, 2001; Theissen, 2001). Class E or SEPALLATA1/2/3/4 genes are a class of genes that are involved in regulating the identity of floral organs in combination with all other classes (Ditta et al., 2004). This suggests that *Ptl* might have a master role in regulating floral organ identity.

LIST OF REFERENCES

1. Abeles, F. B., Morgan, P. W., & Jr, M. E. S. (2012). *Ethylene in Plant Biology*. Academic Press.
2. Alvarez-Buylla, E. R., Liljegren, S. J., Pelaz, S., Gold, S. E., Burgeff, C., Ditta, G. S., ... Yanofsky, M. F. (2000). MADS-box gene evolution beyond flowers: expression in pollen, endosperm, guard cells, roots and trichomes. *The Plant Journal: For Cell and Molecular Biology*, 24(4), 457–466.
3. Ambrose, B. A., Lerner, D. R., Ciceri, P., Padilla, C. M., Yanofsky, M. F., & Schmidt, R. J. (2000). Molecular and Genetic Analyses of the Silky1 Gene Reveal Conservation in Floral Organ Specification between Eudicots and Monocots. *Molecular Cell*, 5(3), 569–579. [https://doi.org/10.1016/S1097-2765\(00\)80450-5](https://doi.org/10.1016/S1097-2765(00)80450-5)
4. Angenent, G. C., & Colombo, L. (1996). Molecular control of ovule development. *Trends in Plant Science*, 1(7), 228–232. [https://doi.org/10.1016/1360-1385\(96\)86900-7](https://doi.org/10.1016/1360-1385(96)86900-7)
5. Bartlett, M. E., Williams, S. K., Taylor, Z., DeBlasio, S., Goldshmidt, A., Hall, D. H., ... Whipple, C. J. (2015). The Maize PI/GLO Ortholog Zmm16/sterile tassel silky ear1 Interacts with the Zygomorphy and Sex Determination Pathways in Flower Development. *The Plant Cell*, 27(11), 3081–3098. <https://doi.org/10.1105/tpc.15.00679>
6. Becker, A., & Theissen, G. (2003). The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Molecular Phylogenetics and Evolution*, 29(3), 464–489.
7. Bittner-Eddy, P., Can, C., Gunn, N., Pinel, M., Tör, M., Crute, I., ... Beynon, J. (1999). Genetic and physical mapping of the RPP13 locus, in Arabidopsis, responsible for specific recognition of several Peronospora parasitica (downy mildew) isolates. *Molecular Plant-Microbe Interactions: MPMI*, 12(9), 792–802. <https://doi.org/10.1094/MPMI.1999.12.9.792>
8. Bittner-Eddy, P. D., Crute, I. R., Holub, E. B., & Beynon, J. L. (2000, January 1). RPP13 is a simple locus in Arabidopsis thaliana for alleles that specify downy mildew resistance to different avirulence determinants in Peronospora parasitica. <https://doi.org/10.1046/j.1365-3113x.2000.00664.x>
9. Bleecker, A. B., & Kende, H. (2000). Ethylene: A Gaseous Signal Molecule in Plants. *Annual Review of Cell and Developmental Biology*, 16(1), 1–18. <https://doi.org/10.1146/annurev.cellbio.16.1.1>
10. Bommert, P., Je, B. I., Goldshmidt, A., & Jackson, D. (2013). The maize Ga gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size. *Nature*, 502(7472), 555–558. <https://doi.org/10.1038/nature12583>
11. Bommert, P., & Whipple, C. (2018). Grass inflorescence architecture and meristem determinacy. *Seminars in Cell & Developmental Biology*, 79, 37–47. <https://doi.org/10.1016/j.semcdb.2017.10.004>
12. Bortiri, E., Chuck, G., Vollbrecht, E., Rocheford, T., Martienssen, R., & Hake, S. (2006). ramosa2 Encodes a LATERAL ORGAN BOUNDARY Domain Protein That Determines the Fate of Stem Cells in Branch Meristems of Maize. *The Plant Cell*, 18(3), 574–585. <https://doi.org/10.1105/tpc.105.039032>

13. Brand, U., Fletcher, J. C., Hobe, M., Meyerowitz, E. M., & Simon, R. (2000). Dependence of Stem Cell Fate in Arabidopsis on a Feedback Loop Regulated by CLV3 Activity. *Science*, 289(5479), 617–619. <https://doi.org/10.1126/science.289.5479.617>
14. Cheng, P. C., Greyson, R. I., & Walden, D. B. (1983). Organ initiation and the development of unisexual flowers in the tassel and ear of zea mays. *American Journal of Botany*, 70(3), 450–462. <https://doi.org/10.1002/j.1537-2197.1983.tb06411.x>
15. Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., ... Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly*, 6(2), 80–92. <https://doi.org/10.4161/fly.19695>
16. Clark, S. E., Williams, R. W., & Meyerowitz, E. M. (1997). The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. *Cell*, 89(4), 575–585.
17. Coen, E. S., & Meyerowitz, E. M. (1991). The war of the whorls: genetic interactions controlling flower development. *Nature*, 353(6339), 31. <https://doi.org/10.1038/353031a0>
18. Colasanti, J., & Coneva, V. (2009). Mechanisms of Floral Induction in Grasses: Something Borrowed, Something New. *Plant Physiology*, 149(1), 56–62. Retrieved from JSTOR.
19. De Bodt, S., Raes, J., Van de Peer, Y., & Theissen, G. (2003). And then there were many: MADS goes genomic. *Trends in Plant Science*, 8(10), 475–483. <https://doi.org/10.1016/j.tplants.2003.09.006>
20. Ditta, G., Pinyopich, A., Robles, P., Pelaz, S., & Yanofsky, M. F. (2004). The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. *Current Biology: CB*, 14(21), 1935–1940. <https://doi.org/10.1016/j.cub.2004.10.028>
21. Eveland, A. L., Goldshmidt, A., Pautler, M., Morohashi, K., Liseron-Monfils, C., Lewis, M. W., ... Jackson, D. (2014). Regulatory modules controlling maize inflorescence architecture. *Genome Research*, 24(3), 431–443. <https://doi.org/10.1101/gr.166397.113>
22. Fan, H.-Y., Hu, Y., Tudor, M., & Ma, H. (1997). Specific interactions between the K domains of AG and AGLs, members of the MADS domain family of DNA binding proteins. *The Plant Journal*, 12(5), 999–1010. <https://doi.org/10.1046/j.1365-313X.1997.12050999.x>
23. FelixKrueger. (2019). *A wrapper around Cutadapt and FastQC to consistently apply adapter and quality trimming to FastQ files, with extra functionality for RRBS data: FelixKrueger/TrimGalore* [Perl]. Retrieved from <https://github.com/FelixKrueger/TrimGalore> (Original work published 2016)
24. Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., ... Rokhsar, D. S. (2012). Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research*, 40(D1), D1178–D1186. <https://doi.org/10.1093/nar/gkr944>
25. Heijmans, K., Morel, P., & Vandenbussche, M. (2012). MADS-box genes and floral development: the dark side. *Journal of Experimental Botany*, 63(15), 5397–5404. <https://doi.org/10.1093/jxb/ers233>
26. Hua, J., & Meyerowitz, E. M. (1998). Ethylene Responses Are Negatively Regulated by a Receptor Gene Family in Arabidopsis thaliana. *Cell*, 94(2), 261–271. [https://doi.org/10.1016/S0092-8674\(00\)81425-7](https://doi.org/10.1016/S0092-8674(00)81425-7)
27. Jiao, Y., Peluso, P., Shi, J., Liang, T., Stitzer, M. C., Wang, B., ... Ware, D. (2017). Improved maize reference genome with single-molecule technologies. *Nature*, 546(7659), 524–527. <https://doi.org/10.1038/nature22971>

28. Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F., & Donoghue, M. J. (2007). *Plant Systematics: A Phylogenetic Approach*. Sunderland, MA: Sinauer Associates.
29. Kaufmann, K., Melzer, R., & Theißen, G. (2005). MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. *Gene*, 347(2), 183–198. <https://doi.org/10.1016/j.gene.2004.12.014>
30. Kellogg, E. A. (2007). Floral displays: genetic control of grass inflorescences. *Current Opinion in Plant Biology*, 10(1), 26–31. <https://doi.org/10.1016/j.pbi.2006.11.009>
31. Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: a fast-spliced aligner with low memory requirements. *Nature Methods*, 12. <https://doi.org/10.1038/nmeth.3317>
32. Korbie, D. J., & Mattick, J. S. (2008). Touchdown PCR for increased specificity and sensitivity in PCR amplification. *Nature Protocols*, 3(9), 1452–1456. <https://doi.org/10.1038/nprot.2008.133>
33. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
34. Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 550. <https://doi.org/10.1186/s13059-014-0550-8>
35. Marini, F., & Binder, H. (2017). Development of Applications for Interactive and Reproducible Research: a Case Study. *Genomics and Computational Biology*, 3(1), e39–e39. <https://doi.org/10.18547/gcb.2017.vol3.iss1.e39>
36. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, 17(1), 10–12. <https://doi.org/10.14806/ej.17.1.200>
37. Mena, M., Ambrose, B. A., Meeley, R. B., Briggs, S. P., Yanofsky, M. F., & Schmidt, R. J. (1996). Diversification of C-Function Activity in Maize Flower Development. *Science*, 274(5292), 1537–1540. <https://doi.org/10.1126/science.274.5292.1537>
38. Nelson, O. E., & Postlethwait, S. N. (1954). VARIATIONS IN ATYPICAL GROWTH IN A MAIZE MUTANT. *American Journal of Botany*, 41(9), 739–748. <https://doi.org/10.1002/j.1537-2197.1954.tb14405.x>
39. Ng, M., & Yanofsky, M. F. (2001). Function and evolution of the plant MADS-box gene family. *Nature Reviews. Genetics*, 2(3), 186–195. <https://doi.org/10.1038/35056041>
40. Pautler, M., Eveland, A. L., LaRue, T., Yang, F., Weeks, R., Lunde, C., ... Jackson, D. (2015). *FASCIATED EAR4* Encodes a bZIP Transcription Factor That Regulates Shoot Meristem Size in Maize. *The Plant Cell Online*, 27(1), 104–120. <https://doi.org/10.1105/tpc.114.132506>
41. Pelaz, S., Gustafson-Brown, C., Kohalmi, S. E., Crosby, W. L., & Yanofsky, M. F. (2001). APETALA1 and SEPALLATA3 interact to promote flower development. *The Plant Journal*, 26(4), 385–394. <https://doi.org/10.1046/j.1365-313X.2001.2641042.x>
42. Postlethwait, S. N., & Nelson, O. E. (1964). Characterization of Development in Maize Through the Use of Mutants. I. The Polytypic (Pt) and Ramosa-1 (ra1) Mutants. *American Journal of Botany*, 51(3), 238–243. <https://doi.org/10.2307/2440292>
43. Riechmann, J. L., Krizek, B. A., & Meyerowitz, E. M. (1996). Dimerization specificity of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA, and AGAMOUS. *Proceedings of the National Academy of Sciences*, 93(10), 4793–4798. <https://doi.org/10.1073/pnas.93.10.4793>

44. Ringnér, M. (2008). What is principal component analysis? *Nature Biotechnology*, 26(3), 303–304. <https://doi.org/10.1038/nbt0308-303>
45. Robinson, J. T., Thorvaldsdóttir, H., Wenger, A. M., Zehir, A., & Mesirov, J. P. (2017). Variant Review with the Integrative Genomics Viewer. *Cancer Research*, 77(21), e31–e34. <https://doi.org/10.1158/0008-5472.CAN-17-0337>
46. Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., & Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, 29, 24–26. <https://doi.org/10.1038/nbt.1754>
47. Sang, T. (2009). Genes and Mutations Underlying Domestication Transitions in Grasses. *Plant Physiology*, 149(1), 63–70. <https://doi.org/10.1104/pp.108.128827>
48. Schmidt, R. J., Veit, B., Mandel, M. A., Mena, M., Hake, S., & Yanofsky, M. F. (1993). Identification and molecular characterization of ZAG1, the maize homolog of the Arabidopsis floral homeotic gene AGAMOUS. *The Plant Cell*, 5(7), 729–737.
49. Schoof, H., Lenhard, M., Haecker, A., Mayer, K. F., Jürgens, G., & Laux, T. (2000). The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell*, 100(6), 635–644.
50. Shore, P., & Sharrocks, A. D. (1995). The MADS-box family of transcription factors. *European Journal of Biochemistry*, 229(1), 1–13.
51. Sommer, H., Beltrán, J. P., Huijser, P., Pape, H., Lönnig, W. E., Saedler, H., & Schwarz-Sommer, Z. (1990). Deficiens, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homology to transcription factors. *The EMBO Journal*, 9(3), 605–613.
52. Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Zuloaga, F. O., Judziewicz, E. J., ... Morrone, O. (2015). A worldwide phylogenetic classification of the Poaceae (Gramineae). *Journal of Systematics and Evolution*, 53(2), 117–137. <https://doi.org/10.1111/jse.12150>
53. Takacs, E. M., Li, J., Du, C., Ponnala, L., Janick-Buckner, D., Yu, J., ... Scanlon, M. J. (2012). Ontogeny of the Maize Shoot Apical Meristem. *The Plant Cell*, 24(8), 3219–3234. <https://doi.org/10.1105/tpc.112.099614>
54. Theissen, G. (2001). Development of floral organ identity: stories from the MADS house. *Current Opinion in Plant Biology*, 4(1), 75–85.
55. Theissen, G., Kim, J. T., & Saedler, H. (1996). Classification and phylogeny of the MADS-box multigene family suggest defined roles of MADS-box gene subfamilies in the morphological evolution of eukaryotes. *Journal of Molecular Evolution*, 43(5), 484–516. <https://doi.org/10.1007/BF02337521>
56. Theissen, G., Melzer, R., & Rümpler, F. (2016). MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. *Development*, 143(18), 3259–3271. <https://doi.org/10.1242/dev.134080>
57. Thompson, B. (2014). Chapter Nine - Genetic and Hormonal Regulation of Maize Inflorescence Development. In F. Fornara (Ed.), *Advances in Botanical Research* (pp. 263–296). <https://doi.org/10.1016/B978-0-12-417162-6.00009-2>
58. Thompson, B. E., Bartling, L., Whipple, C., Hall, D. H., Sakai, H., Schmidt, R., & Hake, S. (2009). bearded-ear Encodes a MADS Box Transcription Factor Critical for Maize Floral Development. *The Plant Cell*, 21(9), 2578–2590. <https://doi.org/10.1105/tpc.109.067751>
59. Thompson, B. E., Basham, C., Hammond, R., Ding, Q., Kakrana, A., Lee, T.-F., ... Hake, S. (2014). The dicer-like1 homolog fuzzy tassel is required for the regulation of meristem

- determinacy in the inflorescence and vegetative growth in maize. *The Plant Cell*, 26(12), 4702–4717. <https://doi.org/10.1105/tpc.114.132670>
60. Thompson, B. E., & Hake, S. (2009). Translational biology: from Arabidopsis flowers to grass inflorescence architecture. *Plant Physiology*, 149(1), 38–45. <https://doi.org/10.1104/pp.108.129619>
 61. Thorvaldsdóttir, H., Robinson, J. T., & Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in Bioinformatics*, 14(2), 178–192. <https://doi.org/10.1093/bib/bbs017>
 62. Vollbrecht, E., Springer, P. S., Goh, L., Iv, E. S. B., & Martienssen, R. (2005). Architecture of floral branch systems in maize and related grasses. *Nature*, 436(7054), 1119. <https://doi.org/10.1038/nature03892>
 63. Weigel, D., & Meyerowitz, E. M. (1994). The ABCs of floral homeotic genes. *Cell*, 78(2), 203–209. [https://doi.org/10.1016/0092-8674\(94\)90291-7](https://doi.org/10.1016/0092-8674(94)90291-7)
 64. Whipple, C. J., Hall, D. H., DeBlasio, S., Taguchi-Shiobara, F., Schmidt, R. J., & Jackson, D. P. (2010). A Conserved Mechanism of Bract Suppression in the Grass Family. *The Plant Cell*, 22(3), 565–578. <https://doi.org/10.1105/tpc.109.073536>
 65. Whipple, Clinton J., Ciceri, P., Padilla, C. M., Ambrose, B. A., Bandong, S. L., & Schmidt, R. J. (2004). Conservation of B-class floral homeotic gene function between maize and Arabidopsis. *Development*, 131(24), 6083–6091. <https://doi.org/10.1242/dev.01523>
 66. Whipple, Clinton J., & Schmidt, R. J. (2006). Genetics of Grass Flower Development. In *Developmental Genetics of the Flower: Vol. 44. Advances in Botanical Research* (pp. 385–424). [https://doi.org/10.1016/S0065-2296\(06\)44010-6](https://doi.org/10.1016/S0065-2296(06)44010-6)
 67. Zhao, Y., Li, X., Chen, W., Peng, X., Cheng, X., Zhu, S., & Cheng, B. (2011). Whole-genome survey and characterization of MADS-box gene family in maize and sorghum. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 105(2), 159–173. <https://doi.org/10.1007/s11240-010-9848-8>

