Characterizing the Role of Pectin in Cell Wall Composition and Organ Initiation in Maize

By

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Abstract

Plants are constantly developing new organs throughout their life and these organs arise from stem cell pools called meristems. Organ primordia are generated on the flanks of meristems when stem cells differentiate into new cell types. Newly differentiated cells are unable to move freely because they are surrounded by a rigid cell wall. To allow for proper organ outgrowth, the cell wall must be modified to allow for cell expansion. Organ initiation is closely linked to, and regulated by, distributions of the plant hormone auxin. The cell wall polysaccharide, pectin, is modified at the sites of auxin accumulation and is required for organ outgrowth, at least in some plants. Previous work in maize showed that floral meristems have differing localization of pectin that may coincide with differences in organ fate. We characterized meristems in the female inflorescence which give rise to different structures and different number of primordia. Mutants with altered floral meristem determinacy were assessed to determine if changes in floral meristem activity correlated with changes in pectin localization. The shoot apical meristem was assessed, and we found that pectin localization is not uniform between vegetative meristems and floral meristems. We found that meristems of the inflorescence localize pectin differently and that pectin modification is necessary for some organ development. Mutants that lose determinacy localize pectin differently which suggests that pectin composition in the cell wall is intimately linked to proper meristem activity and floral organ primordia formation.

Characterizing the Role of Pectin in Cell Wall Composition and Organ Initiation in Maize

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CHAPTER 1: Characterizing cell wall composition in maize

Plants continuously grow and develop new organ structures throughout their life. This growth arises from meristems. Meristems are pools of undifferentiated stem cells that can self-renew and differentiate into new cell types to drive tissue growth and organ formation (Thompson et al. 2009). Plant cells are surrounded by a cell wall, which provides support to the cells and also anchors adjacent cells together. The conjoined nature of plant cells leads to unique challenges in development. For plants to grow organs properly there must be changes in cell wall composition to allow for increases to cell size, changes in cell shape, and an increase in cell number.

All above ground structures of the plant are derived from the shoot apical meristem (SAM) which contains pluripotent stem cells. The shoot apical meristem gives rise to leaves and vegetative organs. At the transition to reproductive development, the SAM transitions to an inflorescence meristem that will ultimately form the tassel, whereas axillary shoot meristems transition to convert inflorescence meristems that will form the ears. The inflorescence meristem is an indeterminate stem cell pool that gives rise to several determinate stem cell pools before initiating floral meristems. Maize plants produce both male and female inflorescences which contain structural similarities that diverge into sex specific differences. Tassel inflorescence meristem first generates a series of axillary meristems known as branch meristems (BM) that make the long branches of the mature tassel (Gallavotti, Yang, et al. 2008). The inflorescence meristem of the ear lacks branch meristems but initiates other meristems in a similar patterning to the tassel. In both the ear and the tassel, the inflorescence meristem will give rise to the spikelet pair meristem (SPM) which will in turn give rise to two spikelet meristems (SM). The spikelet meristems each give rise to two floral meristems (FM) (Figure 1. A) that will give rise to

Figure 1. Cell wall diagram

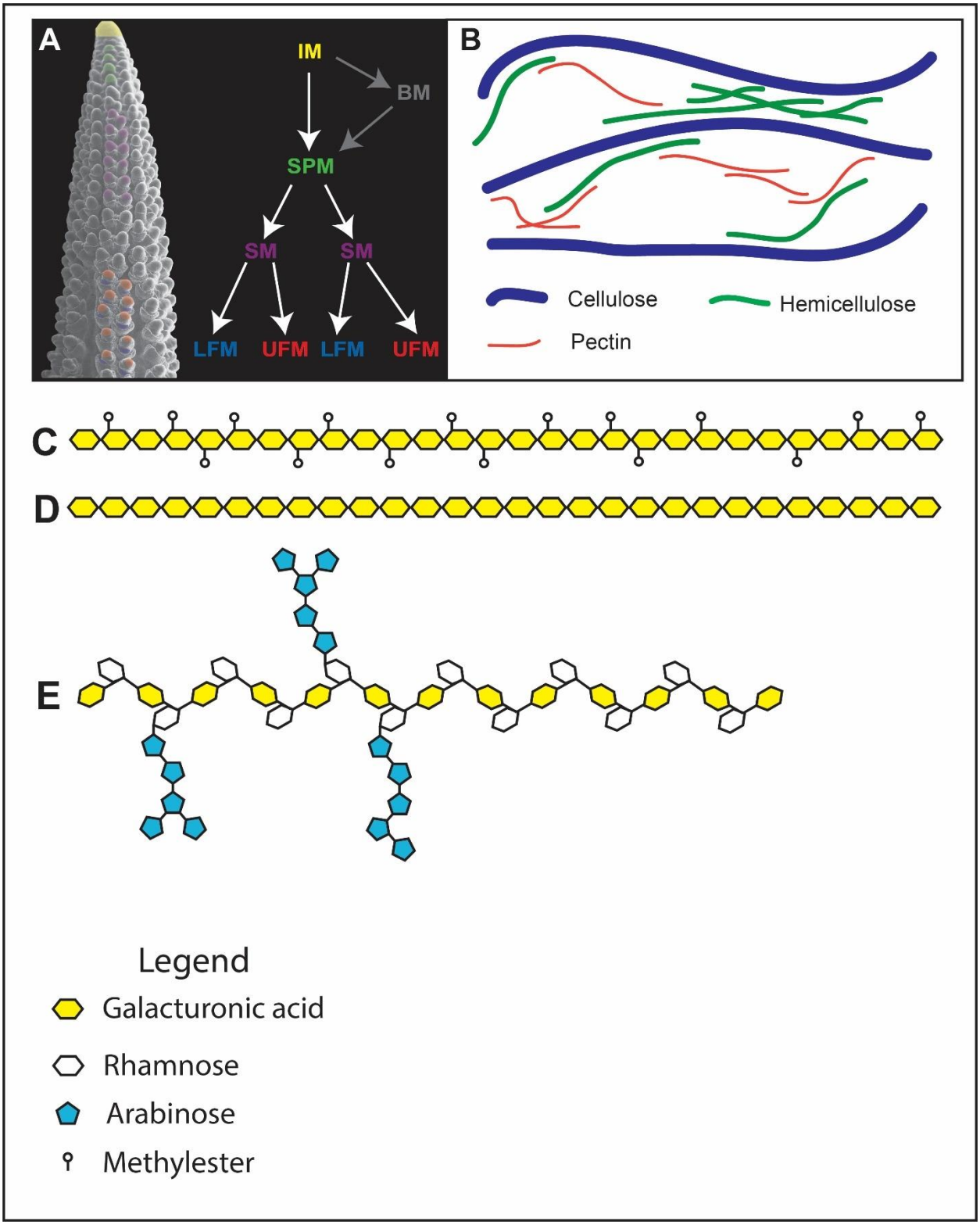

Figure 1. Legend

Overview of inflorescence development and cell wall components in our study. A, scanning electron micrograph of the female inflorescence of the ear. Individual meristems are color coded to match that developmental flow chart. B, overview of primary cell walls consisting of cellulose with a matrix of interconnected pectin and hemicellulose C, native made methylesterified pectin. D, demethylesterified pectin after the removal of methylesters. E, rhamnogalacturonan-I with arabinan side chains in blue.

floral organs (Bommert et al. 2005). In ears, stamens and lodicules arrest and the lower floret aborts, in tassels, the carpels abort (Irish and Nelson 1993). These differences in development lead to the tassel giving rise to male flowers and the ear giving rise to female flowers.

Boundaries play an important role in proper organ patterning and growth throughout inflorescence development (Hibara et al. 2006). Boundaries between meristems and organs display low cell division and expansion rates, parallel oriented microtubules, and relatively stiff cell walls. (Hamant et al. 2008). Several genes that regulate meristem determinacy are expressed in boundary regions. Meristems can be determinate, giving rise to a set number and patterning of primordia, or indeterminate and give rise to an unspecified number of primordia. The gene *barren stalk1* (*ba1*) encodes a transcription factor that acts downstream of auxin transport and leads to maize plants that lack ears and have sterile tassels. *Ba1* is expressed in a boundary pattern that is necessary for axillary meristem formation (Galli et al. 2015). A previously identified gene, Bowman-Birk type trypsin inhibitor (*BBTI*), is expressed in a boundary domain between the upper and lower floret in maize ears (Yang et al. 2022). In a fellow grass, barley, *compositum 1* has been shown to effect spike branching through changing cell wall compositions of meristematic boundary cells (Poursarebani et al. 2020). In some developmental contexts, boundary gene expression is essential to maintaining the determinacy of meristematic cell pools. The loss of boundary regions can lead to changes in meristem activity such as a loss in meristem determinacy (Satoh-Nagasawa et al. 2006).

Floral meristem determinacy is required for proper floral organ development. In *Arabidopsis thaliana*, the gene *AGAMOUS* is required for proper floral organ development and determinacy (Mena et al. 1996). The *AGAMOUS* homolog in maize, *Zea AG* (*zag1*), is a floral mutant that loses determinacy in the upper floral meristem and initiates additional carpels

(Schmidt et al. 1993). A related gene, *zag3*, better known as *bearded-ear* (*bde*) is a floral development mutant that loses determinacy in both the upper and lower floral meristems. The upper floral meristem will initiate floral organs with improper number or positioning while the lower floral meristem initiates additional floral meristems (Thompson et al. 2009). Comparative analysis of pectin cell wall epitopes between the cell walls of floral meristems that are determinate, and those that have lost determinacy can be conducted. With this type of investigation, a link between pectin modification in the cell wall and changes in meristem activity may be observed.

The cell wall is a dynamic and integral part of plant physiology and exists in two major divisions, the primary and the secondary cell walls. The primary cell wall is a pliable cell wall that forms during cell division and is filled with pectic polysaccharide depositions from the Golgi apparatus (Bringmann et al. 2012). The primary cell wall is thin, loosely packed, with the major component being cellulose in a matrix of hemicellulose and pectin cross linkages that can be reversibly modified (Figure 1. B). The secondary cell wall is thick, tightly packed, and consists mostly of cellulose with lower amounts of pectin than are found in the primary cell wall. The secondary cell wall is deposited after cells stop dividing but my work focuses on the primary cell wall. The higher concentration of pectin in the primary cell wall is not universal across all plant species. There are two types of primary cell wall, type I and type II. The distinction between the two types of primary cell walls is based on chemical components and biosynthetic pathways of cell wall components (Carpita 1996). Type I cell walls are found in dicots and non-commelinid monocots (Barnes and Anderson 2018). In contrast, type II cell walls are found in commelinid monocots, which include grasses like maize, and contain less pectin in exchange for more hemicellulose. Almost all the work linking growth and meristem activity has been done in

Arabidopsis, which has type I cell walls. While cell wall traits in Arabidopsis have been used as a basis for investigation of the cell wall in maize, the role of modifications to the cell wall in meristems is very understudied in grasses. It is unclear if the findings in Arabidopsis and the role of cell wall modification will hold true in maize based on the difference in primary cell wall types.

Pectin modification is required to increase cell wall extensibility and promote primordia outgrowth in Arabidopsis (Peaucelle et al. 2008). Pectins are a complex class of cell wall polysaccharides consisting of covalently linked domains of different galactan-rich polysaccharides (Harholt, Suttangkakul, and Vibe Scheller 2010). The domains of pectin consist of xylogalacturonan, homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II, each with their own specific chemistry, biomechanical properties, and localizations during development. The most abundant of these domains is homogalacturonan (HG) which consist of α-d-(1-4)-galacturonic acid (GalA) residues, which can be methyl-esterified, acetylated, and/or substituted with xylose to form xylogalacturonan (Driouich et al. 2012). Homogalacturonan is secreted from the golgi apparatus in a highly methylesterified state (Figure 1. C) and once embedded in the cell wall, it can be selectively modified. One such modification is PECTIN METHYL ESTERASE (PME)-mediated demethylesterification, which removes methylesters from the galacturonic acid backbone (Figure 1. D). Another abundant pectin form is rhamnogalacturonan-I (RG-I), which is the only pectin not built upon an exclusively galacturonic acid rich backbone but instead has the sugar rhamnose interspersed in a disaccharide repeat fashion αGalA(1—>2)-α-L-rhamnose(1—>4). RG-I domains have side chains on the rhamnose sugar consisting of xylan, galactan, arabinogalactan, and arabinan sugars (Höfte and Voxeur 2017). Arabinan side chains can be unbranched or branched (Figure 1. E); long, unbranched

chains of arabinan have been shown to bind cellulose (Zykwinska et al. 2005) and that cellulose microfibrils interact with a stiff arabinan gel (Vignon et al. 2004). An alternate finding suggests that arabinan side chains of RG-I keep HG domains from interacting and forming stiff cross-linkages, increasing extensibility (Jones et al. 2003). The role of pectin in organ initiation and tissue outgrowth is an ongoing field of study and the correlation between RG-I and cell wall extensibility is little understood.

Organ initiation is closely linked to, and regulated by, distributions of the hormone auxin (Reinhardt, Mandel, and Kuhlemeier 2000). Changes to individual cells is the basis for organ initiation and development in a process called organogenesis. Organogenesis occurs on the periphery of meristems that contain stem cells that can differentiate into new cell types. Cells change size, shape, or number for growth to take place, each of these changes requires modification of the cell wall to allow for this expansion. The modifications to the cell wall to drive organogenesis are context dependent and are mediated through multiple forces acting on the cell. There is the combination of turgor pressure, deposition of cortical microtubules to drive cellulose synthesis which determines the axis of elongation, and selective modification to the cell wall in response to auxin (Khadka et al. 2019; Peaucelle et al. 2011). Auxin is a plant growth hormone that regulates many biological functions in plants through the generation of auxin maxima. (Weller et al. 2017). The formation of an auxin maxima leads to changes in cell wall composition to allow for tissue growth. The generation of an auxin maxima relies on auxin transport but also may be supplemented by local auxin biosynthesis, which helps to drive development of proper organ formation (Vernoux, Besnard, and Traas 2010). Auxin maxima leads to changes in gene expression and can induce the functioning of PECTIN METHYLESTERASE to begin selective modification of the cell wall to allow for growth. Over-

expression of PMEs in shoot apical meristems leads to ectopic and additional organ initiation while over-expression of PECTIN METHYLESTERASE inhibitors prevents organ initiation in the shoot apical meristem (Peaucelle et al. 2011). Shoot apical meristems with PECTIN METHYLESTERASE over-expression exhibited more elastic cell walls, while shoot apical meristems with PECTIN METHYLESTERASE inhibitor over-expression exhibited more rigid cell walls (Peaucelle et al. 2011). This indicates that modification of the cell wall is a key mechanism by which auxin regulates primordia initiation and outgrowth.

The link between pectin, auxin, and organ initiation has been further explored using the *pin1* mutant in Arabidopsis. *Pin1* mutants fail to initiate most organ primordia, but exogenous application of PECTIN METHYLESTERASE leads to pectin demethylation and tissue outgrowth. Additionally, the plants with inhibited PECTIN METHYLESTERASE activity exhibit disorganized PIN1 localization (Braybrook and Peaucelle 2013). These experiments indicated that auxin mediated changes of organ initiation cannot occur in the shoot apical meristem without homogalacturonan modification to reduce cell wall rigidity. In *pin1* mutants, cell wall rigidity was inferred by (HG) demethylation status, the greater the degree of HG demethylation the less rigid the cell wall. These assumptions were made based on previous AFM data in Arabidopsis. These experiments were performed in dicots with type I cell walls, much less is known about the role of pectin in grasses such as maize that have type II cell walls.

Previous work in our lab identified the cell wall as an expected regulator of floral development. Laser capture microdissection of the upper and lower floral meristems followed by an RNA sequencing and differential gene expression analysis found that cell wall genes are differentially regulated between the upper and lower floral meristem (Yang et al. 2022). This began the investigation into pectin which found that specific pectin epitopes have differing

patterns of accumulation in the upper and lower floral meristems. Demethylesterified pectin accumulates during organ initiation and remains during organ outgrowth in some organ primordia. This is seen in the initiating glume of the spikelet meristem and the floral organ primordia of the upper floral meristem. This suggests that demethylesterified pectin is important for at least some organogenesis in grasses. Methylesterified pectin exhibits a very different localization pattern, suggesting that both types of pectin are important and doing developmentally separate tasks. In maize roots, immunohistochemistry staining has shown the localization of a variety of pectin epitopes in meristematic or elongating zones. Methylesterified pectin is found in meristematic regions but not in the elongating regions while demethylesterified pectin is found in the early and late elongation zones (Kozlova et al. 2020). These results further suggest that pectin modification is necessary for organ development in maize. These differences in floral meristem cell wall composition raise many questions about the role of the cell wall throughout meristematic development in maize.

The role of pectin and cell wall modification in maize meristems is still unknown. Most of the literature linking cell wall modification and meristem activity has been performed with dicots that have type I cell walls. Maize, with type II cell walls, provides a unique organism to study cell walls in grasses. The maize inflorescence has many different meristems with different activities, which allows us to probe the links between cell wall modifications and meristem activity. We examined pectin dynamics throughout ear development as well as mutants that affect specific aspects of inflorescence development. With maize shoot meristems we can assess pectin dynamics in a meristem that drives vegetative growth. With the combination of many meristem types we can assess pectin dynamics in both reproductive and vegetative meristems. To investigate cell wall dynamics during meristem development, we analyzed the localization of

three pectic epitopes in a combination of normal and mutant meristems. This investigation has provided insight and evidence that cell walls of meristematic tissue are dynamic during development and that different meristems exhibit differing cell wall compositions. Furthermore, mutants that have changes in meristem activity have changes in their localization of pectin that correlate with the altered activity they display. Furthermore, we found that cell wall composition is not uniform in all aerial meristems as the shoot apical meristem exhibits a very different pattern of cell wall composition when compared to inflorescence meristems.

Chapter 2: Results

Methylesterified and Demethylesterified pectin are Dynamically regulated during floral development

Previous work showed that the pectic epitopes recognized by LM19 (demethylesterified pectin) and LM20 (methylesterified pectin) are dynamically regulated during floral development in the ear (Yang et al. 2022). The LM19 antibody binds to low methylesterified homogalacturonan while the LM20 antibody binds highly methyesterified homogalacturonan (Verhertbruggen, Marcus, Haeger, Ordaz-Ortiz, et al. 2009). To repeat what has been seen previously, we characterized wildtype B73 ear floral meristem at early and later stages. In the early stages of floral meristem development, there is strong localization of demethylesterified pectin in the internal layers of the upper floret. The LM19 antibody staining in early-stage floral meristem development is diffuse at the base of the floret (Figure 2. A) but will strongly stain cells at the site of some organ primordia. LM19 staining of initiating organs is most prominent in developing carpels in the upper floret (Figure 2. B). LM19 staining is detected at the base of the lower floret but is not detected in the lower floret with the same intensity or frequency as the upper floret. Ten biological replicates were used to ensure the reliability of our staining protocol and to reproduce the results previously published.

LM20 antibody staining of methylesterified pectin in early-stage floral meristems localizes on the apical surface of the L1 layer of the upper floral meristem and the abaxial side of the glume (Figure 2. C). As the florets age, LM20 staining is predominantly on the apical surface of the upper floret, floral organ primordia such as the carpal, and the abaxial side of the glume (Figure 2. D). The staining pattern of LM20 is much less intense on the apical surface of the lower floral meristem and is confined to cell-to-cell junction this characterization was done

Figure 2. WT ear floral meristem characterized with LM19, LM20, and LM13

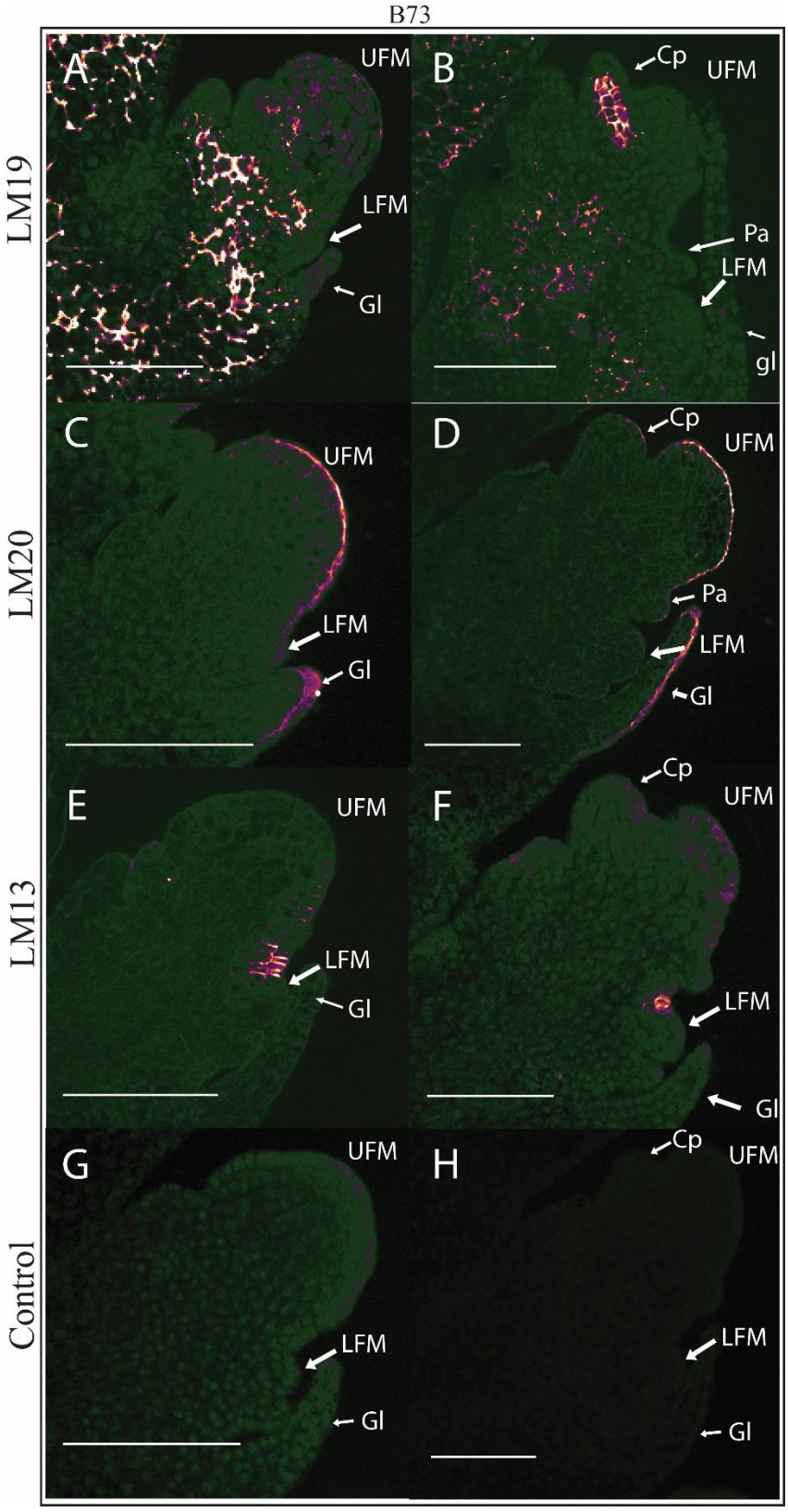

Figure 2. Legend

Wildtype ear floral meristems characterized with LM19, LM20,and LM13. LM19 immunostaining of demethylesterified pectin in early (A) and late (B) stage ear florets. LM20 immunostaining of methylesterified pectin in early (C) and late (D) stage ear florets. LM13 immunostaining of alpha 1,5-linked arabinan side chains of RG-I in early (E) and late (F) stage ear florets. Fluorescence controls in early (G) and late (H) stage ear florets. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. UFM, upper floral meristem; LFM, lower floral meristem; Gl, glume; Cp, carpel primordia; Le, lemma. Scale bars = 100 µm

done using six biological replicates.

This dynamic regulation prompted us to explore the localizations of other cell wall epitopes with commercially available antibodies. The next antibody we assessed in wild type floral meristems is LM13 which binds to the arabinan side chains of RG-I (Verhertbruggen, Marcus, Haeger, Verhoef, et al. 2009). LM13 antibody staining in B73 ear florets shows accumulation in a small group of cells at the boundary between the upper floret and the initiating lower floral meristem (Figure 2. E). At times, staining is seen weakly extending along the L1 of the upper floret above the outgrowth of the palea but is rare. As florets age and the lower floral meristem develops, the staining pattern reduces further but remains in the boundary between the upper and lower floral meristems (Figure 2. F). Staining is consistent in the group between upper and lower floret throughout developmental stages and is variable but mostly absent from the apical surface of the lower floral meristem. To fully characterize this antibodies localization in B73 ear, 15 biological replicates were performed to set a baseline for comparison. Fluorescence controls of B73 ear florets at similar growth stages were included to assess the autofluorescence of the tissue (Figure 2. G, H).

Mutants with altered floral meristem activity have altered pectin dynamics

The upper and lower florets have different fates and growth dynamics. The differential accumulation of pectin in upper and lower florets raised the intriguing possibility that pectin may be linked to meristem activity. To test this hypothesis, we examined pectin accumulation in mutants with altered meristem activity. Specifically, we examined *bde* mutants, in which both the upper and lower floral meristems are indeterminate and *zag1*, in which the upper floral meristem is indeterminate, but the lower floral meristem aborts normally. The first mutant assessed is *bde*, which has abnormal development in both the upper and lower floral meristems

(Thompson et al. 2009). Similar to wild-type, LM19 stained the base of upper florets in *bde* mutants and initiating organ primorida. However, while LM19 did not stain lower florets in wild-type spikelets, LM19 consistently stained the lower floret of *bde* mutant spikelets (Figure 3. A). In later stage floral meristems, there is a large accumulation of demethylesterified pectin in the lower floral meristem that is not seen in wild type floral meristems (Figure 3. B). characterization was completed using five biological replicates.

I also observed altered accumulation of LM20 in *bde* mutants compared to wild-type. Similar to wild-type, LM20 stained the apical surface of the upper florets in *bde* mutants. However, while LM20 did not strongly stain the apical surface of the lower floret, in wild-type spikelet, LM20 consistently stained the apical surface of the lower floret in *bde* mutant spikelets. LM20 staining displayed a similar distribution of methylesterified pectin on the apical surface of young floral meristems (Figure 3. C). In older floral meristems, the signal is strongest at the apex of the upper floret, similar to that seen in wildtype. In *bde* spikelets, LM20 antibody signal persists down the adaxial side of the apical surface of the upper floret and is consistently observed on the apical surface of the lower floral meristem (Figure 3. D). Characterization was done using six biological replicates.

Finally, I examined the LM13 staining in *bde* mutants which binds to arabinan side chains of RG-1. At early stages of floral meristem development, LM13 staining extends from the top of the initiating lower floral meristem along the apical surface of the meristem to the top of the upper floret. The accumulation is more robust at the top of the lower floral meristem and reduces in intensity at the top of the upper floral meristem (Figure 3. E). The tip of the developing glume also exhibits localization in a small group of cells. In older floral meristems with a more developed lower floret, the signal extends unbroken from the top of the lower floret

Figure 3. *bde* floral meristems have increases in pectin

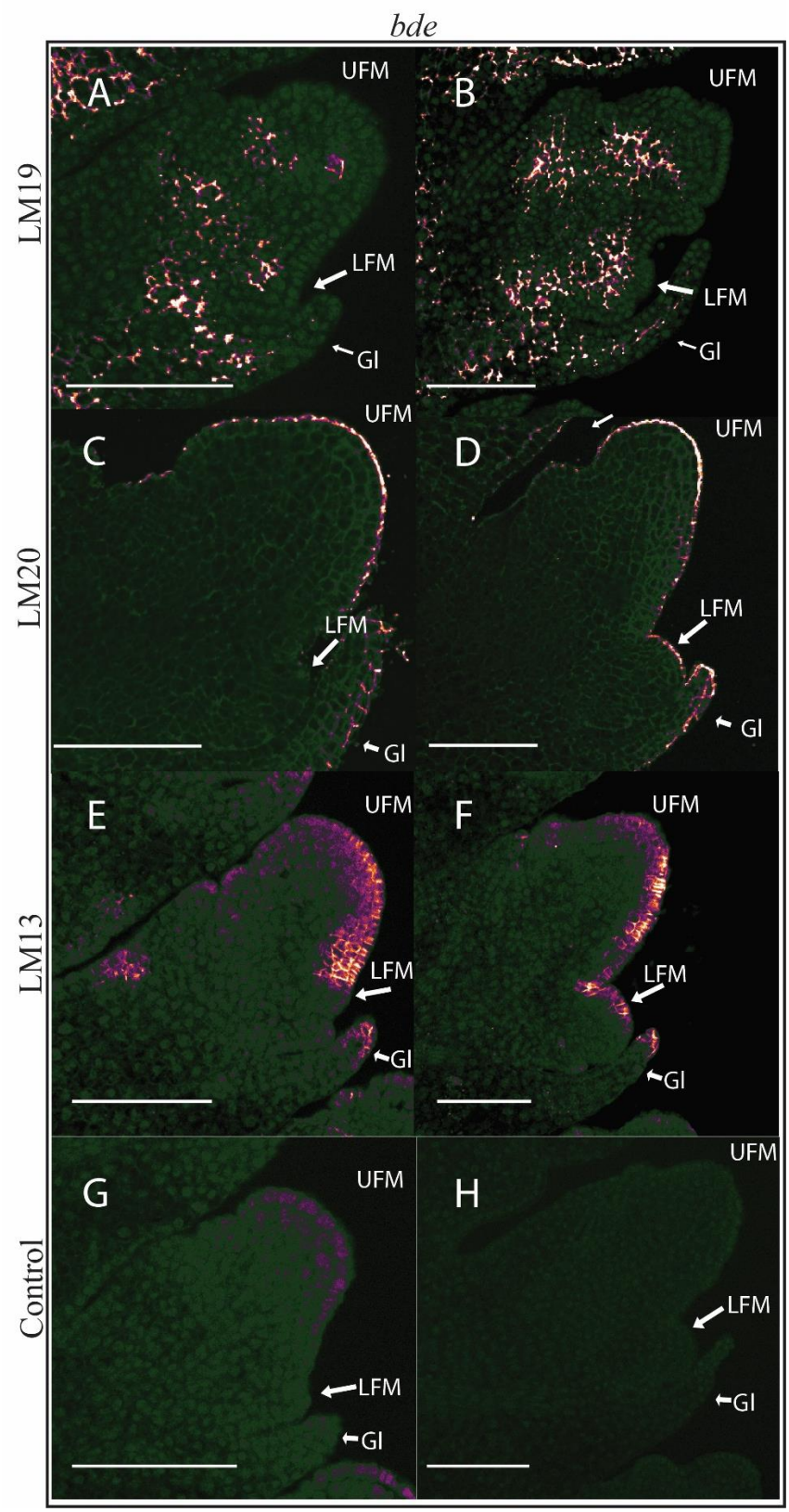

Figure 3. Legend

bde floral meristems have increases in pectin. A, early-stage floral meristems exhibit demethylesterifed pectin (LM19) at incipient organs. B, Demethylesterified pectin can be seen in the LFM. C, methylesterified pectin(LM20) accumulates on the apical surface of the UFM and D, in older spikelets it accumulates on the apical surface of both the UFM and the LFM. E, arabinan side chains of RG-1(LM13) have an expanded localization on the apical surface above the LFM. F, in older spikelets this expansion extends onto the apical surface of the LFM. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. UFM, upper floral meristem; LFM, lower floral meristem; Gl, glume; Cp, carpel primordia; Le, lemma. Scale bars = 100 µm.

along the apical surface to the top of the upper floret. Signal extends along the apical surface of the LFM in older, more developed lower florets (Figure 3. F). Characterization was done with eight biological replicates. Fluorescence controls were included of *bde* ear floral meristems (Figure 3. G, H).

Results found in *bde* suggest that cell wall dynamics are integrated with meristem activity. To further test this hypothesis, we examined *zag1* mutant spikelets. *Zag1* maintains determinacy in the lower floral meristem, but the upper floral meristem will initiate additional carpels (Schmidt et al. 1993). LM19 staining of demethylesterified pectin in *zag1* is similar to wild-type and is seen in the upper floral meristem but absent from the lower floral meristem (Figure 4. A). The altered pectin in the lower floral meristem of *bde* seems to be linked to altered meristem activity of floret growth. In older florets, LM19 staining is diffuse throughout the upper floret but no signal is detected in the lower floral meristem (Figure 4. B). Immunohistochemistry was done with seven biological replicates.

LM20 staining in *zag1* ear florets is indistinguishable from wildtype. Early-stage floral meristems display signal on the apical surface of L1 at the apex of the upper floral meristem (Figure 4. C). In older floral meristems, the LM20 staining is restricted to a small group of cells at the tip of the upper floral meristem but is absent from the apical surface of the lower floral meristem (Figure 4. D). Glume staining is seen on the abaxial side throughout at later stages. Four biological replicates were performed.

LM13 staining of arabinan side chains in early-stage *zag1* spikelets shows LM13 binds a small group of cells sitting above the initiating lower floral meristem. The LM13 staining begins at the top of the lower floral meristem and extends up the apical surface of the upper floret (Figure 4. E). LM13 staining in early stages of spikelet development in *zag1* is similar to that

Figure 4. *zag1* floral meristems have partial changes to pectin composition

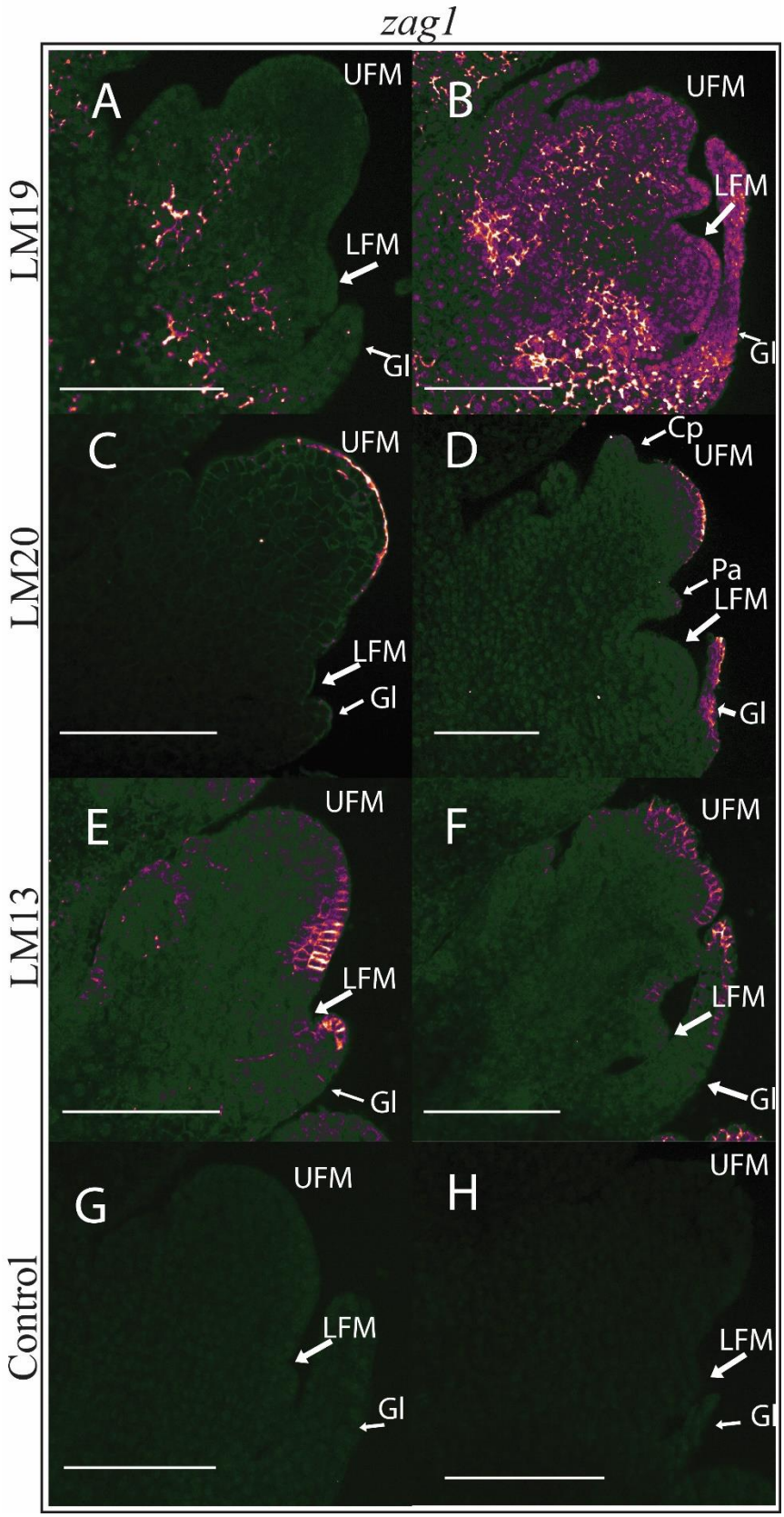

Figure 4. Legend

zag1 ear floral meristems have partial changes to pectin composition Demethylesterified pectin (LM19) localization is normal in the upper floral meristem and lower floral meristem A, B. Methlyesterified pectin (LM20) stains the apical surface of the upper floral meristem and the abaxial side of the glume C,D. Arabinan side chains of RG-I (LM13) have a more diffuse localization pattern along the apical surface of the upper floret, beginning at the top of the initiating lower floral meristem E. Older spikelets exhibit increases in LM13 signal on the apical surface of the upper floret F. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. UFM, upper floral meristem; LFM, lower floral meristem; Gl, glume; Cp, carpel primordia; Le, lemma; Pa, palea. Scale bars = 100 μm.

seen in *bde* mutants. In older spikelets, the staining is seen along the apical surface at the top of the upper floret where additional carpals initiate but does not extend down the apical surface of the lower floral meristem (Figure 4. F). Staining on the glume persists through all stages and becomes more diffuse as the glume develops. LM13 staining of *zag1* spikelets does not extend down into the apical surface of the lower floral meristem as seen in the *bde* mutant. This suggests that arabinan side chains are associated with meristem determinacy. *Bde* has increased LM13 staining in both the upper and lower floral meristems that lose determinacy while *zag1* only shows increased in LM13 in the upper floral meristem that loses determinacy. The control images were taken of *zag1* ears concurrently as a measure of background autofluorescence (Figure 4. G,H). characterization was completed with five biological replicates.

Mutants with altered meristem activity have changes in boundary gene expression

After seeing disruptions in LM13 staining patterns in mutants that lose determinacy, we were curious if this disruption was specific to pectin or if other boundary markers are disrupted. Previous work in our lab identified three genes that specifically localize to the boundary between the upper and lower florets, including *BBTI*, which encodes a Bowman-Birk type trypsin inhibitor (Yang et al. 2022). We decided to assess if these boundary genes would also have an expansion in localization in mutants with altered meristem activity. RNA in situ hybridization with *BBTI* was performed on wildtype (Figure 5. A, A') and *bde* ear florets (Figure 5. B, B'). *BBTI* displays an expansion in its hybridization localization in *bde* ear florets, extending up the upper floret but not extending down into the lower floret. LM13 immunostaining in *bde* ears (Figure 5. C, C') reveals a localization pattern that mirrors the RNA in situ results with *BBTI* in *bde*.

Figure 5. Comparison between *BBTI* in situ signal and LM13 localization

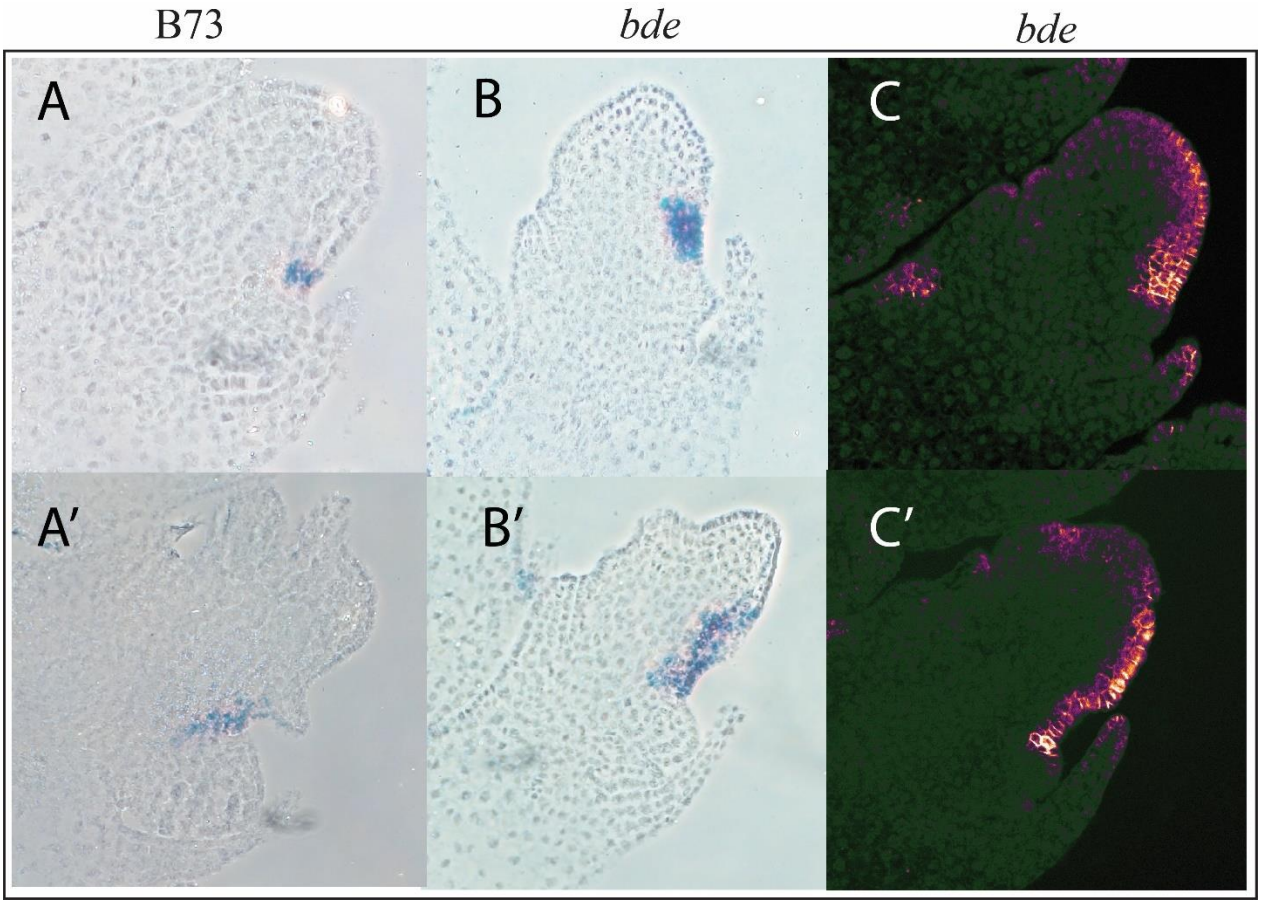

Figure 5. Legend

Comparison between *bbti* in situ signal and LM13 localization. *bbti* hybridization signal in WT ear florets at early stage (A) and late stage (A’) compared to *bde* mutant ear florets in situ at early stage (B) and late stage (B’). LM13 immunostaining of alpha 1,5-linked arabinan side chains of RG-I in *bde* ear florets at early (C) and late (C’) stages showing similar localization pattern to *bbti*. Images in part C were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity

Meristems of the inflorescence exhibit dynamic localizations of pectin

Analysis of floral meristems suggest that the accumulation of at least three pectic epitopes varies and is intimately linked to meristem activity. To more broadly investigate the links between pectin dynamics and meristem activity, we examined other reproductive and vegetative meristems. Reproductive development begins with the inflorescence meristem, an indeterminate meristem which gives rise to the spikelet pair meristem. The spikelet pair meristem is determinate and gives rise to two spikelet meristems. Spikelet meristems give rise to a glume and an upper and lower floral meristem. The upper and lower floral meristems will give rise to floral organs, but the lower floral meristem will abort. We began our assessment of reproductive meristems with the first meristem of the ear, the inflorescence meristem. The LM19 antibody detects demethylesterified pectin and stains the L1 layer of the inflorescence meristem dome but is not seen at the internal layers of the dome (Figure 6. A). Staining continues on the apical surface of the spikelet pair meristems and is seen in the internal pith of the ear. (Figure 6. B). The transition from spikelet pair meristem to spikelet meristem begins the start of demethylesterified pectin accumulation at the site of organ outgrowth in the glume and remains on the apical surface (Figure 6. C) LM19 staining in floral meristems shows strong signal at the site of organ primordia such as the carpel but is absent from the lower floral meristem (Figure 6. D, E). Ten biological replicates were performed to validate the staining protocol and previous staining results in maize floral meristem (Yang et al. 2022).

LM20 detects methylesterified pectin and localizes on the apical surface of the inflorescence meristem dome, but not in the internal layers (Figure 6. F). LM20 stains spikelet pair meristems on the apical surface but is not continuous, the staining is strongest on the center but absent from the flanks of developing spikelets. (Figure 6. G). This continues into spikelet

Figure 6. Meristem of the inflorescence exhibit dynamic localizations of pectin

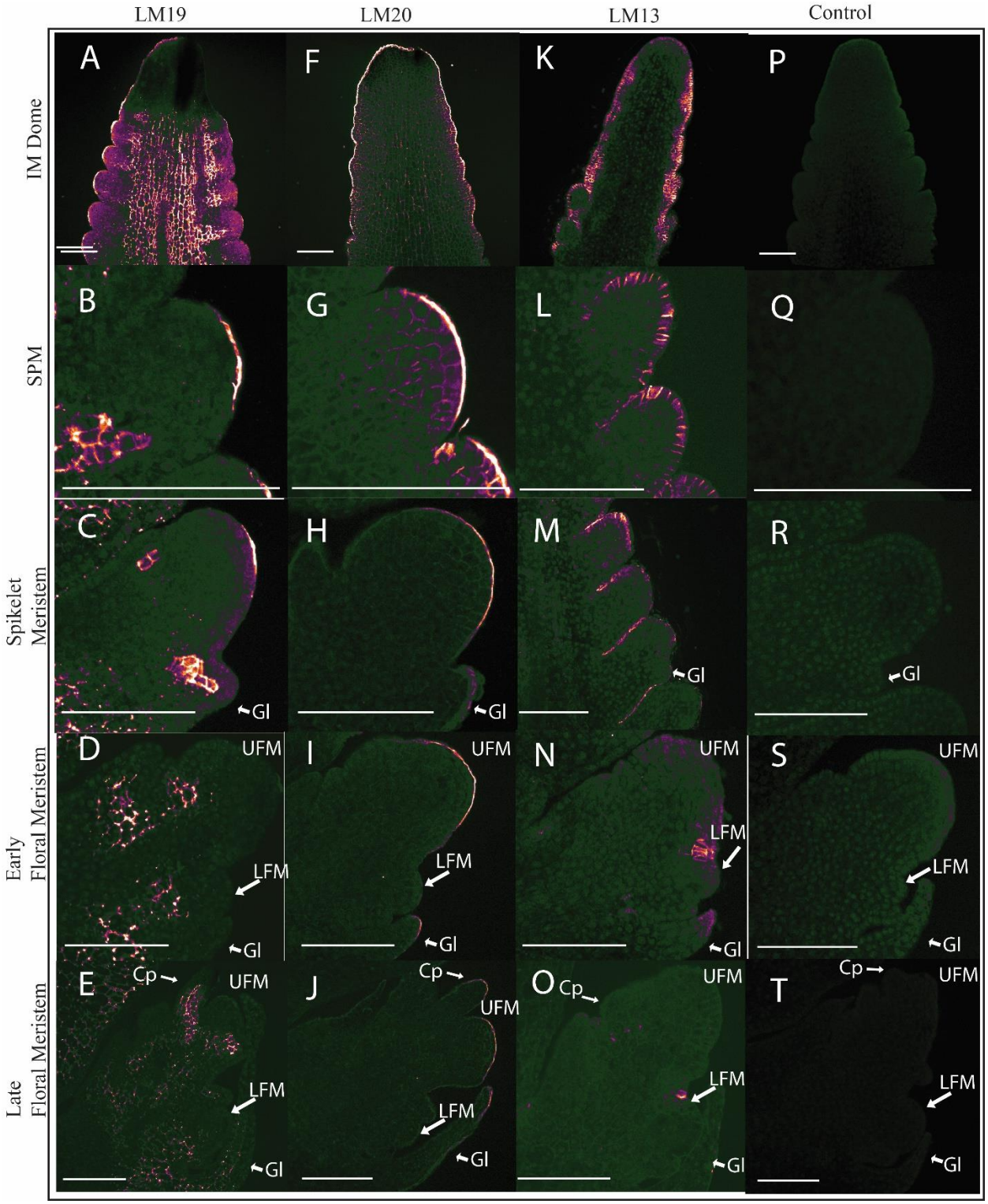

Figure 6. Legend

WT ear development series to assess cell wall composition throughout the ear. A-E, developmental series showing localization of demethylesterified pectin (LM19). F-J, developmental series showing localization of methylesterified pectin (LM20). K-O, developmental series showing the localization of alpha 1,5-linked arabinan side chains of RG-I(LM13). P-T, fluorescence controls comparable growth stages. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. UFM, upper floral meristem; LFM, lower floral meristem; Gl, glume; Cp, carpel primordia; Le, lemma; Pa, palea. Scale bars = 100 µm

meristem development and LM20 staining is seen along the abaxial side of the glume (Figure 6. H). LM20 staining in floral meristems is restricted to the apical surface of the upper floral meristem and some floral organs like the carpel. The staining is mostly absent from the lower floral meristem and when seen is restricted to cell-to-cell junctions (Figure 6. I, J). This staining pattern was consistent across seven biological replicates

Next, we assessed how localization of arabinan side chains of RG-1 might change throughout meristems of the inflorescence. We began our assessment of the ear with the inflorescence meristem, we found that LM13 staining is seen on the L1 of the inflorescence meristem dome but not in the internal layers of the dome. Below the inflorescence meristem, LM13 signal is seen on the apical surface and extending deeper into the ear from the apical surface at the site of incipient spikelet pair meristems (Figure 6. K). In older spikelet pair meristems, LM13 staining reduces to the L1 and is not seen extending multiple cells in depth from the surface (Figure 6. L). LM13 stains the apical surface of spikelet meristems on the flanks but not in the center, which is more restricted than seen in spikelet pair meristems (Figure 6. M). Floral meristems have a very reduced staining pattern of LM13. Early-stage floral meristems have a small group of cell above the lower floral meristem that have LM13 staining (Figure 6. N). In older stage floral meristems, the LM13 staining further restricts in the same region between the upper and lower floral meristem (Figure 6. O) Sixteen biological replicates were performed to characterize the LM13 antibody. Control images were taken of wildtype B73 ears that were not treated with a primary antibody as a measure of background autofluorescence (Figure 6. P- T). The dynamic nature of the cell wall and its ability to change throughout development is a complex area of study but these results begin to shed light on the changes that happen to allow for proper meristem development. Mutants with altered meristem activity

exhibit changes to their cell walls that correlate with changes in meristem determinacy. To further explore the cell wall, we assessed mutants with altered auxin transport or synthesis.

Auxin mutants display an increase in methylesterified pectin

Auxin has been linked to pectin dynamics in Arabidopsis and we observe at least LM19 staining (demethylesterified pectin) is associated with some initiating primordia. To further investigate the links between auxin and pectin in maize, we examined LM19, LM20 and LM13 staining in the auxin biogenesis mutant *sparse inflorescence1* (*spi1*). *Spi1* has a dramatic reduction in axillary primordia, including SM, SPM and FM (Gallavotti, Barazesh, et al. 2008). Because *spi1* mutants initiate so few axillary meristems, we were not able to characterize all meristem types. During floral meristem development, the localization of all three pectic epitopes was assessed. LM19 staining of demethylesterifed pectin revealed a similar localization pattern to wildtype, floral meristems had diffuse staining throughout the upper floret (Figure 7. A). LM20 staining of methylesterified pectin revealed similar patterns on the apical surface of developing florets but also showed punctate fluorescence at cell-cell junctions throughout the tissue (Figure 7. B). LM13 staining is present in the boundary between upper and lower floret and is present on the apical surface of the upper floral meristem (Figure 7. C). Fluorescence control images were taken of *spi1* ear spikelets with no primary antibody applied (Figure 7. D)

LM20 antibody immunostaining revealed a possible link between auxin deficient mutants and cell wall composition. The tassels of *ba1* plants are almost entirely devoid of initiated or developing spikelets. In wildtype sibling tassels, LM20 staining localizes on the apical surface of the inflorescence meristem tip but is not continuous from the tip to the developing spikelets (Figure 8. A). *ba1* tassels have a continuous layer of methylesterified pectin on the apical surface from the inflorescence meristem to regions where spikelets are trying to develop (Figure 8. B).

Figure 7. Antibody localizations in *spi1* mutant ears

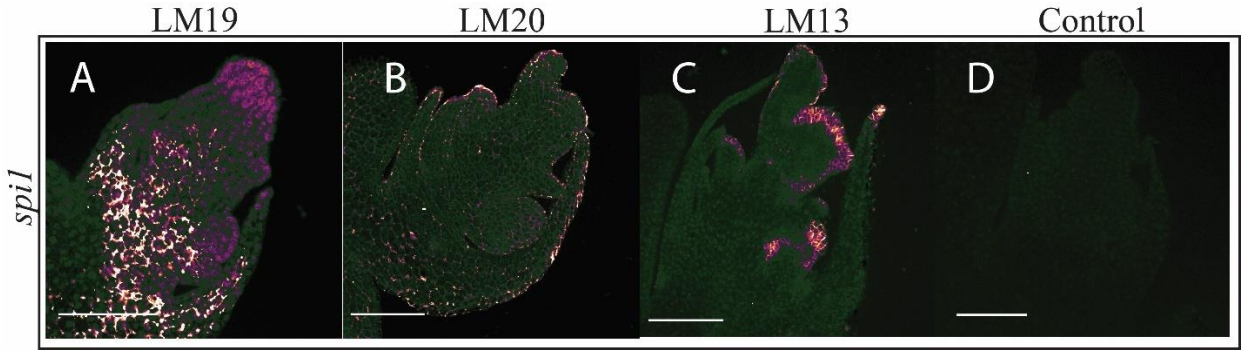

Figure 7 Legend

Antibody localizations in spi1 mutant ears. A, immunostaining of demethylesterified pectin (LM19) in late stage ear floret. B, immunostaining of methylesterified pectin (LM20) in late-stage ear floret. C, immunostaining of alpha 1,5-linked arabinan side chains of RG-I (LM13) in late-stage ear floret. D, fluorescence control of ear floret at similar growth stage. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 µm.

fluorescence controls of a *ba1* stalk with no primary antibody application is included (Figure 7. C). Wild type sibling tassels have distinct LM20 localization patterns, along the apical surface of the developing spikelets but not in the regions between spikelets (Figure 8. D). *ba1* tassels have a continuous localization extending down the length of the tassel (Figure 8. E). Fluorescence control of *ba1* tassel further down the inflorescence was included to highlight real signal seen (Figure 8. F)

Vegetative meristems have differing localizations of pectin than reproductive meristems

After assessing the reproductive meristems of the ear, we decided to also assess meristems that give rise to vegetative growth. Shoot apical meristems give rise to vegetative growth such as leaves. Interestingly, pectin localization was dramatically different in shoot apical meristems from what we observed in the inflorescence. LM19 staining of demethylesterified pectin shows a line of signal at the base of the stem cell containing portion of the shoot (Figure 9. A). The shoot apical meristem exhibits a lack of LM20 localization on the apical surface which stands in contrast to LM20 staining which is seen on the apical surface of the inflorescence meristem, spikelet pair meristem, spikelet meristem, and upper floral meristem. The only LM20 signal that can bee seen is on the apical surface of the abaxial side of the outer leaf (Figure 9. B). LM13 has strong localization on the buds of axillary meristems that will be repressed during growth (Figure 9. C) suggesting that the epitope recognized by LM13 may play a role in specifying boundaries. A fluorescence control was included that did not receive a primary antibody (Figure 9. D).

Figure 8. Auxin signaling mutant *ba1* exhibits increased methylesterified pectin

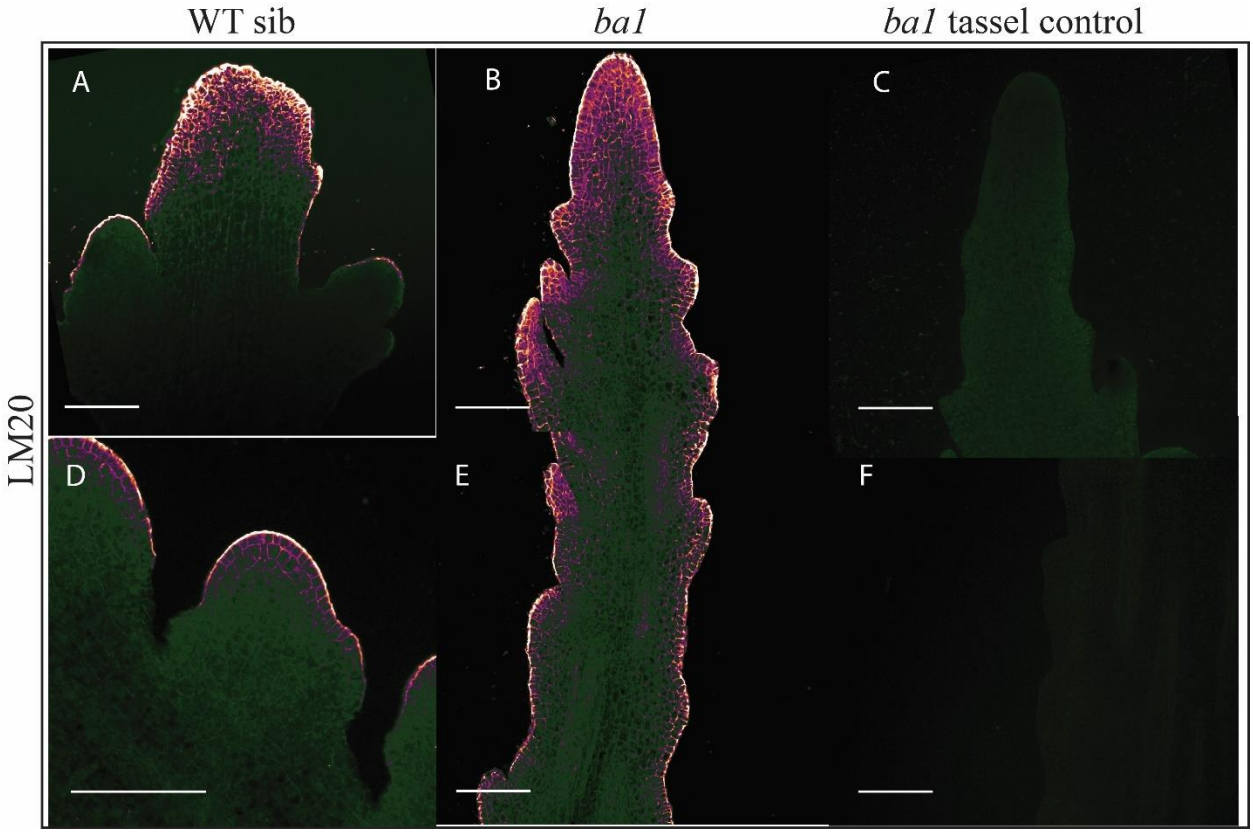

Figure 8 Legend

Auxin signaling mutant *ba1* exhibits increased cell wall rigidity. A, LM20 staining of methylesterifeid pectin WT sib tassel inflorescence meristems have non- continuous staining between IM and developing spikelets while B, *ba1* tassels exhibit continuous staining of the apical surface. C, is a control IM with no primary AB. D, developing spikelets exhibit staining exclusively on the apical surface of the spikelet but not between spikelets. *ba1* mutants fail to develop full spikelets and have continuous signal along the apical surface. F, fluorescence control of a *ba1* stalk. Scale bars = 100 µm

Figure 9. Antibody localizations in B73 shoot apical meristems

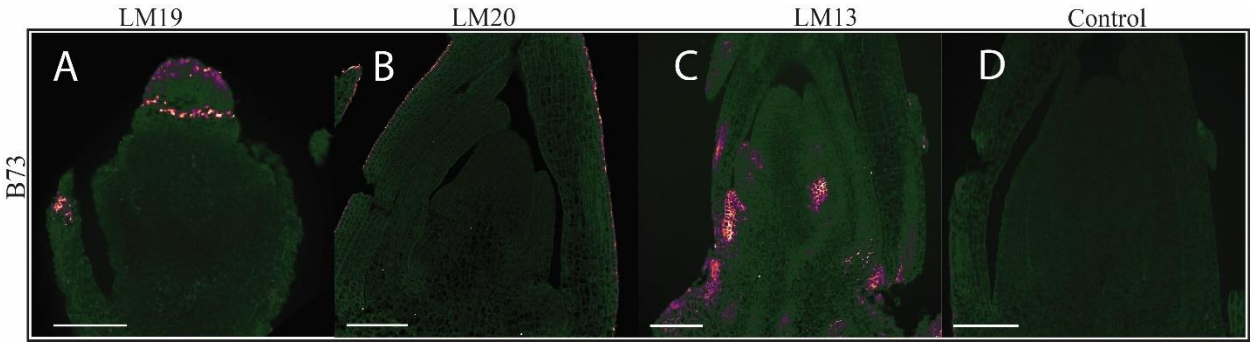

Figure 9 Legend

Antibody localizations in B73 shoot apical meristems. A, immunostaining of demethylesterified pectin (LM19). B, immunostaining of methylesterified pectin (LM20). C, immunostaining of alpha 1,5-linked arabinan side chains of RG-I(LM13). Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 µm.

Chapter 3: Discussion

Cells walls have long been appreciated for their roles in tissue growth and structural support, but very little is known about how the cell wall is integrated with the earliest stages of development in meristems. We have found that meristems display unique localization patterns of three pectin cell wall epitopes. Ear meristems localize pectin in a way that is completely different from what is seen in shoot apical meristems. Even within one tissue, such as the ear, meristems display distinct changes in pectin composition in cell walls. The inflorescence meristem is different when compared to the spikelet pair meristem and so on. The floral meristems in the ear, the upper floral meristem and the lower floral meristem, have different cell wall compositions that may be linked to the difference in fate that exists between them. The upper continues to maturity and the lower aborts. We can link changes in cell wall activity to changes in meristem activity but we cannot say that changes to pectin are causative to changes in meristem activity. There is a pattern that meristems with more pectin experience more organ development, such as we saw in *bde* (Figure 3).

Demethylesterified pectin and methylesterified pectin may work together to drive proper organ outgrowth. Proper outgrowth relies upon the interactions of many changes to cells. Methylesterified pectin provides a rigid external surface that resists changes in shape to build turgor pressure inside the developing organ. At the same time, selective demethylesterification occurs at the site of an auxin maxima to allow expansion of cells at internal layers to drive organ outgrowth (Khadka, Julien, and Alim 2019). *Bde* ears exhibit more demethylesterified and methylesterified pectin in the lower floret. This increase in pectin correlates with changes in lower floral meristem activity that gives rise to ectopic floral meristems. Inversely, *zag1*, which

has a normal lower floral meristem does not exhibit any changes to methylesterified or demethylesterifed pectin in the lower floral meristem.

Maize mutants that exhibit losses of floral meristem determinacy and changes in meristem activity have changes in the localization of certain cell wall pectin epitopes. *Bde*, which loses determinacy in both the upper and lower floral meristem, has changes in pectin localization in both the upper and lower florets. There is an increase in methylesterified pectin on the apical surface of the lower floret, an increase in demethylesterified pectin in the lower floret, and an expansion of the arabinan side chain localizations between the upper and lower floral meristem. In *zag1*, which loses determinacy in the upper floret, there are changes in arabinan side chain localization which is increased in the upper floret. To further assess the role of mutants that lose determinacy, *ids1* and *sid1* can be used. These mutants have spikelets that lose determinacy and generate additional florets (Chuck et al. 2008). These mutants can be assessed to determine if there are changes in cell wall composition that correlate with the loss of determinacy of the spikelet meristem. We hypothesize that increases in demethylesterified pectin may been seen at internal layers, which could correlate with additional floret development characteristic of these mutants.

In plants, including maize, auxin is required for proper organ outgrowth and positioning (Galli et al. 2015). In Arabidopsis, auxin maxima precedes pectin demethylesterification (Peaucelle et al. 2011), but the relationship between auxin and pectin is unclear in other species. The auxin-related mutants *ba1* and *spi1*, both of which have dramatically reduced lateral primordia (Galli et al. 2015) and exhibit altered pectin distribution. In *ba1*, methylesterified pectin, which has been associated with increased cell wall rigidity in Arabidopsis, is continuous on the apical surface of tassel primordia (Figure 8), whereas in wild type the LM20 staining was

restricted to the apical surface of developing spikelets and is non-continuous. One possibility is that auxin signaling is required for PECTIN METHYLESTERASE-mediated demethylesterification of pectin, which promotes cell wall loosening and expansion in the internal layers. In both *spi1*, and *ba1* there is an increase of methylesterified pectin on the apical surface of inflorescence primordia. In contrast, wildtype inflorescences have a non-continuous staining pattern beginning with spikelet pair meristem development. Moving forward, transgenic lines that have the auxin efflux transporter tagged with YFP (PIN1-YFP) can be used to assess if there is an overlapping localization pattern of the PIN1 protein and the LM19 antibody that binds demethylesterified pectin. It would be of considerable use to provide evidence that auxin, pectin demethylesterification, and organ outgrowth collectively function the same way in grasses like maize as they have been found to function in Arabidopsis.

The LM13 antibody localization in wildtype ears closely mirrors the RNA expression patterns of several boundary genes in maize ear florets characterized in (Yang et al. 2022). Interestingly, both LM13 staining and expression of at least one boundary gene is perturbed in *bde* mutants, suggesting that the boundary is perturbed in *bde* mutants and that it is not *bde* that is having an effect on pectin. Future work to examine RNA expression patterns of additional boundary genes in floral determinacy mutants can be used to assess if the boundary is perturbed and how this disruption of the boundary may be leading to abnormal pectin. We will continue to assess the localization of boundary genes via RNA in situ hybridization while also continuing to assess the localization of arabinan side chains with RG-I to provide further evidence of the correlation. The shoot apical meristem also contains LM13 immunostaining of the axillary bud that is repressed. It is possible that LM13 staining may be associated with general growth repression in both boundary regions and in other primordia with repressed growth. To further

assess boundaries in the shoot apical meristem we can characterize LM13 staining in mutants that do not repress axillary buds. *Grassy tillers1* (*gt1*) and *teosinte branched1* (*tb1*) which is expressed in axillary buds, in a region that appears to overlap with LM13 staining, repress axillary bud outgrowth (Whipple et al. 2011). LM13 immunostaining of *gt1* and *tb1* can be performed to determine if changes in axillary bud outgrowth will coincide with different localization patterns of arabinan side chains of RG-I. Another boundary that can be assessed would be the ligule that separates the proximal blade sheath from the distal blade leaf. Mutants such as *liguless1* (*lg1*) the ligule does not form and leads to altered leaf development (Becraft et al. 1990). Assessments can be conducted in plants with both functional and non-functional mutants to determine if arabinan side chains are broadly associated with boundaries and necessary for proper developmental patterning.

Our results have begun to tease apart the connections between cell wall composition and organ initiation in maize. While most work characterizing the cell wall has been done in Arabidopsis, the findings in maize are novel. In Arabidopsis, pectin modification precedes organ outgrowth and that demethylesterified pectin accumulates at internal layers during primordia formation as visualized with immunohistochemistry (Peaucelle et al. 2008). Our results mirror these findings, and we see that pectin demethylesterification occurs at the internal layers of floral organ primordia. LM20 staining has not been performed in Arabidopsis but we have found that LM20 staining is not seen on the shoot apical meristem but is seen in all meristems of the female inflorescence of the ear. Some aspects of pectin dynamics seem to be conserved with Arabidopsis but there are important meristem and species-specific differences. We have also found links between cell wall composition and changes in meristem activity. In meristems that lose determinacy, and grow ectopically, have an increase in some or all of the assessed pectin

epitopes that were investigated. Furthermore, alpha 1,5-linked arabinan side chains of RG-I, as visualized with LM13, may be boundary associated. Our findings show that in wildtype ear florets it localizes like a boundary gene, and in mutants with changes in meristem activity, this boundary localization is expanded in the florets. In shoot apical meristems, LM13 signal is seen on repressed buds and there may be a link between arabinan side chains and growth repression. While much work remains to be done, characterizing cell wall epitopes throughout development is a necessary first step to begin understanding the role the cell wall plays in organogenesis.

Chapter 4: Materials and methods

Genotyping

Seeds from mutant maize lines were planted in Fafard Sungro #3B potting soil, grown in flats for ~8 days before plants were labeled and sampled. Tissue samples were taken from young leaf tissue and genomic DNA was extracted from these samples. PCR amplifications performed with gene specific primer sets and specific thermocycler conditions (Table 1.). PCR products were run on a gel and band placements were used to identify homozygous mutants, heterozygous individuals, and wildtype siblings for comparative immunohistochemistry analysis (Table 1). Plants of interest were transplanted into larger pots that were 50% Fafard Sungro #3B potting soil and Turface. Plants were grown until inflorescences were large enough for dissection. To harvest tassel inflorescences, plants were grown for 5 weeks. To harvest ear inflorescences, plants were grown for 10 weeks. Weather conditions, such as sunlight hours, can affect growth times so time frames are only an approximation of the time it takes to reach proper inflorescence growth stages.

Table 1. Genotyping Primers

Mutant Genotype	*bde*	*zag1*	*spi1*	*ba1* wt hets and homos	*ba1* mutant hets and homos
Forward Primer	BT235 5'-CAGCAGCATGGATCACAGAT -3'	umc1187F 5'-AGAGCTACCACCCCTCTTACCC -3'	SPI 8F 5'-GCAAGGAGGAGCAGTTCGACGCAA T-3'	Ba1_F2 5'-TCTCCTTTTAAGCTAAGCTACTGT-3'	ba05 5'-TCCTAGACATGCATATCTGAACCAGAGCT-3'
Reverse Primer	BT237 5'-TGCCATGGAGAGAGAACCTC-3'	umc1187 5'-ATGTGCATGCTCTTGTTCCATCA -3'	SPI 3R 5'-GGTCAAAACGAGTCAGACGACAGC-3'	Ba1_R2 5' -CTATGGGTTTGGGAGGAGCC -3'	Ba1_R1 5'-TGTTGGCTTTGCTGGTTTCA -3'
Thermocycler	94° x 2:00min 35 cycles	95° x 1:00min 10 cycles 95° x 30s	94° x 2:00min 35 cycles 94° x 30s	94° x 2:00min 35 cycles 94° x 30s	94° x 2:00min 35 cycles 94° x 30s

Conditions	94° x 30s 55° x 30s 72° x 1:00min last step 72° x 5:00min	65° x 30s 72° x 1:00min 35 cycles 95° x 30s 55° x 30s 72° x 45s last step 72° x 5:00min	60° x 30s 72° x 30s last step 72° x 5:00min	55.7° x 30s 72° x 1:00min last step 72° x 5:00min	55.7° x 30s 72° x 1:00min last step 72° x 5:00min
Gel Type	1.5% agarose gel	3.5% metaphor agarose	3% agarose	1.5% agarose	1.5% agarose
Gel Electrophoresis Conditions	120v for 60 minutes	120v for 80 minutes	120v for 85 minutes	120v for 45 minutes	120v for 45 minutes
Interpreting Results	upper band is mutant lower band is wildtype	upper band is wildtype lower band is mutant	upper is WT lower band is mutant het gives multiple bands	One band of 449 base pairs indicates presence of WT DNA. Used to identify WT heterozygotes and homozygotes.	One band of 460 base pairs indicates presence of transposon. Used to identify mutant heterozygotes and homozygotes.

Plant Growth and Tissue Fixation

Inflorescence primordia of the ear and tassel were grown (1-2 cm) before being dissected while shoot apical meristems were grown for 14 days before being dissected. Tissue was grown in a greenhouse (16-h light at 27°C, 8-h dark at 21°C) and upon dissection was immediately fixed with Formaldehyde-acetic acid alcohol and embedded in Parafin wax as described in (Thompson et al. 2009). Tissues were sectioned (10µm) using a microm HM315 Microtome.

RNA in situ and Immunohistochemistry.

RNA in situ hybridization was performed as described in (Yang et al. 2022) and slides were imaged with Olympus BX-41 compound light microscope. Immunofluorescence labeling,

microscopy, and image processing were performed as previously described in (Yang et al. 2022) with the following modifications; blocking buffer incubation in 5% Bovine Serum Albumin in 1x Phosphate Buffered Saline was increased to 45 minutes and secondary antibody incubation was increased to 3 hours at 20°C in humidified chamber. Procedure was performed as follows, slides were dewaxed in histoclear, rehydrated in an ethanol series, and then blocked in a BSA blocking buffer. Primary antibodies LM13 (Kerafast ELD010), LM19(Kerafast ELD001), LM20 (Kerafast ELD003) were diluted 1:10 in 5% Bovine Serum Albumin in 1x Phosphate Buffered Saline for a final volume of 250 µL for each slide sandwich formed. Slides were incubated at 4°C overnight in a sealed container dampened with 5% Bovine Serum Albumin in 1x Phosphate Buffered Saline. After primary incubation, slides were rinsed in 1x Phosphate Buffered Saline with rotation before secondary antibody application. Alexa Fluor 488 Goat anti-Rat secondary antibody (ThermoFisher Scientific Catalog # A-11006) was diluted 1:100 in 5% Bovine Serum Albumin in 1x Phosphate Buffered Saline for a final volume of 250 µL. The incubation of secondaries lasted three hours in dark chamber at 20°C in humidified chamber with 5% Bovine Serum Albumin in 1x Phosphate Buffered Saline. Slides were then rinsed in 1x Phosphate Buffered Saline, quenched in 0.05% toluidine blue O in 1x Phosphate Buffered Saline for five minutes, rinsed two times in 1x Phosphate Buffered Saline, covered in anti-fade mounting media (Hinnant et al. 2017), covered with a cover slide, and sealed with nail polish.

Mutant Analysis

All mutants were analyzed alongside normal siblings, which were grown concurrently in the greenhouse, fixed, processed, and stained concurrently. Mutant analysis was conducted on a minimum of three separate inflorescences with LM19, LM13, LM20. In each round of immunohistochemistry, 1 to 3 mutant inflorescences would be used, and each inflorescence

would have multiple sections imaged. Each section contains many spikelets, with the most recently initiated at the top of the inflorescence and the most developed at the bottom of the inflorescence. Pseudo replication, or the process of taking multiple samples from one biological replicate, was employed to assess all spikelets of appropriate growth stage on a given inflorescence across serial sections of the same inflorescence. There are many spikelets that develop on the maize inflorescences and to best characterize cell wall composition of the spikelets serial images were taken. Serial images are images of the same spikelet that can be seen on successive sections that have been mounted to a slide. This allows for better understanding of the immunohistochemistry staining pattern that is observed. The only mutant that did not receive as many replications is *spi1* due to limited availability of tissue. Shoot apical meristem data had fewer replications with an n value of 2, see table 2

Table 2. Biological Replicates

Genotype	Antibody Used	# of Inflorescences Examined	Sex
B73	LM19	10	Female (Ear)
B73	LM20	7	Female (Ear)
B73	LM13	16	Female (Ear)
bde	LM19	5	Female (Ear)
bde	LM20	6	Female (Ear)
bde	LM13	8	Female (Ear)
zag1	LM19	7	Female (Ear)
zag1	LM20	4	Female (Ear)
zag1	LM13	5	Female (Ear)
spi1	LM19	3	Female (Ear)
spi1	LM20	2	Female (Ear)
spi1	LM13	1	Female (Ear)
ba1	LM19	2	Male (Tassel)
ba1	LM20	3	Male (Tassel)

ba1	LM13	2	Male (Tassel)
B73 SAM	LM19	13	n/a
B73 SAM	LM20	3	n/a
B73 SAM	LM13	2	n/a

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References

Barnes, William J., and Charles T. Anderson. 2018. “Release, Recycle, Rebuild: Cell-Wall Remodeling, Autodegradation, and Sugar Salvage for New Wall Biosynthesis during Plant Development.” *Molecular Plant* 11 (1): 31–46.

Becraft, P. W., D. K. Bongard-Pierce, A. W. Sylvester, R. S. Poethig, and M. Freeling. 1990. “The Liguleless-1 Gene Acts Tissue Specifically in Maize Leaf Development.” Developmental Biology 141 (1): 220–32.

Bommert, Peter, Namiko Satoh-Nagasawa, David Jackson, and Hiro-Yuki Hirano. 2005. “Genetics and Evolution of Inflorescence and Flower Development in Grasses.” *Plant & Cell Physiology* 46 (1): 69–78.

Braybrook, Siobhan A., and Alexis Peaucelle. 2013. “Mechano-Chemical Aspects of Organ Formation in Arabidopsis Thaliana: The Relationship between Auxin and Pectin.” *PloS One* 8 (3): e57813.

Bringmann, Martin, Benoit Landrein, Christian Schudoma, Olivier Hamant, Marie-Theres Hauser, and Staffan Persson. 2012. “Cracking the Elusive Alignment Hypothesis: The Microtubule-Cellulose Synthase Nexus Unraveled.” *Trends in Plant Science* 17 (11): 666–74.

Carpita, Nicholas C. 1996. “STRUCTURE AND BIOGENESIS OF THE CELL WALLS OF GRASSES.” *Annual Review of Plant Physiology and Plant Molecular Biology* 47 (June): 445–76.

Chuck, George, Robert Meeley, and Sarah Hake. 2008. “Floral Meristem Initiation and Meristem Cell Fate Are Regulated by the Maize AP2 Genes ids1 and sid1.” Development 135 (18): 3013–19.

Driouich, Azeddine, Marie-Laure Follet-Gueye, Sophie Bernard, Sumaira Kousar, Laurence Chevalier, Maïté Vicré-Gibouin, and Olivier Lerouxel. 2012. “Golgi-Mediated Synthesis and Secretion of Matrix Polysaccharides of the Primary Cell Wall of Higher Plants.” *Frontiers in Plant Science* 3 (April): 79.

Gallavotti, Andrea, Solmaz Barazesh, Simon Malcomber, Darren Hall, David Jackson, Robert J. Schmidt, and Paula McSteen. 2008. “Sparse inflorescence1 Encodes a Monocot-Specific YUCCA-like Gene Required for Vegetative and Reproductive Development in Maize.” Proceedings of the National Academy of Sciences of the United States of America 105 (39): 15196–201.

Gallavotti, Andrea, Yan Yang, Robert J. Schmidt, and David Jackson. 2008. “The Relationship between Auxin Transport and Maize Branching.” *Plant Physiology* 147 (4): 1913–23.

Galli, Mary, Qiujie Liu, Britney L. Moss, Simon Malcomber, Wei Li, Craig Gaines, Silvia Federici, et al. 2015. “Auxin Signaling Modules Regulate Maize Inflorescence Architecture.” *Proceedings of the National Academy of Sciences of the United States of America* 112 (43): 13372–77.

Hamant, Olivier, Marcus G. Heisler, Henrik Jönsson, Pawel Krupinski, Magalie Uyttewaal, Plamen Bokov, Francis Corson, et al. 2008. “Developmental Patterning by Mechanical Signals in Arabidopsis.” *Science* 322 (5908): 1650–55.

Harholt, Jesper, Anongpat Suttangkakul, and Henrik Vibe Scheller. 2010. “Biosynthesis of Pectin.” *Plant Physiology* 153 (2): 384–95.

Hibara, Ken-Ichiro, Md Rezaul Karim, Shinobu Takada, Ken-Ichiro Taoka, Masahiko Furutani, Mitsuhiro Aida, and Masao Tasaka. 2006. “Arabidopsis CUP-SHAPED COTYLEDON3 Regulates Postembryonic Shoot Meristem and Organ Boundary Formation.” *The Plant Cell* 18 (11): 2946–57.

Hinnant, Taylor D., Arturo A. Alvarez, and Elizabeth T. Ables. 2017. “Temporal Remodeling of the Cell Cycle Accompanies Differentiation in the Drosophila Germline.” *Developmental Biology* 429 (1): 118–31.

Höfte, Herman, and Aline Voxeur. 2017. “Plant Cell Walls.” *Current Biology: CB* 27 (17): R865–70.

Irish, Erin E., and Timothy M. Nelson. 1993. “Development of Tassel Seed 2 Inflorescences in Maize.” *American Journal of Botany* 80 (3): 292–99.

Jones, Louise, Jennifer L. Milne, David Ashford, and Simon J. McQueen-Mason. 2003. “Cell Wall Arabinan Is Essential for Guard Cell Function.” *Proceedings of the National Academy of Sciences of the United States of America* 100 (20): 11783–88.

Khadka, Jason, Jean-Daniel Julien, and Karen Alim. 2019. “Feedback from Tissue Mechanics Self-Organizes Efficient Outgrowth of Plant Organ.” *Biophysical Journal* 117 (10): 1995–2004.

Kozlova, Liudmila V., Alsu R. Nazipova, Oleg V. Gorshkov, Anna A. Petrova, and Tatyana A. Gorshkova. 2020. “Elongating Maize Root: Zone-Specific Combinations of Polysaccharides from Type I and Type II Primary Cell Walls.” *Scientific Reports* 10 (1): 10956.

Mena, M., B. A. Ambrose, R. B. Meeley, S. P. Briggs, M. F. Yanofsky, and R. J. Schmidt. 1996. “Diversification of C-Function Activity in Maize Flower Development.” *Science* 274 (5292): 1537–40.

Peaucelle, Alexis, Romain Louvet, Jorunn N. Johansen, Herman Höfte, Patrick Laufs, Jérome Pelloux, and Grégory Mouille. 2008. “Arabidopsis Phyllotaxis Is Controlled by the Methyl-Esterification Status of Cell-Wall Pectins.” *Current Biology: CB* 18 (24): 1943–48.

Peaucelle, Alexis, Siobhan A. Braybrook, Laurent Le Guillou, Emeric Bron, Cris Kuhlemeier, and Herman Höfte. 2011. “Pectin-Induced Changes in Cell Wall Mechanics Underlie Organ Initiation in Arabidopsis.” *Current Biology: CB* 21 (20): 1720–26.

Poursarebani, Naser, Corinna Trautewig, Michael Melzer, Thomas Nussbaumer, Udda Lundqvist, Twan Rutten, Thomas Schmutzer, et al. 2020. “COMPOSITUM 1 Contributes to the

Architectural Simplification of Barley Inflorescence via Meristem Identity Signals." *Nature Communications* 11 (1): 5138.

Reinhardt, D., T. Mandel, and C. Kuhlemeier. 2000. "Auxin Regulates the Initiation and Radial Position of Plant Lateral Organs." *The Plant Cell* 12 (4): 507–18.

Satoh-Nagasawa, Namiko, Nobuhiro Nagasawa, Simon Malcomber, Hajime Sakai, and David Jackson. 2006. "A Trehalose Metabolic Enzyme Controls Inflorescence Architecture in Maize." *Nature* 441 (7090): 227–30.

Schmidt, R. J., B. Veit, M. A. Mandel, M. Mena, S. Hake, and M. F. Yanofsky. 1993. "Identification and Molecular Characterization of ZAG1, the Maize Homolog of the Arabidopsis Floral Homeotic Gene AGAMOUS." *The Plant Cell* 5 (7): 729–37.

Thompson, Beth E., Linnea Bartling, Clint Whipple, Darren H. Hall, Hajime Sakai, Robert Schmidt, and Sarah Hake. 2009. "Bearded-Ear Encodes a MADS Box Transcription Factor Critical for Maize Floral Development." *The Plant Cell* 21 (9): 2578–90.

Verhertbruggen, Yves, Susan E. Marcus, Ash Haeger, José J. Ordaz-Ortiz, and J. Paul Knox. 2009. "An Extended Set of Monoclonal Antibodies to Pectic Homogalacturonan." *Carbohydrate Research* 344 (14): 1858–62.

Verhertbruggen, Yves, Susan E. Marcus, Ash Haeger, René Verhoef, Henk A. Schols, Barry V. McCleary, Lauren McKee, Harry J. Gilbert, and J. Paul Knox. 2009. "Developmental Complexity of Arabinan Polysaccharides and Their Processing in Plant Cell Walls." The Plant Journal: For Cell and Molecular Biology 59 (3): 413–25.

Vernoux, Teva, Fabrice Besnard, and Jan Traas. 2010. "Auxin at the Shoot Apical Meristem." *Cold Spring Harbor Perspectives in Biology* 2 (4): a001487.

Vignon, M. R., L. Heux, M-E Malainine, and M. Mahrouz. 2004. "Arabinan–cellulose Composite in Opuntia Ficus-Indica Prickly Pear Spines." *Carbohydrate Research* 339 (1): 123–31.

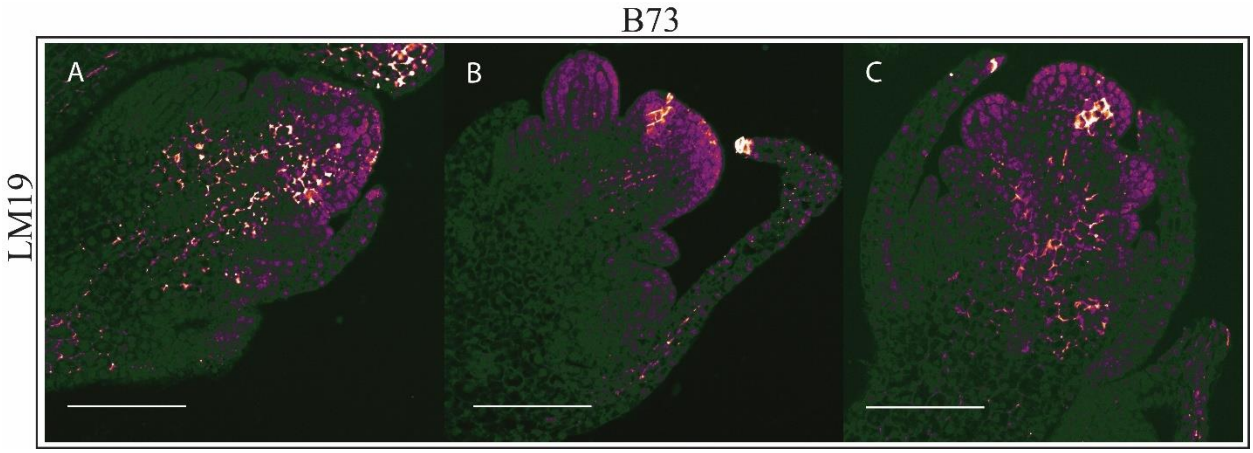
Weller, Benjamin, Melina Zourelidou, Lena Frank, Inês C. R. Barbosa, Astrid Fastner, Sandra Richter, Gerd Jürgens, Ulrich Z. Hammes, and Claus Schwechheimer. 2017. "Dynamic PIN-FORMED Auxin Efflux Carrier Phosphorylation at the Plasma Membrane Controls Auxin Efflux-Dependent Growth." *Proceedings of the National Academy of Sciences of the United States of America* 114 (5): E887–96.

Whipple, Clinton J., Tesfamichael H. Kebrom, Allison L. Weber, Fang Yang, Darren Hall, Robert Meeley, Robert Schmidt, John Doebley, Thomas P. Brutnell, and David P. Jackson. 2011. "Grassy tillers1 Promotes Apical Dominance in Maize and Responds to Shade Signals in the Grasses." Proceedings of the National Academy of Sciences of the United States of America 108 (33): E506–12.

Yang, Hailong, Kate Nukunya, Queying Ding, and Beth E. Thompson. 2022. “Tissue-Specific Transcriptomics Reveal Functional Differences in Floral Development.” *Plant Physiology* 188 (2): 1158–73.

Zykwinska, Agata W., Marie-Christine J. Ralet, Catherine D. Garnier, and Jean-François J. Thibault. 2005. “Evidence for in Vitro Binding of Pectin Side Chains to Cellulose.” *Plant Physiology* 139 (1): 397–407.

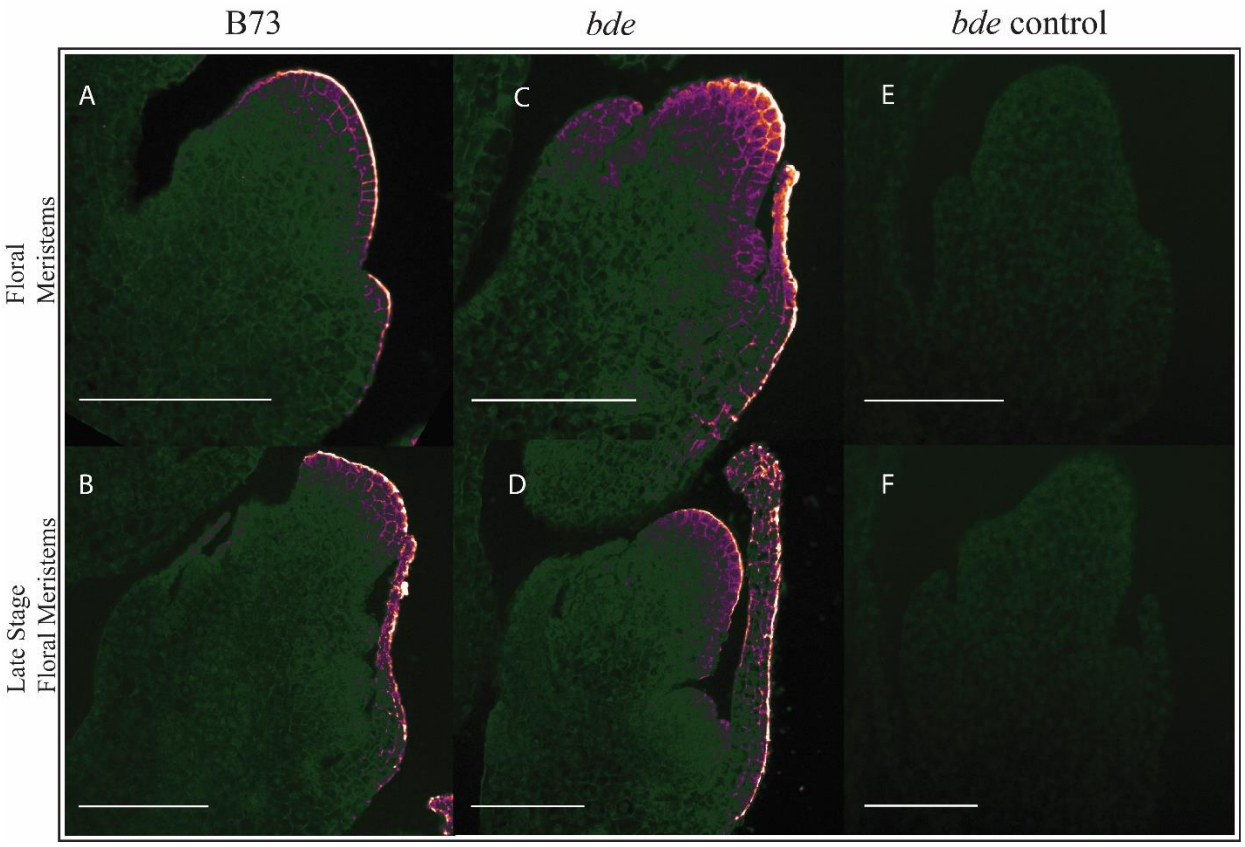
APPENDIX A: LM19 STAIN IN B73 TASSELS



Appendix A Legend

LM19 immunostaining of demethylesterified pectin in three tassel florets A-C. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 μm.

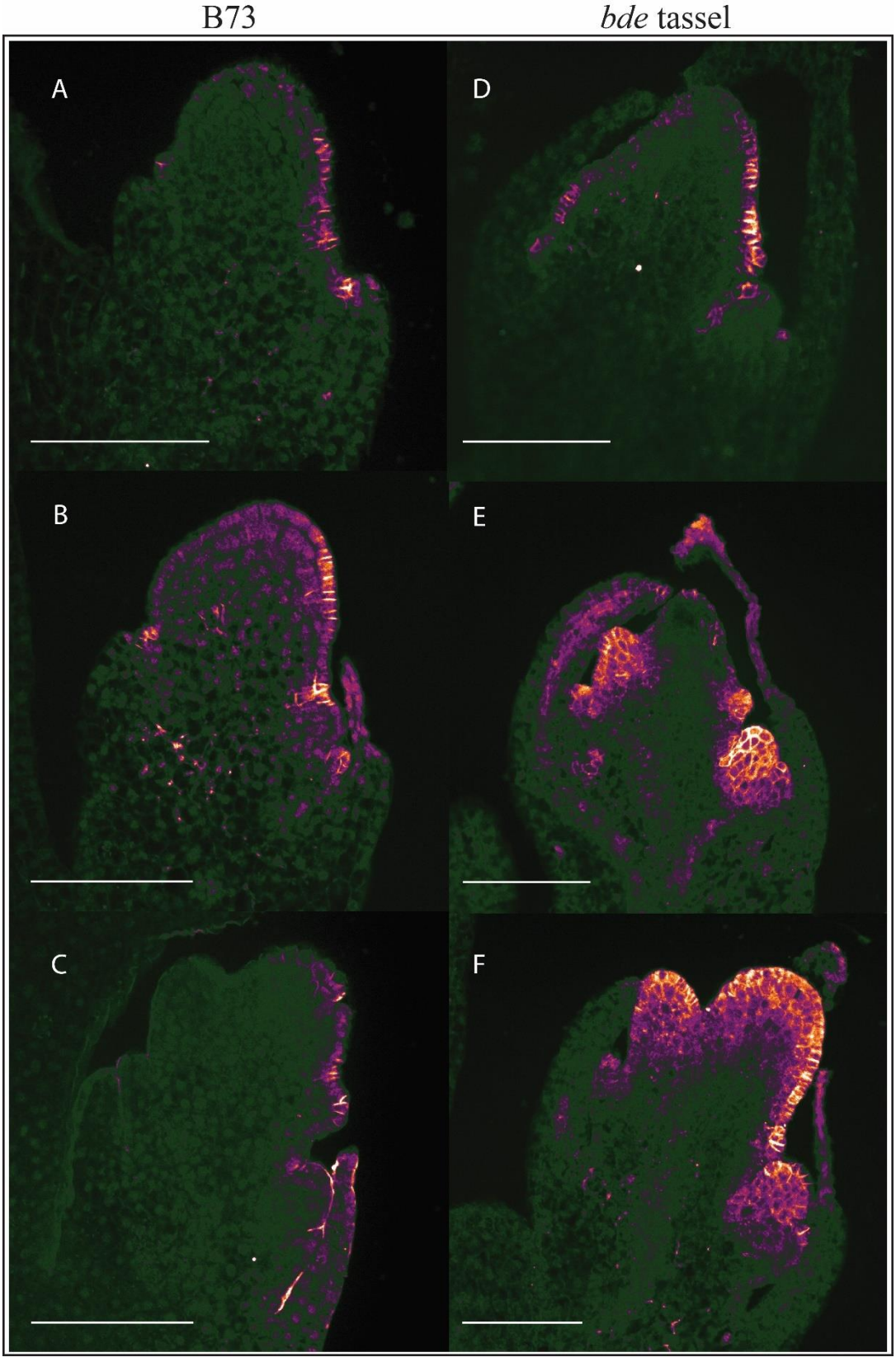
APPENDIX B: LM20 STAIN IN B73 AND *bde* TASSELS



Appendix B Legend

LM20 immunostaining of methylesterified pectin in early (A) and late (B) stage tassel florets. *Bde* mutant tassel florets were assessed with LM20 stain in early (C) and late (D) stages in tassel florets. Fluorescence controls of *bde* tassels were imaged in (E) and (F). Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 µm.

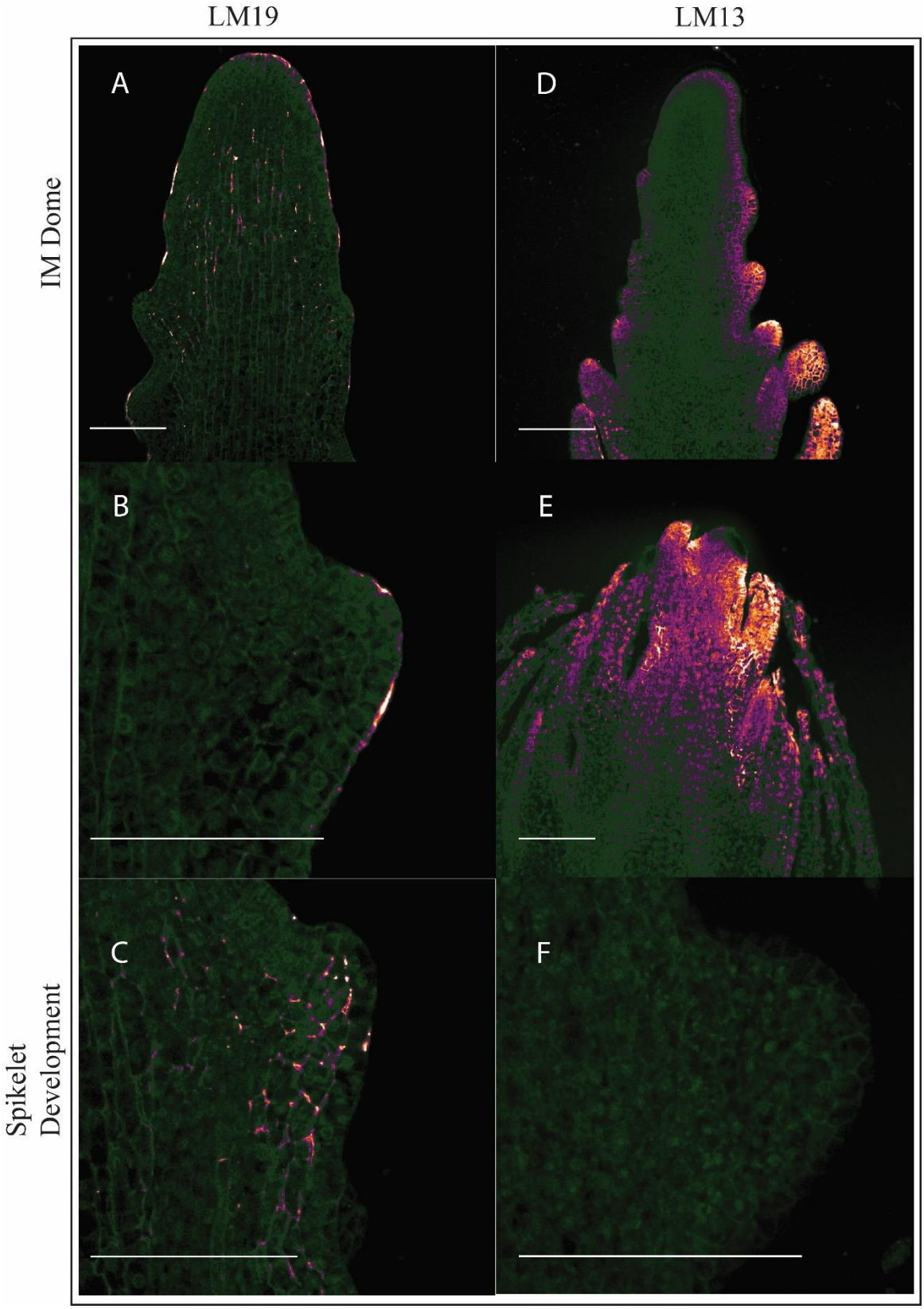
APPENDIX C: LM13 STAIN IN B73 AND *bde* TASSELS



Appendix C Legend

LM13 immunostaining of arabinan side chains of RG-I in early (A, B) and late (C) stage tassel florets. *Bde* mutant tassel florets were assessed with L130 stain in early (D) and late (E, F) stages in tassel florets. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 µm.

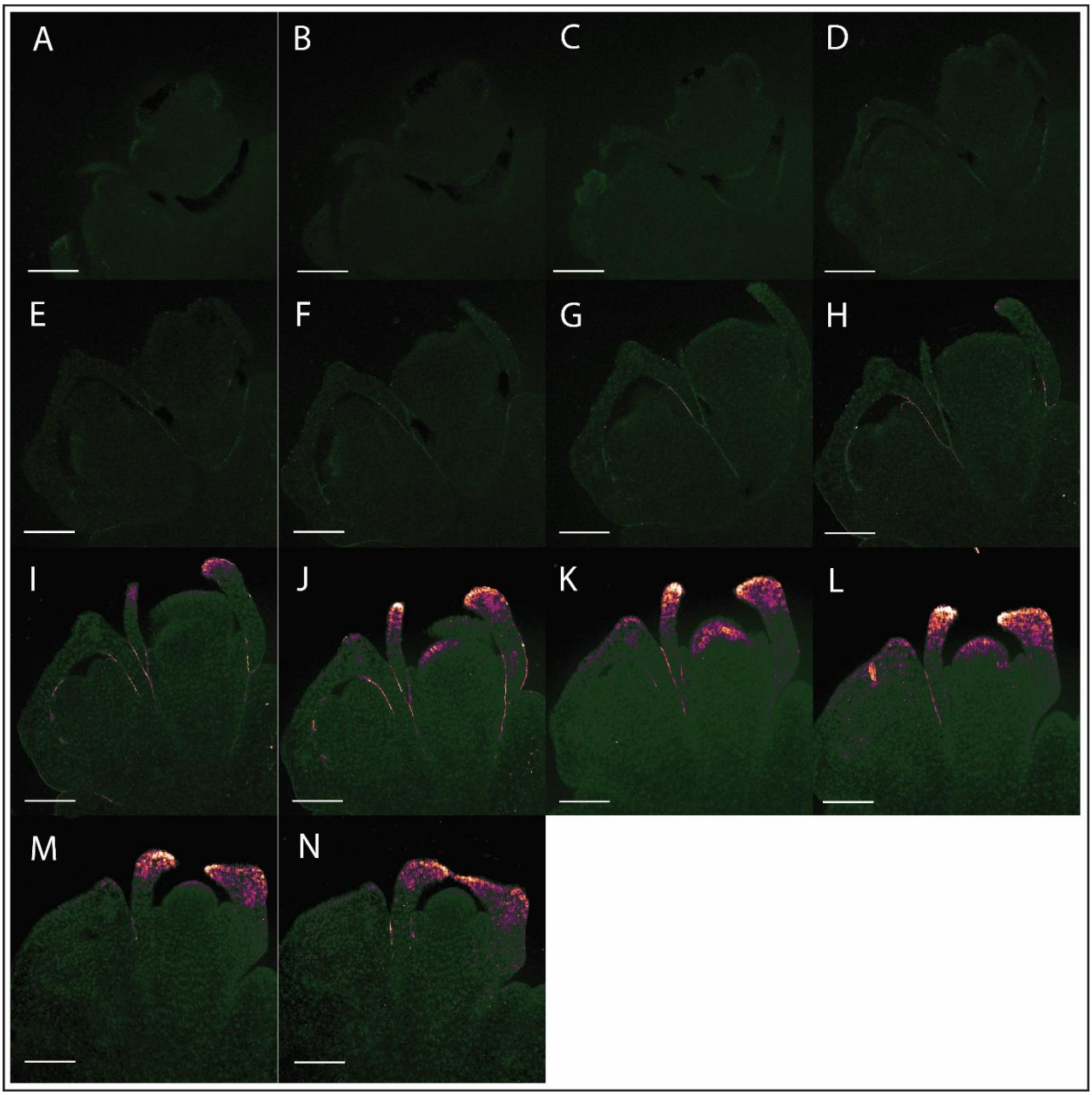
APPENDIX D: LM19 and LM13 STAIN IN *ba1* TASSELS



Appendix D Legend

LM19 immunostaining of demethylesterified pectin in *ba1* mutant tassels in the inflorescence meristem dome (A) and at the site of organ initiation (B, C). LM13 immunostaining of arabinan side chains of RG-I in the inflorescence meristem dome (D) and in a floret initiated at the inflorescence meristem tip (E) and at the site of organ primordia (F). Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 µm.

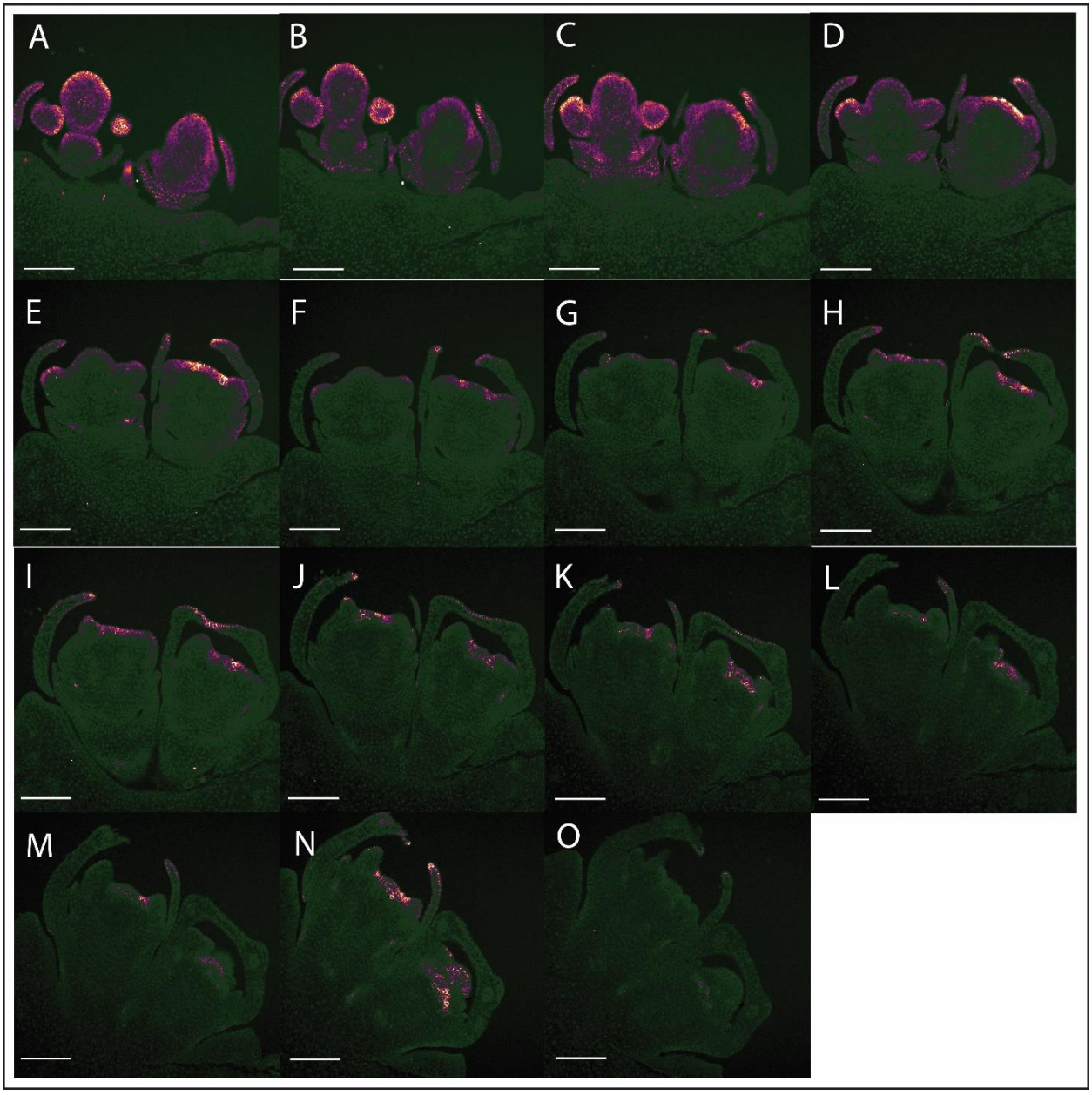
APPENDIX E: LM13 STAIN IN TRANSVERSE SECTIONS OF B73 EARS



Appendix E Legend

LM13 immunostaining of arabinan side chains of RG-I in B73 inflorescence fter transverse sections have been taken. A-N, LM13 staining of transverse serial sections of B73 ears. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 µm.

APPENDIX F: LM13 STAIN IN TRANSVERSE SECTION OF *bde* EARS



Appendix F Legend

LM13 immunostaining of arabinan side chains of RG-I in *bde* mutant inflorescence after transverse sections have been taken. A-O, LM13 staining of transverse serial sections of *bde* ears. Micrographs were false colored using ImageJ (Orange Blue icb look-up table) to visualize signal intensity. Scale bars = 100 μm.