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Spring 2014

Microtubule organization and cell-to-cell communication in *Arabidopsis thaliana*

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Microtubule Organization and

Cell-to-Cell Communication in *Arabidopsis thaliana*

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A Project Presented to

the Faculty of the Undergraduate

College of Science and Mathematics

James Madison University

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in Partial Fulfillment of the Requirements

for the Degree of Bachelor of Science

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by Lauren Michelle Schulte

May 2014

Accepted by the faculty of the Department of Biology, James Madison University, in partial fulfillment of the requirements for the Degree of Bachelor of Science.

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Acknowledgements

I would like to acknowledge and thank my faculty committee, Dr. Alexandra Bannigan, Dr. Jonathan Monroe, and Dr. Susan Halsell. Without any of you this thesis would have never been completed. You all kept me on task, even when time zone differences began to complicate matters.

I would also like to thank the Department of Biology at James Madison University for funding this project through grants and the Anonymous Scholarship that I was awarded in the Summer of 2013. Without this funding I wouldn't have been able to perform all of my various experiments needed to complete this thesis.

Lastly, I would like to thank the Honors Program at James Madison University for allowing me to enter into your program my freshman year at James Madison University and staying with me through the course of my college career.

Abstract

The presence of a cell wall means that plant cells require great coordination in their growth because of their inability to move relative to each other. In order to coordinate cell growth, plants have a variety of ways to communicate cell-to-cell, including the plasmodesmata, tiny channels that connect the cytoplasm of neighboring cells. Important developmental proteins travel between cells using the plasmodesmata network. CAPRICE (CPC) is a signaling protein in the root epidermis that travels between atrichoblast (non-hair) and trichoblast (hair) cells using the plasmodesmata. The *radially swollen 6* (*rsw6*) mutant is a microtubule organization mutant that is believed to have reduced cell-to-cell communication, based on previous work involving the microinjection of fluorescent tracers. In this experiment, confocal microscopy was conducted in order to compare the distribution of CPC:GFP in *rsw6* and wild-type plants as a way of investigating the intercellular movement of naturally occurring proteins in this mutant. A pavement cell analysis was also performed to compare the amount of cell convolution between *rsw6* and wild-type plants using perimeter-to-area ratios. The pavement cell analysis was also expected to be a measure of cell-to-cell movement of signaling proteins, as intercellular coordination is needed for the formation of pavement cells. It was determined that the cell-to-cell movement of naturally occurring signaling protein was not affected in the *rsw6* mutant in either experiment. The most likely explanation for the difference between these results and the previous microinjection study is that endogenous signaling proteins generally have an intercellular movement domain that could override a general reduction in cell-to-cell transport through targeted protein trafficking.

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Introduction

Coordination of Growth

Any growth that takes place in plant cells requires more coordination than in animal cells because the presence of a cell wall prevents them from moving relative to each other. The cell wall is a rigid structure found in plants that separates cells, provides structural support, and confines a cell to a particular position (Van Norman *et al*, 2011). For normal plant growth and development to occur, cell division and expansion must be tightly coordinated across tissues and organs (Overall *et al*, 2001). Such coordination is achieved by various kinds of cell-to-cell communication between neighboring cells as well as between cells and the environment. Environmental cues can affect the way a plant grows and that information is passed between cells (Van Norman *et al*, 2011). One mode of cell-to-cell communication is via the plasmodesmata.

Plasmodesmata & Signaling Proteins

The plasmodesmata are cell wall pores that connect the cytoplasm of neighboring cells and allow for the transport of small molecules between them. Plasmodesmata maintain specific size exclusion limits, which allow transport of molecules up to a particular size and which can be modified to allow transport of selected molecules that exceed the size limit (Kurata *et al*, 2005). Various signaling proteins move cell-to-cell through the plasmodesmata. Some mobile developmental proteins, such as KNOTTED 1 (KN1) and LEAFY (LFY), are involved in either the regulation of cell shape or cytoskeleton organization over multiple cells (Overall *et al*, 2001). The tobacco mosaic virus, like many other viruses, is also known to travel through the plasmodesmata with the aid of the P30 protein, which increases the size exclusion limit of the plasmodesmata so that the virus can move between cells and achieve systemic infection

(Citovsky *et al*, 1993). Other proteins that are known to move through the plasmodesmata include ENHANCER OF TRY AND CPC3 (ETC3) (Digiuni *et al*, 2008) (Wester *et al*, 2009) and TRANSPARENT TESTA GLABRA (TTG1) (Bouyer *et al*, 2008) (Pesch & Hulskamp, 2009), both of which affect trichome patterning in leaves; GLABRA3 (GL3) (Savage *et al*, 2008) and CAPRICE (CPC) (Kurata *et al*, 2005), both of which affect root hair patterning; and SHORT-ROOT (SHR) (Gallagher *et al*, 2004) (Gallagher & Benfey, 2009), which affects radial patterning and maintenance of the root apical meristem (Wu & Gallagher, 2011).

Cell Shape & Growth

Plant cell shape and growth rate are determined, in large part, by microtubule organization. Microtubules are hollow filamentous protein polymers that perform a variety of roles in all eukaryotic cells (Smith & Oppenheimer, 2005). In elongating plant organs, such as the root, interphase microtubules are usually organized in strict parallel transverse arrays perpendicular to the axis of cell expansion (Smith, 2003). In particular, cortical microtubules influence plant cell shape and growth rate through their relationship with the cell wall (Tiwari *et al*, 1984). Cellulose microfibrils typically mirror the cortical microtubules in expanding cells because the movement of cellulose synthase enzyme complexes in the cell membrane is restricted through microtubule interactions (Smith & Oppenheimer, 2005). Cortical microtubules guide the deposition of cellulose microfibrils into the cell wall, which in turn restricts cell expansion to the direction perpendicular to their orientation (Smith & Oppenheimer, 2005). Therefore, the organization of cortical microtubules can affect cell shape. Pavement cells illustrate a different relationship between microtubules and cell expansion. These pavement cells appear as interlocking jigsaw puzzle-shaped cells found in the leaf epidermis in *Arabidopsis* (Figure 1) (Fu *et al*, 2005). Cortical microtubule bundles are arranged transversely across the

"neck" regions in the cells and are believed to restrict expansion in the direction of their general

orientation (Fu *et al*, 2005).
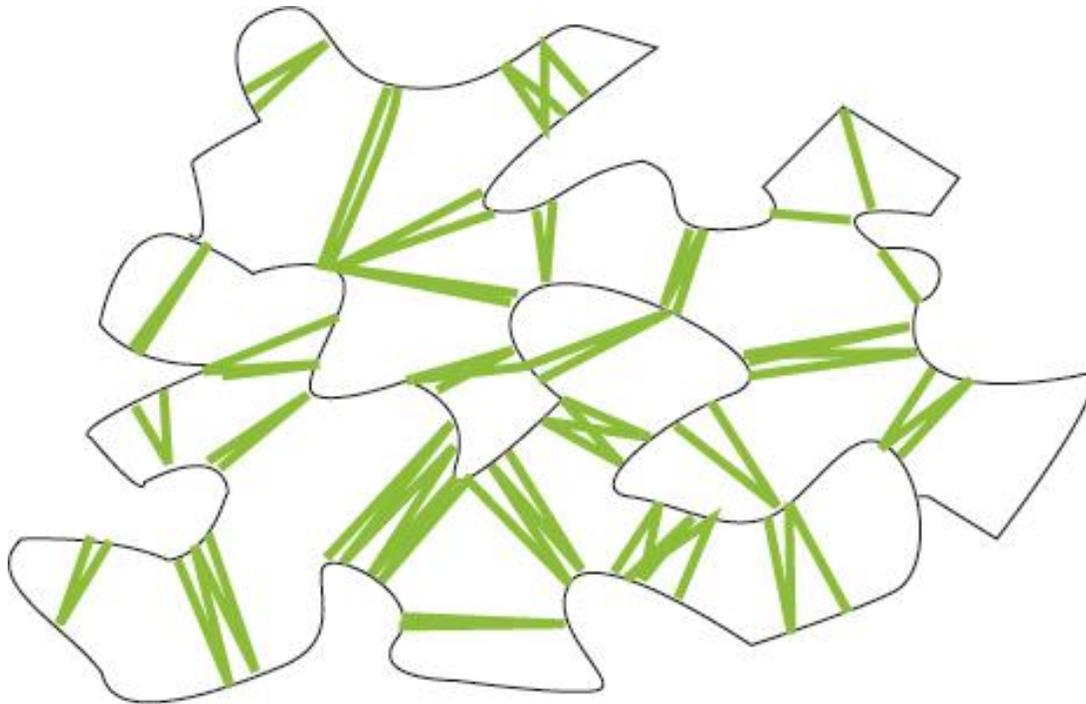
Figure 1. A schematic drawing of pavement cells (cells outlines in black) with their microtubules highlighted in green. Note that the microtubules bundle across the "neck' regions in the cells (Figure adapted from Fu *et al*, 2002).

Examples of Intercellular Microtubule Coordination

Microtubules have been seen coordinated across cell boundaries in several different cases.

One such case has been observed in the cells surrounding a wound site. When a wound occurs in

a plant, the microtubules of the cells surrounding the wound can realign to become parallel to the

edge of the wound; this new orientation allows for cell expansion to be redirected toward the

center of the wound to replace the lost tissue (Overall et al, 2001). Another case has been

observed in the *tangled 1* maize mutant. The *tangled 1* mutant has disrupted spatial control of

cell division in leaf development, leading to abnormally shaped cells. Surprisingly, the overall

shape of the leaves is normal. On closer examination, the microtubules in the abnormally shaped

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cells were aligned across cells (Figure 2) in an orientation that would be expected during normal

leaf development (Cleary & Smith, 1998). The coordination of microtubule organization across

the leaf could account for the normal leaf appearance despite the abnormal shape of individual

cells.
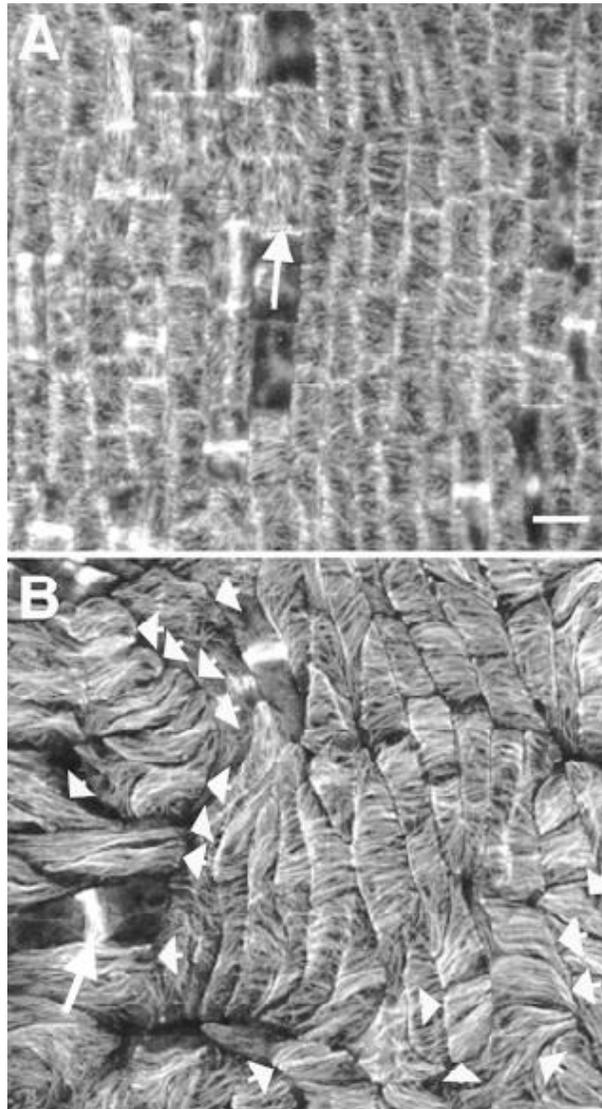
Figure 2. (A) Microtubules observed in leaf epidermal cells of wild-type plants. Most interphase cortical microtubules are transverse to the cell's long axis. Occasionally, microtubules are aligned parallel to the long axis of the cell (arrow). (B) In *tangled1*, microtubules are aligned normally within most cells. Arrowheads indicate cells whose microtubules are better aligned with those of neighboring cells than they are with the cell's own transverse axis. Bar in = 10 μm (Figure taken from Cleary & Smith, 1998).

Cytoskeletal organization in pavement cells (Figure 1) is known to be regulated through signaling molecules that travel between cells (Fu *et al*, 2005). An outgrowth in one cell is promoted through the ROP2 pathway, and is coordinated with the inhibition of outgrowth in a neighboring cell by the RIC1 pathway, forming a jigsaw-like appearance (Fu *et al*, 2005). Without communication between neighboring cells, this interlocking growth would not be possible.

Microtubule Mutants in *Arabidopsis thaliana*

A large number of microtubule mutants have been identified in *Arabidopsis thaliana*. The microtubules in these mutants can be fragmented, disorganized, or display tissue-wide reorganization. In the temperature sensitive mutant *microtubule organization 1* (*mor1*), the microtubules become fragmented and disorganized. *mor1* displays normal cortical microtubule organization at 21°C, but at 29°C the microtubules shorten and lose their usual transverse parallel alignment (Figure 3). The cells no longer have control over their expansion direction because of the disordered microtubules (Wasteneys, 2002).
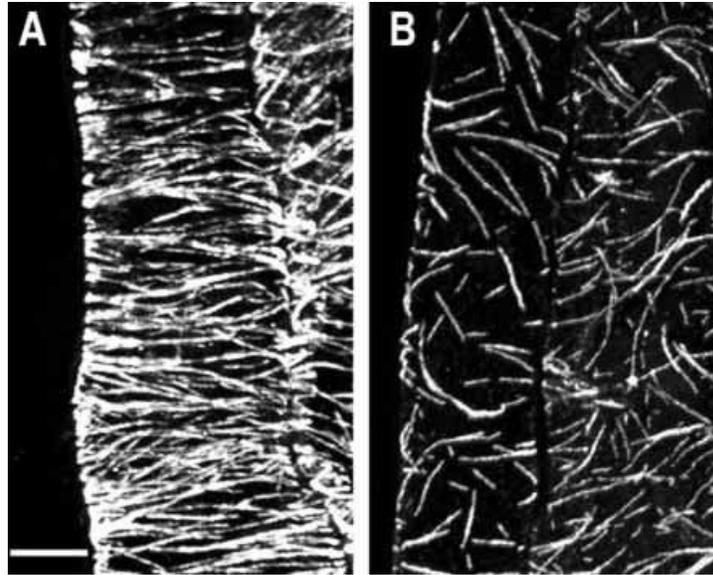
Figure 3. Microtubule patterns in the epidermis of *Arabidopsis thaliana* cotyledons after 4 hours at 29°C. (A) Cortical microtubules are abundant and transversely oriented in wildtype. (B) In the *mor1* mutant, microtubules appear short and disorganized. Bar, 10 µm (Figure and legend taken from Wasteneys, 2002).

In the mutant *fragile fiber2* (*fra2*), the microtubules become disorganized, the amount of cellulose in the cell wall is reduced, and the pattern of microfibrils is disrupted (Wasteneys, 2004). In the right-twisting mutant called *spiral1* (*spr1*), the microtubules are organized into parallel arrays, but the arrays are obliquely oriented. *spiral1* roots twist to form right-handed helices as they grow (Nakajima *et al*, 2004). In the left-twisting helical mutant called *lefty*, the microtubules are organized into parallel oblique arrays oriented in the opposite direction of *spiral1* (Figure 4) (Thitamadee *et al*, 2002).
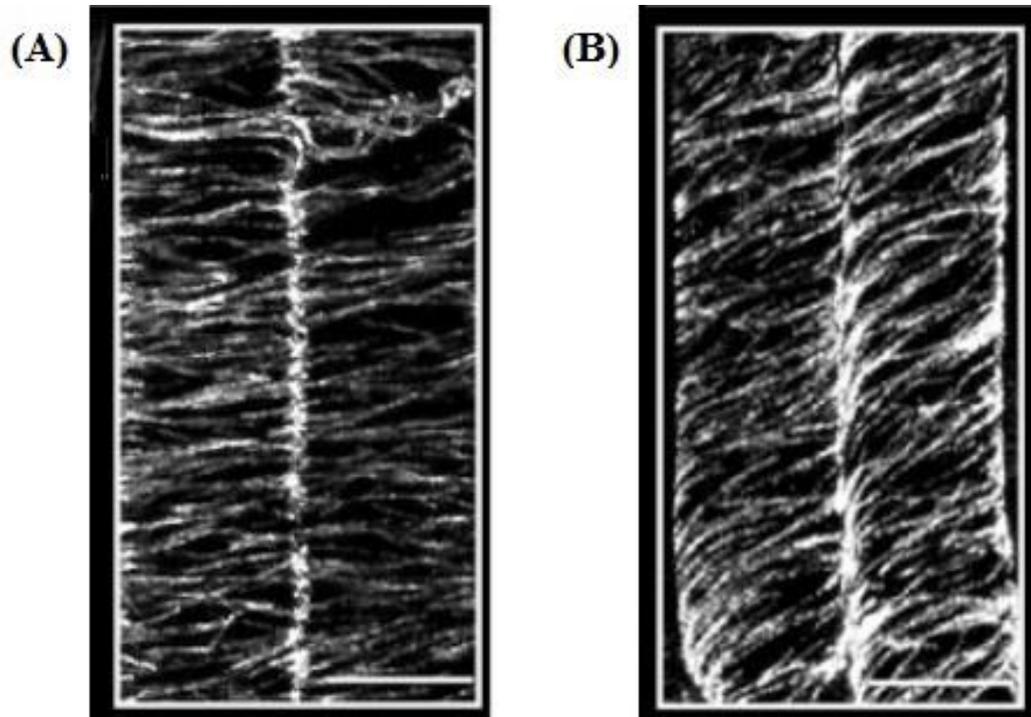
Figure 4. (A) Cortical microtubules in wild-type roots are transversely oriented. (B) In *lefty1*, cortical microtubules are right-handed oblique. Scale bars = 10 µm (Figure adapted from Thitamadee *et al*, 2002).

rsw6 **Mutant**

The *Arabidopsis thaliana* temperature sensitive mutant called *radially swollen 6* (*rsw6*) has a phenotype only observed in the cells found in the root epidermis (Bannigan *et al*, 2006) and is caused by a β-tubulin-3 (TUB3) point mutation (Bannigan, unpublished). The microtubules in *rsw6* are organized into parallel arrays within cells, but the orientation of the microtubule arrays is different between neighboring cells (Figure 5).
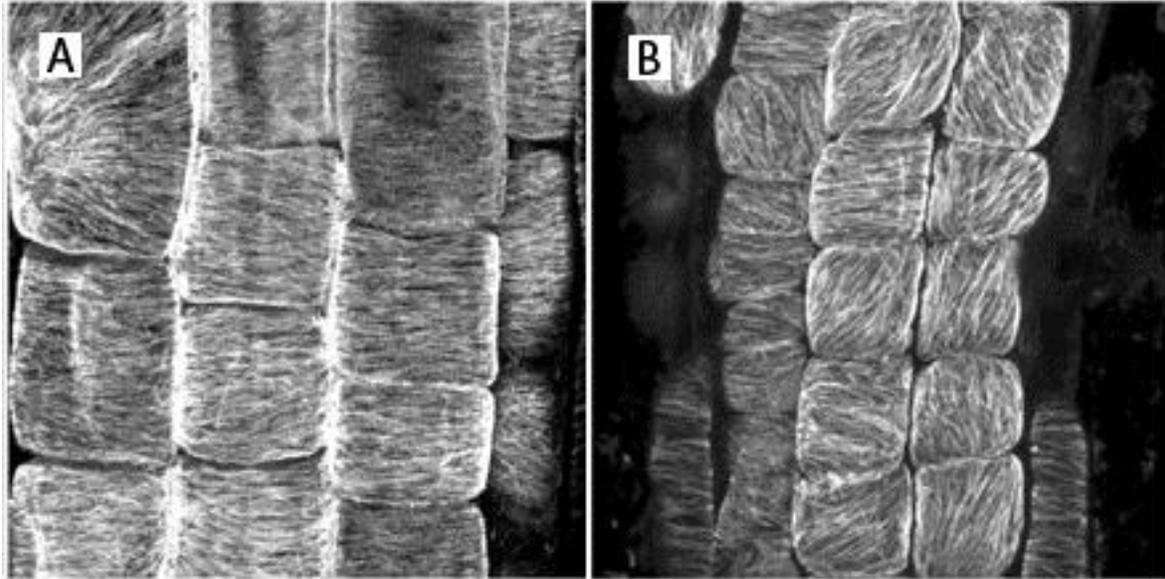
Figure 5. Confocal microscope images of wild-type (A) and *rsw6* (B) microtubules in cells of the root epidermis after 6 hours at 30°C. Note that wild-type cells generally have horizontally oriented microtubules, while the *rsw6* cells have microtubules that are arranged in parallel arrays of various orientations (Figure adapted from Bannigan *et al*, 2006).

The *rsw6* mutant displays normal cortical microtubule organization at 19°C, but at 30°C the cells become swollen and the microtubules lose alignment with neighboring cells. This is the only known *Arabidopsis* microtubule mutant where microtubules show local (cellular) organization, but global (tissue wide) disorganization (Figure 5). Dr. Bannigan has previously found that cell-to-cell movement of injected dyes is reduced in this mutant (unpublished).

Hypothesis

In this project we hypothesize that *rsw6* has reduced cell-to-cell communication, which affects the movement of developmentally important signaling proteins, including CAPRICE (CPC). CPC is a signaling protein that positively regulates root hair (trichoblast) formation and is known to pass through the plasmodesmata in root epidermal cells (Kurata *et al*, 2005). *CPC* mRNA expression has only been detected in atrichoblasts (hairless cells), but the protein also appears in trichoblast cells. Thus, the CPC protein must move from the atrichoblasts to the trichoblasts (Figure 6). In wild-type *Arabidopsis* plants, CPC accumulates in the nuclei of both

the atrichoblasts and trichoblasts. In *cpc* mutants, CPC movement is disrupted and remains in the

cytoplasm of the atrichoblasts (Figure 6) (Kurata *et al*, 2005). Figure 6 (B) and (C) show

examples of defective CPC movement caused by the truncation of its Myb domain. Since CPC is

a known signaling protein in the root epidermis, it can be used to test our hypothesis that *rsw6*

has reduced cell-to-cell communication. Figure 6 (B) and (C) shows examples of what we might

expect to see in *rsw6* at 30°C.
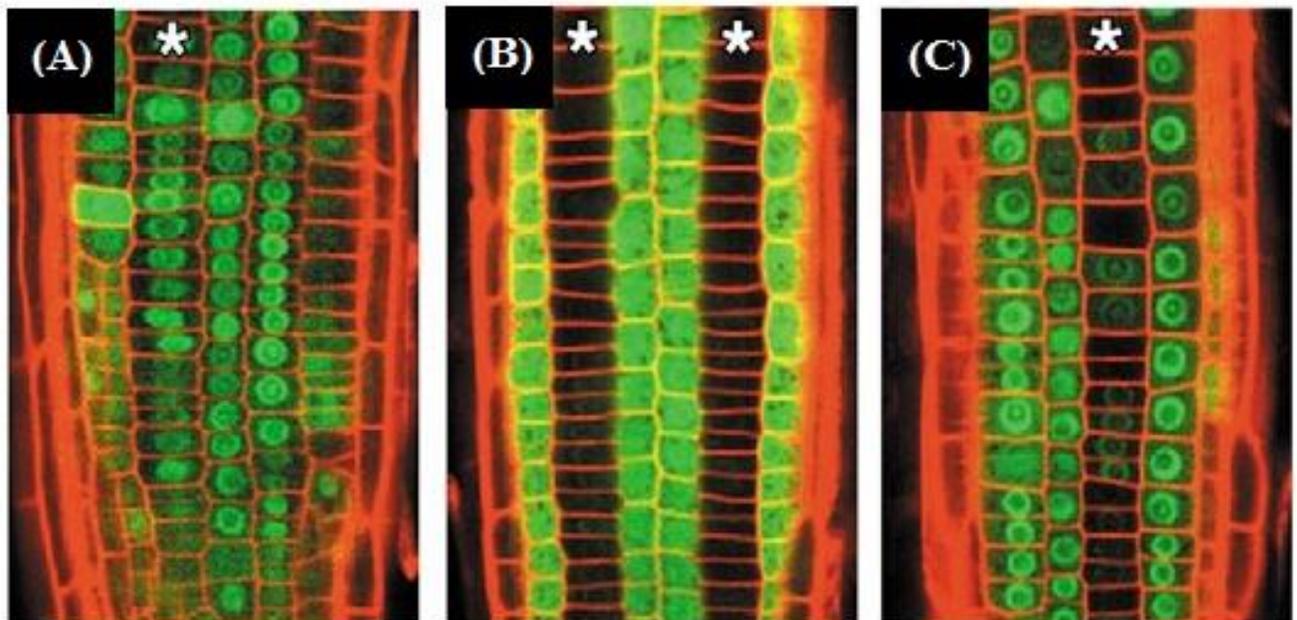
Figure 6. Images tracking the movement of different CPC:GFP constructs in root epidermal cells in 5-day-old seedlings with GFP fluorescence (green) showing the location of CPC and propidium iodide fluorescence (red) showing cell walls. (A) A CPC plant that showed CPC movement identical to wild-type *Arabidopsis* plants, where CPC moved from atrichoblasts to trichoblasts (file of trichoblasts is indicated by an asterisk) and accumulated in the nuclei of both cell types. (B) A CPC plant that showed movement identical to a *cpc* mutant, where CPC remained distributed throughout the cytoplasm of the atrichoblasts. (C) A partial defect in CPC movement in this plant was indicated by the occurrence of the GFP signal only in a fraction of the hair cells, where CPC accumulated in the cell nuclei. (Figure and legend adapted from Kurata *et al*, 2005).

Growing Plants on Plates

In order to view the CPC:GFP expression in wild-type and *rsw6* plants, the seeds were

plated on growth agar plates. To make the plates, ~500 mL of autoclaved growth medium (495

mL of ddH2O, 2 mL of 1M KNO_3, 1 mL of 1M KH_2PO_4, 0.5 mL of 1M $Ca(NO_3)$, 0.5 mL of

89.4 mM Fe citrate, 0.5 mL of micronutrients, 150 µL of 1M $MgSO_4$, 5 g of Bacto-agar, 5 g of

sucrose) was poured into sterile plates inside a laminar flow hood. The seeds were surface

sterilized for 10 minutes in 25% bleach within a microcentrifuge tube. After the 10 minutes, the

seeds were rinsed with sterile ddH_2O three times for 5 minutes each time. Using a sterile glass

pipet and bulb, the seeds were plated on to the growth medium in two rows across the plate, the

plate was sealed with parafilm, and placed in a growth chamber set to 20°C for 7 days. Plants to

be viewed at 30°C were transferred to 30°C 6 hours before viewing to allow expression of the

rsw6 mutant phenotype.

Screening for *rsw6* Phenotype and CPC:GFP Expression

CPC:GFP plants were previously crossed to the *rsw6* mutant plants by Dr. Shuang Wu at

the University of Pennsylvania. The seeds acquired were first generation (F1) plants. In order to

verify the *rsw6* phenotype and the CPC:GFP expression, the F1 *rsw6* x CPC:GFP seeds received

from Dr. Wu were plated on growth medium plates and placed in the 20°C growth chamber for 7

days and then transferred into 30°C growth chamber for 6 hours, by which time *rsw6* roots

would become visibly swollen. The F1 seedlings were screened for GFP fluorescence and root

swelling on the epifluorescence microscope. Seedlings confirmed as *rsw6* x CPC:GFP were

planted in soil and the seed collected. The F2 seeds were harvested, the siliques were allowed to

desiccate at room temperature, and then the seeds were placed in a refrigerator until they were used.

Determining the Wild-Type CPC:GFP Distribution

CPC:GFP wild-type plants were viewed under the Nikon confocal microscope to determine the seedling age and location on the root to use in order to acquire the best live image of CPC:GFP distribution. First the CPC:GFP wild-type seeds were plated on growth medium and placed in the 20°C growth chamber. For 7 days the seedlings were monitored daily and viewed under the Nikon confocal microscope.

Plant Microscopy

To image the CPC:GFP distribution in wild-type and *rsw6* x CPC:GFP plants, half of the week-old seedlings of each genotype were placed in 30°C for 6 hours and the other half remained at 20°C for 6 hours as a control group. The cell outlines are difficult to view, so the roots were treated with 10% propidium iodide for 1 minute to stain the cell walls. Plants were carefully transferred to a small petri dish with a cover glass bottom (Mat Tek) and secured with a small piece of 2% Hoagland's agar (growth medium without sucrose). The plants were observed on the confocal microscope using the 488 nm laser, the green detector, the 546 nm laser, and the red detector with 60x 1.2 NA objective. The channel series function was used to prevent signal bleed through from the green to the red channel. The 30°C plants not in immediate use were kept in an incubator set to 30°C and a heated stage set to 30°C was used for viewing the 30°C plants under the microscope, in order to maintain the restrictive condition.

Image Analysis

A total of 35 roots were used to perform an image analysis for each treatment. For every root image, the GFP distribution was scored in the trichoblasts and atrichoblasts separately as

one of the following: nucleus, cytoplasm, both nucleus and cytoplasm, or no visible expression. The relative frequency of GFP detection in the different cell types and in the different sub-cellular locations was summarized and the different treatments were compared.

Assessing Pavement Cell Shape in *rsw6* Plants

To observe the pavement cells in the leaves of the wild-type *Arabidopsis* plants and *rsw6* plants, leaves from 28 day old plants were cleared of chlorophyll by submerging them in 10 % glacial acetic acid and 90% ethanol at room temperature for one hour or until the leaf was cleared. Then the leaves were mounted on slides in water and photographed using a DIC microscope (Zeiss Axioskop) with a 20x NA 0.45 objective. The perimeter-to-area ratio for the pavement cells was determined by tracing the outline of the cells using Elements software (Figure 7). To find the perimeter-to-area ratio, a microscope image was placed under a 100 μm grid in Elements, and cells in alternating grid squares were selected and traced by hand using Elements tools to measure the perimeter (μm) and area (μm^2). By alternating grid squares bias to certain cells could be avoided. Guard cells and incomplete cells were also avoided. Perimeter-to-area ratios were calculated, and an average perimeter-to-area ratio was found for each treatment. Different treatments were compared using a Student's t-test with P=0.05.
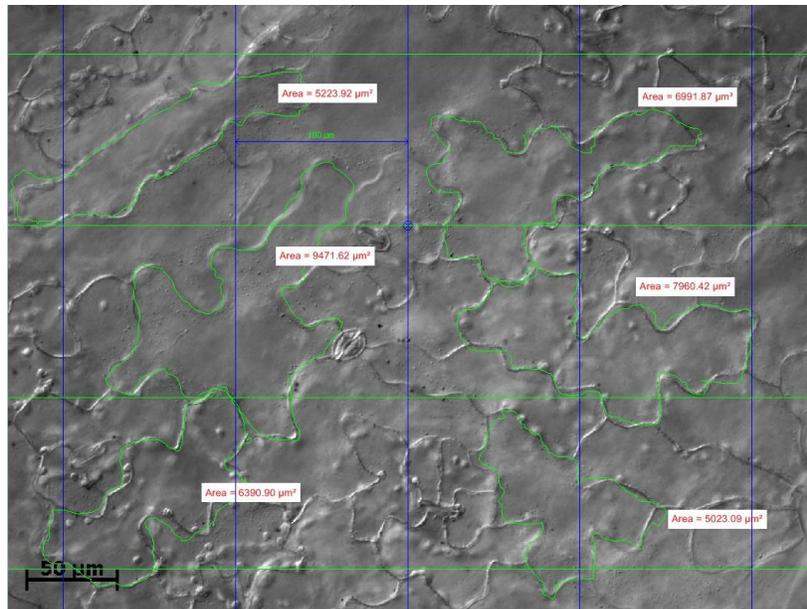
Figure 7. A pavement cell image open in Elements software with a 100 µm grid. The cells that were measured for perimeter (µm) and area (µm^2) were selected from alternating grid squares in order to acquire cell measurements in an unbiased manner. Guard cells and incomplete cells were avoided.

Confirming the Mutant Gene

The *rsw6* phenotype is believed to be caused by a mutation in the β-tubulin 3 (TUB3) gene. The gene was previously cloned and the wild-type allele transformed into mutant *rsw6* plants by Dr. Bannigan. These *rsw6*:TUB3 plants have a wild-type phenotype with the presumed presence of the wild-type transgene in the *rsw6* background. The presence of the wild-type gene from the plasmid as well as the mutant gene must be verified to check that the screened plants were not simply mislabeled wild-type plants. The *rsw6*:TUB3 seeds were plated on a growth agar plate and placed in the 20°C growth chamber for 2 weeks. After 2 weeks the plants were harvested from the plate using forceps, placed in a microcentrifuge tube, and stored in the -80°C freezer.

Primers were designed to allow the wild-type TUB3 to be distinguished from the mutant *RSW6* gene (Figure 8). Because the wild-type gene was transformed into an *rsw6* background via

Gateway cloning, one primer set was designed that would amplify the ATTB regions flanking

the insert as well as the gene, giving a PCR product of 3 Kb. The primer for the *RSW6* gene was

designed to recognize regions on chromosome 5 outside of the sequence included in the plasmid,

giving a larger product of 5.3 Kb (Figure 9). The two products should appear as separate bands

on a gel. The bands can then be excised from the gel, the DNA extracted, and sent for

sequencing to confirm the presence of both the mutant and wild-type genes. In this study, the

primer design was completed, but the confirmation of the gene through gel extraction and

sequencing was not.

Primers for Plasmid Genes:

Forward Primer (At5G62700):

5' - GGG GAC AAG TTT GTA CAA AAA AGC AGG CTG GTC TTC TAT CCT CAT CTT TCT GC - 3'
Tm (50 mM NaCl) = 82.15°C

Reverse Primer (At5G62700 ATTB2):

5' - GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG AAA ATG GGC CGA GCT CTT A - 3'
Tm (50 mM NaCl) = 84.8°C

Primers for TUB3 Gene in Chromosome 5:

Forward Primer:

TTTTCTTCTGTGTGCAGTTG

Located at 25182801, Tm = 55°C

Reverse Primer:

AATTGCAAAATTCTGGCTTA

Located at 25188100, Tm = 55°C

Figure 8. These are the four primers that were used in order to run the PCR.
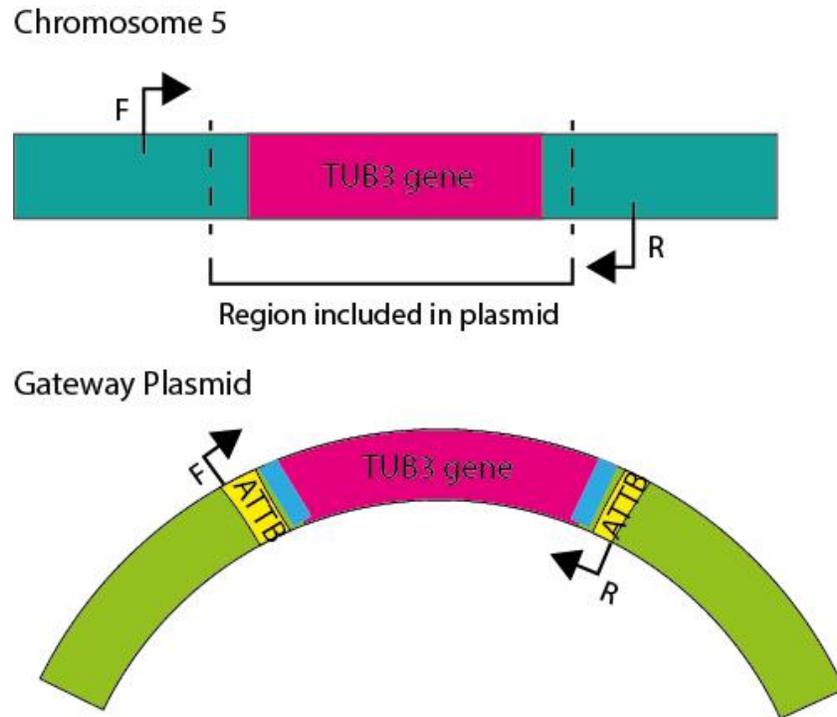
Figure 9. A schematic representation of the primer design for wild-type and mutant TUB3 in transformed *rsw6* plants. The mutant gene is amplified from its normal location on chromosome 5, giving a PCR product of 5.3 Kb. The primers for the wild-type gene recognize small sections of sequence from the plasmid on either side of the gene, giving a product of 3 Kb. These two products can then be separated on a gel and sent for sequencing.

<div align="center">

Results

</div>

Wild-type CPC:GFP Distribution

After viewing the wild-type CPC:GFP seedlings under the confocal microscope for various intervals over the course of 7 days, it was determined that the 7 day old seedlings illustrated the most pronounced fluorescence. This fluorescence was most visible in the beginning of the elongation zone in the root tip of the seedlings.

CPC:GFP Distribution in Wild-Type and *rsw6* x CPC:GFP Plants

The GFP distribution pattern in atrichoblast and trichoblast cells was observed under the confocal microscope in wild-type and *rsw6* x CPC:GFP roots at 20°C and 30°C (Figure 10). The wild-type atrichoblasts and trichoblasts had pronounced fluorescence in the nuclei with occasional expression in the cytoplasm at both 20°C and 30°C (Figures 10 & 11). The *rsw6* x CPC:GFP atrichoblasts and trichoblasts also had pronounced fluorescence in the nuclei with occasional expression in the cytoplasm at both 20°C and 30°C (Figures 10 & 11). The trichoblasts had a higher fluorescence compared to the atrichoblasts in every treatment performed; otherwise there was little difference in expression between plant types or temperature treatments (Table 1 & Figure 11).

Table 1. Overall distribution of GFP between trichoblast and atrichoblast cells in the different treatments.

Cell Type	Treatment			
	Wild-type at 20°C	Wild-type at 30°C	*rsw6* x CPC:GFP at 20°C	*rsw6* x CPC:GFP at 30°C
Atrichoblast	63%	57%	57%	57%
Trichoblast	37%	43%	43%	43%
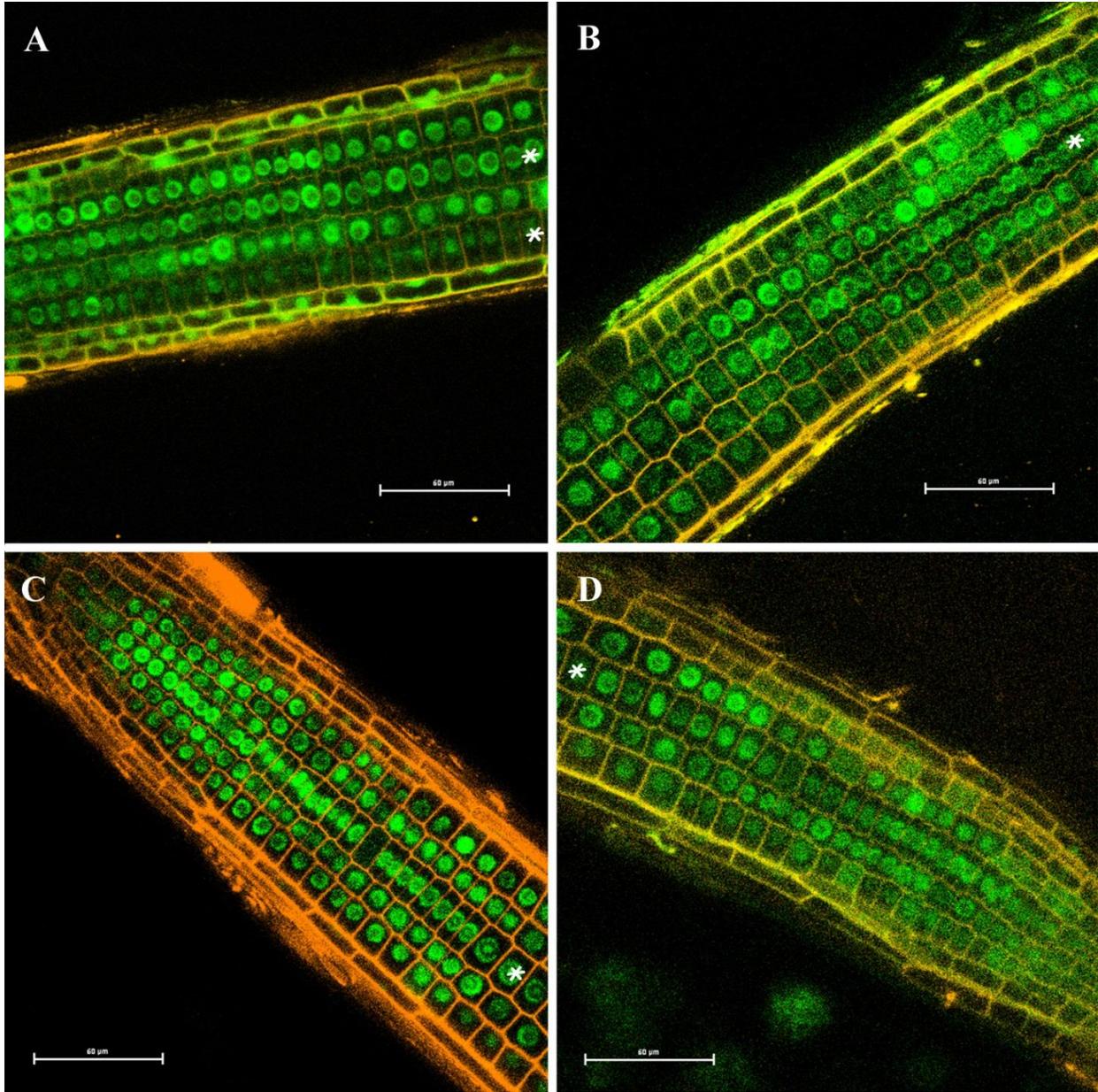
Figure 10. Confocal microscope images of a (A) wild-type root at 20°C, (B) wild-type root at 30°C, (C) *rsw6* x CPC:GFP root at 20°C, and (D) *rsw6* x CPC:GFP root at 30°C. The CPC:GFP protein is in green and propidium iodide has stained the cell membranes in orange. The asterisk marks the trichoblast cell files, while all other cell files are atrichoblasts. Scale bar is 60 µm.
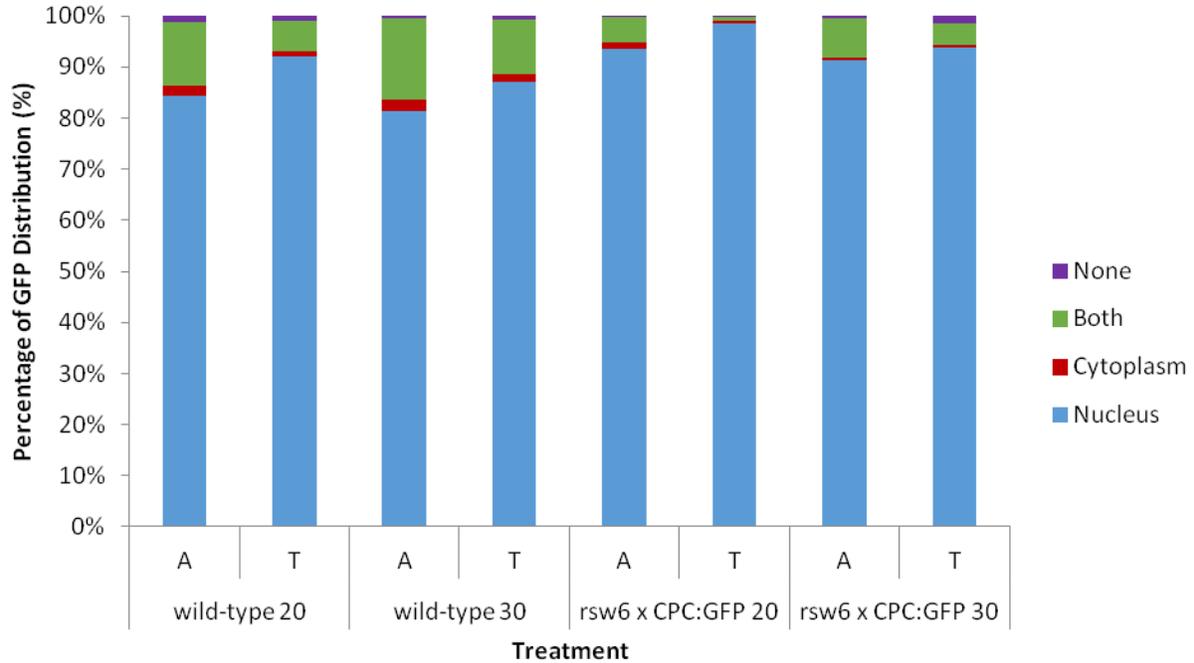
Figure 11. The GFP distribution (%) in atrichoblasts (marked A) and trichoblasts (marked T) in wild-type and *rsw6* x CPC:GFP plants at 20°C and 30°C. GFP was observed in the nucleus, cytoplasm, both the nucleus and cytoplasm, or in neither the nucleus nor cytoplasm in these cells.

Pavement Cell Analysis

Pavement cells on the leaves of wild-type and *rsw6* plants at 20°C and 30°C were analyzed. A total of 84 leaves were imaged and measured for each treatment. From these measurements perimeter-to-area ratios were determined (Figure 12). The highest perimeter-to-area ratios were seen in wild-type at 20°C and *rsw6* at 30°C. However, these ratios illustrated no significant difference between any of the treatment types when a two-tailed t-test was performed with a P-value of 0.05.
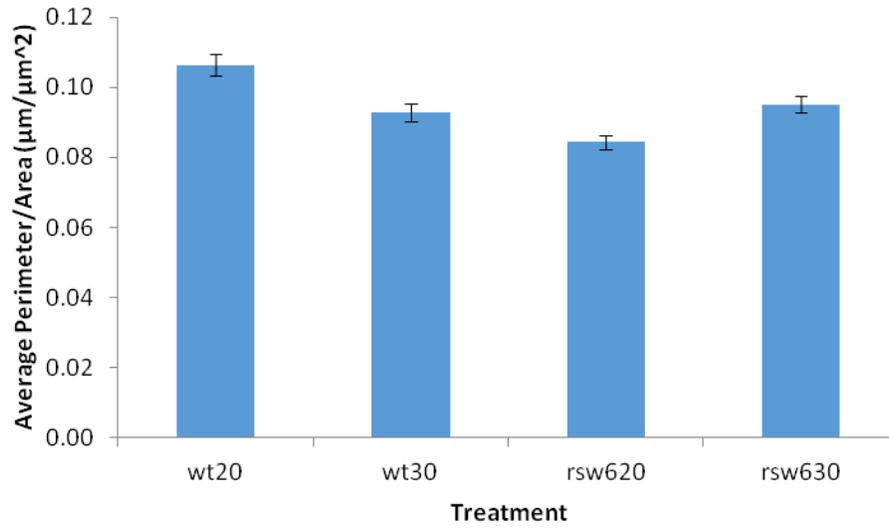
Figure 12. The average perimeter-to-area ratios of pavement cells in wild-type and *rsw6* plants treated at 20°C and 30°C.

Discussion

CPC:GFP Movement in *rsw6*

The pattern of GFP expression in the wild-type and *rsw6* x CPC:GFP roots was found to be very similar. The wild-type GFP expression pattern at 30°C and the *rsw6* x CPC:GFP GFP expression patterns at both temperatures were, in fact, identical. These results do not support the hypothesis that *rsw6* has reduced cell-to-cell communication, affecting the movement of developmentally important signaling proteins, like CPC. This could be because CPC has its own movement domain, which allows the protein to be selectively transported through plasmodesmata. Kurata *et al* (2005) demonstrated that if CPC's Myb domain is removed or truncated, cell-to-cell movement of CPC is disrupted. In previous studies showing reduced cell-to-cell communication in *rsw6* (Bannigan, unpublished), a simple fluorescent dye was used, which would have been restricted in its movement by the plasmodesmata's size exclusion limit alone. CPC has the ability to increase the size exclusion limit of the plasmodesmata (Kurata *et al*, 2005). This could have affected the GFP expression pattern in the *rsw6* x CPC:GFP roots by elevating the amount of cell-to-cell communication of that protein to normal levels in this experiment.

Pavement Cell Shape in *rsw6*

The perimeter-to-area ratio was calculated for pavement cells in wild-type and *rsw6* leaves at 20°C and 30°C as a measure of how convoluted the cell shapes were. This allowed for the quantification of lobe formation in pavement cells. When the pavement cells on the leaves of wild-type and *rsw6* plants were observed and measured, it was found that the perimeter-to-area ratios were not significantly different between any of the treatments. This was unexpected, since preliminary experiments performed by Dr. Bannigan found that the perimeter-to-area ratio was

reduced in *rsw6* at 30°C. There are several possible explanations for this. One is that the pavement cells in the area on the leaves used to determine the perimeter-to-area ratios had already finished expanding into the characteristic jigsaw shapes by the time they went into the heated growth chamber. Ideally, the plants would be grown at 30°C from germination to harvest, but the plants become sick if left in such a high temperature any longer than 4 days' time. Another possible explanation for the different result is that the current study looked at a larger sample size than the preliminary study, so it might, in fact, be a more accurate picture. Lobe formation may be able to take place even without cell-to-cell communication of the ROP/RIC signaling proteins, but a more likely explanation is that, like CPC, the ROP/RIC proteins are not affected by reduced cell-to-cell communication because of their targeted transport mechanisms.

TUB3 Gene Confirmation

Primers were designed to selectively amplify TUB3 in *rsw6* plants transformed with the wild-type TUB3 gene. In order to confirm that these phenotypically wild-type plants were in fact *rsw6* rescued by transformation with the *RSW6* gene (TUB3), the primers had to be designed in such a way that the mutant gene and the wild-type gene could be amplified separately out of the same DNA sample and sent for sequencing. The primers were designed but were unfortunately not tested due to time constraints. The primers and seeds will be sent to Dr. Tobias Baskin at the University of Massachusetts to complete the work.

Conclusion

In conclusion, the hypothesis that *rsw6* has reduced cell-to-cell communication was not supported. The CPC signaling protein did not appear to have any deficiency in its ability to move between cells. CPC:GFP distribution appeared to be the same in both wild-type and *rsw6* x CPC:GFP roots, though there is a possibility that the specific properties of CPC that allow it to move between cells under normal circumstances was the cause of this similarity in GFP expression distribution pattern. The pavement cells in wild-type and *rsw6* plants also illustrated no significant difference. The *rsw6* mutant appears to have normal communication of the ROP/RIC pathway proteins involved in pavement cell development on par with wild-type plants. The previous work showing reduced cell-to-cell communication in *rsw6* involved the microinjection of fluorescent dye into cells. This study aimed to investigate intercellular transport without microinjection, which can perturb the cells, so a second method that does not use microinjection is desirable. This thesis has shown that native proteins that have their own movement domain do not mirror the reduction in cell-to-cell movement seen in microinjection studies. Instead, another technique, such as light-activated fluorescent probes, could prove to be a more successful approach.

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