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The Effects of 1-Butanol on Cellulose Synthase Dynamics and Cellulose Production in *Arabidopsis*

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ABSTRACT

The cellulose synthase (CESA) complex is responsible for the production of cellulose, a potential energy resource for the future. The cellulose microfibril-microtubule alignment hypothesis suggests that cellulose synthase-interactive protein 1 (CSI1) is postulated to be a link between the CESA complex and cortical microtubules. 1-butanol, with the known effect of dissociating cortical microtubules from the plasma membrane by inhibition of phospholipase D (PLD), was applied to distort the cortical microtubule organization. The *Arabidopsis* seedling morphology was examined upon 1-butanol treatment and reduction in root and hypocotyl elongation rates were observed. Then *Arabidopsis* seedlings with florescence protein labeled cellulose synthase complexes, CSI1, and tubulin subunits were treated with 1-butanol and observed with confocal microscopy. Measurements suggest that the velocity of both the CESA complex and CSI1 was decreased probably due to the detachment of cortical microtubules. Consistent with the reduction of CESA velocity, the cellulose content was also reduced in *Arabidopsis* dark-grown hypocotyls upon 1-butanol treatment. These results suggest that the association of cortical microtubules to the plasma membrane is important to maintain the proper motility of the CESA complex as well as CSI1. Overall, the broader interest of this experiment is in the analysis of the relationship between CESA complex, CSI1, and cortical microtubules on the primary cell wall cellulose synthesis in plants. The study of detailed mechanisms of the cellulose synthesis process is closely related to the current world popular issue, the development of alternative energy. With the ability of

genetically manipulating the cellulose synthesis process, the sustainable plant bio-fuel will be more feasible in the future.

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INTRODUCTION

Plant cell wall, a very rigid sub-cellular structure, not only provides architectural structure and shape of plant body, but also allows plants to defend against various environmental stresses and pathogens[1]. Two different types of plant cell walls, primary and secondary cell wall, were characterized and identified by their distinctive structure, composition and function. Both primary and secondary cell walls are composed mainly of polysaccharides, with some glycosylated proteins[2]. Cellulose, a hydrogen-bonded β-1, 4-linked glucan microfibril, is a major carbohydrate-based polymer in the cell wall structure embedded in the aqueous extracellular matrix[3]. The cellulose synthase (CESA) complex is the enzyme complex responsible for the production of cellulose, the most abundant organic polymer on earth[4]. Research on the cellulose synthesis process will be beneficial to lignocellulose-based biofuel development and other industries that require cellulose as raw materials (e.g. textiles and paper industries). For future applications, the basic research conducted in the laboratory and the knowledge generated will serve as a crucial guidance for the genetic manipulation of energy crop and cellulose production in general.

Cellulose fibers are believed to be synthesized at the plasma membrane by hexametric cellulose synthase complexes, which hypothetically hold 36 individual CESA subunits. Each of the CESA subunits has a diameter about 25-30 nm forming a symmetrical rosette structure, based on the freeze fracture electron microcopy observation[5]. In *Arabidopsis*, the synthesis of primary cell wall requires at least three non-redundant CESA isoforms: CESA1, CESA3, and CESA6 (or CESA6-like

proteins), whereas CESA4, CESA7, and CESA8 are required for secondary plant cell wall synthesis[6, 7]. In addition, many other proteins were also required for the primary cell wall cellulose biosynthesis, including cellulose synthase-interactive protein 1 (CSI1) which has been demonstrated to interact with CESA1, CESA3 and CESA6 by yeast two-hybrid assays. *Arabidopsis* mutants in *csi1* show defects in the control of anisotropic expansion, having a phenotype of short and swollen hypocotyls, and a significant reduction of cellulose content in primary cell wall. Further, *csi1* mutants display a distortion of CESA movement and distribution in the plasma membrane, as revealed by florescence-labeled live-cell imaging[8].

Recent studies on simultaneous observation of cortical microtubule organization and the CESA complex movement suggested that microtubules guide the movement of the CESA complex[9, 10]. By labeling CESA with florescent proteins in transgenic *Arabidopsis*, the process of cellulose deposition can be monitored[11, 12]. The time-lapse images from spinning-disk confocal microscopy showed the trajectories of CESA complex movement and linear alignments of underlying cortical microtubules were colocalized[13, 14]. The result revealed that cortical microtubules might be responsible for setting linear tracks for the CESA complex to move along. The oryzalin treatment, which introduces depolymerization of microtubules, is able to alter dynamics of the CESA complex, which further confirms the hypothesis that cortical microtubules provide guidance for cellulose deposition[14]. CSI1 has been reported to bind to microtubules by a vitro microtubule-binding assay. The co-alignment of the CESA complex with cortical microtubules is dependent on CSI1, indicating CSI1 may bridge

the interaction between the CESA complex and cortical microtubules[15, 16].

Phospholipase D (PLD) has recently been shown to decorate the cortical microtubules in plant cells. Studies in tobacco plants revealed that PLD activation triggers the reorganization of microtubules[17]. PLD is an enzyme that catalyzes the removal of the head group of structural phospholipids, forming a transient phosphatidyl-PLD intermediate. PLD then cleave the phosphatidyl, forming water and phosphatidic acid (PA) [18]. Phosphatidyl-PLD intermediates are responsible for anchoring cortical microtubules to the plasma membrane, and evidence suggests that the production of PA by PLD is required for normal microtubule organization and normal *Arabidopsis* seedling growth. In the presence of 1-butanol, the alcohol is used as an alternative trans-phosphatidylation substrate, resulting in the formation of phosphatidylbutanol. Phosphatidylbutanol does not have any known biological activity, thus 1-butanol is considered a very good inhibitor for PLD[19].

Since PLD-dependent PA production is required for normal microtubule organization, application of 1-butanol will result in the dissociation of cortical microtubules from the plasma membrane. Hence, 1-butanol treatment is expected to have effects on plant seedling growth. Studies showed 1-butanol can cause strong suppression to the emergence of radicles and cotyledons in *Arabidopsis*. Also, the germination of *Arabidopsis* was completely blocked when seeds were treated with high concentrations of 1-butanol[20]. Further, immunofluorescence microscopy studies showed that 1-butanol is also able to introduce disturbance in the morphology of root hair by disrupting the cortical microtubules array. Hence, 1-butanol treatment not only

retards seed germination, but also suppresses root elongation. However, butanol isomers, 2- and 3- butanols, do not have the ability to alter the reaction pathway of PLD-dependent PA production. 2-butanol activates PLD through G-protein signaling pathway but is not trans-phosphatidylated, whereas 3-butanol neither activates PLD nor acts as a substrate for trans-phosphatidylation. Thus 2- and 3- butanols show no effect on seed germination, seedling growth, or microtubule organization. Interestingly, 1-butanol has been found to have an effect on fragmentation of microtubules at high concentrations, but not 2- or 3- butanol. Therefore 1-butanol can affect the cortical microtubule organization in two ways: detaching the cortical microtubules from the plasma membrane by altering the normal function of PLD, or serving as a depolymerizing agent that fragments cortical microtubules.

In the experiment, the effects of 1-butanol on the motility of the CESA complex and CSI1 were examined. The primary cell wall cellulose level of dark-grown hypocotyls with 1-butanol treatment was measured by a cellulose content assay. The result revealed upon 1-butanol treatment the cellulose production in the dark-grown *Arabidopsis* hypocotyls was significantly reduced. For most of primary cell wall cellulose biosynthesis deficient mutants, plant seedlings often show abnormal expansion and restricted elongation[21]. Thus, root and hypocotyl growth rates were then examined to confirm that 1-butanol was able to induce insufficient cellulose deposition on primary cell wall. In the presence of 1-butanol, light grown *Arabidopsis* seedlings showed a reduction in the root elongation rate, whereas dark-grown *Arabidopsis* seedlings showed a reduction in the hypocotyl growth rate. In general,

Arabidopsis seedlings with 1-butanol treatment had a typical cellulose defect phenotype, displaying anisotropic cell expansion[22]. Florescent protein labeled CESA subunit, CSI1, and tubulin subunit, were then observed by live-cell imaging. Results revealed that there was a reduction in the velocity of the CESA complex and CSI1 upon 1-butanol treatment in dark-grown *Arabidopsis* hypocotyls. These expected results implicated the decline in cellulose production might due to the reduction of the primary cell wall CESA movement caused by 1-butanol induced modification of cortical microtubules. Further, the conclusion of this experiment can provide some evidence to support the existence of the bridging network of the CESA complex, CSI1, and cortical microtubules involved in the primary cell wall cellulose synthesis process.

MATERIALS AND METHODS

Plant Materials and Growth Conditions: *Arabidopsis thaliana* seeds were surface-sterilized using 30% (v/v) household bleach (sodium hypochlorite solution, 1% available chorine; 15 min), and sterile distilled water was used to wash the seeds (5 X 5min). Sterilized seeds were stratified at 4 ^{o}C for 4 days. *Arabidopsis thaliana* seeds were then plated on Murashige and Skoog (MS) plates ((1/2xMS salts, 0.8% agar, 0.05% MES, PH 5.7, either 1% (v/v) sucrose or no sucrose).

Cellulose Content Assay

Arabidopsis thaliana Columbia and *procuste* seeds were surface-sterilized and plated

on sucrose-free MS plates, and grown vertically at 22 ^{o}C in darkness for 4 days.

Drug Treatment: One set of *Arabidopsis Columbia* seeds were germinated and grown on agar plates containing 0.06% (v/v) 1- and 2- butanols. Another set of *Arabidopsis Columbia* and *procuste* seeds were germinated and grown on butanol-free agar plates.

Cellulose Measurement: Cellulose content was measured from 4-day-old dark-grown seedlings using the Updegraff method (with 620 nm absorbance wavelength) [22].

Assessments of Seedling Morphology

Arabidopsis thaliana Columbia and *csi1* mutant seeds were surface-sterilized. For dark-grown hypocotyl growth rate measurement, seeds were plated on sucrose-free MS plates, and grown vertically at 22 ^{o}C in darkness for 4 days. For light-grown root elongation rate measurement, seeds were plated on 1% (v/v) sucrose MS plates, and grown vertically at 22 ^{o}C in *Arabidopsis* growth chamber under a 16-hour light and 8-hour dark cycle for 7 days.

Drug Treatment: *Arabidopsis* germinated and grown on agar plates containing different concentrations of 1- and 2- butanols (0%, 0.02%, 0.04%, 0.06%, and 0.08% (v/v)).

Length Measurement: Plates were scanned on a flat-bed scanner, with image analysis processed in Adobe Photoshop and lengths determined in ImageJ software (version 1.36b; W. Rasband, National Institution of Health, Bethesda, MD). For dark-grown hypocotyl growth rate measurement, images were taken after 4 days of growth. For light-grown root elongation rate measurement, images were taken after 3, 4, 5, and 6

days of growth. For germination rate determination, images were taken 3 days after seeds were plated from light-grown samples.

Live-Cell Imaging

Arabidopsis thaliana Columbia seeds were surface-sterilized and plated on sucrose-free MS plates, and grown vertically at 22 ^{o}C in darkness for 3 days. Transgenic Lines: GFP-CESA3 and GFP-CESA6 seeds were provided by H. Hofte (institute Jean-pierre Bourgin, Versailles, France). GFP-CESA3 and GFP-CESA6 were crossed with RFP-TUA5 plants to create double-florescence-labeled transgenic lines. RFP-CSI1seeds were crossed with GFP-TUA5 to create double-florescence-labeled transgenic lines. YFP-CESA6 and mCherry-TUA5 double-florescence-labeled lines were provided by R. Gutierrez (Carnegie Institution for Science, Stanford, CA). All above *Arabidopsis* transgenic lines were prepared by L. Lei (Penn State University, University Park, PA).

Confocal Microscopy: Live cell imaging was performed on a Yokagawa CSUX1 spinning disk system featuring a DMI6000 Leica motorized microscope, and a Photometrics QuantEM: 512SC CCD camera, and a Leica 100x/1.4 n.a. oil objective. An ATOF laser with three laser lines (440/491/561 nm) was used to enable fast shuttering and switching between different excitations. Band-pass filters (485/30 nm for CFP; 520/50 nm for GFP; 535/30 nm for YFP; 620/60 nm for RFP) were used for emission filtering. Imaging analysis was performed by using Metamorph (Molecular Devices), ImageJ software (version 1.36b; W. Rasband, National Institution of Health,

Bethesda, MD).

Drug Treatment: 3-day-old dark grown seedlings were mounted in MS liquid medium containing 0.1 % (v/v) 1- or 2-butanol and incubated in darkness for various time points.

RESULTS

Cellulose Content Assay

The cellulose content assay was done on four different experimental groups: wild type *Arabidopsis* without butanol treatment, *Arabidopsis procuste (prc)* mutant without butanol treatment, wild type *Arabidopsis* with 1-butanol treatment, and wild type *Arabidopsis* with 2-butanol treatment. Wild type *Arabidopsis* without butanol treatment, *Arabidopsis procuste* without butanol treatment, and wild type *Arabidopsis* with 2-butanol treatment all served as control groups for this experiment. The wild type *Arabidopsis* with 2-butanol treatment (0.06%, v/v) controlled for whether indirect effects of butanol, such as altered G-protein dependent PLD activation, inhibit seedling growth. *Arabidopsis procuste* has a mutation in the CESA6 gene which is required for primary cell wall cellulose synthesis. The *procuste* mutant has a well-documented reduction in cellulose synthesis and inhibition in seedling elongation[22]. In the experiment, *Arabidopsis procuste* seedlings showed a phenotype of short and swollen hypocotyls. Also, hypocotyls of dark-grown *Arabidopsis procuste* mutants had a significant reduction in cellulose content, compared to wild type dark-grown *Arabidopsis* hypocotyls.

In the cellulose content assay, data suggested dark-grown hypocotyls of wild type *Arabidopsis* without butanol treatment had the cellulose content of 14.208% (m/m) (Fig.1), also the cellulose content for 2-butanol treated *Arabidopsis* dark-grown hypocotyls showed a similar result (14.04%). In other words, 2-butanol does not have any effect on the cellulose production in *Arabidopsis* and the G-protein dependent activation of PLD may not affect the cortical microtubule organization. However, wild type *Arabidopsis* with 1-butanol treatment (0.06%, v/v) showed a significant cellulose content reduction in dark-grown hypocotyls. Wild type *Arabidopsis* with 1-butanol treatment had the cellulose content of 12.40% (m/m), which indicated a 1.77% reduction in the cellulose comparing with wild type *Arabidopsis* without butanol treatment. The statistical evaluation also suggested this reduction value was significant, with p-value of 0.043 (<0.050). As a result, it can be concluded that 1-butanol can cause a significant reduction in the primary cell wall cellulose production.

Figure #1. The Cellulose Content Assay

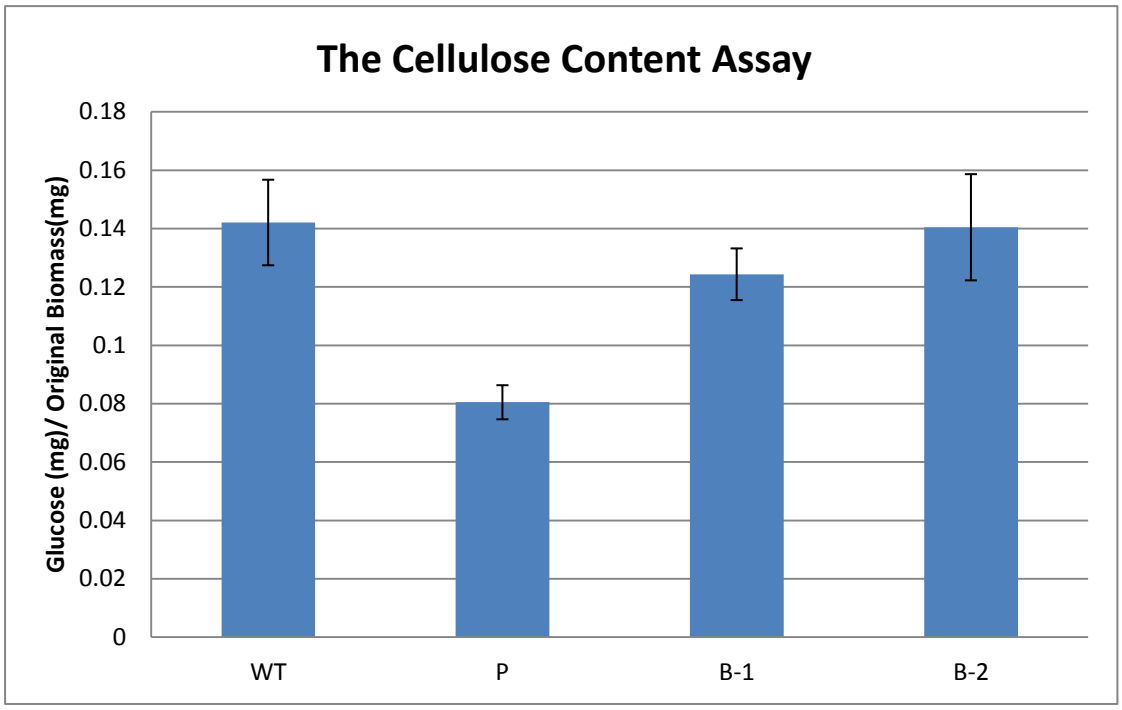


Fig.1. The cellulose content assay of dark-grown hypocotyls of wild type *Arabidopsis* without butanol treatment (WT), *Arabidopsis procuste* (*prc*) mutant without butanol treatment (P), wild type *Arabidopsis* with 1-butanol treatment (0.06%, v/v) (B-1), and wild type *Arabidopsis* with 2-butanol treatment (0.06%, v/v) (B-2). Hypocotyls of 3-day dark-grown wild type *Arabidopsis* with 1-butanol treatment had a statistically significant reduction in the primary cell wall cellulose content, with p-value=0.043.

Figure #2. The Glucose Standard Curve for the Cellulose Content Assay

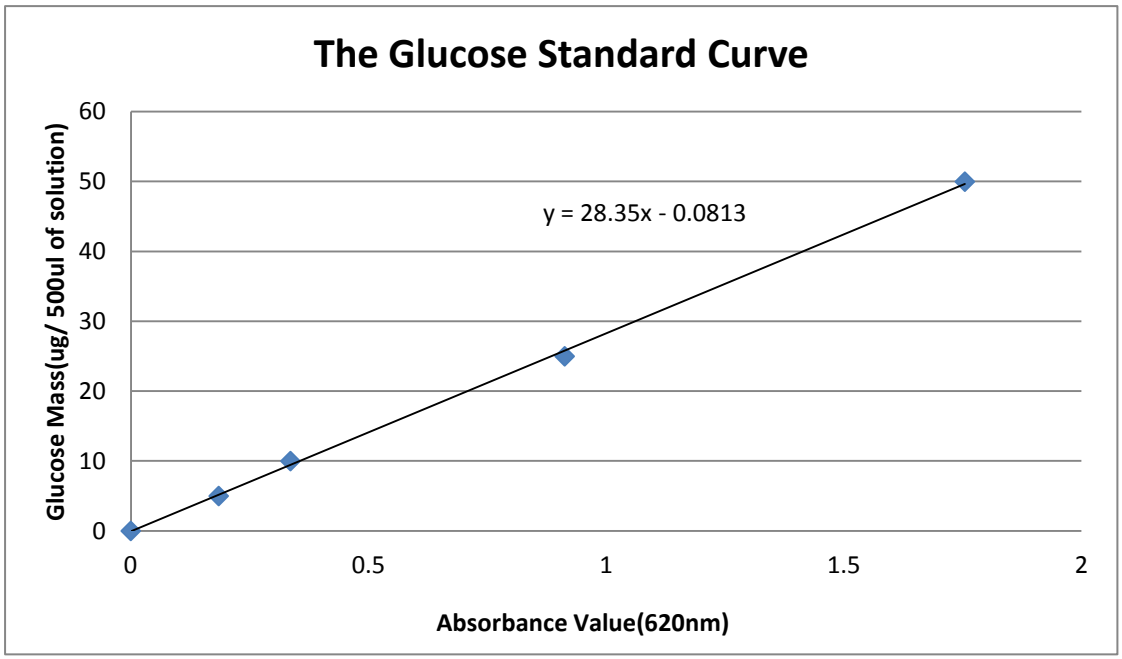


Fig.2. The glucose standard curve for the cellulose content assay with the absorption wavelength of 620 nm.

Assessment of Seedling Morphology of Hypocotyls and Roots

The length of 4-day dark-grown hypocotyls of wild type and *csi1* mutant *Arabidopsis* seedlings upon 1- and 2- butanol treatments with various concentrations were measured (Fig.3). The phenotype for both wild type and csi1 mutant *Arabidopsis* upon 1-butanol treatment was having short and swollen hypocotyls and *csi1* mutant appeared to be more sensitive to 1-butanol treatment. The hypocotyl length was identical for both wild type and *csi1* mutant *Arabidopsis* seedlings upon 2-butanol treatment. However, for 1-butanol treated wild type and *csi1* mutant *Arabidopsis*, the hypocotyl length had a continuous reduction as the concentration of 1-butanol increased. This phenomenon was more obvious in *csi1* mutant *Arabidopsis* seedlings.

Figure #3. The Hypocotyl Growth

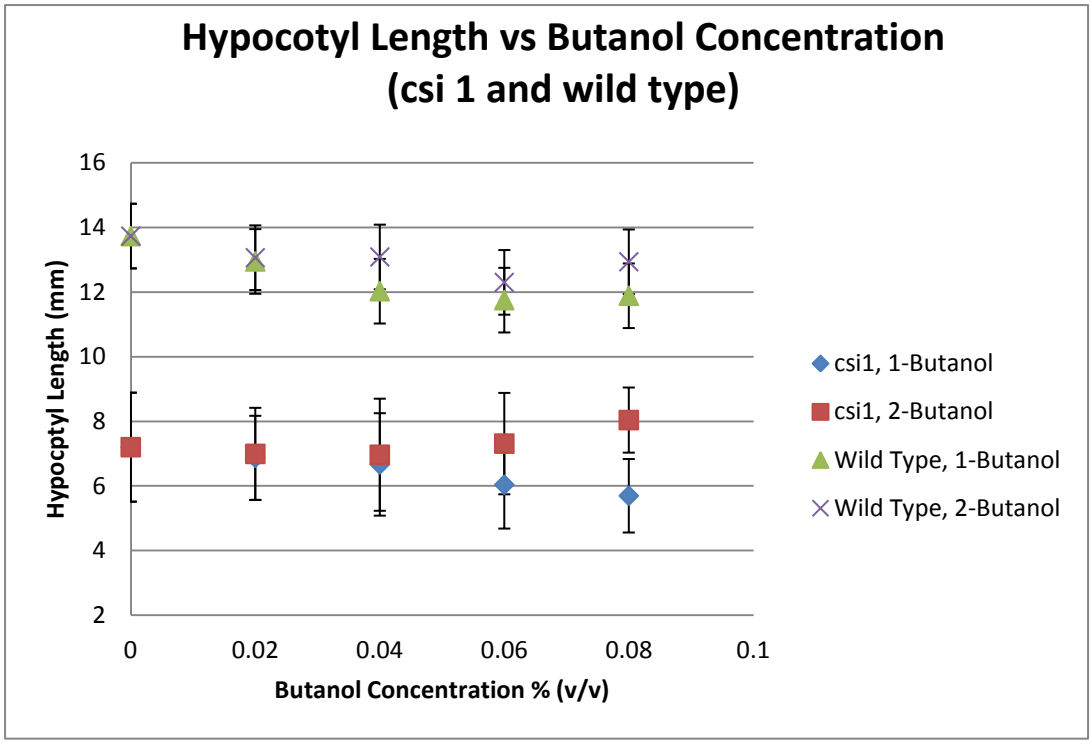
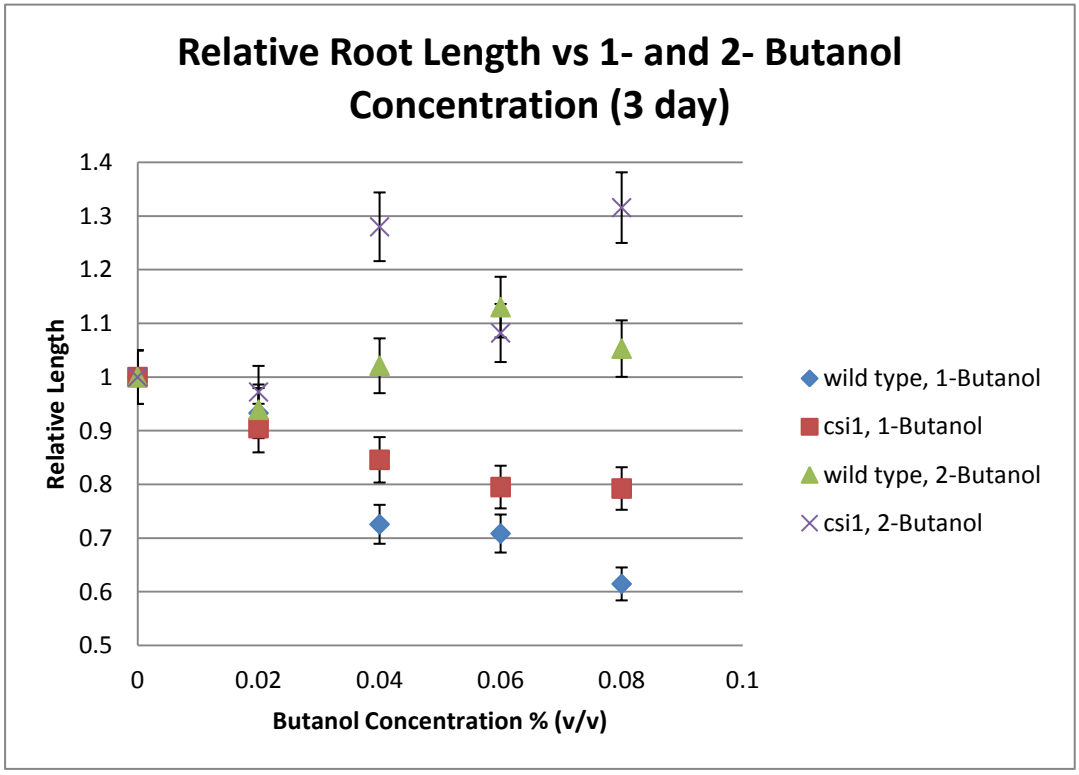


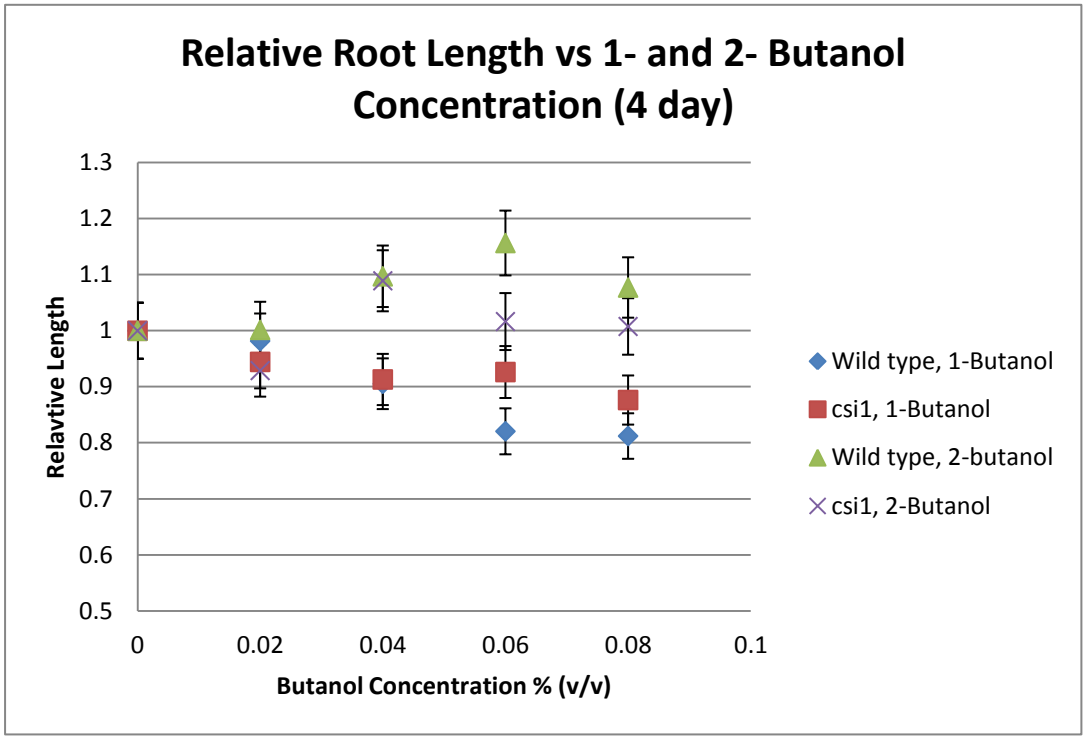
Fig.3. The hypocotyl length measurement of 4-day dark-grown *Arabidopsis* seedlings upon treatments of 1- and 2-butanol with various concentrations (0%, 0.02%, 0.04%, 0.06%, 0.08% (v/v)). The hypocotyl length for 2-butanol treated wild type and *csi1* mutant *Arabidopsis* seedlings were identical as the concentration of 2-butanol increased. However, for 1-butanol treated wild type and *csi1* mutant *Arabidopsis*, the hypocotyl length had a continuous reduction as the concentration of 1-butanol increased.

The root elongation rate was then measured for wild type and *csi1* mutant *Arabidopsis* seedlings upon 1- and 2-butanol treatments (3-5 days). The relative root elongation rate for wild type and *csi1* mutant *Arabidopsis* with 2-butanol treatment was not affected by the increase of drug concentration during the 5-day treatment

period (Fig.4). However, 1-butanol treatment was able to inhibit the root elongation. Figure #4 revealed the relative root elongation rate had a decline when the concentration of 1-butanol increased. The 1-butanol induced reduction of the relative root elongation rate was more obvious at the early stage of seedling growth (3-day). The 3-day light-grown wild type *Arabidopsis* appeared to show a 40% reduction in the relative root elongation rate when treated with 0.08% (v/v) 1-butanol. Overall, the wild type and *csi1* mutant *Arabidopsis* seedlings showed a similar rate of reduction in the relative root length with increase of 1-butanol concentration.

Figure #4. The Relative Root Elongation





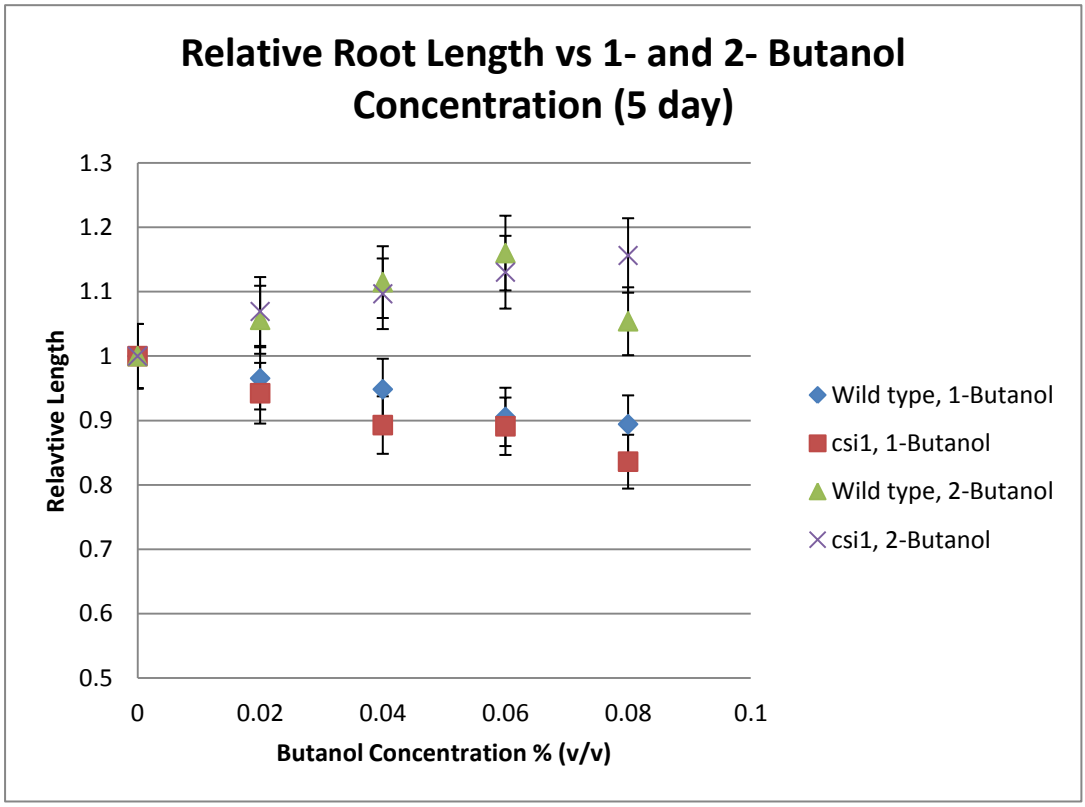


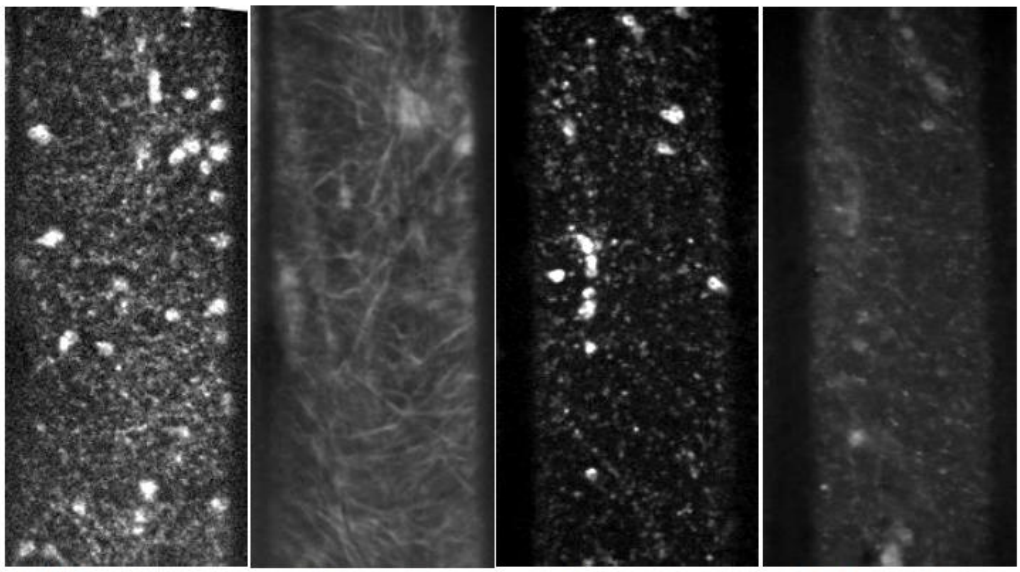
Fig.4. Relative root elongation rates for wild type *Arabidopsis* and *csi1* mutant *Arabidopsis* upon treatments of 1- and 2-butanol with different concentrations (3-5 days). Upon 2-butanol treatment the relative root elongation rate was not affected by

the increase of drug concentration during the 5-day treatment period. However, 1-butanol treatment was able to inhibit the root elongation rate.

Live-Cell Imaging

The live-cell imaging result suggested that the velocity of both CSI1 and the CESA complex was significantly reduced upon 1-butanol treatment (Fig.5 and Fig.6). In order to observe the dynamics of the CESA complex and CSI1, YFP-CESA6 and RFP-CSI1 markers were used. With the application of these two marker lines, the CESA complex and CSI1 can be observed at the plasma membrane as distinctive punctuate particles that move at constant rates along linear tracks. For 1-butanl treatment, 3-day dark-grown hypocotyls of wild type *Arabidopsis* were immersed in 0.1% (v/v) 1-butanol solution for 50 minutes, whereas for 2-butanol treatment same dark-grown hypocotyls were immersed in 0.1% (v/v) 2-butanol solution for 90 minutes. The mean for the velocity of CESA6 particles with 1-butanol treatment was 329.7 nm/min, whereas the mean for the velocity of CESA6 particle with 2-butanol treatment was 399.8 nm/min. Also, the mean for the velocity of CESA 6 particles with 1-butanol treatment was 248.5 nm/min, whereas the mean for the velocity of CESA6 particle with 2-butanol treatment was 406.2 nm/min. The result revealed 1-butanol is able to reduce the dynamics of both the CESA complex and CSI1.

Figure #5. The CESA Complex Velocity



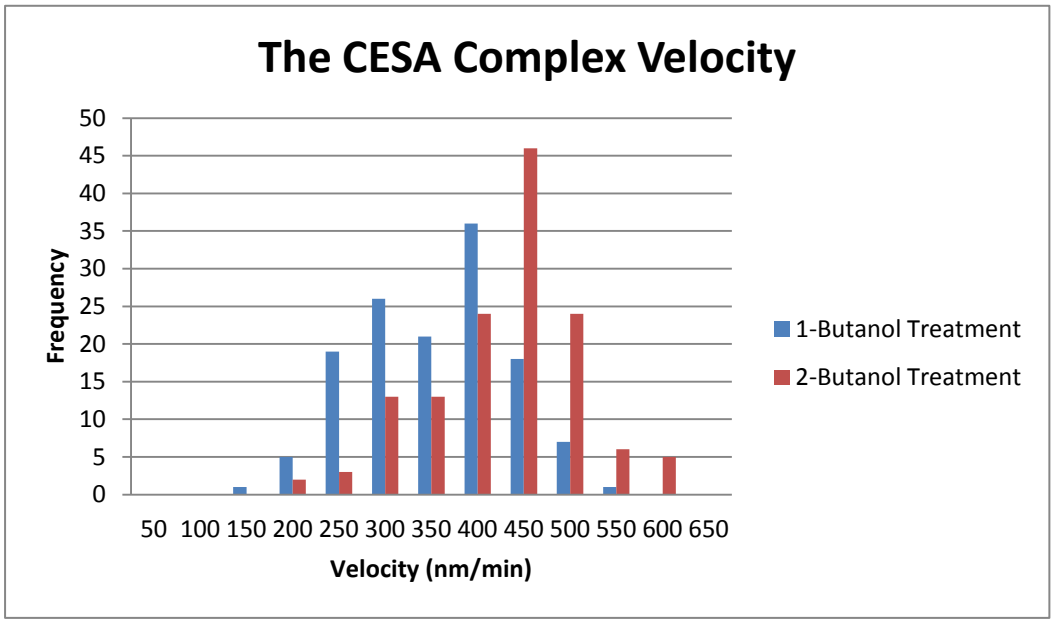
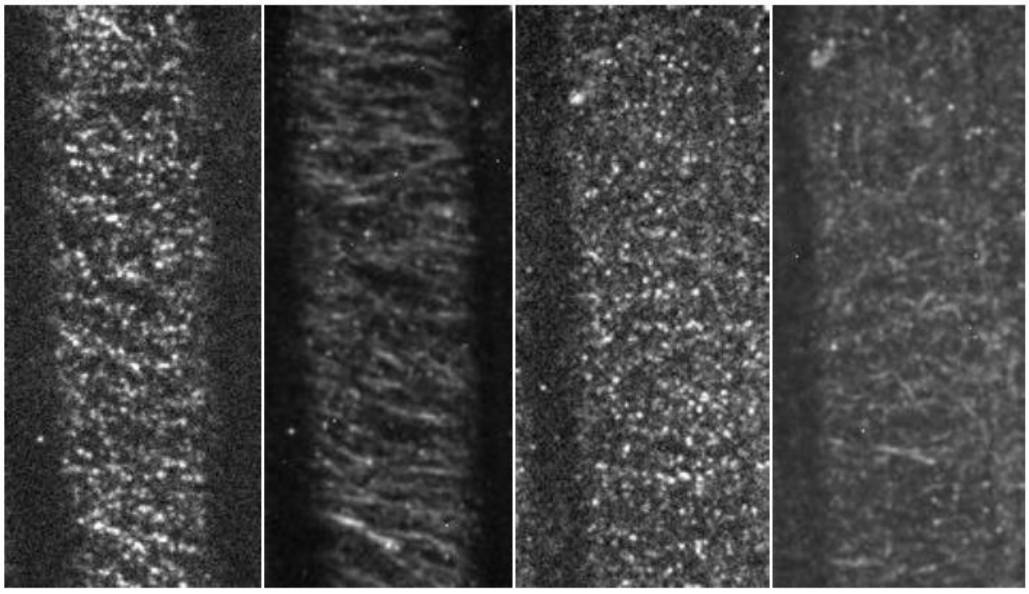


Fig.5. (Top) 3-day-old dark-grown seedlings coexpressing YFP-CESA6 and RFP-TUA5 were incubated in Murashige and Skoog liquid solution containing 0.1% (v/v) 1- or 2- Butanol. Single frame shows distribution of RFP-CSI1. Time Average of 61 frames (5-min duration, 5-s interval) shows linear trajectories of YFP-CESA6. (Bottom) Histogram of measured YFP-CESA6 particle velocities. 3-day dark-grown

hypocotyls of wild type *Arabidopsis* were treated with 0.1 % of 1- and 2-butanol for 50 minutes and 90 minutes respectively. The mean for the velocity of CESA6 particles with 1-butanol treatment was 329.7 nm/min, whereas the mean for the velocity of CESA6 particle with 2-butanol treatment was 399.8 nm/min.

Figure #6. The CSI1 Velocity with 1- and 2-Butanol Treatment



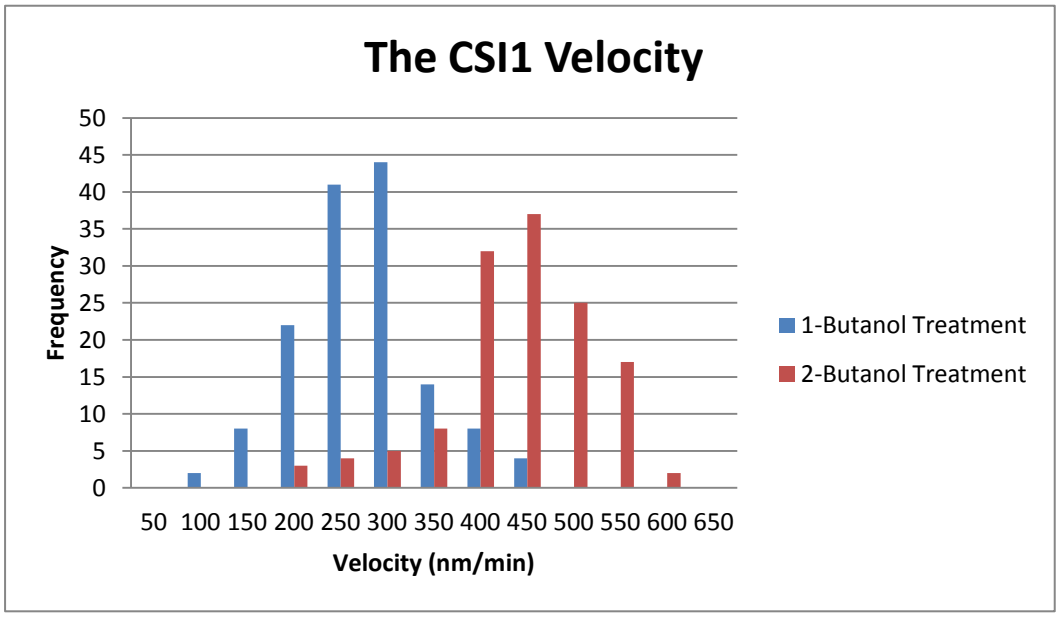


Fig.6. (Top) 3-day-old dark-grown seedlings coexpressing RFP-CSI1 and GFP-TUA5 were incubated in Murashige and Skoog liquid solution containing 0.1% (v/v) 1- or 2-Butanol. Single frame shows distribution of RFP-CSI1. Time Average of 61 frames (5-min duration, 5-s interval) shows linear trajectories of RFP-CSI1. (Bottom) Histogram of measured RFP-CSI1 particle velocities. 3-day dark-grown hypocotyls of

wild type *Arabidopsis* were treated with 0.1 % of 1- and 2-butanol for 50 minutes and 90 minutes respectively. The mean for the velocity of CSI1 particles with 1-butanol treatment was 248.5 nm/min, whereas the mean for the velocity of CSI1 particle with 2-butanol treatment was 406.2 nm/min.

Assessment of Germination Rates

The 1-butanol's effect on the germination rate of *Arabidopsis* seeds was tested at the beginning of this experiment. Since high concentrations of 1-butanol can inhibit the germination rate for *Arabidopsis* seeds, the effect of low concentrations of 1-butanol on the seed germination was examined to ensure that all seeds were able to germinate at the same time. The germination rate for wild type *Arabidopsis* seeds upon 1-and 2-butanol treatments with different concentrations showed identical results with values around 95%. A similar germination rate, around 90%, was observed for both 1- and 2-butanol treatments on *csi1* mutant *Arabidopsis* seeds. The 2-butanol treatment was used as the control for the butanol's effects of the G-protein dependent PLD activation on seedling growth. The data (Fig.7) suggested that there was no obvious change in seed germination rates for either wild type or *csi1* mutant *Arabidopsis*. Overall, 1- and 2-butanol treatments with concentrations less than 0.08% were considered having no effects on seed germination rates. Thus, for further experiments on the root elongation rate and the hypocotyl growth rate, seeds can be germinated and seedlings can be measured on the same butanol-containing plates.

Figure #7. Germination Rates for Wild Type and csi1 Mutant *Arabidopsis*

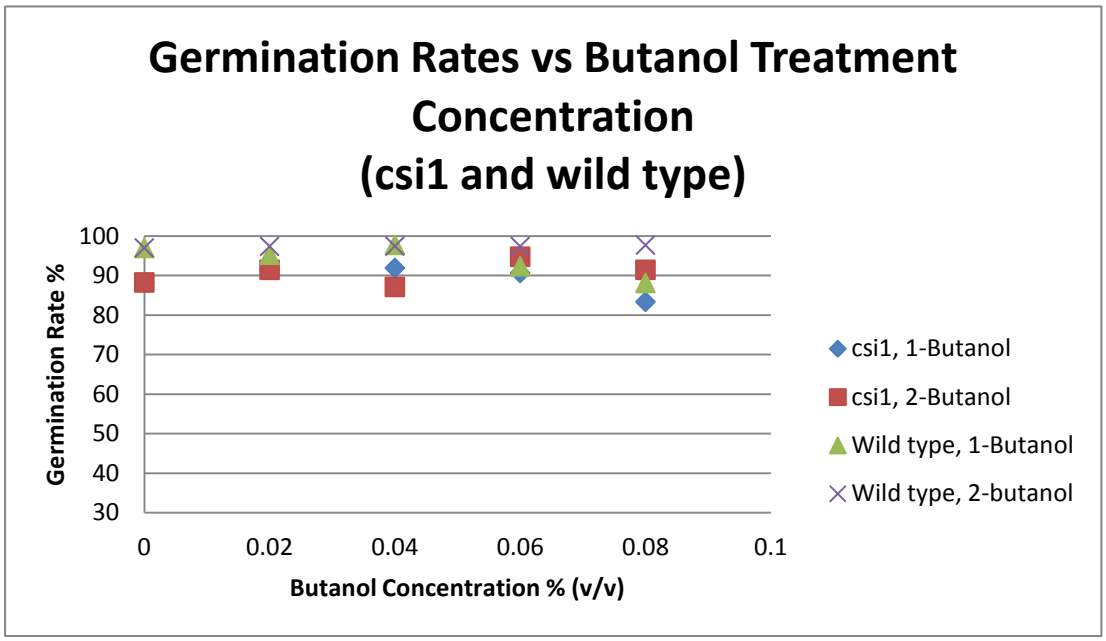


Fig.7. Seed germination rates of wild type and *csi1* mutant *Arabidopsis* upon 1- and 2-butanol treatments with different concentrations (0%, 0.02%, 0.04%, 0.06%, 0.08% (v/v)). Treatments of 1- and 2- butanol with concentrations less than 0.08% were considered having no effect on the *Arabidopsis* seed germination rate.

DISCUSSION

The experimental design for this study is based on the hypothesis that 1-butanol induced distortion of the cortical microtubule organization will affect dynamics of the CESA complex and CSI1 at the plasma membrane, hence decreasing the primary cell wall cellulose production. CSI1 is hypothesized to be the scaffold between the CESA complex and the cortical microtubule. The CESA complex, CSI1, and cortical microtubules may form a close-related network, which is necessary for efficient cellulose deposition in the primary cell wall. Also, studies have shown that 1-butanol

treatment can cause the detachment of cortical microtubules from the plasma membrane[14]. The velocity of the CESA complex and CSI1 at the plasma membrane is expected to be significantly decreased upon 1-butanol treatment, since 1-butanol can introduce disruption to the cortical microtubule organization, further disturbing the CESA-CSI1-microtubules bridging system. As the movement of the CESA complex is reduced, the efficiency of cellulose production cannot be retained. As a result, a reduction in cellulose production for seedlings with 1-butanol treatment is expected due to the decrease in velocity of the CESA complex. Sufficient cellulose deposition is required for supporting the seedling elongation. The lack of cellulose is expected to cause a decrease in the seedling growth rate and alter the seedling morphology. Thus, 1-butanol treated *Arabidopsis* seedlings are expected to have a phenotype of a typical cellulose deficient mutant, which displays anisotropic cell expansion.

Results from this experiment indicate the reduction of cellulose production might due to the reduction of the primary CESA complex velocity, caused by 1-butanol induced modification of cortical microtubules. Data from the cellulose content assay suggested the cellulose production was significantly reduced in the dark grown *Arabidopsis* hypocotyls upon 1-butanol treatment. For most of the primary cellulose biosynthesis deficient mutants, plant seedlings often show abnormal expansion and restricted elongation. Thus, the root and hypocotyl elongation rates were then examined to confirm that 1-butanol was able to induce insufficient cellulose deposition on primary cell wall. In the presence of 1-butanol, light grown *Arabidopsis* seedlings showed a reduction in the root elongation rate, where as dark grown

Arabidopsis seedlings showed a reduction in the hypocotyl elongation rate. For both dark-grown and light-grown *Arabidopsis*, 1-butanol treatment is able to cause anisotropic cell expansion displaying short and swollen seedlings, which is a typical phenotype for cellulose deficient mutants. Florescent protein labeled CESA subunit, CSI1, and tubulin subunit were then observed by live-cell imaging. Results revealed there was a reduction in the velocity of the CESA complex and CSI1 upon 1-butanol treatment in dark grown *Arabidopsis* hypocotyls, as compared to 2-butanol treatment. These expected results implicated the reduction of cellulose production might due to the reduction of the primary CESA movement caused by 1-butanol induced modification of cortical microtubules.

In this experiment, low concentrations of 1-butanol (< 0.08%) were applied because of two reasons. The first reason is 1-butanol treatment has effects of both dissociating cortical microtubules from the plasma membrane and depolymerizing microtubules. The alteration of the phospholipase D reaction, which is responsible for detaching cortical microtubules from the plasma membrane, can be achieved with the application of relatively low concentrations of 1-butanol. However, 1-butanol's function to depolymerize microtubule can only be observed with relatively high concentrations. Theoretically, with low concentrations of 1-butanol the cortical microtubules can be detached from the plasma membrane without being depolymerized, which is the desired condition for the designed experiment. The second reason is a high concentration of 1- butanol can cause delayed seed germination in *Arabidopsis*. With low concentrations of 1-butanol the *Arabidopsis*

seeds do not exhibit germination defects, which eliminates variation between treated and untreated seedlings.

Further, the conclusion of this experiment can provide some evidence to support the existence of the bridging network involving the CESA complex, CSI1, cortical microtubules involved in the primary cell wall cellulose synthesis. The bridging structure hypothesis of the CESA complex, CSI1, and cortical microtubules can be used to explain the reduction of cellulose content in this experiment when the seedlings were treated with 1-butanol. Since 1-butanol dissociates cortical microtubules from the plasma membrane by inhibition of phospholipase D, the cortical microtubule organization will be distorted at the presence of 1-butanol. As the hypothesis suggests efficient cellulose deposition requires a functionally close-related network of the CESA complex, CSI1 and microtubules, inhibition of any of them will cause a reduction in the cellulose production. When cortical microtubules are detached from the plasma membrane, the motility of both CSI1 and the CESA complex at the plasma membrane will decrease. As a result of reduction in velocity of the CESA complex, cellulose cannot be efficiently produced at the plasma membrane, thus the cellulose content will decrease.

Overall, the experiment result suggests 1-butanol is able to cause significant reduction in primary cell wall cellulose production, implicating the association of microtubules to the plasma membrane is important to maintain the proper motility of the CESA complex as well as CSI1. Overall, the broader interest of this experiment is in the analysis of the relationship between CESA complex, CSI1, and cortical

microtubules and their effects on primary cell wall cellulose synthesis in plants. The study of detailed mechanisms of the cellulose synthesis process is closely related to the current world issue, the development of alternative energy resources. With the ability of genetically manipulating the cellulose synthesis process, the sustainable plant bio-fuel will be more feasible in the future.

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EDUCATION:

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EXPERIENCE:

- **Undergraduate Lab Assistant, University Park, PA** Aug. 2010-Present
  - Conduct thesis research and practice lab techniques in a botanical biochemistry lab
  - Provide assistance with various on-going research projects
  - Experienced in making gene construct, molecular cloning and protein expression

- **Tutor for Pref Program, University Park, PA** Jun.-Aug. 2011
  - Worked as a tutor for physics in a program designed for first year students in engineering or science majors.
  - Provide detail analysis for physics problems and scientific methods of examining such problems

- **Tutor in the Academic Excellent Center, University Park, PA** Sep. 2010- Present
  - Provide academic assistance in various science subjects including mathematics, physics, chemistry
  - Experienced in providing logical explanations behind scientific problems

- **Grader for Chemistry Department, University Park, PA** Sep. 2010-May 2011
  - Work with chemistry professors and instructors to provide exam and homework grades for elementary organic chemistry courses.

- **Notes Taker for University Disability Service, University Park, PA** Sep. 2011- Present
  - Provide class and exam preparation notes for university disability service to help students in need

- **American Heart Association** May 2009-May 2010
  - Certificated Healthcare Provider (Basic Life Support)
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- ***Operation Smile*, Hangzhou, China** May-Jul. 2009
  - Participate medical activities with professional physicians
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- **Union Street Student Employee, University Park, PA** Sep. 2008-Jun. 2010
  - Provide a wide range of services optimized the satisfactory of customers
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