Touch-triggered bZIP translocation regulates elongation and secondary wall biosynthesis

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AUTHOR CONTRIBUTIONS
JHC and SH conceived and designed the study. JHC, IM, JJC, MH-R, PH, developed techniques and reagents. GT, IM, JHC, JL, JM, JV, KB, LL, NL, PH, RK, , and RO acquired the data. JHC, KM, and SH analyzed and interpreted the data. JHC, KM, IM, SH drafted the manuscript. SH agrees to serve as the author responsible for contact and ensures communication.

Abstract

Plant growth is mediated by the integration of internal and external cues, perceived by cells and transduced into a developmental program that gives rise to cell division, elongation, and wall thickening. Extra-, inter-, and intra- physical cellular forces contribute to this regulation. Across the plant kingdom thigmomorphogenesis is widely observed to alter plant morphology by reducing stem height and increasing stem diameter. The transcriptome is highly responsive to touch, including components of calcium signaling pathways and cell wall modification. One aspect of this cascade involves gibberellins (GA) and bZIP family transcription factors. Here, we present data connecting thigmomorphogenesis with secondary cell wall synthesis by GA inactivation and bZIP translocation into the nucleus. *Brachypodium distachyon* SECONDARY WALL INTERACTING bZIP (SWIZ) protein translocated into the nucleus in response to mechanical stimulation. This translocation was mitigated by supplementing with exogenous bioactive GA, and induced by chemical inhibition of GA synthesis. Misregulation of *SWIZ* expression resulted in plants with reduced stem and root elongation, and following mechanical stimulation, increased secondary wall thickness. Classical touch responsive genes were upregulated in *B. distachyon* following touch, and we observe significant induction of the glycoside hydrolase 17 family, which may be indicative of a facet of grass thigmomorphogenesis. SWIZ protein binding to an E-box variant in exons and introns was associated with immediate activation followed by repression of gene expression. Thus, SWIZ is a transcriptional regulatory component of thigmomorphogenesis, which includes the thickening of secondary cell walls.

INTRODUCTION

Forces both internal and external to a cell influence growth. Turgor pressure in conjunction with anisotropic cell wall dynamics determine direction of growth and thus cell shape. Force perception between neighboring cells plays a critical role in the development and maintenance of tissue form and function, such as the lobed, interlocking, pavement cells on the leaf epidermis, or the developmental hotspots in the apical meristem (Hamant et al., 2008; Uyttewaal et al., 2012; Bidhendi et al., 2019). Specific inter-cell forces result in dynamic remodelling of the cortical cytoskeleton, with subsequent changes in cellulose microfibril alignment and alterations to other cell wall components such as pectin methyl esterification (Hamant et al., 2008; Uyttewaal et al., 2012; Bidhendi and Geitmann, 2018; Altartouri et al., 2019; Bidhendi et al., 2019). The classic hallmarks of touch responsive growth, or thigmomorphogenesis, include reduced plant height, increased radial growth in plants with a cambial meristem, increased branching, and delayed flowering time (Jaffe, 1973; Biro et al., 1980; Jaffe et al., 1980; Braam, 2004). These attributes have been leveraged by farmers for hundreds of years. As early as 1680 records show Japanese farmers tread on young wheat and barley seedlings to elicit increased branching, spikes per plant,

and grain weight per plant, along with stronger roots (Iida, 2014). This practice, known as mugifumi, continues today with mechanized rollers.

Calcium is a critical cellular signaling molecule, and cytosolic Ca^{2+} fluctuations are induced by mechanical stimuli and implicated in mechanosignaling (Monshausen et al., 2009; Szczegielniak et al., 2012). Mechanical stimulus can significantly remodel gene expression (Braam and Davis, 1990; Braam, 2004; Lee et al., 2005). The so-called *TOUCH* (*TCH*) genes in *Arabidopsis thaliana*, encode calmodulin (*AtTCH1*/*AtCaM2*), calmodulin-like proteins (*AtTCH2*/*AtCML24*, *AtTCH3*/*CML12*), and a xyloglucan endotransglucosylase/hydrolase (*AtTCH4*/*AtXTH22*) (Braam and Davis, 1990). Touch responsive gene expression patterns often overlap with other stimuli such as dark, cold, and hormone treatment (Polisensky and Braam, 1996; Lee et al., 2005). In addition to calcium binding and signaling, genes related to cell wall modification and a variety of transcription factors and kinases are regulated by mechanical stimulus, as well as genes involved in hormone homeostasis and signaling.

The dynamic control of bioactive cellular gibberellic acid (GA) is linked to thigmomorphogenesis. GA is a growth promoting hormone derived through multiple enzymatic steps from the diterpene pathway, with interconvertible bioactive and inactive species. The bioactive forms of GA interact with protein receptors that regulate transcription factors controlling growth. Mechanical force application results in the inactivation of cellular GA by GA20 oxidases (Lange and Lange, 2015). The addition of exogenous bioactive GA to mechanically stressed plants remediates their height phenotype, and mutations in *Atga20ox* do not show thigmomorphogenic phenotypes under mechanical stress treatment (Lange and Lange, 2015). Recent work studying the translocation dynamics of several bZIP family transcription factors has provided new links for calcium and GA signaling in mechanoperception signaling.

Group I bZIPs are also implicated in mechanosensing. VIRE2-INTERACTING PROTEIN 1 (AtVIP1) and related Group I bZIP proteins translocate from the cytoplasm to the nucleus in response to a variety of biotic and abiotic stimuli, including hypo-osmotic conditions (Tsugama et al., 2012; Tsugama et al., 2014; Tsugama et al., 2016). This translocation appears to be dependent on protein phosphorylation, either from MITOGEN ACTIVATED PROTEIN KINASE 3 during pathogen invasion, or via calcium dependent protein kinases (CDPK). AtVIP1 interacts with calmodulins, the calcium binding proteins involved in many calcium signaling events. The Group I bZIP *Nt REPRESSOR OF SHOOT GROWTH* (*NtRSG*) in tobacco plays a role in maintaining GA homeostasis, wherein it translocates from the cytoplasm to the nucleus in response to cellular bioactive GA levels (Igarashi et al., 2001; Ishida et al., 2008; Fukazawa et al., 2010). Under standard conditions, NtRSG is largely localized in the cytoplasm, and to some extent in the nucleus, where it directly activates expression of *NtEnt-kaurene oxidase*, an enzyme at an early step in the GA biosynthesis pathway. After cellular GAs were chemically inhibited, NtRSG translocated to the nucleus and also activated *NtGA20oxidase1* (*NtGA20ox1*), an enzyme further downstream in the GA biosynthesis pathway that is in part responsible for converting GA species to their bioactive form (Fukazawa et al., 2010; Fukazawa et al., 2011). This suggests that

nuclear-localized NtRSG acts to promote bioactive GA synthesis in response to low GA conditions. As with AtVIP1, NtRSG translocation relies on CDPK mediated phosphorylation (Ishida et al., 2008; Ito et al., 2017). Both AtVIP1 and NtRSG associate with 14-3-3 proteins in the cytoplasm while phosphorylated (Ishida et al., 2004; Ito et al., 2014; Ito et al., 2017; Tsugama et al., 2018). Phosphatase activity causes NtRSG and the related AtbZIP29 to dissociate from the 14-3-3 protein and enter the nucleus (Van Leene et al., 2016). *NtRSG* is named for the dwarf phenotype observed when a dominant negative form of the protein is overexpressed. In these dwarf plants, there were reduced levels of bioactive GA and reduced elongation of stem internode cells (Fukazawa et al., 2000).

The development of secondary cell walls is a distinct and critical developmental process in plant growth. Following cellular elongation, some cells, particularly in vascular and structural tissues, increase their mechanical strength by depositing a thick layer of rigid material between the plasma membrane and primary cell wall. These secondary walls are made of crystalline cellulose interwoven with hemicelluloses and phenolic lignin polymers. Although functionally similar, secondary walls in monocotyledonous plants have key differences from those in eudicots, including distinct hemicellulose chemistry and differences in lignin biosynthesis. Grasses also produce mixed-linkage glucans (MLGs), a wall polysaccharide that is rarely found outside the commelinid monocots (Coomey et al., 2020). The synthesis and deposition of secondary wall polymers is tightly controlled spatially and temporally by a well defined transcriptional regulatory network. Evidence in both eudicots and grasses suggest a system of feed-forward loops regulating transcription of wall synthesizing enzymes, with NAC family transcription factors activating wall synthesis genes as well as MYB family and other transcription factors that further promote secondary wall synthesis (McCahill and Hazen, 2019). These networks are very similar between grasses and eudicots, with some components in each that have yet to be described in the other. There are currently no bZIP family members in any secondary wall regulatory model.

Beyond differences at the chemical and regulatory levels, grasses employ a fundamentally different growth mechanism than eudicots. Grasses do not have a cambium layer, and thus no lateral meristem. Stem elongation comes from cellular division and elongation in a discrete series of intercalary meristems, called nodes, with one internode region elongating and pushing up subsequent nodes. Detailed studies of thigmomorphogenesis have been conducted almost exclusively in eudicots However, recent work in the model cereal grass *Brachypodium distachyon* shows general overlap with conventional dicot thigmomorphogenesis, albeit with some notable differences, such as no change in stem diameter and increased time to flower (Gladala-Kostarz et al., 2020).

Thigmomorphogenesis is a widely observed phenomenon that results in reduced height, increased radial growth, and increased branching. The mechanisms behind this form of growth are not yet fully understood, but involve aspects of hormone regulation, Ca^{2+} signaling, Group I bZIP intracellular translocation, and changes in gene expression. Here we describe the

transcriptional response to mechanical stimulation and the function of a *B. distachyon* bZIP transcription factor, SECONDARY WALL ASSOCIATED bZIP (Bradi1g17700) and its role in touch response and cell wall biosynthesis.

RESULTS

SWIZ is a Group I bZIP transcription factor and candidate cell wall regulator

To identify genes involved in the regulation of secondary cell wall thickening, transcript abundance was measured in *B. distachyon* leaf, root, and stem tissue (Trabucco et al., 2013). A gene annotated as a bZIP transcription factor, Bradi1g17700, was highly expressed in root and stem relative to leaf (**Fig 1A**). Bradi1g17700 is also a member of a 112 gene coexpression network (**Supplemental Table S1**) that includes genes highly expressed in the peduncle (Sibout et al., 2017). Phylogenetic analysis of Bradi1g17700, hereinafter referred to as *SECONDARY WALL INTERACTING bZIP* (*SWIZ*), amino acid sequence shows it to be an ortholog of the *A. thaliana* Group I bZIPs (Jakoby et al., 2002; Dröge-Laser et al., 2018), and closely related to *AtbZIP18* and *AtbZIP52* (**Fig 1B**).

SWIZ translocates into the nucleus in response to mechanical stimulus

Group I bZIPs in *A. thaliana* have been described as playing intersectional roles in regulating growth and development, and in doing so display dynamic translocation between the cytosol and the nucleus in response to external cellular force. As an ortholog of these mechanotropic proteins, we hypothesized that SWIZ protein may similarly translocate within the cell in response to mechanical force and contribute to plant growth regulation. To test this, the roots of transgenic plants overexpressing either *SWIZ* fused to *GFP* (*SWIZ:GFP-OE*) or *GFP* alone (*GFP-OE*) were observed following a mechanical stimulus (**Fig 2A**). In control plants, GFP signal was present in both the cytosol and nucleus, which remained constant over the imaging period (**Fig 2B**). GFP signal was mostly observed in the cytosol in *SWIZ:GFP-OE* plants, but following mechanical stimulus, nuclear GFP signal increased substantially, reaching a peak around 30 min post stimulus and returned to near basal levels by about 60 min (**Fig 2B**).

The dynamics and repeatability of SWIZ nuclear translocation were further investigated by sequential stimulus treatments. Touch response to stimulus can saturate at a certain number of treatments (Martin et al., 2010; Leblanc-Fournier et al., 2014; Moulia et al., 2015). To test if SWIZ translocation dynamics varied after repeated treatments, we applied mechanical force to *SWIZ:GFP-OE* roots at regular intervals. A second stimulus was given 90 min after the first, and a third at 180 min. Following each mechanical stimulation, SWIZ consistently translocated from cytoplasm to nucleus (**Fig 2D**). This suggests that SWIZ translocation dynamics are not impacted by repeated stimulus events 90 min apart.

When conducting these translocation assays, root tissue in the field of view was treated with mechanical force and nuclei in that region were tracked and quantified for fluorescence. To determine if the signal triggering SWIZ translocation is spread beyond the specifically stimulated region, two regions of the same *SWIZ:GFP-OE* root separated by 3 cm were simultaneously observed. The stimulated region showed typical SWIZ:GFP nuclear signal accumulation and in the region below no translocation was observed (**Fig 2C**). At 120 min, the treatments were

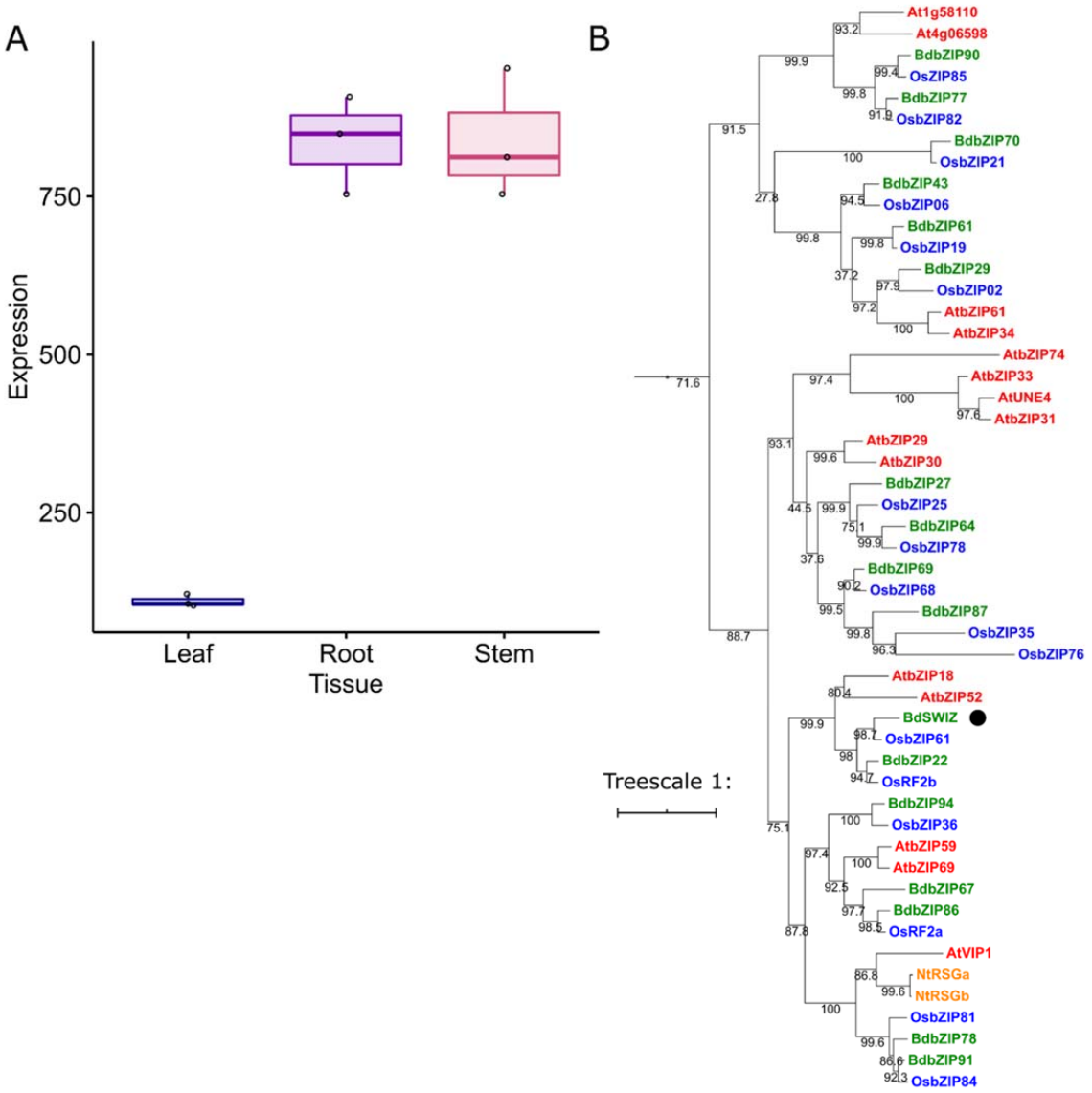

Figure 1. SWIZ is a Group I bZIP highly expressed in maturing stem and root. (A) *SWIZ* transcript abundance in *Brachypodium distachyon* leaf, root, and stem tissue measured by microarray. Mean +/- standard deviation of three biological replicates. (B) Phylogeny analysis of amino acid sequences from *B. distachyon* (green), *Oryza sativa* (blue), *Arabidopsis thaliana* (red), *Nicotiana tabacum* (orange) shows *SWIZ* (black dot). The phylogeny was reconstructed using maximum likelihood analysis with a bootstrap resampling value of 1000. The numbers labeled on the branches are the posterior probability to support each clade. The protein sequences, full locus IDs, and plant species with genome source are provided in Supplemental File 1.

192 reversed, with the lower root region receiving a stimulus while the upper region was
193 unperturbed. The lower region showed SWIZ:GFP nuclear translocation while the upper region
194 did not. Thus, the touch stimulus perceived by SWIZ is localized directly to the perturbed region.

195 **Transcriptional response to touch**

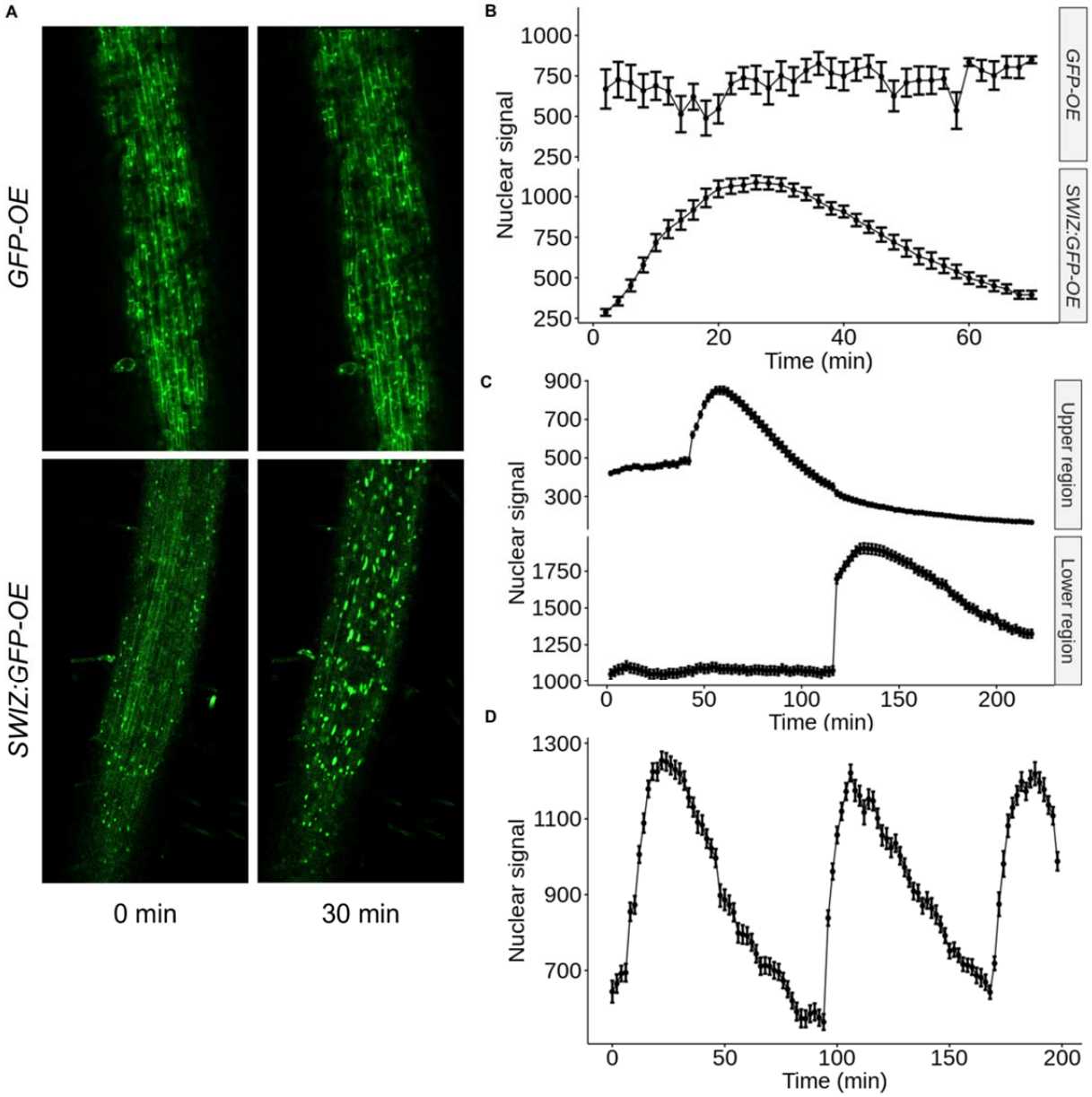

Figure 2. SWIZ translocates to the nucleus in response to mechanical stimulus, specifically in regions directly stimulated. (A) Image of *SWIZ:GFP-OE* and *GFP-OE* roots prior to stimulus and 30 min post stimulus. Roots were observed immediately following mechanical perturbation. (B) Quantification of nuclear signal. n = 14-20 nuclei. (C) SWIZ translocation occured in the local area of the stimulus. At 30 min, stimulus was applied to an upper region of the root, while at 120 min it was applied to a lower region approximately 3 cm below. n = 109, 184 nuclei respectively for upper and lower regions. (D) *SWIZ:GFP-OE* roots were imaged by confocal microscopy with stimulus applied in the field of view at 0, 90, and 180 min. n = 126 nuclei. (B-D) Images were taken every 2 min. Nuclear GFP signal was quantified in selected nuclei at each time point. The average nuclear GFP signal is represented by the line with error bars indicating standard error of the mean.

Having established the nuclear translocation of SWIZ in response to touch, we then investigated what effect touch and SWIZ overabundance during touch may have on gene expression. To assess this, we measured transcript abundance by sequencing mRNA from wildtype and *SWIZ-*

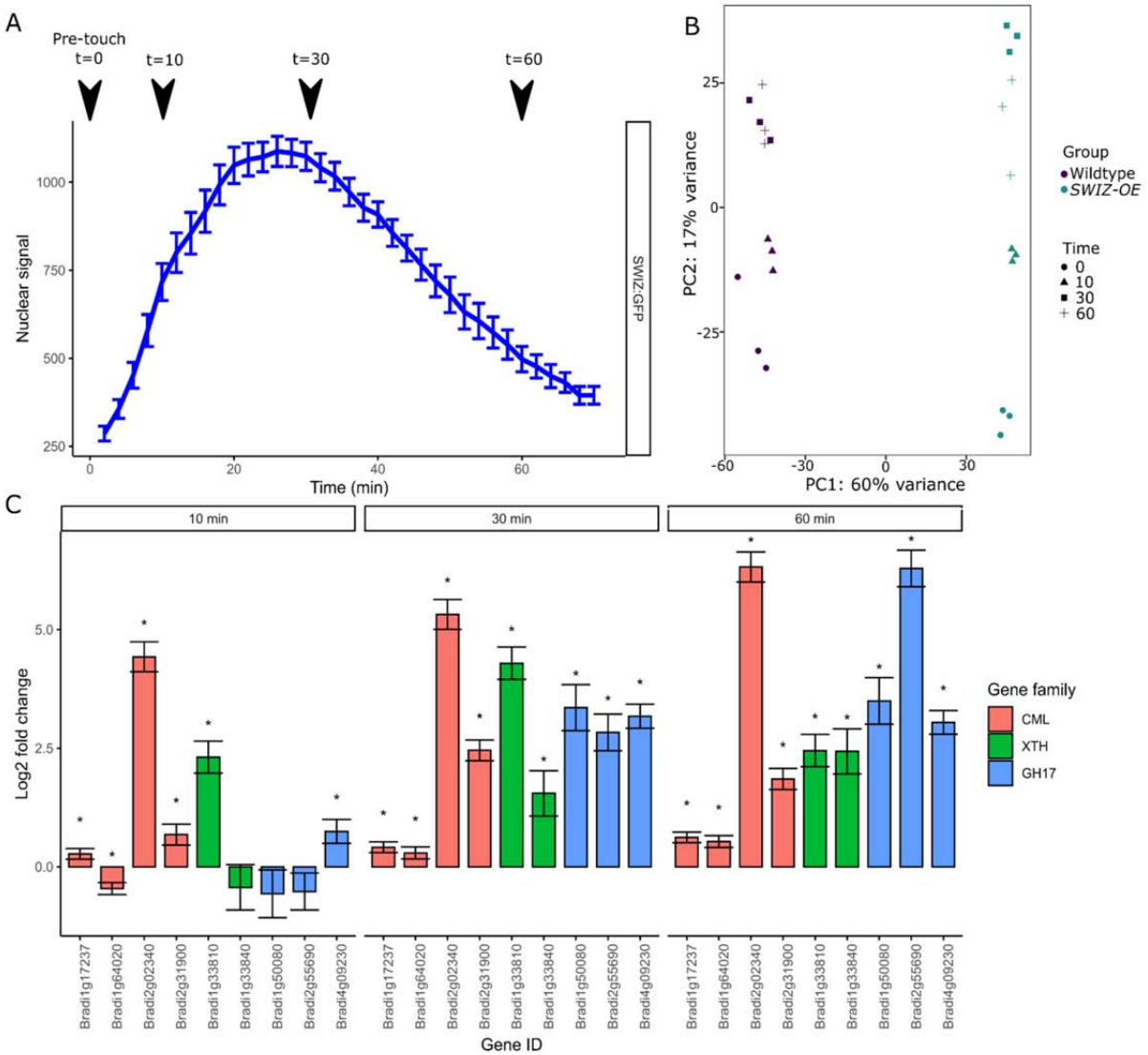

Figure 3. Transcriptome analysis of touch response in *Brachypodium distachyon* roots. (A) Root tissue was sampled just prior to touch (t = 0), and at 10, 30, and 60 min following touch treatment in wildtype and *SWIZ-OE*. (B) Principal component analysis of gene expression across samples shows the greatest difference corresponding to genotype and the second greatest corresponding to time after touch. (C) Canonical, as well as novel touch responsive genes are upregulated in *B. distachyon* following touch. Closest orthologs of the *Arabidopsis thaliana TOUCH* genes encoding calmodulin-like (CML) and xyloglucan endo-transglycosylase/hydrolases (XTH) are upregulated following touch, as are previously unreported members of the glycosyl hydrolase 17 (GH17) family. Full list of gene expression for *TOUCH* and glycoside hydrolases defined by Tyler et al. (2010) in Supplemental Tables 5, 6,and 7. Significance denoted by * reflecting q < 0.1 compared to expression at t = 0, with q-values representing Wald test p-values adjusted for false discovery rate.

199 *OE* root tissue just prior to touch (0 min) and at 10, 30, and 60 min following touch treatment
200 (**Fig 3A**). Principal component analysis of transcript abundance shows the greatest amount of
201 variance is attributed to the first principal component, 60%, where samples cluster based on

genotype (**Fig 3B**). The second principal component, which accounts for 17% of the variance, distinguished between pre-touch and 10 min following touch, with the last two time points clustering similarly.

The wildtype transcriptome was massively remodeled in response to touch, with 8,902 transcripts differentially expressed ($q < 0.1$) at 10 min post touch, 5,682 transcripts at 30 min, and 7,672 transcripts at 60 min (**Supplemental Tables 2-4**). Touch response studies in *A. thaliana* describe similar changes that include numerous calmodulin (CAM), calmodulin-like (CmL), and xyloglucan endo-transglycosylase/hydrolase (XTH) activity (**Fig 3C**)(Braam and Davis, 1990; Lee et al., 2005). Based on homology with *TCH1*, *TCH2*, and *TCH3*, 79 CAM and CmL genes were identified, the majority of which showed upregulation in wildtype plants following touch treatment (**Supplemental Table 5**). Similarly, homology with *TCH4* identified 37 *B. distachyon XTH* and *XTH-like* genes. Of these, 8 genes were upregulated by touch in wildtype plants, including the two with the highest similarity to *TCH4* (Bradi1g33810 and Bradi33840) (**Fig 3C**, **Supplemental Table 6**). Since xyloglucans are in relatively low abundance in grass cell walls compared to eudicots, we also investigated the effect of touch on other families of the glycosyl hydrolase superfamily (Tyler et al., 2010). In particular, GH17 genes were differentially regulated following touch. The GH17 family is broadly described as modifying β-1,3-glucans. We observed measurable transcript abundance for 35 of the 53 annotated GH17 family members, with 8, 11, and 16 members showing differential expression at 10, 30 and 60 min respectively. Fisher's exact test determined significant enrichment of GH17 expression at time 60 ($p = 9.307e-3$) (**Fig 3C**, **Supplemental Table 7**). Online resources are available to explore the RNA-seq data at (https://hazenlab.shinyapps.io/swiztc/), which provides an interactive platform for interrogating the *B. distachyon* touched-root transcriptome.

Cell wall genes are downregulated immediately following touch, then induced concurrent with SWIZ nuclear translocation and more strongly in *SWIZ-OE*

Genes related to secondary cell wall synthesis were immediately downregulated in wildtype plants following touch, but were subsequently upregulated (**Fig 4**). Xylan, cellulose, MLG, and callose synthesis associated genes were upregulated 30 and 60 min following touch, while lignin biosynthesis and BAHD acyltransferase genes were upregulated at 60 min. Network analysis of gene expression patterns over time using the iDREM software showed that in *SWIZ-OE* plants, cell wall genes were more rapidly induced (**Supplemental Fig 2A-B**). Prior to touch, nearly all of the cell wall associated genes investigated were significantly downregulated relative to wildtype, then significantly upregulated following touch (**Fig 4**). In *SWIZ-OE*, cell wall related terms were enriched in paths that had stronger upregulation earlier than the same terms in wildtype (**Supplemental Fig 2A-B**). Notably, aspects of cell wall polysaccharide biosynthesis, particularly glucan biosynthesis, were significantly enriched in *SWIZ-OE* path D, while not significantly enriched in wildtype (**Supplemental Table 8**). This is mirrored when looking at specific gene expression, with primary wall associated CESAs, MLG, and callose synthesis

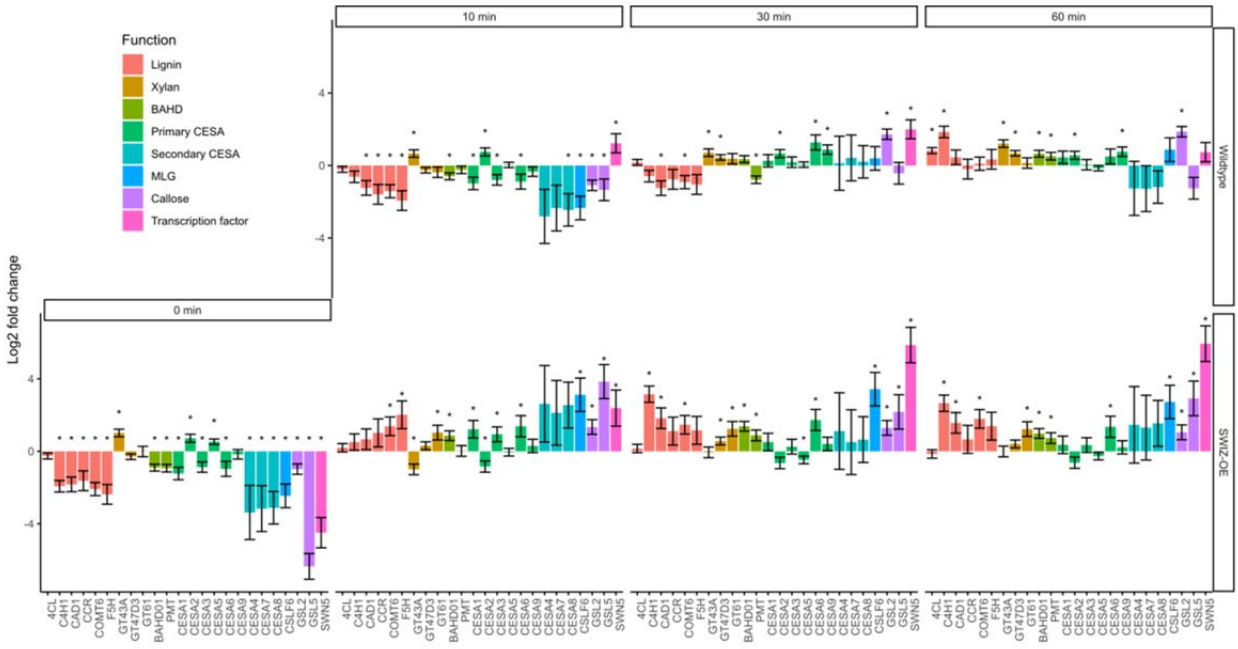

Figure 4. Gene expression analysis of cell wall related genes. Log fold-change of gene expression measured by RNA-seq in wildtype and *SWIZ-OE*, presented as relative to wildtype expression at time 0, pre-touch. Bar color indicates class of cell wall gene. Error bars indicate standard deviation of three biological replicates. Significance denoted by * reflecting q < 0.1 compared to wildtype expression at t = 0, with q-values representing Wald test p-values adjusted for false discovery rate.

genes all significantly upregulated following touch in *SWIZ-OE*. Thus, cell wall genes were immediately repressed by touch, followed by significant activation. That activation occurs more rapidly in *SWIZ-OE* plants and is associated with the timing of SWIZ protein nuclear translocation.

SWIZ protein binding in the gene body is associated with dynamic changes in gene expression

Next we investigated the direct binding targets of SWIZ protein by performing DNA affinity purification sequencing (DAP-seq) with whole genomic DNA. In total, SWIZ interacted with 2,455 distinct positions in the genome. Those regions were significantly enriched for two sequence motifs, (A/C)CAGNCTG(T/G) and (A/C)CAGCTG(T/G) that are variants of the E-box motif (**Fig 5A**). A plurality of the binding sites were between the translational start and stop sites with 21% of the peaks in introns and 21% in exons (**Fig 5B**). Fewer than 10% of the peaks occurred in UTRs and the promoter (defined as 5 kb upstream of the 5 'UTR) and intergenic regions each accounted for 25% of the binding sites. Thus, SWIZ protein is preferentially bound to an E-box like motif and often in the gene body (**Fig 5C**).

To investigate the effects of possible SWIZ binding on gene expression, we compared the genes differentially expressed in the *SWIZ-OE* touch response RNA-seq time courses with the promoter and gene body binding targets identified by the DAP-seq assay. Prior to touch, genes with

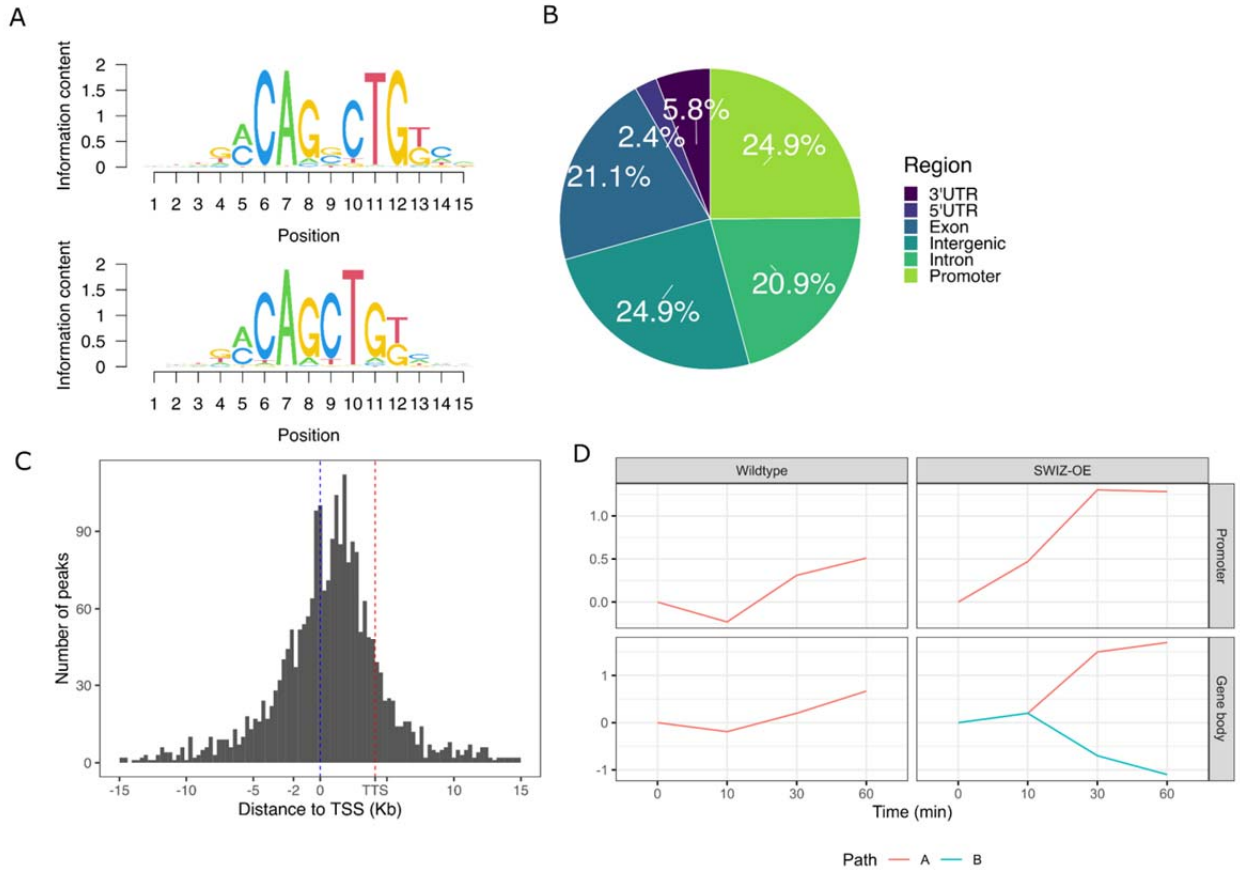

Figure 5. DNA affinity purification sequencing to determine SWIZ binding sites. (A) Top two most statistically enriched sequence motifs in SWIZ binding sites. (B) Distribution of binding sites across genomic features, relative to primary transcripts of the *Brachypodium distachyon* annotation v 3.1. (C) Relative distribution of binding sites centered on the transcriptional start site (TSS, blue dashed line), transcriptional termination site (TTS, red dashed line) represents the average length of all annotated transcripts, approximately 4.5 kb away from the TSS. (D) Path determinations from iDREM time course analysis of differentially expressed genes that also have DAP-seq binding sites. Each line represents a set of genes with similar expression level patterns over the time course relative to time 0, pre-touch.

promoter-SWIZ protein interactions were most often downregulated in *SWIZ-OE* relative to wildtype (**Table 1**). However, some SWIZ targets behaved differently following touch. SWIZ promoter binding targets were most often upregulated in *SWIZ-OE* in all three post-touch time points. Thus, SWIZ promoter binding was coincident with repression, but following a touch stimulus, activation was more prominent. However, this difference between untreated and touched was not as pronounced in gene body targets, with more upregulated genes 10 min following touch and more downregulated 30 and 60 min following touch. To further explore these trends we conducted iDREM cluster analysis of the differentially expressed genes that were also SWIZ protein targets (**Fig 5D**). For both wildtype and *SWIZ-OE*, the network analysis

revealed a pathway to increased gene expression by 60 min following touch. The network pathway trend for both promoter and gene binding targets in wildtype was immediate repression at 10 min followed by increased expression at 30 and 60 min post-stimulus. A unique pathway was observed among gene body binding targets in *SWIZ-OE*; transcript abundance was reduced following touch. Thus, SWIZ binding to a promoter or gene body was more strongly associated with increased expression with the exception of gene body targets in touched *SWIZ-OE* plants, which were more strongly repressed.

Among the genes differentially expressed in response to both touch, *SWIZ-OE* transgene, and bound by SWIZ in the first intron is the mixed glucan synthase *CSLF6* (**Fig 6A**). The transcript abundance of *GA2ox3*, which inactivates bioactive GA, was bound by SWIZ protein in both the gene body and 3'UTR and expression significantly increased 30 min after touch in *SWIZ-OE* (**Fig 6B**). An example of gene body binding repression is Bradi1g30252, the closest ortholog to a wheat transcription factor induced by hypoxia, *TaMYB1* (Lee et al., 2006). This gene was touch induced in wildtype and repressed in *SWIZ-OE* with a binding site in the 5'UTR (**Fig 6C**). A membrane-associated transcription factor, NAC35, exhibited a similar expression to *SWAM3 (Handakumbura et al., 2018)* and SWIZ bound the promoter region (**Fig 6D**).

B. distachyon displays classic thigmomorphogenic phenotypes

We next investigated the effect of touch treatment on aboveground tissue. Wildtype plants were perturbed with a metal bar once every 90 min for either two or three weeks (**Supplemental Fig 3, 4**). After the treatment period, all plants were allowed to recover and grow undisturbed until senescence (**Supplemental Fig 4**). Two week stressed plants were significantly shorter than control plants, and three week stressed plants were shorter still (**Supplemental Fig 4A-B**). Despite this difference in height, there was not a significant difference between the groups in terms of aboveground plant weight (**Supplemental Fig 4C)**. Three week stressed plants had significantly more branches ($p < 0.05$), with a non-significant increase in two week stressed plants (**Supplemental Fig 4D**). Transverse stem cross sections in the third elongated internode and pedncule did not show a significant difference in cell wall thickness or phloroglucinol staining in response to touch (**Supplemental Fig 4E-G**).

Root morphology is altered by *SWIZ-OE* and touch treatment

Given the SWIZ protein mechanotropic response and regulatory influence, we then investigated how SWIZ might impact plant growth in response to touch. We challenged wildtype and *SWIZ-OE* roots with the same style of touch treatment used in the translocation and gene expression experiments, but repeated twice daily for a period of five days. In both touch and control conditions, *SWIZ-OE* roots were significantly shorter than wildtype, suggesting a dwarfing effect from *SWIZ* overabundance (**Fig 7**). Mechanical challenges to roots have been reported to impact root straightness, a trait that has also been described as being impacted in other bZIP studies (Van Leene et al., 2016; Zha et al., 2016). In response to touch treatment, we observed that

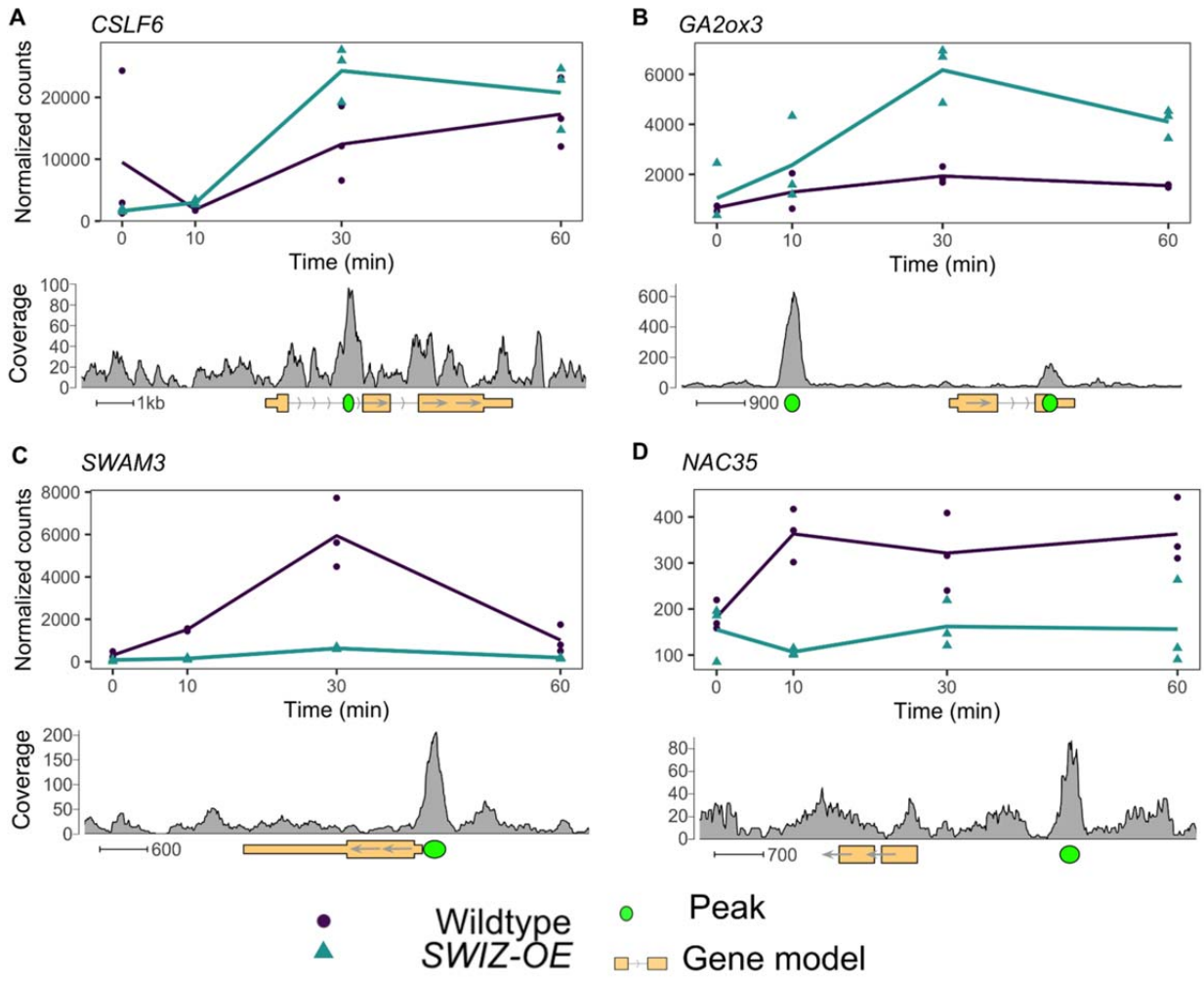

Figure 6. SWIZ binding targets differentially expressed in response to touch and *SWIZ-OE*. Gene expression over time of selected genes with SWIZ binding sites. Line graphs are the average transcript abundance of three biological replicates for each time point. Binding site determined as peaks of sequence alignment. Scale bar unit is bases. Direction of transcription is shown with arrows on the gene model, 5' and 3' UTRs are depicted by narrowed rectangles on the gene model.

SWIZ-OE roots were significantly less straight than wildtype, while this was not observed in control conditions (**Fig 7**). We further tested the mechano-response of *SWIZ-OE* roots by growing seedlings on plates with increasing degrees of plate angle at 10°, 20°, 30°, and 40° from vertical (**Fig 8**). Greater angles result in more direct contact between the root tip and media and thus increased mechanical stimulation in *A .thaliana (Oliva and Dunand, 2007; Zha et al., 2016).*

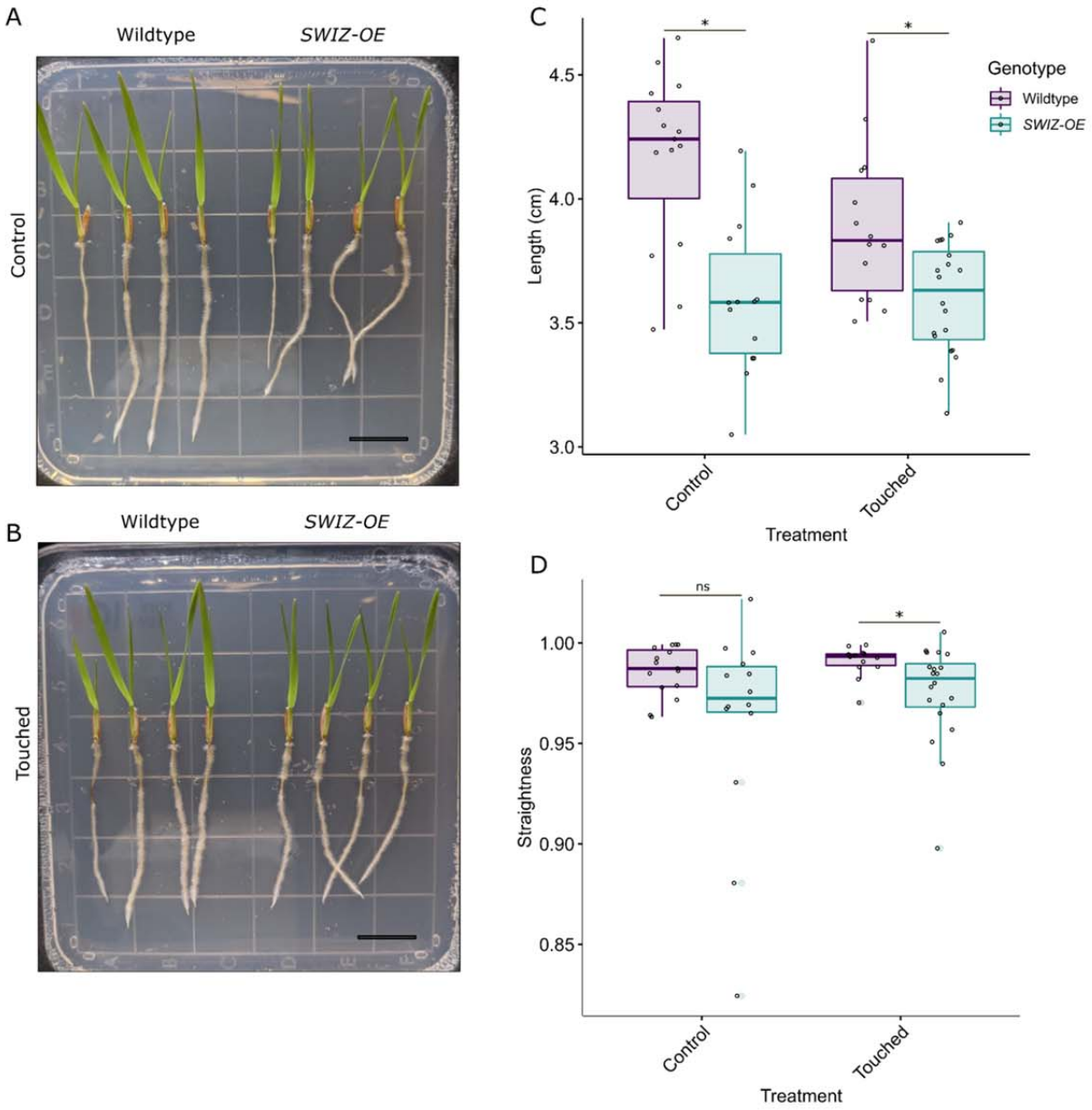

Figure 7. *SWIZ-OE* roots are shorter than wildtype and less straight in response to direct touch. Wildtype and *SWIZ-OE* seedlings grown on plates under control (A) or touched (B) conditions for five days. Touched plants were probed with a pipette tip twice a day. (C) Quantification of root length in control and touched conditions. Significance denoted by * reflecting t-test p-value < 0.05, ns = not significant. (D) Quantification of root straightness in control and touch conditions. Significance denoted by * reflecting Wilcoxon sign-ranked test for non-parametric data with p-value < 0.05, ns = not significant.. Scale bar = 1 cm.

Wildtype *B. distachyon* roots displayed decreasing root length with increasing plate angle. *SWIZ-OE* roots were shorter than wildtype at all plate angles tested (**Fig 8**). Root straightness did not show any significant differences. Thus, *SWIZ-OE* roots were shorter than wildtype regardless of direct touch stimulus or increasing plate angle, and significantly less straight under direct touch.

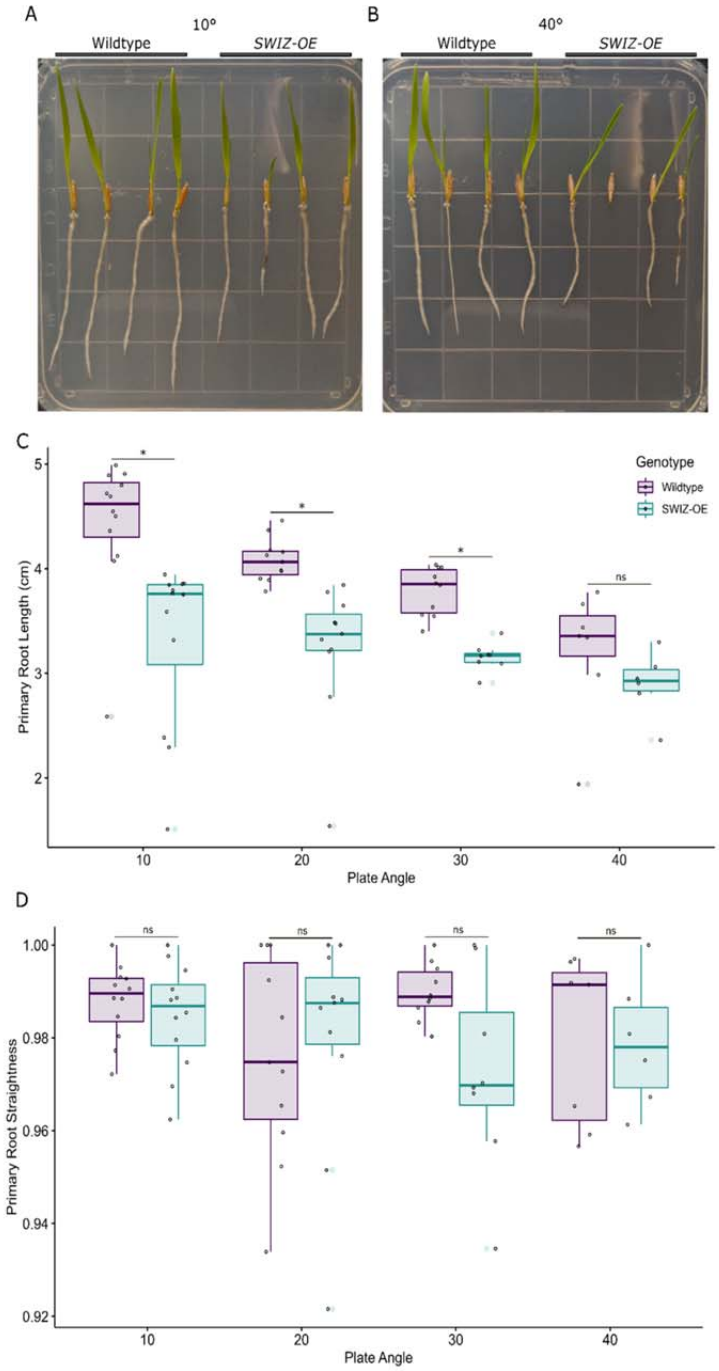

Figure 8. Wildtype roots shorten with increasing plate angle, while *SWIZ-OE* roots are consistently short, with no significant difference in straightness. Wildtype and *SWIZ-OE* seedlings grown on plates at (A) 10°, 20°, 30°, and (B) 40° incline from vertical. (C) Quantification of root length. (D) Quantification of primary root straightness. Significance denoted by * reflecting t-test p-value < 0.05, ns = not significant.Scale bar = 1 cm.

321 **Stem height and cell wall thickening are affected by *SWIZ-OE* and touch treatment**

To test the role of *SWIZ* in regulating thigmomorphogenesis and secondary cell wall development, we tested wildtype and *SWIZ-OE* under two weeks of mechanical perturbation as described above. In control conditions, there were no differences among genotypes in height, weight, or branching. Touch significantly shortened both widltype and *SWIZ-OE* stems relative to control conditions, but not relative to each other. (**Fig 9A, Supplemental Fig 5**). Thus, *SWIZ-OE* stems are inherently shorter than wildtype.

Transverse sections of the stem were made in the peduncle, the last elongated stem internode where the touch treatment occurred during stem elongation, and stained with phloroglucinol-HCl. *SWIZ-OE* touched peduncles showed significantly thicker interfascicular fiber cell walls (**Fig 9B-C**). Thus, overabundance of *SWIZ* transcript sensitizes wall thickening in response to touch treatment.

SWIZ translocation is sensitive to cellular GA levels, and may act in maintaining GA homeostasis following mechanical stimuli

Mechanical forces have been shown to inactivate cellular GA, and that cellular GA levels dictate cytosolic versus nuclear localizations of *N. benthamiana* Group I bZIPs similar to SWIZ (Fukazawa et al., 2010; Lange and Lange, 2015). To investigate this in relation to SWIZ, *SWIZ:GFP-OE* roots were treated with bioactive GA or paclobutrazol, an inhibitor of GA synthesis. SWIZ:GFP signal amplitude was reduced in roots treated with bioactive GA, particularly at the 50 and 100 mM level. After 60 min the nuclear signal had returned to near pre-stimulus levels, and a second stimulus was applied (**Fig 10A**). This time all GA treated roots showed a similar reduction in nuclear signal, nearly half of the untreated control. These results suggest that the addition of exogenous GA reduces SWIZ translocation to the nucleus following mechanical force application.

Roots treated with 10 mM paclobutrazol did not show altered translocation dynamics compared to the untreated control, but roots treated with 50 or 100 mM paclobutrazol initially showed greater SWIZ:GFP nuclear localization, even prior to mechanical stimulus (**Fig 10B**). SWIZ:GFP signal in the 100 mM paclobutrazol treated roots was high prior to treatment and remained high even after the other nuclear signals diminished. This suggests that pharmacological reduction of bioactive GA levels triggers SWIZ nuclear translocation even without the application of mechanical force.

Given the role of GA in touch responsive bZIP translocation dynamics, we measured how GA related gene expression changed in response touch. In wildtype following touch, GA synthesis and GA activating genes *KS*, *KAO4*, and *GA20ox4* were downregulated, while the genes encoding GA inactivating enzymes GA2ox4 and GA2ox9 were significantly upregulated (**Fig 10C**). In *SWIZ-OE*, *KS* was upregulated prior to touch and 10 min post touch. *GA20ox4*, a gene responsible for synthesizing bioactive GA, was downregulated prior to touch in *SWIZ-OE* plants, then significantly upregulated following touch. At 30 and 60 min *GA2ox4* and *GA2ox9* were upregulated, along with the DELLA protein Rht-1 (**Fig 10C**). Here we show consistent patterns

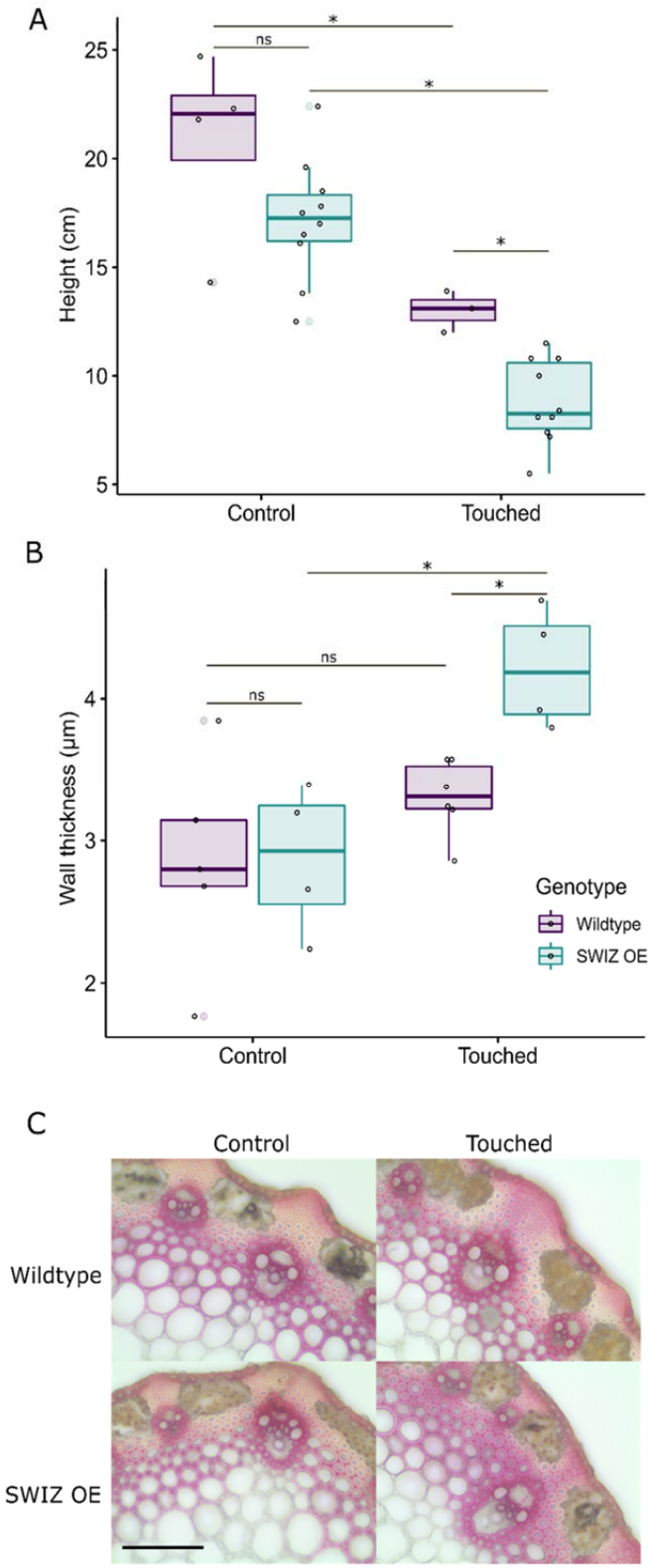

Figure 9. *SWIZ-OE* enhanced thigmomorphogenic response in stems. Wildtype and *SWIZ-OE* plants were placed under control conditions or received two weeks of mechanical stress every 90 min. (A) Quantification of main stem height at senescence. (B) Quantification of interfascicular fiber wall thickness in transverse cross sections of the peduncle. n = 4 to 6 plants per genotype, per treatment. (C) Representative cross sections stained with phloroglucinol-HCl. Scale bar = 100 µm. Significance denoted by * reflecting t-test p-value < 0.05, ns = not significant.

of gene expression associated with the touch inactivation of GA and that SWIZ protein promotes expression of GA synthesis and GA activation genes following mechanical perturbation.

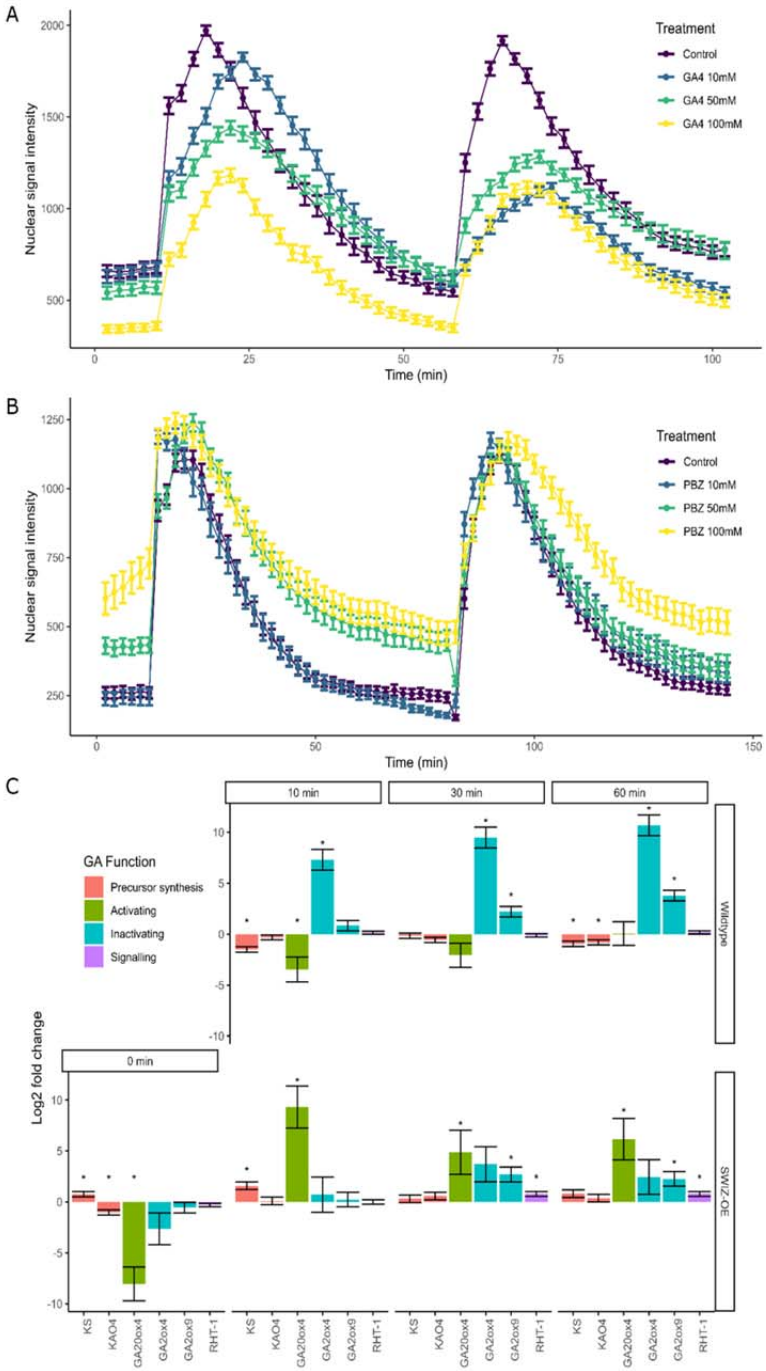

Figure 10. SWIZ translocation is dependent on bioactive GA levels and impacts GA related gene expression. (A) SWIZ translocation to the nucleus is dampened by the addition of exogenous GA4. (B) SWIZ translocation to the nucleus is enhanced by the addition of paclobutrazol (PBZ), a GA synthesis inhibitor. The average nuclear GFP signal is represented by the trend line, with error bars indicating standard deviation of the mean. Imaging was initiated at t = 0 min, while touch treatment was initiated at t = 10 min. n = 85-125 (A) and n = 64-142 (B) nuclei per treatment. Gene expression analysis of GA related genes. (C) Log fold change of gene expression relative to their expression at time 0, pre-touch. Bar color indicates relevant function in GA synthesis and signalling. Significance denoted by * reflecting q < 0.1 compared to wildtype expression at t = 0, with q-values representing Wald test p-values adjusted for false discovery rate.

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DISCUSSION

Touch stimulus is generally an inhibitor of plant elongation growth, but promotes branching and radial expansion (Jaffe, 1973; Braam, 2004; Chehab et al., 2009). The majority of this work has been done in dicots, where increased radial growth has been associated with greater deposition of secondary wall forming cells, particularly in woody species such as poplar (Biro et al., 1980; Coutand et al., 2009; Börnke and Rocksch, 2018; Roignant et al., 2018; Niez et al., 2019). Our understanding of thigmomorphogenesis in grasses is limited, and mostly in the agricultural context of lodging (Shah et al., 2019). While these studies highlight the importance of stem strength and associated cell wall defects, they do relatively little to elucidate the mechanosensitive response. Recent work by Gladala-Kostarz et al (2020) describes grass touch response to both wind and direct mechanical treatment, with an emphasis on cell walls and stem anatomy. Touch treatment significantly increased lignin content, and also altered the relative abundance of wall bound hydroxycinnamates. Wall polysaccharide content, particularly glucose and galactose, also increased. Consistent with increased secondary wall lignification, touched plants had increased wall stiffness and decreased saccharification.

Specific gene expression patterns have become molecular hallmarks of plant touch response, most notably induction of the *TCH* and similar genes. Orthologs of wall modifying XTHs and CAM/CML signaling genes are upregulated in response to mechanical stimulation across species, and as we present here, in *B. distachyon*. Touch elicits major global changes in gene expression, with 2.5% to 10% of the genes assayed in *A. thaliana* and poplar differentially expressed (Lee et al., 2005; Pomiès et al., 2017; Van Moerkercke et al., 2019). In sorghum (*Sorghum bicolor*), leaf sheath imposes mechanical constraints on emerging buds, and removing this dramatically altered gene expression, with 42% of genes differentially expressed over a 9 h period (Liu and Finlayson, 2019). Our observations of short term (<2 h) touch responses within stress related pathways align with observations from poplar and *A. thaliana*, with rapid induction of *TCH*, biotic, and abiotic stress related genes (Pomiès et al., 2017). Canonical touch responsive genes such as the orthologs of *TCH1-4* and related *XTH* and *CML* genes were upregulated immediately following stimulus. However, we also note the previously unreported touch-induction of GH17 family genes, as well as *CSLF6*, suggesting a role for MLG and β-1,3 glucan modification in grass touch response.

In both poplar and in our analysis of *B. distachyon* touch responses, touch-regulated expression of secondary cell wall related transcripts differed between early and late timepoints. Upon touch, we observed immediate repression of many key cell wall biosynthetic enzymes, followed by significant upregulation one hour after stimulus. In poplar, no significant repression of these transcripts was reported, but terms related to wood or cell wall synthesis were also not enriched among touch-regulated transcripts until 24 or 72 hours after touch (Pomiès et al., 2017). While differences in the method used to quantify gene expression, and in the temporal sampling scheme

complicate direct comparisons of plant touch response experiments, this may suggest that delayed induction of secondary cell wall transcripts is a common feature of plant touch responses.

Thigmomorphogenesis, GA physiology, and secondary cell walls

Our understanding of touch responsive gene expression is further informed by the dynamics of SWIZ nuclear translocation. The timing of SWIZ nuclear accumulation following mechanical stimulus was consistent with observations of *A. thaliana* Group I bZIPs (Tsugama et al., 2014; Tsugama et al., 2016). However, by adopting a finer temporal sampling scheme, and quantifying nuclear signal sooner after touch (2 vs. 30 min), our results clarify the rapid and immediate nature of this translocation. Furthermore, we show that the speed and magnitude of SWIZ nuclear accumulation is not diminished over three successive touch treatments. Notably, poplar transcriptional responses to a second touch stimulus were significantly attenuated relative to a single treatment, and in separate experiments, trees subjected to a daily touch treatment over four days no longer showed touch-induced increases in their rates of radial growth (Martin et al., 2010; Pomiès et al., 2017). Thus, if *B. distachyon* is likewise desensitized to repeated touch, the high repeatability of SWIZ translocation might implicate a mechanism downstream of touch perception and bZIP translocation in mediating that shift. Like the touch-responsive transcription factor AtVIP1, SWIZ translocation occurred in the specific area of touch treatment, suggesting a local response to stimulus rather than a tissue or whole organism response. SWIZ also displays a highly similar pattern of GA responsive translocation to the well-studied bZIP NtRSG (Ishida et al., 2004; Fukazawa et al., 2010; Fukazawa et al., 2011). Exogenous bioactive GA dampened SWIZ nuclear translocation, while inhibition of GA synthesis with paclobutrazol induced translocation, even prior to touch. Furthermore, like NtRSG, SWIZ appears to both respond to and regulate GA bioactivity. In wildtype, GA inactivating enzymes GA2ox4 and GA2ox9 were upregulated following touch, consistent with previous reports (Lange and Lange, 2015). In *SWIZ-OE* we also observe touch induced expression of *GA20ox4*, a GA activating enzyme, and as with NtRSG, this response can restore bioactive GA following touch induced degradation (Ishida et al., 2004; Fukazawa et al., 2010; Fukazawa et al., 2011). Altered GA pathways are a well described mechanism for reduced stem elongation (Lange and Lange, 2006; Nagai et al., 2020), and dwarfing has been described for dominant negative mutants of Group I bZIPs *NtRSG* and *OsRF2A* (Fukazawa et al., 2000; Dai et al., 2003). *SWIZ-OE* stems were significantly shorter following touch treatment, suggesting that GA misregulation may contribute to this thigmomorphogenic phenotype. *SWIZ-OE* roots were shorter than wildtype under control, direct touch, and multiple plate growth angles. This may suggest that *SWIZ-OE* roots grown in agar media experience a sufficient degree of mechanical input to reduce elongation. It is also conceivable that misregulation of GA related genes by *SWIZ* overabundance inherently results in shorter tissues.

Another component of this reduced elongation may be increased secondary cell wall deposition, as this could terminate elongation. Prior to touch, almost the entire monolignol biosynthetic pathway, including laccase genes that radicalize monolignols, and BAHD acyltransferases that add hydroxycinnamic acids to wall polymers, are significantly downregulated in *SWIZ-OE* plants. Following touch, most of these genes become significantly upregulated. This activity is consistent with our observations of increase in wall thickness in touched *SWIZ-OE* peduncles, and aligns with the compositional data presented by Gladala-Kostarz et al. (2020). This activation may be a result of direct binding of SWIZ to modulate expression, or an indirect effect from another transcriptional regulator such as the NAC transcription factor *SWN5* that is significantly upregulated in *SWIZ-OE* following touch and capable of activating the full developmental program for secondary cell wall synthesis (Valdivia et al., 2013). GA has also been implicated in regulating cell wall thickening, and the interactions between SWIZ and touch mediation of GA levels may contribute to this aspect of thigmomorphogenesis (Huang et al., 2015; Falcioni et al., 2018; Wang et al., 2021).

While the E-box (CANNTG) was initially described as a bHLH binding motif, many bZIP groups in *A. thaliana* have been shown to bind similar sequences. The two closets *A. thaliana* orthologs of SWIZ , bZIP18 and 52, both bind (A/C)CAG(G/C)(T/C), while VIP1 binds (A/C)CAGCT(G/A) (O'Malley et al., 2016). Using the same technique, we identified the SWIZ binding motifs as (A/C)CAGNCTG(T/G) and (A/C)CAGCTG(T/G). Both of these sequences are similar to those bound by *A. thaliana* orthologs, although the presence of an ambiguous nucleotide in the core of one variant is not previously reported for other Group I bZIPs. Among genes bound by SWIZ *in vitro,* we observed both activation and repression following touch, suggesting a complex regulatory function for SWIZ. Furthermore, SWIZ direct binding sites were found in both promoter regions and in the body of genes and most often in genes activated by touch. SWIZ gene body binding targets tended to be repressed in *SWIZ-OE* plants following touch, without either being clearly associated with up or down regulation. AtVIP1 and AtbZIP29 have both been described as activators, particularly of genes related to biotic and abiotic stress response, cell cycle control, and development. (Yin et al., 1997; Ringli and Keller, 1998; Pitzschke et al., 2009; Van Leene et al., 2016). AtbZIP18 is suggested to have repressive roles (Tsugama et al., 2012; Gibalová et al., 2017).

bZIPs are known to homo- and heterodimerize through their leucine zipper domains (Schütze et al., 2008), and in *A. thaliana* the combinatorial interactions of different bZIP groups have been fairly well described (Deppmann et al., 2004; Ehlert et al., 2006; Grigoryan and Keating, 2006; Weltmeier et al., 2006; Schütze et al., 2008). These interactions can have a synergistic effect on transcriptional activity, and can result in unique binding interactions (Schütze et al., 2008; Van Leene et al., 2016). It is conceivable that this heterodimerization may explain some aspects of our results, such as why so many of the cell wall genes that are upregulated in *SWIZ-OE* after touch are significantly downregulated prior to touch. One hypothesis is that SWIZ, as with many other bZIPs, can regulate targets as a heterodimer. The abundance of SWIZ protein in the

cytoplasm in *SWIZ-OE* plants prior to touch may act as a sink, sequestering interacting partners that would otherwise be regulating targets independent of a touch stimulus. Further experimentation is needed to elucidate this bZIP regulatory network in response to touch and other stimuli.

CONCLUSIONS

Proteins orthologous to SWIZ in other species have been implicated in cell wall development and remodeling, but not cell wall thickening. SWIZ may influence root length inherently, and stem height and cell wall thickening in response to mechanical stimuli, which regulates SWIZ translocation into the nucleus by acting on the cellular bioactive GA pool. Touch has been shown to decrease cellular bioactive GA levels in *A. thaliana*, and Group I bZIPs are known to translocate to the nucleus in response to touch and to low levels of bioactive GA. Direct mechanical stimulation and chemical inhibition of GA biosynthesis both caused SWIZ to translocate from the cytoplasm into the nucleus. This translocation can be reduced with exogenous bioactive GA. GA biosynthesis and signaling genes were upregulated in *SWIZ-OE* lines following touch, indicating that SWIZ may act in managing GA homeostasis following mechanical perturbation. Altering *SWIZ* function may impact GA homeostasis and result in reduced elongation. Touch significantly remodeled the *B. distachyon* transcriptome, with notable changes in wall polysaccharide biosynthetic gene expression not previously reported. Enhanced *SWIZ* function through overexpression amplified the expression of cell wall related genes and synthesis of interfascicular fiber walls in touched tissues. Greater secondary wall synthesis in response to touch may also explain reduced stem elongation in *SWIZ-OE*. Together, the evidence presented here connects mechanotropic bZIP dynamics with changes in cellular GA levels that have been observed separately in other systems, and shows a link between touch response and secondary cell wall transcriptional regulation (**Fig 11**).

MATERIALS AND METHODS

Phylogenetic analysis

Protein sequences described for *A. thaliana*, *B. distachyon*, and *O. sativa* (Liu and Chu, 2015) as Group II bZIPs were selected and searched against the most recent genome annotations in Phytozome v12.1 (https://phytozome.jgi.doe.gov). The *Nicotiana tabacum* homologs NtRSGa and NtRSGb were also added to the analysis. Protein sequences were aligned using the MAFFT service for multiple sequence alignment with the iterative refinement method L-INS-I (Katoh et al., 2019). The alignment was used to construct the phylogenetic tree using a maximum likelihood analysis with a bootstrap resampling value of 1000 in W-IQ-TREE (Trifinopoulos et al., 2016). All of the proteins included in the phylogenetic analysis are described in **Supplemental File 1**.

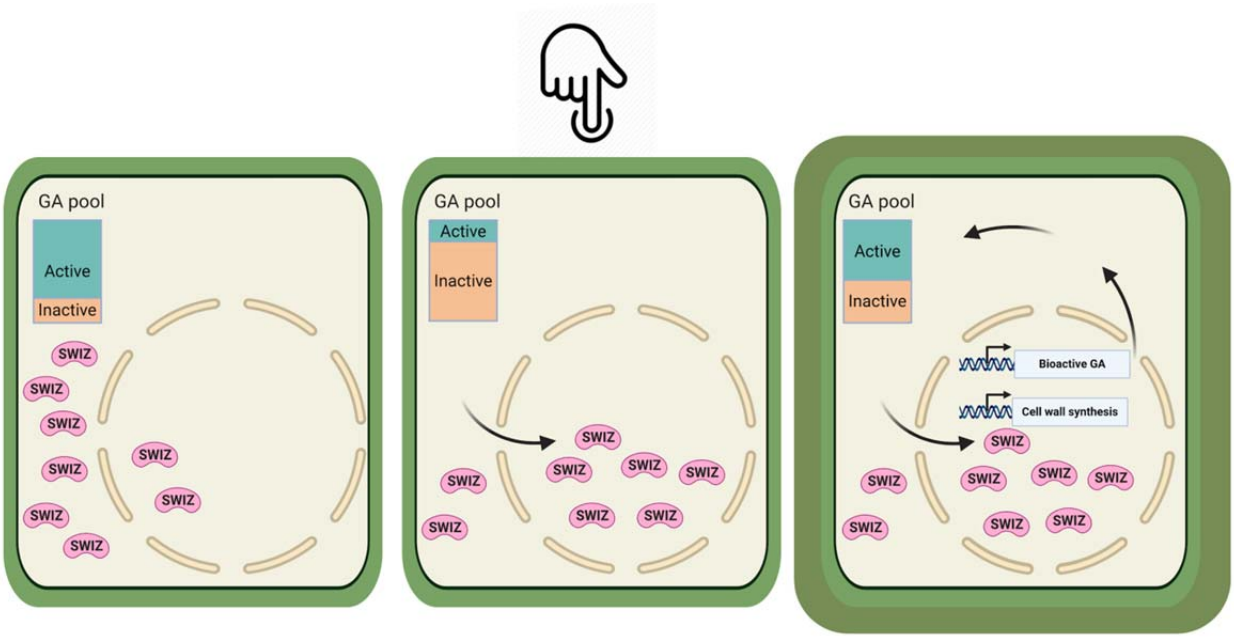

Figure 11. SWIZ regulates cell wall synthesis and GA homeostasis in response to mechanical stimulus. Prior to touch, SWIZ is localized to the cytosol and partially in the nucleus. The bioactive GA pool is depleted in response to touch, triggering SWIZ to translocate to the nucleus where it positively regulates cell wall synthesis and GA activating enzymes.

Plant transformation

Overexpression and artificial microRNA transgenes and transgenic plants were constructed as previously described (Handakumbura et al., 2013). The full length coding sequence of Bradi1g17700 was amplified from cDNA and cloned into the pOL001 ubigate ori1 binary expression vector (Handakumbura et al., 2018) to make the *SWIZ-OE* trangene. The coding sequence lacking the stop codon was amplified and fused in frame with the coding sequence of *Aequorea victoria* enhanced GFP in the pOL001 vector to generate *SWIZ:GFP-OE*.

Translocation assay

Seeds were surface sterilized and grown vertically on 1X MS media, pH 5.7, without sucrose for 6d at 28°C in the dark. After 6 d, seedlings were moved to treatment plates containing 1X MS media, pH 5.7, plus varying concentrations (0, 10, 50, or 100 mM) of GA4, paclobutrazol. Roots were positioned such that they were completely submerged in media and relatively flat along the imaging plane After a 6 h incubation, plants were stimulated and imaged.

All observations were made on a Nikon A1R scanning confocal microscope using a Plan Apo 10x 0.5NA objective. Root areas to be observed were located by eye using transmitted light and then confirmed under confocal conditions using a 488 nm laser and green filtered PMT detector. The X, Y, and Z coordinate stage locations of each region were programmed into the Nikon NIS

539 Elements 5.2 Advanced Research V6 software package for automated imaging. After all target
540 regions were programmed, 30 min of imaging began pre-treatment, with images captured every 2
541 min.

542 To elicit the touch response, the observed root region was gently probed for 5 sec with a blunt
543 metal probe while observing through the eyepiece. Images were captured for 60-90 min post
544 treatment. For experiments with multiple stimulus events, the timelapse sequence was paused
545 and roots were probed as described for the relevant stimulus events.

546 Analysis of GFP signal was done using the Nikon NIS Elements Advanced Research V5
547 software package. For each time series, the frame showing maximal nuclear signal was selected
548 and used as a reference point. Using the General Analysis tool, a channel for GFP signal was
549 established and thresholded for intensity and particle size to identify the nuclear regions. These
550 regions were added to the timelapse image series as a binary layer and then converted to static
551 regions of interest for quantification. The GFP intensity under each nuclear region of interest was
552 calculated for the course of the timelapse and the average signal from each nucleus was plotted
553 for intensity over time.
554

555 **Thigmomatic construction and operation**

556 The Thigmomatic is a basic robotic device that sweeps plants with a metal bar at regular
557 intervals to elicit a touch response. The device was constructed from aluminum V-Slot linear rail
558 (Openbuilders Partstore, Monreoville, NJ) and bracket joints for the upright supports (20x20
559 mm), cross bars (20x20 mm), and tracks (20x40 mm). Two gantry carts ride along the 20x40 mm
560 V-Slot linear rails, connected by a 6.35 mm diameter metal rod bolted to the carts. Their
561 movement is powered by a belt driven linear actuator system using a NEMA 17 stepper motor
562 with a 12V 18W AC/DC power supply. The stepper motor provides fine spatial control over the
563 gantry cart position with bi-directional motion. Motor function is controlled by a Raspberry Pi
564 3B microcomputer equipped with a stepper motor HAT (Adafruit Industries, New York). The
565 Thigmomatic was programmed to cover a specified distance in one direction once every 90 min.
566 Accession Bd21-3 was used for these experiments. Seeds were stratified on wet paper towel
567 wrapped in foil to exclude light for 10 days at 4°C before being planted in Promix BX potting
568 mix in SC10 Ray Leach Cone-tainers (Stuewe & Sons Inc). Plants were grown in a Percival
569 PGC-15 growth chamber with day/night conditions of 20 h light at 24°C and 4 h dark at 18°C,
570 respectively.
571

572 **Transverse stem sections and histology**

573 The main stem of senesced plants was taken and the internode of interest removed and embedded
574 in 8% agarose. A Leica VT1000 Vibratome was used to make 55 µm thick transverse sections.
575 Histochemical staining was carried out using phloroglucinol-HCl as previously described (Matos
576 et al., 2013). Images were obtained at 4, 10, and 20X using a Nikon Eclipse E200MV R light
577 microscope and PixeLINK 3 MP camera. Transverse sections imaged at 20x were used for cell

wall thickness measurements. Interfascicular fiber cells separated by one cell layer from the mestome cells on the phloem side of major vascular bundles were targeted for measurement. Using ImageJ, lines were drawn across two walls of adjoining cells. The resulting line length was divided by two to give one cell wall width. Approximately 15 measurements were made for each plant.

RNA extraction and quantification and analysis

Seedlings were grown for 6 d on vertical 1X MS agar plates. Touch treatment was performed as described above using a metal probe along the entire length of the root. Untouched samples were collected immediately before touch treatment began, and touched samples were collected after the designated time. Three roots were pooled per biological replicate, and RNA was extracted using the Qiagen RNeasy Plant Mini Kit with on-column DNA digestion with RNase-free DNase I (Qiagen). Strand-specific libraries were prepared using the Illumina TruSeq kit. Libraries were sequenced using Illumina technology. Sequences were processed as previously described (MacKinnon et al., 2020) and then checked for differential expression using the likelihood ratio test. Specific contrasts (i.e., wildtype vs *SWIZ-OE*) were identified and compared using the Wald test. Statistical enrichment of gene families was assessed using Fisher's exact test. Raw read data were deposited in the European Nucleotide Archive for public access (Accession no.: E-MTAB-10084).

DNA affinity purification sequencing

DNA affinity purification was carried out as previously described (Handakumbura et al., 2018). In brief, transcription factor coding sequences were HALO tagged and mixed with Bd21 genomic DNA for *in vitro* binding. Protein-DNA was crosslinked, fragmented, immunoprecipitated using the HALO antibody, barcoded, and sequenced. Reads were mapped back to the Bd21 genome using HiSat2 (Kim et al., 2015) to identify binding target loci. Peak calling and motif analysis was done using HOMER v4.10 (Hypergeometric Optimization of Motif EnRichment) suite (Heinz et al., 2010). Motif enrichment was calculated against the hypergeometric distribution; the significance threshold was set to $p < 0.05$. The nearest annotated gene to a bound peak was used for GO analysis. Raw read data were deposited in the European Nucleotide Archive for public access (Accession no.: E-MTAB-10066).

Gene Ontology analysis

Phytozome was used to find orthologs for all *B. distachyon* v3.1 genes as the reciprocal best match to *A. thaliana* TAIRv10 protein sequences. Arabidopsis gene identifiers were submitted to g:Profiler (Raudvere et al., 2019) for KEGG and Wiki pathway enrichment analysis.

iDREM network analysis

Probabilistic graphical models that predict diverging gene expression paths and the points at which regulators may influence those paths were generated using iDREM (Ding et al., 2018). Briefly, this software applies an input-output hidden Markov model to time course gene

616 expression data overlaid with static regulatory information, in this case SWIZ protein-DNA
617 interactions identified from DAP-seq. GO analysis, described above, was also applied to the gene
618 sets in each path identified by iDREM.
619
620 **Root Touch Experiment**
621 Seeds were surface sterilized and plated on 1x MS, pH 5.7, containing 0.05 % MES as a
622 buffering agent and 1% plant agar (Gold Bio). Seeds were stratified on plates in the dark at 4 °C
623 for 2 days and then transferred to a Percival PGC-15 growth chamber with day/night conditions
624 of 16h light at 24 °C and 8h dark at 18 °C, respectively and grown at a ~10° angle from vertical.
625 After 2 days of preliminary growth, a touch location was designated by selecting the lower of 1
626 cm up from the root tip or 1 cm down from the seed and marked on the plate. Each day for 5
627 days, this marked spot was treated twice a day, two hours before and two hours after chamber
628 midday (ZT6 and ZT10). Touch treatment consisted of 5 firm presses with the side of a sterile
629 pipette tip, just hard enough to cause elastic deformation of the media underlying the root when
630 viewed through a dissecting microscope. To ensure unbiased application of touch treatment and
631 control for any blocking effects, each plate contained one group of four *SWIZ-OE* seeds and one
632 group of four Bd21-3 wildtype seeds, with the experimenter blinded to their respective positions
633 for the duration of the experiment.
634
635 **Root length and straightness measurement**
636 Plates were photographed at the conclusion of the experiment. To generate high contrast images
637 suitable for root tracing, plate images were separated into hue, saturation and brightness
638 channels, and the saturation channel was selected and inverted. Semi-automated measurements
639 of root length were performed using the Smart Roots plugin for ImageJ (Lobet et al., 2011).
640 Straightness was quantified as described in (Swanson et al., 2015); the straight line distance
641 between root tip and the base of the seed was measured, and this value was divided by the traced
642 length of the root.

643 **SUPPLEMENTAL MATERIALS**

644 **Supplemental Table S1. Cluster 56 from network analysis of gene expression atlas (Sibout**
645 **et al. 2017) includes Bradi1g17700.**

646 **Supplemental Table S2. Differentially expressed genes in wildtype Bd21-3 root tissue 10**
647 **min after touch treatment.**
648

649 **Supplemental Table S3. Differentially expressed genes in wildtype Bd21-3 root tissue 30**
650 **min after touch treatment.**
651

652 **Supplemental Table S4. Differentially expressed genes in wildtype Bd21-3 root tissue 60**
653 **min after touch treatment.**

654 **Supplemental Table S5. Calmodulin and calmodulin-like touch responsive gene expression**
655 **in wildtype Bd21-3 root tissue.**

656 **Supplemental Table S6. XTH and XTH-like touch responsive gene expression in wildtype**
657 **Bd21-3 root tissue.**

658 **Supplemental Table S7. Glycoside hydrolase touch responsive gene expression in wildtype**
659 **Bd21-3 root tissue.**

660 **Supplemental Table S8. GO analysis of genes in iDREM identified pathways derived from**
661 **differential gene expression following touch in wildtype, swiz-amiRNA, and SWIZ-OE root**
662 **tissue.**
663

664 **Supplemental Figure 1. Description of *SWIZ* transgenic reagents. (A) Diagram of *SWIZ***
665 **overexpression transgenes.** The *ZmUbi* promoter was used to drive expression of the *SWIZ*
666 coding sequence, either alone or fused in frame with *eGFP*. (B) Relative level of *SWIZ* transcript
667 measured by RT-qPCR in whole stem tissue was collected 1 d after inflorescence emergence in
668 *SWIZ:GFP-OE*. LB, left border; *ZmUbi* prom, maize ubiquitin promoter; Hyg, hygromycin
669 phosphotransferase gene; NOS, nopaline synthase terminator; RB, right border. ns: $p > 0.05$, : *:
670 $p < 0.05$.

671

672 **Supplemental Figure 2. Network analysis of differential gene expression paths and**
673 **enriched GO terms.** (A) Path determinations from iDREM time course analysis. Each line
674 represents a set of genes with similar expression level patterns over the time course relative to
675 time 0, pre-touch. (B) Heatmap of selected GO term enrichment of genes from iDREM path
676 analysis. Color indicates qvalue score, with a cutoff of 0.1. Terms without a colored block are
677 not significantly enriched in that path.

Supplemental Figure 3. Thigmomatic, an automated system to administer touch stimulus. (A) Overview of Thigmomatic inside a Percival PGC-15 growth chamber showing linear rail based frame (1), gantry carts (2), NEMA17 stepper motor (3), 12V 18W AC/DC power supply (4), and Raspberry Pi 3b microcomputer (5). (B) Thigmomatic making contact with a *Brachypodium distachyon* plant.

Supplemental Figure 4. *Brachypodium distachyon* displays classic thigmomorphogenic phenotypes. One-week-old wildtype plants were treated with touch stimulus every 90 min. (A) Left to right, plants that experienced no stress, two weeks stress, three weeks stress and were then allowed to recover, imaged one week after the end of treatment (B) height, (C) aboveground non-grain biomass weight, and (D) branch number were measured at senescence. ns: $p > 0.05$, *: $p < 0.05$, n = 5 plants per treatment. (E) Transverse sections of the peduncle or third internode were take from control, 2 week stressed, and 3 week stressed plants and stained with phloroglucinol-HCl. (F) Quantification of interfascicular fiber wall thickness. Scale bar = 100 µm. n = 3 plants per treatment.

Supplemental Figure 5. Biomass and branching in SWIZ-OE under touch treatment shows no difference from wildtype. Wildtype and *SWIZ-OE* plants were placed under control conditions or received two weeks of mechanical stress every 90 min. After senescence, aboveground biomass weight (A) and branch number (B) were quantified. p value calculated by student t-test.

Supplemental File 1. Gene names and amino acid sequences used in the SWIZ bZIP Group I phylogenetic analysis.

ACCESSION NUMBERS

AtbZIP18 (At2g40620), AtbZIP29 (At4g38900), AtbZIP52 (At1g06850), AtTCH1/AtCaM2 (At2g41110), AtTCH2/AtCML24 (At5g37770), AtTCH3/CML12 (At2g41100), AtTCH4/AtXTH22 (At5g57560), CAD1 (Bradi3g17920), CESA4 (Bradi4g28350), COMT6 (Bradi3g16530), CSLF6 (Bradi3g16307), GA2ox3 (Bradi2g50280), MYB44 (Bradi1g30252), NAC35 (Bradi2g28020), NtRSGa (Niben101Scf01150g00005), NtRSGb (Niben101Scf02191g00014), SWIZ (Bradi1g17700), VIP1 (At1g43700).

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Table 1. The count of differentially expressed genes relative to untouched wildtype plants that were SWIZ DAP-seq targets in either the promoter or gene body regions.

Time after touch	Promoter		Gene body	
	Up	Down	Up	Down
(min)	percent (count)			
0	42.0 (103)	58.0 (142)	48.0 (286)	52.0 (310)
10	57.0 (73)	43.0 (55)	53.0 (167)	47.0 (148)
30	59.4 (79)	40.6 (54)	41.2 (136)	58.8 (194)
60	64.2 (77)	35.8 (43)	45.7 (128)	54.3 (152)

FIGURE LEGENDS

Figure 1. SWIZ is a Group I bZIP highly expressed in maturing stem and root. (A) *SWIZ* transcript abundance in *Brachypodium distachyon* leaf, root, and stem tissue measured by microarray. Mean +/- standard deviation of three biological replicates. (B) Phylogeny analysis of amino acid sequences from *B. distachyon* (green), *Oryza sativa* (blue), *Arabidopsis thaliana* (red), *Nicotiana tabacum* (orange) shows *SWIZ* (black dot). The phylogeny was reconstructed using maximum likelihood analysis with a bootstrap resampling value of 1000. The numbers labeled on the branches are the posterior probability to support each clade. The protein sequences, full locus IDs, and plant species with genome source are provided in Supplemental File 1.

Figure 2. SWIZ translocates to the nucleus in response to mechanical stimulus, specifically in regions directly stimulated. (A) Image of *SWIZ:GFP-OE* and *GFP-OE* roots prior to stimulus and 30 min post stimulus. Roots were observed immediately following mechanical perturbation. (B) Quantification of nuclear signal. n=14-20 nuclei. (C) SWIZ translocation occured in the local area of the stimulus. At 30 min, stimulus was applied to an upper region of the root, while at 120 min it was applied to a lower region approximately 3 cm below. n=109, 184 nuclei respectively for upper and lower regions. (D) *SWIZ:GFP-OE* roots were imaged by confocal microscopy with stimulus applied in the field of view at 0, 90, and 180 min. n=126 nuclei. (B-D) Images were taken every 2 min. Nuclear GFP signal was quantified in selected nuclei at each time point. The average nuclear GFP signal is represented by the line with error bars indicating standard error of the mean.

Figure 3. Transcriptome analysis of touch response in *Brachypodium distachyon* roots. (A) Root tissue was sampled just prior to touch (t = 0), and at 10, 30, and 60 min following touch treatment in wildtype and *SWIZ-OE*. (B) Principal component analysis of gene expression across samples shows the greatest difference corresponding to genotype and the second greatest corresponding to time after touch. (C) Canonical, as well as novel touch responsive genes are upregulated in *B. distachyon* following touch. Closest orthologs of the *Arabidopsis thaliana TOUCH* genes encoding calmodulin-like (CML) and xyloglucan endo-transglycosylase/hydrolases (XTH) are upregulated following touch, as are previously unreported members of the glycosyl hydrolase 17 (GH17) family. Full list of gene expression for *TOUCH* and glycoside hydrolases defined by Tyler et al. (2010) in Supplemental Tables 5, 6, and 7. Significance denoted by * reflecting $q < 0.1$ compared to expression at t = 0, with q-values representing Wald test p-values adjusted for false discovery rate.

Figure 4. Gene expression analysis of cell wall related genes. Log fold-change of gene expression in wildtype, *SWIZ-OE*, and *swiz-amiRNA* relative to wildtype expression at time 0, pre-touch. Bar color indicates the class of cell wall gene. Error bars indicate standard deviation of three biological replicates. Significance denoted by * reflecting $q < 0.1$ compared to wildtype expression at t = 0, with q-values representing Wald test p-values adjusted for false discovery rate.

Figure 5. DNA affinity purification sequencing to determine SWIZ binding sites. (A) Top two most statistically enriched sequence motifs in SWIZ binding sites. (B) Distribution of binding sites across genomic features, relative to primary transcripts of the *Brachypodium distachyon* annotation v 3.1. (C) Relative distribution of binding sites centered on the transcriptional start site (TSS, blue dashed line), transcriptional termination site (TTS, red dashed line) represents the average length of all annotated transcripts, approximately 4.5 kb away from the TSS. (D) Path determinations from iDREM time course analysis of differentially expressed genes that also have DAP-seq binding sites. Each line represents a set of genes with similar expression level patterns over the time course relative to time 0, pre-touch.

Figure 6. SWIZ binding targets are differentially expressed in response to touch and SWIZ. Gene expression over time of selected genes with SWIZ binding sites. Line graphs are the average transcript abundance of three biological replicates for each time point. Binding site determined as peaks of sequence alignment. Scale bar unit is bases. Direction of transcription is shown with arrows on the gene model, 5' and 3' UTRs are depicted by narrowed rectangles on the gene model.

Figure 7. *SWIZ-OE* roots are shorter than wildtype and less straight in response to direct touch. Wildtype and *SWIZ-OE* seedlings grown on plates under control (A) or touched (B)

conditions for five days. Touched plants were probed with a pipette tip twice a day. (C) Quantification of root length in control and touched conditions. Significance denoted by * reflecting t-test p-value < 0.05, ns = not significant. (D) Quantification of root straightness in control and touch conditions. Significance denoted by * reflecting Wilcoxon sign-ranked test for non-parametric data with p-value < 0.05, ns = not significant.. Scale bar = 1 cm.

Figure 8. Wildtype roots shorten with increasing plate angle, while *SWIZ-OE* roots are consistently short, with no significant difference in straightness. Wildtype and *SWIZ-OE* seedlings grown on plates at (A) 10°, 20°, 30°, and (B) 40° incline from vertical. (C) Quantification of root length. (D) Quantification of primary root straightness. Significance denoted by * reflecting t-test p-value < 0.05, ns = not significant.Scale bar = 1 cm.

Figure 9. *SWIZ-OE* enhanced thigmomorphogenic response in stems. Wildtype and *SWIZ-OE* plants were placed under control conditions or received two weeks of mechanical stress every 90 min. (A) Quantification of main stem height at senescence. (B) Quantification of interfascicular fiber wall thickness in transverse cross sections of the peduncle. n = 4 to 6 plants per genotype, per treatment. (C) Representative cross sections stained with phloroglucinol-HCl. Scale bar = 100 µm. Significance denoted by * reflecting t-test p-value < 0.05, ns = not significant.

Figure 10. SWIZ translocation is dependent on bioactive GA levels and impacts GA related gene expression. (A) SWIZ translocation to the nucleus is dampened by the addition of exogenous GA4. (B) SWIZ translocation to the nucleus is enhanced by the addition of paclobutrazol (PBZ), a GA synthesis inhibitor. The average nuclear GFP signal is represented by the trend line, with error bars indicating standard deviation of the mean. n= 85-125 (A) and n= 64-142 (B) nuclei per treatment. Gene expression analysis of GA related genes. (C) Log fold change of gene expression in wildtype samples, relative to their expression at time 0, pre touch. (D) Log fold change of gene expression in SWIZ OE and swiz amiRNA samples, relative to their expression in wildtype at the same time points. Bar color indicates relevant function in GA synthesis and signaling. Significance denoted by * reflecting q < 0.1 compared to wildtype expression at t = 0, with q-values representing Wald test p-values adjusted for false discovery rate.

Figure 11. SWIZ regulates cell wall synthesis and GA homeostasis in response to mechanical stimulus. Prior to touch, SWIZ is localized to the cytosol and partially in the nucleus. The bioactive GA pool is depleted in response to touch, triggering SWIZ to translocate to the nucleus where it positively regulates cell wall synthesis and GA activating enzymes.

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