Feedback Microtubule Control and Microtubule-Actin Cross-talk in Arabidopsis Revealed by Integrative Proteomic and Cell Biology Analysis of KATANIN 1 Mutants*

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Microtubule organization and dynamics are critical for key developmental processes such as cell division, elongation, and morphogenesis. Microtubule severing is an essential regulator of microtubules and is exclusively executed by KATANIN 1 in Arabidopsis. In this study, we comparatively studied the proteome-wide effects in two KATANIN 1 mutants. Thus, shotgun proteomic analysis of roots and aerial parts of single nucleotide mutant fra2 and T-DNA insertion mutant ktn1-2 was carried out. We have detected 42 proteins differentially abundant in both fra2 and ktn1-2. KATANIN 1 dysfunction altered the abundance of proteins involved in development, metabolism, and stress responses. The differential regulation of tubulins and microtubule-destabilizing protein MDP25 implied a feedback microtubule control in KATANIN 1 mutants. Furthermore, deregulation of profilin 1, actin-depolymerizing factor 3, and actin 7 was observed. These findings were confirmed by immunoblotting analysis of actin and by microscopic observation of actin filaments using fluorescently labeled phalloidin. Results obtained by quantitative RT-PCR analysis revealed that changed protein abundances were not a consequence of altered expression levels of corresponding genes in the mutants. In conclusion, we show that abundances of several cytoskeletal proteins as well as organization of microtubules and the actin cytoskeleton are amended in accordance with defective microtubule severing. Molecular & Cellular Proteomics 16: 10.1074/mcp.M117.068015, 1591–1609, 2017.

Microtubules are tubulin filamentous polymers involved in cell division and expansion (1, 2). They are capable of rapid elongation or shortening (polymerization and depolymerization), which is known as dynamic instability. This dynamic instability together with other mechanisms, including nucleation, branching, severing, and bundling, determine the spatiotemporal organization of microtubule arrays, which is crucial for plant growth and development (3–5). Microtubule dynamics and organization are controlled mainly by microtubule-associated proteins (MAPs),1 kinesins, plus-end binding (EB1) proteins, microtubule-severing protein katanin, microtubule-destabilizing protein 25 (MDP25), phospholipase D, and others (6–8). Some of these proteins might be regulated by signaling molecules such as mitogen-activated protein kinases (9, 10), Rop GTPases, calcium, and phosphatidic acid (11–13). Such interactions couple microtubules to the external environment and mediate their developmental or conditional rearrangements.

KATANIN 1 is a microtubule-severing AAA-ATPase assembled from a catalytic subunit of 60 kDa (p60) and a structural 80-kDa subunit (p80 (14)). It is capable of severing microtubules in an ATP-dependent manner (15). At the cellular level, the severing activity of KATANIN 1 was shown to regulate plant microtubule organization (16). Except for microtubule severing, KATANIN 1 activity favors microtubule bundle formation (17) and can be modulated by other microtubule-binding proteins like SPIRAL2 (18). Moreover, KATANIN 1 severing activity is induced by Rho-GTPase signaling, thus connecting hormonal and external stimuli to microtubule dynamics (19).

1 The abbreviations used are: MAP, microtubule-associated protein; ABA, abscisic acid; ABP, actin-binding protein; ADF, actin-depolymerizing factor; GA, gibberellic acid; IAA, indole-3-acetic acid; MDP25, microtubule-destabilizing protein 25; TUB4, TUBULIN β-4; TSN, TUDOR-staphylococcal nuclease protein; WPP2, WPP domain-containing protein 2; FDR, false discovery rate; BSE, benzoyl-2-hydroxysuccinimide ester; ANOVA, analysis of variance; PI, precursor ion intensity; M-MLV, Moloney murine leukemia virus; GO, gene ontology.
The importance of KATANIN 1 for plant development is manifested by multiple developmental defects reported in KATANIN 1 mutants such as fra2 or lue1. They exhibit reduced root, hypocotyl, stem, and leaf growth as well as stubby flower organs with reduced anther length (20–22). KATANIN 1 mutants show reduced fertility and defects in ovule and anther development, and they exhibit aberrant embryogenesis and seed formation (23). It is generally accepted that these phenotypes are caused by reduced cell expansion (20, 21). It was also proposed that fra2 and lue1 mutants exhibit some defects in cell division resulting from altered organization of microtubule arrays showing multipolar spindles and disorientation of the cell division plane (24). Advanced live microscopy of microtubules in ktn1-2 uncovered the contribution of KATANIN 1 to dynamic organization of cortical microtubules as well as a new function in the formation and maturation of preprophase band and rectification of cell division plane (25). Moreover, KATANIN 1 mutants displayed altered gibberellic acid (GA) and ethylene responses (22, 26) pointing to the role of KATANIN 1 in controlling microtubule reorganization in response to hormones.

Integrative bioinformatics analyses of Leishmania flagellar genes and proteins revealed that katanin along with profilin and formin are important actin-interacting proteins, which are involved in flagellum assembly, disassembly, and dynamics (27). However, actin-binding properties of katanin have not been experimentally proved so far. In addition, targeted proteomic analysis of mammalian katanin subunits in HeLa cell lines was used for creation of mammalian katanin interaction network (Katan-ome), which plays an important role in microtubule severing (28).

Although some developmental and cellular roles of KATANIN 1 in plants were relatively well established, a comprehensive proteome-wide study on KATANIN 1 mutants was not performed yet. Therefore, the present proteomic dissection of fra2 and ktn1-2 mutants provides an important survey of new proteins linked to phenotypic and microtubule defects of these mutants.

**EXPERIMENTAL PROCEDURES**

**Experimental Design and Statistical Rationale**—Proteomics analyses were carried out with four biological replicates for each of the six biological samples (roots and aerial parts of Col-0, fra2, and ktn1-2). Each replicate contained at least 30 seedlings. Pooling of the specimens was necessary to limit the effects of variations between individual plants. The number of replicates was sufficient to ascertain statistical significance, when analysis of variance (ANOVA) was used to test the differences of protein abundances between biological samples. Because a single factor (wild type and mutants represent one factor) was evaluated and the protein abundance datasets exhibit normal (Gaussian) distribution, it was appropriate to apply one-way ANOVA analysis.

**Plant Material**—Seeds of ktn1-2, fra2, and wild type Arabidopsis thaliana (ecotype Col-0) were surface-sterilized and placed on half-strength MS culture medium (pH 5.7) containing 1% (w/v) sucrose and 0.8% (w/v) phytagel. Plates with seeds were stored at 4 °C for 48 h to break seed dormancy, and afterward kept vertically in a culture chamber under 16 h light/8 h dark at 22 °C. fra2 is a single nucleotide mutant in the seventh exon of KATANIN 1 (At1g80350), where the A at nucleotide residue 2329 is deleted (21). ktn1-2 is a knockout mutant with T-DNA inserted after the 147th nucleotide in the 5th exon of KATANIN 1 (16). Fourteen-day-old seedlings were used for proteomic and immunoblotting analyses. For whole-mount immunolabeling, 3-day-old seedlings of ktn1-2 and fra2 mutants and Col-0 were used.

**Protein Extraction for Proteomic Analysis**—Roots and aerial parts of mutant and control plants were subjected to phenol protein extraction, trypsin digestion, and peptide purification as described previously (29).

Fresh material (250 mg) was homogenized in liquid nitrogen with 500 μl of cold extraction medium (0.9 m sucrose, 0.1 m Tris-HCl (pH 8.8), 10 mM EDTA, 100 mM KCl, and 0.4% (v/v) 2-mercaptoethanol) and an equal amount of Tris-HCl-buffered phenol (pH 8.1). The mixture was incubated for 30 min at 4 °C. Then, the protein-enriched phenol phase was separated from the aqueous phase by 5 min of centrifugation at 6000 × g at 4 °C. Phenol phase was subjected to ammonium acetate/methanol precipitation at −20 °C overnight. The precipitate was then pelleted by centrifugation at 13,000 × g at 4 °C for 20 min followed by two washes with ice-cold 80% (v/v) acetone and 1 wash in 70% (v/v) ethanol. Precipitate suspensions were stored at −20 °C for 15 min for each washing step. Finally, the pellets were resuspended in 80% (v/v) acetonitrile, centrifuged, and air-dried for 10 min. Subsequently they were dissolved in 6 μl urea in Tris-HCl buffer (pH 7.4). After protein content determination (with Bradford assay), equal amounts of proteins were used for in solution digestion. Prior to trypsin application, protein extracts were subjected to a reduction step (by the addition of 50 mM DTT and incubation for 1 h at room temperature), alkylation step (by addition of 50 mM iodoacetamide and incubation at room temperature for 1 h), and the urea concentration was lowered to less than 1 M. The trypsin digestion (1 μg of sequencing grade modified trypsin from Promega per 50 μg of proteins) was performed by permanent gentle shaking at 37 °C overnight. After stopping trypsin digestion by acetic acid, peptides were cleaned on C18 cartridges (Bond Elut C18; Agilent Technologies, Santa Clara, CA) according to manufacturer’s instructions. Peptides eluted by 90% (v/v) acetonitrile were dried using SpeedVac and used for LC-MSMS.

**Detailed Description of Liquid Chromatography, Mass Spectrometry, Protein Identification, and Relative Quantitative Analysis**—Two μg of protein tryptic digest resuspended in 0.1% (v/v) formic acid, 5% (v/v) acetonitrile were loaded on reverse phase fused silica C18 column measuring 75 μm × 150 mm (Thermo Fisher Scientific, Waltham, MA). Peptides were separated and eluted at a constant flow rate of 0.3 μl·min⁻¹ by a 170-min long nonlinear gradient of acetonitrile (in 0.1% formic acid) as follows: 2–55% for 125 min, 95% for 15 min, and 2% for 30 min. The mass spectra were obtained in the data-dependent acquisition mode, with dynamic exclusion being applied, in 18 scan events: one MS scan (m/z range, 300–1700) followed by 17 MSMS scans for the 17 most intense ions detected in MS scan. Other critical parameters were set as given here: normalized collision energy, 35%; automatic gain control “on” with MS² target 4 × 10⁴, isolation width (m/z), 1.5; capillary temperature, 170 °C; spray voltage, 1.97 kV. The method and raw spectral files were created and generated, respectively, by Xcalibur 2.1 (Thermo Fisher Scientific).

The raw files were searched using the SEQUEST algorithm of the Proteome Discoverer 1.1.0 software (Thermo Fisher Scientific) with selection of parameters as follows: minimum and maximum precursor mass, 300 and 6,000 Da, respectively; precursor mass tolerance, 1.5 Da; fragment mass tolerance, 0.8 Da; intensity threshold, 1000; minimum ion count, 7; minimum S/N ratio, 3; enzyme, trypsin; maximum missed cleavages, 2; FDR = 0.01; dynamic (variable) modifications,
cysteine carbamidomethylation (+57.021), methionine oxidation (+15.995), and methionine dioxidation (+31.990).

The spectral data were matched against target and decoy data-bases for more stringent approach to calculating FDR, compared with single search of concatenated database. The NCBI (www.ncbi.nlm.nih.gov) Arabidopsis genus taxonomy referenced protein database (67,924 entries as of November, 2013) served as the target database, and its reversed copy (created automatically by the software) served as a decoy database. The search results were filtered by Xcorr values pertinent to +1, +2, and +3 charged peptides, resulting in FDR <1%. Identified proteins were grouped by default parameters of the soft-ware, defining the group as proteins strictly necessary to explain the presence of identified peptides. A representative/master protein of the group is the protein with the highest score, spectral count, and number of matched peptides. If those parameters are equal, the protein with the longest sequence is designated as a master protein. Proteins listed in the supplemental materials are master proteins; however, all groups proteins, their accession numbers, respective peptides, and annotated spectra are included in “.msf” files (see below for how to view them). If the peptide can be attributed to more than one protein, it is indicated by multiple protein accession num-bers allocated to a given peptide.

The relative quantitative analysis was based on sums of precursor ion intensities (PII) of filtered peptides attributed to given proteins. PII values were extracted from raw files and exported to spread sheets by Proteome Discoverer software. Even though the peptide-respective experimental PII values are not strictly stoichiometric for a given protein, they are commonly accepted for label-free mass spectrometry-based relative protein quantification. Intensities were summed for each identified master protein in each replicate using in house Xcell script. All data points were considered, and no outliers were excluded. Summed intensities pertinent to proteins in individual replicates were normalized by factors that were calculated to equalize total intensity of all master proteins across all biological samples and replicates. Normalized average protein intensities were used to calcu-late fold changes when comparing biological samples. The ANOVA analysis of four replicates for each biological sample was performed, and \( p \leq 0.05 \) was used to filter statistically significant results.

Bioinformatic Evaluation of Proteomic Data—Venn diagram was created using Venny 2.1 on-line application (http://bioinfopg.cnb.csic.es/tools/venny/index.html). Differentially abundant proteins were annotated using Gene Ontology annotation analysis by Blast2Go software (30). Blast was performed against Arabidopsis thaliana NCBI database allowing 1 BLAST Hit. The annotation was performed by using these parameters: E value hit filter, \( 1.0E^{-11002} \)/H11002, Blast2Go software (30). Blast was performed against Arabidopsis (clone YOL1/34; ABD Serotec), or rabbit anti-KNOLLE (31) primary antibodies diluted 1:75, 1:300, and 1:2000, respectively, in 3% (w/v) BSA in PBS at 4 °C overnight. In the case of KNOLLE and tubulin, co-localization a double immunolabeling was performed. Secondary antibodies included Alexa-Fluor 488 goat anti-rat and Alexa-Fluor 546 goat anti-rabbit IgGs (Thermo Fisher Scientific) and were diluted 1:500 in PBS containing 3% (w/v) BSA for 3 h at 37 °C and 1 h at room temperature. Where necessary, nuclei were counterstained with DAPI. Microscopic analyses of immunolabeled samples were examined with a Zeiss 710 LSM platform (Carl Zeiss, Jena, Germany), using excitation lines at 405, 488, and 561 nm from argon, HeNe, diode, and diode-pumped solid-state lasers. Images were processed using ZEN 2010 software, Photoshop 6.0/CS, and Mi-crosoft PowerPoint. Fluorescence intensity was evaluated using ZEN 2010 software (Carl Zeiss). Maximum intensity projections from Z-stack images (15 \( \mu \)m thick) of root epidermal cells were used for measurements. At least five individual root tips were analyzed. Student’s \( t \) test was applied to evaluate the statistical significance of differences. Mitotic tubule orientation and degree of isotropy were eval-uated using CytoSpectre software (35).

Visualization of Actin Using Alexa-labeled Phalloidin—Actin visualization was performed according Panteris et al. (36) with a small modification: after actin stabilization with 200 \( \mu \)m m-maleimido-benzoyl-N-hydroxysuccinimide ester (BSE), whole seedlings were fixed in a mixture of 2.5% (w/v) paraformaldehyde, 0.5% glutaraldehyde, 10 \( \mu \)m BSE, and 0.1 \( \mu \)m Alexa-Fluor 568 phalloidin in microtubule stabilizing buffer (MTSB; 25 mm K-PIPES (pH 6.8), 2.5 mm EGTA and 2.5 mm MgSO4, 1% (v/v) DMSO, 1% (v/v) Triton X-100 in MTSB for 60 min. After washing in MTSB (three times for 2 min), seedlings were extracted in extraction buffer containing 5% (v/v) DMSO, 1% (v/v) Triton X-100 in MTSB for 15 min. Finally, seedlings were stained with 10% (v/v) Alexa-Fluor 488 phalloidin in MTSB for 60 min in the dark. Microscopic analysis of immunolabeled samples was performed using a Zeiss LSM710. Alexa-Fluor 488 phalloidin was excited at 488 nm, and fluorescence was detected between 499 and 566 nm. Images were processed using ZEN 2010 software, Photoshop 6.0/CS, and Microsoft PowerPoint. Actin fil-a-ment orientation and degree of isotropy were evaluated using Cyto-Spectre software (35).
## Table I

List of proteins with significantly different abundances consistently found in fra2 and ktn1-2 mutants as compared with wild type (Col-0). NA = not applicable.

| Accession no. | Description | Sample | Normalized intensity average in Col-0 roots | Normalized intensity average in fra2 roots | Normalized intensity average in ktn1-2 roots | Fold change fra2 vs Col-0 | Fold change ktn1-2 vs Col-0 | p value fra2 vs Col-0 | p value ktn1-2 vs Col-0 |
|---------------|-------------|--------|--------------------------------------------|-------------------------------------------|---------------------------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
| gi5233111     | Cysteine synthase C1 | Roots  | 52115.82                                   | 185447.46                                 | 136112.56                                  | 3.56                      | 2.61                      | 0.05                     | 0.01                     |
| gi5239010     | Aspartate semialdehyde dehydrogenase | Roots  | 55508.12                                   | 230059.47                                 | 125986.93                                  | 4.14                      | 2.27                      | 0.05                     | 0.05                     |
| gi8402225     | Granulin repeat cysteine protease family protein | Roots  | 75658.44                                   | 343248.11                                 | 484897.32                                  | 4.54                      | 5.93                      | 0.01                     | 0.00                     |

### Amino acid metabolism

| Accession no. | Description | Sample | Normalized intensity average in Col-0 roots | Normalized intensity average in fra2 roots | Normalized intensity average in ktn1-2 roots | Fold change fra2 vs Col-0 | Fold change ktn1-2 vs Col-0 | p value fra2 vs Col-0 | p value ktn1-2 vs Col-0 |
|---------------|-------------|--------|--------------------------------------------|-------------------------------------------|---------------------------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
| gi15223910    | Aspartate semialdehyde dehydrogenase | Roots  | 55508.12                                   | 230059.47                                 | 125986.93                                  | 4.14                      | 2.27                      | 0.05                     | 0.05                     |

### Protein synthesis and translation

| Accession no. | Description | Sample | Normalized intensity average in Col-0 roots | Normalized intensity average in fra2 roots | Normalized intensity average in ktn1-2 roots | Fold change fra2 vs Col-0 | Fold change ktn1-2 vs Col-0 | p value fra2 vs Col-0 | p value ktn1-2 vs Col-0 |
|---------------|-------------|--------|--------------------------------------------|-------------------------------------------|---------------------------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
| gi18310833    | 50S ribosomal protein L31 | Aerial parts | 6417292.09                               | 2298501.19                                 | 4254324.45                                  | 0.36                      | 0.66                      | 0.007                    | 0.023                    |
| gi18399110    | Aspartate semialdehyde dehydrogenase | Roots  | 55508.12                                   | 230059.47                                 | 125986.93                                  | 4.14                      | 2.27                      | 0.05                     | 0.05                     |

### Protein folding, chaperones

| Accession no. | Description | Sample | Normalized intensity average in Col-0 roots | Normalized intensity average in fra2 roots | Normalized intensity average in ktn1-2 roots | Fold change fra2 vs Col-0 | Fold change ktn1-2 vs Col-0 | p value fra2 vs Col-0 | p value ktn1-2 vs Col-0 |
|---------------|-------------|--------|--------------------------------------------|-------------------------------------------|---------------------------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
| gi334185828   | Clp ATPase | Roots  | 107823.61                                   | 37454.35                                  | 48712.02                                   | 0.35                      | 0.45                      | 0.02                     | 0.03                     |
| gi8415982     | Tetratricopeptide repeat protein | Roots  | 222702.14                                   | 427357.63                                 | 486498.43                                  | 1.92                      | 2.18                      | 0.03                     | 0.02                     |
| gi5237739     | Cyclophilin ROC7 | Aerial parts | 10957142.37                               | 3607894.57                                 | 3546535.96                                  | 0.33                      | 0.32                      | 0.002                    | 0.004                    |
Quantitative Analysis of mRNA Transcript Levels by Real-time PCR—Total RNA was extracted from roots and aerial parts of 14-day-old seedlings of Col-0, fra2, and ktn1-2 mutants using TRI Reagent® (Sigma-Aldrich) according to the manufacturer’s protocol. After DNase I digestion, RNA concentration and purity were determined with NanoDrop Lite spectrophotometer (Thermo Fisher Scientific). Template-primer mix for reverse transcription was composed of 1 μl of oligo(dT) primers (0.5 μg per reaction), 1.5 μg of RNA, and PCR-grade distilled water in total volume of 20 μl. The mixture was denatured at 65 °C for 5 min. The following components were added: 4 μl M-MLV reverse transcriptase 5X reaction buffer (Promega), 2 μl of deoxynucleotide mix (10 mM), 1 μl (40 units) RNasin® Plus RNase inhibitor (Promega), 1 μl (100 units) of M-MLV reverse transcriptase (Promega), and PCR-grade distilled water, in a total volume of 20 μl. PCRs were performed at 42 °C for 1 h followed by inactivation at 70 °C for 10 min. After reverse transcription reaction, the mixture was diluted four times. Quantitative RT-PCRs were performed in a 96-well plate with StepOnePlus Real-time PCR system (Applied Biosystems, Foster City, CA) using SYBR® Green to monitor dsDNA synthesis. Reaction contained 5 μl of Power SYBR® Green PCR master mix (Thermo Fisher Scientific), 0.75 μl of cDNA (corresponds to 140 ng of RNA before reverse transcription), and 0.5 μl gene-specific primers (supplemental Table S1). The following standard thermal profile was used for all PCRs: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Experiments were run in three biological replicates, and the intra-assay variability was determined with technical triplicates. The expression data were normalized to the expression of EF1α (ELONGATION FACTOR 1-α; At5g60390) as a reference gene, and relative gene expression was calculated by \(2^{-\Delta\Delta Cq}\) method. Specificity of the target amplification was further verified by melting curve analysis of reaction products.

RESULTS

Overview of Proteomic Analysis and Functional Classification of the Differential Proteome—In addition to complete mass spectrometry/proteomics data deposited to PRIDE (see above), the information pertinent to protein identification can be found in the supplemental material in the form of common Excel files given for each individual sample. We compared the proteomes of roots and aerial parts of fra2 and ktn1-2 mutants with Col-0 quantitatively. Only those proteins that differed in abundance more than 1.5-fold between wild type and both mutants have been considered. Forty two proteins, 22 in roots and 20 in aerial parts, were differentially abundant in both mutants and showed a consistent trend in abundance difference (Table I). Twenty six of them were down-regulated, and 16 were up-regulated. Quantification details of all proteins identified in the mutants are presented in supplemental Table S2 (roots) and supplemental Table S3 (aerial parts). We have detected 69 and 45 differentially abundant proteins in roots of fra2 and ktn1-2, respectively. In aerial parts, 53 and 51 differentially regulated proteins were identified in fra2 and ktn1-2 (Fig. 1, A and B). Among them, seven proteins in roots and two proteins in aerial parts were identified only in Col-0, but they were not identified in the mutants. It is likely that differences in proteomes of these two mutants might arise from distinct types of mutations (mentioned under “Experimental Procedures”).

### TABLE I—Continued

| Sample          | Description                                      | Accession no.    | Description                                      |
|-----------------|--------------------------------------------------|------------------|--------------------------------------------------|
| Aerial parts    | NAD(P)-binding Rossmann-fold-containing protein  | gi18404496       | Unknown function                                 |
| Roots           | NAD(P)-binding Rossmann-fold-containing protein  | gi15237739, gi15224648 |                     |
| Roots           | Membrane-associated progesterone binding protein | gi13366748       |                     |
| Aerial parts    | Vacuolar calcium-binding protein-like protein     | gi15237739, gi30682601 |                     |
| Roots           | Membrane transport                               | gi15237739, gi18397991 |                     |
| Aerial parts    | MD-2-related lipid recognition domain-containing protein | gi15237739, gi18397991 |                     |

| Sample          | Description                                      | Accession no.    | Description                                      |
|-----------------|--------------------------------------------------|------------------|--------------------------------------------------|
| Roots           | Unknown function                                 | gi18404496       |                     |
| Aerial parts    | Unknown function                                 | gi15237739       |                     |
| Roots           | Unknown function                                 | gi15224648       |                     |
| Aerial parts    | Unknown function                                 | gi13366748       |                     |


Proteome-wide Dissection of Arabidopsis KATANIN 1 Mutants

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We used gene ontology (GO) annotation to evaluate the impact of defective microtubule severing on the *Arabidopsis* proteome. According to biological process, the highest number of differentially abundant proteins was annotated to diverse metabolic processes (Fig. 2A). A significant number of proteins was also denoted as involved in the response to biotic and abiotic stimuli. Last but not least, GO annotations connected to cellular component organization and development showed changed abundances in both mutants (Fig. 2A). According to molecular functions, differentially abundant proteins were involved in binding to proteins, organic and inorganic compounds, as well as binding to small molecules (Fig. 2B). GO annotation according to cell compartment showed different abundances of proteins localized to plastids, cytosol, mitochondria, ribosomes, nuclei, and vacuoles (Fig. 2C). A detailed GO annotation performed separately for differentially
regulated proteins in roots and aerial parts is provided in supplemental Figs. S1–S6.

Analysis of protein interaction networks occurring among these proteins using STRING application showed deregulation of proteins involved in photosynthesis, carbon metabolism, and translation in both mutants (Fig. 3). Other less abundant networks were annotated to purine nucleotide and amino acid synthesis and chloroplast thylakoid development. The majority of proteins involved in photosynthesis showed decreased abundance in the mutants, whereas carbon metabolism appears to be enhanced. Two proteins contributing to thylakoid development showed unequivocal decreased abundance.
dance. Abundances of proteins involved in translation did not show uniform changes in the mutants. Similar protein networks were predicted when both mutants were analyzed separately (supplemental Fig. S7).

KATANIN1 Mutants Exert Altered Abundances of MAPs, Disturbed Microtubule Organization, and Abnormal Nuclear Shape—Disrupted microtubule severing resulted in severe developmental defects of fra2 and ktn1-2 mutants (21, 23, 26). Several proteins related to microtubule severing may contribute to these developmental phenotypes. Therefore, by performing differential proteomics on fra2 and ktn1-2 mutants, we focused on proteins involved in plant development as classified by gene ontology annotation (supplemental Tables S4–S7) or experimentally (Table II). These proteins are mostly involved in embryogenesis, germination, root growth, and hypocotyl elongation.

Our proteomic analysis revealed several cytoskeletal and cytoskeleton-related proteins. Interestingly, these proteins form a functionally interconnected network consisting of both microtubule-binding as well as actin-binding and regulatory proteins, as predicted by STRING (Fig. 4). Among them, tubulin β-4 was down-regulated in aerial parts of fra2 (Table II). To validate such down-regulation, we carried out an immunoblotting analysis of fra2 and ktn1-2 roots using anti-β-tubulin antibody. In agreement with proteomic data, β-tubulin showed decreased abundances in both mutants (Fig. 5, A–C). As expected, a similar trend was observed in the case of α-tubulin (Fig. 5, D–F). Whole-mount immunofluorescence la-

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**TABLE II**

Differentially abundant proteins having developmental roles in fra2 and ktn1-2 mutants

Proteins annotated in GO cellular component organization (GO:0016043), single organism developmental process (GO:0044767), anatomical structure development (GO:0048856), and proteins reported to have developmental roles are listed.

| Accession no. | Description | Sample | Fold change fra2 vs Col-0 | Fold change ktn1-2 vs Col-0 | GO annotation | Experimental evidence |
|---------------|-------------|--------|--------------------------|-----------------------------|---------------|-----------------------|
| gi15221692    | Pyruvate dehydrogenase E1 component subunit α-2 (IAR4) | Roots  | 0.22                     | 0.26                        | Auxin homeostasis (67, 68) |
| gi42570831    | S-Akyl-thiohydroximate lyase SUR1 | Roots  | Unique in Col-0          | Unique in Col-0            | Auxin homeostasis (66)   |
| gi183897991   | MD-2-related lipid recognition domain-containing protein | Roots  | Unique in Col-0          | Unique in Col-0            | Gravitropism, auxin transport (72) |
| gi18413181    | 14-3-3-like protein GP14 γ | Roots  | Unique in Col-0          | Unique in Col-0            | Regulation of ethylene synthesis (62) |
| gi18402225    | Granulin repeat cysteine protease family protein | Roots  | 4.54                     | 5.93                        | ABA signaling (73)       |
| gi33418582    | Clp ATPase | Roots  | 0.35                     | 0.45                        | GO:0016043     | Photosystem biogenesis, thylakoid membrane biogenesis, leaf development (74) |
| gi7324564     | 40S ribosomal protein S5-1 | Roots  | 0.38                     | 0.43                        | GO:0016043     | Delayed embryo development, abnormal venation (75) |
| gi73917147    | 50S ribosomal protein L4 | Aerial parts | 0.25                     | 0.29                        | Embryo development (76) |
| gi2326646     | TUDOR-SN protein 1 | Roots  | 1.50                     |                             | Embryogenesis and fertility (78) |
| gi15243052    | TUDOR-SN protein 2 | Roots  | 4.48                     | 3.12                        | Embryogenesis and fertility (78) |
| gi15257739    | Cyclophilin ROC7 | Aerial parts | 0.33                     | 0.32                        |                      |
| gi30684767    | ATP-dependent zinc metalloprotease FTSH 2 (VAR2) | Aerial parts | 0.52                     | 0.59                        |                      |
| gi334187997   | Uncharacterized protein | Aerial parts | Unique in Col-0 | Unique in Col-0 | GO:0016043     | Photosystem biogenesis, thylakoid membrane biogenesis, leaf development (74) |
| gi15236386    | Selenium-binding protein 2 | Aerial parts | Unique in Col-0 | Unique in Col-0 | GO:0016043     | Photosystem biogenesis, thylakoid membrane biogenesis, leaf development (74) |
| gi145329204   | Triose-phosphate isomerase | Roots  | 2.47                     | 2.06                        |                      |
| gi15233111    | cysteine synthase C1 | Roots  | 3.56                     | 2.61                        |                      |
| gi15250529    | Chlorophyll a-b-binding protein CP26 | Aerial parts | 2.02                     | 1.49                        |                      |
| gi7325183     | Plasma membrane-associated calmodulin-binding protein 1 (MDP25) | Roots  | 1.61                     |                             |                      |
| gi15241472    | Tubulin β-4 chain | Roots  | 0.20                     |                             |                      |
| gi18401616    | WPP domain-containing protein 2 | Aerial parts | 0.46                     |                             | Cell division and elongation (5), seed imbition (41, 77) |
| gi15224838    | Profilin 1 | Roots  | 1.09                     | 1.48                        |                      |
| gi30697298    | Actin-depolymerizing factor 3 | Aerial parts | 2.09                     | 0.30                        | Cell elongation (79), embryo development and germination (80) |
| gi15245216    | Actin 7 | Aerial parts | 2.09                     | 0.30                        | Tip growth (81)       |
| gi15222929    | V-type proton ATPase subunit B1 | Aerial parts | 1.23                     |                             | Root growth (82), seed imbition (41, 83) |
| gi15219901    | Patellin 2 | Roots  | 0.22                     |                             |                      |
| gi18391066    | 2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase 1 | Roots  | 1.78                     |                             | Actin stabilizing (84) |

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Proteome-wide Dissection of Arabidopsis KATANIN 1 Mutants
beling of microtubules performed on root cells revealed reorganization and randomization of microtubules in root epidermal cells of fra2 and ktn1-2 mutants (Fig. 6). Furthermore, plasma membrane-associated cation-binding protein 1, also called microtubule-depolymerizing protein 25 (MDP25) (37, 38), showed an increased abundance in the fra2 mutant (Table II). In addition, we have found proteins controlling microtubule-dependent processes such as cell division and cell plate formation (patellin 2 and WPP domain-containing protein 2; Table II). This is in agreement with the obliquely oriented cell plates in fra2 and lue1 mutants as it was previously proposed due to the aberrantly oriented phragmoplasts (24). Our more detailed whole-mount immunolabeling of cell plates using specific antibody recognizing cell plate marker protein KNOLLE (34) showed obliquely oriented and misaligned cell plates in the ktn1-2 mutant (supplemental Fig. S8). It seems that patellin 2 and WPP2 are co-regulated with KATANIN 1 to control phragmoplast and cell plate formation. Because WPP2 is also a nuclear envelope localized protein (39), its down-regulation might indicate altered nuclear shape in KATANIN 1 mutants. Consistent with this assumption, vi-

Fig. 4. Depiction of functional protein association networks predicted by STRING among cytoskeletal proteins found to be differentially abundant in fra2 and ktn1-2 as compared with Col-0. Different line colors represent types of evidence used in predicting associations: gene fusion (red); neighborhood (green); co-occurrence across genomes (blue); co-expression (black); experimental (purple); association in curated databases (light blue); or co-mentioned in PubMed abstracts (yellow). ↑ means increased abundance; ↓ means decreased abundance. TUB4 is tubulin β-4 chain; TSN1 is TUDOR-staphylococcal nuclease protein 1, TSN2; MDP25 is microtubule-destabilizing protein 25; PATL2 is patellin 2; PRF1 is profilin 1; ACT7 is actin 7; ADF3 is actin-depolymerizing factor 3; ANNAT1 is annexin 1; and VAB1 is V-type proton ATPase subunit B1.

Fig. 5. Immunoblotting analysis of β-tubulin and α-tubulin in roots of Arabidopsis wild type Col-0 and KATANIN 1 mutants fra2 and ktn1-2. A and D, immunoblots probed with anti-β-tubulin (A) and anti-α-tubulin (D) antibodies. B and E, visualization of proteins transferred on nitrocellulose membranes. C and F, optical density quantifications of respective bands in A and D. Asterisks indicate significant differences between mutants and wild type at p < 0.05 according to Student’s t test. Error bars represent standard deviations.
visualization of nuclei by DAPI revealed that nuclei of fra2 and ktn1-2 root epidermal cells showed aberrant shapes (supplemental Fig. S9).

We have also detected increased abundances of TUDOR-staphylococcal nuclease protein 1 (TSN1) and TSN2 in the fra2 mutant (Table II). These proteins are co-localizing with and move along cortical microtubules (32). They are involved in the formation of stress granules, which is dependent on microtubule dynamics (32). Thus, our results support a tight link between TSN proteins and microtubules. Immunoblotting analysis using primary antibody recognizing both TSN1 and TSN2 verified increased abundances of these two isoforms in fra2 and ktn1-2 roots (Fig. 7). This was further confirmed by immunolocalization of TSN proteins in intact roots of fra2 and ktn1-2 mutants (Fig. 8). Root cells of both mutants showed prominent accumulation of TSN proteins preferentially at cell peripheries and in the whole cytoplasm that was not so prominent and was quantitatively less abundant in the control Col-0. Altogether, these data indicate a feedback mechanism controlling microtubule organization in KATANIN 1 mutants.

Defective Microtubule Severing Altered Actin Regulatory Proteins and Actin Organization—Notably, defective microtubule severing in the mutants affected abundances of actin and actin-binding proteins (ABPs). Thus we have found up-regulation of profilin 1 in roots of the ktn1-2 mutant and up-regulation of actin 7 in fra2 aerial parts (Table II). The abundance of actin-binding vacuole-type proton ATPase subunit B1 was also increased in aerial parts of fra2 (Table II). Verifi-
cation of actin abundances in the mutants and Col-0 control using immunoblotting with an antibody recognizing denatured monomeric G-actin approved the proteomic data (Fig. 9). In addition, actin-depolymerizing factor 3 was negatively regulated in the ktn1-2 aerial parts (Table II). Importantly, visualization of F-actin by staining with fluorescently labeled phalloidin showed that proteomic changes of actin 7 isoform (up-regulation) and actin-depolymerizing factor 3 (ADF3, down-regulation) led to reorientation and disorganization of actin filaments in leaves of fra2 and ktn1-2 mutants. Thus, actin filaments in the leaf epidermal cells of both mutants were more distorted, less abundant, and also less bundled in these cells (Fig. 10). These results suggested a tight cross-talk between microtubules and organization of the actin cytoskeleton in KATANIN 1 mutants.

Abundances of Cytoskeletal Proteins and Transcript Levels of Corresponding Genes Are Not Correlated in the KATANIN1 Mutants—KATANIN 1 mutants exhibit severe developmental defects that may cause general transcriptional reprogramming. This may imply that the changes of cytoskeletal proteins in KATANIN 1 mutants resulted from altered transcriptional regulation and not from direct effects of impaired microtubule-severing activity. Therefore, we quantitatively examined expression levels of genes encoding the most-important 11 cytoskeletal and cytoskeleton-related proteins found by proteomic analysis. In all cases, except TUBULIN β-4 (TUB4) in fra2, we have found only minor insignificant changes in the mRNA levels of both KATANIN 1 mutants as compared with the control (Fig. 11). This indicates that changes in protein abundances were not caused by transcriptional alterations in the mutants.

Proteins Involved in Hormonal Homeostasis Showed Altered Abundances in KATANIN 1 Mutants—Generally, it is known that microtubule cytoskeleton is sensitive to hormones (40), whereas abundance of TUBULIN α2 (TUA2) is controlled by gibberellic acid in Arabidopsis (41). In addition, KATANIN 1 participates in the regulation of microtubule reorganization induced by gibberellic acid and ethylene (26). Interestingly, we have found several proteins involved in the regulation of auxin, ethylene (Table II), and gibberellic acid (Tables III and IV) homeostasis. Moreover, a screening of abscessic acid-responsive elements in the promoter sequences of genes encoding identified differentially regulated proteins indicated that proteins involved in ABA response might be also affected by impaired microtubule severing (Table V). Thus, our study also provides new protein candidates for the cross-talk between hormones and microtubules.

DISCUSSION

Although proteomics are effective in identification of proteins regulating cytoskeletal organization, it has been only rarely exploited for this aim in current plant-oriented research. Targeted proteomic approaches were used to identify microtubule-binding proteins (42), whereas proteomic analysis of detergent-resistant and -sensitive membranous fractions combined with cytoskeletal inhibitor treatments revealed the cytoskeleton-dependent distribution of plasma membrane proteins (43). Recently, changes in the abundance of proteins involved in the vesicular transport, RNA nuclear export, and ABA response were reported in response to the actin-depolymerizing drug latrunculin B (44). In mammalian cells, where microtubule severing is more complex as in plants, a proteomic approach was adopted to define the protein interaction module consisting of katanin, katanin-like protein isoforms, and various microtubule-associated proteins (28). However in plants, neither altered cytoskeletal protein profiles nor cross-talk to the actin cytoskeleton was reported in the mutants defective in microtubule-associated protein so far. This study combining genetic (mutants), proteomic, biochemical, and cell biological approaches provides new evidence that defects in microtubule severing by KATANIN 1 caused significant changes in abundances of tubulins, MAP, actin, and ABPs concomitant to global reorganization of microtubules and actin cytoskeleton. In this way, this study revealed a feedback mechanism in the regulation of microtubule organization and uncovered a novel cross-talk mechanism between microtubule and actin cytoskeleton in Arabidopsis.

Defective Microtubules and Actin Cytoskeleton in KATANIN 1 Mutants—It is generally accepted that defective microtubule severing impairs cell elongation and promotes isotropic growth leading to profound phenotypic manifestations in KATANIN 1 mutants (20, 21, 26). KATANIN 1 is activated by Rho GTPase ROP6 via binding to the activator RIC1 (19). Microtubule severing by KATANIN 1 is also regulated by the microtubule-associated protein SPIRAL2 defining where the severing occurs (18). Our proteomic analysis revealed that roots of fra2 mutant exerted a decreased abundance of TUB4 and increased abundance of TSN1, TSN2, as well as microtubule-severing protein MDP25.

TSN proteins are components of cytoplasmic messenger ribonucleoprotein (mRNP) complexes called stress granules. Such stress granules are sites of post-transcriptional gene
silencing, and TSN proteins are important for stress-induced mRNA decapping thus modulating the abiotic stress responses. Importantly, stress granules assemble under stress conditions in a KATANIN 1-dependent manner, and TSN proteins co-localize and move along cortical microtubules (32). This might suggest their potential role in the regulation of microtubule organization. Our data indicate that abundances and subcellular accumulation of TSN proteins are dependent on microtubule severing. MDP25 is a plasma membrane-associated protein, which dissociates from the membrane in a calcium-dependent manner exerting inhibition of microtubule polymerization (37). Interestingly, MDP25 overexpression leads to reduced cell elongation and cortical microtubule reorientation (37), similarly to KATANIN 1 mutants. Along with reorganization of microtubules in both KATANIN 1 mutants, it suggests a feedback microtubule control and possible link between MDP25 and microtubule severing in Arabidopsis. Moreover, MDP25 is also capable of calcium-dependent binding to F-actin and its severing (38). Another link to the actin cytoskeleton is provided by co-expression of TSN2 with ACTIN7 (Fig. 4). Our proteomic analysis revealed alterations in actin and important ABPs (PRF1 and ADF3) in KATANIN 1 mutants, very likely contributing to disturbances in the actin organization. This was consistent with reoriented and distorted actin filaments, which we observed in leaf epidermal cells of fra2 and ktn1-2 mutants. Moreover, we found up-regulation of annexin 1 in aerial parts of both mutants. Interestingly, phosphorylation of annexin A2 (closely related to

**Fig. 8.** A, immunolocalization of TSN1/2 (TUDOR-staphylococcal nuclease protein 1/2) in root epidermal cells of Arabidopsis wild type Col-0 and KATANIN 1 mutants fra2 and ktn1-2. B, fluorescence intensity quantification of immunolabeled TSN1/2 in root epidermal cells of wild type Col-0 and fra2 and ktn1-2 mutants. Maximum intensity projections from z-stack images (15 μm thick) of root epidermal cells were used for measurements. At least five individual root tips were analyzed. Differences between both mutants and Col-0 were statistically significant (p ≤ 0.05) according to Student’s t test. Bar, 20 μm.
 annexin 1) was found to be essential for actin cytoskeleton dynamics (45). Cross-talk between microtubule and the actin cytoskeleton was widely documented (46–48). Quantitative live cell imaging showed that reorganization and reassembly of actin microfilaments is dependent on microtubules following drug-induced depolymerization (49). Altered actin organization related to microtubule severing was not described yet. Here, we show that disturbed microtubule severing in the ktn1-2 mutant might also have direct impact on the actin organization, which is mediated by increased abundances of MDP25 and profilin 1 but decreased abundance of ADF3. In fact, the equilibrium of profilins and ADFs directs the actin filaments to polymerization or depolymerization (50). MDP25 is a promising candidate potentially linking KATANIN 1 with actin microfilaments. Further targeted analyses would be beneficial to experimentally study interactions between katanin and ABPs.

Katanin Mutants Exerts Aberrant Nuclear Shape Likely Caused by Deregulation of WPP2 and HSC70-1—Roots of fra2 mutant possess substantially decreased levels of WPP domain-containing protein 2 (WPP2). WPP2 is localized to the nuclear envelope in interphase cells and to immature cell plates during cytokinesis (39). This was consistent with changed nuclear shapes in both KATANIN 1 mutants as revealed by DAPI staining. WPP proteins bind to WPP domain-interacting tail-anchored protein (WITs) and facilitate their nuclear envelope targeting (51). The same binding and targeting activity were assigned to heat shock cognate protein 70-1 (HSP70-1), which also interacts with WPPs (51). HSP70-1, unlike WPP2, showed an increased abundance in the fra2 roots, suggesting an altered equilibrium in these two mechanisms of WIT nuclear targeting. WITs are constituents of plant Klarlicht/ANC-1/Syne-1 homology (KASH)—Sad1/UNC-84 (SUN) complex controlling nuclear morphology and movement (52). Recently, it was shown that WIT2 proteins interact and recruit myosin XI-1 to the nuclear envelope and link KASH–SUN complexes with actin cytoskeleton (53), which is reorganized in the KATANIN 1 mutants. Our results suggest that actin-dependent nuclear shape control in plants through WPP2 and WIT might be linked to microtubule severing by KATANIN 1.

Proteins Associated with Cell Plate Alterations in KATANIN 1 Mutants—In dividing cells, WPP2 might contribute to the emergence of obliquely oriented and misaligned cell plates in KATANIN 1 mutants (this study and Ref. 21), which was tested here by co-immunolocalization of KNOLLE, a bona fide cell plate marker (34), and microtubules in the phragmoplast. Additionally, patellin 2 (down-regulated in the ktn1-2 mutant roots) is a cell plate associated protein involved in the maturation of the cell plate (54). Recently, it was identified as a phosphorylation target of mitogen-activated protein kinase 4 (MPK4), whereas patellin phosphorylation by MPK4 altered its binding to phosphoinositides (55). The role of MPK4 in cell division is more complex, because it is also involved in phragmoplast formation through phosphorylation of phragmoplast-localized microtubule-associated protein MAP65-1 (9, 56–58). Together, WPP2 and patellin 2 may represent protein candidates co-regulated with KATANIN 1 during cytokinesis. In conclusion, phenotypes of fra2 and ktn1-2 mutants are likely determined by wider deregulation of developmentally important cytoskeletal and cell plate proteins.

Proteins Associated with Hormone Homeostasis in KATANIN 1 Mutants—It is well known that microtubule organization is responsive to plant hormones (40). KATANIN 1 was proposed as a mediator of GA- and ethylene-induced microtubule rearrangements in Arabidopsis (26). Mutant lue1 exhibits increased AtGA20ox1 expression levels, a key oxidase enzyme in the gibberellin biosynthesis. Hormonal responses of lue1 to ethylene and gibberellins caused inappropriate cortical microtubule reorientation during cell growth (22). Other proteins regulating GA biosynthesis in Arabidopsis, like TSN1 and TSN2 and glycine-rich RNA-binding protein 7 (GRP7) (59, 60), were differentially abundant in KATANIN 1 mutants. Although TSN1 expression positively correlates with AtGA20ox1 in Arabidopsis (59), GRP7 appears to have a negative role (60).

In fra2 we encountered also changed abundances of proteins involved in ethylene biosynthesis. Thus, 1-amino-cyclopropane-1-carboxylic acid oxidase 2, catalyzing oxygen-dependent conversion of 1-amino- cyclopropane-1-carboxylic acid to ethylene (61), was up-regulated. Moreover, 14-3-3-like protein GF14ω, found in Col-0 but not in the KATANIN 1 mutants, was reported to control ethylene synthesis through down-regulation of ubiquitin ligases targeting 1-amino-cyclopropane-1-carboxylic synthase for degradation (62). In contrast, enzymes controlling synthesis of ethylene precursor methionine, such as methionine synthase, S-adenosylmethionine synthetase 4, as well as 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (cobalamin-independent methionine synthase) (63), were down-regulated. Methionine is a precursor of S-adenosyl-L-methionine being a major methyl group donor for trans-methylation reactions (63). Adenosine kinase maintaining general
S-adenosyl-L-methionine-dependent methylation activities (64, 65) was up-regulated in the fra2 mutant.

Our results point also to altered auxin homeostasis in KATANIN 1 mutants. S-Alkyl-thiohydroximate lyase SUR1 (also known as SUPERROOT1), a protein involved in auxin biosynthesis, was detected only in wild-type roots suggesting down-regulation in the KATANIN 1 mutants. Suppression of SUR1 results in heavy accumulation of auxin in Arabidopsis (66), and thus lower abundance in KATANIN 1 mutants may indicate some defects in auxin homeostasis. This might be also influenced by pyruvate dehydrogenase E1 component subunit α-2, also called IAA-ALANINE-RESISTANT 4 (IAR4), which was strongly decreased in abundance in both KATANIN 1 mutants, and it is implicated

Fig. 10. Filamentous actin (F-actin) organization in leaf epidermal cells of Arabidopsis wild type Col-0 and KATANIN 1 mutants fra2 and ktn1-2. A, Alexa phalloidin was used for F-actin visualization. B, F-actin orientation and degree of isotropy analyzed by CytoSpectre software. Note reorientation of F-actin and higher anisotropy in both mutants in contrast to Col-0. Moreover, actin filaments are less prominent and look distorted in both mutants. Bar, 20 μm.
in IAA homeostasis (67, 68). Altogether, we show that, except for ethylene and GA, microtubule severing is likely linked to homeostasis of abscisic acid and auxin. The mechanism of hormonal regulation by KATANIN 1 is not known.

One possible explanation is suggested by changes in enzymes responsible for methylation in the KATANIN 1 mutants, because they have been shown to control hormone homeostasis in plants (69).

**TABLE III**

List of differentially abundant proteins in fra2 and ktn1-2 mutants (as compared with Col-0 wild type; p ≤ 0.05), which are responsive to gibberellic acid as reported in transcriptomic study (86)

| Accession  | Protein name                                      | Fold change fra2 vs. Col-0 | Fold change ktn1-2 vs. Col-0 |
|------------|--------------------------------------------------|----------------------------|-----------------------------|
| gi18391066 | 2,3-Bisphosphoglycerate-independent Mutase 1      | 1.78 (root)                | 1.78 (root)                 |
| gi334182565| Salt tolerance-related protein                    | 0.57 (aerial parts)        | 0.55 (root)                 |
| gi79313261 | PYK10-binding protein 1                           | 0.54 (root)                | 0.55 (root)                 |
| gi15235401 | Glutathione S-transferase F2                      | 2.4 (root)                 | 3.27 (root)                 |
| gi79326500 | Putative cinnamyl alcohol dehydrogenase 9         | 2.95 (aerial parts)        | 2.95 (aerial parts)         |

Stomatal movements, pollen development (85)
Salt tolerance (87)
Defense (88)
Stress tolerance (89)
Lignification (90)
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Because of the substantial impact on agriculturally important crop traits, cytoskeleton and cytokinetic-associated proteins serve as perspective subjects of genetic engineering for biotechnological applications (70). Our study strengthens this view and provides proteome framework for development-related defects related to KATANIN 1 function. Thus, genetic modification of KATANIN 1 may be considered as a tool to modify plant growth and development.

In conclusion, genetic disruption of microtubule severing in fra2 and ktn1-2 mutants shows a strong impact on the abundance of tubulins, MAP, actin, and ABPs and on the organization of both microtubules and actin filaments. Thus, our study opens a door to investigate these new aspects of feedback microtubule control and cross-talk between microtubules and actin cytoskeleton in plants involving KATANIN 1.

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DATA AVAILABILITY

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (71) partner repository with the dataset identifier PXD005917 (http://www.ebi.ac.uk/pride/archive/). In addition to “.raw” data files, the “.msf” results files are available to download. They can be viewed free of charge using Proteome Discoverer demo/viewer (https://portal.thermo-brims.com/).

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