Research Article

Identification of Aluminum Responsive Genes in Al-Tolerant Soybean Line PI 416937

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Soybean is one of the most aluminum (Al) sensitive plants. The complex inheritance of Al tolerance trait has so far undermined breeding efforts to develop Al-tolerant soybeans. Discovering the genetic factors underlying the Al tolerance mechanisms would undoubtedly accelerate the pace of such endeavor. As a first step toward this goal, we analyzed the transcriptome profile in roots of Al-tolerant soybean line PI 416937 comparing Al-treated and untreated control plants using DNA microarrays. Many genes involved in transcription activation, stress response, cell metabolism and signaling were differentially expressed. Patterns of gene expression and mechanisms of Al toxicity and tolerance suggest that Cys2His2 and ADR6 transcription activators, cell wall modifying enzymes, and phytosulfokines growth factor play role in soybean Al tolerance. Our data provide insights into the molecular mechanisms of soybean Al tolerance and will have practical value in genetic improvement of Al tolerance trait.

1. Introduction

Aluminum (Al) toxicity is a major constraint of crop production on acid soils. In view of the fact that 40% of world's arable land is acidic [1, 2], Al toxicity remains a major hurdle for increasing world food, fiber, and fuel production particularly via expansion of cultivation into acid soils. Aluminum inflicts a wide range of cellular injuries in plants that ultimately result in reduced root growth, nutrient and water uptake, and productivity [1, 2]. Plants possess some degree of tolerance to Al toxicity that varies among species and genotypes [1, 3–6]. Al tolerance mechanisms include exclusion and internal detoxification. Al exclusion via rhizosphere Al-organic acid anion complex formation is the most widely documented physiological mechanism of Al tolerance in cultivated and wild plants alike [1, 7]. Root-exuded citrate, malate, and oxalate are the key organic acid anions involved in such mechanism. Genes involved in Al-induced root exudation of malate and citrate have been cloned in wheat [8] and sorghum [5], and their variants are being discovered in several plant species. Internal detoxification mechanisms involve the formation of Al complexes with organic acids, acidic polypeptides, and/or proteins and subsequent sequestration of Al in organelles away from sensitive sites in the cell [9, 10]. The genetic components of the internal detoxification pathways are yet to be elucidated.

In soybean, Al tolerance is a complex trait perhaps involving several genes and pathways [11, 12]. Quantitative trait loci (QTL) mapping in a population derived from Al tolerant PI 416937 and Al sensitive Young has revealed five DNA markers associated with Al tolerance [11]. Most of the alleles were derived from Al-tolerant PI 416937. Other reported soybean Al tolerance genes include phosphoenolpyruvate carboxylase (PEPC), homolog of translationally controlled tumor proteins (TCTPs), inosine 5′-monophosphate dehydrogenases (IMPDHs) [13], aluminum-induced 3-2 (Sali3-2), and aluminum-induced 5-4a (Sali 4-5a) [14]. Ermolayev
et al. [13] and Ragland and Soliman [14] used gene expression as a tool to identify the above genes but the techniques used in these experiments were not sensitive enough to detect large number of genes that might be expected from the quantitative nature of soybean Al tolerance trait. The objective of this study was to discover putative Al tolerance genes in Al-tolerant soybean line PI 416937 using DNA microarrays—a robust genome-wide transcript profiling technology. Such an approach was recently employed in wheat [15, 16], maize [17], Arabidopsis [18], and Medicago truncatula [19, 20] to discern the molecular basis of Al tolerance in the respective species.

2. Materials and Methods

2.1. Plant Genotype and Growth Conditions. An Al-tolerant soybean plant introduction (PI 416937) highly characterized for Al response [12, 21] was used in this experiment. Seeds were surface sterilized with 20% household bleach (Clorox) in water for 12 min, rinsed with distilled-deionized water several times, and were germinated in deionized water moistened standard germination paper at 25°C in an incubator for 72 h. Seedlings uniform in tap root length were transferred to black-painted pots filled with approximately 4L of 800 μM CaCl₂ background solution with 10 μM Al added (treated) or no Al added (control) in a Conviron growth chamber (16/8 h light/dark cycle with respective duration of the experiment. After 2, 12, 48, or 72 h of Al treatment 1cm sections of the primary root tips of approximately 15 plants/pot were harvested, immediately flash frozen in liquid nitrogen, and stored at −70°C for RNA extraction. Three independent replications were used per treatment.

2.2. RNA Extraction, Microarray Procedure, and Data Analysis. Total RNA was extracted from 100 mg root tissue samples using Qiagen RNeasy plant RNA isolation kit following the manufacturer’s protocol (Qiagen, Inc.). The Affymetrix GeneChip Soybean Genome Array with over 68,000 probe sets, Glycine max L. and wild soybean combined, was used for microarray analysis of the soybean genome for Al tolerance. Three chips were used per treatment. Detailed procedures for RNA labeling and array analysis are described in the Manufacturer’s GeneChip Expression Technical Manual (Affymetrix). Briefly, the quality of total RNA was determined using the RNA 6000 Nano Chip on Agilent BioAnalyzer 2100 prior to double-stranded cDNA synthesis. Total RNA in the amount of 2 μg was used for double-stranded cDNA generation by linear amplification using oligo dT-T7 primer and reverse transcriptase (RT). Subsequently, biotin-labeled cRNA was synthesized by in vitro transcription (IVT) using the ENZO High Yield IVT kit (ENZO). Quality and quantity of cRNA were assessed using the RNA 6000 Nano chip on Agilent BioAnalyzer 2100. Fifteen-microgram cRNA was used for hybridization.

Arrays were hybridized overnight at 45°C for 16 h in GeneChip Hybridization Oven 640 (Affymetrix). The next day, arrays were washed and stained in the Fluidics Station 450 (Affymetrix) and scanned by the High Resolution GeneChip Scanner 3000 (Affymetrix).

Gene expression values were determined using the GeneChip Operating Software (GCOS 1.1, Affymetrix). The expression levels were subjected to data query and data mining in Data Mining Tool (DMT). Statistical Analysis of the data was conducted using the software packages ArrayAssist Enterprise together with Pathway Assist (Strata-gene/Agilent, Santa Clara, CA). The raw GeneChip files from GeneChip Operating Software (GCOS, Affymetrix, CA) were uploaded, background-subtracted, variance stabilized, and normalized with GC-RMA method [22]. The control group was used as a baseline to calculate the intensity ratio/fold changes of the treatment versus control. The ratio was log-transformed before further statistical analysis. The P-values were obtained by an unpaired t-test assuming unequal variance. Significantly upregulated and downregulated genes were annotated using protein databases accessed by blastx at National Center for Biotechnology Information (NCBI).

2.3. Quantitative Real-Time PCR. Quantitative real-time PCR quantification of transcript levels for representative genes [Gma. 20326: F-5'-tcacctcctccattctatgg-3', R-5'-tcatggttggtggtggtt-3'; Gma. 6948: F-5'-ttatctccggcgaaacctct-3', R-5'-tctggtctgtctgatgttaag-3'; Gma.12326: F-5'-agggcactcaagttggctggc-3', R-5'-tctcctctcctcctcctc-3'; Gma. 24062: F-5'-tgccaagatctcttaac-3', R-5'-cgaggtatggtctgg-3'; Gma.26937: F-5'-tacccaaagccgggact-3', R-5'-ggccggaatttaccccacatc-3'; Gma.4156: F-5'-ttcaactgcatcttctc-3', R-5'-tagggacactctgcccact-3'; Gma.2577: F-5'-acgccgctaggtgtaaac-3', R-5'-acatcagggcatacct-3'] from microarray experiments was conducted using the Roche Diagnostics Light Cycler 480 System with SYBR green detection (Roche Diagnostic, Corp) using beta-tubulin gene (beta-tubulin: F-5'-ccatgctcataaatggttcagc-3', R-5'-tgccgaaggatcatctcaac-3') as internal control. mRNA was isolated from plants grown under similar experimental conditions as in the microarray experiments. mRNA extraction and quality test was as described above. RNA samples were treated with Applied Biosystems Turbo DNA-free DNase (Ambion, Inc.) to remove DNA contamination. Briefly, 2 μl 10x DNase I buffer and 1 μl rDNase I were added to 20 μl RNA sample, and the mix was incubated at 37°C for 30 minutes in water bath. Subsequently, 2 μl resuspended DNase inactivation reagent was added and the samples mixed well and incubated at room temperature for 3 minutes. Samples were then centrifuged at 10 000 g for 1.5 min (Eppendorf centrifuge 5415 D) in 1.6 ml centrifuge tubes and supernatants transferred to fresh tubes.

cDNA was synthesized from 1 μg DNase-treated RNA samples using the Roche Diagnostics Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Corp) according to manufacturer’s protocol. cDNA concentration and quality was determined using NanoDrop Spectrophotometer brand ND-1000 (NanoDrop Technologies, Inc.). cDNA samples were diluted with nuclease-free water in
varying ratios ranging from 1:4 to 1:10 depending on sample concentration. A total reaction volume of 11 µl comprising 2 µl cDNA sample, 2 µl each of the reverse and forward primers at 0.2 µM concentration, and 5 µl SYBR mix was prepared in 96-well plates (Roche Diagnostics) in two biological and three technical replicates for each gene. A real-time PCR profile of preincubation at 95°C for 5 min, a 45-cycle amplification at 95°C for 10 second, 55°C for 20 second, and 72°C for 20 second, melting at 95°C for 1 min, 65°C for 1 min, and 95°C continuous, and cooling at 40°C for 30 seconds was used to amplify the samples. Negative controls in which cDNA sample was replaced with PCR grade water for each primer pair were included in each run. Sample wells were individually assessed for data quality by evaluating amplification curves and PCR product specificity was verified by melting curve analysis. The expression level of target genes was normalized using in-run beta-tubulin gene as internal control, and transcript concentration ratios were calculated using the ΔΔC_T-Method [23]. The change in gene expression levels (fold change) was calculated as treatment to control ratio and compared with results from microarray.

3. Results and Discussion

3.1. Gene Expression in Response to 2-Hour Al Treatment. A total of 38 genes were identified as differentially expressed in the 10 µM Al-treated experimental plants compared to no Al added controls at 2 h post Al treatment (Figure 1). Thirty-four of them were upregulated and 4 were downregulated with a fold change ranging from 3.08 to 32.55 (Table 1).

3.2. Gene Expression in Response to 12- and 72-Hour Al Treatment. At 12 and 72 h post treatment only one gene each showed significant change in expression in response to Al treatment (Figure 1 and Table 1).

3.3. Gene Expression in Response to 48-Hour Al Treatment. The highest number of differentially expressed genes was detected at 48 h post Al treatment (Figures 1 and 2). A total of 542 genes (97.2% upregulated and 2.8% downregulated) were detected. Those exceeding 13-fold changes are presented in Table 2. The marked fold differences observed in the current research are substantially higher in comparison with results obtained by most authors but are comparable to results of [18, 24]. There were two genes in common between the set of genes detected at 2 h and 48 h post treatment (Gma.2577, 7-fold downregulated at 2 h and 8-fold upregulated at 48 h and Gma.26937, 8-fold downregulated at 2 h and 115 upregulated at 48 h). Similar patterns of gene expression were observed in Arabidopsis roots under Al stress with few overlaps between sets of genes detected at 6 h and 48 h post Al treatment [18].

The temporal pattern of Al-induced gene expression changes observed in this study diverges from results of other authors. At 12 and 72 h, almost no genes were differentially expressed or detected. The virtually no detection of Al-regulated genes at 12 and 72 h post treatment seems a little odd but it is what is expressed in this soybean genotype to Al stress. Among the few reported Al microarray studies, the results of Kumari et al. [18] in Arabidopsis is the closest to ours with regard to the number of genes detected at early and late time points. They detected 127 genes at 6 h post treatment and 733 genes at 48 h post treatment using a threshold of a 2-fold change whereas we detected 38 genes at 2 h and 542 at 48 h using a 3-fold change.

All of the differentially expressed genes that were functionally annotated by the Genbank nonredundant protein database were grouped into five functional categories based
on their putative cellular function. The functional classification showed that stress- and metabolism-related genes constitute the major fractions of Al-regulated genes (Figure 3).

3.4. Quantitative Real-Time PCR Validation of Microarray Expression Levels. The microarray gene expression levels were validated with quantitative real-time PCR for representative genes (Figure 4). In general, the microarray results were in agreement with qRT-PCR but in a few cases quantitative RT-PCR gave higher levels of expression compared to microarray. Such results are obtained by a number of investigators [16, 20, 25]. Detail discussion of factors contributing to the discrepancy between microarray and RT-PCR gene expression levels is covered in [26]. Many authors attribute the phenomenon to the high dynamic range and greater sensitivity of PCR detection. It is worth noting that the gene expression kinetics depicted in Figure 1 shows the efficacy of our experimental design in capturing the full dynamic range of gene expression profiles in the soybean genotype studied. Gene expression peaks at 2 and 48 h suggesting that major savings in microarray experimental expenditure could be realized by limiting sampling to these time points in future experiments.

3.5. Differentially Regulated Genes by Functional Category

3.5.1. Genes Related to Transcription Factors. A number of transcription factors including bZIP, WRKY, MYB, ADR6, and NAc were highly upregulated in the present study (Tables 1 and 2). Members of these families of transcription factors were previously detected under Al stress in several plant species [16, 18–20, 27]. Cys2His2-type zinc finger (bZIP) and auxin downregulated (ADR6) factors are particularly interesting from Al tolerance perspective. Cys2His2-type zinc finger (bZIP) protein coregulates molecular response to proton and Al toxicities [28]. It controls the expression of ALMT1—a malate transporter protein that acts in Al exclusion mechanism. In this study, Cys2His2 (Gma.4526, Table 2) was upregulated 51-fold at 48 h post treatment suggesting that malate plays a major role in Al tolerance mechanism of PI 416937 soybean. Earlier physiological study by Silva et al. [29] showed that Al stress increases exudation of both malate and citrate during the first 6 h of exposure to Al in both tolerant and sensitive soybean types. But they concluded that the sustained accumulation and exudation of citrate is mainly responsible for the genotypic differences in Al tolerance. In the present work, 48 h after Al exposure the malate transporter regulator protein was highly expressed in contrast with the observation of Silva et al. [29]. We postulate that Cys2His2 might regulate the expression of other Al tolerance genes in addition to malate transporter. It is also possible that malate biosynthesis becomes a limiting step or malate might indeed play a major role in soybean Al-tolerance contrary to earlier conclusions. ADR6 transcription factors were previously reported as Al tolerance genes [14, 18]. In the present study, ADR6 was highly upregulated (14-fold, Table 2). The plant hormone auxin and ADR6 exhibit opposite behavior in plant roots under Al stress. Al has been shown to inhibit auxin biosynthesis and transport genes as one possible mechanism of its toxicity [18]. On the contrary, ADR6—an auxin downregulated transcription factor is induced under Al stress perhaps mimicking auxin’s role of promoting root growth. These observations suggest that Cys2His2 and ADR6 transcription factors are important modulators of soybean molecular response to Al stress.

3.5.2. Genes Related to Transporters. Transporters, specifically malate (ALMTs) and citrate (MATE) transporters are the first Al tolerance genes cloned in plants and represent the
Table 1: Aluminum-regulated genes in soybean genotype PI 416937 2 h post aluminum treatment.$^\dagger$

| Unigene ID | Fold change   | Average ± SD | Functional category | Annotation                                                                 | e-value |
|------------|---------------|--------------|---------------------|---------------------------------------------------------------------------|---------|
| Gma.18664  | 32.55 (down)  | −0.88 ± 0.06 | Stress response     | **Anionic peroxidase/oxidative stress**                                    | $6e − 66$ |
| Gma.4152   | 29.89 (up)    | 4.39 ± 0.13  | Stress response     | **Trypsin and protease inhibitor**                                         | $2e − 20$ |
| Gma.17961  | 29.13 (up)    | 4.63 ± 0.14  | Stress response     | **Soybean oleosin isoform B**                                              | $2e − 22$ |
| Gma.26984  | 27.751 (up)   | 3.96 ± 0.05  | Stress response     | **Putative protease inhibitor**                                            | $4e − 35$ |
| Gma15007   | 24.75 (up)    | 3.77 ± 0.24  | Metabolism          | **Ferredoxin 2 protein**                                                   | $2e − 46$ |
| Gma.29855  | 11.98 (up)    | 1.81 ± 0.15  | Metabolism          | **Ribulose-1, 5-bisphosphate carboxylase**                                 | $5e − 100$ |
| Gma.18110  | 11.36 (up)    | 2.87 ± 0.19  | Unclassified        | **Cp12-2 Protein/peptide cross-linking**                                  | $9e − 29$ |
| Gma.26937  | 8.29 (down)   | −0.61 ± 0.03 | Unclassified        | **Unknown**                                                               | —       |
| Gma.10987  | 8.03 (up)     | 2.76 ± 0.09  | Metabolism          | **Ribulose bisphosphate carboxylase**                                      | $1e − 33$ |
| BE024005   | 7.58 (up)     | 3.41 ± 0.27  | Stress response     | **Glutaredoxin family protein/glutathione-dependent reductase**            | $6e − 24$ |
| Gma.2577   | 7.3 (down)    | −0.86 ± 0.02 | Metabolism          | **Hydrolase family protein**                                               | $7e − 30$ |
| Gma.21354  | 6.51 (up)     | 2.59 ± 0.12  | Transcription factor | **NAc1 domain protein Plant development protein Zinc finger**                | $1e − 61$ |
| Gma.4156   | 6.41 (up)     | 1.26 ± 0.24  | Transcription factor | **protein/transcription factor (CCCH-type family)**                        | $4e − 10$ |
| Gma.32658  | 5.78 (up)     | 2.25 ± 0.31  | Unclassified        | **Hypothetical protein**                                                   | $1e − 44$ |
| Gma.12121  | 5.36 (up)     | 2.23 ± 0.07  | Unclassified        | **Hypothetical protein**                                                   | $9e − 13$ |
| Gma.6487   | 4.62 (up)     | 2.33 ± 0.19  | Unclassified        | **Unknown**                                                               | —       |
| Gma.15538  | 4.47 (up)     | 2.16 ± 0.21  | Stress response     | **Glutaredoxin family protein (arsenate reductase)**                       | $7e − 39$ |
| Gma.28376  | 4.43 (down)   | −0.02 ± 0.28 | Stress response     | **Syringolide-induced protein B13-1-9 hypersensitive response**            | $6e − 102$ |
| Gma.12481  | 4.39 (up)     | 2.52 ± 0.19  | Unclassified        | **Hypothetical protein**                                                   | $2e − 44$ |
| Gma.4226   | 4.36 (up)     | 1.53 ± 0.16  | Stress response     | **ATTP2-A13 protein/wound response nod33 protein (putative phosphatase)**  | $2e − 44$ |
| Gma.1248   | 4.09 (up)     | 2.50 ± 0.16  | Signaling           | **My family transcription factor**                                         | $5e − 14$ |
| BQ629821   | 3.97 (up)     | 2.01 ± 0.17  | Transcription factor | **My family transcription factor**                                         | $5e − 14$ |
| Gma.1043   | 3.86 (up)     | 1.03 ± 0.07  | Unclassified        | **Hypothetical protein**                                                   | $6e − 21$ |
| Gma.31382  | 3.79 (up)     | 2.04 ± 0.07  | Transcription factor | **Bzip transcription factor (bzip 105)**                                   | 0.0     |
| BQ785779   | 3.75 (up)     | 1.68 ± 0.13  | Unclassified        | **Unknown**                                                               | —       |
| Gma.4710   | 3.54 (up)     | 1.63 ± 0.21  | Unclassified        | **Hypothetical protein**                                                   | $3e − 19$ |
well-characterized Al tolerance mechanism in a wide range of plant species [5, 8]. None of the family members of these two genes were detected in the present study which could be due to constitutive expression. In contrast, an ABC transporter, a multidrug resistance glutathione-S-transferase-conjugated toxin to the vacuole from sensitive plants, and the heavy metal binding proteins upregulated here might function in such pathway.

Lipid and sugar transport proteins are among other transporters detected. Lipid transport proteins transport lipids to cell wall for biosynthesis of cutin layers and surface waxes as a defense mechanism against pathogen attack [33]. They are also induced by abiotic stresses including aluminum [15, 33]. Lipid transport proteins loosen cell wall in a nonhydrolytic mode and enhance cell elongation, a role traditionally attributed to expansins [34]. Aluminum stress inhibits root growth by restricting cell wall extension [1]; hence, there should be a significance to the upregulation of lipid transport proteins under Al stress. Plant sugar transporters have been reported to be induced by pathogen attack and Al stress [18, 35], as is the case in the present study (Gma.11888, Table 2).

3.5.3. Genes Related to Stress Response. Aluminum toxicity has been shown to elicit a wide range of stress-related proteins [19, 20, 36, 37]. In this study, genes known to be responsive to pathogens, oxidative stress, toxins, or Al were classified under this category. Several pathogenesis-related proteins including syringolide-induced protein, acidic endo-

Table 1: Continued.

| Unigene ID   | Fold change | Average ± SD | Functional category | Annotation                                             | e-value |
|--------------|-------------|--------------|---------------------|--------------------------------------------------------|---------|
| Gma.27015    | 3.53 (up)   | 1.67 ± 0.08  | Unclassified        | Octicosapeptide PB1 domain protein                     | 2e−34   |
| Gma.23849    | 3.51 (up)   | 1.01 ± 0.05  | Unclassified        | Unknown                                                | —       |
| Gma.4216     | 3.39 (up)   | 3.01 ± 0.01  | Metabolism          | Endo-xyloglucan transferase/hydrolase                  | 3e−64   |
| AW733463     | 3.38 (up)   | 1.86 ± 0.11  | Unclassified        | Unknown                                                | —       |
| CF807342     | 3.29 (up)   | 2.45 ± 0.20  | Unclassified        | Hypothetical protein                                   | 2e−18   |
| Gma.32376    | 3.27 (up)   | 2.38 ± 0.02  | Transcription factor| BLH1 (embryo sac develop arrest 29)                   | 4e−21   |
| Gma.27837    | 3.16 (up)   | 1.66 ± 0.05  | Unclassified        | Hypothetical protein                                   | 7e−29   |
| Gma.4149     | 3.13 (down) | 0.42 ± 0.04  | Unclassified        | Unknown                                                | —       |
| Gma.19917    | 3.12 (up)   | 1.48 ± 0.02  | Metabolism          | CTP synthase/biosynthesis                              | 3e−54   |
| Gma.34551    | 3.08 (up)   | 1.71 ± 0.04  | Signaling           | MARD1 (mediator of ABA-regulated Dormancy1)           | 2e−20   |
| Gma.3429     | 3.02 (up)   | 2.01 ± 0.10  | Metabolism          | 2-oxoisovalerate dehydrogenase                         | 3e−54   |
| Gma.32595††† | 5.25 (up)   | 1.32 ± 0.45  | Stress response     | Glutathione s-transferase                             | 7e−111  |
| Gma.20326††† | 3.2 (down)  | −1.44 ± 0.20 | Unknown             | —                                                     | —       |

†Significance threshold (P < .01, Fold change >= 3); †† from the 12 h post treatment; ††† from 72 h post treatment; up: upregulated; down: downregulated; e-value: the probability that the match between the gene and its annotation has no biological basis. Fold change: absolute value of the ratio of gene expression under Al to gene expression of untreated control. SD: standard deviation.
| Unigene ID   | Fold change | Average ± SD | Functional category | Annotation                                      | e-value |
|--------------|-------------|--------------|---------------------|------------------------------------------------|---------|
| Gma.6089     | 226.57 (up) | 6.48 ± 1.80  | Unclassified        | Unknown                                        | —       |
| BM139970     | 176.22 (up) | 5.91 ± 0.80  | Unclassified        | Unknown                                        | —       |
| Gma.2586     | 154.32 (up) | 5.86 ± 0.94  | Unclassified        | Unknown                                        | —       |
| Gma.1654     | 130.42 (up) | 4.69 ± 0.64  | Transport           | Coatamer protein complex subunit 2 protein transporter | 2e − 51 |
| Gma.26937    | 115.43 (up) | 5.43 ± 1.37  | Unclassified        | Unknown                                        | —       |
| Gma.35222    | 113.94 (up) | 4.67 ± 0.56  | Stress response     | Syringolide-induced protein B13-1-9 defense protein | 4e − 64 |
| Gma.24062    | 89.44 (up)  | 5.15 ± 1.37  | Unclassified        | Unknown                                        | —       |
| Gma.8048     | 79.07 (up)  | 5.03 ± 0.94  | Unclassified        | Unknown                                        | —       |
| Gma.27466    | 72.87 (up)  | 4.96 ± 1.57  | Unclassified        | Unknown                                        | —       |
| Gma.12326    | 71.14 (up)  | 4.84 ± 1.90  | Unclassified        | Unknown                                        | —       |
| Gma.6948     | 69.75 (up)  | 4.69 ± 1.27  | Unclassified        | Unknown                                        | —       |
| BU5151397    | 65.63 (up)  | 4.37 ± 0.70  | Unclassified        | Hypothetical protein                           | 2e − 15 |
| Gma.2523     | 61.59 (up)  | 4.78 ± 0.96  | Stress response     | Secretory protein (R14 protein soybean-defense protein) | 6e − 64 |
| Gma.35601    | 59.51 (up)  | 3.61 ± 0.43  | Transport           | Heavy-metal transport/detoxification           | 2e − 19 |
| Gma.6649     | 58.09 (up)  | 4.45 ± 0.98  | Unclassified        | Unknown                                        | —       |
| Gma.16246    | 55.90 (up)  | 4.27 ± 0.59  | Unclassified        | BAP2 (BON associated protein 2)                | 1e − 15 |
| Gma.30731    | 54.34 (up)  | 4.14 ± 0.90  | Unclassified        | Unknown                                        | —       |
| Bf967874     | 53.43 (up)  | 4.4 ± 1.35   | Unclassified        | Unknown                                        | —       |
| Gma.4526     | 50.59 (up)  | 4.38 ± 1.02  | Transcription factor | Zinc finger (C2H2 family protein)              | 7e − 27 |
| Gma.25191    | 47.41 (up)  | 5.06 ± 1.11  | Unclassified        | Unknown                                        | —       |
| Gma.9397     | 47.24 (up)  | 4.28 ± 0.98  | Stress response     | Syringolide-induced protein B13-1-9 defense protein | 1e − 53 |
| Gma.25462    | 46.34 (up)  | 4.34 ± 0.65  | Transcription factor | WRKY19 DNA-binding protein 19                  | 1e − 62 |
| BU579058     | 45.35 (up)  | 4.44 ± 1.44  | Metabolism          | N-acetyltransferase activity                   | 2e − 40 |
| Gma.28852    | 43.12 (up)  | 4.10 ± 0.58  | Metabolism          | Cytochrome P450                                | 0.0     |
| Gma.27514    | 41.91 (up)  | 3.91 ± 0.78  | Stress response     | Basic secretory protein/defense protein        | 3e − 69 |
| Gma.23347    | 41.67 (up)  | 4.08 ± 1.79  | Unclassified        | Unknown                                        | —       |
| Gma.22079    | 41.46 (up)  | 4.34 ± 0.70  | Stress response     | Glutathione s-transferase                     | 5e − 57 |
| Gma.32994    | 40.77 (up)  | 3.71 ± 0.62  | Stress response     | Acidic endochitinase (chitinase III-A)        | 7e − 93 |
| Gma.27743    | 39.93 (up)  | 4.14 ± 0.98  | Unclassified        | Unknown                                        | —       |
| Gma.1622     | 39.56 (up)  | 3.93 ± 1.02  | Unclassified        | Hypothetical protein/ABC transporter like      | 8e − 31 |
| Gma.36756    | 36.68 (up)  | 4.27 ± 1.08  | Transcription factor | WRKY17 protein/transcription factor           | 1e − 101|
| Gma.5622     | 32.77 (up)  | 4.29 ± 0.75  | Unclassified        | Unknown                                        | —       |
| Gma.26204    | 32.06 (up)  | 3.82 ± 0.46  | Metabolism          | Transferase/transferase activity               | 2e − 29 |
| Gma.27239    | 31.70 (up)  | 3.83 ± 1.35  | Unclassified        | Unknown                                        | —       |
| Gma.36287    | 31.08 (up)  | 4.56 ± 0.48  | Metabolism          | Carboxylesterase/lipase activity               | 3e − 64 |
| Gma.28246    | 30.45 (up)  | 3.54 ± 0.64  | Unclassified        | Unknown                                        | —       |
| Gma.36753    | 30.41 (up)  | 4.50 ± 0.74  | Transcription factor | WRKY 30(DNA binding protein)                      | 1e − 104|
| UniGene ID | Fold change | Average ± SD | Functional category | Annotation | e-value |
|------------|-------------|--------------|---------------------|------------|---------|
| Gma.8565   | 29.01 (up)  | 3.45 ± 0.70  | Metabolism          | Hydrolase /xyloglucan endotransglycosylase      | 5e − 18  |
| Gma.7861   | 28.48 (up)  | 3.48 ± 1.34  | Unclassified        | Unknown    | 5e − 20  |
| Gma.32790  | 28.00 (up)  | 3.63 ± 0.99  | Stress response     | Band 7 family protein/hypersensitive inducible reaction protein 1 | 3e − 27  |
| Gma.7697   | 27.48 (down)| −3.66 ± 0.81 | Unclassified        | Hypothetical protein/31 kDa glycoprotein        | 2e − 137 |
| Gma.31827  | 27.44 (up)  | 4.22 ± 0.65  | Transcription factor| WRKY70/DNA-binding protein 70                   | 5e − 87  |
| Gma.21022  | 27.36 (up)  | 4.13 ± 1.18  | Unclassified        | Unknown    | —       |
| Gma.28273  | 27.29 (up)  | 3.84 ± 0.87  | Transcription factor| NAC6 /NAC domain protein/apical elongation plant development protein | 2e − 156 |
| Gma.35830  | 27.20 (up)  | 3.48 ± 0.77  | Signaling           | Regulation of gene silencing/calcium-sensor     | 9e < ?bhlt? > e < ?ehlt? > 33 |
| Gma.8262   | 26.92 (up)  | 3.73 ± 1.33  | Stress response     | AGc 2-1(oxidative signal-inducible kinase)       | 3e − 57  |
| Gma.14080  | 26.50 (up)  | 3.81 ± 0.80  | Transport           | Similar to ATMRP3/multidrug resistance glutathione s-conjugate-exporting ATPase | 8e − 11  |
| Gma.27371  | 25.99 (up)  | 3.78 ± 1.33  | Unclassified        | Unknown    | —       |
| BM523736   | 25.34 (up)  | 3.76 ± 0.83  | Metabolism          | Transferase family protein                      | 1e − 23  |
| Gma.28330  | 25.32 (up)  | 3.84 ± 1.53  | Unclassified        | Calcium-binding protein                         | 7e − 23  |
| Gma.34717  | 24.87 (up)  | 3.83 ± 1.15  | Unclassified        | Unknown    | —       |
| Gma.26682  | 24.58 (up)  | 3.95 ± 1.15  | Unclassified        | Unknown    | —       |
| Gma.17184  | 24.42 (up)  | 3.77 ± 0.52  | Transport           | Glycolipid-binding/transport protein             | 2e − 57  |
| Gma.11888  | 24.39 (up)  | 3.19 ± 0.25  | Transport           | ATPP2-B10 (phloem protien2) carbohydrate binding | 6e − 27  |
| Gma.4222   | 23.57 (up)  | 3.14 ± 0.97  | Unclassified        | Hypothetical protein                            | 2e − 57  |
| Gma.26712  | 23.39 (up)  | 2.82 ± 0.18  | Unclassified        | Unknown    | —       |
| Gma.33327  | 23.16 (up)  | 3.56 ± 0.29  | Transcription factor| Transcription factor                            | 2e − 166 |
| Gma.17019  | 23.08 (up)  | 3.67 ± 1.54  | Unclassified        | Unknown    | —       |
| Gma.33178  | 23.07 (up)  | 3.81 ± 1.54  | Unclassified        | Plastocyanin-like domain-containing copper ion binding | 3e − 33  |
| Gma.35364  | 22.81 (up)  | 3.55 ± 0.40  | Stress response     | FAD-linked oxidoreductase 1/carbohydrate-oxidase | 1e − 52  |
| Gma.15839  | 22.63 (down)| −4.00 ± 0.36 | Metabolism          | GDSL-motif lipase/hydrolase                    | 2e − 39  |
| Gma.6948   | 22.54 (up)  | 3.73 ± 1.24  | Unclassified        | Unknown    | —       |
| Gma.2821   | 22.36 (up)  | 3.40 ± 0.95  | Stress response     | PR-5 protein (pathogenesis related)             | 4e − 134 |
| Gma.8628   | 21.20 (up)  | 3.19 ± 1.11  | Unclassified        | Unknown protein                                 | —       |
| BQ473604   | 20.51 (up)  | 3.43 ± 0.83  | Unclassified        | Hypothetical protein                            | 8e − 46  |
| Gma.9913   | 20.34 (up)  | 3.81 ± 0.44  | Unclassified        | Unknown protein                                 | —       |
| Gma.144    | 20.01 (up)  | 3.72 ± 1.00  | Transport           | Nodulin protein/transport function               | 0.0      |
| Unigene ID  | Fold change | Average ± SD | Functional category | Annotation                                           | e-value |
|------------|-------------|--------------|---------------------|------------------------------------------------------|---------|
| Gma.34099  | 19.67 (up)  | 2.93 ± 0.93  | Unclassified        | Hypothetical protein                                 | 2e − 77 |
| Gma.4305   | 19.63 (up)  | 3.82 ± 0.62  | Stress response     | Glutathione s-transferase (GST 15)                   | 7e − 128|
| Gma.4336   | 19.56 (up)  | 3.16 ± 0.98  | Unclassified        | Unknown                                              | —       |
| Gma.24625  | 19.40 (up)  | 3.50 ± 1.12  | Transport           | Heavy metal transport/detoxification                 | 2e − 20 |
| Gma.7726   | 19.18 (up)  | 3.36 ± 1.22  | Signalling          | Calcium-binding EF hand family protein               | 4e − 25 |
| Gma.26531  | 18.95 (up)  | 3.55 ± 1.31  | Transcription factor| Zinc finger (C3HC4-type ring family)                 | 2e − 26 |
| Gma.34717  | 18.56 (up)  | 3.46 ± 1.24  | Unclassified        | Unknown                                              | —       |
| BE322282   | 18.64 (up)  | 2.87 ± 0.66  | Unclassified        | Unknown                                              | —       |
| Gma.27062  | 18.22 (up)  | 2.96 ± 0.95  | Transcription factor| NAc domain containing protein 2 plant development/apical elongation | 3e − 103|
| Gma.4478   | 18.03 (up)  | 3.54 ± 1.03  | Unclassified        | Hypothetical protein                                 | 2e − 10 |
| Gma.24807  | 18.01 (up)  | 3.09 ± 0.76  | Unclassified        | Unknown                                              | —       |
| BK000119.1 | 17.98 (up)  | 3.36 ± 0.87  | Cell cycle          | Phytosulfokines 4 precursor/growth factor cell differentiation, cell proliferation | 3e − 19 |
| Gma.29479  | 17.92 (up)  | 3.16 ± 0.52  | Unclassified        | Unknown                                              | —       |
| Gma.10956  | 17.76 (up)  | 3.32 ± 0.74  | Stress response     | Similar to pathogenesis-related protein (STH-2)      | 1e − 40 |
| BI967589   | 17.25 (up)  | 3.08 ± 0.49  | Unclassified        | Unknown                                              | —       |
| Gma.17184  | 17.16 (up)  | 2.94 ± 0.11  | Transport           | Heavy-metal-associated domain containing protein metal ion transport | 1e − 10 |
| Gma.24561  | 17.08 (up)  | 3.15 ± 0.31  | Unclassified        | Unknown                                              | —       |
| Gma.21739  | 17.05 (up)  | 3.33 ± 0.82  | Metabolism          | AAA-type ATPase protein/ATPase activity              | 5e − 39 |
| Gma.17184  | 16.89 (up)  | 3.27 ± 0.63  | Transport           | Glycolipid-binding protein/glycolipid transport      | —       |
| Gma.4366   | 16.62 (up)  | 3.29 ± 1.27  | Metabolism          | VTc2 (Vitamin C defective 2)/L-ascorobic-acid biosynthesis | 1e − 36 |
| Gma.26405  | 15.37 (up)  | 3.04 ± 0.83  | Unclassified        | Unknown                                              | —       |
| DQ222982   | 15.09 (up)  | 3.42 ± 1.04  | Transport           | Lipocalcin/ fatty acid transport                     | 2e − 107|
| Gma.17929  | 15.05 (up)  | 3.12 ± 0.58  | Metabolism          | Transferase family protein                           | 8e − 76 |
| Gma.21512  | 14.99 (up)  | 2.71 ± 0.78  | Unclassified        | Unknown                                              | —       |
| BE440732   | 14.85 (up)  | 3.48 ± 0.97  | Unclassified        | Unknown                                              | —       |
| Gma.29663  | 14.64 (up)  | 2.52 ± 1.04  | Unclassified        | Unknown                                              | —       |
| Gma.31861  | 14.60 (up)  | 3.09 ± 1.07  | Unclassified        | Unknown                                              | —       |
| Gma.29655  | 14.52 (up)  | 3.00 ± 0.34  | Metabolism          | CytochromeP50 subfamily B polypeptide 1              | 3e − 58 |
| Gma.11257  | 14.51 (up)  | 2.65 ± 0.86  | Unclassified        | Hypothetical protein exo-1, 3-beta-glucanase precursor | 9e − 64 |
| Gma.26640  | 14.48 (up)  | 2.93 ± 1.18  | Unclassified        | Unknown                                              | —       |
| Gma.28243  | 14.18 (up)  | 3.28 ± 0.85  | Unclassified        | Unknown                                              | —       |
| CD394418   | 14.12 (up)  | 3.08 ± 1.24  | Metabolism          | Ribulose-1, 5-bisphosphate carboxylase               | 2e − 30 |
| Gma.1527   | 14.09 (down)| −2.26 ± 1.40 | Metabolism          | Dihydropyronol reductase (anthocyanin biosynthesis)  | 0.0     |
| Gma.25234  | 14.08 (up)  | 2.96 ± 0.18  | Transcription factor| WRKY43 protein                                       | 4e − 131|
protein STH-2, and proteinase inhibitors were upregulated at 48 h post Al treatment (Table 2). The confluence between plant molecular response to aluminum toxicity and pathogen infection likely arises from the fact that both cause oxidative stress. However, the role of pathogenesis-related proteins in Al tolerance is equivocal. Overexpression of peroxidase- and proteinase inhibitor genes in Arabidopsis did not improve Al tolerance for the transformed plants relative to controls [38]. On the other hand, overexpressing pepper basic pathogenesis-related protein 1 gene in tobacco resulted in enhanced tolerance to heavy metal cadmium and pathogen infection [39].

Other Al-upregulated stress-related genes included carbohydrate oxidase, glutathione-S-transferase, and glutathione-based reductase (Tables 1 and 2). Carbohydrate oxidase and cell wall peroxidases have been reported to provide protection against pathogens by generating hydrogen peroxide from carbohydrate substrates in the apoplast [14]. Hydrogen peroxide has antimicrobial property and also acts as a signal molecule for defense genes expression. In the case of aluminum, the activity of these enzymes is correlated with plant Al sensitivity [40, 41]. Glutathione-S-transferase and glutathione-based reductase are the key enzymes of cellular antioxidation system. Oxidative stress is one aspect of Al toxicity, and maintenance of cellular ascorbate homeostasis has been reported to be an essential component of plant Al tolerance [16]. Cytochrome P450 may serve as monoxygenase in the biosynthetic pathways for lignin, defense compounds, hormones, pigments, fatty acids, and signaling molecules or in the detoxification pathway to catalyze the breakdown of numerous endogenous and exogenous toxic compounds [44]. We detected two genes (Gma.28852 upregulated 43-fold and Gma.29655- upregulated 15-fold) which code for cytochrome P450 (Table 2). Gma.28852 encodes protein involved in pathways of ascorbate metabolism, coumarine and phenylpropanoid biosynthesis, and gamma hexachlorohexane degradation. Endoxyloglucan hydrolases are cell wall metabolism enzymes. Members of this family of enzymes have been implicated in Al tolerance [16, 18–20]. There is a causal relationship among endoxyloglucan hydrolases, cell wall composition, and Al tolerance. Al induced increases in cell wall pectin and hemicellulose increases plant Al sensitivity [43]. Pectin and hemicellulose form complexes with Al resulting in increased cell wall rigidity and reduced cell extension and growth [27, 43, 45]. Endoxyloglucan hydrolases appear to relax the Al-rigidified cell wall presumably by hydrolyzing the Al-sugar complexes.

### Table 2: Continued.

| UniGene ID | Fold change | Average ± SD | Functional category | Annotation | e-value |
|-----------|-------------|--------------|---------------------|------------|---------|
| Gma.28057 | 13.62 (up)  | 2.52 ± 0.68  | Transcription factor | Sali5-4a protein (ADR6) | 8e−60  |
| Gma.8480  | 13.58 (up)  | 3.20 ± 0.69  | Stress response     | Resistance protein LM12 | 0.0    |
| Gma.28756 | 13.53 (up)  | 2.89 ± 0.53  | Unclassified        | Unknown    | —       |
| BM177218  | 13.45 (up)  | 3.14 ± 0.45  | Unclassified        | Unknown    | —       |
| BG551078  | 13.37 (up)  | 3.09 ± 0.42  | Unclassified        | Conserved hypothetical protein | 6e−11 |
| Gma.728   | 13.28 (up)  | 2.78 ± 0.99  | Unclassified        | Unknown    | —       |
| Gma.35332 | 13.23 (up)  | 3.45 ± 0.73  | Unclassified        | Unknown    | —       |
| Gma.26712 | 13.17 (up)  | 3.09 ± 1.01  | Unclassified        | Unknown    | —       |

†Significance threshold (P < .01, Fold change >= 3); up: upregulated, down: downregulated; e-value: the probability that the match between the gene and its annotation has no biological basis. Fold change: absolute value of the ratio of gene expression under Al to gene expression of untreated control. SD: standard deviation.
3.5.5. Genes Related to Cell Signaling. Perception of stress signal by the cell is the starting point for cascade of events leading to gene expression and change in cell metabolism in response to a stress factor. Aluminum perception and signaling is currently poorly understood. Cell wall-associated receptor kinase (WAK1) was the first Al signaling gene discovered [46], but there is no evidence that demonstrate, WAK1’s major role in Al tolerance [1]. Microarray analyses have shown kinases, phosphates, and EF hand Ca$^{2+}$ binding proteins as possible components of Al signaling pathway [16, 18]. In the present work, a Ca$^{2+}$ sensor protein (Gma.35830), calcium-binding EF hand family protein (Gma.7726), oxidative signal kinase (Gma.8262), and a gene for growth factor phytosulfokines precursor (BK0001191) were upregulated 48 h post Al treatment (Table 2). The phytosulfokines growth factor is a novel Al-induced gene, and it is involved in cell proliferation and growth, characteristics that confer Al tolerance.

4. Conclusion

We conducted a transcriptome analysis in Al-tolerant soybean line PI 416937 to identify potential genetic factors underlying Al tolerance trait. Our results uncovered several genes which might potentially have influence on soybean Al tolerance. Among these, two transcription factors, cell wall metabolism enzymes and a cell proliferation gene are particularly interesting from perspective of the physiological and molecular mechanisms of plant Al tolerance. The first transcription factor, Cys2His2 zinc finger protein, coregulates molecular response to proton and aluminum toxicities, the major acid soil stress factors [28]. The second transcription activator, ADR6 is an auxin downregulated gene. Al suppresses auxin biosynthesis and transport in root system which might be one possible mechanism of Al induced root growth inhibition [18]. Conversely, ADR6 is triggered under Al stress probably in a parallel pathway to auxin to restore root growth under Al stress. Root cell wall rigidification by Al binding is one principal mechanism of Al toxicity. Cell wall metabolism enzymes and proteins are induced under Al stress and may counteract Al effects on root cell walls. It is increasingly evident that these proteins as well as cell wall pectin and hemicellulose content are important determinants of Al tolerance in cereals [3, 4, 43]. Evidence from this study also implies that cell wall remodeling enzymes and proteins may play role in soybean Al tolerance. Inhibition of cell division and proliferation is another major mechanism of Al toxicity. We identified a novel cell proliferation stimulating gene phytosulfokines growth factor which might reverse this effect of Al. Taken together; our findings provide important insights into the molecular mechanisms of aluminum tolerance in soybean. The genes we identified may guide efforts to improve plant Al tolerance trait.

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References

[1] L. V. Kochian, O. A. Hoekenga, and M. A. Piñeros, “How do crop plants tolerate acidic soils? Mechanisms of aluminum tolerance and phosphorous efficiency,” Annual Review of Plant Biology, vol. 55, pp. 459–493, 2004.
[2] L. V. Kochian, M. A. Piñeros, and O. A. Hoekenga, “The physiology, genetics and molecular biology of plant aluminum resistance and toxicity,” Plant and Soil, vol. 274, no. 1-2, pp. 175–195, 2005.
[3] D. Eticha, A. Staß, and W. J. Horst, “Localization of aluminum in the maize root apex: can morin detect cell wall-bound aluminum?” Journal of Experimental Botany, vol. 56, no. 415, pp. 1351–1357, 2005.
[4] Z. A. K. M. Hossain, H. Koyama, and T. Hara, “Growth and cell wall properties of two wheat cultivars differing in their sensitivity to aluminum stress,” Journal of Plant Physiology, vol. 163, no. 1, pp. 39–47, 2006.
[5] J. V. Magalhaes, J. Liu, C. T. Guimarães et al., “A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum,” Nature Genetics, vol. 39, no. 9, pp. 1156–1161, 2007.
[6] J. L. Yang, Y.Y. Li, Y. J. Zhang et al., “Cell wall polysaccharides are specifically involved in the exclusion of aluminum from the rice root apex,” Plant Physiology, vol. 146, no. 2, pp. 602–611, 2008.
[7] D. A. Samac and M. Tesfaye, “Plant improvement for tolerance to aluminum in acid soils—a review,” Plant Cell, Tissue and Organ Culture, vol. 75, no. 3, pp. 189–207, 2003.
[8] T. Sasaki, Y. Yamamoto, B. Ezaki et al., “A wheat gene encoding an aluminum-activated malate transporter,” The Plant Journal, vol. 37, no. 5, pp. 645–653, 2004.
[9] A. Morita, O. Yanagisawa, S. Takatsu, S. Maeda, and S. Hiradate, “Mechanism for the detoxification of aluminum in roots of tea plant (Camellia sinensis (L.) Kuntze),” Phytochemistry, vol. 69, no. 1, pp. 147–153, 2008.
[10] T. Watanabe, M. Osaki, H. Yano, and I. Rao, “Internal mechanisms of plant adaptation to aluminum toxicity and phosphorus starvation in three tropical forages,” Journal of Plant Nutrition, vol. 29, no. 7, pp. 1243–1255, 2006.
[11] C. M. Bianchi-Hall, T. E. Carter Jr., M. A. Bailey et al., “Aluminum tolerance associated with quantitative trait loci derived from soybean PI 416937 in hydroponics,” Crop Science, vol. 40, no. 2, pp. 538–545, 2000.
[12] H. Nian, Z. Yang, H. Huang, X. Yan, and H. Matsumoto, “Citrate secretion induced by aluminum stress may not be a key mechanism responsible for differential aluminum tolerance of some soybean genotypes,” Journal of Plant Nutrition, vol. 27, no. 11, pp. 2047–2066, 2004.
[13] V. Ermolayev, W. Weschke, and R. Manteuffel, “Comparison of Al-induced gene expression in sensitive and tolerant soybean cultivars,” *Journal of Experimental Botany*, vol. 54, no. 393, pp. 2745–2756, 2003.

[14] M. Ragland and K. M. Soliman, “Two genes induced by Al in soybean roots,” *Plant Physiology*, vol. 114, p. 395, 1997.

[15] P. Guo, G. Bai, B. Carver, R. Li, A. Bernardo, and M. Baum, “Transcriptional analysis between two wheat near-isogenic lines contrasting in aluminum tolerance under aluminum stress,” *Molecular Genetics and Genomics*, vol. 277, no. 1, pp. 1–12, 2007.

[16] M. Houde and A. O. Diallo, “Identification of genes and pathways associated with aluminum stress and tolerance using transcriptome profiling of wheat near-isogenic lines,” *BMC Genomics*, vol. 9, article 400, 2008.

[17] L. G. Maron, M. Kirst, C. Mao, M. J. Milner, M. Menossi, and L. V. Kochian, “Transcriptional profiling of aluminum toxicity and tolerance responses in maize roots,” *New Phytologist*, vol. 179, no. 1, pp. 116–128, 2008.

[18] M. Kumari, G. J. Taylor, and M. K. Deyholos, “Transcriptional response to aluminum stress in roots of Arabidopsis thaliana,” *Molecular Genetics and Genomics*, vol. 279, no. 4, pp. 339–357, 2008.

[19] D. Chandran, N. Sharopova, S. Iwashita, J. S. Gantt, K. A. VandenBosch, and D. A. Samac, “Transcriptome profiling identified novel genes associated with aluminum toxicity, resistance and tolerance in Medicago truncatula,” *Planta*, vol. 228, no. 1, pp. 151–166, 2008.

[20] D. Chandran, N. Sharopova, K. A. Vandenbosch, D. F. Garvin, and D. A. Samac, “Physiological and molecular characterization of aluminum resistance in Medicago truncatula,” *BMC Plant Biology*, vol. 8, article 89, 2008.

[21] I. R. Silva, T. J. Smyth, D. F. Moxley, T. E. Carter, N. S. Allen, and T. W. Rufky, “Aluminum accumulation at nuclei of cells in the root tip. Fluorescence detection using lumogallion and confocal laser scanning microscopy,” *Plant Physiology*, vol. 123, no. 2, pp. 543–552, 2000.

[22] Z. Wu, R. A. Irizarry, R. Gentleman, F. M. Murillo, and F. Spencer, “A model-based background adjustment for oligonucleotide expression arrays,” *Journal of the American Statistical Association*, vol. 99, no. 468, pp. 909–917, 2004.

[23] M. W. Pfaffl, “A new mathematical model for relative quantification in real-time RT-PCR,” *Nucleic Acids Research*, vol. 29, no. 9, article e45, 2001.

[24] T. L. Maguire, S. Grimmond, A. Forrest, I. Iturbe-Ormaetxe, K. Meksem, and P. Gresshoff, “Tissue-specific gene expression in soybean (*Glycine max*) detected by cDNA microarray analysis,” *Journal of Plant Physiology*, vol. 159, no. 12, pp. 1361–1374, 2002.

[25] Z. Wu, K. M. Soliman, J. J. Bolton, S. Saha, and J. N. Jenkins, “Identification of differentially expressed genes associated with cotton fiber development in a chromosomal substitution line (CS-B22sh),” *Functional and Integrative Genomics*, vol. 8, no. 2, pp. 165–174, 2008.

[26] J. S. Morey, J. C. Ryan, and F. M. Van Dolah, “Microarray validation: factors influencing correlation between oligonucleotide microarrays and real-time PCR,” *Biological Procedures Online*, vol. 8, no. 1, pp. 175–193, 2006.

[27] J. Zhang, Z. He, H. Tian, G. Zhu, and X. Peng, “Identification of aluminum-responsive genes in rice cultivars with different aluminum sensitivities,” *Journal of Experimental Botany*, vol. 58, no. 8, pp. 2269–2278, 2007.

[28] S. Iuchi, H. Koyama, A. Iuchi et al., “Zinc finger protein STOP1 is critical for proton tolerance in Arabidopsis and coregulates a key gene in aluminum tolerance,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 23, pp. 9900–9905, 2007.

[29] I. R. Silva, T. J. Smyth, C. D. Raper, T. E. Carter, and T. W. Rufky, “Differential aluminum tolerance in soybean: an evaluation of the role of organic acids,” *Physiologia Plantarum*, vol. 112, no. 2, pp. 200–210, 2001.

[30] P. B. Larsen, M. J. B. Geisler, C. A. Jones, K. M. Williams, and J. D. Cancel, “ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in Arabidopsis,” *The Plant Journal*, vol. 41, no. 3, pp. 353–363, 2005.

[31] T. Sasaki, B. Ezaki, and H. Matsumoto, “A gene encoding multidrug resistance (mdr)-like protein is induced by aluminum and inhibitors of calcium flux in wheat,” *Plant and Cell Physiology*, vol. 43, no. 2, pp. 177–185, 2002.

[32] J. F. Ma, P. R. Ryan, and E. Delhaize, “Aluminum tolerance in plants and the complexing role of organic acids,” *Trends in Plant Science*, vol. 6, no. 6, pp. 273–278, 2001.

[33] J.-C. Kader, “Lipid-transfer proteins: a puzzling family of plant proteins,” *Trends in Plant Science*, vol. 2, no. 2, pp. 66–70, 1997.

[34] J. Nieuwland, R. Feron, B. A. H. Huismans et al., “Lipid transfer proteins enhance cell wall extension in tobacco,” *The Plant Cell*, vol. 17, no. 7, pp. 2009–2019, 2005.

[35] L. E. Williams, R. Lemoine, and N. Sauer, “Sugar transporters in higher plants—a diversity of roles and complex regulation,” *Trends in Plant Science*, vol. 5, no. 7, pp. 283–290, 2000.

[36] M. A. R. Mill, E. D. Butler, A. R. Huete, C. F. Wilson, O. Anderson, and J. P. Gustafson, “Expressed sequence tag-based gene expression analysis under aluminum stress in rye,” *Plant Physiology*, vol. 130, no. 4, pp. 1706–1716, 2002.

[37] K. D. Richards, E. J. Schott, Y. K. Sharma, K. R. Davis, and R. C. Gardner, “Aluminum induces oxidative stress genes in Arabidopsis thaliana,” *Plant Physiology*, vol. 116, no. 1, pp. 409–418, 1998.

[38] B. Ezaki, R. C. Gardner, Y. Ezaki, and H. Matsumoto, “Expression of aluminum-induced genes in transgenic Arabidopsis plants can ameliorate aluminum stress and/or oxidative stress,” *Plant Physiology*, vol. 122, no. 3, pp. 657–665, 2000.

[39] S. Sarowar, Y. J. Kim, E. N. Kim et al., “Over expression of a pepper basic pathogenesis-related protein 1 gene in tobacco plants enhances resistance to heavy metal and pathogen stresses,” *Plant Cell Reporter*, vol. 24, pp. 216–224, 2005.

[40] P. R. S. Boscolo, M. Menossi, and R. A. Jorge, “Aluminum-induced oxidative stress genes in Arabidopsis thaliana,” *Plant Physiology*, vol. 84, no. 7, pp. 661–665, 2002.

[41] G. Delisle, M. Champoux, and M. Houde, “Characterization of oxalate oxidase and cell death in Al-sensitive and tolerant wheat roots,” *Plant and Cell Physiology*, vol. 42, no. 3, pp. 324–333, 2001.

[42] S. Saka, W. Aouchi, and C. Abdenour, “The capacity of glutathione reductase in cell protection from the toxic effect of heated oils,” *Biochimie*, vol. 84, no. 7, pp. 661–665, 2002.

[43] Q. Liu, J. L. Yang, L. S. He, Y. Y. Li, and S. J. Zheng, “Expression of heated oils,” *Biologia Plantarum*, vol. 52, no. 1, pp. 87–92, 2008.

[44] M. A. Schuler and D. Werck-Reichhart, “Functional genomics of P450s,” *Annual Review of Plant Biology*, vol. 54, pp. 629–667, 2003.
[45] C. Mao, K. Yi, and L. Yang, "Identification of aluminum-regulated genes by cDNA-AFLP in rice (*Oryza sativa* L.): aluminum-regulated genes for the metabolism of cell wall components," *Journal of Experimental Botany*, vol. 55, no. 394, pp. 137–143, 2003.

[46] M. Sivaguru, B. Ezaki, Z.-H. He et al., "Aluminum-induced gene expression and protein localization of a cell wall-associated receptor kinase in Arabidopsis," *Plant Physiology*, vol. 132, no. 4, pp. 2256–2266, 2003.