VITOR BATISTA PINTO

TRANSCRIPTIONAL PROFILE OF POPCORN ROOTS UNDER ALUMINUM STRESS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*.

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José Marcelo Soriano Viana
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Aos meus pais Milton e Eliana,
dedico.

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BIOGRAFIA

VITOR BATISTA PINTO, filho de Milton Antônio Caetano Pinto e Eliana Pereira Batista Pinto, nasceu em Sabará, Minas Gerais, no dia 19 de novembro de 1988.

Realizou o ensino médio e o curso técnico em Química Industrial no Centro Federal de Educação Tecnológica de Minas Gerais (CEFET-MG), sendo concluído em 2006. Graduou-se em Agronomia pela Universidade Federal de Minas Gerais (UFMG), na cidade de Montes Claros, Minas Gerais, em julho de 2013. Concluiu o mestrado em Genética e Melhoramento na Universidade Federal de Viçosa em julho de 2015. Ingressou no mês seguinte no doutorado em Genética e Melhoramento na mesma universidade, realizou o doutorado-sanduíche na Univrsità dgli Studi di Padova, na cidade de Padova, Itália e, em julho de 2019, submeteu-se à defesa de tese.

SUMÁRIO

ABSTRACT

PINTO, Vitor Batista, D.Sc., Universidade Federal de Viçosa, July, 2019. **Transcriptional profile of popcorn roots under aluminum stress**. Advisor: José Marcelo Soriano Viana. Co-advisor: Maximiller Dal Bianco Lamas Costa.

Aluminum (Al) is the third most abundant element in the earth's crust. In acid soils, with pH values at or below 5, the phytotoxic species Al^{3+} is solubilized in soil solution and becomes one of the most important abiotic stresses limiting the crops production. The phytotoxic form Al^{3+} inhibits root growth altering water and nutrients absorption and consequently reducing the plant development. In some species, most of this variation is controlled by a single major gene, whereas in other the characteristic is more complex. In maize Al-resistance is considered a quantitative trait. The investigation of Al-responsive genes in maize was focused mainly in an early response, but we believe that a maintenance mechanism is activated in which several metabolic processes are working at the same time during a long term of Al-exposure. This study aimed to investigate new players involved in defense response under Al stress of two contrasting popcorn lines during 72 h under stress using high-throughput RNA sequencing. The specific objectives were: i) to track the expression profile of popcorn inbred lines under Al-stress; ii) to link the RNA-seq data into maize QTLs involved in Al tolerance; iii) to perform a single nucleotide polymorphism (SNP) analysis to detect variants with predicted deleterious substitutions. The contrasting popcorn inbred lines were maintained in control and Al-treatment conditions in a chamber growth during 72h and then the total RNA from three biological replicates, in a total of 12 samples, was extracted from roots. The RNA sequencing was performed using Illumina HiSeq 2500 platform. The cDNA libraries reads were submitted to quality control analysis with FastQC software. The genome assembly was performed with the program Bowtie2 and TopHat using the B73 (RefGenv4) genome as reference. The expression levels and to identification of differentially expressed genes (DEGs) were calculated with the program Cuffdiff. A total of 1,121 DEGs were identified in the Al-sensitive line and 2,872 DEGs in the Al-resistant line. It were shared 384 DEGs in both lines. The most significant gene ontology (GO) modules were clustered in response to a stimulus. The Al-resistant line presented genes that may play a role in an efficient oxidative system against ROS, involved in cell wall stiffening and dynamic changes of the cell wall to prevent the Al ion transport via the symplast. Also, we detected transporters belonging to families already known to perform a role in Al-detoxification and organic acid exclusion, and we proposed a class of SWEET transporters that might be involved in regulation of vacuolar sugar storage under Al-stress. In the study to explore the variation and reduce the number of Al-responsive genes, it was performed the mapping of the DEGs to the chromosomes and identified SNP variants. Reads were mapped to reference genome using BWA-MEM algorithm. The mapping files were processed using Picard software. The variants were called using FreeBayes software. The SNPs were filtered using vcftools and annotated with Variant Effect Predictor. To detect DEGs inside previously Al-tolerance QTLs identified, in silico mapping was performed by a comparison between the positions of the genes and the region flanked by markers in each chromosome. This allowed the identification of genes inside maize Al-tolerance QTLs previously reported in the literature and the detection of variants that may create a defense host mechanism against Al-toxicity. We were able to highlighted some new targets, such as SNF1-related protein kinase, histone deacetylase (HDT1), SWEET transporters, delta (12)-fatty-acid desaturase (FAD), MADS-box, AP2/EREBP, HY5-like, and pathogenesis-related (PR) proteins, that may contribute to maintaining root growth under Al-stress in popcorn.

RESUMO

PINTO, Vitor Batista, D.Sc., Universidade Federal de Viçosa, julho de 2019. **Perfil transcricional de raízes de milho pipoca sob estresse por alumínio**. Orientador: José Marcelo Soriano Viana. Coorientador: Maximiller Dal Bianco Lamas Costa.

Alumínio (Al) é o terceiro elemento mais abundante na crosta terrestre. Em solos ácidos, com valores de pH iguais ou inferiores a 5, a espécie fitotóxica Al^{3+} é solubilizada na solução do solo e se torna um dos mais importantes estresses abióticos que limitam a produção das culturas agrícolas. A forma fitotóxica Al^{3+} inibe o crescimento radicular alterando a absorção de água e nutrientes e, consequentemente, reduzindo o desenvolvimento da planta. Em algumas espécies grande parte dessa variação é controlada por um único gene, enquanto em outras, a característica é mais complexa. Em milho a resistência ao Al é considerada uma característica quantitativa. A investigação de genes responsivos ao Al em milho foi focada, principalmente, em uma resposta precoce. Porém acredita-se que durante a um longo período de exposição ao Al, o mecanismo de manutenção é ativado onde vários processos metabólicos estão trabalhando ao mesmo tempo. Este estudo teve como objetivo investigar novos genes envolvidos na resposta de defesa sob estresse por Al. Para isso, o perfil de expressão de duas linhagens contrastantes de milho-pipoca foi monitorado durante 72h sob estresse, usando o sequenciamento de RNA de alto rendimento. Os dados de RNA-seq foram associados aos QTLs de milho envolvidos em tolerância ao Al. Uma análise de polimorfismo de nucleotídeo único (SNP) foi realizada para detectar variantes com substituições deletérias preditas que podem ser responsáveis por criar um mecanismo de defesa no hospedeiro contra o estresse por Al. As linhagens contrastantes de milho pipoca foram mantidas em condições controladas e sob tratamento por Al em câmara de crescimento durante 72 h e o RNA total das raízes de três réplicas biológicas, totalizando 12 amostras, foi extraído. O sequenciamento do RNA foi realizado usando a plataforma Illumina HiSeq 2500. As *reads* das bibliotecas de cDNA foram submetidas à análise de controle de qualidade com o software FastQC. O alinhamento das *reads* foi realizado com os programas Bowtie2 e TopHat usando o genoma B73 (RefGenv4) como referência. Os níveis de expressão e a identificação dos genes diferencialmente expressos (DEGs) foram calculados com o programa Cuffdiff. Um total de 1.121 DEGs foram identificados na linhagem Al-sensível e 2.872 DEGs na linhagem Al-resistente. Foram compartilhados 384 DEGs em ambas linhagens. Os módulos mais significativos de ontologia gênica (GO) foram agrupados em resposta a estímulos. A linhagem Al-resistente apresentou genes que podem desempenhar um papel na formação de um eficiente sistema oxidativo contra ROS, no enrijecimento da parede celular e nas mudanças da dinâmica da parede celular para prevenir o transporte de íons Al via simplasto. Além disso, detectamos transportadores pertencentes a famílias já conhecidas por desempenharem um papel na detoxificação de Al e na exclusão de ácidos orgânicos, e, propusemos uma classe de transportadores SWEET que podem estar envolvidos na regulação do armazenamento de açúcar vacuolar sob estresse por Al. No estudo para explorar a variação e reduzir o número de genes responsivos ao Al, foi realizado o mapeamento de DEGs nos cromossomos e identificadas variantes SNPs. Reads foram mapeadas no genoma referência utilizando o algoritmo BWA-MEM. Os arquivos de mapeamento foram processados usando o software Picard. A chamada de variantes foi realizada usando o software FreeBayes. Os SNPs foram filtrados utilizando vcftools e anotados com o Variant Effect Predictor. Para detectar DEGs dentro de QTLs para Al-tolerância anteriormente identificados, o mapeamento *in silico* foi realizado pela comparação entre a posição dos genes e a região flanqueada por marcadores em cada cromossomo. Isto permitiu a identificação de genes dentro de QTLs anteriormente reportados na literatura e a detecção de variantes. Fomos capazes de destacar alguns novos alvos como: SNF1-related protein kinase, histone deacetylase (HDT1), transportadores SWEET, delta (12)-fatty-acid desaturase (FAD), MADS-box, AP2/EREBP, HY5-like e proteínas relacionadas à patogênese (PR) que podem contribuir para a manutenção do crescimento radicular de milho pipoca sob estresse por Al.

INTRODUCTION

Abiotic stress is the primary cause of crop loss worldwide, reducing average yields by more than 50% (Rodríguez et al., 2005). Plants alter their physiology, metabolic mechanisms, gene expression, and developmental activities to cope with the environmental stress effects (Rao et al., 2006). The heavy metal pollution is an abiotic stress that is increasing in the environment due to industrial and agricultural activities such as mining and smelting of metalliferous ores, wastewater irrigation, and the irrational use of chemical fertilizers and pesticides (Redondo-Gómez et al., 2010). An excess of essential or nonessential elements in soil solution can have detrimental effects on plant growth and developmental. The survival of plants growing on contaminated soils is the result of tolerance rather than avoidance since no plants have the ability to suppress metal uptake, but can only restrict it in an internal detoxification (Baker, 1981).

Aluminum (Al) is one of the most abundant heavy metals present in the earth's crust. Its toxicity is the primary factor limiting the plant growth and development in acid soils ($pH < 5$), restricting a range of physical, cellular, and molecular processes, causing multiple damaging effects on plants (Kochian et al., 2005). The cellular processes that are adversely affected by Al stress are extensive. Two of the most important are: the inhibition of root growth, since the root meristems are the first sites of accumulation of toxic forms of Al; and the inhibition of root elongation, which is the main evidence of the physiological manifestation of the toxicity by this ion. This lead to a decrease in crop yield caused by drought and mineral deficiency (Kochian et al., 2015; Von Uexküll and Mutert, 1995). Other important processes have already been described are: changes in the physical property, composition, permeability dynamics and structure of the plasma membrane (Illéš et al., 2006; Ishikawa and Wagatsuma, 1998; Wagatsuma et al., 1995; Zhang et al., 1997; Zhao et al., 1987), changes in cell wall structure, Ca^{2+} and other cations uptake (Liu and Luan, 2001; Ryan et al., 1992), signal transduction (Huang et al., 1995; Jones and Kochian, 1995; Rengel and Zhang, 2003), oxidative stress (Boscolo et al., 2003; Yamamoto et al., 2003), and cytoskeleton dynamics (Blancaflor et al., 1998; Sivaguru et al., 2000).

Plants vary considerably in their ability to tolerate toxic concentrations of Al^{3+} ions in acid soils. This variation is driven by the ability to exclude Al from its tissues or by its ability to detoxify Al^{3+} ions once it enters the cytosol (Kochian et al., 2005; Ma et al., 2001). In some species, most of this variation is controlled by a single major gene, whereas in other the characteristic is more complex including multiple genes and mechanisms. These mechanisms can be constitutively expressed and/or induced in the exposure to Al, involving several signaling components (Li et al., 2014). Plants use multiple strategies against Al-stress, and two types of mechanisms are described: Al-exclusion and the tolerant mechanism (Kochian et al., 2015).

The Al-exclusion mechanism is associated with the exudation of organic compounds by the root apex in the presence of activated Al, protecting the intracellular sites sensitive to the ion attack. The mechanism includes the immobilization of aluminum in the cell wall, selective permeability of the plasma membrane, formation of a pH barrier in the rhizosphere, exudation of chelators and efflux of the ion in question. The Al-dependent root exudation of organic acid anions into the rhizosphere is a well-documented Al exclusion mechanism. Organic acids such as malate, citrate and oxalate are able to chelate Al and attenuate Al toxicity (Kochian et al., 2015; Taylor, 1988). Furthermore, phenolic compounds also have the ability to chelate Al^{+3} ions, although they are less effective (Kidd et al., 2001).

The tolerance mechanism consists in neutralize toxic Al^{3+} absorbed by sequester and detoxification in subcellular compartments and/or translocate it away from the root tip (Kochian et al., 2015). Several proteins and compounds can form stable complexes with Al within the cell, including organic acids such as citrate, oxalate and malate (Simões et al., 2012). In addition, the Al^{+3} ion or complexed with chelating agents can be transported to the cellular vacuole, where they are stored without causing toxicity to the plants (Kochian et al., 2004).

Maize is a crop widely grown on acid soils throughout tropics and subtropics (Maron et al., 2013). Al tolerance is considered a quantitative trait in maize (Conceição et al., 2009; Guimaraes et al., 2014; Magnavaca et al., 1987; Ninamango-Cárdenas et al., 2003; Sibov et al., 1999). Although the root citrate release plays an important mechanism against the Al stress (Maron et al., 2013), this mechanism is not well correlate with Al-resistance, suggesting the hypothesis that there are other Al resistance mechanisms operating in maize roots (Piñeros et al., 2005).

Temporal changes in gene expression are the main determinants of metabolic and physiological processes, and also the primary mediators of altered cell properties, defining several states related to biotic and abiotic stresses (Jiang et al., 2000). With the advance of high throughput sequencing and functional genomics tools, the molecular mechanisms underlying abiotic stress tolerance in plants are being unraveled, progressing in the comprehension of stress signal perception and transduction of the associated molecular regulatory networks (Swatismita et al., 2009).

The identification and determination of gene expression patterns in response to stress should enhance the understanding of its functions and provide the necessary basis for effective strategies to improve the adaptation of cultivars to this reality. The use of sensitive and high throughput techniques and the application in comparative studies is crucial for revealing the role of differential gene regulation in Al toxicity and tolerance (Maron et al., 2008). The diversity of biological process affected by Al and its complexity evidences the large number of genes that may be involved in tolerance mechanisms.

Although there are some studies of the characterization of genes involved in resistance to Al in maize, the investigation of the roles of these genes in hydroponic system has been focused in a short time of Al exposure (Maron et al., 2010). This provided identify genes through microarray data involved in a fast response of Al toxicity. In this way, the approach using the new generation RNA sequencing technology in the evaluation of differential expression has not yet been performed and it is believed that after a long term of Al exposure (e.g. 72 h) a maintenance mechanism may be created to alleviate the Al-damages and promote the root growth and plant development.

This study aimed to identify new players involved in Al-resistance using RNA-sequencing. The specific objectives were: i) to track the gene expression of two contrasting popcorn inbred lines (one resistant to and the other sensitive to Al stress) under a long time of Al exposure (72 h); ii) to link the RNA-seq data into previous maize QTLs involved in Al tolerance to explore and reduce the genes associated with these regions; iii) to detect variants with predicted deleterious substitutions.

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CHAPTER 1

HIGH-THROUGHPUT TRANSCRIPTOMIC ANALYSIS REVEALS NEW PLAYERS IN POPCORN (*Zea mays*) ALUMINUM RESISTANCE

High-throughput transcriptomic analysis reveals new players in popcorn (*Zea mays*) aluminum resistance

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Abstract

At the date, the investigation of genes involved in Al resistance has focused mainly in gene expression profiles based on microarray and in short periods of exposure to Al. Under a long term of Al exposure, such as 72 h, resistance maintenance mechanisms might be created in which several metabolic processes are working simultaneously. To investigate new players involved in defense response under Al stress, we tracked the expression profile of two Al-contrasting popcorn inbreed lines during 72 h under Al-stress using Illumina high-throughput mRNA sequencing technology. A total of 1,121 differentially expressed genes (DEGs) were identified in the Al-sensitive line and 2,872 DEGs in the Al-resistant line, of which 384 were shared in both lines. Genes categorized in lipid metabolic process and oxidation-reduction were present in both lines, but lipid metabolic process was up-regulated in the Al-resistant line but down-regulated in the Al-sensitive line. The biological process category revealed that the down-regulated genes on the Al-resistant line were involved in signaling. The most significant GO modules were clustered in response to a stimulus. Differentially expressed transporters were also found in both lines, including ABC transporters, Nramp, aquaporins, SWEET transporters, Al-activated malate transporter (ALMT), and multidrug and toxic compound extrusion (MATE). Several transcription factors and genes involved in cell wall modifications were also detected. The Al-resistant line presented genes that may play a role in an efficient oxidative system against ROS, involved in cell wall stiffening and dynamic changes of the cell wall to prevent the Al ion transport via the symplast. Also, we detected transporters belonging to families already known to perform a role in Al-detoxification and organic acid exclusion, and we proposed a class of SWEET transporters that might be involved in regulation of vacuolar sugar storage under Al-stress. Although the exact functions of these genes remain to be investigated, these results provide a platform for functional analysis of the defense response against Al-stress in maize.

Keywords: Aluminum stress; RNA-seq; roots; transcriptome; *Zea mays*

Introduction

Aluminum (Al) is the third most abundant element in the earth's crust. In acid soils, with pH values at or below 5, the phytotoxic species Al^{3+} is solubilized in soil solution and becomes one of the most important abiotic stresses limiting crop production. Al stress occurs in approximately 30% of world's arable soils and in more than 50% of potentially arable land. From this total, approximately 60% is located in tropical and subtropical regions, and negatively impacting the food supply chain. The phytotoxic form Al^{3+} inhibits root growth, thereby altering water and nutrients absorption and consequently reducing plant development (Kochian et al., 2002; Kochian et al., 2015; Von Uexküll and Mutert, 1995).

Plants use multiple strategies against Al-stress, and two types of mechanisms are described: 1) Al-exclusion, preventing the Al entrance in the root apex, and 2) the tolerant mechanism, where Al enters the plant and is detoxified and sequestered (Kochian et al., 2015). The well-characterized exclusion mechanism is dependent on organic acid (OA) exudation from the root apex (Kochian et al., 2004; Ma et al., 2001). Although the root citrate release plays an important mechanism against the Al-stress in maize, as identified from the citrate transporter ZmMATE1 (Maron et al., 2010), this mechanism is not well correlated with Al-resistance, suggesting that others Al resistance mechanisms are operating in maize roots (Piñeros et al., 2005). Organic acids such as malate, citrate, and oxalate can chelate Al and attenuate Al toxicity (Kochian et al., 2015). The Al exposure induced malate secretion in wheat (*Triticum aestivum*) (Delhaize et al., 1993), *Arabidopsis thaliana* (Hoekenga et al., 2006), rape (*Brassica napus*) (Ligaba et al., 2006), citrate secretion in sorghum (*Sorghum bicolor*) (Magalhaes et al., 2007), barley (*Hordeum vulgare*) (Furukawa et al., 2007), rice bean (*Vigna umbellata*) (Yang et al., 2011b), rice (*Oryza sativa*) (Yokosho et al., 2011), wheat (Ryan et al., 2009; Tovkach et al., 2013) and maize (*Zea mays*) (Maron et al., 2013), and oxalate secretion in buckwheat (*Fagopyrum esculentum*) (Ma et al., 1997), spinach (*Spinacia oleracea*) (Yang et al., 2005) and tomato (Yang et al., 2011a).

RNA sequencing technology (RNA-Seq) has been revolutionary in the study of transcript expression. To understand the elements involved in response to biotic and abiotic stresses, the whole transcriptome has been used to identify new plant defense responses. Using RNA-Seq technology, Zhu et al. (2015) have identified genes involved in the cell wall toxicity and oxidative stress during 6 h of Al-stress in buckwheat. In roots and leaves of *Hydrangea macrophylla*, were found many transporters involved in the Al-citrate complex transporting during 4 h of Al-stress (Chen et al., 2015). In *Stylosanthes* sp., Jiang et al. (2018) reported that Al-resistance involves multiple strategies and enhancement of citrate anabolism during 24 h of exposure. During 48 h of exposure of two contrasting soybean lines under Al-stress, RNA-Seq analysis revealed that citrate metabolism and secretion are preferentially expressed in the root of Al-resistance soybean, and the jasmonate biosynthesis and signaling are induced in leaves of Al-sensitive soybean (Huang et al., 2017). Li et al. (2017), following a gradient Al-level exposure, found ideal candidates involved in the Al resistance or accumulation in tea plant tracked by RNA-Seq.

In maize, no RNA-Seq studies tracking the expression profile of Al resistance responsive genes have been performed yet. Maron et al. (2008) performed the first microarray study of global transcriptional regulation under Al-stress in maize in hydroponic system in a short period of Al exposure. Likewise, Mattiello et al. (2010) characterized the gene expression profile of maize roots grown in acid soil. This results also provided the integration of microarray data and previously published Al tolerance maize QTLs, allowing the identification of potential Al-resistance genes (Mattiello et al., 2012).

As previously observed, the search of Al-responsive genes in maize was focused mainly in an early response. It is believed that in a long term of Al exposure, such as 72 h, a robust maintenance mechanism is activated in which several metabolic processes are working at the same time. New players involved in defense response under Al stress were investigated tracked by the expression profile of two popcorn lines (one resistant to and the other sensitive to Al stress) during 72 h under stress by looking for genes that are important for the resistance, and not only signaling. This study presents the first high-throughput RNA sequencing in popcorn and new components regulating the resistance mechanism that will expand knowledge about this trait and might help to identify new markers of Al-resistance in crops.

Results

A range of 38.5 ~ 46.5 million clean reads wre after the quality process for each sample and obtained around 52.87% of GC content. An average of 80.10% of the reads was mapped, and from this total, only 6.33% presented multiple alignments to the B73 reference genome using the default parameters (Additional file: Table S1).

The results showed that the control and +Al treatment were clustered together for both lines, but the lines were grouped into separated clusters, revealing differences on the genetic background between both lines (Fig. 1) as previously reported by Rahim et al. (2019). A total of 1,121 differentially expressed genes (DEGs) for the Al-sensitive line (527 were down-regulated, and 594 were up-regulated) and 2,872 DEGs in the Al-resistant line (1,469 down-regulated and 1,403 up-regulated) were detected at an FDR of $q < 0.01$ (Fig 2; Additional file: Figure S1; Additional file: Table S2). The different number of expressed genes in both lines reveal that the Al-resistant line has a broader response to Al stress, regulating multiple maintenance pathways against the Al damage (Fig. 2).

Furthermore, were found 384 common expressed genes in both lines under Al-stress (Fig. 2; Additional file: Figure S2). Among those, 57 were up-regulated while 51 were down-regulated in both lines. Many of the genes with the same differential expression pattern are involved in response to a stimulus, playing an important role in the defense system. Besides, 145 up-regulated genes in the Al-sensitive line were down-regulated in the Al-resistant line, and many of them are related to "oxidation-reduction" class. Meanwhile, 131 genes had the opposite behavior: down-regulated in the sensitive line and up-regulated in the Al-resistant line, and many of them are involved in lipid metabolic process, catalytic activity, metal binding, and oxidoreductase activity (Fig. 2; Additional file: Table S3).

The Gene Ontology (GO) analysis categorized DEGs into different groups. For both of the tested lines, many of the differentially expressed genes were classed as belonging to the "metabolic process" category (Fig. 3; Fig. 4). In the biological process category, "response to stimulus" and "oxidation-reduction" were also found highly enriched, "membrane" was enriched in the cellular component category, and "catalytic activity" and "metal ion binding" in molecular function category (Additional files: Figures S3 and S4).

The GO enrichment analysis revealed that genes categorized in lipid metabolic process, oxidation-reduction, oxidoreductase activity, peroxidase activity, and antioxidant activity were present in both lines (Fig. 3; Fig. 4), but the lipid metabolic process was up-regulated in the Al-resistant line but down-regulated in the Al-sensitive line. In the Al-resistant line, we only found significantly up-regulated genes categorized as involved in glutathione transferase (GST) activity (Table 1).

Additionally, peroxidase (POD), catalase (CAT), and some reductase (RE) playing a role in reactive oxygen species (ROS) scavenging were detected only in the Al-resistant line, but cytochrome P450 genes with oxidoreductase activity were found in both lines (Additional files: Table S4; Table S5). Among the GST genes expressed in both lines, 11 were up-regulated in the Al-resistant line, contrasting with only three in the Al-sensitive line (Table 1). Down-regulated GST genes were detected: GST34 (Zm00001d029696) and GST U16 (Zm00001d029702) in 11-133 and 11-60, respectively.

The biological process category revealed that the down-regulated genes on the Al-resistant line were involved in signaling. The most significant GO modules were clustered in response to a stimulus. Also detected genes involved in phosphorylation, oxidation-reduction, metabolic sugar process (hexose, fructose, glucose), organic and carboxylic acid metabolic processes, and carbohydrate and lipid metabolic process. However, the up-regulated genes were involved in cellulose, glucan, and suberin biosynthetic processes. Up-regulated genes on cell wall categories were observed only in the Al-resistant line, which was consistent with the pattern of enriched terms on biological process network analysis conducted in Cytoscape (Additional file: Figure S5). Significant terms found on this analysis were cell wall organization, primary cell wall biogenesis, and polysaccharides responsible for cell wall stiffening (Additional file: Figure S5). On the other hand, GO analysis in the Al-sensitive line identified 30 cell wall enriched genes (Additional file: Figure S3), and no significant enriched terms were reported on protein-protein interaction network, contrasting with 106 genes in the Al-resistant line (Additional file: Figure S5).

The predicted metabolic pathway analysis revealed that the metabolic routes in the Al-resistant line may highly modified under aluminum stress in comparison with the Al-sensitive line (Additional files: Figure S6; Figure S7). After 72 h of Al exposure, this prediction indicated that the brassinosteroid biosynthesis route was induced in the Al-resistant line and deactivated in the sensitive line (Additional files: Figure S6; Figure S7).

Likewise, part of the tricarboxylic acid (TCA) cycle may have been induced in the resistant line. In this predicted map, was visualized a deactivation in 2-oxoglutarate production, and consequently, a reduction in the amino acid metabolism route dependent on this compound. Also was observed an exclusive induction of oxaloacetate production in the Al-resistant line. However, in the Al-sensitive line, isocitrate on the TCA cycle was used to produce glyoxylate and induce the glyoxylate cycle. It is noteworthy that the lines showed an opposite pattern when comparing glycine, serine, and threonine metabolism. These pathways were up-regulated in the Al-resistant line and down-regulated in the Al-sensitive line (Additional files: Figure S6; Figure S7).

The fatty acid biosynthesis and degradation route may have been induced only in the Al-resistant line (Additional files: Figure S6; Figure S8). A curious pattern of alternation in fatty acid biosynthesis between the seven main enzymes was found. Beta-ketoacyl synthase I, beto-ketoacyl synthase II, fatty acid synthase, fatty acyl-CoA synthase, and 3-hydroxyacyl dehydratase were up-regulated while 3-oxoacyl reductase, fatty acid synthase, fatty acyl-CoA synthase and enoyl reductase (NADPH Si-specific, NADPH and NADH) were down-regulated. However, the final step possibly induced the production of octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid and, mainly, hexadecanoic acid. This compound is responsible for inducing cutin, suberine and wax biosynthesis route and might play an important role in cell wall stiffening. In Al-sensitive line, was predicted just an inactivation at the end of the lipid biosynthesis route, probably reducing the production of hexadecanoic acid (Additional files: Figure S7). Also was observed that the sphingolipid, linoleic acid, arachidonic acid, and glycerolipid metabolism may have been altered in Al-resistant line, showing an opposite way on the Al-sensitive line.

Differentially expressed transporters were also found in both lines (Table 2; Table 3), including ABC transporters, Nramp, aquaporins, SWEET transporters, Al-activated malate transporter (ALMT), and multidrug and toxic compound extrusion (MATE). The majority of transporters in both lines were ABC transporters, of both the G and B families. The most expressive in the Al-resistant line was the ABC G member 29 and ABC B member 19. Nrat1 was up-regulated, while Nramp6 were down-regulated in the Al-sensitive line. Four genes encoding aquaporin proteins were found only in the Al-resistant line. PIP2-2 (Zm00001d005410 and Zm00001d014285) was up-regulated and TIP3-1 (Zm00001d048520), and an ortholog PIP2-2 (Zm00001d022608), was down-regulated. Seven SWEET transporters were also identified of which one was down-regulated in the Al-resistant line, and three were up-regulated in the Al-sensitive line.

Several transcription factors (TFs) were also detected among the differentially expressed genes found in this study. The number of TFs in the Al-sensitive line were 32 and 23 down- and up-regulated, respectively, and the Al-resistant line presented 89 and 38, also down- and up-regulated, respectively (Additional files: Table S6; Table S7). TFs exclusively overexpressed were found in the Al-resistant line, including ZF-HD, CO-like, ARF, and E2F/DP families.

Four transposable elements were found in both lines (Additional file: Table S2). In Al-sensitive line was found a gene that encodes a retrotransposon protein (Zm00001d029444) and in Al-resistant line was found a gene that encodes a transposon protein CACTA%2C En/Spm sub-class (Zm00001d039524). Moreover, a transposon protein (Zm00001d041987) was found down-regulated in Al-sensitive, but up-regulated in Al-resistant.

Discussion

Aluminum toxicity is one of the main factors limiting crop cultivation on acidic soils (Vitorello et al., 2005). For more than 50 years, breeders have explored the genetic diversity to improve Al-resistance in several crops, especially in tropical breeding programs (Kochian et al., 2015). Some critical Al toxicity events are initiated at the transcriptional, biochemical, and physiological levels (Xu et al., 2017). To date, several Al-tolerance mechanisms have been described, but much more needs to be discovered to reveal the complex response to Al-stress. Here, was provided a comprehensive RNA-sequencing analysis revealing new important players involved with Al-resistance in a high concentration of Al^{3+} (160 µM) under a long exposure time (72 h).

In this study, the RNA-Seq data revealed a large number of differentially expressed genes regulated by Al-stress in both genotypes. Also, the number of genes differentially expressed in the Al-resistant line was higher than in the Al-sensitive line, suggesting several maintenance responses playing a tolerance role in the Al-resistant genotype. Moreover, under normal conditions, the Al-resistant popcorn has an expression pattern that is clearly distinct from the sensitive genotype, and the basal separation between both popcorn lines by PCA analyses indicates that the resistance is clearly intrinsic to the Al-resistant genotype.

Transporters are one of the most important players in plant Al-resistance, having a role in the plasma membrane and the tonoplast, acting in the exclusion and tolerance mechanisms (Kochian et al., 2015). The aluminum-activated malate transporter 10 (ALMT10) was up-regulated in the Al-sensitive (Zm00001d026102) and Al-resistant (Zm00001d046029) lines, but a putative MATE efflux family protein (Zm00001d009494) was down-regulated in the Al-resistant line. Although it is clear that Al-resistance is related to higher MATE1 gene copy in maize (Maron et al., 2013), at the time point of our analysis, it is not possible to track the response of this gene, possibly because it was already activated during initial steps of Al-stress or perhaps a different allele is present in our Al-resistant genotype. Although there are multigenes members of ALMT in maize genome, the only functional study has been made on the ZmALMT, showing that this member is not play a role in malate exudation in maize (Piñeros et al., 2008). Based on this, the ALMT gene response between both genotypes may acts in other response not exactly in the organic acid production which needs further functional investigation.

The predicted metabolic pathway analysis in Al-resistant line, has been showed a probable deactivation of 2-oxoglutarate production from isocitrate in the TCA cycle and induction of oxaloacetate production from the malate (Additional files: Figure S6). This pattern might be due to the release of citrate from maize roots or the high production of malate acting as an exclusion mechanism against Al. Citrate release might affect the availability of substrate to increase levels of isocitrate and consequently 2-oxoglutarate. However, further metabolic analysis need to be made to quantify these compounds in popcorn roots. The organization of carboxylic acid metabolism in plants is highly dependent on the metabolic and physiological demands of the cell (Sweetlove et al., 2010) and the Al-stress condition might induce this non-cyclic flux.

The in silico analysis showed that possibly were no responses detected in the TCA cycle in the Al-sensitive line. However, in this study was observed a supposed partial activation in part of the glyoxylate cycle. Since isocitrate serves as a substrate for activating this cycle (Additional files: Figure S5), this may work as an extra production of succinate and posterior conversion to malate to supply the TCA cycle with metabolites or a new energy source to the cell from acetyl-CoA. To date, no evidence has been presented demonstrating glyoxylate cycle change in plants under Al-stress, and this hypothesis needs further investigation.

Other transporters play an important role in the tolerance mechanism by detoxifying the Al ion inside the cells. The ABC transporters families found in both lines suggest that these transporters work together with other detoxifying systems to increase the tolerance response in Al-resistant line. Nrat1 (Nramp aluminum transporter 1) is a specific Al transporter identified in rice that uptakes Al to cells for sequestration to vacuoles, and it is required for the initial steps of internal Al-detoxification (Xia et al., 2010). The best candidate gene as homolog of OsNrat1, showed an up-regulation pattern in Al-sensitive line. We proposed that, although our sensitive plant can respond with multiple mechanisms against the absorption of Al ion in popcorn roots, this is not sufficient to support this stress due to the metabolic unbalance caused by the Al toxicity condition. Aquaporins are a group of highly conserved membrane proteins that facilitate water transport across biological membranes (Jang et al., 2004) and, in this study, the results suggest that tonoplast aquaporins are likely involved in the Al-tolerance response, but it remains unclear whether PIP proteins might play an important role in response to Al-stress in popcorn.

Cellular efflux of sugar plays important roles in the maintenance of sugar efflux in phloem loading, nectar secretion, for the supply such as mycorrhiza, and as maternal efflux for filian tissue development (Chen, 2014). Plants need to maintain a rigid regulation in the storage and transport of vacuolar sugar to deal with environmental adverse conditions (Chandran, 2015). The role of SWEET in Al-tolerance responses remains unclear, but its play an important role in adverse conditions, such as tolerance to osmotic stress, including cold, high salinity and drought (Seo et al., 2011) and a mechanism to accumulate sugars in vacuoles as a tolerance mechanism to freezing stress has been described (Yuanyuan et al., 2009). Based on this, we suggest that these SWEET transporters play a role in maintaining the tight regulation on vacuolar sugar storage.

Plants under aluminum stress might produce ROS in mitochondria, chloroplast, and peroxisome (Kochian et al., 2005) and to defend themselves, they might control the ROS levels by deploying a complex antioxidant defense system comprised of enzymes and non-enzymatic metabolites that might accumulate in various stresses conditions and scavenging the ROS (Vranova et al., 2002).

Testing two contrasting maize lines under different concentration of ion aluminum, Giannakoula et al. (2010) showed that the anionic POD isoforms and superoxide dismutase (SOD) isoforms increased with increasing of Al stress in the tolerant line, and that this antioxidant system up-regulation might provide considerable protection to roots against oxidative damage. In the same way, the CAT enzyme acts as an auxiliary antioxidant working selectively either with SOD or POD during the peroxidation caused by Al stress, being the major enzyme responsible for root growth (Wang et al., 2015), thereby corroborating with our results.

Also acting as detoxification enzymes, GSTs are positioned in the central glutathione network, playing a role against plant oxidative damage (Labrou et al., 2015). Overexpressing the tobacco par B, which encodes an antioxidant phi class GST enzyme, in *A. thaliana* demonstrated that the transgenic plants were more tolerant than the wild-type under aluminum and copper stress, and able to significantly decrease the lipid peroxidation (Ezaki et al., 2000; Ezaki et al., 2001). Cançado et al. (2005) described how an up-regulation of GST27.2 might play a role in maize roots alleviation against Al toxicity. These results support the observations that GSTs might reduce the oxidative damage caused by Al stress in our Al-resistant genotype.

The function of cytochromes P450 involved in heavy metal stress is not totally clear. Cytochrome P450 might act as monooxygenases in the biosynthetic pathways of lignin, defense compounds, hormones, pigments, fatty acids, and signaling molecules. Moreover, there is a function in the detoxification pathway to catalyzes several endogenous and exogenous toxic compounds in both the cytoplasm and the endoplasmic reticulum A decrease in ROS production has been reported following the interaction between NADPH-dependent cytochrome P450 oxidoreductase with human Bax inhibitor-1, and this results in the electron uncoupling between this P450 reductase and cytochrome P450 2E1, reducing the source of ROS at the endoplasmic reticulum membrane (Ishikawa et al., 2011). The differential expression of several genes that encode cytochrome P450 proteins in both lines might indicate an important adaptive mechanism, which relieves the endoplasmic reticulum through a decrement of oxidative load under Al-stress.

In maize, the ROS production might trigger a signal transduction cascade which induces callose production and inhibits the Al ion migration into the cortex via symplast and, consequently, the apoptosis of the epidermal layers (Jones et al., 2006). Moreover, the cell wall represents a physical barrier against the entry into the symplastic compartment, and plants might remove Al ion from the protoplasts by sequestration and compartmentalize in the cell wall (Krzesłowska, 2011; Ovečka and Takáč, 2014).

Cell wall modification response under abiotic stresses might involve maintenance of cell wall plasticity due to an increased level in xyloglucan endotransglucosylase/hydrolase and expansin proteins associated with an increase in the degree of rhamnogalacturonan I or by the increase of hemicellulose and lignin deposition contributing to the reinforcement of secondary wall and, consequently, cell wall thickening (Le Gall et al., 2015).

Eticha et al. (2005) demonstrated that Al accumulates primarily in the maize root apoplast, where Al^{3+} can bind to the negatively-charged bindings sites provided by non-methylesterified pectin in the cell wall, and the degree of pectin methylation might contribute to genotypic differences in aluminum tolerance in maize. In rye, it was reported that the pectin biosynthesis induced by Al is directly related tolerance, which is related to the relative pectin methylesterase (PME) expression levels (El-Moneim et al., 2014). In *Medicago truncatula*, pectin esterase and pectin esterase precursors were up-regulated following treatment with Al, while the silencing of genes that encode pectin acetylesterase and annexin increased the sensitivity of the plant to Al (Chandran et al., 2008). Unlike these authors, in our RNA-seq analysis, we found only one gene encoding a PME that was down-regulated in the resistant genotype, as well as putative pectin-esterases and pectin-lyases in both genotypes, but without a regulation pattern, probably due to the relatively long exposure time of our experiment.

Expansins modify the cellulose and non-cellulosic components of the cell wall, thereby loosening and modifying the plant cell wall during growth and adaptation to biotic and abiotic stress (Cosgrove, 2005), but the role of expansins in Al-stress remains unclear. Tenhaken (2015) proposed that expansin and xyloglucan endotransglucosylases/hydrolases frequently show differential expression under abiotic stress conditions and consequently increasing presence of ROS, ultimately pausing the plant growth. In our study, the differentially expressed expansins were EXPANSIN A11 (EXPA11) and EXPANSIN B4 (EXPB4), up-regulated in the Al-resistant line, but with opposite regulation in the Al-sensitive line, suggesting that these specific expansins play an important role in the cell wall modification under Al-stress.

With a role in the construction of barriers to the environment, as components of cellular membranes, suberin and cutin waxes (Beisson et al., 2007), fatty acids are among the main components responsible for membrane integrity and function, which is determined by structure and fluidity (Mikami and Murata, 2003). The changes induced by abiotic stress in the fatty acid composition of plant membrane lipids occur due to the regulated activities of fatty acid desaturases and the ability to adjust membrane lipid fluidity by changing the level of unsaturated fatty acids (Upchurch, 2008).

Growing in nutritive solution, seedlings of Al-tolerant sorghum cultivar demonstrated an increase of linolenic and palmitic acids in the plasma membrane under Al-stress, but the concentration of these fatty acids decreased in the Al-sensitive line, and these fatty acids might be useful to indicate Al tolerance in sorghum (Peixoto et al., 2001).

In predicted metabolic pathway maps, we reported the induction of fatty acid biosynthesis that may have been produced hexadecanoic acid activating the cutin, suberin, wax biosynthesis route, and down-regulation in part of the fatty acid degradation route in Al-resistant (Supplemental Figure 6). However, the Al-sensitive line demonstred a predicted down-regulation in the fatty acid biosynthesis pathways

(Supplemental Figure 7), demonstrating that fatty acids might contribute to membrane integrity under Al-stress.

Root growth and development response, which are also involved with cell enlargement, were up-regulated in the Al-resistant line. Our results indicate that the majority of transcripts associated with hormones were related to cytokinin, auxin (IAA), and abscisic acid (ABA). Abscisic acid might regulate Al resistance in soybean through the possible involvement with citrate release (Shen et al., 2004). In Arabidopsis, the AtALMT1 expression levels are up-regulated by ABA, suggesting that this hormone might activate ALMT1 expression as malate transport activity and then inducing organic acid expression (Kobayashi et al., 2013). Furthermore, using a microarray approach, Al-stress was shown to induce the expression of ABA-related genes (DREB1A and DREB1A) in *A. thaliana* (Sawaki et al., 2016).

The transcription of Al-tolerance genes might also be activated by IAA (Kobayashi et al., 2013). In Arabidopsis mutants with internal Al-detoxification mechanisms, it was verified that endogenous IAA suppress the transport of symplastic Al^{3+} to the vacuole, thereby negatively regulating Al-tolerance (Zhu et al., 2013), but exogenous IAA induced high expression levels in AtALMT1, as well as a slight increase in AtMATE expression levels (Kobayashi et al., 2013).

We also found a class of steroid hormones, the brassinosteroids (BRs), that might minimize the toxic effects caused by aluminum and other heavy metals, thereby reducing the accumulation of these elements due to their capacity to regulate the uptake of ions inside plant cells and inhibit the degradation of lipids resulting from the overproduction of ROS. Furthermore, BRs trigger the accumulation of apoplastic H_2O_2, which up-regulates the antioxidant system, thereby increasing the stress tolerance (Bajguz and Hayat, 2009; Jiang et al., 2012; Soares et al., 2016).

Little is known about the effect of BRs in Al stressed plants, but most evidence suggests that BRs improve the response of the antioxidant system. Treating mung bean seedlings with exogenous BRs, 28-homobrassinolide or 24-epibrassinolide (EBL), Ali et al. (2008) evidenced an increase in proline content and the activities of SOD, CAT, and guaicol peroxidase in response to Al stress, indirectly contributing to the improvement of plant growth and photosynthesis. Additionally, EBL significantly increased the chlorophyll content and fresh masses of shoots and roots.

Here, was reported a possible differential response in BRs biosynthesis route between the contrasting lines under aluminum stress for 72 h through in silico metabolic analysis (Supplemental Figure 6; Supplemental Figure 7). Two reaction paths may have been induced in the Al-resistant line in the formation of 3-dehydro-6-deoxoteasterone and typhasterol, this one closer to the final path in the production of brassinolide. On the other hand, this same route had four paths that may have been disabled related to the production of 6-deoxocathasterone and 3-dehydroteasterone in the Al-sensitive line, which supported these previous observations that BRs might induce a response against Al-stress. These components might be involved in a defense mechanism carried out by brassinosteroids in our Al-resistant line.

The importance of TFs under Al-stress have been investigated. Kumari et al. (2008) identified AP2/EREBP, MYB, and bHLH as predominant families of TFs responsive to Al-stress in *Arabidopsis thaliana* and Xu et al. (2017) found an increased expression of 27 TFs in buckwheat, where most were categorized in the NAC family, but only three down-regulated (bHLH1, OVATE family protein 17, and MYB19). In rice, some authors reported ASR (Abscisic Acid, Stress and Ripening) as involved in Al and other stresses (Arenhart et al., 2014). MYB proteins were found to be regulated under Al-stress in common bean (Yang et al., 2012) and playing a role in the cleavage mediated by miRNA, essential for responding to Al-stress, in wild soybean (Zeng et al., 2012).

Members of the WRKY family might develop various responses under Al-stress. Ding et al. (2013) reported that WRKY46 acts as a repressor of expression of AtALMT1 in Arabidopsis. WRKY22 has a positive role in increasing the expression of OsFRDL4, a gene that encodes a citrate transporter, enhancing the Al-tolerance in rice by citrate secretion (Li et al., 2018).

The zinc finger-associated to a homeodomain (ZF-HD) family regulates diverse plant-biological processes, such as development and responses to phytohormones and abiotic stresses (Wang et al., 2016). An increase in the expression response of BraZF-HD under heat, cold, and salt stress was found in Chinese cabbage (Wang et al., 2016). CONSTANS-like (CO-like) has been found to respond by the overexpression in ABA and salt stress as a positive regulator in Arabidopsis (Min et al., 2015). Zeng et al. (2012) reported an important role for miR160 in the cleavage of Auxin Response Factor (ARF) transcripts in response to auxin, which might regulate the inhibition of root development under Al-stress. Little is known about the role of E2F/DP in abiotic stress but acts as a sub-category in response to heat stress in Arabidopsis and rice (Ueda et al., 2012). The relationships between ZF-HD, CO-like, and E2F/DP and aluminum stress are still poorly understood, although it is believed that this TFs might play diverse roles in transcriptional regulation of key genes in Al-stress response.

Some important TFs that regulate the expression of genes related to Al tolerance have been identified. ART1 encodes a C2H2-type zinc finger TF required for Al resistance in rice (Yamaji et al., 2009), ART2 an ART1 homologs (Che et al., 2018) and STOP1 that is involved in signal transduction pathways regulating aluminum responsive gene expression (Sawaki et al., 2009). However, these TFs were not found in our analysis probably due the long term of exposure of Al toxicity levels. These TFs were induced in a short period of Al-exposure, regulating the expression of initial responsive genes in previous work. For these reasons, we believe that these TFs have been already expressed in initial steps in popcorn roots under Al-stress not being detected by the RNA-seq analysis.

Transposable elements (TE) are a source of spontaneous mutations (Bennetzen, 2000; Lambert et al., 1988; Paquin and Williamson, 1986) and play an important role in responding to environmental adverse conditions (Casacuberta and González, 2013). TEs may enhance plant adaptation to acid soils increasing the expression of Al-responsive genes or the gene copy number due to the increase of beneficial mutations (Pereira and Ryan, 2018). On the other hand, TEs may reduce the expression of key genes affecting splicing patterns or altering protein function (Pereira and Ryan, 2018). The TEs found in our data may contribute to activate or inactivate important genes that may confer tolerance to Al toxicity. However, further investigations have been made to characterize these TEs expressed under Al-stress.

Moreover, up-regulated genes expressed only in Al-resistant lines might be useful as a marker to screening Al-resistance in maize, which might help identify key players in future studies. Transporters that presented positive and high fold change values, such as ABC transporter B family member 9 (Zm00001d044564 and Zm00001d043766) and member 19 (Zm00001d024600), ABC transporter G family 29 (Zm00001d036986), SWEET 2 (Zm00001d009365), SWEET 4A (Zm00001d015905), SWEET 13C (Zm00001d041067), SWEET 13A (Zm00001d023677), SWEET 14B (Zm00001d049252), Aluminum-activated malate transporter (Zm00001d046029), and aquaporin PIP2-2 (Zm00001d005410), must be involved in specific mechanisms of defense response against Al ion to our Al-resistant line. Once the transporters have an important role in Al-tolerance, these genes must be potential markers linked to Al tolerance candidate genes, and future studies should be performed to investigate the segregation of these genes.

Conclusions

In this study, were identified new players involved in Al-resistance response in popcorn using RNA-sequencing technology that were not found in previous studies using hydroponic experiments, thereby increasing the understanding of responses to Al-stress. Here, were found that the Al-resistant inbred line needs more than one effective mechanism to alleviate the damages caused by Al-stress and that the combination of several mechanisms cause Al resistance in popcorn. The up-regulation of genes involved in cell wall and fatty acid biosynthesis (important for the dynamic changes of the cell wall and membrane integrity) and an efficient oxidative system that increase the defense machinery against ROS might be performing an autophagy role to alleviate the damages and inducing a cell wall stiffening, thereby preventing Al ion transport via the symplast. Moreover, the presented transporters family are already known to perform a role in Al-detoxification and organic acid exclusion, and we proposed a class of SWEET transporters that might be involved in regulation of vacuolar sugar storage under Al-stress. These results open new avenues to further investigate the specific function of these genes, associating them with maize QTLs correlated with aluminum resistance and comparing these data with Al-resistance genes already identified as candidates for Al-resistance in popcorn.

Methods

Plant materials and growth conditions

The two contrasting popcorn inbred lines were developed by Popcorn Breeding Program of the Universidade Federal de Viçosa. From the screening of 18 inbred lines evaluated for relative root growth (RRG), hematoxylin staining, Al content, and external morphology of roots, Rahim et al. (2019) identified that the inbred line 11-133 was the most Al-resistant presenting statistical significance exhibiting greatest RRG (0.15 to 0.37), no damages on root apices, lower hematoxylin staining score and low Al accumulation (926.4 µg/g). The inbred line 11-60 was the most Al-sensitive presenting lowest RRG (0.02 to 0.06), strong hematoxylin staining and epidermal degradation and high Al accumulation (1,660.3 µg/g).

Initially, were treated seeds with fungicide (Captan-400®) and germinated at 25°C ± 1°C in a growth chamber for 7 days. Seedlings with uniform growth were picked randomly and transferred to a nutritive solution with constant aeration to acclimated for 24 h. The nutrient solution composition was: 1 mM KCl, 1.5 mM NH_4NO_3, 1 mM $CaCl_2$, 45 µM KH_2PO_4, 200 µM $MgSO_4$, 500 µM $Mg(NO_3)_2$, 155 µM $MgCl_2$, 11.8 µM $MnCl_2.4H_2O$, 33 µM H_3BO_3, 3.06 µM $ZnSO_4.7H_2O$, 0.8 µM $CuSO_4.5H_2O$, 1.07 µM $Na_2MoO_4.H_2O$, and 77 µM Fe-EDTA (Famoso et al., 2010; Magnavaca et al., 1987). Then, the treatment group was submitted to aluminum stress with 540 µM of $AlCl_3$ (160 µM Al^{3+}) at pH 4.5 for 72 h. The seedlings were maintained in a growth chamber at 25ºC with 12/12 h light/dark cycle. Total roots from three biological replicates were collected and immediately frozen in liquid nitrogen.

RNA isolation and transcriptome sequencing

The total root RNA from the twelve samples was isolated with Trizol LS reagent (Invitrogen, USA) according to the manufacturer's protocol. The RNA was treated with DNase I Amp Grade (Thermo Scientific) to remove contaminated DNA, and then quantified by spectrophotometry (NanoDrop 2000c, Thermo Scientific). The RNA integrity was verified by electrophoresis on 1.6% agarose gel in the presence of ethidium bromide. After quantification, the RNA samples were sent to Macrogen Inc. (Seoul, South Korea) where transcriptome sequencing library was generated using the TruSeq Stranded mRNA kit and sequenced using the Illumina HiSeq 2500 platform.

Reads preprocessing and differential expression analysis

Quality control was initially performed using the FastQC program (version 0.11.8) (Andrews, 2010) to check the sequencing quality and identify reads with adaptors contamination. Then, the raw reads were trimmed, filtered , and adapters were removed using Trimmomatic (version 0.38) (Bolger et al., 2014). The clean reads of all twelve samples were aligned to the maize reference genome (B73 RefGenv4) using Bowtie2 (version 2.3.3.1) (Langmead and Salzberg, 2012) and TopHat (version 2.1.1) (Kim et al., 2013), with default settings for all parameters. The Cuffdiff (v2.2.1) program (Trapnell et al., 2012) was used with default parameters to calculate gene expression levels and to identify differentially expressed genes (DEGs) in terms of fragments per kilobase per million mapped reads (FPKM). We considered DEGs those showing a $FDR < 0.01$ (false discovery rate) and a log2 fold change value (treated/control) > 1 to up-regulated genes and < -1 to down-regulated genes. Aiming to assess the line groups, a principal component analysis (PCA) was conducted using stats (version 3.4.4) R-package and plotted in Prism 5 (GraphPad).

Gene function annotation and metabolic pathway analysis

Functional enrichment of all DEGs in both lines was conducted using agriGO (http://systemsbiology.cau.edu.cn/agriGOv2) (Tian et al., 2017). Singular Enrichment Analysis (SEA) with *Zea mays* AGPv3.30 as the reference genome background was performed and the significant enriched GO terms were selected ($q < 0.05$). Protein sequences from each DEGs were submitted to a similarity search against the UniRef Enriched KEGG Orthology (UEKO) database (http://maxixe.icb.ufmg.br/ueko/) using BLAST (version 2.7.1) (Altschul et al., 1990), and a script was developed to parse the output and return the KO (KEGG Ontology) ID from each corresponding gene in both lines. The Interactive Pathways (ipath) analysis was carried out via Interactive Pathways Explorer (version 3) (https://pathways.embl.de/) using the KO ID.

Biological network analysis

The DEGs from each line were used to construct an interaction network. The first-degree of interaction was retrieved from STRING (version 10.5) (https://string-db.org). The resulting protein-protein interaction network was used as an input for downstream analysis in Cytoscape (version 3.4.0) (Shannon et al., 2003), and the Moduland (version 2.0.2) and BiNGO (version 3.0.3) plugins were used to evaluate and validate the significant overrepresentation genes involved in biological processes (Maere et al., 2005).

Declarations

Abbreviations

ABA – Abscisic acid

Al – Aluminum

ALMT – Al-activated malate transporter

BRs – Brassinosteroids

CAT – Catalase

DEGs – Differentially expressed genes

EBL – 24-epibrassinolide

FPKM – Fragments per kilobase per million mapped reads

GO – Gene Ontology

GST – Glutathione transferase

IAA – Auxin

MATE – Multidrug and toxic compound extrusion

Nrat1 – Nramp aluminum transporter 1

OA – Organic acid

PME – Pectin methylesterase

POD – Peroxidase

RE – Reductase

RNA-Seq – RNA sequencing technology

ROS – Reactive oxygen species

RRG – Relative root growth

SOD – Superoxide dismutase

TCA – Tricarboxylic acid

TEs – Transposable elements

TFs – Transcription factors

ZF-HD – Zinc finger-associated to a homeodomain

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Table 1 Glutathione transferases proteins identified in RNA sequencing of Al-sensitive and Al-resistant popcorn lines under 72 hours of Al-stress.

Gene ID	GST protein	Fold change
	Al-sensitive	
Zm00001d029706	*gst39*	2.3630
Zm00001d029696	*gst34*	1.4779
Zm00001d029702	Glutathione S-transferase U16	-1.2121
Zm00001d048558	gst25	1.2982
	Al-tolerant	
Zm00001d048353	*gst13*	2.0340
Zm00001d029707	*gst38*	1.8619
Zm00001d042104	*gst7*	1.6635
Zm00001d027539	*gst11*	1.3049
Zm00001d029699	*gst42*	1.2658
Zm00001d029801	*gst14*	1.2620
Zm00001d048354	*gst9*	1.1963
Zm00001d027540	*gst12*	1.0799
Zm00001d038192	*gst41*	1.0730
Zm00001d028692	Glutathione S-transferase L2 chloroplastic	1.0698
Zm00001d047765	Glutathione S-transferase L2 chloroplastic	1.0270
Zm00001d029696	*gst34*	-1.1529

Table 2 Aluminum responsive transporters related in Al-sensitive popcorn.

Class	**Gene ID**	**Transporter**	**Fold change**
ABC transporter	Zm00001d043598	ABC transporter G family member 29	-2.4384
	Zm00001d032279	ABC2 homolog 15	-1.4484
	Zm00001d025703	ABC transporter B family member 15	-1.3762
	Zm00001d011315	ABC transporter G family member 40	1.8044
	Zm00001d026041	ABC transporter C family member 9	1.7880
	Zm00001d021647	ABC transporter G family member 34	1.0676
SWEET	Zm00001d050577	SWEET 15a	2.2081
	Zm00001d015914	SWEET 4b	2.1945
	Zm00001d010440	SWEET 3a	1.0330
Nramp	Zm00001d019327	Metal transporter Nramp6	-1.0948
	Zm00001d014391	*Nrat1*	1.7826
ALMT	Zm00001d026102	Aluminum-activated malate transporter 10	1.9831

Table 3 Aluminum responsive transporters related in Al-resistant popcorn.

Class	Gene ID	Transporter	Fold change
ABC transporter	Zm00001d011315	ABC transporter G family member 40	-2.8548
	Zm00001d044442	ABC transporter G family member 40	-2.1104
	Zm00001d028870	ABC transporter family protein	-1.6613
	Zm00001d046225	ABC transporter family protein	-1.4889
	Zm00001d046226	*mrpa1* (ABC) transporter	-1.3632
	Zm00001d021647	ABC transporter G family member 34	-1.3278
	Zm00001d004361	ABC transporter C family member 4	-1.0053
	Zm00001d043598	ABC transporter G family member 29	2.5981
	Zm00001d024600	ABC transporter B family member 19	2.5639
	Zm00001d043766	ABC transporter B family member 9	1.8129
	Zm00001d036986	ABC transporter G family member 29	1.3984
	Zm00001d044564	ABC transporter B family member 9	1.1888
	Zm00001d042953	ABC transporter G family member 6	1.1345
SWEET	Zm00001d011299	SWEET 6b	-3.5525
	Zm00001d029135	SWEET 12a	3.4175
	Zm00001d023673	SWEET 13b	2.7135
	Zm00001d015905	SWEET 4a	1.5050
	Zm00001d041067	SWEET 13c	1.4285
	Zm00001d010440	SWEET 3a	1.0593
	Zm00001d015914	SWEET 4b	1.0114
Aquaporin	Zm00001d022608	Aquaporin PIP2-2	-2.0717
	Zm00001d048520	Aquaporin TIP3.1	-1.8133
	Zm00001d005410	Aquaporin PIP2-2	1.5432
	Zm00001d014285	Aquaporin PIP2-2	1.1855
ALMT	Zm00001d046029	Aluminum-activated malate transporter 10	1.0719
Heavy metal transporters	Zm00001d002496	Heavy metal transport/detoxification superfamily protein	1.6792
	Zm00001d026298	Putative heavy metal transport/detoxification protein	1.2951
MATE	Zm00001d009494	Putative MATE efflux family protein	-2.9624

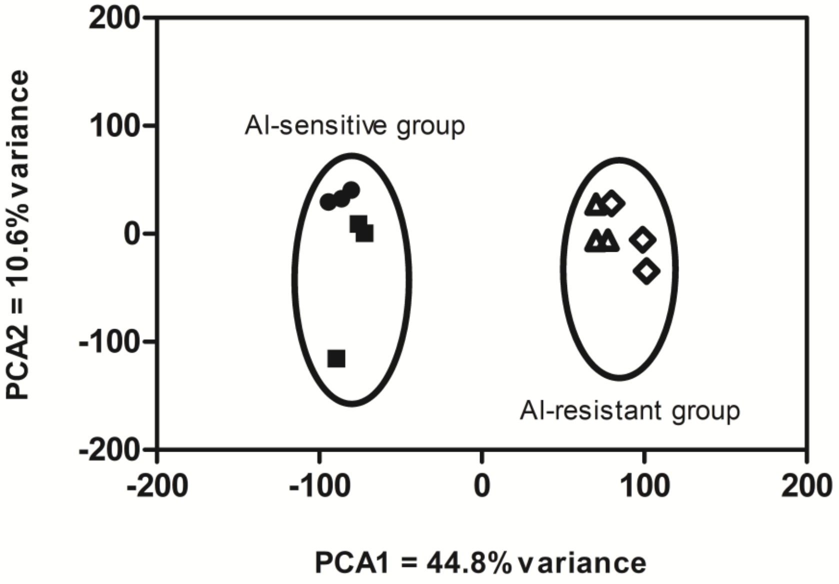


Figure 1 PCA plot analysis using FPKM values and performed in stats R-package and ploted with Prism 5 (●: sensitive control; ■: sensitive treatment; : tolerant control; ◊: tolerant treatment).

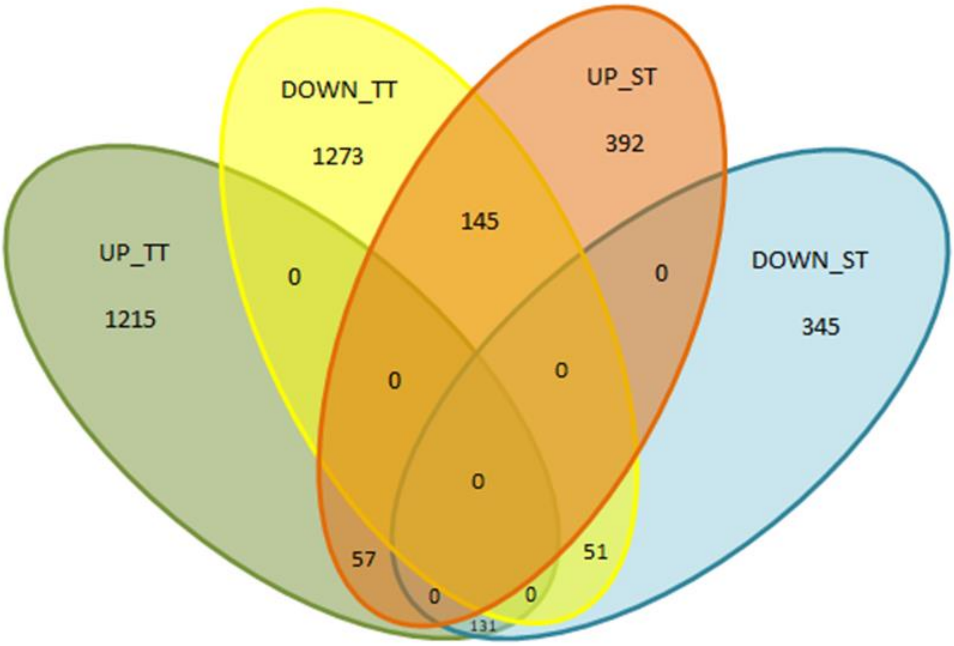


Figure 2 Venn diagram of DEGs under Al-stress (UP_TT: up-regulated genes in Al-resistant; DOWN_TT: down-regulated genes in Al-resistant; UP_ST: up-regulated genes expressed in Al-sensitive; DOWN_ST: down-regulated genes expressed in Al-sensitive).

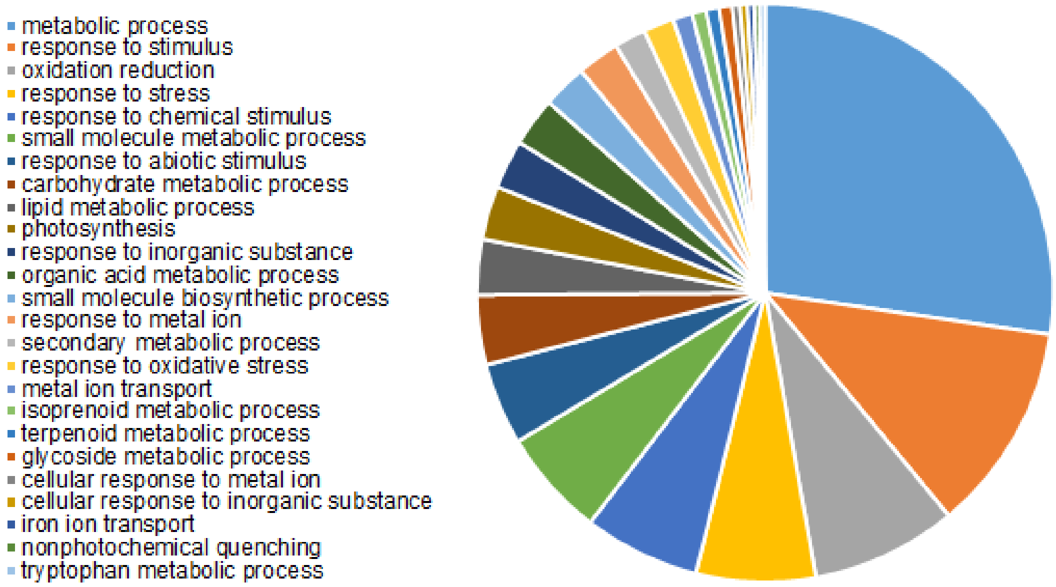


Figure 3 Gene ontology (GO) enrichment of biological process in Al-resistant popcorn line.

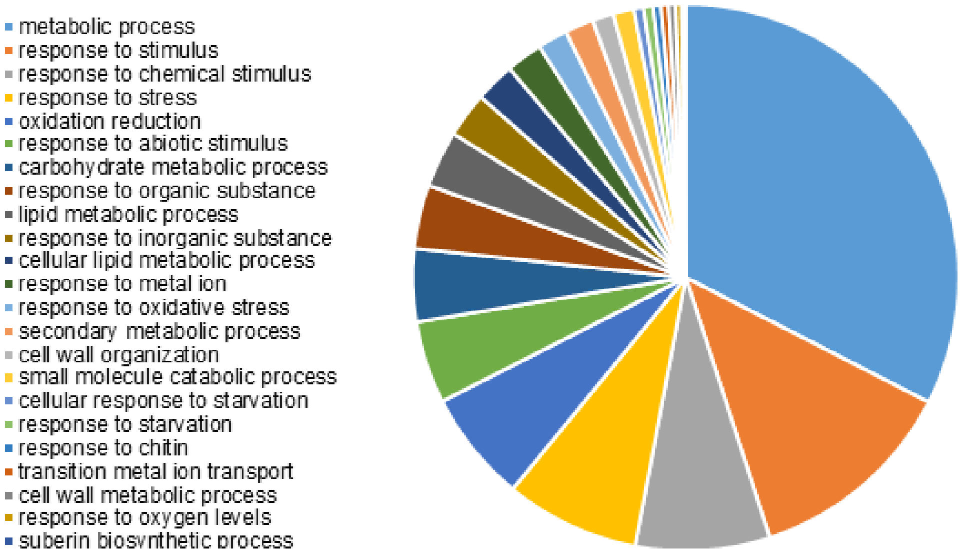
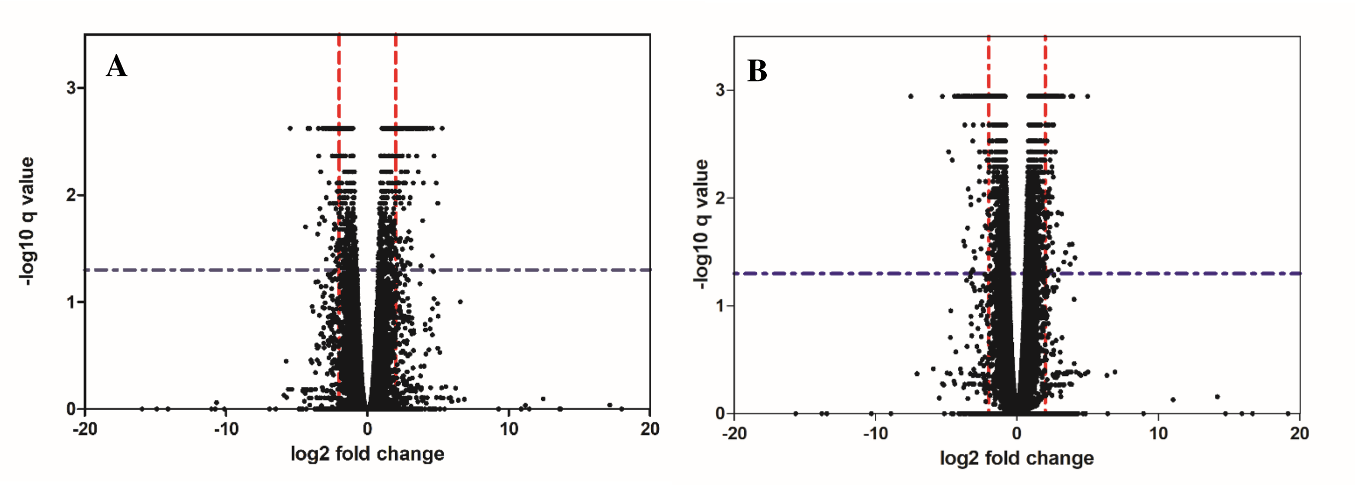
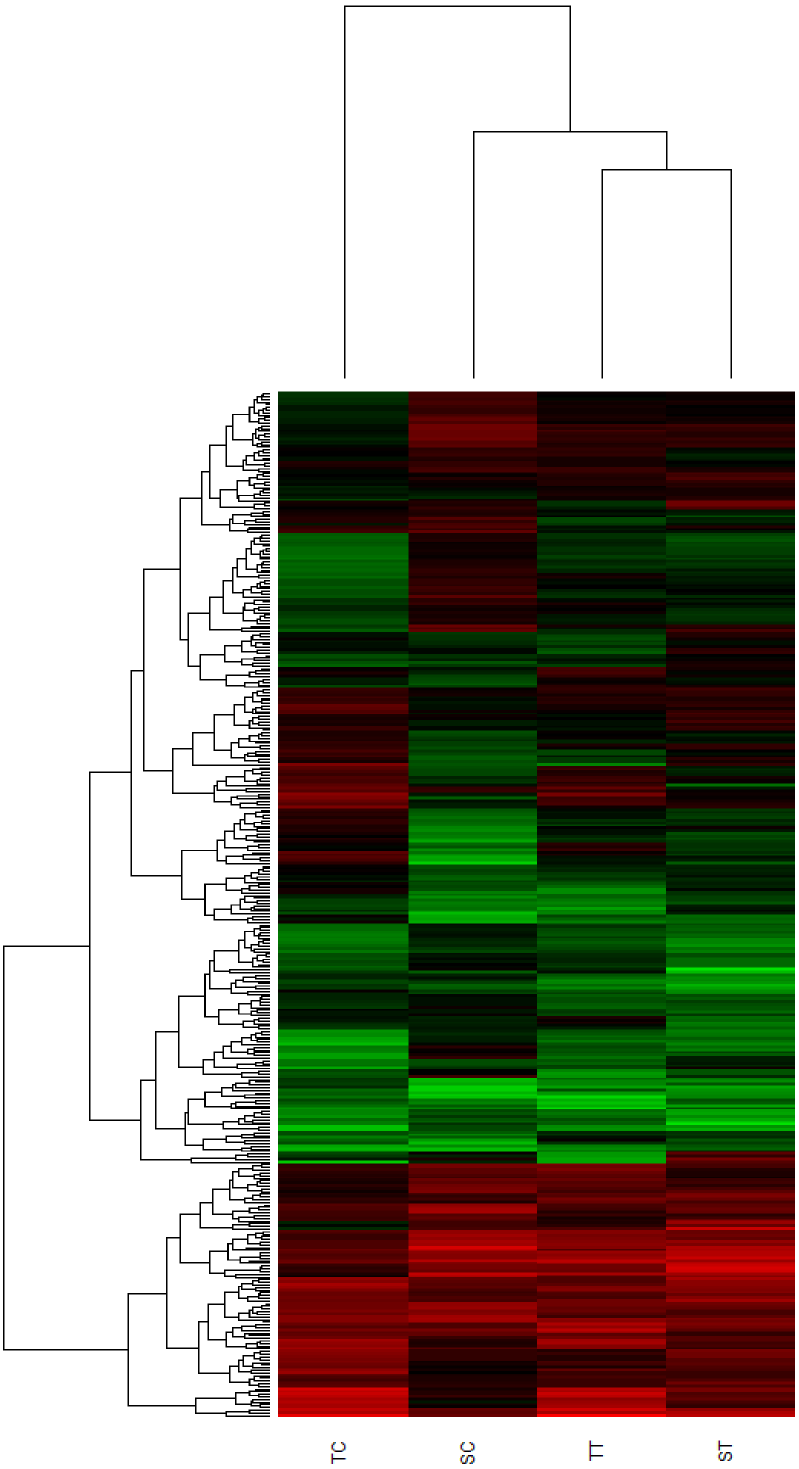


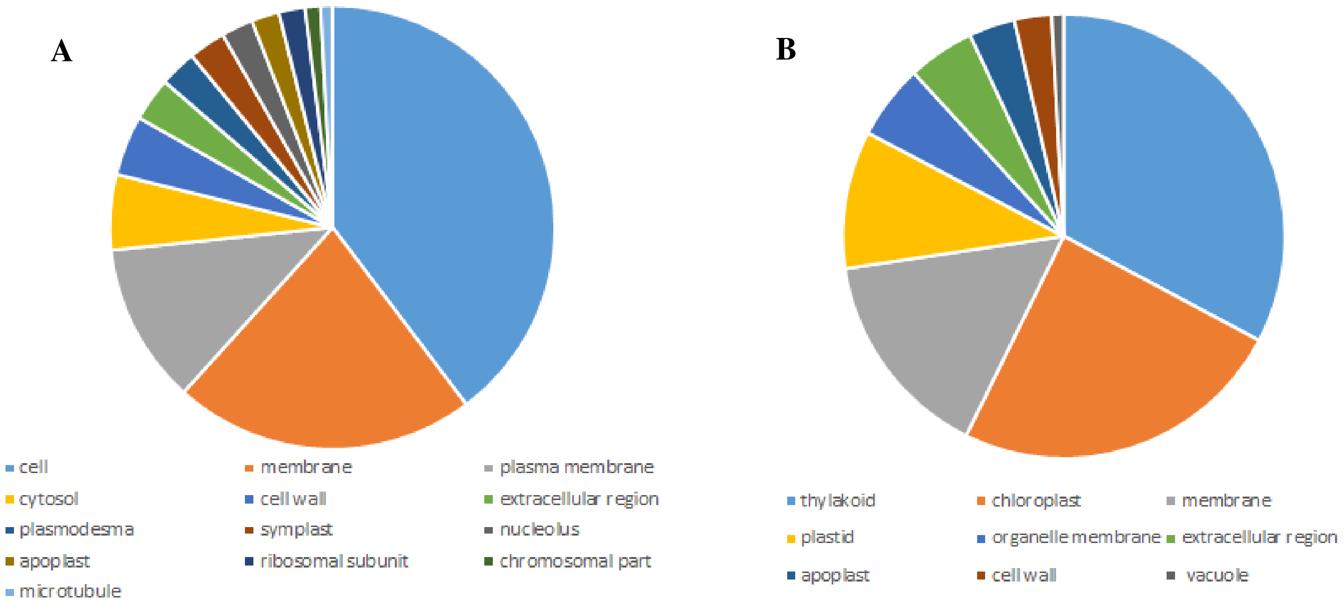
Figure 4 Gene ontology (GO) enrichment of biological process in Al-sensitive popcorn line.



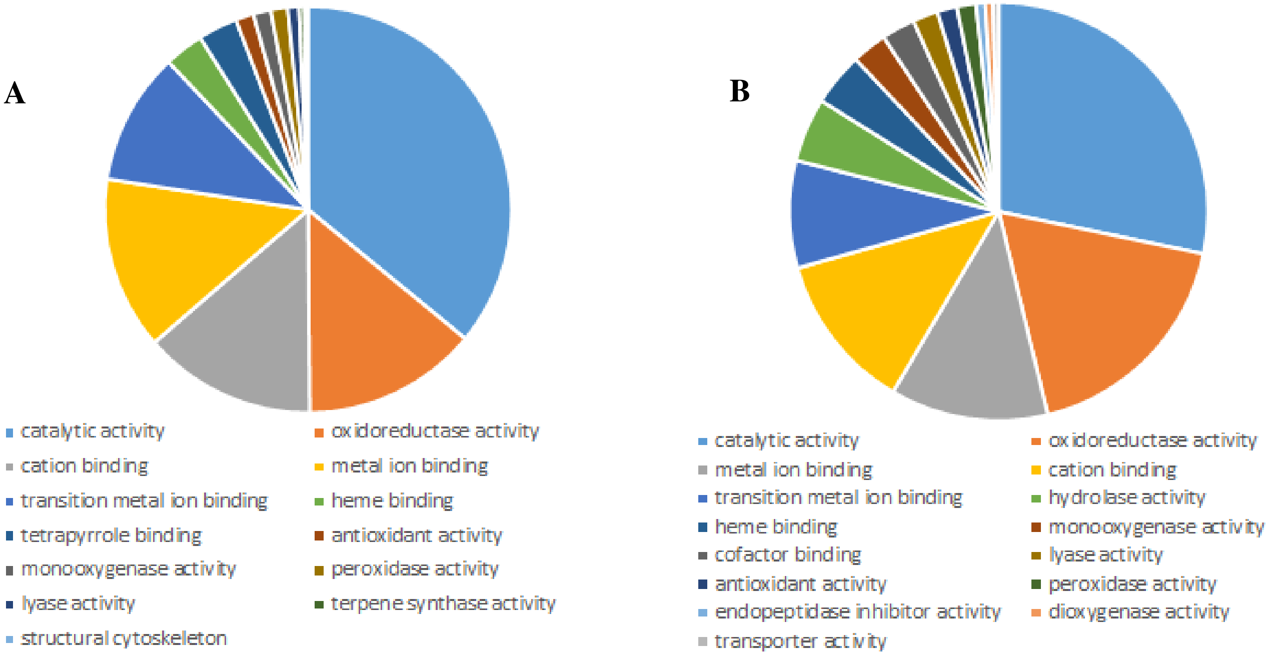
Supplementary Figure S1 Volcano plot of genes differentially expressed. **(A)** Al-senstive profile. **(B)** Al-resistant profile. Points marked above the horizontal dashed line represent significant observations (q value ≤ 0.05).



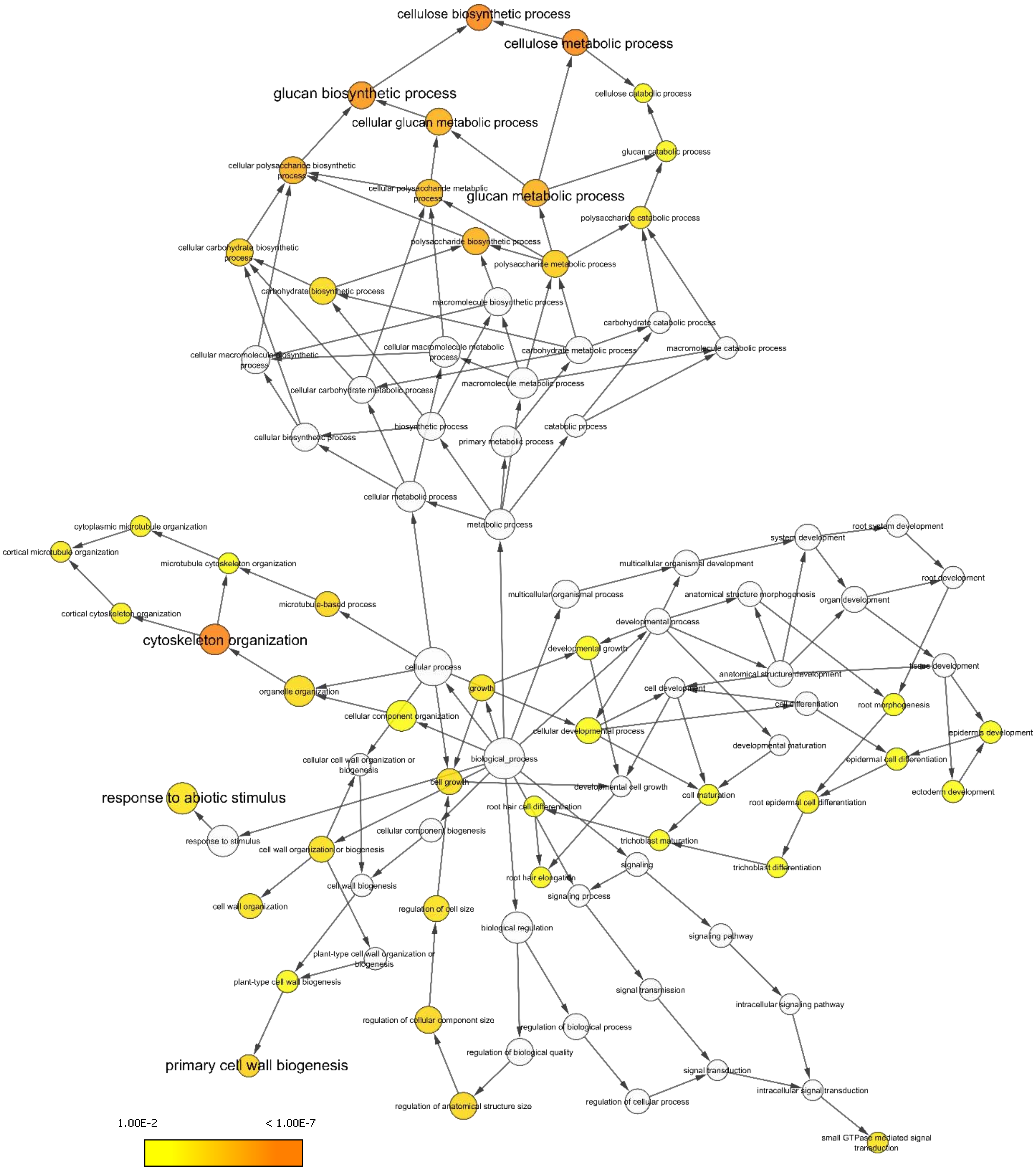
Supplementary Figure S2 Heatmap of all differentially expressed genes shared in both Al-resistant and Al-sensitive popcorn lines under 72 hours of Al-stress (SC – sensitive control, ST – sensitive treatment, TC – tolerant control, TT – tolerant treatment).



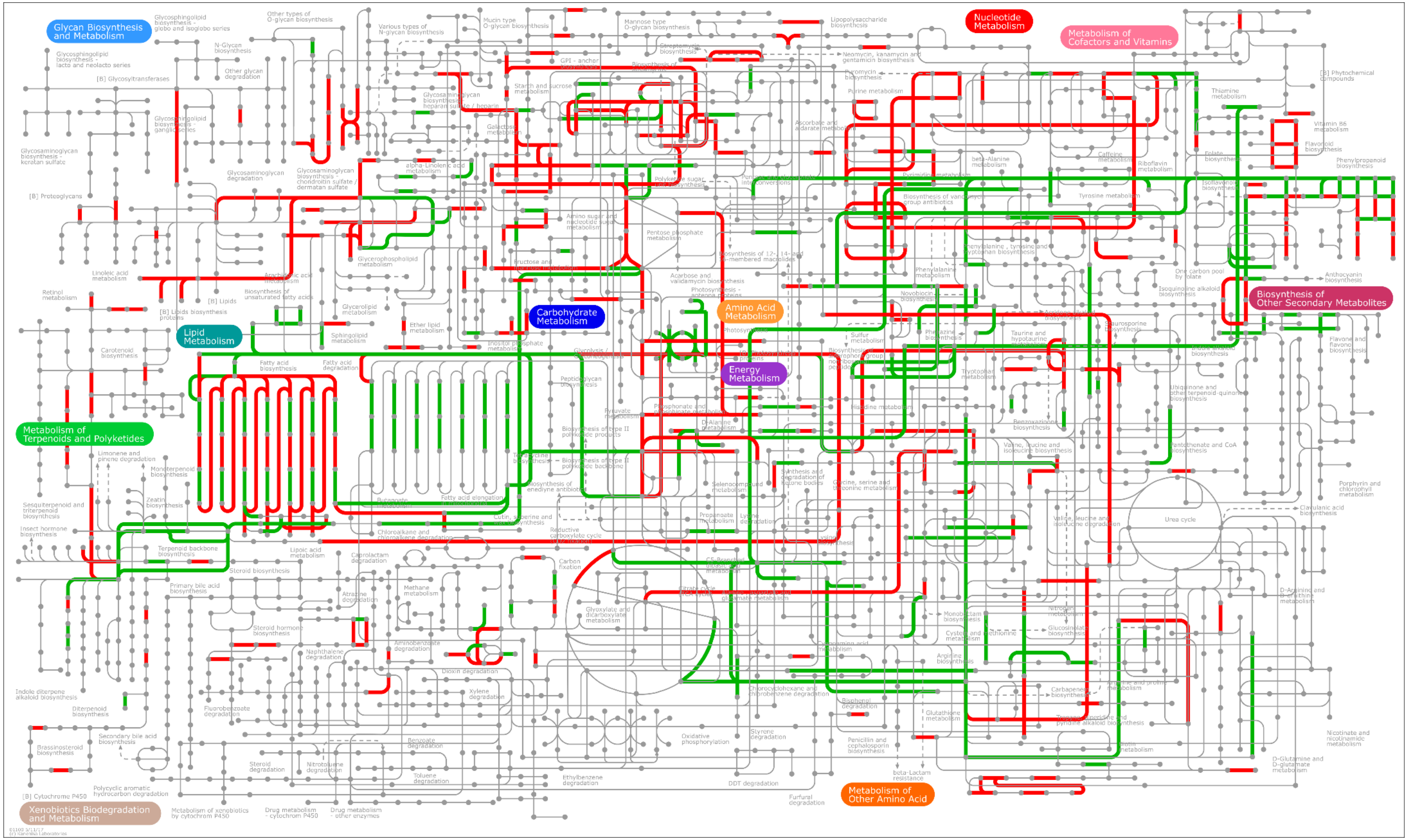
Supplementary Figure S3 Gene ontology enrichment of cellular component in **(A)** Al-resistant and **(B)** Al-sensitive.



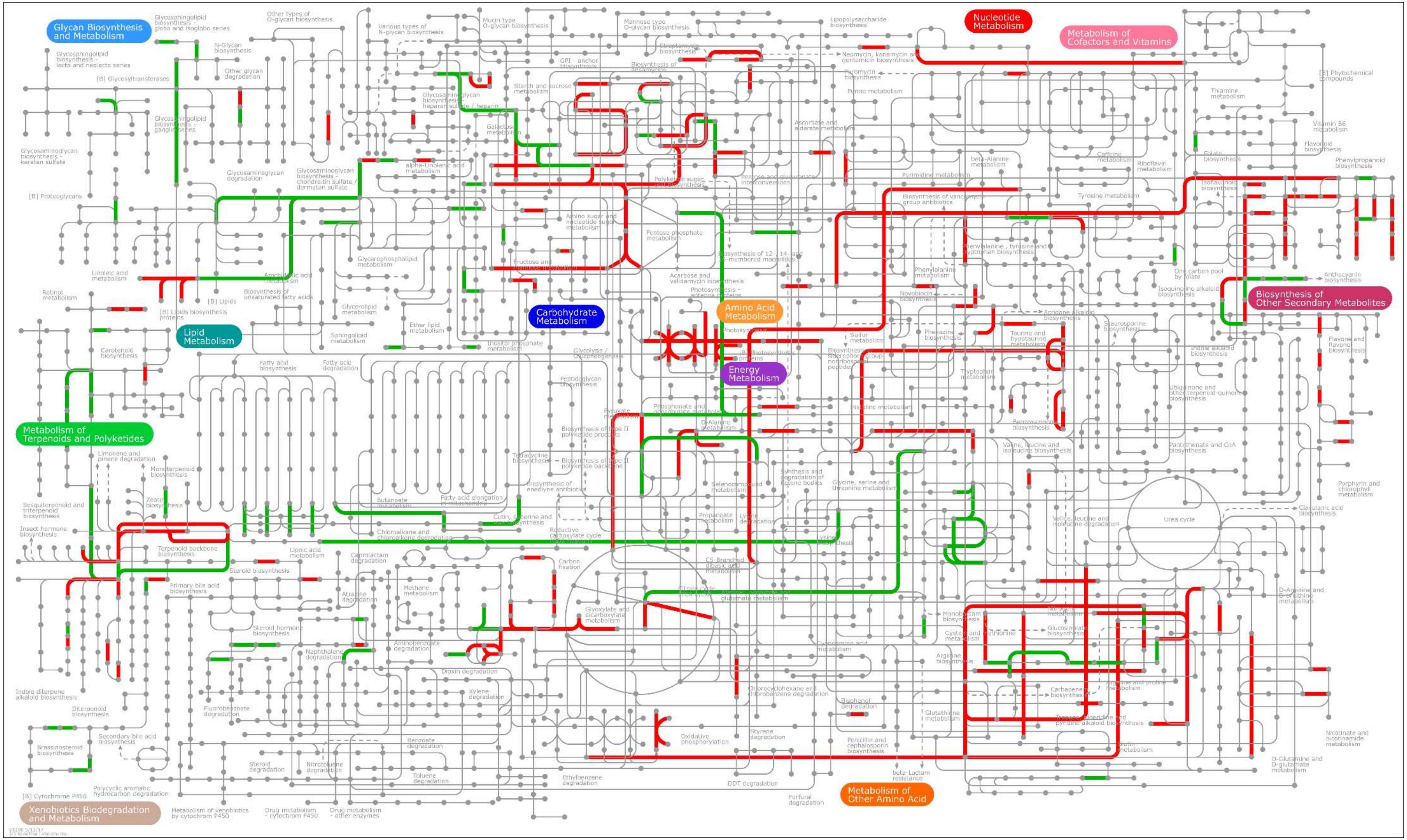
Supplementary Figure S4 Gene ontology enrichment of molecular function in **(A)** Al-resistant and **(B)** Al-sensitive.



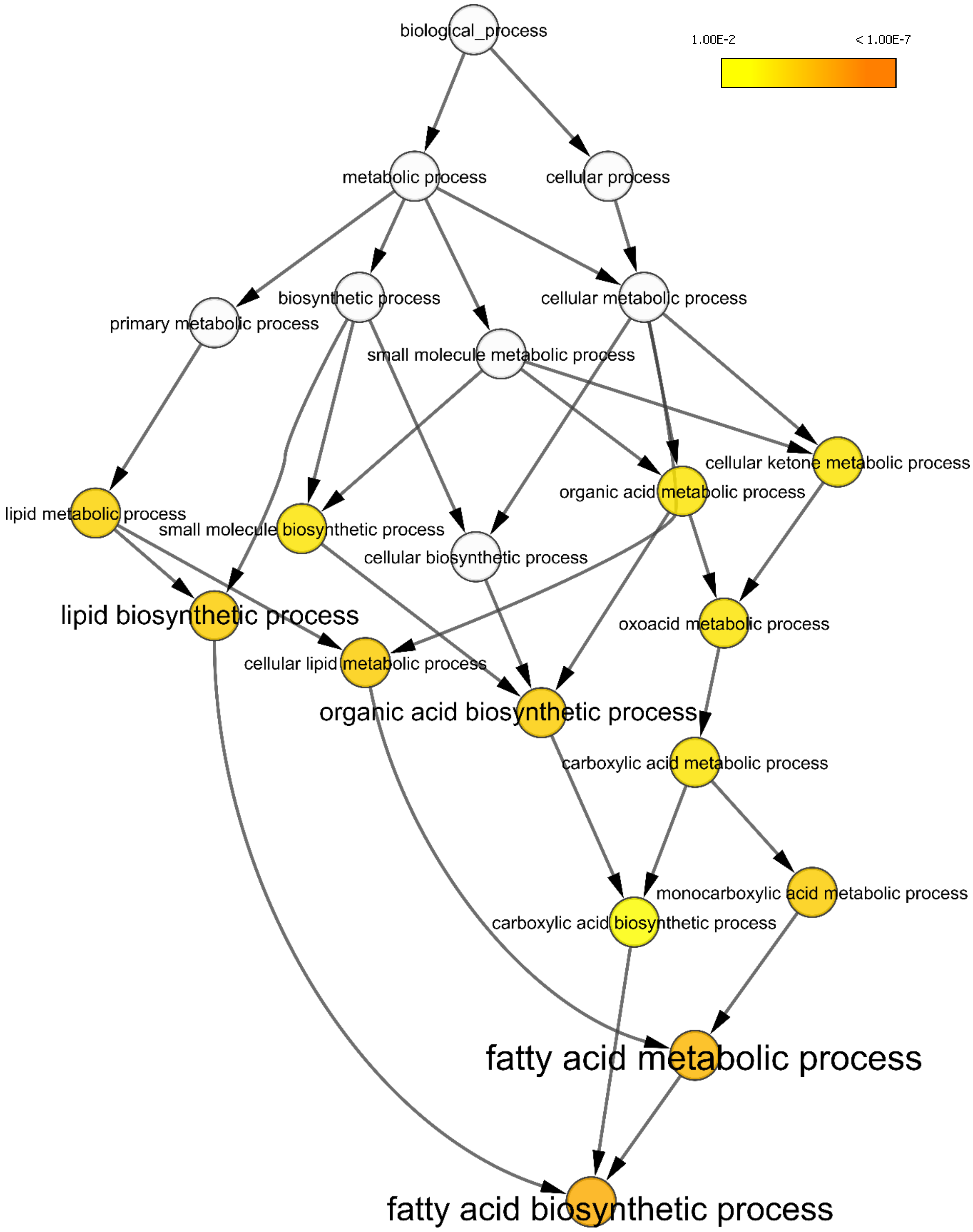
Supplementary Figure S5 Protein-protein interaction network of up-regulated genes differentially expressed in Al-resistant modulated in cell-wall modification.



Supplementary Figure S6 Predicted metabolic pathway analysis in Al-resistant. Red lines indicate activated paths and green lines indicate deactivated paths.



Supplementary Figure S7 Metabolic pathway analysis in Al-sensitive. Red lines indicate activated paths and green lines indicate deactivated paths.



Supplementary Figure S8 Protein-protein interaction network of up-regulated genes differentially expressed in Al-resistant modulated in fatty acid biosynthesis.

Supplementary Table 1 Reads generated by Illumina HiSeq 2500 sequencing

Sample	Total bases (bp)	Total reads	GC(%)	Q30(%)	Processed reads	Mapped	Multiple alignments
TC_01	4,444,714,070	44,007,070	52.916	95.217	43,947,680	33,703,244	2,324,056
TC_02	4,414,719,696	43,710,096	52.637	95.160	43,654,340	33,558,664	3,010,175
TC_03	3,934,680,028	38,957,228	53.428	95.254	38,903,164	30,745,289	1,872,546
TT_04	3,902,792,712	38,641,512	53.058	95.108	38,583,814	30,923,936	1,907,004
TT_05	4,702,968,646	46,564,046	52.860	95.198	46,500,580	38,402,919	3,137,437
TT_06	4,459,818,620	44,156,620	53.066	95.329	44,090,584	36,285,123	2,282,727
SC_07	4,693,011,258	46,465,458	52.778	95.155	46,408,556	38,360,870	4,500,362
SC_08	3,996,676,858	39,571,058	53.244	95.110	39,520,564	32,502,598	2,198,710
SC_09	4,275,555,028	42,332,228	52.893	95.237	42,277,156	35,214,177	2,556,341
ST_10	4,078,236,378	40,378,578	51.818	94.986	40,326,152	29,140,521	1,855,519
ST_11	4,052,052,936	40,119,336	52.461	94.927	40,065,116	32,446,465	2,339,842
ST_12	4,445,754,976	44,017,376	53.306	95.148	43,950,194	36,102,695	4,567,153

Supplementary Table 3 List of genes shared in both genotypes under Al-stress.

Gene ID	Gene function
	Down-regulated in Al-tolerant and up-regulated in Al-sensitive
Zm00001d032873	Metal ion binding protein
Zm00001d002410	Probable xyloglucan endotransglucosylase/hydrolase protein 21
Zm00001d018737	Pathogenesis-related protein 1
Zm00001d042148	Cytokinin oxidase 2
Zm00001d021208	Dehydration-responsive element-binding protein 1B
Zm00001d018966	Acidic endochitinase
Zm00001d041382	Expressed protein; Os12g0594800 protein
Zm00001d028930	myb domain protein 112
Zm00001d023811	Pathogenesis-related protein STH-21
Zm00001d046055	No annotated
Zm00001d043299	Photosystem II reaction center W protein chloroplastic
Zm00001d041711	Resistance to sugarcane mosaic virus 2
Zm00001d039520	Cytokinin oxidase 1
Zm00001d031158	Pathogenesis related protein5
Zm00001d022192	Trehalose-6-phosphate phosphatase 9
Zm00001d038300	Putative cytochrome P450 superfamily protein
Zm00001d044291	Actin binding protein family
Zm00001d026060	1-aminocyclopropane-1-carboxylate synthase7
Zm00001d027924	No annotated
Zm00001d022461	ERF-like protein
Zm00001d045392	Early nodulin 93
Zm00001d032283	No annotated
Zm00001d009726	alpha/beta-Hydrolases superfamily protein
Zm00001d026599	Light harvesting chlorophyll a/b binding protein6
Zm00001d042611	Gibberellin 20-oxidase3
Zm00001d042022	Peroxidase 12
Zm00001d001811	Tetraspanin-19
Zm00001d006256	syringolide-induced protein 14-1-1
Zm00001d012603	Methylesterase 3
Zm00001d029373	Serine carboxypeptidase-like 40
Zm00001d028307	Probable inactive poly [ADP-ribose] polymerase SRO1
Zm00001d034095	cytochrome P450 family 81 subfamily D polypeptide 8
Zm00001d040682	Sequence-specific DNA binding transcription factors
Zm00001d036164	Salicylate/benzoate carboxyl methyltransferase
Zm00001d011315	ABC transporter G family member 40
Zm00001d022084	B12D protein
Zm00001d009296	Pathogenesis-related protein PRMS
Zm00001d028816	Pathogenesis-related protein 10
Zm00001d047252	No annotated
Zm00001d033846	No annotated
Zm00001d036535	Oxygen evolving complex
Zm00001d037656	Xylanase inhibitor protein 1
Zm00001d052518	Pollen Ole e 1 allergen and extensin family protein

Zm00001d052206	Protein EXORDIUM
Zm00001d007924	Cytochrome P450 family 93 subfamily D polypeptide 1
Zm00001d026603	Mg chelatase subunit H 1
Zm00001d013146	Photosystem I reaction center subunit III chloroplastic
Zm00001d001857	Light harvesting complex photosystem II subunit 6
Zm00001d024210	Terpene synthase6:
Zm00001d025860	No annotated
Zm00001d010588	Pyruvate decarboxylase1
Zm00001d028986	WUSCHEL-related homeobox 11
Zm00001d001960	Naringenin2-oxoglutarate 3-dioxygenase
Zm00001d022193	Probable trehalose-phosphate phosphatase J
Zm00001d029696	Glutathione transferase 34
Zm00001d031345	ssr bnlg2057
Zm00001d048307	No annotated
Zm00001d031127	Cupin2C RmlC-type
Zm00001d035888	Extensin-like protein
Zm00001d025916	E3 ubiquitin-protein ligase RMA1
Zm00001d043121	Osmotin-like protein OSM34
Zm00001d011237	Putative WRKY DNA-binding domain superfamily protein
Zm00001d048949	Hevein-like preproprotein
Zm00001d023774	Erg28 like protein
Zm00001d023859	Respiratory burst oxidase homolog protein B
Zm00001d028651	Wusl1032
Zm00001d020351	Alpha-amylase 1
Zm00001d039495	Zinc finger CCCH domain-containing protein 23
Zm00001d035854	Alpha-dioxygenase 1
Zm00001d047981	Glutamate decarboxylase 1
Zm00001d046138	No annotated
Zm00001d019277	Defensin-like protein 6
Zm00001d031449	Lipoxygenase 13
Zm00001d037550	Peroxidase 5
Zm00001d029648	Copalyl diphosphate synthase2
Zm00001d045051	Zinc finger nuclease2
Zm00001d024207	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Zm00001d030577	Light-inducible protein CPRF-2
Zm00001d031813	Patatin-like protein 2
Zm00001d045390	Early nodulin-related
Zm00001d005392	Lecithin-cholesterol acyltransferase-like 1
Zm00001d017381	(+)-neomenthol dehydrogenase
Zm00001d020006	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha 2
Zm00001d009619	Putative WRKY DNA-binding domain superfamily protein
Zm00001d016691	Copper transport protein CCH
Zm00001d047942	Actin-depolymerizing factor 5
Zm00001d018414	IAA9-auxin-responsive Aux/IAA family member
Zm00001d049630	Galactose oxidase/kelch repeat superfamily protein
Zm00001d047192	5-pentadecatrienyl resorcinol O-methyltransferase

Zm00001d007363	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1
Zm00001d036197	HXXXD-type acyl-transferase family protein
Zm00001d016471	Trans-cinnamate 4-monooxygenase
Zm00001d029842	Divalent ion symporter
Zm00001d023768	Delta(12)-fatty-acid desaturase
Zm00001d031135	GATA zinc finger family protein
Zm00001d037607	Probable WRKY transcription factor 50
Zm00001d037689	Hexokinase7
Zm00001d014945	Lactate/malate dehydrogenase family protein
Zm00001d017615	RING/U-box superfamily protein
Zm00001d024960	Bowman-Birk type trypsin inhibitor
Zm00001d049288	No annotated
Zm00001d014613	Xyloglucan endotransglucosylase/hydrolase protein 23
Zm00001d034097	Cytochrome P450 family 81 subfamily D polypeptide 8
Zm00001d048690	Serine carboxypeptidase-like 50
Zm00001d021647	ABC transporter G family member 34
Zm00001d023769	Delta(12)-fatty-acid desaturase
Zm00001d036409	OSJNBa0013K16.15 protein
Zm00001d043727	Farnesyl diphosphate synthase3
Zm00001d051938	Cinnamoyl-CoA reductase 1
Zm00001d014121	Ent-cassadiene C2-hydroxylase
Zm00001d023617	Laccase-7
Zm00001d020686	1-aminocyclopropane-1-carboxylate oxidase2
Zm00001d038163	Pyruvate, orthophosphate dikinase1
Zm00001d018229	Adenine nucleotide alpha hydrolases-like superfamily protein
Zm00001d021207	DRE-binding protein 3
Zm00001d028761	Tetratricopeptide repeat (TPR)-like superfamily protein
Zm00001d020652	Plant viral-response family protein-like
Zm00001d044460	Alpha/beta-Hydrolases superfamily protein
Zm00001d014134	Ent-cassadiene C2-hydroxylase
Zm00001d009022	NmrA-like negative transcriptional regulator family protein
Zm00001d043179	PIN-formed protein9
Zm00001d044683	Probable chalcone--flavonone isomerase 3
Zm00001d033862	1-aminocyclopropane-1-carboxylate synthase6
Zm00001d031877	No annotated
Zm00001d013193	CCG-binding protein 1
Zm00001d043651	Tryptophan aminotransferase-related protein 4
Zm00001d037005	Putative FAD-binding Berberine family protein
Zm00001d042244	Potasium ion uptake permease 1
Zm00001d011461	No annotated
Zm00001d023941	Carbohydrate transporter/ sugar porter
Zm00001d037724	Gibberellin 2-beta-dioxygenase
Zm00001d046342	Kaurene oxidase2
Zm00001d005383	Lecithin-cholesterol acyltransferase-like 1
Zm00001d011628	PR5-like receptor kinase
Zm00001d044192	Probable GABA transporter 2

Zm00001d007765	Probable prolyl 4-hydroxylase 12
Zm00001d028968	Chaperone protein dnaJ 20 chloroplastic
Zm00001d010375	ATP-dependent 6-phosphofructokinase 4 chloroplastic
Zm00001d052333	Hypoxia-responsive family protein
Zm00001d009118	Adenine nucleotide alpha hydrolases-like superfamily protein
Zm00001d049113	Senescence-specific cysteine protease SAG12
Zm00001d034096	Cytochrome P450 family 81 subfamily D polypeptide 8
Zm00001d036950	No annotated
Zm00001d038061	Probable calcium-binding protein CML25
Zm00001d019409	OSJNBb0004G23.8 protein
	Up-regulated in Al-tolerant and down-regulated in Al-sensitive
Zm00001d045420	Root cap-specific protein
Zm00001d044821	GDSL esterase/lipase
Zm00001d044327	Cytokinin riboside 5'-monophosphate phosphoribohydrolase LOG7
Zm00001d006775	Hydroxyproline-rich glycoprotein family protein
Zm00001d026251	Probable xyloglucan endotransglucosylase/hydrolase protein 21
Zm00001d044652	Nucleotide sugar transporter-KT 1
Zm00001d004614	Protease inhibitor/seed storage/LTP family protein
Zm00001d051389	Phosphoribosylanthranilate transferase
Zm00001d010585	UTP--glucose-1-phosphate uridylyltransferase 3 chloroplastic
Zm00001d018422	Glycosyltransferase family protein 2
Zm00001d041987	Transposon protein
Zm00001d034415	Phospholipid transfer protein homolog5
Zm00001d031428	Monogalactosyldiacylglycerol synthase 2 chloroplastic
Zm00001d011657	Plasma membrane associated protein
Zm00001d018926	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin
Zm00001d022669	No annotated
Zm00001d017455	Uclacyanin-3
Zm00001d010434	Arabinogalactan peptide 20
Zm00001d002370	HXXXD-type acyl-transferase family protein
Zm00001d002496	Heavy metal transport/detoxification superfamily protein
Zm00001d007345	11-beta-hydroxysteroid dehydrogenase-like 5
Zm00001d038126	No annotated
Zm00001d048418	Expansin-A11
Zm00001d039695	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Zm00001d037336	GDSL esterase/lipase
Zm00001d018324	Pathogenesis-related protein-1-like
Zm00001d022280	Peroxidase
Zm00001d037182	12-oxo-phytodienoic acid reductase3
Zm00001d022478	Protein SRC2 homolog
Zm00001d049129	Late embryogenesis abundant protein-related / LEA protein-related
Zm00001d007351	Esterase
Zm00001d020614	ZIM-transcription factor 28
Zm00001d027645	Leucine-rich repeat receptor-like protein kinase PXL1
Zm00001d018855	Probable carboxylesterase 15
Zm00001d001575	No annotated

Zm00001d039697	Cytochrome P450 monooxygenase
Zm00001d046491	Putative alcohol dehydrogenase superfamily protein
Zm00001d004231	No annotated
Zm00001d005148	Myristoyl-acyl carrier protein thioesterase
Zm00001d043019	Laccase-13
Zm00001d006875	Alanine aminotransferase10
Zm00001d004635	Leucine-rich repeat protein kinase family protein
Zm00001d034414	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein
Zm00001d022057	Hydroxyproline-rich glycoprotein family protein
Zm00001d051333	Blue copper protein
Zm00001d022496	Anther-specific proline-rich protein APG
Zm00001d002687	Cytochrome P450 86A7
Zm00001d032083	OSJNBb0026E15.9 protein
Zm00001d011274	Metal ion binding protein
Zm00001d017454	No annotated
Zm00001d031161	No annotated
Zm00001d052068	Pathogenesis-related protein-1-like
Zm00001d027855	Transcription factor PRE3
Zm00001d041984	Root-cap periphery1
Zm00001d014971	Leucine-rich repeat receptor-like protein kinase PXL1
Zm00001d017785	Alpha/beta-Hydrolases superfamily protein
Zm00001d029699	Glutathione transferase42
Zm00001d009765	No annotated
Zm00001d011912	Putative transmembrane protein kinase family protein
Zm00001d038068	ATPase
Zm00001d019669	Cinnamoyl CoA reductase2
Zm00001d025541	Eukaryotic aspartyl protease family protein
Zm00001d019364	Pathogenesis-related protein 1
Zm00001d042723	Omega-hydroxypalmitate O-feruloyl transferase
Zm00001d046834	Low temperature and salt responsive protein
Zm00001d037359	Peroxidase 11
Zm00001d018024	PIN-formed protein2
Zm00001d041985	Late embryogenesis abundant protein-related / LEA protein-related
Zm00001d049690	Putative cytochrome P450 superfamily protein
Zm00001d038598	Peroxidase 2
Zm00001d002773	Cupredoxin superfamily protein
Zm00001d006111	Cytochrome b561 and DOMON domain-containing protein
Zm00001d025446	Pentatricopeptide repeat-containing protein
Zm00001d007832	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin
Zm00001d034546	Cupredoxin superfamily protein
Zm00001d025141	Basic helix-loop-helix (bHLH) DNA-binding superfamily protein
Zm00001d004630	ZCN17
Zm00001d033225	Pyruvate dehydrogenase E1 component subunit beta-3 chloroplastic
Zm00001d020400	Probable cinnamyl alcohol dehydrogenase 6
Zm00001d044541	SPX domain-containing protein 3
Zm00001d032443	DUF538 family protein

Zm00001d039698	Cytochrome P450 family 706 subfamily A polypeptide 5
Zm00001d027700	Phosphate transporter protein9
Zm00001d004855	Actin-7
Zm00001d026412	No annotated
Zm00001d013089	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin
Zm00001d031657	Probable inactive purple acid phosphatase 27
Zm00001d007160	Peroxidase 66
Zm00001d015921	Plasma membrane-associated cation-binding protein 1
Zm00001d034839	Purple acid phosphatase 15
Zm00001d014758	Phospholipid transfer protein homolog6
Zm00001d028714	Probable RNA-binding protein ARP1
Zm00001d032883	Expansin-A11
Zm00001d033595	Lipid transfer protein
Zm00001d027511	Catalase isozyme 2
Zm00001d005986	Probable LRR receptor-like serine/threonine-protein kinase
Zm00001d034461	Tryptophan synthase alpha chain chloroplastic
Zm00001d012274	Hydroxycinnamoyltransferase10
Zm00001d017494	Expansin-B4
Zm00001d004443	Probable cinnamyl alcohol dehydrogenase 9
Zm00001d043929	Fasciclin-like arabinogalactan protein 6
Zm00001d014101	No annotated
Zm00001d051791	GDSL esterase/lipase
Zm00001d003707	Peroxidase 72
Zm00001d050201	Probable xyloglucan endotransglucosylase/hydrolase protein 21
Zm00001d033585	Leaf permease1
Zm00001d008600	Sulfoquinovosyl transferase SQD2
Zm00001d006774	Hydroxyproline-rich glycoprotein family protein
Zm00001d026395	Micronuclear linker histone polyprotein-like protein
Zm00001d018618	Peroxidase 1
Zm00001d002901	Peroxidase 12
Zm00001d028367	Purple acid phosphatase 3
Zm00001d036366	Basic endochitinase B
Zm00001d013979	Cytochrome P450 86B1
Zm00001d028689	ATPase
Zm00001d038366	Glycerol-3-phosphate acyltransferase 5
Zm00001d009373	Peroxidase 72
Zm00001d034416	Lipid binding protein
Zm00001d026205	No annotated
Zm00001d025873	Low-molecular-weight cysteine-rich protein LCR70
Zm00001d012394	Phytoene synthase2
Zm00001d018625	Calcineurin-like metallo-phosphoesterase superfamily protein
Zm00001d024925	No annotated
Zm00001d010038	CASP-like protein 5
Zm00001d032821	No annotated
Zm00001d003841	Xanthoxin dehydrogenase
Zm00001d051659	No annotated

Zm00001d043598	ABC transporter G family member 29
Zm00001d018097	MYB domain protein 93
Zm00001d042209	Methyl esterase 10
Zm00001d013038	Cupredoxin superfamily protein
Up-regulated in Al-tolerant and Al-sensitive	
Zm00001d028599	Laccase-17
Zm00001d020264	No annotated
Zm00001d053960	3-isopropylmalate dehydratase large subunit
Zm00001d033747	Glutamine synthetase root isozyme 2
Zm00001d049916	Protein WALLS ARE THIN 1
Zm00001d025103	Amine oxidase1
Zm00001d026628	Dof zinc finger protein DOF1.6
Zm00001d023516	Salt stress-induced protein
Zm00001d034756	No annotated
Zm00001d023559	Fructose-bisphosphate aldolase
Zm00001d028348	Peroxidase 53
Zm00001d043610	No annotated
Zm00001d038891	Phosphoethanolamine N-methyltransferase 3
Zm00001d048703	Benzoxazinone synthesis4
Zm00001d039914	Metallothionein-like protein type 2
Zm00001d031971	Vignain
Zm00001d012972	Chalcone flavanone isomerase3
Zm00001d037894	Dehydrin DHN1
Zm00001d027488	Glyceraldehyde phosphate dehydrogenase B1
Zm00001d011477	Phospholipase A1-IIgamma
Zm00001d040730	GDSL esterase/lipase LIP-4
Zm00001d008529	Endonuclease 2
Zm00001d010521	Flavonoid 3'-monooxygenase
Zm00001d029604	Peroxidase 2
Zm00001d002343	Zinc transporter 1
Zm00001d015133	Metal transporter Nramp6
Zm00001d015914	Sugars will eventually be exported transporter4b
Zm00001d019936	No annotated
Zm00001d052336	Peroxidase 67
Zm00001d007718	Benzoxazinone synthesis13
Zm00001d045101	Cinnamoyl-CoA reductase 1
Zm00001d025059	Germin-like protein subfamily 1 member 8
Zm00001d049330	No annotated
Zm00001d047276	Brittle stalk2
Zm00001d048709	Benzoxazinless1
Zm00001d025533	NAD(P)-linked oxidoreductase superfamily protein
Zm00001d020133	No annotated
Zm00001d018751	Amino acid permease 6
Zm00001d028219	ATMAP70-2
Zm00001d048705	Benzoxazinone synthesis5
Zm00001d013956	Tonoplast intrinsic protein3

Zm00001d003039	Actin-related protein 2/3 complex subunit 2B
Zm00001d041495	SRP40 carboxy-terminal domain protein
Zm00001d047723	Tetratricopeptide repeat (TPR)-like superfamily protein
Zm00001d028718	Hydrophobic protein RCI2B
Zm00001d027749	Sulfate transporter 3.1
Zm00001d045884	ADPATP carrier protein 1 mitochondrial
Zm00001d041842	Amino acid permease 2
Zm00001d040173	Isoflavone reductase-like1
Zm00001d010440	Sugars will eventually be exported transporter3a
Zm00001d044156	Cytochrome P450 family 72 subfamily A polypeptide 8
Zm00001d032230	Terpene synthase 7
Zm00001d042619	Amino acid permease 2
Zm00001d044024	Ustilago maydis induced8
Zm00001d048710	Benzoxazinone synthesis2
Zm00001d044061	F-box domain containing protein
Zm00001d048702	Benzoxazinone synthesis3
Down-regulated in Al-tolerant and Al-sensitive	
Zm00001d026605	Endoglucanase 5
Zm00001d002071	DNA topoisomerase 2
Zm00001d022032	Ribosome-inactivating protein2
Zm00001d045571	Expansin-A7
Zm00001d041111	Transporter of mugineic acid1
Zm00001d029195	Terpene synthase8
Zm00001d046243	Putative ferroportin-domain family protein
Zm00001d022965	No annotated
Zm00001d004671	No annotated
Zm00001d047639	Nicotianamine synthase6
Zm00001d040163	Deoxy xylulose reductoisomerase1
Zm00001d002974	Probable metal-nicotianamine transporter YSL7
Zm00001d045965	Formate dehydrogenase chloroplastic/mitochondrial
Zm00001d028888	Nicotianamine synthase2
Zm00001d051634	Ribose-phosphate pyrophosphokinase 4
Zm00001d053281	Nicotianamine aminotransferase1
Zm00001d011847	Putative HLH DNA-binding domain superfamily protein
Zm00001d053320	Probable xyloglucan endotransglucosylase/hydrolase protein 26
Zm00001d010662	SNF1-related protein kinase regulatory subunit beta-1
Zm00001d047656	Nicotianamine synthase8
Zm00001d037198	LMBR1-like membrane protein
Zm00001d041769	Serine carboxypeptidase-like 20
Zm00001d033957	Helix-loop-helix DNA-binding domain containing protein
Zm00001d040292	Protein induced upon tuberization
Zm00001d035028	Putative glycolipid transfer protein (GLTP) family protein
Zm00001d035561	Mannose-specific jacalin-related lectin
Zm00001d012456	Jasmonate-regulated gene 21
Zm00001d036072	Zinc finger (C3HC4-type RING finger) family protein
Zm00001d041662	OSJNBa0024J22.14 protein

Zm00001d032084	Chaperone DnaJ-domain superfamily protein
Zm00001d019221	Nitrile-specifier protein 1
Zm00001d016705	ATPase inhibitor
Zm00001d016760	Abscisic acid stress ripening6
Zm00001d050043	Wound responsive protein
Zm00001d025957	Double B-box zinc finger protein12
Zm00001d028472	Far-red impaired responsive (FAR1) family protein
Zm00001d003365	No annotated
Zm00001d042062	Transcription factor ORG3
Zm00001d051397	U-box domain-containing protein 33
Zm00001d053220	MYB transcription factor 42
Zm00001d028360	NAD(P)-linked oxidoreductase superfamily protein
Zm00001d013326	No annotated
Zm00001d049191	Enolase-phosphatase E1
Zm00001d005823	Flavonoid 3-monooxygenase
Zm00001d027947	Tyrosine decarboxylase 1
Zm00001d006115	Anthocyanin-related membrane protein 1 (Anm1)-like
Zm00001d042684	Protein NRT1/ PTR FAMILY 5.10
Zm00001d017429	Iron-phytosiderophore transporter yellow stripe 1
Zm00001d028887	Nicotianamine synthase9
Zm00001d038296	Putative bZIP transcription factor superfamily protein
Zm00001d047115	Extensin-like protein

Supplementary Table 4 List of Cytochrome P450 proteins identified in Al-sensitive popcorn line under 72 hours of Al-stress

Gene ID	**Cytochrome P450 protein**	**Fold change**
	Down-regulated	
Zm00001d023210	Putative cytochrome P450 superfamily protein	-3.4586
Zm00001d029526	*cyp18*	-3.1985
Zm00001d036763	Putative cytochrome P450 superfamily protein	-2.3865
Zm00001d050628	Putative cytochrome P450 superfamily protein	-2.2775
Zm00001d053586	Putative cytochrome P450 superfamily protein	-2.2170
Zm00001d019414	71B10	-2.0864
Zm00001d013979	86B1	-1.6806
Zm00001d048819	Putative cytochrome P450 superfamily protein	-1.5612
Zm00001d030856	CYP81N4	-1.5137
Zm00001d023213	Cytochrome P450 family 87 subfamily A	-1.5001
Zm00001d049690	Putative cytochrome P450 superfamily protein	-1.4353
Zm00001d039697	Cytochrome P450 monooxygenase	-1.3715
Zm00001d024515	Putative cytochrome P450 superfamily protein	-1.3016
Zm00001d002687	86A7	-1.2175
Zm00001d039698	Cytochrome P450 family 706 subfamily A	-1.2130
Zm00001d018488	*cyp14*	-1.2015
Zm00001d032035	Cytochrome P450 family 706 subfamily	-1.0389
Zm00001d003283	71B10	-1.0369
	Up-regulated	
Zm00001d044159	*cyp11*	3.6729
Zm00001d034097	Cytochrome P450 family 81 subfamily D	3.5272
Zm00001d034096	Cytochrome P450 family 81 subfamily D	3.4790
Zm00001d006947	CYP709C14	2.6940
Zm00001d034095	Cytochrome P450 family 81 subfamily D	2.4572
Zm00001d044156	Cytochrome P450 family 72 subfamily A	2.3538
Zm00001d027601	Cytochrome P450 family 96 subfamily A	2.2579
Zm00001d004555	Cytochrome P450 family 93 subfamily D	2.1461
Zm00001d029290	CYP709H1	2.0548
Zm00001d007924	Cytochrome P450 family 93 subfamily D	1.7835
Zm00001d038300	Putative cytochrome P450 superfamily protein	1.6491
Zm00001d043174	98A3	1.2410
Zm00001d038064	Cytochrome P450 family 94 subfamily D	1.1282

Supplementary Table 5 List of Cytochrome P450 proteins identified in Al-resistant popcorn line under 72 hours of Al-stress

Gene ID	**Cytochrome P450 protein**	**Fold change**
	Down-regulated	
Zm00001d044147	Cytochrome P450 family 72 subfamily A	-3.31612
Zm00001d022262	709B2	-2.89096
Zm00001d016151	Cytochrome P450 family 93 subfamily D	-2.10089
Zm00001d014335	Cytochrome P450 family 87 subfamily A	-2.06617
Zm00001d034096	Cytochrome P450 family 81 subfamily D	-1.85268
Zm00001d007924	Cytochrome P450 family 93 subfamily D	-1.82429
Zm00001d052367	714A1	-1.82271
Zm00001d038300	Putative cytochrome P450 superfamily protein	-1.78663
Zm00001d034095	Cytochrome P450 family 81 subfamily D	-1.63015
Zm00001d034097	Cytochrome P450 family 81 subfamily D	-1.61428
Zm00001d038555	98A3	-1.43478
Zm00001d030372	Cytochrome P450 family 76 subfamily C	-1.37619
Zm00001d006649	Cytochrome P450 family 87 subfamily A	-1.35799
Zm00001d052530	94C1	-1.3545
Zm00001d032458	Cytochrome P450 family 87 subfamily A	-1.33467
Zm00001d025200	Putative cytochrome P450 superfamily protein	-1.11094
Zm00001d012304	Pfutative cytochrome P450 superfamily protein	-1.00014
	Up-regulated	
Zm00001d002687	P450 86A7	2.58876
Zm00001d049690	Putative cytochrome P450 superfamily protein	2.41258
Zm00001d022075	*cyp17*	2.31326
Zm00001d011420	Putative cytochrome P450 superfamily protein	2.25039
Zm00001d012326	86A1	2.23315
Zm00001d017528	CYP86A35	2.1516
Zm00001d002629	Putative cytochrome P450 superfamily protein	2.01732
Zm00001d013979	86B1	1.87403
Zm00001d039698	Cytochrome P450 family 706 subfamily A	1.56698
Zm00001d005822	*cyp12*	1.33883
Zm00001d003349	Cytochrome P450 family 724 subfamily A	1.31667
Zm00001d030851	CYP81N5	1.27433
Zm00001d042814	86A1	1.26734
Zm00001d014554	718	1.19819
Zm00001d044156	Cytochrome P450 family 72 subfamily A	1.12622

Supplementary Table 6 List of aluminum responsive transcription factors related in Al-sensitive popcorn

Gene ID	**Fold change**	**Category**	**Transcription factor protein**
Zm00001d002121	N/A[1]	bHLH	Transcription factor PRE3
Zm00001d017444	N/A[2]	WRKY	Probable WRKY transcription factor 12
Zm00001d006094	4.86129	MIKC_MADS	AGL2 MADS box family
Zm00001d034891	4.59606	MYB_related	RAD-like 3
Zm00001d041277	3.63828	YABBY	Putative axial regulator YABBY 2
Zm00001d002794	3.05265	WRKY	WRKY36-superfamily
Zm00001d023294	2.52101	NAC	NAC transcription factor 29
Zm00001d022461	2.39094	ERF	ERF-like protein
Zm00001d028986	2.38698	WOX	WUSCHEL-related homeobox 11
Zm00001d011237	2.31134	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d040682	2.13782	Trihelix	Sequence-specific DNA binding transcription factors
Zm00001d009619	2.08409	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d008399	2.01316	NAC	Putative NAC domain transcription factor superfamily protein
Zm00001d030577	1.74771	bZIP	Light-inducible protein CPRF-2
Zm00001d049485	1.66272	NF-YB	Nuclear transcription factor Y subunit B-3
Zm00001d052180	1.6538	MIKC_MADS	Tunicate1
Zm00001d026317	1.58702	bHLH	PIF4
Zm00001d007842	1.50468	TALE	Homeobox protein knotted-1-like 4
Zm00001d018742	1.44826	TALE	Rough sheath 1
Zm00001d037607	1.4298	WRKY	Probable WRKY transcription factor 50
Zm00001d028930	1.40685	MYB	MYB domain protein 112
Zm00001d016119	1.29598	NAC	Protein SOMBRERO
Zm00001d010634	1.196	G2-like	Putative MYB DNA-binding domain superfamily protein
Zm00001d026628	1.1593	Dof	Dof zinc finger protein DOF1.6
Zm00001d053220	-1.0568	MYB	MYB transcription factor 42
Zm00001d020938	-1.06508	bZIP	bZIP transcription factor family protein
Zm00001d026542	-1.14522	G2-like	MYB family transcription factor PHL5
Zm00001d011847	-1.28981	bHLH	Putative HLH DNA-binding domain superfamily protein
Zm00001d025205	-1.31014	bHLH	Putative HLH DNA-binding domain superfamily protein
Zm00001d038296	-1.32288	bZIP	Putative bZIP transcription factor superfamily protein
Zm00001d035195	-1.38235	LSD	Protein LSD1
Zm00001d044117	-1.39789	MYB_related	DNA binding protein
Zm00001d043420	-1.41676	bZIP	Basic leucine zipper 34
Zm00001d017366	-1.42066	ERF	Ethylene-responsive transcription factor ERF053

Zm00001d002452	-1.42216	WRKY	Probable WRKY transcription factor 35
Zm00001d033957	-1.53354	bHLH	Helix-loop-helix DNA-binding domain containing protein
Zm00001d029896	-1.66473	GATA	GATA transcription factor 2
Zm00001d016154	-1.67837	bZIP	Basic leucine zipper 34
Zm00001d025957	-1.75766	DBB	Double B-box zinc finger protein12
Zm00001d028526	-1.77858	GRAS	Scarecrow-like protein 26
Zm00001d042062	-1.81141	bHLH	Transcription factor ORG3
Zm00001d006110	-1.94673	bHLH	Transcription factor bHLH137
Zm00001d033005	-1.96495	HD-ZIP	Homeobox-leucine zipper protein HAT7
Zm00001d038013	-1.99743	LBD	Putative LOB domain-containing family protein
Zm00001d043062	-2.07345	WRKY	Probable WRKY transcription factor 30
Zm00001d018097	-2.22923	MYB	MYB domain protein 93
Zm00001d035918	-2.24211	MYB	Putative MYB DNA-binding domain superfamily protein
Zm00001d028445	-2.3412	MYB_related	RAD-like 3
Zm00001d053442	-2.62526	bZIP	Putative bZIP transcription factor superfamily protein
Zm00001d052804	-2.66282	MYB	Putative MYB DNA-binding domain superfamily protein
Zm00001d027855	-2.67624	bHLH	Transcription factor PRE3
Zm00001d021214	-3.00227	ERF	Ethylene-responsive transcription factor RAP2-11
Zm00001d024522	-4.20718	bHLH	Putative HLH DNA-binding domain superfamily protein
Zm00001d038288	-4.37553	MYB_related	Putative MYB DNA-binding domain superfamily protein
Zm00001d025141	-5.4748	bHLH	Basic helix-loop-helix DNA-binding superfamily protein

N/A[1]: Fold change value not available due to expression only in control condition. N/A[2]: Fold change value not available due to expression only in stress condition.

Supplementary Table 7 Aluminum responsive transcription factors related in Al-resistant popcorn

Gene ID	**Fold change**	**Category**	**Transcription factor protein**
Zm00001d017243	3.30523	MYB	R2R3MYB-domain protein
Zm00001d053163	2.56781	GRAS	Scarecrow-like protein 26
Zm00001d044074	2.48556	C3H	Zinc finger CCCH domain-containing protein 14
Zm00001d018435	2.47202	NAC	NAC domain containing protein 74
Zm00001d031759	2.15219	C2H2	Protein SHOOT GRAVITROPISM 5
Zm00001d045463	2.11847	NAC	NAC domain-containing protein 43
Zm00001d044081	2.09531	HD-ZIP	Homeobox-leucine zipper protein ATHB-4
Zm00001d017422	1.92427	HD-ZIP	Homeobox-leucine zipper protein ATHB-6
Zm00001d044662	1.9007	ZF-HD	ZHD9 ZF-HD type transcription factor
Zm00001d022259	1.82151	MYB	Transcription factor MYB46
Zm00001d026197	1.79402	MYB	MYB transcription factor 9
Zm00001d031270	1.74632	MYB	MYB domain protein 93
Zm00001d038761	1.69672	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d027855	1.68958	bHLH	Transcription factor PRE3
Zm00001d025141	1.65582	bHLH	Basic helix-loop-helix DNA-binding superfamily protein
Zm00001d052591	1.62046	B3	B3 domain-containing protein
Zm00001d041549	1.59773	Dof	Dof zinc finger protein DOF2.2
Zm00001d043729	1.46302	MYB	Transcription factor MYB86
Zm00001d038878	1.45538	MYB	Transcription factor MYB86
Zm00001d036050	1.39105	NAC	Putative NAC domain transcription factor superfamily protein
Zm00001d003293	1.33773	NAC	NAC domain containing protein 44
Zm00001d027335	1.33249	Trihelix	Trihelix transcription factor GT-2
Zm00001d005740	1.28741	bHLH	Transcription factor AIG1
Zm00001d009030	1.28619	C2H2	DNA binding protein
Zm00001d018097	1.28339	MYB	MYB domain protein 93
Zm00001d054080	1.28093	Trihelix	Trihelix transcription factor GTL2
Zm00001d043921	1.2721	NAC	NAC domain-containing protein 73
Zm00001d011669	1.25542	MYB	Putative MYB DNA-binding domain superfamily protein
Zm00001d052288	1.2346	E2F/DP	E2F transcription factor-like E2FE
Zm00001d038801	1.23366	GATA	GATA transcription factor 12
Zm00001d011969	1.20059	NAC	NAC domain containing protein 84
Zm00001d035835	1.13951	ERF	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d015468	1.12831	CO-like	Zinc finger protein CONSTANS-LIKE 5

Zm00001d026628	1.10833	Dof	Dof zinc finger protein DOF1.6
Zm00001d001945	1.08581	ARF	Auxin response factor 4
Zm00001d003414	1.06294	NAC	NAC domain-containing protein 79
Zm00001d049137	1.06134	C2H2	No annoted
Zm00001d012092	1.00287	C2H2	Histone deacetylase HDT1
Zm00001d031135	-1.02733	GATA	GATA zinc finger family protein
Zm00001d040621	-1.02902	MYB	MYB transcription factor40
Zm00001d037607	-1.03227	WRKY	Probable WRKY transcription factor 50
Zm00001d044409	-1.03405	MYB_related	MYB-related transcription factor
Zm00001d002762	-1.03555	ERF	Ethylene-responsive transcription factor 2
Zm00001d036726	-1.04403	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d009619	-1.05405	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d025727	-1.06175	bHLH	U-box domain-containing protein 7
Zm00001d028986	-1.06324	WOX	WUSCHEL-related homeobox 11
Zm00001d019207	-1.08706	NAC	NAC domain-containing protein 2
Zm00001d036392	-1.10772	bZIP	BZIP transcription factor TRAB1
Zm00001d026447	-1.12669	ERF	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d050195	-1.12815	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d038843	-1.14578	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d010399	-1.1514	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d049173	-1.1569	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d028930	-1.17057	MYB	MYB domain protein 112
Zm00001d034145	-1.17475	C2H2	Zinc finger protein AZF2
Zm00001d024324	-1.19426	ERF	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d010676	-1.21522	ERF	Ethylene-responsive transcription factor 12
Zm00001d042580	-1.22612	NAC	NAC domain-containing protein 35
Zm00001d033987	-1.22713	HSF	Heat stress transcription factor A-6b
Zm00001d005798	-1.26504	ERF	Ethylene-responsive transcription factor RAP2-2
Zm00001d002867	-1.27841	ERF	Ethylene-responsive transcription factor ERF053
Zm00001d010175	-1.28614	ERF	Ethylene-responsive transcription factor ABR1
Zm00001d011297	-1.31402	MYB_related	Putative MYB DNA-binding domain superfamily protein
Zm00001d014029	-1.3156	MYB	MYB domain protein 15
Zm00001d012482	-1.33205	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d053746	-1.33240	WRKY	Probable WRKY transcription factor 40
Zm00001d031164	-1.34688	NAC	JUNGBRUNNEN 1
Zm00001d033957	-1.35449	bHLH	Helix-loop-helix DNA-binding domain containing protein

Zm00001d013208	-1.36274	C2H2	Zinc finger protein AZF2
Zm00001d012285	-1.3791	MYB_related	Putative MYB DNA-binding domain superfamily protein
Zm00001d016298	-1.40892	TALE	Homeobox protein ATH1
Zm00001d038296	-1.41119	bZIP	Putative bZIP transcription factor superfamily protein
Zm00001d010751	-1.41935	LBD	Bn15D17A
Zm00001d004007	-1.42276	bHLH	Transcription factor FER-LIKE IRON DEFICIENCY-INDUCED
Zm00001d043094	-1.46154	NAC	Pentatricopeptide repeat-containing protein mitochondrial
Zm00001d037941	-1.4863	ERF	Ethylene-responsive transcription factor RAP2-3
Zm00001d011614	-1.50346	MYB	Putative MYB DNA-binding domain superfamily protein
Zm00001d027987	-1.50461	bHLH	Transcription factor bHLH99
Zm00001d008812	-1.50898	C3H	C3H47 C3H type transcription factor
Zm00001d017462	-1.51719	ERF	Ethylene-responsive transcription factor 1A
Zm00001d024545	-1.53853	B3	B3 domain-containing transcription factor NGA2
Zm00001d052254	-1.54597	bHLH	Transcription factor PRE3
Zm00001d011237	-1.54649	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d030577	-1.55843	bZIP	Light-inducible protein CPRF-2
Zm00001d051149	-1.5711	MYB	MYB transcription factor39
Zm00001d002545	-1.63852	MYB_related	REVEILLE 1
Zm00001d013003	-1.74607	NAC	NAC domain-containing protein 2
Zm00001d038717	-1.75561	LBD	Bn15D17A
Zm00001d039459	-1.78127	bHLH	Putative transcription factor bHLH041
Zm00001d011847	-1.80534	bHLH	Putative HLH DNA-binding domain superfamily protein
Zm00001d031728	-1.8205	ERF	Dehydration-responsive element-binding protein 1B
Zm00001d053195	-1.84311	ERF	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d010232	-1.86042	MIKC_MADS	Zea mays MADS29
Zm00001d008853	-1.86542	bHLH	Putative HLH DNA-binding domain superfamily protein
Zm00001d042482	-1.90512	bHLH	bHLH-transcription factor 132
Zm00001d037749	-1.91759	bHLH	Putative HLH DNA-binding domain superfamily protein
Zm00001d012527	-1.91997	NAC	NAC domain-containing protein 2
Zm00001d032024	-1.93652	MYB	MYB transcription factor38
Zm00001d022437	-2.01975	WRKY	Probable WRKY transcription factor 30
Zm00001d029506	-2.03867	LBD	LOB domain-containing protein 42
Zm00001d013151	-2.0679	NAC	JUNGBRUNNEN 1
Zm00001d046805	-2.13229	WRKY	Putative WRKY DNA-binding domain superfamily protein
Zm00001d040004	-2.14959	LBD	LOB domain-containing protein 42
Zm00001d043699	-2.2273	bHLH	Transcription factor bHLH87

Zm00001d030232	-2.23736	bHLH	Transcription factor bHLH18
Zm00001d048229	-2.2738	bHLH	Putative HLH DNA-binding domain superfamily protein
Zm00001d017268	-2.39455	MYB	R2R3MYB-domain protein
Zm00001d012296	-2.51375	bZIP	ABA response element binding factor
Zm00001d022227	-2.54393	MYB	Fused leaves 1
Zm00001d009103	-2.58549	ERF	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d020492	-2.60987	WRKY	Probable WRKY transcription factor 40
Zm00001d034920	-2.75139	ERF	Ethylene-responsive transcription factor 1B
Zm00001d009160	-2.77477	Trihelix	Sequence-specific DNA binding transcription factors
Zm00001d042062	-2.89808	bHLH	Transcription factor ORG3
Zm00001d029587	-2.95095	C2H2	Zinc finger protein AZF2
Zm00001d005055	-2.969	MYB_related	Transcription factor MYB86
Zm00001d027924	-3.00681	ERF	No annoted
Zm00001d024725	-3.04336	MYB	Putative MYB DNA-binding domain superfamily protein
Zm00001d007329	-3.06049	WRKY	Probable WRKY transcription factor 75
Zm00001d039531	-3.45512	WRKY	Probable WRKY transcription factor 50
Zm00001d040651	-3.58944	ERF	Ethylene-responsive transcription factor RAP2-2
Zm00001d047334	-3.6847	C2H2	Zinc finger protein AZF2
Zm00001d040682	-4.18814	Trihelix	Sequence-specific DNA binding transcription factors
Zm00001d022002	-4.37513	C2H2	Zinc finger protein AZF2
Zm00001d022461	-4.38569	ERF	ERF-like protein
Zm00001d047330	-4.57431	C2H2	Zinc finger protein AZF2

CHAPTER 2

E-SNP ANALYSIS HIGHLIGHT GENES ASSOCIATED TO AL RESISTANCE IN POPCORN (*Zea mays*)

e-SNP analysis highlight genes associated to Al resistance in popcorn (*Zea mays*)

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Abstract

Aluminum (Al) is the third most abundant element in the earth's crust and in acid soils limiting crop production. The Al phytotoxic form inhibits the growth and development of the roots altering the water and nutrients absorption and consequently reducing the plant development**.** Robust techniques, such as high-throughput mRNA sequencing, may facilitate the comprehension of this complex trait. In our previous work, were identified genes that may contribute to Al tolerance during a long term exposure to Al toxicity using RNA-sequencing. SNP call analysis were performed to map these genes on the chromosomes and identify variants. Reads were mapped to reference genome using BWA-MEM algorithm and the mapping files were processed using Picard software. The variants were called using FreeBayes software and the SNPs were filtered using vcftools and annotated using Variant Effect Predictor. This allowed the identification of genes inside maize Al-tolerance QTLs previously reported in the literature and the detection of variants. The results of this study allowed highlighted some new targets, such as SNF1-related protein kinase, histone deacetylase (HDT1), SWEET transporters, delta (12)-fatty-acid desaturase (FAD), MADS-box, AP2/EREBP, HY5-like, and pathogenesis-related (PR) proteins, that may contribute to maintaining root growth under Al-stress in popcorn.

Keywords: Aluminum stress; RNA-seq; QTL; SNP

Introduction

Acid soils are widely distributed around the world (Ma et al., 2014) and cover 30-40% of the world's arable land, of which approximately 60% comprise the areas of tropical and subtropical regions (Kochian et al., 2004; Von Uexküll and Mutert, 1995). Soils can be naturally acidic but the agricultural process can accelerate this acidity by the intense use of fertilizers with an acidifying effect.

Aluminum (Al) is one of the most abundant elements in the earth's crust. In acid soils, with pH values at or below 5, the phytotoxic species Al^{3+} are solubilized in soil solution and become one of the major problems of crop cultivation by interfering in a root function and reducing plant development (Kochian, 1995; Kochian et al., 2015). In Al-sensitive cereal genotypes, the small root system leads to reduced water and nutrient absorption, making the plant more susceptible to diseases, water stress, and temperature changes, consequently reducing grain yields (Ma et al., 2014).

Some plants have evolved mechanisms to grow on Al-toxic environments and tolerate high levels of this metal. The exclusion mechanism prevents the Al^{3+} from entering the root apex through the regulation of organic acid release from the root tip, which chelates Al^{3+}, forming stable nontoxic complexes. The tolerance mechanism consists of neutralize toxic Al^{3+} absorbed by sequester and detoxification in subcellular compartments and/or translocate it away from the root tip (Kochian et al., 2015).

Al tolerance is considered a quantitative trait in maize. Quantitative trait loci (QTL) mapping studies for Al tolerance in maize, using root growth as the phenotypic index, identified genomic regions associated with this trait on the chromosomes 2, 3, 4, 5, 6, 8 and 10 (Conceição et al., 2009; Guimaraes et al., 2014; Krill et al., 2010; Maron et al., 2010; Ninamango-Cárdenas et al., 2003; Sibov et al., 1999). Despite the agronomic importance of Al tolerance in maize, only the *ZmMATE1* has been characterized (Maron et al., 2010), but other genes and mechanisms involved in the tolerance to the phytotoxic Al ion are currently unknown.

Some studies linking previous reported Al-tolerance QTLs to expression data have been made to reduce the number of Al responsive genes in previous gene expression analysis using microarray data. Maron et al. (2010) and Mattiello et al. (2012) used this approach for additional exploration of microarray data in gene expression studies made in hydroponic (Maron et al., 2008) and acid soil (Mattiello et al., 2010) conditions.

Recently, our group tracked the gene expression profile of two contrasting popcorn lines for Al resistance under 72 h of stress using a high throughput RNA sequencing, aiming to reveal maintenance mechanisms involved in a later response in long term stress. Our results revealed several differentially expressed genes involved in cell wall modification, fatty acid biosynthesis and degradation, response to reactive oxygen species, brassinosteroids biosynthesis, transporters, and transcription factors already known to be related to Al resistance. Moreover, in the first chapter of this thesis, were identified genes not previously described in hydroponic and soil experiments that may play an important role in suppressing the Al effects on plant growth.

In this work, the RNA-seq data previously obtained were linked into maize QTLs involved in Al tolerance (Conceição et al., 2009; Guimaraes et al., 2014; Maron et al., 2010; Ninamango-Cárdenas et al., 2003; Sibov et al., 1999) to explore and reduce the genes associated with these regions. Besides that, was performed a single nucleotide polymorphism (SNP) analysis to detect variants with predicted deleterious substitutions that may be responsible to maintaining the root growth and plant development under Al-stress conditions and which may be useful as possible markers in popcorn breeding programs.

Material and methods

RNA isolation and RNA-seq

In the first chapter , Al-tolerant inbred line (11-133) and Al-sensitive inbred line (11-60) (Rahim et al., 2019) were cultivated in the hydroponic system during 72 hours. Total roots from three biological replicates were collected and immediately frozen in liquid nitrogen. Total root RNA from the twelve samples was isolated and the transcriptome sequencing library was generate using the TruSeq Stranded mRNA kit. The libraries were sequenced using the Illumina HiSeq 2500 platform.

Selection of differentially expressed genes

The raw reads were trimmed, filtered, and adapters were removed. The clean reads of the twelve samples, three biological replicates from each genotype that has been grown in the control and Al stress conditions, were aligned to the maize (*Zea mays*) reference genome (B73 RefGenv4) (Jiao et al., 2017) using Bowtie2 (version 2.3.3.1) (Langmead and Salzberg, 2012) and TopHat (version 2.1.1) (Kim et al., 2013). The Cuffdiff (v2.2.1) program (Trapnell et al., 2012) was used to calculate gene expression levels and to identify differentially expressed genes (DEGs) in terms of fragments per kilobase per million mapped reads (FPKM). The DEGs were identified by a false discovery rate (FDR) < 0.01 and by significant Q value calculated from the P-values.

SNP discovery

The genome of maize version B73 RefGenv4 (Jiao et al., 2017) was downloaded at Phytozome database (https://phytozome.jgi.doe.gov). Reads were mapped to reference genome using BWA-MEM algorithm of BWA version 0.7.17 (http://bio-bwa.sourceforge.net/) (Li and Durbin, 2009), and a flag identifying the respective popcorn sample was added to each mapping file. Then, the mapping files were processed using SortSam, MarkDuplicates and BuildBamIndex tools of Picard version 2.18.27 (https://github.com/broadinstitute/picard/). Variants were called using FreeBayes version 1.2.0 (https://github.com/ekg/freebayes) (Garrison and Marth, 2012) with a minimum mapping quality of 20, minimum base quality of 20, and minimum coverage of 20 reads at every position in the reference genome. After variant calling, SNPs were filtered using vcftools version 0.16.15 (https://vcftools.github.io/index.html) and annotated using Variant Effect Predictor (McLaren et al., 2016) available at Ensembl Plants web server (http://plants.ensembl.org/Tools/VEP). Then, the SNP effects were predicted as deleterious or neutral mutation based on Protein Variation Effect Analyzer (http://provean.jcvi.org/index.php).

In silico mapping

The positions of each delimiting QTL marker (Table 1) were examined using the MaizeGDB database to verify the DEGs observed in both lines inside previously identified Al tolerance QTLs. Then, a comparison between the positions of the genes and the region flanked by markers in each chromosome allowed the identification of all DEGs inside the QTLs and a manual comparison with the NCBI database using BlastX algorithm was performed.

Results and discussion

DEGs mapped inside QTLs

The first chapter of this thesis revealed new players involved in maintenance mechanisms that were not identified in previous studies using hydroponics and soil experiments. The Al-tolerant inbred line rescues several mechanisms to alleviate the damages caused by Al-stress and the combination of them may be responsible for the Al tolerance in popcorn. Considering the relevance of Al tolerance studies to understand this complex trait, in this work were highlighted new genes related to Al-resistance through the linking of our RNA-seq data to previous QTLs identified for Al-tolerance in maize and the identification of variants with predicted deleterious effect in the biological function of the protein.

Al-tolerance QTLs that have been identified in early studies were used in this work (Table 1) to identify DEGs inside genomic regions important to Al-tolerance. A total of 632 genes was identified inside QTLs in the Al-tolerant inbred line (11-133) (Suppl. Table S1), and 234 genes in the Al-sensitive inbred line (11-60) (Figure 1; Suppl. Table S2). The majority of DEGs mapped inside QTLs were categorized in response to stimulus, response to stress and oxidoreductase activity.

The chromosome 2 and 5 mapped two aquaporins PIP2-2 in Al-tolerant inbred line and over the chromosome 10 were mapped three ABC transporter genes (Suppl. Table S1). One of the most important mechanisms of plant tolerance to Al has been the role of Al transporters in the plasma membrane and in the tonoplast (Kochian et al., 2015). Aquaporin probably has an important role in Al-tolerance since the overexpression of the *HmVALT* from *Hydrangea macrophylla* in *Arabidopsis* conferred tolerance to Al toxicity (Negishi et al., 2012). The ABC transporters are involved in diverse processes in plant growth and development and in response to abiotic stress (Kang et al., 2011). Thus, these results suggest that aquaporin and ABC transporters may play an important role in increasing the tolerance response in 11-133.

Five SWEET transporters were mapped in QTLs only in 11-133 (Suppl. Table S1). However, two SWEET transporters presented a constitutive pattern in both lines (Suppl. Table S3). In the first chapter, was proposed that the class of SWEET transporters might be involved in the regulation of vacuolar sugar storage under Al-stress. To deal with environmental stress conditions, plants need to maintain a rigid regulation in the storage and transport of vacuolar sugar (Chandran, 2015). The SWEET genes play an important role in adverse conditions, such as tolerance to osmotic stress, including cold, high salinity and drought (Seo et al., 2011). The presence of these genes mapped in QTLs present in chromosomes 5 (Maron et al., 2010), in QTL_4 (Ninamango-Cárdenas et al., 2003) and in the loci *Alm1* (Sibov et al., 1999) suggest that SWEET transporter may contribute to the Al-tolerant phenotype overcoming the stress effect maintaining the tight regulation on vacuolar sugar storage.

The chromosome 6 shown two SNP variation with amino acid change in a putative MADS-box transcription factor mapped in QTL_2 identified by Ninamango-Cárdenas et al. (2003) (Table 2). The role of MADS-box has been studied in Al-stress. Four MADS-box genes were induced in tomato roots under Al-stress and were detected using microarray hybridization (Zhou et al., 2008). The MADS-box proteins abundance was also increased in Al-tolerant rice under stress conditions (Wang et al., 2013). Another transcription factor (TF) with amino acid change identified was the AP2/EREBP. This gene was mapped inside QTL_4 (Ninamango-Cárdenas et al., 2003) (Table 2). This TF was one of the most predominant families identified in *Arabidopsis* under Al-stress (Kumari et al., 2008), and known to mediate process in hormone and redox signaling in abiotic stress conditions (Dietz et al., 2010). These data indicate that

MADS-box and AP2/EREBP may be involved in regulation of downstream expression of genes involved in Al tolerance in 11-133.

The cell wall modification under abiotic stresses might involve maintenance of cell wall plasticity (Le Gall et al., 2015). Here, were possible identify some genes that modulate the cell wall in 11-133 and 11-60, indicating that both lines have a cell wall response under Al-stress. Two of these genes that encode a cellulose synthase, showed non-synonymous substitutions in 11-133 (Table 2). The synthesis of cellulose is regulated by the plant cell to adapt its growth to environmental stress (Kesten et al., 2017). A SNP in this gene may confer an adaptive advantage contributing to the cell wall modification in 11-133 under Al-stress.

Plants under aluminum stress might produce reactive oxygen species (ROS) in mitochondria, chloroplast and peroxisome (Kochian et al., 2005). To control this stress, plants deploy a complex antioxidant defense system (Vranova et al., 2002). Several genes involved in defense system against ROS were mapped inside the QTLs in both lines (Suppl. Table S1, Suppl. Table S2). Some of them presented SNPs with predicted neutral variation in both lines (Table 2, Table 3). Based on this, these plants respond to the ROS created due to the Al ion present in the cell. Some enzymes scavenging the ROS under Al-stress were identified in different levels at different times in roots of two contrasting maize genotypes (Wang et al., 2015).

None of the DEGs found in 11-133 were mapped on the QTLs found by Conceição et al. (2009) on chromosomes 6 and 8, by Maron et al. (2010) on chromosome 6, and in the locus *qAlt6* on chromosome 6 by Guimaraes et al. (2014). The DEGs in 11-60 were also not mapped in these same regions, plus those in the loci *qAlt6* and *qAlt8* on chromosomes 6 and 8, respectively (Guimaraes et al., 2014), and on chromosome 10 (Conceição et al., 2009).

DEGs mapped inside QTLs shared in both inbred lines

Eighty DEGs were shared in both inbred lines, which of them 62 genes were mapped inside 11 QTLs regions (Figure 2; Table 4), with differential expression pattern due to the genetic background, and the remaining 18 were considered as constitutive genes (Suppl. Table S3).

The majority of the genes up-regulated in 11-60, but down-regulated in 11-133 were found inside the region mapped by Sibov et al. (1999) in chromosome 10 (Table 4). One of these genes was the delta (12)-fatty-acid desaturase (FAD), which acts in the synthesis of polyunsaturated fatty acids (FA) in plant cells converting oleic acid (18:1) to linoleic acid (18:2) (Okuley et al., 1994) and modulated by a variety of abiotic stresses (Yuan et al., 2012). Aluminum stress was able to pronounce the effect on the degree of FA unsaturation (Peixoto et al., 2001), and influenced the FA composition of the root system in sorghum under Al-stress condition (Peixoto et al., 2001). Together with other genes involved in lipid metabolic process, these results indicate that Al-stress may affect the membrane fluidity in 11-60 line.

Up-regulated pathogenesis-related (PR) proteins were found in 11-60, but down-regulated in 11-133 (Table 4). These genes were mapped in chromosomes 8 (Ninamango-Cárdenas et al., 2003) and 10 (Sibov et al., 1999). PR proteins have been reported to have multiple functions in adaptation to abiotic stress, like enhancing heavy metal tolerance (Sarowar et al., 2005). The expression of these genes related to defense response may be an important mechanism to induce others genes related to Al toxicity in 11-60. Moreover, with the same expression pattern, were identified genes involved in reactive oxygen species metabolic process, response to oxidative stress, lignin catabolism process, and cell wall biogenesis, mapped over the QTL regions (Table 4).

These results suggest that the Al-sensitive inbred line, even with a response machinery against the Al toxicity, is not sufficient for its development in Al-stress condition.

The 23 DEGs with opposite expression pattern (up-regulated in 11-133 and down-regulated in 11-60) were mapped among the chromosomes 2 (Ninamango-Cárdenas et al., 2003), 4 (Conceição et al., 2009), 5 (Guimaraes et al., 2014; Maron et al., 2010), 6 and 8 (Ninamango-Cárdenas et al., 2003), and 10 (Sibov et al., 1999), but four of them have unknown function (Table 4).

Were mapped inside QTLs genes that encode enzymes involved in ROS defense. The up-regulation of these genes in 11-133 may be responsive to create a decrement of oxidative load under Al-stress and provide considerable protection to roots against oxidative damage.

Genes involved in several biological functions as response to hormones (e.g. CASP-like protein 5), transmembrane transport (e.g. ATPase) and defense response (e.g. LCR70) may act in conjunction with other unmapped DEGs inside QTLs contributing to Al tolerance in 11-133.

DEGs with SNP

Once that were extracted the SNPs presents among the DEGs, were identified 339 and 77 non-synonymous SNPs with amino acid change in Al-tolerant and –sensitive inbred lines, respectively. From this data, were identified predicted deleterious mutation in 31 genes in 11-133 (Table 5), and 17 in 11-60 (Suppl. Table S4).

In the previous Al QTL studies (Conceição et al., 2009; Guimaraes et al., 2014; Maron et al., 2010; Ninamango-Cárdenas et al., 2003; Sibov et al., 1999), the chromosomes 1 and 7 have been never mapped in Al-tolerance QTL regions (Table

5). We considered that, due to the different genetic background between our present popcorn inbred lines, the genotypes used in the QTL mapping works, and the reference genome used to map the RNA-seq reads, these chromosomes may also have important regions that control the Al tolerance trait in popcorn, mainly such as maintenance response.

On chromosome 1, among the 10 genes detected with predicted deleterious variation, the peroxidase 1 (POD) is one of the enzymes that play a role in ROS alleviation (Table 5). As mentioned on the last section above, POD may be one of the major enzymes responsible for maize root growth working in the antioxidant system activated during the peroxidation caused by Al stress (Wang et al., 2015). Also acting in maize antioxidant system, was detected a variation in a phenylalanine ammonia-lyase 1 (PAL). Phenylalanine ammonia-lyase is involved in the first step of the phenylpropanoid pathway, which produces precursors with a variety of functions, including protection from abiotic stresses (Huang et al., 2010). Gholizadeh and Kohnehrouz (2010) have suggested that PAL may be a promising target to an antioxidant system strategy during salt stress in maize.

On chromosome 3 were found variations in cytochrome P450 monooxygenase and glutathione transferase 7 (GST). Glutathione transferases are the main cellular detoxification enzymes acting against the plant oxidative damage (Labrou et al., 2015) and its function has been demonstrated playing a role in maize root alleviation against Al toxicity (Cançado et al., 2005). On the other hand, the function of cytochromes P450 involved in aluminum stress is not totally clear, but P450 monooxygenases act in detoxification pathway to catalyze several endogenous and exogenous toxic compounds in both the cytoplasm and the endoplasmic reticulum (Schuler and Werck-Reichhart, 2003).

These variations found in enzymes involved in ROS scavenging suggest that these genes may be antioxidants of crucial importance to Al tolerance in popcorn.

Predicted deleterious variation inside QTLs

In the Al-tolerant inbred line, were found two genes inside the QTL_5 (Ninamango-Cárdenas et al., 2003) on chromosome 8 that encode a histone deacetylase (HDT1) and an enzyme desaturase stearoyl-acyl-carrier-protein 9 (Table 6).

The *Arabidopsis* knockout of HDT1/2 resulted in an earlier switch and caused the reduction of root meristem cell number, being an important part of the mechanism that modulates root growth in response to environmental factors (Li et al., 2017). Moreover, histone deacetylation gives a tag for epigenetic repression. Specific histone modification sites and modifiers regulate key stress-responsive genes, but the entire correlation network between abiotic stress responses and epigenetic information remain unclear (Kim et al., 2015). The complexity of this trait in 11-133 may be correlated also with epigenetic regulation, mainly because some DEGs such as histone methyltransferases, lysine demethylase, and dicer-like mapped inside QTLs in chromosomes 5 and 6 (Suppl. Table S1). Furthermore, the same HDT1 gene presented five amino acid changes and a histone methyltransferase two amino acid changes, both with predicted neutral variations (Table 2).

Stearoyl-acyl carrier protein desaturase (SAD) plays an essential role in determining the overall content of unsaturated fatty acids (Hernandez et al., 2019), thus catalyzes the first desaturation step conducting to oleic acid, which can be desaturated in next steps in linoleic and α-linolenic acids (Shanklin and Somerville, 1991). In Al-tolerant sorghum cultivars have been demonstrated a reduction in the concentration of stearic, oleic, and linoleic acids, but an increase in the concentration of palmitic, palmitoleic and linolenic acids under stress condition (Peixoto et al., 2001). In addition to being inside a QTL, the down-regulated SAD variant found in 11-133 may play a role, possibly, in the changes in fatty acid composition modulating the lipid fluidity of the tolerant line and acting in the retention of ions and metabolites and/or to limit the entry of toxic Al ions into roots.

In the Al-sensitive inbred line were found five genes with predicted deleterious variations among four QTLs (Table 7), however three genes did not have any known function. The SNF1-related protein kinase (SnRK1) is a key regulator in the adjustment of cellular metabolism during stress condition, starvation and growth-promoting conditions (Wurzinger et al., 2018). During diverse abiotic and biotic stress conditions, the SnRK1 can be activated causing an energy deficit, consequently affecting photosynthesis, respiration, or carbon allocation. However, the activity of this kinase complex is repressed by sugars (Baena-González et al., 2007).

Biochemical analyses of SnRK1 mutants in *Arabidopsis thaliana* have been shown the connection to the regulatory function of SnRK1 to abscisic acid (ABA) signaling (Jossier et al., 2009). Additionally, in knocked-out SnRK1 lines of *Arabidopsis* under extended darkness conditions, the content of tricarboxylic (TCA) cycle was altered as malate, fumarate and succinate levels increased, whereas citrate levels decreased (Pedrotti et al., 2018), reveling the crucial function of SnRK1 in metabolic control (Pedrotti et al., 2018). The best hit in *Arabidopsis* for the gene that encodes a SnRK1 regulatory subunit beta-1 (Zm00001d010662) is the *AKINβ1*, that is involved in the specific regulation of nitrogen metabolism and sugar signaling (Li et al., 2009).

This gene presented a constitutive pattern (Suppl. Table S3), but the predicted deleterious SNP was found only in the 11-66. It is clear that SnRK1 modulates the plant metabolism under abiotic stress, but until the date there is no investigation about the role of SnRK1 in Al-stress conditions. Its constitutive expression indicate that the SnRK1 regulatory subunit beta-1 may be involved in continuous signal transduction cascade, carbohydrate metabolism, and organic acid production being relevant for Al tolerance. However, the SnRK1 activity under Al-stress needs further functional investigations.

Another predicted deleterious mutation was found in ATPase inhibitor gene mapped inside the QTL presents in chromosome 5 (Maron et al., 2010). It is have been shown that phytotoxic Al^{3+} concentration results in H^{+}-ATPase inhibition in maize (Façanha and Okorokova-Façanha, 2002) and soybean roots (Shen et al., 2005). Besides that, in squash roots, Al inhibited the H^{+}-ATPase activity by the alteration in plasma membrane surface (Ahn et al., 2001). These results indicate that Al ions may affected the surface charge of the plasma membrane in sensitive line by the inhibition of ATPase activity.

Non sense variation effect

The SNP analysis revealed three non-sense variations only in transcripts found in 11-133 (Table 5). These genes were not mapped inside previous QTLs, but receive attention due to the adaptive advantage they can offer to the Al-tolerant genotype. The stop codon lost was found in a coatomer subunit zeta-2 (Zm00001d020783). The coatomer complex plays an essential role in plant growth and survival by maintaining the Golgi apparatus and modulating cell formation (Ahn et al., 2015), but its physiological function is until unclear in environment stress conditions.

The start codon lost was found in a transcription factor HY5-like (Zm00001d039658) and a mitochondrial outer membrane protein porin 4 (Zm00001d048362). The *HY5* is a positive regulator of ABA signaling (Lau and Deng, 2010) and regulates the transcription of some photosynthesis-related genes (Chattopadhyay et al., 1998) that may be involved in the photosynthesis regulation under adverse environmental conditions (Saibo et al., 2008). Besides that, in *Arabidopsis* under high nitrogen condition it acts as one of the major transcription factors required for the expression of genes involved in detoxification (Bellegarde et al., 2018). Thus, the defective transcript may act in the photosynthesis pathway, detoxification process and ABA signaling in 11-133 under Al toxicity conditions.

The ion transporters level are altered during abiotic stresses, such as the voltage-dependent anion channel (*VDAC*) and the outer mitochondrial membrane porin (Jacoby et al., 2010). Besides that, the millet *VDAC* expressed in transgenic rice plants contribute to the tolerance to salinity stress, suggesting that the enhancement of *VDAC* expression may lead to tolerance against environmental stresses (Desai et al., 2006). The *Arabidopsis* orthologous, At5g57490, was one of the four VDAC found down-regulated under high salt stress but not affected upon cold and drought stresses (Lee et al., 2009). In this context, the start codon lost in this gene may play an adaptive role in plant development and in stress adaptation. Together with other genes, these variants found in our work may contribute to tolerance to Al toxicity in popcorn.

Conclusions

Here, the first RNA-seq data from contrasting popcorn inbred lines cultivated in nutritive solution under Al stress was integrated with previously published maize QTL data. Moreover, a genotyping analysis was performed and predicted deleterious variations were identified among these data. This approach allowed to identify several genes with a wild range of functions correlated with Al stress response, such as enzymes involved in ROS alleviation, cell wall modification, stress response, lipid and fatty acid biosynthesis, transporters, and transcription factors, that may work together contributing to the QTLs role thus enabling the root growth and plant development in Al toxicity conditions. In addition, genes with predicted deleterious mutations and non-sense variation may play a key role in adaptive advantage to survive under Al stress. According to Mattiello et al. (2012), Al-tolerance QTLs comprise larges regions in maize genome, because of that false positives may appear with this approach. On the other hand, the genetic background between these genotypes and our popcorn inbred lines may allow new regions that control Al-tolerance in chromosomes not yet mapped. Using further investigation techniques, such as functional genomics, the number of potential genes inside QTLs and with predicted neutral/deleterious variations can be reduced allowing a better understanding of this complex trait. Based on our results, we highlighted some new targets, such as SNF1-related protein kinase, histone deacetylase (HDT1), SWEET transporters, delta (12)-fatty-acid desaturase (FAD), MADS-box, AP2/EREBP, HY5-like, and pathogenesis-related (PR) proteins, that may work together and contribute to maintaining root growth under Al-stress in popcorn.

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Table 1 Al-tolerance QTLs identified in previous work

QTL	CHROMOSOME	MARKERS	REFERENCE
1	6	umc85 / umc59	Sibov, et al. (1999)
2	10	umc130 / npi232	
3	2	umc139 / p-bnlg198	Ninamango-Cardenas, et al. (2003)
4	6	p-phi126 / p-phi077	
5	6	mmc0241 / nc013	
6	8	umc103 / p-bnlg162	
7	8	p-bnlg1031 / p-phi080	
8	4	umc1550	Conceição, et al. (2009)
9	5	bnlg1382	
10	6	bnlg1154	
11	8	umc1202	
12	10	umc1318	
13	6	p-phi126 / umc1018	Maron, et al. (2010)
14	5	bnlg105 / umc1019	
15	2	S2_212940514	Guimarães, et al. (2014)
16	3	S3_187460236	
17	5	S5_30301926	
18	6	ZmMATE1	
19	8	S8_22681622	

Table 2 Neutral missense variation mapped in tolerance aluminum QTLs in Al-resistant inbred line

GENE	QTL	CHR	FUNCTION	Consequence	Variant	PROVEAN score
Zm00001d024939	2	10	Phosphoglycerate mutase-like protein	missense_variant	258,N,Y	-1.246
Zm00001d024943	2	10	Putative cytochrome P450 superfamily protein	missense_variant	366,A,V	2.633
Zm00001d025103	2	10	amo1;amine oxidase1: single copy rice cDNA rz69	missense_variant	314,R,G	-1.903
Zm00001d025528	2	10	NAD(P)-linked oxidoreductase superfamily protein	missense_variant	302,Q,E	-1.271
Zm00001d005451	3	2	Cellulose synthase A catalytic subunit 5 [UDP-forming]	missense_variant	912,D,E	-0.153
Zm00001d005951	3	2	Homeobox-leucine zipper protein ATHB-6	missense_variant	13,A,T	0.28
Zm00001d006016	3	2	Multiple stress-responsive zinc-finger protein ISAP1	missense_variant	94,A,V	-0.603
Zm00001d035053	4	6	Putative MADS-box transcription factor family protein	missense_variant	60,S,T	-1.059
Zm00001d035053	4	6	Putative MADS-box transcription factor family protein	missense_variant	76,R,G	3.062
Zm00001d035028	4	6	Putative glycolipid transfer protein (GLTP) family protein	missense_variant	102,T,A	-0.789
Zm00001d035028	4	6	Putative glycolipid transfer protein (GLTP) family protein	missense_variant	26,G,S	0.931
Zm00001d009795	5	8	Cellulose synthase1	missense_variant	902,A,AQ	-2.342
Zm00001d008850	5	8	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	missense_variant	100,S,P	-2.379
Zm00001d009103	5	8	Putative AP2/EREBP transcription factor superfamily protein	missense_variant	31,E,D	0.621
Zm00001d009567	5	8	Fasciclin-like arabinogalactan protein 7	missense_variant	15,L,V	-1.13
Zm00001d009567	5	8	Fasciclin-like arabinogalactan protein 7	missense_variant	25,A,S	-0.554
Zm00001d009567	5	8	Fasciclin-like arabinogalactan protein 7	missense_variant	45,A,S	-0.674
Zm00001d009837	5	8	RING/U-box superfamily protein	missense_variant	447,T,A	-0.119
Zm00001d010728	5	8	PLASMODESMATA CALLOSE-BINDING PROTEIN 2	missense_variant	36,A,V	0.333
Zm00001d010751	5	8	Seed specific protein Bn15D17A	missense_variant	294,K,Q	-0.154
Zm00001d012086	6	8	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	missense_variant	570,G,D	-1.541
Zm00001d012092	6	8	Histone deacetylase HDT1	missense_variant	19,G,A	-2.167
Zm00001d012092	6	8	Histone deacetylase HDT1	missense_variant	41,A,V	2.378
Zm00001d012092	6	8	Histone deacetylase HDT1	missense_variant	171,V,L	-0.316
Zm00001d012092	6	8	Histone deacetylase HDT1	missense_variant	175,T,A	0.147
Zm00001d012092	6	8	Histone deacetylase HDT1	missense_variant	249,A,S	0.632

Zm00001d012254	6	8	Putative patellin family protein	missense_variant	233,N,S	-0.319
Zm00001d012254	6	8	Putative patellin family protein	missense_variant	83,E,K	-0.259
Zm00001d016225	14	5	U-box domain-containing protein 17	missense_variant	601,H,Y	-1.253
Zm00001d013830	14	5	cytochrome P450 family 76 subfamily C polypeptide 7	missense_variant	323,G,A	1.108
Zm00001d013934	14	5	soluble epoxide hydrolase	missense_variant	171,G,C	-0.579
Zm00001d013934	14	5	soluble epoxide hydrolase	missense_variant	136,M,I	-0.297
Zm00001d014134	14	5	Ent-cassadiene C2-hydroxylase	missense_variant	348,T,R	0.533
Zm00001d014134	14	5	Ent-cassadiene C2-hydroxylase	missense_variant	503,V,D	-1.438
Zm00001d014203	14	5	F-box/kelch-repeat protein SKIP11	missense_variant	36,D,G	-0.327
Zm00001d015361	14	5	Transducin/WD40 repeat-like superfamily protein	missense_variant	897,N,S	-1.296
Zm00001d015420	14	5	DUF581 domain containing protein expressed	missense_variant	166,E,D	-1.367
Zm00001d015971	14	5	Probable LRR receptor-like serine/threonine-protein kinase	missense_variant	69,S,I	-2.398
Zm00001d016225	14	5	U-box domain-containing protein 17	missense_variant	601,H,Y	-1.253
Zm00001d016299	14	5	mcf1;mitochondrial carrier family protein1	missense_variant	308,C,W	2.744
Zm00001d016483	14	5	MACPF domain-containing protein	missense_variant	348,R,K	-1.102
Zm00001d016582	14	5	SAUR-like auxin-responsive protein family	missense_variant	148,Q,H	-0.076
Zm00001d016736	14	5	2-Cys peroxiredoxin BAS1 chloroplastic	missense_variant	54,T,I	-0.208
Zm00001d016736	14	5	2-Cys peroxiredoxin BAS1 chloroplastic	missense_variant	24,V,A	0.436
Zm00001d016804	14	5	histone methyltransferases	missense_variant	1715,Y,H	1.057
Zm00001d016804	14	5	histone methyltransferases	missense_variant	1673,I,T	-0.085
Zm00001d016902	14	5	Bifunctional riboflavin biosynthesis protein RIBA 1 chloroplastic	missense_variant	558,L,Q	0.221
Zm00001d016902	14	5	Bifunctional riboflavin biosynthesis protein RIBA 1 chloroplastic	missense_variant	536,R,K	-1.852
Zm00001d016902	14	5	Bifunctional riboflavin biosynthesis protein RIBA 1 chloroplastic	missense_variant	123,T,S	0.654
Zm00001d017090	14	5	#N/D	missense_variant	75,L,V	1.933
Zm00001d017139	14	5	hds1;hydroxymethylbutenyl diphosphate synthase1:	missense_variant	554,G,V	0.689
Zm00001d017139	14	5	hds1;hydroxymethylbutenyl diphosphate synthase1:	missense_variant	16,H,R	0.254

Table 3 Neutral missense variation mapped in tolerance aluminum QTLs in Al-sensitive inbred line

GENE	QTL	CHR	FUNCTION	CONSEQUENCE	VARIANT	PROVEAN score
Zm00001d023941	2	10	Carbohydrate transporter/ sugar porter	missense_variant	143,A,T	-0.32
Zm00001d023941	2	10	Carbohydrate transporter/ sugar porter	missense_variant	160,G,V	0.789
Zm00001d024960	2	10	Bowman-Birk type trypsin inhibitor	missense_variant	41,R,K	0.094
Zm00001d024996	2	10	Bowman-Birk type trypsin inhibitor	missense_variant	64,V,I	-0.259
Zm00001d025166	2	10	Putative quinone-oxidoreductase homolog chloroplastic	missense_variant	368,G,A	-1.255
Zm00001d025166	2	10	Putative quinone-oxidoreductase homolog chloroplastic	missense_variant	251,T,S	0.834
Zm00001d025354	2	10	incw5;invertase cell wall5:	missense_variant	557,I,L	1.013
Zm00001d025354	2	10	incw5;invertase cell wall5:	missense_variant	34,T,A	0.836
Zm00001d005392	3	2	Lecithin-cholesterol acyltransferase-like 1	missense_variant	59,E,A	1.31
Zm00001d005392	3	2	Lecithin-cholesterol acyltransferase-like 1	missense_variant	88,R,H	1.422
Zm00001d005392	3	2	Lecithin-cholesterol acyltransferase-like 1	missense_variant	91,V,A	0.533
Zm00001d005392	3	2	Lecithin-cholesterol acyltransferase-like 1	missense_variant	362,E,D	1.004
Zm00001d008911	6	8	sodium/calcium exchanger family protein / calcium-binding EF hand family protein	missense_variant	50,P,A	0.437
Zm00001d008911	6	8	sodium/calcium exchanger family protein / calcium-binding EF hand family protein	missense_variant	77,A,T	-0.243
Zm00001d009140	6	8	Peroxidase 64	missense_variant	28,G,A	1.532
Zm00001d009702	6	8	P-loop containing nucleoside triphosphate hydrolases superfamily protein	missense_variant	279,R,K	-1.231
Zm00001d012091	7	8	#N/D	missense_variant	144,A,T	-1.5
Zm00001d013193	9	5	CCG-binding protein 1	missense_variant	67,I,V	-0.711
Zm00001d015569	14	5	Vacuolar H+-translocating inorganic pyrophosphatase	missense_variant	655,I,V	0.096
Zm00001d016724	14	5	#N/D	missense_variant	77,S,R	0

Table 4 DEGs shared in both lines mapped inside Al-tolerance QTLs regions

GENE	FOLDCHANGE 11-60	FOLDCHANGE 11-133	QTL	FUNCTION
Zm00001d036409	2.09963	-2.6963	1	OSJNBa0013K16.15 protein
Zm00001d024925	-0.904463	0.782892	2	#N/D
Zm00001d025541	-0.973001	0.962743	2	Eukaryotic aspartyl protease family protein
Zm00001d025873	-1.21212	1.03219	2	Low-molecular-weight cysteine-rich protein LCR70
Zm00001d025446	-1.98214	1.31399	2	Pentatricopeptide repeat-containing protein
Zm00001d025141	-5.4748	1.65582	2	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
Zm00001d023617	4.60072	-1.31596	2	Laccase-7
Zm00001d024210	4.42321	-3.20108	2	tps6;terpene synthase6:
Zm00001d024207	3.88066	-3.01802	2	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Zm00001d025916	1.74025	-2.69493	2	E3 ubiquitin-protein ligase RMA1
Zm00001d023859	1.64738	-2.66456	2	Respiratory burst oxidase homolog protein B
Zm00001d023769	1.17171	-1.20525	2	Delta(12)-fatty-acid desaturase
Zm00001d023774	1.16129	-1.70175	2	Erg28 like protein
Zm00001d023768	1.10341	-1.30818	2	Delta(12)-fatty-acid desaturase
Zm00001d025860	0.99344	-2.35184	2	#N/D
Zm00001d023941	0.918634	-0.935743	2	Carbohydrate transporter/ sugar porter
Zm00001d024960	0.886773	-1.73123	2	Bowman-Birk type trypsin inhibitor
Zm00001d023811	0.863863	-1.38069	2	Pathogenesis-related protein STH-21
Zm00001d005986	-1.09105	0.809041	3	Probable LRR receptor-like serine/threonine-protein kinase
Zm00001d006111	-1.58273	2.05336	3	Cytochrome b561 and DOMON domain-containing protein
Zm00001d005383	1.64992	-2.46263	3	Lecithin-cholesterol acyltransferase-like 1
Zm00001d005392	1.03075	-0.773041	3	Lecithin-cholesterol acyltransferase-like 1
Zm00001d036366	-1.23266	1.11585	4	Basic endochitinase B
Zm00001d035888	3.48583	-1.54718	4	extensin-like protein
Zm00001d035854	2.90108	-0.857225	4	Alpha-dioxygenase 1
Zm00001d036164	2.25561	-2.82145	4	Salicylate/benzoate carboxyl methyltransferase

Zm00001d036197	2.16101	-2.0121	4	HXXXD-type acyl-transferase family protein
Zm00001d038068	-1.28697	1.25025	5	ATPase%2C coupled to transmembrane movement of substance
Zm00001d038126	-1.83634	1.21167	5	#N/D
Zm00001d038061	2.69105	-2.3243	5	Probable calcium-binding protein CML25
Zm00001d009373	-0.802607	1.14798	6	Peroxidase 72
Zm00001d010585	-1.10568	1.41812	6	UTP--glucose-1-phosphate uridylyltransferase 3 chloroplastic
Zm00001d010038	-1.12898	2.18267	6	CASP-like protein 5
Zm00001d009765	-1.47902	0.857895	6	#N/D
Zm00001d010434	-1.55358	1.17172	6	Arabinogalactan peptide 20
Zm00001d009726	3.27219	-2.27691	6	alpha/beta-Hydrolases superfamily protein
Zm00001d009619	2.08409	-1.05405	6	Putative WRKY DNA-binding domain superfamily protein
Zm00001d010588	1.97414	-3.85025	6	pdc1;pyruvate decarboxylase1
Zm00001d010375	1.73889	-2.61153	6	ATP-dependent 6-phosphofructokinase 4 chloroplastic
Zm00001d009118	1.34864	-3.18487	6	Adenine nucleotide alpha hydrolases-like superfamily protein
Zm00001d009296	1.34351	-1.05309	6	Pathogenesis-related protein PRMS
Zm00001d009022	0.91816	-0.706664	6	NmrA-like negative transcriptional regulator family protein
Zm00001d012274	-1.72713	1.5745	7	hct10;hydroxycinnamoyltransferase10:
Zm00001d012394	-2.28134	1.5653	7	psy2;phytoene synthase2
Zm00001d049129	-1.55688	1.21869	8	late embryogenesis abundant protein-related / LEA protein-related
Zm00001d049113	2.53008	-1.42202	8	Senescence-specific cysteine protease SAG12
Zm00001d013193	1.07525	-2.60953	9	CCG-binding protein 1
Zm00001d014758	-0.949654	1.47075	14	plt6;phospholipid transfer protein homolog6:
Zm00001d015921	-1.53774	2.00807	14	Plasma membrane-associated cation-binding protein 1
Zm00001d013979	-1.68058	1.87403	14	Cytochrome P450 86B1
Zm00001d014101	-1.90834	1.30566	14	#N/D
Zm00001d014971	-2.35508	1.00819	14	Leucine-rich repeat receptor-like protein kinase PXL1
Zm00001d014121	3.75337	-1.88102	14	Ent-cassadiene C2-hydroxylase
Zm00001d014945	2.44687	-2.74164	14	Lactate/malate dehydrogenase family protein
Zm00001d016691	2.04952	-1.22594	14	Copper transport protein CCH
Zm00001d016471	1.72178	-4.19007	14	Trans-cinnamate 4-monooxygenase

Zm00001d014613	1.07418	-0.80763	14	Xyloglucan endotransglucosylase/hydrolase protein 23
Zm00001d014134	0.996542	-1.27344	14	Ent-cassadiene C2-hydroxylase
Zm00001d013979	-1.68058	1.87403	17	Cytochrome P450 86B1
Zm00001d014101	-1.90834	1.30566	17	#N/D
Zm00001d014121	3.75337	-1.88102	17	Ent-cassadiene C2-hydroxylase
Zm00001d014134	0.996542	-1.27344	17	Ent-cassadiene C2-hydroxylase

Table 5 Predicted deleterious variations in differentially expressed genes in Al-resistant line

CHR	GENE	FUNCTION	CONSEQUENCE	VARIANT	PROVEAN score
1	Zm00001d030888	Probable disease resistance protein	missense_variant	225,G,C	-2.706
1	Zm00001d030915	#N/D	missense_variant	66,N,T	-3.111
1	Zm00001d032060	Probable GTP diphosphokinase RSH2 chloroplastic	missense_variant	479,I,T	-2.798
1	Zm00001d032292	#N/D	missense_variant	13,T,S	-4
1	Zm00001d032405	Peroxidase 1	missense_variant	243,D,N	-2.916
1	Zm00001d032857	Protein kinase superfamily protein	missense_variant	332,D,N	-2.524
1	Zm00001d033286	Phenylalanine ammonia-lyase 1	missense_variant	381,D,N	-4.537
1	Zm00001d034032	Protein STRICTOSIDINE SYNTHASE-LIKE 3	missense_variant	162,G,S	-5.333
1	Zm00001d034077	#N/D	missense_variant	125,R,C	-8
1	Zm00001d034440	60S ribosomal protein L4-1	missense_variant	201,N,S	-4.73
2	Zm00001d002865	Transducin/WD40 repeat-like superfamily protein	missense_variant	655,H,N	-3.311
3	Zm00001d039658	Transcription factor HY5-like	start lost	1,M,I	-
3	Zm00001d039697	Cytochrome P450 monooxygenase	missense_variant	517,H,Y	-4.02
3	Zm00001d042104	Glutathione transferase 7	missense_variant	24,R,Q	-2.559
3	Zm00001d043291	Purple acid phosphatase 10	missense_variant	163,G,S	-4.339
4	Zm00001d051659	#N/D	missense_variant	65,L,V	-3
4	Zm00001d051659	#N/D	missense_variant	45,G,C	-9
4	Zm00001d053212	E3 ubiquitin ligase BIG BROTHER-related	missense_variant	222,V,A	-3.209
7	Zm00001d020783	Coatomer subunit zeta-2	stop_gained	4,W,*	-
7	Zm00001d020623	Copper transporter 5	missense_variant	103,V,F	-3.233
7	Zm00001d020783	Coatomer subunit zeta-2	missense_variant	56,P,L	-6.333
7	Zm00001d020783	Coatomer subunit zeta-2	missense_variant	40,H,R	-8
7	Zm00001d021698	Receptor-like protein kinase RK20-1	missense_variant	359,E,K	-2.829
7	Zm00001d021698	Receptor-like protein kinase RK20-1	missense_variant	56,A,P	-3.268
7	Zm00001d021929	Geranylgeranyl pyrophosphate synthase 3	missense_variant	354,L,F	-3.194
8	Zm00001d012092	Histone deacetylase HDT1	missense_variant	254,P,T	-3.577

8	Zm00001d012221	Stearoyl-acyl-carrier-protein desaturase 9	missense_variant	213,T,M	-5.867
9	Zm00001d048362	Mitochondrial outer membrane protein porin 4	start lost	1,M,T	-
9	Zm00001d044754	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit beta 2	missense_variant	562,G,D	-6.361
9	Zm00001d044754	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit beta 2	missense_variant	370,E,V	-3.174
9	Zm00001d046472	Hemoglobin-like protein HbO	missense_variant	130,D,G	-4.239
9	Zm00001d046952	Allantoate deiminase	missense_variant	225,A,G	-3.268
10	Zm00001d026287	UDP-N-acetylglucosamine diphosphorylase 2	missense_variant	261,G,E	-5.537

Table 6 Deleterious variation mapped in Al-tolerance QTLs in Al-resistant inbred line

GENE	QTL	LOG2 FOLDCHANGE	FUNCTION	CONSEQUENCE	VARIANT	PROVEAN score
Zm00001d012092	7	1.00287	Histone deacetylase HDT1	missense_variant	254,P,T	-3.577
Zm00001d012221	7	-2.26334	Stearoyl-acyl-carrier-protein desaturase9	missense_variant	213,T,M	-5.867

Table 7 Deleterious variation mapped in Al-tolerance QTLs in Al-sensitive line

GENE	QTL	LOG2 FOLDCHANGE	FUNCTION	CONSEQUENCE	VARIANT	PROVEAN score
Zm00001d010662	6	-1.13469	SNF1-related protein kinase regulatory subunit beta-1	missense_variant	149,I,F	-2.902
Zm00001d012091	7	-1.97108	#N/D	missense_variant	112,G,A	-2.6
Zm00001d012091	7	-1.97108	#N/D	missense_variant	151,G,A	-3.6
Zm00001d024925	2	-0.904463	#N/D	missense_variant	185,F,L	-4.063
Zm00001d016705	14	-1.53341	ATPase inhibitor	missense_variant	36,R,W	-8
Zm00001d016724	14	1.24504	#N/D	missense_variant	26,A,S	-3

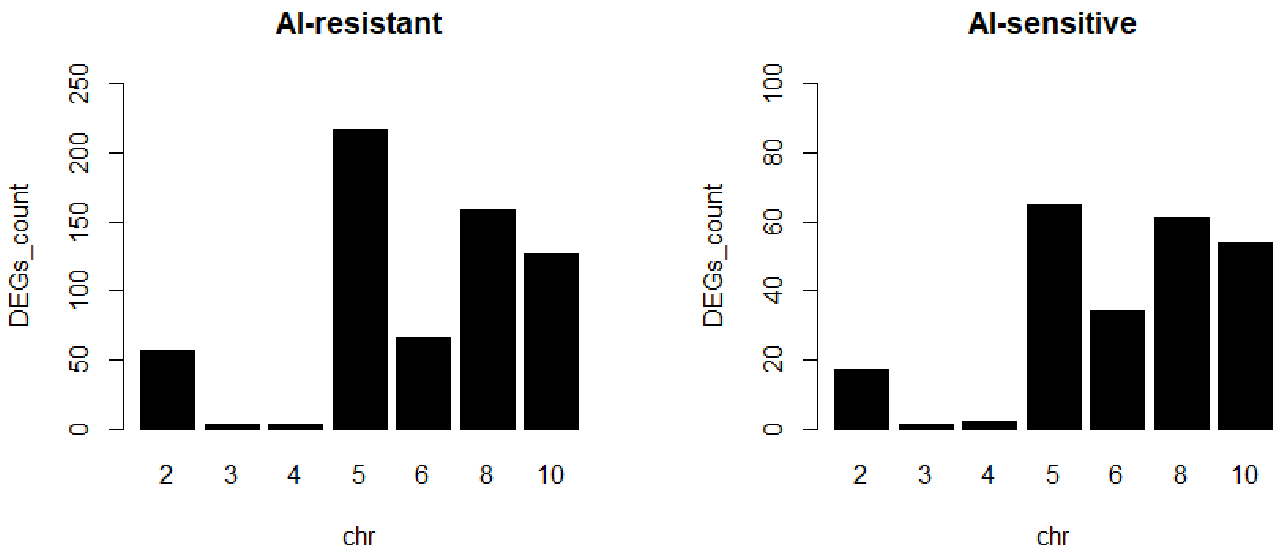

Figure 1 Count of differentially expressed genes mapped inside previous Al-tolerance QTLs in Al-resistant and –sensitive inbred lines.

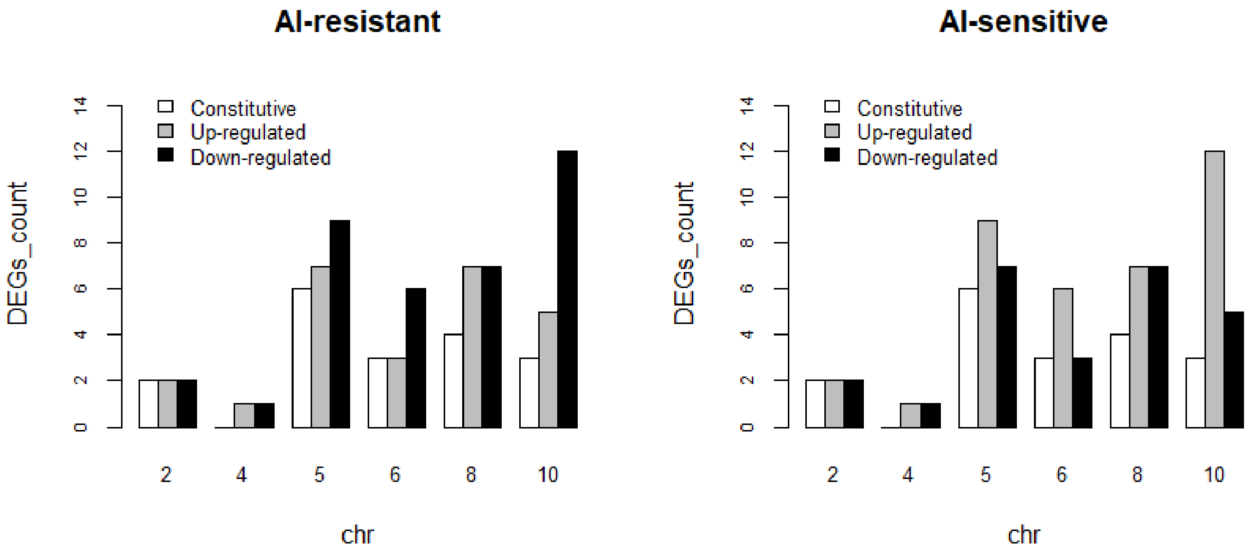

Figure 2 Differentially expressed genes shared in both lines mapped inside previous Al-tolerance

Supplementary Table 1 Differentially expressed genes mapped in aluminum tolerance QTLs for Al-resistant inbred line

GENE	FOLDCHANGE	QTL	CHR	FUNCTION
Zm00001d036409	-2.6963	1	6	OSJNBa0013K16.15 protein; protein
Zm00001d036416	-0.701883	1	6	cdpk13;calcium dependent protein kinase13
Zm00001d024664	-4.26164	2	10	Tyrosine decarboxylase 1
Zm00001d024210	-3.20108	2	10	tps6;terpene synthase6:
Zm00001d025872	-3.11662	2	10	senescence regulator
Zm00001d024665	-3.05585	2	10	Tyrosine decarboxylase 1
Zm00001d024725	-3.04336	2	10	Putative MYB DNA-binding domain superfamily protein
Zm00001d024207	-3.01802	2	10	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Zm00001d024208	-2.95183	2	10	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Zm00001d024726	-2.69791	2	10	myb domain protein 112
Zm00001d025916	-2.69493	2	10	E3 ubiquitin-protein ligase RMA1
Zm00001d023859	-2.66456	2	10	Respiratory burst oxidase homolog protein B
Zm00001d025081	-2.58959	2	10	Rhodanese-like domain-containing protein 19 mitochondrial
Zm00001d025860	-2.35184	2	10	#N/D
Zm00001d024969	-2.35152	2	10	Dirigent protein 11
Zm00001d024852	-2.2659	2	10	acco3;1-aminocyclopropane-1-carboxylate oxidase3:
Zm00001d024591	-2.23023	2	10	SnRNP core Sm protein Sm-X5-like protein
Zm00001d023588	-1.94277	2	10	Protein kinase protein with adenine nucleotide alpha hydrolases-like domain
Zm00001d024598	-1.75459	2	10	Factor of DNA methylation 1
Zm00001d024843	-1.73884	2	10	acco31;1-aminocyclopropane-1-carboxylate oxidase15:
Zm00001d024960	-1.73123	2	10	Bowman-Birk type trypsin inhibitor
Zm00001d023774	-1.70175	2	10	Erg28 like protein
Zm00001d025490	-1.65263	2	10	zar1;Zea mays ARGOS1: affects yield, and response to drought
Zm00001d025360	-1.62655	2	10	Probable F-box protein At1g60180

Zm00001d023779	-1.60293	2	10	3-hydroxyisobutyryl-CoA hydrolase 1
Zm00001d024853	-1.58889	2	10	acco15;1-aminocyclopropane-1-carboxylate oxidase15:
Zm00001d024545	-1.53853	2	10	B3 domain-containing transcription factor NGA2
Zm00001d024593	-1.53593	2	10	Sm-like protein LSM2
Zm00001d024753	-1.53233	2	10	peroxidase 35
Zm00001d025799	-1.4879	2	10	Calmodulin-binding receptor-like cytoplasmic kinase 3
Zm00001d024458	-1.46139	2	10	#N/D
Zm00001d024752	-1.41888	2	10	Peroxidase 64
Zm00001d025325	-1.41858	2	10	#N/D
Zm00001d024352	-1.39073	2	10	OSJNBb0022F16.11 protein; protein
Zm00001d023811	-1.38069	2	10	Pathogenesis-related protein STH-21
Zm00001d024412	-1.32619	2	10	NADPH--cytochrome P450 reductase
Zm00001d025474	-1.32319	2	10	Chloride channel protein CLC-c
Zm00001d023617	-1.31596	2	10	Laccase-7
Zm00001d023768	-1.30818	2	10	Delta(12)-fatty-acid desaturase
Zm00001d024702	-1.29154	2	10	Tryptophan synthase beta chain 2%2C chloroplastic
Zm00001d025581	-1.22848	2	10	loricrin-related
Zm00001d023995	-1.21899	2	10	Sec14p-like phosphatidylinositol transfer family protein
Zm00001d023769	-1.20525	2	10	Delta(12)-fatty-acid desaturase
Zm00001d024324	-1.19426	2	10	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d025050	-1.18829	2	10	etr2;ethylene receptor homolog2:
Zm00001d024939	-1.18089	2	10	Phosphoglycerate mutase-like protein
Zm00001d025200	-1.11094	2	10	Putative cytochrome P450 superfamily protein
Zm00001d025727	-1.06175	2	10	U-box domain-containing protein 7
Zm00001d024723	-1.02343	2	10	Long chain acyl-CoA synthetase 1
Zm00001d024621	-1.01508	2	10	Phloem-specific lectin
Zm00001d025015	-1.00806	2	10	Glucose-6-phosphate 1-dehydrogenase

Zm00001d023815	-0.943555	2	10	Putative lipase class 3 family protein
Zm00001d023941	-0.935743	2	10	Carbohydrate transporter/ sugar porter
Zm00001d023694	-0.882445	2	10	2-oxoisovalerate dehydrogenase subunit alpha 2 mitochondrial
Zm00001d024762	-0.83697	2	10	Putative CBL-interacting protein kinase family protein
Zm00001d023843	-0.830127	2	10	Calmodulin-binding protein 60 G
Zm00001d025235	-0.820141	2	10	Calmodulin-binding transcription activator 4
Zm00001d024275	-0.814681	2	10	Zinc finger BED domain-containing protein DAYSLEEPER
Zm00001d024821	-0.8089	2	10	Sucrose-phosphatase 2
Zm00001d023868	-0.808381	2	10	Galactose oxidase/kelch repeat superfamily protein
Zm00001d025654	-0.794661	2	10	#N/D
Zm00001d025012	-0.778563	2	10	ABC transporter G family member 34
Zm00001d025551	-0.775133	2	10	Polyadenylate-binding protein RBP47B
Zm00001d025526	-0.720234	2	10	serine carboxypeptidase 1
Zm00001d023590	-0.713691	2	10	Putative zinc transporter
Zm00001d024940	-0.713366	2	10	Zinc finger (C3HC4-type RING finger) family protein
Zm00001d025431	-0.70987	2	10	OSJNBa0088K19.7 protein
Zm00001d024755	-0.697285	2	10	PTI1-like tyrosine-protein kinase 3
Zm00001d023680	-0.69059	2	10	Pollen Ole e 1 allergen and extensin family protein
Zm00001d024715	-0.635461	2	10	EYES ABSENT homolog
Zm00001d025798	-0.631514	2	10	calcium-binding EF hand family protein
Zm00001d024943	-0.625681	2	10	Putative cytochrome P450 superfamily protein
Zm00001d025059	0.847503	2	10	Germin-like protein subfamily 1 member 8
Zm00001d025091	1.84809	2	10	#N/D
Zm00001d025103	1.1415	2	10	amo1;amine oxidase1: single copy rice cDNA rz69
Zm00001d025136	1.29052	2	10	Protein SMAX1-LIKE 4
Zm00001d025141	1.65582	2	10	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
Zm00001d025149	0.724409	2	10	Kinesin-like protein KIN-12A

Zm00001d025208	0.951196	2	10	myosin heavy chain-related
Zm00001d025247	1.78823	2	10	Beta-13-N-Acetylglucosaminyltransferase family protein
Zm00001d023592	1.59194	2	10	Amino acid permease 2
Zm00001d025338	1.30161	2	10	#N/D
Zm00001d025369	1.1943	2	10	Protein SMAX1-LIKE 3
Zm00001d025381	0.749585	2	10	Histone H3.2
Zm00001d023606	1.05478	2	10	Dihydrolipoyllysine-residue acetyltransferase
Zm00001d025446	1.31399	2	10	Pentatricopeptide repeat-containing protein
Zm00001d025475	1.79276	2	10	Endoglucanase 9
Zm00001d025528	0.779497	2	10	NAD(P)-linked oxidoreductase superfamily protein
Zm00001d025533	0.736491	2	10	NAD(P)-linked oxidoreductase superfamily protein
Zm00001d025541	0.962743	2	10	Eukaryotic aspartyl protease family protein
Zm00001d025547	1.75506	2	10	Fucosyltransferase 7
Zm00001d025670	1.78882	2	10	Glycerophosphodiester phosphodiesterase GDPDL3
Zm00001d025721	1.25623	2	10	Mitotic spindle checkpoint protein MAD2
Zm00001d025724	2.10031	2	10	Acid phosphatase/vanadium-dependent haloperoxidase protein
Zm00001d023630	1.60814	2	10	TPX2 (targeting protein for Xklp2) protein family
Zm00001d025754	4.09853	2	10	#N/D
Zm00001d025847	2.64446	2	10	Beta-glucosidase 47
Zm00001d025869	1.59251	2	10	Nucleotide/sugar transporter family protein
Zm00001d025873	1.03219	2	10	Low-molecular-weight cysteine-rich protein LCR70
Zm00001d023655	0.682811	2	10	pale yellow 9
Zm00001d023673	2.71345	2	10	sweet13b;sugars will eventually be exported transporter13b:
Zm00001d023677	0.768726	2	10	sweet13a;sugars will eventually be exported transporter13a:
Zm00001d023692	0.967853	2	10	selenium binding
Zm00001d023810	0.929974	2	10	Putative cellulose synthase-like family protein
Zm00001d023895	1.15909	2	10	Mitochondrial substrate carrier family protein

Zm00001d023899	1.48965	2	10	Peroxidase 24
Zm00001d023901	0.735531	2	10	Histone H2A
Zm00001d023950	1.25978	2	10	Glucuronoxylan 4-O-methyltransferase 2
Zm00001d024004	0.971638	2	10	#N/D
Zm00001d024047	0.822489	2	10	Leucine-rich repeat protein kinase family protein
Zm00001d024170	1.27297	2	10	Micronuclear linker histone polyprotein-like protein
Zm00001d024178	1.1614	2	10	Xyloglucan glycosyltransferase 4
Zm00001d024281	1.35928	2	10	pao1;polyamine oxidase1: cDNA and genomic clones, low copy number
Zm00001d024364	0.649446	2	10	Cysteine-type endopeptidase/ ubiquitin thiolesterase
Zm00001d024382	1.16972	2	10	Xyloglucan endotransglucosylase/hydrolase protein 2
Zm00001d024386	1.15056	2	10	xth1;xyloglucan endo-transglycosylase/hydrolase1:
Zm00001d024497	0.708193	2	10	ABC transporter G family member 14
Zm00001d024600	2.56389	2	10	ABC transporter B family member 19
Zm00001d024676	2.12648	2	10	#N/D
Zm00001d024717	1.56532	2	10	#N/D
Zm00001d024925	0.782892	2	10	#N/D
Zm00001d024927	0.906037	2	10	RING/U-box superfamily protein
Zm00001d024963	0.798737	2	10	gst22;glutathione transferase22:
Zm00001d024982	1.73149	2	10	Stress responsive protein
Zm00001d024995	0.650411	2	10	Expressed protein; protein
Zm00001d025002	0.928577	2	10	Putative leucine-rich repeat receptor protein kinase family protein
Zm00001d024850	#NOME?	2	10	1-aminocyclopropane-1-carboxylate oxidase 5
Zm00001d005383	-2.46263	3	2	Lecithin-cholesterol acyltransferase-like 1
Zm00001d005748	-2.36233	3	2	CIPK-like protein 1
Zm00001d006078	-1.71365	3	2	RING/U-box superfamily protein
Zm00001d005823	-1.3582	3	2	Flavonoid 3-monooxygenase
Zm00001d005798	-1.26504	3	2	Ethylene-responsive transcription factor RAP2-2

Zm00001d005752	-1.25175	3	2	DUF868 family protein
Zm00001d005679	-1.24706	3	2	TIM-barrel signal transduction protein isoform 2
Zm00001d006016	-1.13206	3	2	Multiple stress-responsive zinc-finger protein ISAP1
Zm00001d006115	-1.1013	3	2	Anthocyanin-related membrane protein 1 (Anm1)-like
Zm00001d005962	-0.96102	3	2	basic leucine-zipper 58
Zm00001d005783	-0.891742	3	2	Glutamate receptor 2.7
Zm00001d005966	-0.819065	3	2	Phosphatidylinositol 4-kinase gamma 7
Zm00001d006022	-0.80936	3	2	Transcription factor bHLH130
Zm00001d005392	-0.773041	3	2	Lecithin-cholesterol acyltransferase-like 1
Zm00001d005501	-0.692448	3	2	Serine/threonine protein phosphatase 2A 59 kDa regulatory subunit B' eta isoform
Zm00001d005951	-0.687804	3	2	Homeobox-leucine zipper protein ATHB-6
Zm00001d005504	-0.667836	3	2	Ras-related protein RABA4c
Zm00001d005764	-0.664617	3	2	#N/D
Zm00001d005394	1.16803	3	2	Fiber protein Fb34
Zm00001d005410	1.54319	3	2	Aquaporin PIP2-2
Zm00001d005451	0.868267	3	2	Cellulose synthase A catalytic subunit 5 [UDP-forming]
Zm00001d005456	1.36583	3	2	UDP-glycosyltransferase 76C1
Zm00001d005570	1.80731	3	2	TPX2 (targeting protein for Xklp2) protein family
Zm00001d005624	0.943053	3	2	Delta(8)-fatty-acid desaturase 2
Zm00001d005696	0.775519	3	2	NPK1-related protein kinase 2
Zm00001d005715	3.87815	3	2	Vacuolar iron transporter 1
Zm00001d005740	1.28741	3	2	Transcription factor AIG1
Zm00001d005775	2.11691	3	2	Cellulose synthase A catalytic subunit 7 [UDP-forming]
Zm00001d005789	0.927933	3	2	his2b3;histone 2B3: genomic sequence selected with his2B cDNA probe
Zm00001d005822	1.33883	3	2	cyp12;cytochrome P450 12:
Zm00001d005860	0.815007	3	2	Eukaryotic aspartyl protease family protein
Zm00001d005889	1.4087	3	2	abh5;abscisic acid 8'-hydroxylase5:

Zm00001d005905	2.26055	3	2	Probable carboxylesterase 2
Zm00001d005945	0.802484	3	2	#N/D
Zm00001d005986	0.809041	3	2	Probable LRR receptor-like serine/threonine-protein kinase
Zm00001d006009	1.29535	3	2	Fasciclin-like arabinogalactan protein 7
Zm00001d006010	0.830146	3	2	#N/D
Zm00001d006064	0.747288	3	2	60S ribosomal protein L32-1
Zm00001d006100	0.695582	3	2	60S ribosomal protein L7a-1
Zm00001d006111	2.05336	3	2	Cytochrome b561 and DOMON domain-containing protein
Zm00001d036164	-2.82145	4	6	Salicylate/benzoate carboxyl methyltransferase
Zm00001d036245	-2.03987	4	6	Protein-tyrosine sulfotransferase
Zm00001d036197	-2.0121	4	6	HXXXD-type acyl-transferase family protein
Zm00001d035133	-1.91034	4	6	LRR receptor-like serine/threonine-protein kinase GSO2
Zm00001d036159	-1.81343	4	6	Phenolic glucoside malonyltransferase 1
Zm00001d036357	-1.74125	4	6	Glycosyltransferase family 92 protein
Zm00001d035561	-1.59718	4	6	Expressed protein; Mannose-specific jacalin-related lectin; protein
Zm00001d035888	-1.54718	4	6	extensin-like protein
Zm00001d035079	-1.46162	4	6	zar5;Zea mays ARGOS5:
Zm00001d035080	-1.44696	4	6	#N/D
Zm00001d036256	-1.39968	4	6	Phospholipase A1-Igamma1 chloroplastic
Zm00001d036216	-1.39712	4	6	F-box protein MAX2
Zm00001d035236	-1.3881	4	6	Plant calmodulin-binding protein-related
Zm00001d035200	-1.27933	4	6	Putative AMP-dependent synthetase and ligase superfamily protein
Zm00001d035367	-1.18041	4	6	#N/D
Zm00001d035263	-1.1181	4	6	Probable protein phosphatase 2C 30
Zm00001d036392	-1.10772	4	6	BZIP transcription factor TRAB1
Zm00001d035297	-1.09829	4	6	Protein CLT2 chloroplastic
Zm00001d035652	-1.0687	4	6	Putative clathrin assembly protein

Zm00001d035542	-1.01918	4	6	GPRP
Zm00001d035246	-0.993548	4	6	Taxadien-5-alpha-ol O-acetyltransferase
Zm00001d036072	-0.938498	4	6	Zinc finger (C3HC4-type RING finger) family protein
Zm00001d035753	-0.926245	4	6	Polygalacturonase inhibitor
Zm00001d035710	-0.920625	4	6	Protein translation factor SUI1-like protein
Zm00001d035028	-0.909298	4	6	Putative glycolipid transfer protein (GLTP) family protein
Zm00001d035854	-0.857225	4	6	Alpha-dioxygenase 1
Zm00001d035162	-0.847033	4	6	LHT1
Zm00001d036298	-0.791946	4	6	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d035199	-0.787827	4	6	WPP domain-associated protein
Zm00001d035029	-0.786148	4	6	Protein transporter%3B Putative VHS/GAT domain
Zm00001d034986	-0.784846	4	6	Cytochrome P450 71A26
Zm00001d035214	-0.733178	4	6	Ureide permease 5
Zm00001d035775	-0.677342	4	6	Putative phospholipid-transporting ATPase 9
Zm00001d036296	-0.62696	4	6	Histone-lysine N-methyltransferase EZ1
Zm00001d035304	0.772177	4	6	LIM domain-containing protein WLIM1
Zm00001d035312	0.72864	4	6	40S ribosomal protein S29
Zm00001d035337	0.698574	4	6	GDP-L-galactose phosphorylase 1
Zm00001d035053	0.870099	4	6	Putative MADS-box transcription factor family protein
Zm00001d035067	1.31247	4	6	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Zm00001d035631	1.31303	4	6	Cation/H(+) antiporter 20
Zm00001d035682	2.40287	4	6	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Zm00001d035094	0.68868	4	6	40S ribosomal protein S13-1
Zm00001d035835	1.13951	4	6	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d035963	1.22808	4	6	#N/D
Zm00001d034977	1.08083	4	6	VAMP protein SEC22
Zm00001d036050	1.39105	4	6	Putative NAC domain transcription factor superfamily protein

Zm00001d036177	0.940007	4	6	Nucleosome assembly protein 1;2
Zm00001d036250	0.798004	4	6	Histone H3-like 5
Zm00001d036283	0.883588	4	6	Late embryogenesis abundant protein group 2
Zm00001d036322	0.782283	4	6	Ribonucleoside-diphosphate reductase
Zm00001d036360	0.991156	4	6	Cyclin-B2-4
Zm00001d036366	1.11585	4	6	Basic endochitinase B
Zm00001d038061	-2.3243	5	6	Probable calcium-binding protein CML25
Zm00001d038003	-1.89583	5	6	#N/D
Zm00001d038070	-1.85475	5	6	#N/D
Zm00001d037973	-1.27617	5	6	Serine/threonine-protein kinase
Zm00001d038023	-0.737662	5	6	Putative WRKY DNA-binding domain superfamily protein
Zm00001d038005	1.20509	5	6	Putative patellin family protein
Zm00001d038021	0.701341	5	6	ARM repeat superfamily protein
Zm00001d038029	1.73473	5	6	senescence associated gene 20
Zm00001d038068	1.25025	5	6	ATPase%2C coupled to transmembrane movement of substance
Zm00001d038101	0.61837	5	6	#N/D
Zm00001d038126	1.21167	5	6	#N/D
Zm00001d038153	2.15477	5	6	Leucine-rich repeat (LRR) family protein
Zm00001d008960	-4.8401	6	8	Cupredoxin superfamily protein
Zm00001d010588	-3.85025	6	8	pdc1;pyruvate decarboxylase1: cDNA and genomic clone, SSR UMC1163
Zm00001d009118	-3.18487	6	8	Adenine nucleotide alpha hydrolases-like superfamily protein
Zm00001d010800	-3.17632	6	8	Zinc finger CCCH domain-containing protein 54
Zm00001d010885	-3.07954	6	8	#N/D
Zm00001d009494	-2.96236	6	8	Putative MATE efflux family protein
Zm00001d009160	-2.77477	6	8	sequence-specific DNA binding transcription factors
Zm00001d009666	-2.67458	6	8	NAD(P)-binding Rossmann-fold superfamily protein
Zm00001d010375	-2.61153	6	8	ATP-dependent 6-phosphofructokinase 4 chloroplastic

Zm00001d009103	-2.58549	6	8	Putative AP2/EREBP transcription factor superfamily protein
Zm00001d009659	-2.31087	6	8	Cwf21
Zm00001d009726	-2.27691	6	8	alpha/beta-Hydrolases superfamily protein
Zm00001d010218	-2.23025	6	8	Peroxidase 53
Zm00001d008853	-1.86542	6	8	Putative HLH DNA-binding domain superfamily protein
Zm00001d010232	-1.86042	6	8	zmm29;Zea mays MADS29
Zm00001d009755	-1.84106	6	8	Putative calmodulin-binding family protein
Zm00001d008843	-1.72833	6	8	Grx_A2-glutaredoxin subgroup III
Zm00001d008974	-1.71565	6	8	Mitochondrial arginine transporter BAC2
Zm00001d010385	-1.69939	6	8	Protein UPSTREAM OF FLC
Zm00001d010520	-1.51773	6	8	Glycerol-3-phosphate acyltransferase 5
Zm00001d009705	-1.5119	6	8	S-type anion channel SLAH3
Zm00001d008812	-1.50898	6	8	C3H47 C3H type transcription factor
Zm00001d009112	-1.4995	6	8	cAMP-regulated phosphoprotein 19-related protein
Zm00001d009328	-1.43992	6	8	Glycosyltransferase
Zm00001d010751	-1.41935	6	8	Seed specific protein Bn15D17A
Zm00001d008923	-1.39029	6	8	#N/D
Zm00001d010175	-1.28614	6	8	Ethylene-responsive transcription factor ABR1
Zm00001d009660	-1.2337	6	8	Protein kinase superfamily protein
Zm00001d010676	-1.21522	6	8	Ethylene-responsive transcription factor 12
Zm00001d010201	-1.19256	6	8	Transcription repressor MYB6
Zm00001d010399	-1.1514	6	8	Putative WRKY DNA-binding domain superfamily protein isoform 1
Zm00001d010445	-1.15109	6	8	Abscisic acid receptor PYL9
Zm00001d009837	-1.11145	6	8	RING/U-box superfamily protein
Zm00001d009619	-1.05405	6	8	Putative WRKY DNA-binding domain superfamily protein
Zm00001d009296	-1.05309	6	8	Pathogenesis-related protein PRMS
Zm00001d010284	-1.04256	6	8	Probable carboxylesterase 17

Zm00001d010243	-0.96545	6	8	Protein root UVB sensitive 6
Zm00001d010671	-0.936139	6	8	Probable glucan endo-13-beta-glucosidase BG1
Zm00001d010662	-0.896902	6	8	SNF1-related protein kinase regulatory subunit beta-1
Zm00001d010572	-0.888659	6	8	E3 ubiquitin-protein ligase UPL4
Zm00001d010200	-0.87665	6	8	ATP binding protein
Zm00001d009108	-0.7659	6	8	RING/U-box superfamily protein
Zm00001d010172	-0.763132	6	8	Fructose-16-bisphosphatase cytosolic
Zm00001d008816	-0.755732	6	8	ATP-dependent 6-phosphofructokinase 4 chloroplastic
Zm00001d010066	-0.745532	6	8	#N/D
Zm00001d009022	-0.706664	6	8	NmrA-like negative transcriptional regulator family protein
Zm00001d008794	-0.679467	6	8	Putative WRKY DNA-binding domain superfamily protein
Zm00001d009077	-0.670671	6	8	Mono-/di-acylglycerol lipase N-terminal;Lipase class 3
Zm00001d010269	-0.664791	6	8	Putative disease resistance RPP13-like protein 1
Zm00001d010478	-0.618732	6	8	Peroxisomal adenine nucleotide carrier 1
Zm00001d010180	1.07811	6	8	Thioesterase superfamily protein
Zm00001d010231	1.19857	6	8	Amino acid carrier
Zm00001d010255	0.613004	6	8	Guanosine nucleotide diphosphate dissociation inhibitor 2
Zm00001d010287	2.76865	6	8	WAT1-related protein
Zm00001d010406	0.975598	6	8	mcm6;minichromosome maintenance6
Zm00001d010431	0.730272	6	8	Elongation factor 1-beta
Zm00001d010434	1.17172	6	8	Arabinogalactan peptide 20
Zm00001d010440	1.05927	6	8	sweet3a;sugars will eventually be exported transporter3a:
Zm00001d010442	1.41275	6	8	Putative wall-associated receptor protein kinase family protein
Zm00001d010446	1.32055	6	8	Endoglucanase 6
Zm00001d010499	1.56639	6	8	Sec14p-like phosphatidylinositol transfer family protein
Zm00001d010512	0.700291	6	8	ARM repeat superfamily protein
Zm00001d010521	0.973091	6	8	Flavonoid 3'-monooxygenase

Zm00001d010529	0.719235	6	8	Probable mediator of RNA polymerase II transcription subunit 37c
Zm00001d010545	1.59027	6	8	Lung seven transmembrane receptor family protein
Zm00001d010579	0.739642	6	8	Putative calcium-dependent protein kinase family protein
Zm00001d010585	1.41812	6	8	UTP--glucose-1-phosphate uridylyltransferase 3 chloroplastic
Zm00001d010625	0.679665	6	8	Transducin/WD40 repeat-like superfamily protein
Zm00001d010656	0.989756	6	8	Cyclin1
Zm00001d010689	0.940539	6	8	Cytokinin-O-glucosyltransferase 1
Zm00001d010703	0.807772	6	8	Secretory carrier-associated membrane protein 3
Zm00001d010728	0.886417	6	8	PLASMODESMATA CALLOSE-BINDING PROTEIN 2
Zm00001d010731	0.813077	6	8	Mitochondrial import inner membrane translocase subunit TIM50
Zm00001d010736	0.854619	6	8	O-methyltransferase ZRP4
Zm00001d010832	3.9036	6	8	Fasciclin-like arabinogalactan protein 11
Zm00001d010840	0.749042	6	8	triacylglycerol lipase-like 1
Zm00001d008700	0.61217	6	8	vt2;vanishing tassel2: inflorescence development; TRP-depenedent auxin synthesis
Zm00001d008715	0.94769	6	8	Putative leucine-rich repeat receptor protein kinase family protein
Zm00001d008730	1.23442	6	8	seed gene 3
Zm00001d008850	1.30362	6	8	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
Zm00001d008982	0.98533	6	8	Reticulon-like protein B4
Zm00001d008996	0.834376	6	8	RNA polymerase I-associated factor PAF67
Zm00001d009030	1.28619	6	8	DNA binding protein%2C mRNA
Zm00001d009032	1.24113	6	8	Stigma-specific STIG1-like protein 4
Zm00001d009071	0.729939	6	8	sweet17b;sugars will eventually be exported transporter17b:
Zm00001d009084	0.698108	6	8	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Zm00001d009365	0.778276	6	8	sweet2;sugars will eventually be exported transporter2:
Zm00001d009373	1.14798	6	8	Peroxidase 72
Zm00001d009374	0.876884	6	8	DNA replication licensing factor MCM4
Zm00001d009399	2.82384	6	8	Protein NRT1/ PTR FAMILY 6.2

Zm00001d009417	0.703498	6	8	60S ribosomal protein L28-1
Zm00001d009506	1.7961	6	8	Cytochrome b561 and DOMON domain-containing protein
Zm00001d009509	0.775831	6	8	60S ribosomal protein L37a
Zm00001d009567	0.920066	6	8	Fasciclin-like arabinogalactan protein 7
Zm00001d009652	0.957927	6	8	arpp2a-2;acidic ribosomal protein P2a-2:
Zm00001d009653	0.676602	6	8	60S acidic ribosomal protein P2A
Zm00001d009693	0.816507	6	8	Protein REDUCED WALL ACETYLATION 3
Zm00001d009765	0.857895	6	8	#N/D
Zm00001d009795	0.800881	6	8	Cellulose synthase1
Zm00001d009810	0.87656	6	8	Core-2/I-branching beta-16-N-acetylglucosaminyltransferase family protein
Zm00001d009908	0.936108	6	8	UDP-glucuronic acid decarboxylase 4
Zm00001d009932	1.08365	6	8	Plasma membrane associated protein
Zm00001d009957	0.898015	6	8	#N/D
Zm00001d009967	1.06842	6	8	UDP-sulfoquinovose synthase chloroplastic
Zm00001d010009	0.667084	6	8	ribosomal protein L17a
Zm00001d010012	1.53077	6	8	UNE1
Zm00001d010016	0.848438	6	8	60S ribosomal protein L11-1
Zm00001d010033	0.670023	6	8	Putative DUF231 domain containing family protein
Zm00001d010038	2.18267	6	8	CASP-like protein 5
Zm00001d010043	0.819202	6	8	Triose phosphate/phosphate translocator TPT chloroplastic
Zm00001d010049	1.15465	6	8	Putative patellin family protein isoform 1
Zm00001d012296	-2.51375	7	8	Putative bZIP transcription factor superfamily protein
Zm00001d012221	-2.26334	7	8	sacd9;stearoyl-acyl-carrier-protein desaturase9:
Zm00001d012257	-2.03514	7	8	#N/D
Zm00001d012430	-1.91365	7	8	Zinc finger protein ZAT11
Zm00001d012333	-1.49504	7	8	HXXXD-type acyl-transferase family protein
Zm00001d012254	-1.49353	7	8	Putative patellin family protein

Zm00001d012321	-1.40606	7	8	Nematode resistance protein-like HSPRO2
Zm00001d012285	-1.3791	7	8	Putative MYB DNA-binding domain superfamily protein
Zm00001d012482	-1.33205	7	8	Putative WRKY DNA-binding domain superfamily protein
Zm00001d012024	-1.16143	7	8	Nucleotide-diphospho-sugar transferase family protein
Zm00001d012456	-1.05357	7	8	jasmonate-regulated gene 21
Zm00001d012206	-1.01179	7	8	#N/D
Zm00001d012459	-1.01109	7	8	Farnesol kinase chloroplastic
Zm00001d012258	-1.00521	7	8	Expressed protein; protein
Zm00001d012304	-1.00014	7	8	Putative cytochrome P450 superfamily protein
Zm00001d012255	-0.996733	7	8	Putative MYB DNA-binding domain superfamily protein
Zm00001d012407	-0.946737	7	8	triose phosphate isomerase 5
Zm00001d012069	-0.922208	7	8	DUF506 family protein
Zm00001d012197	-0.913816	7	8	dmes2;diphosphocytidyl methyl erythritol synthase2:
Zm00001d012142	-0.85972	7	8	Acid phosphatase/vanadium-dependent haloperoxidase-related protein
Zm00001d012128	-0.828409	7	8	Putative two-component response regulator family protein
Zm00001d011969	1.20059	7	8	NAC domain containing protein 84
Zm00001d012086	0.618078	7	8	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2
Zm00001d012092	1.00287	7	8	Histone deacetylase HDT1
Zm00001d012165	0.985467	7	8	#N/D
Zm00001d012166	1.18191	7	8	Faciata 1-like protein
Zm00001d012167	1.65457	7	8	SF16 protein
Zm00001d012274	1.5745	7	8	hct10;hydroxycinnamoyltransferase10:
Zm00001d012279	0.918608	7	8	Putative RING zinc finger domain superfamily protein
Zm00001d012320	1.63741	7	8	Cell division control protein 6 homolog B
Zm00001d012326	2.23315	7	8	Cytochrome P450 86A1
Zm00001d012378	1.1535	7	8	SOUL heme-binding family protein
Zm00001d012387	1.12782	7	8	Embryogenesis transmembrane protein-like; protein

Zm00001d012394	1.5653	7	8	psy2;phytoene synthase2
Zm00001d012395	0.911627	7	8	Mannose-1-phosphate guanylyltransferase 1
Zm00001d012423	0.741384	7	8	Histone H2B
Zm00001d012477	1.39444	7	8	Laccase-13
Zm00001d049113	-1.42202	8	4	Senescence-specific cysteine protease SAG12
Zm00001d049125	-0.787455	8	4	Transcriptional corepressor SEUSS
Zm00001d049129	1.21869	8	4	late embryogenesis abundant protein-related / LEA protein-related
Zm00001d013193	-2.60953	9	5	CCG-binding protein 1
Zm00001d013175	-1.57383	9	5	#N/D
Zm00001d023398	1.53695	12	10	Probable xyloglucan 6-xylosyltransferase 5
Zm00001d023399	1.92123	12	10	Probable xyloglucan 6-xylosyltransferase 5
Zm00001d014494	-4.42498	14	5	#N/D
Zm00001d016471	-4.19007	14	5	Trans-cinnamate 4-monooxygenase
Zm00001d014852	-3.71972	14	5	Ser/Thr-rich protein T10 in DGCR region
Zm00001d013909	-3.32615	14	5	#N/D
Zm00001d016730	-2.86324	14	5	E3 ubiquitin-protein ligase PUB23
Zm00001d014945	-2.74164	14	5	Lactate/malate dehydrogenase family protein
Zm00001d016142	-2.64223	14	5	RING-H2 finger protein ATL5H
Zm00001d016242	-2.45282	14	5	Probable leucine-rich repeat receptor-like serine/threonine-protein kinase
Zm00001d017268	-2.39455	14	5	R2R3MYB-domain protein
Zm00001d016237	-2.24982	14	5	NOD26-like membrane intrinsic protein1
Zm00001d016401	-2.21529	14	5	#N/D
Zm00001d013945	-2.14946	14	5	Ripening-related protein 3
Zm00001d016151	-2.10089	14	5	cytochrome P450 family 93 subfamily D polypeptide 1
Zm00001d014335	-2.06617	14	5	cytochrome P450 family 87 subfamily A polypeptide 6
Zm00001d014649	-2.02002	14	5	Disease resistance protein RPM1
Zm00001d014121	-1.88102	14	5	Ent-cassadiene C2-hydroxylase

Zm00001d014493	-1.85813	14	5	Wound-responsive family protein
Zm00001d014520	-1.80011	14	5	#N/D
Zm00001d016760	-1.75115	14	5	aasr6;abscisic acid stress ripening6:
Zm00001d016121	-1.70432	14	5	#N/D
Zm00001d015616	-1.68197	14	5	Leucine zipper protein-like; protein
Zm00001d014650	-1.65107	14	5	Disease resistance protein RPM1
Zm00001d014081	-1.63311	14	5	RmlC-like cupins superfamily protein
Zm00001d017060	-1.63033	14	5	Hypoxia-responsive family protein
Zm00001d013934	-1.62263	14	5	soluble epoxide hydrolase
Zm00001d017032	-1.61882	14	5	#N/D
Zm00001d016857	-1.54636	14	5	zar7;Zea mays ARGOS7:
Zm00001d014258	-1.48705	14	5	alanine aminotransferase 5
Zm00001d014294	-1.45876	14	5	Acetyl-CoA carboxylase 1
Zm00001d017139	-1.44959	14	5	hds1;hydroxymethylbutenyl diphosphate synthase1:
Zm00001d013786	-1.41535	14	5	transferases;folic acid binding
Zm00001d014499	-1.41349	14	5	Synaptotagmin-3
Zm00001d016298	-1.40892	14	5	Homeobox protein ATH1
Zm00001d016705	-1.39836	14	5	ATPase inhibitor
Zm00001d013795	-1.38797	14	5	Peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein
Zm00001d015844	-1.378	14	5	alpha/beta hydrolase family protein
Zm00001d016009	-1.34689	14	5	L-type lectin-domain containing receptor kinase V.9
Zm00001d015434	-1.34069	14	5	B-box zinc finger family protein
Zm00001d014109	-1.31932	14	5	transmembrane protein-related
Zm00001d014029	-1.3156	14	5	myb domain protein 15
Zm00001d016947	-1.29626	14	5	#N/D
Zm00001d016625	-1.29172	14	5	Os02g0519800 protein
Zm00001d014001	-1.27706	14	5	Flotillin-like protein 2

Zm00001d014134	-1.27344	14	5	Ent-cassadiene C2-hydroxylase
Zm00001d014447	-1.25313	14	5	Serine carboxypeptidase-like 19
Zm00001d016691	-1.22594	14	5	Copper transport protein CCH
Zm00001d015393	-1.21619	14	5	10A19I.15
Zm00001d013997	-1.21398	14	5	Cysteine-rich receptor-like protein kinase 2
Zm00001d016136	-1.14221	14	5	E3 ubiquitin-protein ligase ATL41
Zm00001d016745	-1.13544	14	5	Probable F-box protein At1g60180
Zm00001d017002	-1.10039	14	5	#N/D
Zm00001d016198	-1.09675	14	5	glutamate-oxaloacetic transaminase 3
Zm00001d016483	-1.07357	14	5	MACPF domain-containing protein
Zm00001d014324	-1.04127	14	5	Calcium-binding endonuclease/exonuclease/phosphatase family
Zm00001d017138	-0.992938	14	5	Transcription factor bHLH128
Zm00001d013743	-0.986106	14	5	Zinc finger protein 1
Zm00001d016441	-0.9826	14	5	Tyrosine aminotransferase
Zm00001d016225	-0.958905	14	5	U-box domain-containing protein 17
Zm00001d016772	-0.930119	14	5	Protein FAR1-RELATED SEQUENCE 5
Zm00001d014244	-0.903445	14	5	Esterase
Zm00001d015884	-0.903063	14	5	Protein kinase
Zm00001d016582	-0.888657	14	5	SAUR-like auxin-responsive protein family
Zm00001d014569	-0.885335	14	5	Probable cadmium/zinc-transporting ATPase HMA1 chloroplastic
Zm00001d014609	-0.885051	14	5	RING/U-box superfamily protein
Zm00001d014063	-0.884572	14	5	MDIS1-interacting receptor like kinase 2
Zm00001d015971	-0.871149	14	5	Probable LRR receptor-like serine/threonine-protein kinase
Zm00001d017053	-0.860447	14	5	Calcineurin-like metallo-phosphoesterase superfamily protein
Zm00001d016052	-0.848457	14	5	WRKY transcription factor 22
Zm00001d017152	-0.846914	14	5	endochitinase precursor 4
Zm00001d016066	-0.846059	14	5	F-box/kelch-repeat protein

Zm00001d016379	-0.838212	14	5	#N/D
Zm00001d017210	-0.836092	14	5	SPla/RYanodine receptor (SPRY) domain-containing protein
Zm00001d016586	-0.822462	14	5	hydroxyproline-rich glycoprotein family protein
Zm00001d013787	-0.819322	14	5	Secretory carrier-associated membrane protein 3
Zm00001d014613	-0.80763	14	5	Xyloglucan endotransglucosylase/hydrolase protein 23
Zm00001d014422	-0.805409	14	5	Lysine-specific demethylase REF6
Zm00001d016299	-0.803914	14	5	mitochondrial carrier family protein 1
Zm00001d013620	-0.786601	14	5	#N/D
Zm00001d016438	-0.774661	14	5	3-ketoacyl-CoA synthase
Zm00001d016902	-0.764493	14	5	Bifunctional riboflavin biosynthesis protein RIBA 1 chloroplastic
Zm00001d015420	-0.757137	14	5	DUF581 domain containing protein expressed
Zm00001d016520	-0.746578	14	5	Heat stress transcription factor A-5
Zm00001d013915	-0.744139	14	5	Glutamyl-tRNA reductase 1 chloroplastic
Zm00001d015361	-0.739311	14	5	Transducin/WD40 repeat-like superfamily protein
Zm00001d015319	-0.701982	14	5	Protein SRG1
Zm00001d014203	-0.687299	14	5	F-box/kelch-repeat protein SKIP11
Zm00001d015148	-0.664732	14	5	Uncharacterised conserved protein (UCP030365)
Zm00001d014525	-0.660889	14	5	Glycosyltransferase
Zm00001d014944	-0.64255	14	5	lactate dehydrogenase 1
Zm00001d016804	-0.641775	14	5	histone methyltransferases
Zm00001d013830	-0.629173	14	5	cytochrome P450 family 76 subfamily C polypeptide 7
Zm00001d014953	-0.596294	14	5	Ferrochelatase
Zm00001d015730	1.41529	14	5	Complex 1 LYR protein 45
Zm00001d015737	1.09218	14	5	Ran BP2/NZF zinc finger-like superfamily protein
Zm00001d015802	0.810886	14	5	#N/D
Zm00001d015839	0.788957	14	5	Chaperone protein dnaJ 6
Zm00001d015860	2.59617	14	5	1-aminocyclopropane-1-carboxylate oxidase 1

Zm00001d015863	1.00628	14	5	Mitotic spindle checkpoint protein BUBR1
Zm00001d015905	1.50502	14	5	sweet4a;sugars will eventually be exported transporter4a:
Zm00001d015914	1.01136	14	5	sweet4b;sugars will eventually be exported transporter4b:
Zm00001d015921	2.00807	14	5	Plasma membrane-associated cation-binding protein 1
Zm00001d015969	2.01889	14	5	IQ-domain 21
Zm00001d016031	1.32477	14	5	GDSL esterase/lipase
Zm00001d016082	0.851316	14	5	High mobility group B protein 6
Zm00001d013561	0.714221	14	5	glycosyl hydrolase family 10 protein
Zm00001d016091	1.0576	14	5	UDP-glycosyltransferase 73D1
Zm00001d013566	1.06526	14	5	#N/D
Zm00001d016132	0.995567	14	5	AT-hook motif nuclear-localized protein 26
Zm00001d016185	0.661953	14	5	Peroxidase 52
Zm00001d016203	0.850463	14	5	Actin-related protein 2/3 complex subunit 3
Zm00001d013612	0.622997	14	5	Tubulin beta-4 chain
Zm00001d016255	0.857108	14	5	Heat stress transcription factor C-1
Zm00001d016274	0.915538	14	5	Monosaccharide-sensing protein 2
Zm00001d016276	0.669536	14	5	ribosomal protein S24
Zm00001d016277	0.893678	14	5	Auxin-responsive protein IAA26
Zm00001d016301	2.87718	14	5	Inorganic pyrophosphatase 1
Zm00001d016358	0.709821	14	5	Probable elongation factor 1-gamma 2
Zm00001d013641	0.968876	14	5	HSP20-like chaperones superfamily protein
Zm00001d013654	1.28923	14	5	C2 calcium/lipid-binding plant phosphoribosyltransferase family protein
Zm00001d016533	0.829291	14	5	Electron transfer flavoprotein subunit alpha mitochondrial
Zm00001d016723	0.650305	14	5	Histone H3
Zm00001d016733	1.03479	14	5	NDR1/HIN1-like 1
Zm00001d016736	0.954296	14	5	2-Cys peroxiredoxin BAS1 chloroplastic
Zm00001d016756	1.04081	14	5	NF-180

Zm00001d016758	1.32933	14	5	#N/D
Zm00001d016788	2.4986	14	5	#N/D
Zm00001d016849	1.05869	14	5	TPX2 (targeting protein for Xklp2) protein family
Zm00001d016982	1.46209	14	5	Protein NRT1/ PTR FAMILY 6.4
Zm00001d016997	1.90694	14	5	#N/D
Zm00001d017043	1.19883	14	5	OSJNBb0022F23.8 protein
Zm00001d017090	0.834585	14	5	#N/D
Zm00001d017101	1.22774	14	5	Protein binding protein
Zm00001d017111	0.752306	14	5	Very-long-chain 3-oxoacyl-CoA reductase 1
Zm00001d017242	0.640737	14	5	H/ACA ribonucleoprotein complex subunit 3-like protein
Zm00001d017243	3.30523	14	5	R2R3MYB-domain protein
Zm00001d013758	0.7759	14	5	60S ribosomal protein L24
Zm00001d013796	0.76931	14	5	dcl105;dicer-like 105:
Zm00001d013804	0.8469	14	5	Probable beta-13-galactosyltransferase 4
Zm00001d013873	1.09861	14	5	Actin-2
Zm00001d013926	1.71923	14	5	Formin-like protein 6
Zm00001d013956	1.00616	14	5	tip3a;tonoplast intrinsic protein3:
Zm00001d013979	1.87403	14	5	Cytochrome P450 86B1
Zm00001d014025	0.733737	14	5	Cyclase family protein
Zm00001d014060	1.05678	14	5	Protein NRT1/ PTR FAMILY 5.2
Zm00001d014100	1.86804	14	5	Glycine rich protein 3
Zm00001d014101	1.30566	14	5	#N/D
Zm00001d014102	1.58464	14	5	Divalent ion symporter
Zm00001d014116	0.695876	14	5	pht13;phosphate transporter protein13:
Zm00001d014285	1.18552	14	5	Aquaporin PIP2-2
Zm00001d014319	1.23983	14	5	#N/D
Zm00001d014325	0.924379	14	5	Calcium-binding EF-hand family protein

Zm00001d014355	0.633999	14	5	60S ribosomal protein L27-2
Zm00001d014419	0.905411	14	5	Chalcone-flavanone isomerase family protein
Zm00001d014467	1.03162	14	5	Peroxidase 3
Zm00001d014554	1.19819	14	5	cytochrome P450 family 718
Zm00001d014563	1.63229	14	5	Thaumatin-like protein
Zm00001d014683	1.20506	14	5	ARM repeat superfamily protein
Zm00001d014758	1.47075	14	5	plt6;phospholipid transfer protein homolog6:
Zm00001d014787	0.719956	14	5	DEAD-box ATP-dependent RNA helicase 10
Zm00001d014885	1.12107	14	5	zinc knuckle (CCHC-type) family protein
Zm00001d014947	0.891005	14	5	Beta-fructofuranosidase 1%3B Invertase
Zm00001d014965	1.38349	14	5	Glycosyltransferase
Zm00001d014971	1.00819	14	5	Leucine-rich repeat receptor-like protein kinase PXL1
Zm00001d014983	0.797853	14	5	Serine carboxypeptidase-like 49
Zm00001d015008	0.909118	14	5	UTP--glucose-1-phosphate uridylyltransferase 1
Zm00001d015036	0.704331	14	5	Rho-related protein from plants 9
Zm00001d015064	1.59413	14	5	sdg129;set domain gene129:
Zm00001d015092	1.20132	14	5	Cyclin-U4-2
Zm00001d015126	0.988958	14	5	response to low sulfur 3
Zm00001d015133	0.870219	14	5	Metal transporter Nramp6
Zm00001d015202	2.08613	14	5	Protein STRUBBELIG-RECEPTOR FAMILY 1
Zm00001d015213	0.725737	14	5	leucine-rich repeat/extensin 2
Zm00001d015301	0.751166	14	5	60S ribosomal protein L37a-2
Zm00001d015307	2.25185	14	5	Protein IRX15-LIKE
Zm00001d015348	0.872435	14	5	Tubulin beta-4 chain
Zm00001d015375	0.661244	14	5	60S ribosomal protein L27a-3
Zm00001d015449	0.702678	14	5	Dimethyladenosine transferase
Zm00001d015468	1.12831	14	5	Zinc finger protein CONSTANS-LIKE 5

Zm00001d015474	1.06377	14	5	Vacuolar import/degradation Vid27-related protein
Zm00001d015560	1.09856	14	5	PLATZ transcription factor family protein
Zm00001d006526	-4.8401	15	2	Eukaryotic aspartyl protease family protein
Zm00001d006699	-3.32615	15	2	Ribosome associated membrane protein RAMP4
Zm00001d006591	-2.14946	15	2	F-box domain containing protein
Zm00001d006649	-1.88102	15	2	cytochrome P450 family 87 subfamily A polypeptide 6
Zm00001d006606	-1.86542	15	2	bromodomain 4
Zm00001d006608	-1.72833	15	2	Whole genome shotgun sequence of line PN40024 scaffold_35.assembly12x (Fragment)
Zm00001d006446	-1.63311	15	2	salmon silk2: like sm1
Zm00001d006467	-1.62263	15	2	aprl2;adenosine 5'-phosphosulfate reductase-like2:
Zm00001d006493	-1.50898	15	2	Protein DETOXIFICATION 40
Zm00001d006511	-1.48705	15	2	Glucose-6-phosphate/phosphate translocator 2 chloroplastic
Zm00001d006612	-1.45876	15	2	#N/D
Zm00001d006623	-1.43348	15	2	Polygalacturonase inhibitor 1
Zm00001d006630	-1.42236	15	2	Chalcone isomerase
Zm00001d006639	-1.41535	15	2	#N/D
Zm00001d006640	-1.39029	15	2	O-fucosyltransferase family protein
Zm00001d006658	-1.38797	15	2	Glucan endo-13-beta-glucosidase 4
Zm00001d006697	-1.36665	15	2	Putative polyol transporter 1
Zm00001d043075	-1.35799	16	3	Leucine-rich repeat protein kinase family protein
Zm00001d043047	-1.31932	16	3	Expansin-A2
Zm00001d043058	-1.3156	16	3	Probable LRR receptor-like serine/threonine-protein kinase IRK
Zm00001d013909	-1.27706	17	5	#N/D
Zm00001d013945	-1.27344	17	5	Ripening-related protein 3
Zm00001d014121	-1.21398	17	5	Ent-cassadiene C2-hydroxylase
Zm00001d014081	-0.986106	17	5	RmlC-like cupins superfamily protein
Zm00001d013934	-0.920325	17	5	soluble epoxide hydrolase

Zm00001d014258	-0.903445	17	5	alanine aminotransferase 5
Zm00001d014294	-0.891809	17	5	Acetyl-CoA carboxylase 1
Zm00001d013786	-0.884572	17	5	transferases;folic acid binding
Zm00001d013795	-0.819322	17	5	Peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein
Zm00001d014109	-0.797568	17	5	transmembrane protein-related
Zm00001d014029	-0.755732	17	5	myb domain protein 15
Zm00001d014001	-0.744139	17	5	Flotillin-like protein 2
Zm00001d014134	-0.687299	17	5	Ent-cassadiene C2-hydroxylase
Zm00001d013997	-0.679467	17	5	Cysteine-rich receptor-like protein kinase 2
Zm00001d013743	-0.629173	17	5	Zinc finger protein 1
Zm00001d014244	1.91154	17	5	Esterase
Zm00001d014063	1.03243	17	5	MDIS1-interacting receptor like kinase 2
Zm00001d013787	1.35395	17	5	Secretory carrier-associated membrane protein 3
Zm00001d013915	1.8853	17	5	Glutamyl-tRNA reductase 1 chloroplastic
Zm00001d014203	3.71018	17	5	F-box/kelch-repeat protein SKIP11
Zm00001d013830	0.750047	17	5	cytochrome P450 family 76 subfamily C polypeptide 7
Zm00001d013758	1.27006	17	5	60S ribosomal protein L24
Zm00001d013796	1.3578	17	5	dcl105;dicer-like 105:
Zm00001d013804	0.780945	17	5	Probable beta-13-galactosyltransferase 4
Zm00001d013873	0.884591	17	5	Actin-2
Zm00001d013926	2.10815	17	5	Formin-like protein 6
Zm00001d013956	0.919727	17	5	tip3a;tonoplast intrinsic protein3:
Zm00001d013979	1.68309	17	5	Cytochrome P450 86B1
Zm00001d014025	0.7759	17	5	Cyclase family protein
Zm00001d014060	0.76931	17	5	Protein NRT1/ PTR FAMILY 5.2
Zm00001d014100	0.8469	17	5	Glycine rich protein 3
Zm00001d014101	1.09861	17	5	#N/D

Zm00001d014102	1.71923	17	5	Divalent ion symporter
Zm00001d014116	1.00616	17	5	pht13;phosphate transporter protein13:
Zm00001d014285	1.87403	17	5	Aquaporin PIP2-2
Zm00001d008960	0.733737	19	8	Cupredoxin superfamily protein
Zm00001d008853	1.05678	19	8	Putative HLH DNA-binding domain superfamily protein
Zm00001d008843	1.86804	19	8	Grx_A2-glutaredoxin subgroup III
Zm00001d008812	1.30566	19	8	C3H47 C3H type transcription factor
Zm00001d008923	1.58464	19	8	#N/D
Zm00001d008816	0.695876	19	8	ATP-dependent 6-phosphofructokinase 4 chloroplastic
Zm00001d008794	1.18552	19	8	Putative WRKY DNA-binding domain superfamily protein
Zm00001d008700	0.61217	19	8	vanishing tassel 2
Zm00001d008715	0.94769	19	8	Putative leucine-rich repeat receptor protein kinase family protein
Zm00001d008730	1.23442	19	8	seed gene 3
Zm00001d008850	1.30362	19	8	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family

Supplementary Table 2 Differentially expressed genes mapped in aluminum tolerance QTLs for Al-sensitive line

GENE	FOLDCHANGE	QTL	CHR	FUNCTION
Zm00001d036409	2.09963	1	6	OSJNBa0013K16.15 protein; protein
Zm00001d025059	1.11766	2	10	Germin-like protein subfamily 1 member 8
Zm00001d025103	1.57756	2	10	amo1;amine oxidase1: single copy rice cDNA rz69
Zm00001d025141	-5.4748	2	10	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
Zm00001d025166	-0.902018	2	10	Putative quinone-oxidoreductase homolog chloroplastic
Zm00001d025205	-1.31014	2	10	Putative HLH DNA-binding domain superfamily protein
Zm00001d023596	1.40871	2	10	Amino acid permease 2
Zm00001d025346	2.32214	2	10	Protein TERMINAL FLOWER 1
Zm00001d025354	1.83536	2	10	incw5;invertase cell wall5:
Zm00001d025423	-1.97444	2	10	F1N21.17
Zm00001d025446	-1.98214	2	10	Pentatricopeptide repeat-containing protein
Zm00001d025533	1.73807	2	10	NAD(P)-linked oxidoreductase superfamily protein
Zm00001d025541	-0.973001	2	10	Eukaryotic aspartyl protease family protein
Zm00001d025573	-1.74906	2	10	Sugar transport protein 5
Zm00001d023617	4.60072	2	10	Laccase-7
Zm00001d025703	-1.37618	2	10	ABC transporter B family member 15
Zm00001d025747	1.42296	2	10	Phosphoglycolate phosphatase 1A chloroplastic
Zm00001d025753	1.56031	2	10	ctb1;chitinase B1
Zm00001d025803	1.97154	2	10	Putative DUF869 domain containing family protein
Zm00001d025860	0.99344	2	10	#N/D
Zm00001d025873	-1.21212	2	10	Low-molecular-weight cysteine-rich protein LCR70
Zm00001d025900	1.43431	2	10	Putative GEM-like protein 8
Zm00001d025912	-2.44455	2	10	Transferase/ transferase
Zm00001d025916	1.74025	2	10	E3 ubiquitin-protein ligase RMA1

Zm00001d023753	-1.81961	2	10	#N/D
Zm00001d023768	1.10341	2	10	Delta(12)-fatty-acid desaturase
Zm00001d023769	1.17171	2	10	Delta(12)-fatty-acid desaturase
Zm00001d023774	1.16129	2	10	Erg28 like protein
Zm00001d023811	0.863863	2	10	Pathogenesis-related protein STH-21
Zm00001d023859	1.64738	2	10	Respiratory burst oxidase homolog protein B
Zm00001d023941	0.918634	2	10	Carbohydrate transporter/ sugar porter
Zm00001d024000	3.73893	2	10	glu3;beta-glucosidase3:
Zm00001d024027	1.56916	2	10	prcw1;proline rich cell wall protein1:
Zm00001d024207	3.88066	2	10	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Zm00001d024210	4.42321	2	10	tps6;terpene synthase6:
Zm00001d024250	Inf	2	10	#N/D
Zm00001d024314	2.82115	2	10	Putative cinnamyl-alcohol dehydrogenase family protein
Zm00001d024359	4.00764	2	10	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase
Zm00001d024393	0.923396	2	10	Indole-3-glycerol phosphate synthase chloroplastic
Zm00001d024512	-1.51604	2	10	cpps3;copalyl diphosphate synthase3:
Zm00001d024514	-1.40671	2	10	Ent-kaur-16-ene synthase chloroplastic
Zm00001d024515	-1.30164	2	10	Putative cytochrome P450 superfamily protein
Zm00001d024522	-4.20718	2	10	Putative HLH DNA-binding domain superfamily protein
Zm00001d024597	-1.38803	2	10	#N/D
Zm00001d024691	1.91943	2	10	Uclacyanin-3
Zm00001d024800	1.94474	2	10	#N/D
Zm00001d024802	2.55623	2	10	#N/D
Zm00001d024891	2.05854	2	10	Beta-glucosidase 17
Zm00001d024893	Inf	2	10	#N/D
Zm00001d024897	Inf	2	10	#N/D
Zm00001d024900	Inf	2	10	#N/D

Zm00001d024925	-0.904463	2	10	#N/D
Zm00001d024960	0.886773	2	10	Bowman-Birk type trypsin inhibitor
Zm00001d024996	1.01733	2	10	Bowman-Birk type trypsin inhibitor
Zm00001d025018	-2.01751	2	10	Expansin-A11
Zm00001d005383	1.64992	3	2	Lecithin-cholesterol acyltransferase-like 1
Zm00001d005392	1.03075	3	2	Lecithin-cholesterol acyltransferase-like 1
Zm00001d005446	1.85247	3	2	Photosystem I reaction center subunit IV A
Zm00001d005823	-1.70883	3	2	Flavonoid 3-monooxygenase
Zm00001d005986	-1.09105	3	2	Probable LRR receptor-like serine/threonine-protein kinase
Zm00001d005996	1.61913	3	2	Photosystem I reaction center subunit V
Zm00001d006094	4.86129	3	2	zmm27;Zea mays MADS27: AGL2 MADS box family
Zm00001d006101	-1.69248	3	2	Calmodulin binding protein
Zm00001d006110	-1.94673	3	2	Transcription factor bHLH137
Zm00001d006111	-1.58273	3	2	Cytochrome b561 and DOMON domain-containing protein
Zm00001d006115	-1.50822	3	2	Anthocyanin-related membrane protein 1 (Anm1)-like
Zm00001d035001	2.6884	4	6	fdx1;ferredoxin1: chloroplast
Zm00001d035306	2.03253	4	6	#N/D
Zm00001d035310	-1.53299	4	6	#N/D
Zm00001d035028	-1.24425	4	6	Putative glycolipid transfer protein (GLTP) family protein
Zm00001d035390	1.87266	4	6	scmv1;resistance to sugarcane mosaic virus1
Zm00001d035479	-2.93068	4	6	Putative WAK family receptor-like protein kinase
Zm00001d035561	-2.39709	4	6	Mannose-specific jacalin-related lectin; protein
Zm00001d035562	-1.59572	4	6	Mannose-specific jacalin-related lectin; protein
Zm00001d035640	-2.26949	4	6	Phospholipase A1-IIgamma
Zm00001d035683	-1.3034	4	6	Subtilisin-chymotrypsin inhibitor CI-1
Zm00001d035684	-1.6023	4	6	Subtilisin-chymotrypsin inhibitor CI-1B
Zm00001d035725	-2.41709	4	6	late embryogenesis abundant protein-related / LEA protein-related

Zm00001d035756	0.996614	4	6	Leucine-rich repeat (LRR) family protein
Zm00001d035854	2.90108	4	6	Alpha-dioxygenase 1
Zm00001d035859	2.17792	4	6	ploc1;plastocyanin homolog1: leaf cDNA csu257, similar to plastocyanin
Zm00001d035888	3.48583	4	6	extensin-like protein
Zm00001d035899	1.14832	4	6	Pectin lyase-like superfamily protein
Zm00001d035918	-2.24211	4	6	Putative MYB DNA-binding domain superfamily protein
Zm00001d035962	-1.36166	4	6	CYCLOPS
Zm00001d035134	2.67798	4	6	Zinc finger protein CONSTANS-LIKE 7
Zm00001d034978	1.23301	4	6	Farnesylated protein 2
Zm00001d036072	-1.38102	4	6	Zinc finger (C3HC4-type RING finger) family protein
Zm00001d036164	2.25561	4	6	Salicylate/benzoate carboxyl methyltransferase
Zm00001d036197	2.16101	4	6	HXXXD-type acyl-transferase family protein
Zm00001d036345	1.10288	4	6	y1;yellow endosperm1
Zm00001d036366	-1.23266	4	6	Basic endochitinase B
Zm00001d035195	-1.38235	4	6	Protein LSD1
Zm00001d038013	-1.99743	5	6	Putative LOB domain-containing family protein
Zm00001d038049	-1.45727	5	6	Lichenase-2%3B Putative O-Glycosyl hydrolase superfamily protein
Zm00001d038061	2.69105	5	6	Probable calcium-binding protein CML25
Zm00001d038064	1.12823	5	6	cytochrome P450 family 94 subfamily D polypeptide 2
Zm00001d038068	-1.28697	5	6	ATPase 2C
Zm00001d038126	-1.83634	5	6	#N/D
Zm00001d010136	-1.15181	6	8	Probable ferredoxin-4 chloroplastic
Zm00001d010159	0.835362	6	8	Actin-1
Zm00001d010169	-2.90194	6	8	Cysteine-rich receptor-like protein kinase 40
Zm00001d010185	2.93509	6	8	#N/D
Zm00001d010313	2.81306	6	8	inosine-uridine preferring nucleoside hydrolase family protein
Zm00001d010375	1.73889	6	8	ATP-dependent 6-phosphofructokinase 4 chloroplastic

Zm00001d010434	-1.55358	6	8	Arabinogalactan peptide 20
Zm00001d010440	1.03269	6	8	sweet3a;sugars will eventually be exported transporter3a:
Zm00001d010451	-1.19171	6	8	GDSL esterase/lipase
Zm00001d010494	-1.69745	6	8	#N/D
Zm00001d010521	0.965187	6	8	Flavonoid 3'-monooxygenase
Zm00001d010585	-1.10568	6	8	UTP--glucose-1-phosphate uridylyltransferase 3 chloroplastic
Zm00001d010588	1.97414	6	8	pdc1;pyruvate decarboxylase1: cDNA and genomic clone, SSR UMC1163
Zm00001d010634	1.196	6	8	Putative MYB DNA-binding domain superfamily protein
Zm00001d010655	0.96458	6	8	WAT1-related protein
Zm00001d010662	-1.13469	6	8	SNF1-related protein kinase regulatory subunit beta-1
Zm00001d010715	2.45241	6	8	Photosystem II reaction center W protein chloroplastic
Zm00001d010755	-1.00391	6	8	trps1;trehalose-6-phosphate synthase1:
Zm00001d010814	1.02298	6	8	AtS40-3
Zm00001d010871	2.50102	6	8	Thioredoxin F2 chloroplastic
Zm00001d010903	-1.2808	6	8	#N/D
Zm00001d010911	-1.59558	6	8	Basic endochitinase B
Zm00001d008706	2.0053	6	8	Photosystem I reaction center subunit XI chloroplastic
Zm00001d008721	1.32208	6	8	Adenine nucleotide alpha hydrolases-like superfamily protein
Zm00001d008795	1.59004	6	8	UDP-glycosyltransferase 74B1
Zm00001d008911	-1.35834	6	8	sodium/calcium exchanger family protein / calcium-binding EF hand family protein
Zm00001d009022	0.91816	6	8	NmrA-like negative transcriptional regulator family protein
Zm00001d009057	1.17698	6	8	Pectin lyase-like superfamily protein
Zm00001d009060	-1.48655	6	8	Probable beta-D-xylosidase 5
Zm00001d009118	1.34864	6	8	Adenine nucleotide alpha hydrolases-like superfamily protein
Zm00001d009140	-1.08832	6	8	Peroxidase 64
Zm00001d009224	-2.95941	6	8	#N/D

Zm00001d009296	1.34351	6	8	Pathogenesis-related protein PRMS
Zm00001d009373	-0.802607	6	8	Peroxidase 72
Zm00001d009425	4.12843	6	8	#N/D
Zm00001d009589	2.81564	6	8	Chlorophyll a-b binding protein M9%2C chloroplastic
Zm00001d009619	2.08409	6	8	Putative WRKY DNA-binding domain superfamily protein
Zm00001d009702	1.2305	6	8	P-loop containing nucleoside triphosphate hydrolases superfamily protein
Zm00001d009726	3.27219	6	8	alpha/beta-Hydrolases superfamily protein
Zm00001d009765	-1.47902	6	8	#N/D
Zm00001d009877	2.92873	6	8	Protein plastid transcriptionally active 16 chloroplastic
Zm00001d009899	1.07408	6	8	Probable pectinesterase/pectinesterase inhibitor 7
Zm00001d009927	1.14089	6	8	oxidoreductase 2OG-Fe(II) oxygenase family protein
Zm00001d009929	3.40042	6	8	Beta-propeller domain of methanol dehydrogenase type 3B
Zm00001d010025	Inf	6	8	Subtilisin-chymotrypsin inhibitor CI-1C
Zm00001d010038	-1.12898	6	8	CASP-like protein 5
Zm00001d010061	-1.27105	6	8	#N/D
Zm00001d012063	-2.74284	7	8	Loricrin
Zm00001d012088	1.26524	7	8	#N/D
Zm00001d012091	-1.97108	7	8	#N/D
Zm00001d012138	1.58867	7	8	Protein NRT1/ PTR FAMILY 8.1
Zm00001d012159	-1.08846	7	8	Beta-glucosidase 42
Zm00001d012274	-1.72713	7	8	hct10;hydroxycinnamoyltransferase10:
Zm00001d012394	-2.28134	7	8	psy2;phytoene synthase2
Zm00001d012444	2.5346	7	8	#N/D
Zm00001d012456	-2.5911	7	8	jasmonate-regulated gene 21
Zm00001d012484	2.42476	7	8	Heavy metal transport/detoxification superfamily protein
Zm00001d049113	2.53008	8	4	Senescence-specific cysteine protease SAG12
Zm00001d049129	-1.55688	8	4	late embryogenesis abundant protein-related

Zm00001d013193	1.07525	9	5	CCG-binding protein 1
Zm00001d015636	-1.64451	14	5	GDSL esterase/lipase
Zm00001d015715	-2.16422	14	5	#N/D
Zm00001d015914	2.19454	14	5	sweet4b;sugars will eventually be exported transporter4b:
Zm00001d015921	-1.53774	14	5	Plasma membrane-associated cation-binding protein 1
Zm00001d015977	-1.23448	14	5	Xyloglucan endotransglucosylase/hydrolase protein 26
Zm00001d016100	2.74284	14	5	Rhodanese-like domain-containing protein 4 chloroplastic
Zm00001d016119	1.29598	14	5	Protein SOMBRERO
Zm00001d016120	Inf	14	5	#N/D
Zm00001d016134	1.85862	14	5	ris1;iron-sulfur protein1
Zm00001d016139	-1.47151	14	5	E3 ubiquitin-protein ligase ATL41
Zm00001d016154	-1.67837	14	5	Basic leucine zipper 34
Zm00001d016177	-3.31129	14	5	Peroxidase 52
Zm00001d016189	-2.54021	14	5	Dirigent
Zm00001d013610	1.26808	14	5	Osmotin-like protein OSM34
Zm00001d013651	1.46742	14	5	Chlorophyll(ide) b reductase NOL chloroplastic
Zm00001d016471	1.72178	14	5	Trans-cinnamate 4-monooxygenase
Zm00001d016474	-0.956118	14	5	Probable protein phosphatase 2C 74
Zm00001d016601	-1.525	14	5	Phosphatidylinositol/phosphatidylcholine transfer protein SFH10
Zm00001d016691	2.04952	14	5	Copper transport protein CCH
Zm00001d016705	-1.53341	14	5	ATPase inhibitor
Zm00001d016724	1.24504	14	5	#N/D
Zm00001d016760	-0.858116	14	5	aasr6;abscisic acid stress ripening6:
Zm00001d016893	3.05047	14	5	Thioredoxin-like 2
Zm00001d016896	-1.08092	14	5	UDP-glycosyltransferase 85A7
Zm00001d016926	1.15887	14	5	ATATH13
Zm00001d013725	1.62604	14	5	ga20ox4;gibberellin 20-oxidase4:

Zm00001d017240	1.08332	14	5	Grx_C2.1-glutaredoxin subgroup I
Zm00001d013825	-1.20882	14	5	#N/D
Zm00001d013907	2.53522	14	5	Nonspecific lipid-transfer protein
Zm00001d013937	2.38112	14	5	Protochlorophyllide reductase C chloroplastic
Zm00001d013956	3.65699	14	5	tip3a;tonoplast intrinsic protein3:
Zm00001d013979	-1.68058	14	5	Cytochrome P450 86B1
Zm00001d014101	-1.90834	14	5	#N/D
Zm00001d014121	3.75337	14	5	Ent-cassadiene C2-hydroxylase
Zm00001d014134	0.996542	14	5	Ent-cassadiene C2-hydroxylase
Zm00001d014217	-2.12406	14	5	#N/D
Zm00001d014282	-1.77597	14	5	#N/D
Zm00001d014386	-1.06904	14	5	Patatin-like protein 6
Zm00001d014391	1.78264	14	5	nrat1;nramp aluminum transporter1
Zm00001d014496	-1.81032	14	5	ARM repeat superfamily protein
Zm00001d014564	1.71608	14	5	Oxygen-evolving enhancer protein 1-1 chloroplastic
Zm00001d014600	1.92206	14	5	#N/D
Zm00001d014606	1.48276	14	5	Peroxidase 45
Zm00001d014613	1.07418	14	5	Xyloglucan endotransglucosylase/hydrolase protein 23
Zm00001d014695	1.28042	14	5	Camelliol C synthase
Zm00001d014758	-0.949654	14	5	plt6;phospholipid transfer protein homolog6:
Zm00001d014914	-2.23995	14	5	Leucoanthocyanidin dioxygenase
Zm00001d014945	2.44687	14	5	Lactate/malate dehydrogenase family protein
Zm00001d014971	-2.35508	14	5	Leucine-rich repeat receptor-like protein kinase PXL1
Zm00001d014984	-1.42995	14	5	Glycerol-3-phosphate acyltransferase 8
Zm00001d015032	-1.38731	14	5	Serine/threonine-protein kinase STY46
Zm00001d015133	0.979691	14	5	Metal transporter Nramp6
Zm00001d015385	2.09309	14	5	Chlorophyll a-b binding protein 6 chloroplastic

Zm00001d015569	0.771839	14	5	Vacuolar H+-translocating inorganic pyrophosphatase
Zm00001d006538	1.35351	15	2	O-Glycosyl hydrolases family 17 protein
Zm00001d006540	2.19282	15	2	Oxygen-evolving enhancer protein 3-1
Zm00001d006587	2.8305	15	2	Chlorophyll a-b binding protein CP29.1 chloroplastic
Zm00001d006626	-1.69584	15	2	Remorin family protein
Zm00001d006659	1.03403	15	2	Constitutive photomorphogenesis protein 10
Zm00001d006663	2.23078	15	2	light harvesting complex A1
Zm00001d043062	-2.07345	16	3	Probable WRKY transcription factor 30
Zm00001d013825	-1.20882	17	5	#N/D
Zm00001d013907	2.53522	17	5	Nonspecific lipid-transfer protein
Zm00001d013937	2.38112	17	5	Protochlorophyllide reductase C chloroplastic
Zm00001d013956	3.65699	17	5	tip3a;tonoplast intrinsic protein3:
Zm00001d013979	-1.68058	17	5	Cytochrome P450 86B1
Zm00001d014101	-1.90834	17	5	#N/D
Zm00001d014121	3.75337	17	5	Ent-cassadiene C2-hydroxylase
Zm00001d014134	0.996542	17	5	Ent-cassadiene C2-hydroxylase
Zm00001d014217	-2.12406	17	5	#N/D
Zm00001d014282	-1.77597	17	5	#N/D
Zm00001d008706	2.0053	19	8	Photosystem I reaction center subunit XI chloroplastic
Zm00001d008721	1.32208	19	8	Adenine nucleotide alpha hydrolases-like superfamily protein
Zm00001d008795	1.59004	19	8	UDP-glycosyltransferase 74B1
Zm00001d008911	-1.35834	19	8	sodium/calcium exchanger family protein

Supplementary Table 3 Constitutive genes mapped inside QTLs

GENE	FOLDCHANGE 11-60	FOLDCHANGE 11-133	QTL	FUNCTION
Zm00001d012456	-2.5911	-1.05357	7	jasmonate-regulated gene 21
Zm00001d035561	-2.39709	-1.59718	4	Mannose-specific jacalin-related lectin; protein
Zm00001d005823	-1.70883	-1.3582	3	Flavonoid 3-monooxygenase
Zm00001d016705	-1.53341	-1.39836	14	ATPase inhibitor
Zm00001d006115	-1.50822	-1.1013	3	Anthocyanin-related membrane protein 1 (Anm1)-like
Zm00001d036072	-1.38102	-0.938498	4	Zinc finger (C3HC4-type RING finger) family protein
Zm00001d035028	-1.24425	-0.909298	4	Putative glycolipid transfer protein (GLTP) family protein
Zm00001d010662	-1.13469	-0.896902	6	SNF1-related protein kinase regulatory subunit beta-1
Zm00001d016760	-0.858116	-1.75115	14	aasr6;abscisic acid stress ripening6:
Zm00001d010521	0.965187	0.973091	6	Flavonoid 3'-monooxygenase
Zm00001d015133	0.979691	0.870219	14	Metal transporter Nramp6
Zm00001d010440	1.03269	1.05927	6	sweet3a;sugars will eventually be exported transporter3a:
Zm00001d025059	1.11766	0.847503	2	Germin-like protein subfamily 1 member 8
Zm00001d025103	1.57756	1.1415	2	amo1;amine oxidase1: single copy rice cDNA rz69
Zm00001d025533	1.73807	0.736491	2	NAD(P)-linked oxidoreductase superfamily protein
Zm00001d015914	2.19454	1.01136	14	sweet4b;sugars will eventually be exported transporter4b:
Zm00001d013956	3.65699	1.00616	14	tip3a;tonoplast intrinsic protein3:
Zm00001d013956	3.65699	1.00616	17	tip3a;tonoplast intrinsic protein3:

Supplementary Table 4 Predicted deleterious variations in differentially expressed genes in Al-sensitive line

CHR	GENE	FUNCTION	CONSEQUENCE	VARIANT	PROVEAN score
1	Zm00001d031971	Vignain	missense_variant	185,A,T	-2.506
2	Zm00001d003757	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	missense_variant	89,G,E	-7
3	Zm00001d042367	Selenium-binding protein 3	missense_variant	460,N,S	-4.373
3	Zm00001d044291	actin binding protein family	missense_variant	402,E,D	-2.867
4	Zm00001d048898	F-box/kelch-repeat protein SKIP11	missense_variant	385,D,N	-3.104
4	Zm00001d049288	#N/D	missense_variant	9,T,A	-5
4	Zm00001d049288	#N/D	missense_variant	62,Q,L	-7
4	Zm00001d049288	#N/D	missense_variant	84,H,Y	-6
5	Zm00001d016705	ATPase inhibitor	missense_variant	36,R,W	-8
5	Zm00001d016724	#N/D	missense_variant	26,A,S	-3
5	Zm00001d017746	Tocopherol O-methyltransferase chloroplastic	missense_variant	172,A,G	-2.52
7	Zm00001d020696	Asparate aminotransferase	missense_variant	418,A,V	-2.57
8	Zm00001d010662	SNF1-related protein kinase regulatory subunit beta-1	missense_variant	149,I,F	-2.902
8	Zm00001d012091	Pro-resilin	missense_variant	112,G,A	-2.6
8	Zm00001d012091	Pro-resilin	missense_variant	151,G,A	-3.6
9	Zm00001d048065	Tubulin alpha-1 chain	missense_variant	331,A,V	-3.052
10	Zm00001d024925	#N/D	missense_variant	185,F,L	-4.063