Aluminum Accumulation and The Effects On Proteome Expression In Tomato Seeds.

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Abstract

One of the major factors that affect plant growth in acid soils (pH < 5) is aluminum (Al) toxicity. Aluminum is the most abundant metal in the earth's crust, representing about 8% of total mineral components. Under acidic condition, Al is hydrolyzed into the soluble major phytotoxic form, Al3+. This study was conducted to localize Al in tomato plant tissues, including fruits. Also to determine protein expression in seed tissues of matured green fruits under Al stress. Tomato (Solanum lycopersicum cv. Micro-Tom) plants were grown in hydroponic tanks filled with Magnacava's solution, pH 4.5 - 4.6. For Al treatment, the hydroponic solution was supplemented with 50 µM AlK (SO4)2. 12H2O, and the control was refreshed with only the Magnacava's solution. Cross sections of roots, stems and green fruits were stained with morin (2', 3, 4', 5, 7-pentahydroxyflavone) fluorochrome to track and detect Al in situ. The Al-treated tissues showed brighter green fluorescence than the untreated ones. Differentially expressed proteins between the Al-enriched seeds and controls were identified using two-dimensional gel electrophoresis (DIGE) analysis followed by a procedure of in-gel trypsin digestion-mass spectrometry-database search (the annotated tomato database). Results showed that the identified proteins are involved in gene expression and cell division, chaperones and protectants, metabolic pathways, and phytohormone-biosynthesis. Also, elevated expression level of malic enzymes and antioxidant enzymes was observed. Based on these results, a molecular model of ion toxicity from endogenous Al during seed maturation and germination is being evaluated.

Objectives

This study was performed to test the hypothesis that Al supplemented in roots is transported into the above-ground tissues, and the presence of excess Al in tomato fruits affects cellular metabolism therewith.

Acknowledgement

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Methods

Plant culture and Al treatment: Seedlings at two-leaf stage were transplanted into hydroponic solution containing modified Magnavaca's solution in 30L tanks (Fig 1).

Figure 1: Tomato (*Solanum lycopersicum* cv. Micro-Tom) plants growing in hydroponic solutions in the greenhouse

Al treatment (50 µM Al $(SO_4)_3$. $18H_2O$) was applied nine weeks later when the matured plants started producing fruits. Four replicates of control and Al treatment each containing 12 seedlings were set up. Seeds (containing the pericarp) from the green fruits were collected for the aluminum, proteomic, and enzyme analyses.

Growing tomato plants, and performing Al-treatment

Collecting seed and pericarp tissues from matured green fruits

Seed Al analysis (Morin staining) | Proteomics and bioinformatics | Antioxidant Enzyme assay

Figure 2: Diagrammatic sketch of experimental activities

- **Morin staining**: In order to confirm the upward transportation of Al after root uptake, samples of roots, stems, flower stalks and green matured fruits were collected in biological replicates. Cross-sections of these samples (Fig 3) were made and stained with 100µM morin according to a slightly modified protocol by Tice et al. (1992). Epifluorescence was viewed using excitation and emission wavelengths of 405-445nm and 500-550nm respectively.

Figure 3: Cross sections of 'Micro-Tom' tissues

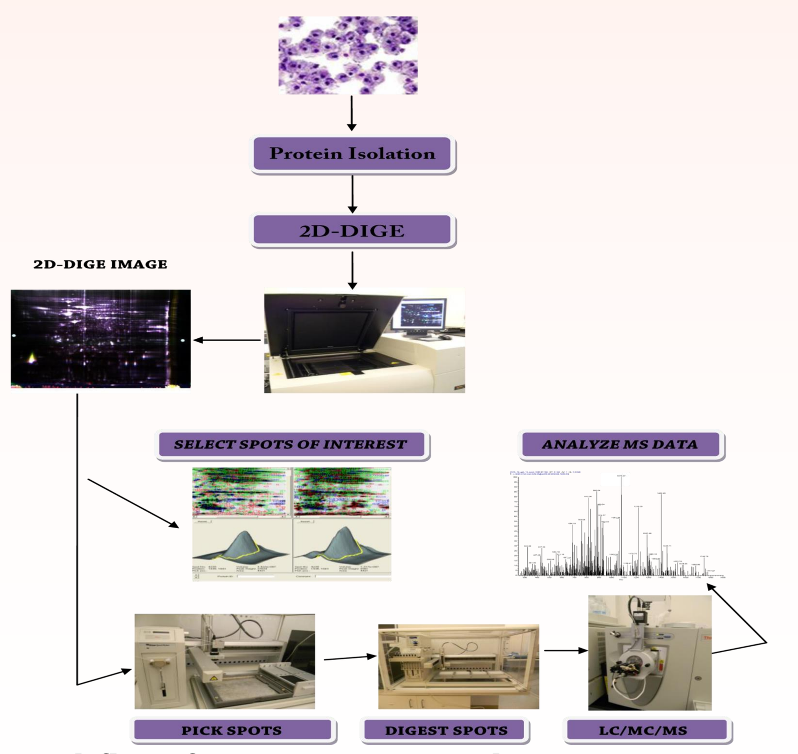

Figure 4: Workflow for proteomics analysis

Results

- **Morin staining:**

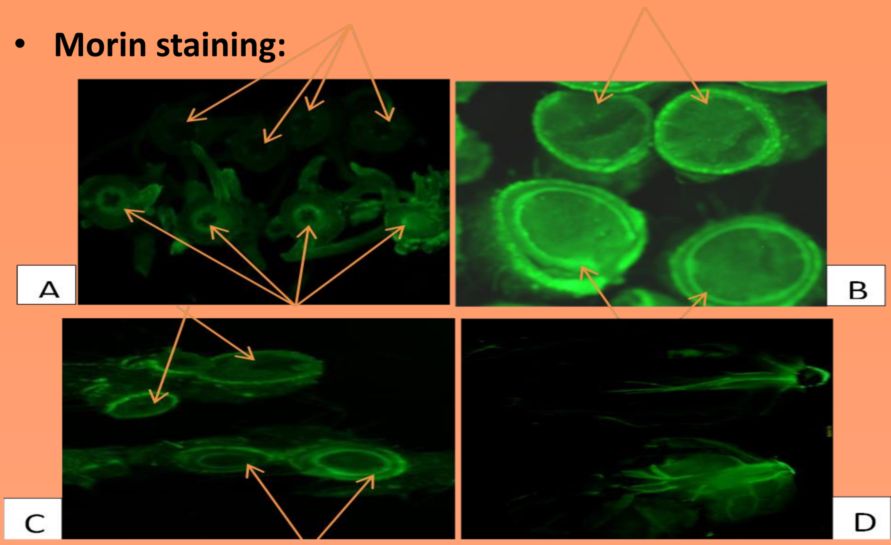

Figure 5: Cross sections of 'Micro-Tom' tissues stained with morin. A: Roots; B: Stems; C: Flower stalks; and D: Green fruits with seeds and pericarp. For each of the tissues, top row- control; and bottom row- Al treated. Arrows point to the vascular tissues of the respective tissues in A, B, and C.

- **Proteomic Analysis:**

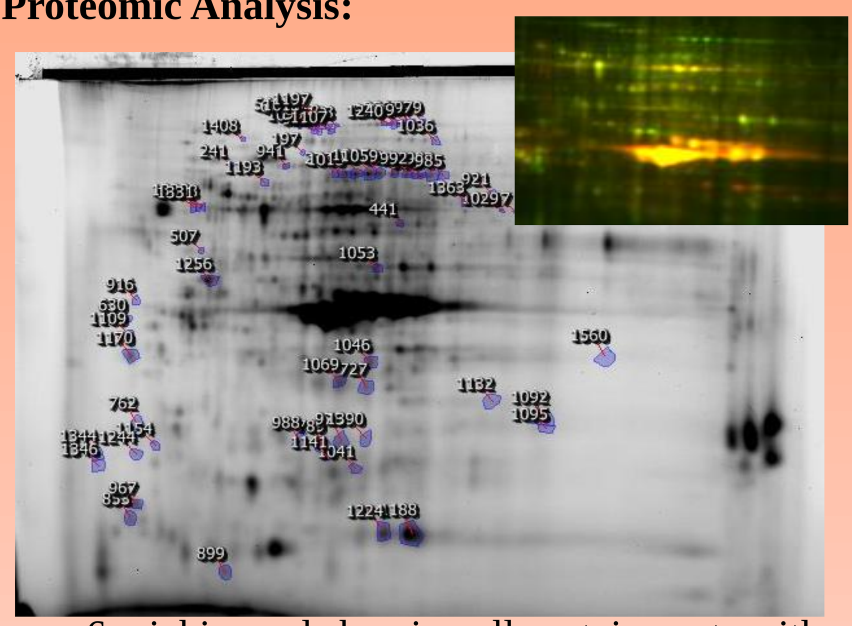

Figure 6: picking gel showing all protein spots with significant difference

Table 1. Al-regulated proteins in tomato seed tissues identified using MALDI-TOF-TOF analysis and database searches

<table>
<tr><th>Spot number</th><th>Accession number</th><th>Protein name</th><th>Fold change</th></tr>
<tr><td colspan="4">Suppressed proteins</td></tr>
<tr><td>762</td><td>gi|350537787|ref|NP_001234566.1|</td><td>Translationally-controlled tumor</td><td>-1.8</td></tr>
<tr><td>1109</td><td>gi|11125685|emb|CAC15504.1|</td><td>B2-type cyclin dependent kinase</td><td>-2.2</td></tr>
<tr><td>789</td><td>gi|350535533|ref|NP_001234711.1|</td><td>Protein suppressor of gene silencing</td><td>-2</td></tr>
<tr><td>1682</td><td>gi|349591296|gb|AEP95307.1|</td><td>Small heat shock protein</td><td>-2</td></tr>
<tr><td>916</td><td>gi|158827644|gb|ABW80999.1|</td><td>PI-phospholipase C PLC5</td><td>-1.5</td></tr>
<tr><td>899</td><td>gi|350536509|ref|NP_001234762.1|</td><td>fruit-specific protein</td><td>-2.0</td></tr>
<tr><td>1029</td><td>gi|312986083|gb|ADR31354.1|</td><td>ABA aldehyde oxidase</td><td>-1.5</td></tr>
<tr><td>630</td><td>gi|365818519|gb|AEX00348.1|</td><td>IAA2</td><td>-1.6</td></tr>
<tr><td>1677</td><td>gi|134612|sp|P14830.2|SODC1_SOLLC</td><td>Superoxide dismutase</td><td>-1.5</td></tr>
<tr><td>978</td><td>gi|349591296|gb|AEP95307.1|</td><td>class I small heat shock protein</td><td>-1.7</td></tr>
<tr><td>1390</td><td>gi|349591294|gb|AEP95305.1|</td><td>class I small heat shock protein 20.1</td><td>-1.5</td></tr>
<tr><td colspan="4">Induced proteins</td></tr>
<tr><td>1701</td><td>gi|315937172|gb|ADU56212.1|</td><td>Gag-pol polyprotein-retrotransposon</td><td>1.4</td></tr>
<tr><td>1224</td><td>gi|36783452|emb|CAE47523.1|</td><td>SANT/MYB protein</td><td>2</td></tr>
<tr><td>1053</td><td>gi|171854577|dbj|BAG16479.1|</td><td>Glutamate decarboxylase isoform</td><td>1.5</td></tr>
<tr><td>1331</td><td>gi|20269121|emb|CAC81817.1|</td><td>Glutamine synthase</td><td>1.6</td></tr>
<tr><td>1736</td><td>gi|68500714|gb|AAY98026.1|</td><td>ASR4</td><td>1.4</td></tr>
<tr><td>1050</td><td>gi|164472579|gb|ABY58971.1|</td><td>Jasmonate ZIM-domain protein 3</td><td>1.3</td></tr>
<tr><td>1059</td><td>gi|111073725|dbj|BAF02551.1|</td><td>Brassinosteroid hydroxylase</td><td>1.5</td></tr>
<tr><td>971</td><td>gi|804817|gb|AAA66051.1|</td><td>Malic Enzyme</td><td>1.5-2.0</td></tr>
<tr><td>1669</td><td>gi|350538691|ref|NP_001234099.1|</td><td>Alcohol dehydrogenase 2</td><td>1.7</td></tr>
<tr><td>1408</td><td>gi|125719302|gb|ABN54441.1|</td><td>Glutathione-S-transferase</td><td>1.5</td></tr>
<tr><td>241</td><td>gi|158635118|gb|ABW76421.1|</td><td>Heat shock protein 70</td><td>1.3</td></tr>
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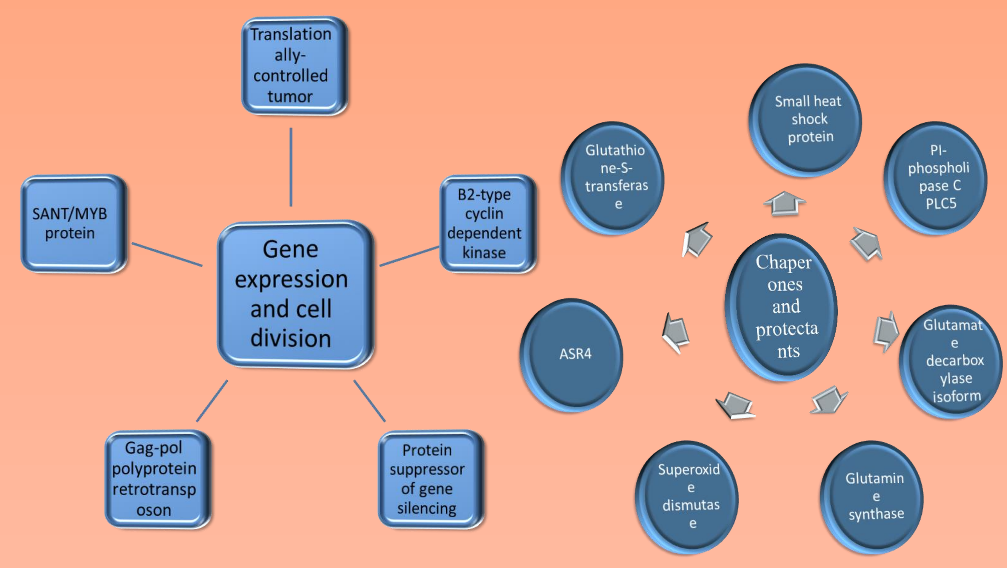
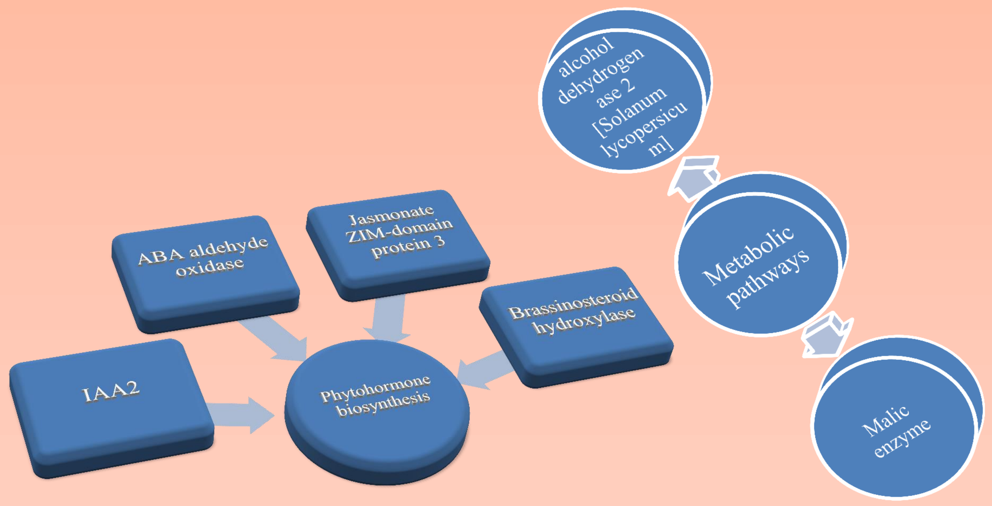
Discussion & Conclusions





When tomato plants are grown under excessive levels of Al, these Al-ions are transported into fruits, and seeds. These seeds develop in an environment of both elevated Al concentration and acidic pH as shown by the morin staining. Thus, they should contain endogenous Al.

However, the results of the morin staining are not conclusive as we have noticed very strong autofluorescence in those tissues.

Protein expression patterns and antioxidant activities support this observation by revealing that the tomato pericarp and/or seed tissues (the protein samples were mixtures of both) showed a change in protein expression (e.g. malic enzyme, gag-pol retrotransposon) that may affect tolerance to toxic ions.

This study provides valuable insights into the molecular mechanisms associated with Al toxicity in tomato. Additional works are proposed to identify mechanisms that will enhance Al-tolerance in tomato seeds, as this trait is very important for withstanding Al toxicity when crops are transplanted to acidic soils.

References

Tice, K. R., D. R. Parker, D. A. DeMason. 1992. Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat. Plant Physiology, 100: 309–318.