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Article Addendum

Aluminum induced proteome changes in tomato cotyledons

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Cotyledons of tomato seedlings that germinated in a 20 μM $AlK(SO_4)_2$ solution remained chlorotic while those germinated in an aluminum free medium were normal (green) in color. Previously, we have reported the effect of aluminum toxicity on root proteome in tomato seedlings (Zhou et al.[1]). Two dimensional DIGE protein analysis demonstrated that Al stress affected three major processes in the chlorotic cotyledons: antioxidant and detoxification metabolism (induced), glyoxylate and glycolytic processes (enhanced), and the photosynthetic and carbon fixation machinery (suppressed).

Different biochemical processes occur depending on the developmental stages of cotyledons. During early seed germination, before the greening of the cotyledons, glyoxysomes enzymes are very active. Fatty acids are converted to glucose via the gluconeogenesis pathway.[2,3] In greening cotyledons, chloroplast proteins for photosynthesis and leaf peroxisomal enzymes in the glycolate pathway for photorespiration are metabolized.[2-4] Enzymes involved in regulatory mechanisms such as protein kinases, protein phosphatases, and mitochondrial enzymes are highly expressed.[3,5,6]

The chlorotic cotyledons are similar to other chlorotic counterparts in that both contains lower levels of chlorophyll, thus the photosynthetic activities are not as active. In order to understand the impact of Al on tomato cotyledon development, a comparative proteome analysis was performed using 2D-DIGE following the as previously described procedure.[1] Some proteins accumulated differentially in Al-treated (chlorotic) and untreated cotyledons (Fig. 1). Mass spectrometry of tryptic digestion fragments of the proteins followed by database search has identified some of the differentially expressed proteins (Table 1). The proteome changes affected by Al stress are summarized in the followings.

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Antioxidant and Detoxification Proteins were Strongly Induced in Al-Treated Chlorotic Cotyledons

Aluminum and other heavy metals activate generation of reactive oxygen species (ros).[7,8] Excessive production of ros may lead to oxidative stress that result in elevated levels of oxidized proteins and oxidized lipids, loss of cell function, and ultimately apoptosis or necrosis. Antioxidant mechanisms are activated to protect against damages produced by ros.[1,9] Mondehydroascorbate reductase (mdar) is an enzymatic component of the ascorbate-glutathione cycle. The tomato homologous protein of mdar was induced 1.78 fold in al treated tissues (spot 7). Superoxide dismutase (sod), an enzyme that repairs cells and reduces the damage done to them by superoxide, and the corresponding enzyme protein (spot 92) were induced 1.29 fold.

Two proteins associated with proline synthesis, important component of stress responses in plants, were induced. Arginine-tRNA ligase, an enzyme that participates in arginine and proline metabolism, (spot 5) was induced 1.96 fold. Glutamate dehydrogenase (spot 15) was increased 1.66 fold in Al treated tissues. Glutamate dehydrogenase was found to be activated by ROS in order to detoxify ammonia and to produce Glu for the synthesis of praline.[10]

The Glyoxylate and Glycolytic Processes were more Active in Al-Treated Seedlings

During the early stages of seed germination, before the onset of photosynthesis, plants rely on stored nutrients to synthesize the various cellular structural molecules essential for cell division, growth, and for the production of ATPs needed to drive these reactions. Conversion of high energy lipids into glucose via the glyoxylate cycle occurs in cotyledons during the early stages of seed germination. Triglyceride lipases promote fatty acid β-oxidation to produce free fatty acids which are further converted to glucose. In Al treated tomato tissues, the lipase class 3 family (spot 3) was induced 2.0 fold, indicating that treated seeds were using fat in order to sustain the growth of seedlings while the control plants had became autotrophic through photosynthesis in the green cotyledons. Triosephosphate isomerase (TPI) plays an important role in glycolysis and is essential for efficient energy production.

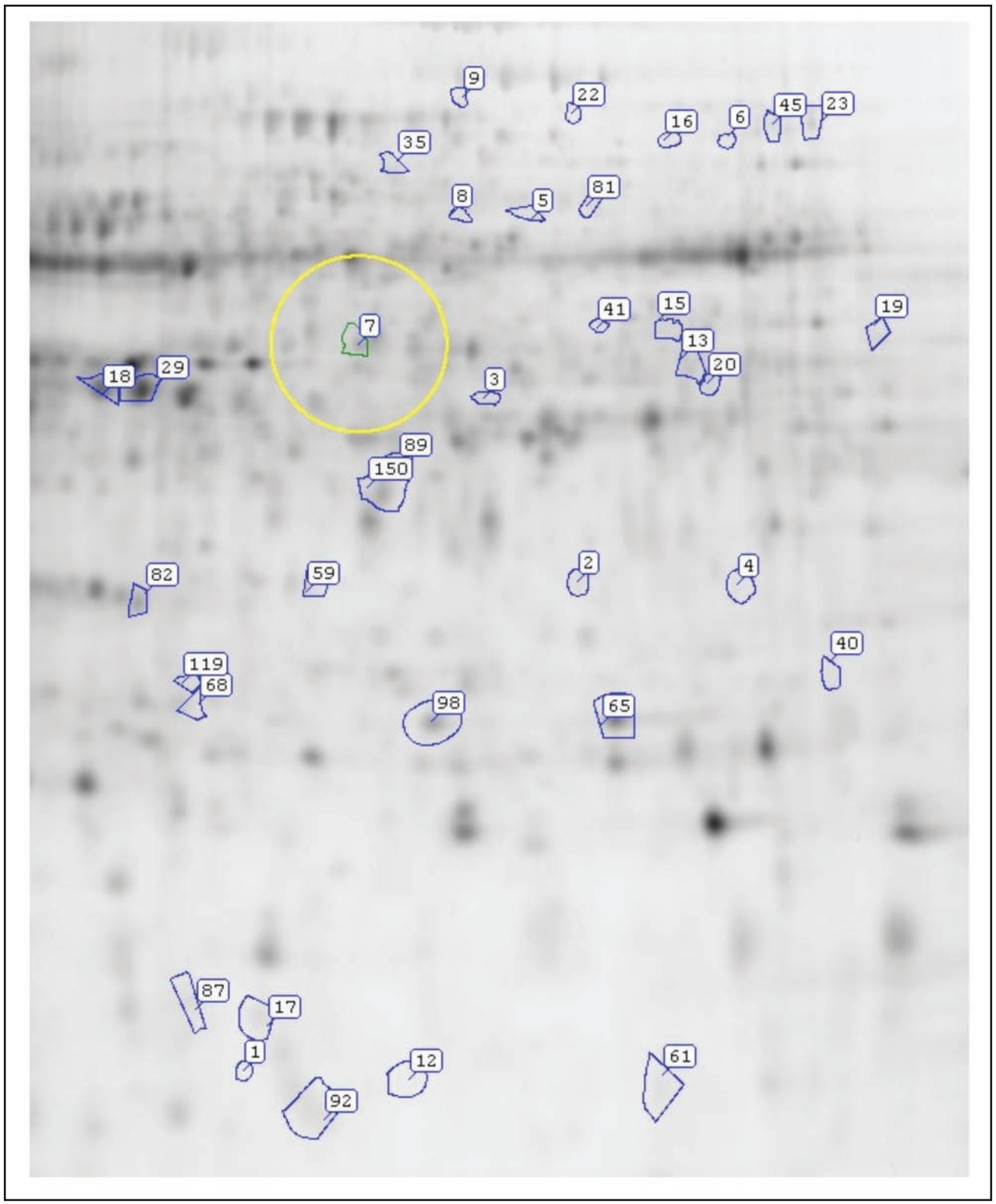

Figure 1. Image of protein spots generated by Samspot analysis of Al treated and untreated tomato cotyledons proteomes separated on 2D-DIGE.

Table 1 **Proteins identified from tomato cotyledons of seeds germinating in Al-solution**

Spot No.	Fold (treated/ctr)	ANOVA (p value)	Annotation	SGN accession
1	2.34	0.001374	12S seed storages protein (CRA1)	SGN-U314355
2	2.13	0.003651	unidentified	
3	2.0	0.006353	lipase class 3 family	SGN-U312972
4	1.96	0.002351	large subunit of RUBISCO	SGN-U346314
5	1.95	2.66E-05	arginine-tRNA ligase	SGN-U316216
6	1.95	0.003343	unidentified	
7	1.78	0.009219	Monodehydroascorbate reductase (NADH)	SGN-U315877
8	1.78	0.000343	unidentified	
9	1.75	4.67E-05	unidentified	
12	1.70	0.002093	unidentified	
13	1.68	0.004522	unidentified	
15	1.66	0.019437	Glutamate dehydrogenase 1	SGN-U312368
16	1.66	0.027183	unidentified	
17	1.62	2.01E-08	Major latex protein-related, pathogenesis-related	SGN-U312368
18	-1.61	0.009019	RUBisCo activase	SGN-U312543
19	1.61	0.003876	Cupin family protein	SGN-U312537
20	1.60	0.000376	unidentified	
22	1.59	0.037216	unidentified	
		0.003147	unidentified	
29	-1.56	0.001267	RUBisCo activase	SGN-U312543
35	1.52	0.001955	unidentified	
40	1.47	0.007025	unidentified	
41	1.47	0.009446	unidentified	
45	1.45	0.001134	unidentified	
59	-1.40	5.91E-05	12 S seed storage protein	SGN-U314355
61	1.39	1.96E-05	MD-2-related lipid recognition domain containing protein	SGN-U312452
65	1.37	0.000608	triosephosphate isomerase, cytosolic	SGN-U312988
68	1.36	0.004225	unidentified	
81	1.32	0.001128	unidentified	
82	-1.31	0.001408	33 kDa precursor protein of oxygen-evolving complex	SGN-U312530
87	1.30	0.002306	unidentified	
89	-1.3	0.000765	unidentified	
92	1.29	0.000125	superoxide dismutase	SGN-U314405
98	1.28	0.000246	triosephosphate isomerase, cytosolic	SGN-U312988

Two isoforms of the cytosolic TPI (spot 65, 1.37 fold, spot 98, 1.28 fold) were identified, and both were induced in Al treated tomato tissues.

The Photosynthetic Machinery was Affected by Aluminum Toxicity

Spot 82, identified to be 33 kDa precursor protein of oxygen-evolving complex, was suppressed (-1.31 fold). Two isoforms of RUBisCo. activase in tomato were suppressed, (spot 29, -1.56 fold, and spot 18, -1.61 fold). The 33 kDa protein is one of the three subunits of the oxygen evolving complex (OEC) responsible for oxidation of water and releasing of molecular oxygen in the PSII complex. Rubisco activase serves as a chaperone for the promotion and maintenance of Rubisco's catalytic activities. The lower amount of such proteins could be related to chlorotic cotyledons. Low chlorophyll contents in chlorotic cotyledons of Al-treated seedlings could result in deficiency in absorbing light energy, consequently, fewer electrons were passed from PS I onto PSII. The Al-induced reduction in the photosynthetic machinery could delay the development of seedlings into mature plants.

The large subunit of RUBISCO (spot 4) was induced 1.96 fold. Several proteins associated with disease and stress resistance were induced. These included major latex protein-related, pathogenesis-related (spot 17, 1.62 fold), cupin family of proteins (spot 19, 1.61 fold) and MD-2-related lipid recognition domain containing protein (spot 61, 1.39 fold).

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