Title:
Proteomics study of COI1-regulated proteins in *Arabidopsis* flower.

Running title:
Proteomics profiles of COI1-regulated male fertility.

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Abstract

Jasmonates (JA) are a new class of plant hormone that regulate expression of diverse genes to mediate various plant responses. The Arabidopsis F-box protein COI1 is required for plant defense and male fertility in JA signal pathway. To further investigate the regulatory role of COI1 in male fertility, we compared the proteomics profiles of Arabidopsis wild-type (WT) flowers with coi1-1 mutant male-sterile flowers using two-dimensional difference gel electrophoresis coupled with MALDI-TOF mass spectrometry. 16 proteins with potential function in specific biological processes such as metabolism processes and defense/stress responses were differentially expressed in WT and coi1-1 mutant flowers. Verification on a phi class glutathione transferase AtGSTF9, one out of these 16 identified proteins, revealed that the expression of AtGSTF9 was severely down-regulated in flowers of coi1-1 mutant compared with that of WT. Further function analyses of these genes would provide new insights into the molecular basis of COI1-regulated male fertility.

Key Word: jasmonate, COI1, male fertility, 2-D DIGE
Introduction

*Arabidopsis* flowers are organized into four concentric whorls: sepals form first, followed by petals, and then by stamen, and finally by carpels. Stamens, the male reproductive organs of flowering plants, coincide with the pistil development to determine the fertility (Smyth et al., 1990). Mutations that impair stamen development such as filament elongation, pollen maturation or anther dehiscence will result in male sterility (Chaudhury, 1993; Taylor et al., 1998). A large of number genes have been found to regulate the stamen development process (McCormick, 2004; Nakayama et al., 2005; Alves-Ferreira et al., 2007). Stamen development is also subjected to hormonal control (Cecchetti et al., 2008; Cheng et al., 2009).

Jasmonates, including jasmonic acid and its derivatives, act as regulators in many plant development and growth processes, and also play pivotal roles in plant responses to abiotic and biotic stresses (McConn et al., 1997; Rao et al., 2000; Sasaki et al., 2001; Cheong and Choi, 2003; Farmer et al., 2003; Howe, 2004; Xiao et al., 2004; Schilmiller and Howe, 2005; Shan et al., 2007; Wasternack, 2007; Kim et al., 2009; Koo and Howe, 2009). In *Arabidopsis*, JA is also essential for plant defense and male fertility. The JA-synthesis deficient mutants including *fad3-2 fad7-2 fad8, dad1, aos, dde1* and *opr3*, all show defects in stamen development resulting in male sterile phenotypes (McConn and Browse, 1996; Sanders et al., 2000; Stintzi and Browse, 2000; Ishiguro et al., 2001; Park et al., 2002). These mutants could be restored to fertility by exogenous application of methyl jasmonate (MeJA) on developing flower buds (McConn and Browse, 1996; Sanders et al., 2000; Stintzi and Browse, 2000; Ishiguro et al., 2001; Park et al., 2002). Using microarray and differential display approaches, Mandaokar et al. found that 38 genes, which were involved in a variety of physiological events such as amino acid metabolism, cell wall modification and defense/stress responses, were differentially expressed between wild-type (WT) and *opr3* mutant anthers (Mandaokar et al., 2003). Furthermore, transcriptome analyses of JA-treated *opr3* mutant anthers revealed that a lot of transcription factors such as *myb21* and *myb24* may regulate the stamen maturation process triggered by JA
The F-box protein COI1 (Xie et al., 1998), as a JA receptor, directly binds to
JA-isoleucine (JA-Ile) (Yan et al., 2009), assembles SCF^{COI1} complex (Xu et al., 2002;
Liu et al., 2004; Ren et al., 2005; Wang et al., 2005) and recruits jasmonate
ZIM-domain proteins (JAZs) for degradation by the 26S proteasome to derepress the
JA primary responsive genes (Chini et al., 2007; Thines et al., 2007; Katsir et al.,
2008). The null mutant coi1-1 displays insensitivity of JA-inhibitory root growth and
susceptibility to insect attack and pathogen infection (Feys et al., 1994; Xie et al.,
1998; Reymond et al., 2000). Microarray gene transcription profiling of WT and coi1
mutant seedlings revealed that COI1 was required for the transcription of many genes
that functioned in JA-regulated secondary metabolism and defense (Devoto et al.,
2005). The coi1-1 mutant also exhibits male sterile phenotypes including retarded
filament elongation and delayed anther dehiscence (Feys et al., 1994; Xie et al.,
1998; Xu et al., 2002). Comparison of expression profiles between Arabidopsis WT flowers
and coi1-1 mutant flowers would give new enlightenment for COI1 function in male
fertility.

Recently, two-dimensional difference gel electrophoresis (2-D DIGE), a new
emerging technology that was build on two-dimensional gel electrophoresis (2-DE)
by adding a highly accurate quantitative dimension, was developed for proteomics
analysis. It has been successfully performed to identify proteins regulated by UV-B
(Casati et al., 2005), salt and osmotic stresses (Ndimba et al., 2005), cold stress
(Aemme et al., 2006), fungal elicitors (Chivasa et al., 2006), gibberellins (Komatsu et
al., 2006), and brassinosteroid (Deng et al., 2007; Tang et al., 2008) in many plant
species. In this study, we used 2-D DIGE and MALDI-TOF mass spectrometry to
compare the proteomics profiles of Arabidopsis WT flowers with coi1-1 mutant
male-sterile flowers. A total of 16 differentially expressed proteins have been
identified. Of these, 12 proteins were up-regulated while 4 were down-regulated in the
flowers of WT compared with that of coi1-1 mutant. These proteins were predicted to
play potential roles in specific biological processes, such as metabolism processes and
defense/stress responses.


Results and Discussion

Analysis of proteomics changes between WT and coi1-l mutant flowers

Similar to previously observations (Feys et al., 1994; Xie et al., 1998; Xu et al., 2002), we found that all the flowers in coi1-1 mutant could not develop normally to set seeds (Figure 1, A and B). Closer examination of WT and coi-1 mutant flowers showed that the filaments of coi1-l mutant could not elongate sufficiently to position the anther onto the stigmatic papilla (Figure 1C). Meanwhile, the anther of coi1-1 mutant could not dehisce at the time of flower opening (Figure 1C).

To examine the regulatory role of COI1 in male fertility, we harvested flowers (Figure 1C) from both 5-week-old WT and coi-1 mutant plants for protein extraction and 2-D DIGE analysis. Comparing the protein profiles between WT and coi1-l mutant showed that the abundance changes of 33 protein spots were significant (at least 50% increase or decrease, \( p<0.05 \)). Peptide mass fingerprinting via MALDI-TOF mass spectrometry on these 33 protein spots successfully generated protein assignments for 23 spots, which represented 16 unique proteins (Figure 2 and Table 1). Among these, 11 spots represented 11 unique proteins; the remaining 12 spots represented 5 proteins. More than two spots represented a single protein in some cases (Figure 2 and Table 1), which probably resulted from the posttranslational modifications that shifted the mobility in 2-DE. The discrepancy between the experimental and theoretical MW and pI of some proteins (Table 1) might be caused by changes in posttranslational modifications, proteolytic degradation of polypeptides, or variability arising from alternate splicing of mRNAs. In WT flowers, 12 out of these 16 proteins were up-regulated whereas 4 were down-regulated compared with coi1-1 mutant male sterile flowers.

Classification of the 16 COI1-regulated proteins

These 16 COI-regulated proteins were grouped following the Gene Ontology (GO) categories based on: (1) the biological process (BP) in which the protein
participates; (2) the cellular component (CC) where the protein can be found; (3) the molecular function (MF) that describes the protein activities at molecular level. Protein distributions according to the GO rules were shown in Figure 3.

Proteins involved in metabolism processes In this study, we identified 7 COI1-regulated proteins that were involved in a variety of metabolism processes. Of these, 5 proteins (At5g26000, At5g25980, At4g08870, At5g24420 and At2g39990) were more strongly expressed in WT flowers whereas 2 proteins (At3g13470 and At2g05990) were more strongly expressed in *coi1-1* mutant male sterile flowers. At5g26000 and At5g25980 encode TGG1 and TGG2 respectively, two known functional myrosinase enzyme involved in glucosinolate catabolic process. TGG1 and TGG2 both catalyze the hydrolysis of glucosinolates into compounds that are toxic to various microbes and herbivores (Xue et al., 1995). At4g08870 encodes an arginase functioned in polyamine metabolic process. At5g24420 encodes a glucosamine/galactosamine-6-phosphate isomerase, which is known to catalyze the reaction in pentose-phosphate shunt of carbohydrate metabolic process. At2g39990 encodes a translation initiation factor eIF2 p47 subunit homolog involved in protein metabolism process. At3g13470 encodes a chaperonin, which also functions in one branch of protein metabolism process: protein folding. At2g05990 encodes an enoyl-ACP reductase that acts as a component of the fatty acid synthase complex (Mou et al., 2000). Anther development has been shown to be involved in an active stage of protein and lipid metabolism for pollen wall formation (Wang et al., 2005). These proteins with predicted roles in carbohydrate, protein and lipid metabolism may function in pollen wall formation or other aspects in stamen development.

Proteins involved in defense/stress responses In this study, we found 9 COI1-regulated proteins that functioned in different kinds of defense/stress responses. Of these, the expression of 7 proteins (At5g24780, At5g24770, At3g16470, At1g52400, At1g52040, At1g19570 and At2g30860) was induced whereas the expression of 2 proteins (At1g04410 and At5g01410) was repressed in WT flowers compared with *coi1-1* mutant male sterile flowers. At5g24780 and At5g24770 encode two vegetative storage proteins VSP1 and VSP2 respectively and their expression is
induced by wounding and JA (Berger et al., 1995). At3g16470 also encodes a JA inducible protein isolog, which functions in plant responses to abiotic stimulus including cold, wounding and salt (Leon et al., 1998; Bae et al., 2003; Jiang et al., 2007). At1g52400, which encodes a member of glycosyl hydrolase family 1, is required in wound-inducible ER body formation and defense response to fungus (Xu et al., 2004). At1g52040 encodes a myrosinase-binding protein MBP1 functioned in plant defense response. At1g19570 encodes a dehydroascorbate reductase that plays an important role in plant ozone tolerance and response to symbiotic fungus (Yoshida et al., 2006; Vadasserya et al., 2009). At2g30860 encodes AtGSTF9, a member of glutathione transferases belonging to the phi class that is important in plant defense responses (Wagner et al., 2002). At1g04410 encodes a malate dehydrogenase involved in salt stress and cadmium exposure response (Sarry et al., 2006; Jiang et al., 2007). At5g01410 encodes a pyridoxine biosynthesis protein (PDX) 1, which is essential for the biosynthesis of vitamin B6, development and stress tolerance in Arabidopsis (Titiz et al., 2006). It has been reported that anther development was an environment sensitive process (Dix et al., 1996). The corresponding stress responses of these proteins might correlate with the process of anther development.

Based on the above analyses, most of these genes encodes enzyme that potentially mediate specific cellular and physiological processes and therefore function as the downstream targets of COI1. Primary COI1-regulated genes such as transcription factors were not detected in this study, which might be caused by less sensitivity of 2-D DIGE in low-abundance protein identification. To detect primary COI1-regulated proteins, protein enrichment should be essential.

**Comparative analysis of proteomics and transcriptomics data**

Changes at RNA level might not necessarily lead to similar changes at protein level (Huber et al., 2004). Gene expression is also regulated by translational or posttranslational processes (Griffin et al., 2002; Tian et al., 2004). By comparison of our proteomics data with previous transcriptomics data (Capella et al., 2001; Devoto et al., 2005), we found that 7 of these 16 COI1-regulated proteins were identified as
COI1-regulated RNAs in Arabidopsis flowers (Table 1). 6 genes, including At5g26000, At4g08870, At5g24770, At3g16470, At1g52040 and At2g30860, were up-regulated in WT flowers compared with coi1-1 mutant male sterile flowers at transcriptional level (Capella et al., 2001; Devoto et al., 2005), which was correlated well with our proteomics data (Figure 2 and Table 1). However, the transcriptional pattern of the remaining gene, At2g05990, was not consistent with its protein expression pattern. The transcripts of At2g05990 were up-regulated (Devoto et al., 2005), whereas its protein was down-regulated in WT flowers compared to coi1-1 mutant male sterile flowers (Figure 2 and Table 1), which might result from posttranscriptional processes.

Confirmation of 2-D DIGE data

To verify the 2-D DIGE data, we used Western blot approach to further examine the expression pattern of a phi class glutathione transferases AtGSTF9, one out of the 16 COI1-regulated proteins. In our proteomics study, we found that the expression level of AtGSTF9 protein was severely reduced in the flowers of coi1-1 mutant compared with that in WT (Table 1). Consistent with our 2-D DIGE results, Western blot analysis also showed that the expression of AtGSTF9 protein was greatly affected in coi1-1 male sterile flowers (Figure 4), which demonstrates that 2-D DIGE analysis yields relatively accurate measurement of protein expression. Molecular and genetic approaches will be used to examine whether AtGSTF9 has possible role in COI1-regulated male fertility.

Conclusion

In this study, we identified 16 COI1-regulated proteins by comparing the proteomics profiles of WT with coi1-1 mutant flowers. This study is the first application of proteomics to study on COI1 function in JA-regulated male fertility. These proteins are predicted to function in specific biological processes such as metabolism processes and defense/stress responses. Further characterization of these
proteins might contribute to the understanding of the molecular mechanism of COI1-regulated male fertility.

Materials and Methods

Plant materials

The *Arabidopsis* mutant *coi1-1* was described previously (Feys et al., 1994). Seeds were surface-sterilized, plated on Murashige and Skoog medium (Sigma, Saint Louis, MO, USA) supplemented with 1% sucrose, chilled at 4°C for 3 days, and then grown under a 16-h-light (23-25°C)/8-h-dark (17-20°C) photoperiod. Same photoperiod was followed for soil-grown plants.

Extraction of total proteins for 2-D DIGE

For 2-D DIGE analysis (Figure 2), flowers from both 5-week-old WT and *coi1-1* mutant plants were harvested for protein extraction. Flowers were homogenized in ice-cold extraction buffer (0.7M sucrose, 0.1M KCl, 0.5M Tris-HCl pH7.4, 50mM EDTA, 1% w/v PVPP, 1mM PMSF, 0.2% β-mercaptoethanol) supplemented with the protease inhibitor cocktail (Roche, Mannheim, Germany) and placed on ice for 30 min. The protein extracts were then mixed with an equal volume of ice-cold phenol (Tris-HCl pH8.0 buffered) and incubated for 30 min at 4°C shaking. After centrifugation at 5000g for 20 min, the proteins in the upper phenol phase were precipitated with 5 volumes of cold 0.1M ammonium acetate in methanol at -20°C overnight and centrifugated at 5000g for 20 min. The pellet was then washed two times with ice-cold 0.1M ammonium acetate in methanol, two times with 80% v/v acetone, once with 70% v/v ethanol and then dissolved in the lysis buffer (7M urea, 2M thiourea, 4% w/v CHAPS, 30mM Tris-HCl pH8.0, pH adjusted to 9). The proteins were cleaned up using 2D clean up kit (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and resuspended in the lysis buffer after the pH was adjusted to about 8.5. 50μg proteins were labeled with 400pmol of DIGE specific Cy3 or Cy5 (GE healthcare, Piscataway, NJ) and incubated on ice in the dark for at least 30 min.
The reaction was stopped by adding 1μl of 10mM lysine under the same condition. The pooled sample internal standard was always Cy2-labeled.

**Two-dimensional gel electrophoresis**

Labeled protein samples to be separated in the same gel were mixed together with an equal volume of rehydration solution (8M urea, 2% w/v CHAPS, 0.002g/ml DTT and 1% IPG buffer). The first dimension isoelectric focusing (IEF) was performed on an Ettan IPGphor IEF system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) with an 24cm, pH gradient from 4 to 7 strip. After a total of 84250 vhs IEF, IPG strips were treated with the equilibration buffer (6M urea, 30% w/v glycerol, 2% w/v SDS, 50mM Tris-HCl pH8.0 and 1% bromophenol blue) first with 0.01g/ml DTT, and then with 0.025g/ml iodoacetamide. The strips were then overlayed onto 12.5% SDS-PAGE gels using the Ettan Dalt six apparatus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and electrophoresed at 1W/gel for an hour followed by 17W/gel at 15°C until the bromophenol blue dye front had run off the bottom of the gels.

**Image scanning and spot analysis**

Cy2-, Cy3-, and Cy5-labeled images of each gel were acquired on a Typhoon laser scanner (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at excitation/emission values of 488/520 nm, 532/580 nm, and 633/670 nm, respectively. Gel analysis was carried out by the Decyder v6.5 software (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). First, the Decyder differential in-gel analysis module was used to perform the spot detection, spot volume quantification and volume ratio normalization of different samples in the same gel. Then, the Decyder biological variation analysis (BVA) software module was used to perform the gel to gel matching and statistical analysis. The statistical significance of quantitative data was determined using the Student’s $t$-test ($n = 3$). A spot with abundance ratio $>1.5$ or $<-1.5$ and $p<0.05$ was set as a threshold to identify differently expressed proteins. Differently expressed protein spots were then subjected to in-gel trypsin digestion and
PMF analysis using a Voyager DE STR MALDI-TOF mass spectrometer (Applied Biosystems) with Proteomics Solution 1 (PS1) software (Applied Biosystems). The obtained spectrum was analyzed with the Data Explorer software. Self-degraded fragments of trypsin were used for internal calibration, while the standard peptide mixtures (angiotensin II, angiotensin I, substance P, bombesin, ACTH clip 1-17, ACTH clip 18-39, and somatostatin 28) were used for external mass calibration.

**Protein identification and database search**

The mass spectrum data were used to search for protein candidates using MS-Fit in the National Center for Biotechnology Information non-redundant (NCBI-nr) database. MS-Fit searching parameters were as follows: molecular weight searched from 1000 to 100000 Da and pI searched from 0 to 14; allowing one trypsin missed cleavage site, four minimum number of peptides to match and a mass accuracy of 50 ppm; N terminus-Hydrogen (H) and C terminus-Free Acid (OH); fixed modifications-carbamidomethylation of Cys (C) and considered modifications-phosphorylation of Ser (S), Thr (T) and Tyr (Y).

**Gene ontology annotation**

Functional categorization of identified proteins was performed according to the GO rules by the program at http://www.arabidopsis.org/tools/bulk/go/index.jsp.

**Western blot**

The Western blot analysis was performed as previously described procedures (Xu et al., 2002). The AtGSTF9 antibody was made by Alpha Diagnostic Intl. Inc. The Ribulose bisphosphate carboxylase oxygenase activase (RCA) antibody was generated by Jiang et al (Jiang et al., 2001).

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Reference


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Figure legends

Figure 1. Phenotypes of WT and coi1-1 mutant.
(A) Main shoots of 5-week-old WT (left) and coi1-1 mutant (right) plants grown in soil.
(B) Fully developed siliques of 5-week-old WT (top) and coi1-1 mutant (bottom) plants grown in soil.
(C) Opened flowers of 5-week-old WT (left) and coi1-1 mutant (right) plants grown in soil. A, anther; F, filament; S, stigma.

Figure 2. A representative superimposed 2-D DIGE image of Cy3-labeled WT, Cy5-labeled coi1-1, and Cy2-labeled internal standard. Differentially expressed proteins between WT and coi1-1 mutant flowers were identified by MALDI-TOF mass spectrometry and marked with spot numbers (their identities were shown on Table 1). The spots marked with white solid and white dashed arrows indicated up-regulated or down-regulated in WT over the coi1-1 mutant respectively.

Figure 3. Functional categorization of the 16 COI1-regulated proteins according to different GO categories: biological processes (BP), cellular components (CC) and molecular functions (MF).

Figure 4. AtGSTF9 expression pattern at protein level in WT flowers and coi1-1 mutant male sterile flowers. The immunoblot was detected with Ribulose bisphosphate carboxylase oxygenase activase (RCA) antibody as a protein loading control.
Table 1 COI1-regulated proteins in *Arabidopsis* flowers.

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Gene Locus</th>
<th>Protein name</th>
<th>Possible function</th>
<th>coi1-1/Col-0 ratio*</th>
<th>coi1-1/Col-0 p-value</th>
<th>Mowse score</th>
<th>No. of Matching Peptides</th>
<th>Protein Coverage</th>
<th>Observed MW (KD)</th>
<th>Theoretical MW (KD)</th>
<th>Observed pl</th>
<th>Theoretical pl</th>
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<tbody>
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<td>At5g26000</td>
<td>Myrosinase</td>
<td>Glucosinolate metabolism</td>
<td>-2.11</td>
<td>3.20E-04</td>
<td>1.80E+13</td>
<td>20</td>
<td>47%</td>
<td>85.0</td>
<td>54.2</td>
<td>5.00</td>
<td>5.33</td>
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<td>Response to cold, salt, and wounding</td>
<td>-7.04</td>
<td>3.50E-04</td>
<td>1.67E+11</td>
<td>19</td>
<td>60%</td>
<td>50.0</td>
<td>48.5</td>
<td>5.00</td>
<td>5.12</td>
</tr>
<tr>
<td>16</td>
<td>At3g16470</td>
<td>Jasmonate inducible protein isolog</td>
<td>Response to cold, salt, and wounding</td>
<td>-8.77</td>
<td>2.70E-03</td>
<td>9.70E+10</td>
<td>18</td>
<td>54%</td>
<td>50.0</td>
<td>48.5</td>
<td>5.00</td>
<td>5.12</td>
</tr>
<tr>
<td>17</td>
<td>At1g32400</td>
<td>Beta-glucosidase homolog 1</td>
<td>Response to fungus</td>
<td>-16.48</td>
<td>1.00E-03</td>
<td>1.14E+12</td>
<td>16</td>
<td>47%</td>
<td>70.0</td>
<td>60.4</td>
<td>6.70</td>
<td>6.75</td>
</tr>
<tr>
<td>18</td>
<td>At1g32400</td>
<td>Beta-glucosidase homolog 1</td>
<td>Response to fungus</td>
<td>-11.85</td>
<td>2.90E-03</td>
<td>4.05E+12</td>
<td>18</td>
<td>41%</td>
<td>70.0</td>
<td>60.5</td>
<td>6.70</td>
<td>6.75</td>
</tr>
<tr>
<td>19</td>
<td>At1g20400</td>
<td>Beta-glucosidase-binding protein</td>
<td>Response to stress</td>
<td>-2.04</td>
<td>1.20E-02</td>
<td>9.11E-07</td>
<td>12</td>
<td>32%</td>
<td>50.0</td>
<td>50.0</td>
<td>5.50</td>
<td>5.38</td>
</tr>
<tr>
<td>20</td>
<td>At1g19570</td>
<td>Dehydroascorbate reductase</td>
<td>Response to ozone and symbiotic fungus</td>
<td>-1.87</td>
<td>5.60E-04</td>
<td>2.38E+08</td>
<td>11</td>
<td>68%</td>
<td>25.0</td>
<td>23.6</td>
<td>5.50</td>
<td>5.56</td>
</tr>
<tr>
<td>21</td>
<td>At2g08660</td>
<td>Glutathione S-transferase, putative</td>
<td>Response to stress</td>
<td>-3.30</td>
<td>1.50E-04</td>
<td>1.29E-05</td>
<td>8</td>
<td>47%</td>
<td>25.0</td>
<td>24.1</td>
<td>6.60</td>
<td>6.17</td>
</tr>
<tr>
<td>22</td>
<td>At1g04410</td>
<td>Malate dehydrogenase</td>
<td>Response to salt and cadmium exposure</td>
<td>1.70</td>
<td>4.90E-04</td>
<td>3.11E+08</td>
<td>11</td>
<td>48%</td>
<td>37.0</td>
<td>35.5</td>
<td>5.90</td>
<td>6.11</td>
</tr>
<tr>
<td>23</td>
<td>At5g01410</td>
<td>Pyridoxine biosynthesis protein-like</td>
<td>Response to stress</td>
<td>1.61</td>
<td>7.00E-03</td>
<td>5.69E+03</td>
<td>7</td>
<td>22%</td>
<td>30.0</td>
<td>33.1</td>
<td>5.80</td>
<td>5.79</td>
</tr>
</tbody>
</table>

16 COI1-regulated proteins in *Arabidopsis* flowers were classified based on the biological process in which the protein participated and their corresponding spots were shown on Figure 2.

a, COI1-regulated genes identified previously in *Arabidopsis* flowers by transcriptomics analysis. b, Average volume ratio quantified by DeCyder 6.5 BVA module.

c, Number of peptide masses matching the top hit from MS-Fit PMF. d, Amino acid sequence coverage for the identified proteins.
Figure 1

A

B

C
Figure 2
Figure 3

BP
- response to stress
- other metabolic processes
- response to abiotic or biotic stimulus
- cellular processes
- other biological processes
- protein metabolism
- cell organization and biogenesis

CC
- other intracellular components
- other cytoplasmic components
- chloroplast
- plastid
- nucleus
- cytosol
- extracellular
- ribosome
- plasma membrane
- mitochondria
- other membranes
- cell wall
- other cellular components
- unknown cellular components

MF
- other enzyme activity
- hydrolase activity
- other binding
- protein binding
- transferase activity
- nucleotide binding
- nucleic acid binding
Figure 4

Col-0  coi1-1

anti-AtGSTF9

anti-RCA