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# DE-FG02-05ER15649: Transcription Factors Expressed in Lateral Organ Boundaries: Identification of Downstream Targets

Lateral organ initiation and patterning involve communication between the shoot apical meristem (SAM) and initiating organ primordia. The goal of this project was to characterize three boundary-expressed genes that encode predicted transcription factors. Specifically, we have studied *LATERAL ORGAN BOUNDARIES* (*LOB*), *LATERAL ORGAN FUSION1* (*LOF1*), and *LATERAL ORGAN FUSION2* (*LOF2*). *LOB* encodes the founding member of the LOB-DOMAIN (LBD) plant-specific DNA binding transcription factor family (1,2) and *LOF1* and *LOF2* encode paralogous MYB-domain transcription factors (3).
We characterized the genetic relationship between these three genes and other boundary and meristem genes. We also used an ectopic inducible expression system to identify direct targets of LOB.

## I. Genetic interaction studies:

Mutations in *LOB* and *LOF1* result in defects in boundary specification or maintenance, leading to fusion between axillary branches and the subtending cauline leaf. In addition, *lof1* mutants lack accessory meristems (a class of axillary meristems that normally form at the base of a primary axillary branch). Mutations in *lof2* do not result in a conspicuous phenotype, but enhance the *lof1* mutation, such that in addition to axillary branch-leaf fusions, *lof1 lof2* mutants exhibit fusions between floral pedicels and the inflorescence stem (3). While the *lob* and *lof1* mutant phenotypes are quite specific, they are limited to only a subset of the regions expressing these genes. Both genes are part of large gene families and a number of other transcription factors are also expressed in the boundary (reviewed in 4). Thus, we hypothesize that genetic redundancy masks phenotypes in many places where these genes are expressed. The enhanced *lof1 lof2* double mutant phenotype is consistent with this hypothesis.

To understand the relationship between *LOB, LOF1, LOF2*, and other genes important for boundary and meristem regulation, we generated and analyzed a number of double mutant combinations between *lob*, *lof1*, *lof2* and genes that function in boundary and meristem establishment or maintenance. Prior to the start of this project, we had observed that double mutants between *lateral suppressor* (*las*) and either *lof1* or *lof2* exhibited dramatically enhanced fusions. We had also observed that ectopic expression of the meristem-specific transcription factor SHOOT MERISTEMLESS (STM) resulted in ectopic expression of *LOF1* and *LOF2*, suggesting that STM positively regulates *LOF1/LOF2* expression. In contrast, *LOF1* and *LOF2* expression was expanded in the *pennywise* (*pny*) mutant, suggesting that PNY contributes to negatively regulating their expression. We had also found that the *lof1* mutant suppresses some aspects of the *pny* single mutant and the *pny poundfoolish* (*pnf*) double mutant. We also had data implicating LOB as a negative regulator of response to the phytohormone brassinosteroid (BR), based on *LOB* ectopic expression. In this project, we explored interactions and our genetic interaction data are summarized in Table 1.

Our data indicate that *LOB* and *LOF1*/*LOF2* define two parallel pathways to control boundary specification or elaboration. In addition, the enhanced meristem defects revealed when *lof1* or *lof2* were combined with *stm-10 (*a weak allele), *las*, *cuc2*, and *cuc3* indicate that *LOF1* and *LOF2* contribute to maintenance of the shoot apical meristem and axillary meristems.

## II. Identification of LOB targets.

We used an ectopic inducible expression system that is based on the steroid-inducible glucocorticoid receptor (GR) to identify LOB targets. A transcription factor (TF) that is fused to the hormone-binding domain of GR is prevented from entering the nucleus in the absence of steroid hormone. Upon application of a steroid hormone such as dexamethasone (DEX), the TF-GR fusion protein is transported to the nucleus to regulate the expression of downstream genes. We generated transgenic Arabidopsis plants that express LOB fused to GR, under control of the ubiquitously expressed CaMV *35S* promoter. To identify downstream targets of LOB, we conducted Affymetrix gene chip experiments with *LOB-GR* plants, following a short period of exposure to DEX. We treated 7-day-old wild-type Columbia and *35S:LOB-GR* seedlings with either mock, DEX, cycloheximide (CHX), or DEX + CHX. Three biological replicates were performed. RNAs were isolated and used in hybridization experiments to the Affymetrix Ath1 chip. These array experiments identified 113 genes whose transcript levels increased or decreased by 2-fold or more ($P \leq 0.01$) in DEX- and DEX + CHX-treated *LOB-GR* plants and were unchanged in wild-type (Table 2). These genes are putative direct targets of LOB.

Approximately 50% of the putative LOB targets are predicted to encode enzymes involved in cell wall modification such as expansins, glycosyl transferases, pectin methyl transferases, hydrolases, and glycosylases. These data indicate that LOB plays a major role in sculpting the composition of the cell wall in the boundary. We also identified a number of genes that are predicted to function in different hormone signaling pathways, including BR, auxin, cytokinin, gibberellin and ethylene, suggesting that LOB may function to integrate multiple hormone responses in the organ boundary

*LOB regulates BR accumulation*

Given our previous data suggesting that *LOB* is a negative regulator of BR responses, we focused our attention on two putative targets that are implicated in BR signaling: *BAS1* and *BRL3*. *BAS1* encodes a cytochrome P450 enzyme that hydroxylates active BRs, resulting in their inactivation (5), while *BRL3* encodes a leucine-rich-repeat receptor kinase that is one of a family of BR receptors (6). Using independent biological replicates and an induction time course, we confirmed that the transcript levels of both genes increase within 30 minutes of LOB-GR activation by DEX, and this positive regulation occurs in the presence of the translation inhibitor cycloheximide, indicating that they are likely to be directly regulated by LOB. Furthermore, two copies of the LOB binding site, in inverted orientation and separated by a few nucleotides, are present in the *BAS1* upstream regulatory region. Using EMSAs, we demonstrated that LOB binds to the *BAS1* promoter *in vitro,* but does not bind when either LOB-binding site is mutated. To examine LOB association with the *BAS1* promoter *in vivo*, we used a LOB antibody in chromatin immunoprecipitation (ChIP) experiments with DEX- and mock-treated *35S:LOB-GR* seedlings. In DEX-treated samples we detected enrichment of a ~200 bp fragment spanning the LOB binding sites in the *BAS1* promoter. These data suggest that LOB directly activates *BAS1* to inactivate BRs. Reduced BR signaling is likely necessary for boundary cell specification, as BRs promote cell division and expansion (7) and boundary cells divide slowly and are relatively unexpanded (3,8).

Assuming *BAS1* regulation is a normal function of LOB, we hypothesized that reduced *BAS1* expression contributed to the fusion phenotype in the *lob* mutant. To test this, we expressed *BAS1* under control of the *LOB* promoter in wild-type and *lob-3* mutant plants. In a wild-type background, *pLOB:BAS1* plants were morphologically normal. In *lob* mutants, *pLOB:BAS1* expression rescued the fusion defect (5 independent single-locus transgenics were analyzed), which could be quantified by measuring the region of contact between the axillary stem and cauline leaf. Thus, our data indicate that expression of *BAS1* in the domain of *LOB* expression is sufficient to rescue the *lob* mutant phenotype.

A target of LOB transcriptional regulation should have expression overlapping that of LOB. To examine the *BAS1* expression pattern, we generated a *pBAS1:BAS1-GUS* construct containing 6.4 kb of upstream *BAS1* sequence. In general, GUS activity in *pBAS1:BAS1-GUS* transgenic plants was observed in regions undergoing active growth, such as young leaves, floral buds, and lateral root primordia. *GUS* expression was generally broader than that of *LOB*, but showed apparent overlap at the base of leaves, base of floral organs, and in paraclade junctions. Mutation of LOB-binding sites in the *pBAS1:BAS1-GUS* construct did not significantly alter expression however, suggesting that other regulatory sites also contribute to *BAS1* expression.

Although *BAS1* can suppress the *lob* mutant phenotype, *bas1* mutants do not exhibit fusion defects. It is likely that the *lob* mutant phenotype results from alteration in the regulation of multiple genes. Consistent with this idea, the *bas1* mutant does not appreciably suppress the phenotypes of *35S:LOB-GR* plants, whereas *bzr1-d,* which produces a stabilized BR-responsive transcription factor (9), partially suppresses the *LOB-GR* phenotype.

To test the hypothesis that boundary specification or integrity requires limited BR signaling, we expressed *DWF4,* which encodes a rate-limiting step in BR biosynthesis (10,11), under control of the *LOB* promoter to increase BR levels. *pLOB:DWF4* plants exhibited fusion phenotypes similar to the *lob* mutant, providing confirmation that BR accumulation or response must be limited in the organ boundary.

Our data indicate that LOB acts as a negative regulator of BR signaling by increasing *BAS1* levels, which results in a reduction in the availability of active BRs. *LOB* expression is also positively regulated by BRs, indicating the presence of a feedback loop involving BR regulation of LOB accumulation and LOB repression of BR accumulation. More puzzling is the positive regulation of *BRL3*, which is presumed to increase sensitivity to BRs. These data seem to indicate that LOB functions to fine-tune BR responses by altering both the levels of active BR and the response. The work demonstrates the developmental importance of regulating BR responses and indicates that formation of a BR minimum in organ boundaries is critical for boundary integrity.

## Publications resulting from project

Lee, D. K., Geisler, M. and Springer, P. S. (2009). *LATERAL ORGAN FUSION1* and *LATERAL ORGAN FUSION2* function in lateral organ separation and axillary meristem formation in *Arabidopsis*. *Development* 136, 2423-2432.

Bell, E. M., Lin, W.-C., Husbands, A., Yu, L., Jaganatha, V., Jablonska, B., Mangeon, A., Neff, M., Girke, T. and Springer, P. S. (2010). Arabidopsis *LATERAL ORGAN BOUNDARIES* negatively regulates brassinosteroids to limit growth in organ boundaries. In preparation.

Lee, D. K., Wu, S., Smith, H. M. S., and Springer, P. S. (2010) The MYB-transcription factor LOF1 functions downstream of STM in meristem maintenance and boundary specification. In preparation.

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**Table 1. Genetic interactions observed in this project.**
$^{\$}$interactions that were observed prior to the start of this project.

| | *LOB* | *LOF1* | *LOF2* |
|---|---|---|---|
| **Boundary genes** | | | |
| *LOB* | -- | Additive$^{\$}$ | Additive$^{\$}$ |
| *LOF1* | Additive$^{\$}$ | -- | Enhanced/additional fusions$^{\$}$ |
| *LOF2* | Additive$^{\$}$ | Enhanced/additional fusions$^{\$}$ | -- |
| *CUP-SHAPED COTYLEDON1 (CUC1)* (12) | Additive | Additive | Additive |
| *CUP-SHAPED COTYLEDON2 (CUC2)* (12) | Additive | Enhanced/additional fusions and meristem defects | Enhanced/additional fusions and meristem defects |
| *CUP-SHAPED COTYLEDON3 (CUC3)* (13) | Additive | Enhanced/additional fusions and meristem defects | Enhanced/additional fusions and meristem defects |
| *LATERAL SUPPRESSOR (LAS)* (14) | Enhanced fusion | Enhanced/additional fusions and meristem defects$^{\$}$ | Enhanced/additional fusions and meristem defects$^{\$}$ |
| *REGULATORS OF AXILLARY MERISTEMS (RAX)* (15,16) | Additive | Additive | Additive |
| *BLADE ON PETIOLE1 (BOP1)* (17) | Additive | Additive | Additive |
| **Meristem genes** | | | |
| *SHOOT MERISTEMLESS (STM)* (18) | Additive | Meristem defect in weak *stm-10* enhanced | Meristem defect in weak *stm-10* enhanced |
| *BREVIPEDICELLUS (BP)* (19) | Additive | Additive | Additive |
| *PENNYWISE (PNY)* | Additive | *pny* phenotype suppressed$^{\$}$ | *pny* phenotype suppressed$^{\$}$ |
| *POUNDFOOLISH (PNF)* (20) | Additive | No interaction with *pnf*, but *pny pnf* double mutant phenotype suppressed$^{\$}$ | No interaction with *pnf*, but *pny pnf* double mutant phenotype suppressed$^{\$}$ |

**Table 2:** Genes differentially expressed in DEX treated *35S:LOB-GR* seedlings compared to mock treated and CHX/DEX treated compared to CHX[1]

[1] Differentially expressed genes in LOB-GR DEX-treated (D) compared to mock-

[2] Arabidopsis Genome Initiative identifier

[3] log2 Fold Change, means of three biological replicates. log2FC ≥1 or ≤-1 in LOB-GR

[4] False discovery rate (FDR) adjusted p-value. P≤0.001 in LOB-GR D/M

[5] Multiple LOB binding sites present in promoter

| AGI[2] | Description | log2FC LOB-GR CD/C[3] | p-value LOB-GR CD/C[4] | log2FC LOB-GR D/M[3] | p-value LOB-GR D/M[4] | Multiple Binding sites[5] |
|---|---|---|---|---|---|---|
| AT3G13380 | leucine-rich repeat family protein / protein kinase family protein, contains Pfam domains PF00560: Leucine Rich Repeat and PF00069: Protein kinase domain | 2.101 | 4.2E-12 | 3.242 | 1.1E-14 | yes |
| AT2G26710 | Encodes a member of the cytochrome p450 family. Involved in brassinolide metabolism. Mediates response to a variety of light signals including hypocotyl elongation and cotyledon expansion. | 4.938 | 6.4E-15 | 3.216 | 1.4E-10 | yes |
| AT5G66460 | (1-4)-beta-mannan endohydrolase, putative, similar to (1-4)-beta-mannan endohydrolase (Coffea arabica) GI:10178872; contains Pfam profile PF00150: Cellulase (glycosyl hydrolase family 5) | 4.186 | 2.2E-16 | 3.180 | 5.5E-14 | |
| AT3G22840 | chlorophyll A-B binding family protein / early light-induced protein (ELIP), identical to early light-induced protein; ELIP (Arabidopsis thaliana) GI:1872544; contains Pfam profile: PF00504 chlorophyll A-B binding protein; identical to cDNA early light-i | 2.660 | 7.6E-12 | 2.219 | 1.1E-09 | yes |
| AT5G57785 | expressed protein | 1.775 | 5.8E-07 | 2.177 | 7.7E-08 | |
| AT3G51660 | macrophage migration inhibitory factor family protein / MIF family protein, contains Pfam profile: PF01187 Macrophage migration inhibitory factor family(MIF) | 3.946 | 6.4E-15 | 1.898 | 1.3E-08 | |
| AT1G64405 | expressed protein | 2.727 | 3.9E-12 | 1.858 | 1.4E-08 | |
| AT5G01600 | ferritin 1 (FER1), identical to ferritin (Arabidopsis thaliana) GI:1246401, GI:8163920 | 1.421 | 2.8E-04 | 1.839 | 2.8E-05 | |
| AT5G02260 | expansin, putative (EXP9), similar to expansin precursor GI:4138914 from (Lycopersicon esculentum); alpha-expansin gene family, PMID:11641069 | 1.223 | 1.3E-07 | 1.832 | 8.4E-10 | |
| AT3G13672 | seven in absentia (SINA) family protein, low similarity to SP:P21461 Developmental protein seven in absentia {Drosophila melanogaster}; contains Pfam profile PF03145: Seven in absentia protein family | 2.879 | 5.2E-11 | 1.828 | 3.1E-07 | |
| AT1G48330 | expressed protein, similar to hypothetical protein GI:9294146 from (Arabidopsis thaliana) | 1.075 | 1.4E-04 | 1.761 | 4.3E-07 | |
| AT1G71030 | Encodes a putative myb family transcription factor. In contrast to most other myb-like proteins its myb domain consists of a single repeat. A proline-rich region potentially involved in transactivation is found in the C-terminal part of the protein. | 2.159 | 1.6E-07 | 1.758 | 7.0E-06 | |
| AT4G28270 | zinc finger (C3HC4-type RING finger) family protein, contains Pfam profile: PF00097 zinc finger, C3HC4 type (RING finger) | 1.129 | 1.8E-06 | 1.682 | 1.4E-08 | |
| AT4G30410 | expressed protein, similar to cDNA bHLH transcription factor (bHLH eta gene) gi:32563007 | 4.023 | 2.2E-14 | 1.633 | 3.5E-07 | |
| AT3G18280 | protease inhibitor/seed storage/lipid transfer protein (LTP) family protein, similar to TED4 (Zinnia elegans) GI:493721; contains Pfam protease inhibitor/seed storage/LTP family domain PF00234 | 1.677 | 1.4E-11 | 1.499 | 7.8E-10 | |
| AT1G74670 | gibberellin-responsive protein, putative, similar to SP:P46690 Gibberellin-regulated protein 4 precursor {Arabidopsis thaliana} GASA4; contains Pfam profile PF02704: Gibberellin regulated protein | 1.132 | 9.7E-04 | 1.478 | 1.2E-04 | |
| AT2G39700 | expansin, putative (EXP4), similar to alpha-expansin 6 precursor GI:16923359 from (Cucumis sativus); alpha-expansin gene family, PMID:11641069 | 2.981 | 1.6E-13 | 1.394 | 2.7E-07 | yes |
| AT4G37295 | expressed protein | 1.751 | 5.0E-10 | 1.370 | 1.0E-07 | |
| AT3G22060 | contains Pfam profile: PF01657 Domain of unknown function that is usually associated with protein kinase domain Pfam:PF00069, however this protein does not have the protein kinase domain | 1.584 | 6.7E-07 | 1.361 | 1.2E-05 | |
| AT4G24780 | pectate lyase family protein, similar to pectate lyase GP:14289169 from (Salix gilgiana) | 2.876 | 1.0E-12 | 1.352 | 1.0E-06 | |
| AT4G11140 | encodes a member of the ERF (ethylene response factor) subfamily B-5 of ERF/AP2 transcription factor family. The protein contains one AP2 domain. There are 7 members in this subfamily. | 3.610 | 3.2E-15 | 1.326 | 3.2E-07 | yes |
| AT3G61160 | shaggy-related protein kinase beta / ASK-beta (ASK2), identical to shaggy-related protein kinase beta SP:O23145 GI:2569931 from (Arabidopsis thaliana) | 1.395 | 1.0E-06 | 1.307 | 5.5E-06 | |

| AGI[2] | Description | log2FC LOB-GR CD/C[3] | p-value LOB-GR CD/C[4] | log2FC LOB-GR D/M[3] | p-value LOB-GR D/M[4] | Multiple Binding sites[5] |
|---|---|---|---|---|---|---|
| AT5G65140 | trehalose-6-phosphate phosphatase, putative, similar to trehalose-6-phosphate phosphatase (AtTPPB) (Arabidopsis thaliana) GI:2944180; contains Pfam profile PF02358: Trehalose-phosphatase | 4.843 | 2.2E-16 | 1.301 | 2.5E-06 | |
| AT1G55610 | protein kinase family protein, contains Prosite:PS00107: Protein kinases ATP-binding region signature | 2.284 | 2.5E-13 | 1.286 | 2.9E-08 | |
| AT5G56320 | expansin, putative (EXP14), similar to alpha-expansin 3 GI:6942322 from (Triphysaria versicolor); alpha-expansin gene family, PMID:11641069 | 1.931 | 4.5E-06 | 1.284 | 1.2E-03 | yes |
| AT4G00080 | invertase/pectin methylesterase inhibitor family protein, low similarity to pectinesterase from Lycopersicon esculentum SP:Q43143, Arabidopsis thaliana SP:Q42534; contains Pfam profile PF04043: Plant invertase/pectin methylesterase inhibitor | 3.323 | 1.4E-09 | 1.283 | 1.6E-03 | |
| AT5G43150 | expressed protein | 1.449 | 1.0E-06 | 1.265 | 1.4E-05 | |
| AT4G31820 | phototropic-responsive NPH3 family protein, contains NPH3 family domain, Pfam:PF03000 (NPY1) | 2.581 | 1.1E-10 | 1.251 | 2.2E-05 | yes |
| AT2G34080 | cysteine proteinase, putative, contains similarity to cysteine protease SPCP1 GI:13491750 from (Ipomoea batatas) | 1.747 | 7.8E-07 | 1.242 | 1.5E-04 | |
| AT5G53900 | expressed protein, similar to unknown protein (gb:AAF34833.1) | 1.663 | 2.8E-09 | 1.239 | 7.2E-07 | |
| AT4G28560 | leucine-rich repeat family protein (fragment), contains leucine rich-repeat domains Pfam:PF00560, INTERPRO:IPR001611; | 2.837 | 1.6E-09 | 1.235 | 5.0E-04 | yes |
| AT1G10650 | expressed protein | 3.493 | 3.9E-12 | 1.212 | 1.4E-04 | |
| AT5G10130 | pollen Ole e 1 allergen and extensin family protein, contains similarity to pollen specific protein C13 precursor (Zea mays) SWISS-PROT:P33050 | 2.766 | 1.8E-10 | 1.178 | 1.6E-04 | yes |
| AT4G27900 | expressed protein | 1.194 | 1.1E-06 | 1.177 | 3.0E-06 | |
| AT2G24550 | expressed protein, weak similarity to MTD1 (Medicago truncatula) GI:9294810 | 2.962 | 4.7E-14 | 1.153 | 1.3E-06 | |
| AT5G04770 | amino acid permease family protein, similar to cationic amino acid transporter-1 (Rattus norvegicus) GI:1589917; contains Pfam profile PF00324: Amino acid permease | 1.912 | 1.6E-07 | 1.138 | 3.6E-04 | yes |
| AT1G15670 | kelch repeat-containing F-box family protein, similar to SP:Q9ER30 Kelch-related protein 1 (Sarcosin) {Rattus norvegicus}; contains Pfam profiles PF01344: Kelch motif, PF00646: F-box domain | 1.728 | 1.2E-09 | 1.114 | 3.0E-06 | yes |
| AT5G53890 | leucine-rich repeat transmembrane protein kinase, putative | 1.381 | 9.4E-09 | 1.093 | 9.0E-07 | |
| AT2G24240 | potassium channel tetramerisation domain-containing protein, contains Pfam profile PF02214: K+ channel tetramerisation domain | 1.397 | 1.2E-07 | 1.088 | 9.8E-06 | |
| AT3G07410 | Ras-related GTP-binding family protein, contains Pfam profile: PF00071 Ras family | 1.574 | 1.6E-09 | 1.060 | 2.0E-06 | |
| AT4G19810 | glycosyl hydrolase family 18 protein, similar to chitinase/lysozyme GI:467689 from (Nicotiana tabacum) | 1.377 | 3.2E-08 | 1.050 | 4.3E-06 | |
| AT3G15357 | expressed protein | 1.122 | 7.0E-06 | 1.031 | 4.1E-05 | |
| AT3G58790 | glycosyl transferase family 8 protein, contains Pfam profile: PF01501 glycosyl transferase family 8; general stress protein gspA, Bacillus subtilis, PIR:S16423 | 1.139 | 4.9E-07 | 1.016 | 5.3E-06 | |
| AT1G26290 | expressed protein | 2.859 | 6.5E-10 | 1.014 | 2.4E-03 | |
| AT1G29440 | auxin-responsive family protein, similar to auxin-induced protein 6B (SP:P33083) (Glycine max) | -1.740 | 5.7E-06 | -1.008 | 5.1E-03 | |
| AT4G11290 | peroxidase, putative, identical to peroxidase ATP19a (Arabidopsis thaliana) gi:1546692:emb:CAA67337 | -1.016 | 7.6E-04 | -1.008 | 1.8E-03 | |
| AT3G43430 | zinc finger (C3HC4-type RING finger) family protein, contains Pfam profile: PF00097 zinc finger, C3HC4 type (RING finger) | -1.017 | 9.3E-06 | -1.021 | 1.8E-05 | |
| AT3G15650 | phospholipase/carboxylesterase family protein, low similarity to lysophospholipase I (Mus musculus) GI:1864159; contains Pfam profile PF02230: Phospholipase/Carboxylesterase family | -1.183 | 7.0E-05 | -1.041 | 6.1E-04 | |
| AT2G25000 | WRKY family transcription factor, contains Pfam profile: PF03106 WRKY DNA -binding domain | -1.868 | 3.6E-10 | -1.051 | 7.6E-06 | |
| AT1G25230 | purple acid phosphatase family protein, contains Pfam profile: PF00149 calcineurin-like phosphoesterase; similar to purple acid phosphatase (GI:20257479) (Arabidopsis thaliana) | -1.154 | 6.1E-05 | -1.055 | 3.6E-04 | |
| AT2G37640 | expansin, putative (EXP3), identical to Alpha-expansin 3 precursor (At-EXP3)(Arabidopsis thaliana) SWISS-PROT:O80932; alpha-expansin gene family, PMID:11641069 | -1.541 | 2.8E-05 | -1.067 | 3.1E-03 | yes |
| AT3G49220 | pectinesterase family protein, contains Pfam profile: PF01095 pectinesterase | -1.205 | 1.2E-07 | -1.082 | 1.4E-06 | yes |

| AGI[2] | Description | log2FC LOB-GR CD/C[3] | p-value LOB-GR CD/C[4] | log2FC LOB-GR D/M[3] | p-value LOB-GR D/M[4] | Multiple Binding sites[5] |
|---|---|---|---|---|---|---|
| AT1G19450 | integral membrane protein, putative / sugar transporter family protein, similar to GB:U43629 GI:1209756 integral membrane protein from (Beta vulgaris); contains Pfam profile PF00083: major facilitator superfamily protein; contains TIGRfam TIGR00879: Suga | -1.408 | 4.9E-09 | -1.088 | 7.2E-07 | |
| AT1G64640 | plastocyanin-like domain-containing protein, contains InterPro:IPR003245 plastocyanin-like domain | -1.482 | 1.8E-05 | -1.093 | 1.2E-03 | |
| AT5G20740 | invertase/pectin methylesterase inhibitor family protein, low similarity to pectinesterase from Arabidopsis thaliana SP:Q42534, Lycopersicon esculentum SP:Q43143; contains Pfam profile PF04043: Plant invertase/pectin methylesterase inhibitor | -1.068 | 1.2E-04 | -1.102 | 1.7E-04 | |
| AT3G57010 | strictosidine synthase family protein, similar to strictosidine synthase (Rauvolfia serpentina)(SP:P15324) | 1.602 | 2.5E-06 | -1.104 | 5.4E-04 | |
| AT5G64330 | non-phototropic hypocotyl 3 (NPH3), identical to non-phototropic hypocotyl 3 (Arabidopsis thaliana) gi:6224712:gb:AAF05914, PMID:10542152 | -1.561 | 7.0E-11 | -1.138 | 6.2E-08 | yes |
| AT2G06925 | phospholipase A2 family protein, similar to secretory low molecular weight phospholipase A2 beta (Arabidopsis thaliana) GI:25992715; contains INTERPRO domain IPR001211 phospholipase A2 | -1.171 | 9.4E-08 | -1.166 | 2.6E-07 | |
| AT5G62920 | two-component responsive regulator / response regulator 6 (ARR6), identical to response regulator 6 (Arabidopsis thaliana) GI:3953601 | -1.078 | 5.9E-06 | -1.168 | 4.2E-06 | yes |
| AT1G62480 | vacuolar calcium-binding protein-related, contains weak similarity to vacuolar calcium binding protein (Raphanus sativus) gi:9049359:dbj:BAA99394 | -1.646 | 6.2E-08 | -1.168 | 1.9E-05 | |
| AT1G58360 | amino acid permease I (AAP1), identical to amino acid permease I GI:22641 from (Arabidopsis thaliana) | -1.398 | 3.4E-05 | -1.179 | 5.0E-04 | |
| AT5G53550 | transporter, putative, similar to iron-phytosiderophore transporter protein yellow stripe 1 (Zea mays) GI:10770865; contains Pfam profile PF03169: OPT oligopeptide transporter protein | -1.119 | 4.7E-07 | -1.182 | 4.6E-07 | |
| AT3G44990 | xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative | -1.683 | 2.6E-07 | -1.184 | 6.8E-05 | |
| AT1G04240 | auxin-responsive protein / indoleacetic acid-induced protein 3 (IAA3), identical to SP:Q38822 Auxin-responsive protein IAA3 (Indoleacetic acid-induced protein 3) {Arabidopsis thaliana}; EST gb:T04296 comes from this gene | -1.134 | 3.1E-07 | -1.189 | 3.5E-07 | |
| AT2G31750 | UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase | -1.056 | 6.2E-07 | -1.212 | 2.0E-07 | |
| AT1G71880 | sucrose transporter / sucrose-proton symporter (SUC1), identical to sucrose-proton symporter SUC1 (Arabidopsis thaliana) GI:407094 | -2.483 | 4.2E-12 | -1.214 | 1.8E-06 | yes |
| AT2G28790 | osmotin-like protein, putative, similar to SP:Q41350 Osmotin-like protein precursor {Lycopersicon esculentum}; contains Pfam profile PF00314: Thaumatin family | -1.131 | 4.9E-06 | -1.237 | 3.0E-06 | |
| AT5G10430 | arabinogalactan-protein (AGP4), identical to gi_3883126_gb_AAC77826 | -1.364 | 1.2E-04 | -1.272 | 5.2E-04 | |
| AT3G23730 | xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative, similar to xyloglucan endotransglycosylase-related protein GI:1244760 from (Arabidopsis thaliana) | -1.205 | 4.0E-07 | -1.289 | 3.4E-07 | |
| AT4G19120 | early-responsive to dehydration stress protein (ERD3), identical to ERD3 protein (Arabidopsis thaliana) GI:15320410; contains Pfam profile PF03141: Putative methyltransferase; identical to cDNA ERD3 GI:15320409 | -1.048 | 7.0E-06 | -1.295 | 7.3E-07 | |
| AT2G14900 | gibberellin-regulated family protein, similar to SP:P46690 Gibberellin-regulated protein 4 precursor {Arabidopsis thaliana} GASA4; contains Pfam profile PF02704: Gibberellin regulated protein | -1.361 | 8.1E-07 | -1.298 | 3.5E-06 | |
| AT3G47510 | expressed protein | -1.247 | 1.8E-06 | -1.309 | 2.0E-06 | |
| AT5G63180 | pectate lyase family protein, similar to pectate lyase GP:14289169 from (Salix gilgiana) | -1.561 | 6.7E-07 | -1.309 | 1.6E-05 | |
| AT3G10720 | pectinesterase, putative, contains similarity to pectinesterase from Vitis vinifera GI:15081598, Prunus persica SP:Q43062; contains Pfam profile PF01095 pectinesterase | -1.023 | 7.0E-06 | -1.311 | 4.5E-07 | yes |
| AT4G03190 | F-box family protein (FBL18), almost identical to GRR1-like protein 1 GI:12658970 from (Arabidopsis thaliana); similar to leucine-rich repeats containing F-box protein FBL3 (GI:5919219) (Homo sapiens); similar to F-box protein FBL2 (GI:6063090) (Homo sap | -1.502 | 2.2E-06 | -1.317 | 2.7E-05 | |
| AT1G13710 | cytochrome P450 family protein, similar to cytochrome P450 78A1 (SP:P48420) GI:349717 from (Zea mays) | -1.058 | 1.1E-04 | -1.338 | 1.2E-05 | |

| AGI[2] | Description | log2FC LOB-GR CD/C[3] | p-value LOB-GR CD/C[4] | log2FC LOB-GR D/M[3] | p-value LOB-GR D/M[4] | Multiple Binding sites[5] |
|---|---|---|---|---|---|---|
| AT1G67810 | Fe-S metabolism associated domain-containing protein, contains Pfam PF02657: Fe-S metabolism associated domain | -1.060 | 1.8E-05 | -1.363 | 1.1E-06 | |
| AT3G02170 | expressed protein | -1.181 | 9.3E-05 | -1.365 | 3.2E-05 | |
| AT5G23010 | 2-isopropylmalate synthase 3 (IMS3), identical to 2-isopropylmalate synthase (IMS3) (Arabidopsis thaliana) GI:15983745; identical to cDNA 2-isopropylmalate synthase (IMS3) GI:15983744 | -1.632 | 4.1E-07 | -1.370 | 1.0E-05 | |
| AT1G11545 | xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative, similar to endo-xyloglucan transferase GI:2244732 from (Gossypium hirsutum) | -1.692 | 1.8E-09 | -1.373 | 1.7E-07 | |
| AT2G31730 | ethylene-responsive protein, putative, similar to ethylene-inducible ER33 protein (Lycopersicon esculentum) gi:5669656:gb:AAD46413 | -1.275 | 2.1E-07 | -1.414 | 1.3E-07 | |
| AT1G20190 | expansin, putative (EXP11), similar to GB:U30460 from (Cucumis sativus); alpha-expansin gene family, PMID:11641069 | -1.262 | 5.1E-03 | -1.467 | 3.4E-03 | yes |
| AT5G45670 | GDSL-motif lipase/hydrolase family protein, similar to family II lipases EXL3 GI:15054386, EXL1 GI:15054382, EXL2 GI:15054384 from (Arabidopsis thaliana); contains Pfam profile PF00657: GDSL-like Lipase/Acylhydrolase | -1.277 | 6.4E-09 | -1.468 | 2.6E-09 | |
| AT1G29500 | auxin-responsive protein, putative, similar to auxin-induced protein 6B (SP:P33083) (Glycine max) | -1.265 | 8.7E-07 | -1.478 | 2.1E-07 | |
| AT2G17740 | DC1 domain-containing protein | -1.613 | 4.8E-06 | -1.489 | 2.8E-05 | |
| AT1G52190 | proton-dependent oligopeptide transport (POT) family protein, contains Pfam profile: PF00854 POT family | -1.675 | 1.2E-06 | -1.491 | 1.3E-05 | |
| AT2G21220 | auxin-responsive protein, putative, similar to auxin-induced protein TGSAUR22 (GI:10185820) (Tulipa gesneriana) | -1.634 | 1.6E-09 | -1.492 | 2.9E-08 | |
| AT4G23810 | WRKY family transcription factor, AR411 - Arabidopsis thaliana (thale cress), PID:g1669603 | -1.761 | 5.1E-06 | -1.531 | 6.3E-05 | yes |
| AT4G36670 | mannitol transporter, putative, similar to mannitol transporter (Apium graveolens var. dulce) GI:12004316; contains Pfam profile PF00083: major facilitator superfamily protein | -1.144 | 6.6E-06 | -1.551 | 2.0E-07 | yes |
| AT1G29430; | [AT1G29430, auxin-responsive family protein, similar to auxin-induced protein 10a 6B {SP:P33083) (Glycine max) ];[AT5G27780, auxin-responsive family protein, similar to Auxin-induced protein 10A5 (SP:P33079) (Glycine max) ] | -2.139 | 2.1E-08 | -1.556 | 5.8E-06 | |
| AT4G00050 | basic helix-loop-helix (bHLH) family protein, contains Pfam profile: PF00010 helix-loop-helix DNA-binding domain | -1.315 | 7.5E-06 | -1.567 | 1.4E-06 | yes |
| AT1G29510 | auxin-responsive protein, putative, similar to auxin-induced protein 6B (SP:P33083) (Glycine max) | -1.469 | 1.2E-06 | -1.586 | 8.7E-07 | |
| AT5G61660 | glycine-rich protein | -1.335 | 8.0E-07 | -1.598 | 1.5E-07 | |
| AT3G07010 | pectate lyase family protein, similar to pectate lyase GP:14531296 from (Fragaria x ananassa) | -1.382 | 7.6E-06 | -1.639 | 1.5E-06 | |
| AT5G46050 | proton-dependent oligopeptide transport (POT) family protein, contains Pfam profile: PF00854 POT family | -1.190 | 4.1E-05 | -1.681 | 7.8E-07 | |
| AT1G62500 | protease inhibitor/seed storage/lipid transfer protein (LTP) family protein, similar to auxin down regulated GB:X69640 GI:296442 from (Glycine max); contains Pfam profile PF00234: Protease inhibitor/seed storage/LTP family | -1.781 | 3.6E-08 | -1.682 | 2.5E-07 | |
| AT1G78970 | lupeol synthase (LUP1) / 2,3-oxidosqualene-triterpenoid cyclase, identical to lupeol synthase GI:1762150 from (Arabidopsis thaliana), 2,3-oxidosqualene-triterpenoid cyclase (Arabidopsis thaliana) GI:2738027; contains Pfam profile PF00432: Prenyltransfera | -1.140 | 1.1E-03 | -1.692 | 2.9E-05 | |
| AT3G50440 | similar to esterase, putative [Arabidopsis thaliana] (TAIR:At2g23620.1); similar to methyl jasmonate esterase [Solanum tuberosum] (GB:AAV87151.1); contains InterPro domain Esterase/lipase/thioesterase (InterPro:IPR000379); contains InterPro domain Alpha/ | -1.123 | 1.1E-04 | -1.751 | 6.9E-07 | |
| AT3G53250 | auxin-responsive family protein, similar to auxin-induced protein TGSAUR22 (GI:10185820) (Tulipa gesneriana and auxin-induced protein 6B (SP:P33083) (PIR:T10942) (Glycine max) | -1.141 | 4.0E-03 | -1.812 | 8.0E-05 | |
| AT2G41090 | calmodulin-like calcium-binding protein, 22 kDa (CaBP-22), identical to SP:P30187 22 kDa calmodulin-like calcium-binding protein (CABP-22) (Arabidopsis thaliana) | -1.093 | 1.4E-05 | -1.815 | 2.5E-08 | |
| AT1G12200 | flavin-containing monooxygenase family protein / FMO family protein, low similarity to FMO2 from Homo sapiens (SP:Q99518); contains Pfam profile: PF00743 Flavin-binding monooxygenase-like | -1.385 | 8.2E-09 | -1.862 | 4.1E-10 | |

| AGI[2] | Description | log2FC LOB-GR CD/C[3] | p-value LOB-GR CD/C[4] | log2FC LOB-GR D/M[3] | p-value LOB-GR D/M[4] | Multiple Binding sites[5] |
|---|---|---|---|---|---|---|
| AT2G39200 | seven transmembrane MLO family protein / MLO-like protein 12 (MLO12), identical to SP:O80961 MLO-like protein 12 (AtMlo12) {Arabidopsis thaliana}, membrane protein Mlo12 (Arabidopsis thaliana) gi:14091594:gb:AAK53805; similar to MLO protein SWISS-PROT:P9 | -1.547 | 2.0E-06 | -1.874 | 2.9E-07 | |
| AT5G64570 | glycosyl hydrolase family 3 protein | -1.186 | 1.6E-05 | -1.889 | 5.9E-08 | |
| AT3G12710 | methyladenine glycosylase family protein, similar to SP:P05100 DNA-3-methyladenine glycosylase I (EC 3.2.2.20) (3-methyladenine-DNA glycosylase I, constitutive) {Escherichia coli}; contains Pfam profile PF03352: Methyladenine glycosylase | -1.009 | 7.1E-04 | -1.895 | 5.9E-07 | |
| AT2G13610 | ABC transporter family protein | -1.061 | 4.1E-05 | -1.897 | 3.0E-08 | |
| AT5G02760 | protein phosphatase 2C family protein / PP2C family protein, similar to Ser/Thr protein phosphatase 2C (PP2C6) (GI:15020818) (Arabidopsis thaliana); similar to protein phosphatase 2C (GI:3608412) (Mesembryanthemum crystallinum); contains Pfam PF00481 : P | -1.505 | 6.9E-08 | -1.899 | 6.0E-09 | |
| AT5G50335 | expressed protein | -1.996 | 4.1E-07 | -2.037 | 6.7E-07 | |
| AT2G40610 | expansin, putative (EXP8), similar to expansin 2 GI:7025493 from (Zinnia elegans); alpha-expansin gene family, PMID:11641069 | -1.247 | 8.6E-03 | -2.061 | 1.8E-04 | yes |
| AT3G51860 | cation exchanger, putative (CAX3), similar to high affinity calcium antiporter CAX1 (Arabidopsis thaliana) gi:9256741:gb:AAB05913; Ca2+:Cation Antiporter (CaCA) Family member PMID:11500563; non-consensus AT-acceptor splice site at intron 1 | -2.347 | 4.2E-09 | -2.092 | 9.2E-08 | |
| AT4G34220 | leucine-rich repeat transmembrane protein kinase, putative, protein kinase TMKL1, Arabidopsis thaliana, PID:E353150 | -1.092 | 1.9E-05 | -2.130 | 2.6E-09 | |
| AT5G01210 | transferase family protein, contains Pfam profile PF02458 transferase family | -2.368 | 9.7E-12 | -2.399 | 1.0E-10 | yes |
| AT5G62280 | expressed protein | -4.056 | 4.2E-12 | -2.838 | 1.1E-08 | yes |
| AT4G02330 | pectinesterase family protein, contains Pfam profile: PF01095 pectinesterase | -1.647 | 1.1E-05 | -2.960 | 5.3E-09 | yes |