

Lateral organ boundaries domain transcription factors

New roles in plant defense

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Over the last two decades, several transcription factor gene families have been identified with some of them characterized in detail for their roles on transcriptional regulation of plant defense responses against pest or pathogen attack. We have recently added another transcription factor gene family to this list through the characterization of the LATERAL ORGAN BOUNDARIES (LOB) DOMAIN (LBD)-CONTAINING PROTEIN20 (LBD20). We showed LBD20 acts as a repressor of a subset of jasmonate mediated defenses and in susceptibility to the root-infecting fungal pathogen *Fusarium oxysporum*. However, possible roles for other members of this gene family in plant defense are currently unknown. Here we searched publicly available microarray expression data and provide an overview of the expression patterns of selected members of the *LBD* gene family for their response to other fungal pathogens and soil nematodes. Distinct expression patterns of the *LBD* genes suggest that certain members of this gene family have previously undescribed roles in plant defense.

The transcriptional control of defense responses following pathogen attack plays a critical role in determining final disease outcome. The amplitude, temporal and spatial expression of these responses is largely controlled through the activity of specific transcription factors, of which some have functions overlapping with other plant processes.^{1,2}

Several well studied plant transcription factor families are associated with defense responses.¹ These include the Ethylene Response Factor (ERF), basic-domain leucine-Zipper (bZIP) TGA, MYB, Whirly, WRKY, NAC, and members of the basic helix-loop-helix (bHLH) transcription factors. Members of these families regulate gene expression in response to a range of biotic stimuli including microbes (fungi, oomycetes, bacteria) and insects, and downstream defense signaling hormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET).^{1,3} Our work focuses on defense and other host responses to the root-infecting fungal pathogen *Fusarium oxysporum*. This pathogen gains entry into roots through wounds or at points of lateral root formation, moving through the root cortex intercellularly and gaining entry into the xylem. From here the pathogen travels upwards, clogging the vascular system and secreting toxins causing chlorosis, wilting and eventually plant death.⁴⁻⁶

In *Arabidopsis*, increased resistance to *F. oxysporum* can be achieved through altering the expression of specific transcriptional activators or repressors.^{4,7,8} For example, positive regulators of defense against *F. oxysporum* include the ERF transcription factors ERF1, AtERF2 and AtERF14,⁹⁻¹¹ while negative regulators include AtERF4,¹⁰ the NAC-domain transcription factor AtAF2,¹² and the bHLH AtMYC2.¹³ Recently, we have identified a member of another transcription factor family, termed LATERAL ORGAN BOUNDARIES (LOB) DOMAIN (LBD),¹⁴ which functions as a susceptibility

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Gene	LBD Class	<i>Alternaria</i>	<i>Botrytis</i>	<i>Phytophthora</i>	<i>Meloidogyne</i>
LBD3	I	1.34	1.00	-1.87	3.70
LBD4	I	1.38	-1.10	-2.10	5.09
LBD12	I	-1.09	1.02	2.66	-1.27
LBD13	I	1.03	-1.02	-2.63	-1.21
LBD16	I	-1.10	1.11	-1.94	-1.83
LBD17	I	1.04	1.04	1.55	-1.03
LBD32	I	-1.08	1.06	1.93	-1.17
LBD35	I	1.11	-1.01	1.89	-1.02
LBD37	II	2.14	-1.53	-1.89	-1.20
LBD38	II	1.63	-1.46	-1.48	-1.48
LBD39	II	1.72	-1.77	-2.61	1.00
LBD40	II	-1.30	1.04	3.55	1.02
LBD41	II	-5.83	2.13	4.42	2.38

Figure 1. Expression profiles of *LBD* genes responsive to selected leaf or root pathogens. Public array data examined through GENEVESTIGATOR²⁰ is presented for eight class I and five class II *LBD* genes in leaf tissue in response to the leaf infecting necrotrophic fungal pathogens *Alternaria brassicicola* or *Botrytis cinerea*, or in root tissue in response to the root pathogen *Phytophthora parasitica* (oomycete) or to the root-knot nematode *Meloidogyne incognita*. Shown are fold changes relative to controls with those > 1.5-fold highlighted in bold font. For data sets with multiple sampled time-points, time-points with the greatest fold changes were selected. This figure was generated using the HeatMapper tool from The Bio-Array Resource for Plant Biology (<http://bar.utoronto.ca/>) with green and red representing repressed or induced gene expression, respectively.

gene to Fusarium disease symptom development.¹⁵ Knockouts of this gene, *LOB DOMAIN-CONTAINING PROTEIN20* (*LBD20*), exhibited increased resistance to *F. oxysporum* and reduced chlorosis following application of *F. oxysporum* culture filtrate. The *lbd20* mutant also showed increased expression of the JA-regulated defense genes *THIONIN2.1* (*THI2.1*) and *VEGETATIVE STORAGE PROTEIN2* (*VSP2*). We further showed that plants overexpressing *LBD20* had repressed *THI2.1* and *VSP2* expression, and exhibited increased susceptibility to *F. oxysporum*. Together, these results suggested that *LBD20* acts as a negative regulator of JA-regulated pathogen defense in Arabidopsis. This is the first time, to our knowledge, that a role for a LBD transcription factor in plant-pathogen interactions has been demonstrated.

To date, 43 *LBD* genes have been identified in Arabidopsis and classified into two main classes.¹⁶ The majority of LBD proteins, including *LBD20*, belong to class I with LOB domains most similar to the defining LOB member containing a cysteine-repeat motif, a conserved glycine residue and leucine zipper sequence. The smaller and less similar class II group have incomplete leucine zippers and are not predicted to form coiled-coil structures required for protein-protein interactions or homodimerization.^{14,16} Currently, the

function of most LBD family members is not known, although diverse functions have been proposed for those characterized to some extent. These roles include regulating organ development, anthocyanin or nitrogen metabolism, or responses to phytohormones such as cytokinin, auxin and gibberellins.^{14,16-18} We now demonstrate a role for *LBD20* in JA-signaling where it functions downstream of COI1 and MYC2,¹⁵ respectively part of the JA receptor complex and a key downstream transcriptional regulator of JA signaling (for a review see ref. 19).

LBD20 expression was predominantly limited to root tissues, the initial site of *F. oxysporum* colonization, where its expression increased following infection.¹⁵ To determine if other LBD transcription factors may function in root or fungal pathogen interactions, we searched public array data from Arabidopsis transcripts (GENEVESTIGATOR²⁰) for response of *LBD* genes (26 members with probesets) to leaf necrotrophs or to root pathogens. The expression of 13 *LBD* genes was either repressed or induced greater than 1.5-fold following inoculation with the leaf fungal necrotrophs *Alternaria brassicicola* or *Botrytis cinerea*, or the oomycete root pathogen *Phytophthora parasitica*, with the greatest response exhibited by *LBD41* (Fig. 1). Nearly half of the *LBD* genes with probesets were responsive to *P. parasitica*,

with six induced and six repressed greater than 1.5-fold. The expression of several *LBD* genes was also responsive to the root-colonizing parasite, root-knot nematode (*Meloidogyne incognita*) (Fig. 1). The diverse expression profiles of these *LBD* genes suggest, in addition to *LBD20*, other LBD proteins may function during plant defense responses to fungal or root pathogens.

Interestingly, the *LBD* genes whose expression was responsive to multiple pathogens belong to the smaller class II of LBD proteins. These are *LBD37* to *LBD42* inclusive, with smaller phylogenetically similar subgroups formed between *LBD37*, 38 and 39, and *LBD40*, 41 and 42.^{14,16} In addition, we found the two subgroups showed distinctly opposite expression patterns in response to different pathogens with *LBD37*, 38 and 39 expression induced by *A. brassicicola* and repressed by *B. cinerea* and *P. parasitica*, and the reverse observed for *LBD41* and to a lesser extent *LBD40* (Fig. 1). It is plausible that these members play opposing roles in response to these stimuli as a means of maintaining the appropriate expression level of downstream defense responses. In addition to *LBD20* expression being induced by *F. oxysporum* infection, we also found its expression was responsive to the defense signaling hormone JA.¹⁵ In GENEVESTIGATOR, we only found *LBD* genes differentially expressed greater than 1.5-fold from controls to be repressed by JA treatment (data not shown). Again, the class II *LBD* genes (*LBD37*, 38, 39 and 41) were most responsive.

Recently the class II LBD proteins *LBD37* and *LBD41* were shown to interact with TOPLESS (TPL) and TPL-related (TPR) co-repressors, potentially through an ERF-associated amphiphilic repression-(EAR) motif.^{21,22} With the exception of *LBD39*, the remaining class II LBD proteins are also predicted to contain EAR repression domains,²³ suggesting that other LBDs may also recruit TPL/TPRs co-repressors to mediate transcriptional repression. We found *LBD20* functions as a repressor of a subset of JA-regulated defense genes however, it is not known whether *LBD20* directly or indirectly represses their transcription.¹⁵ Unlike class I LBD proteins, those characterized so far

from class II do not have any reported roles in development.¹⁶ Their structural difference, including an incomplete leucine zipper, conserved EAR-repression motifs, and increased response to pathogen attack (Fig. 1), suggest they have distinctly different roles from the class I LBD proteins.

With a role for LBD20 proposed in plant defense and JA-responses,¹⁵ and the expression of several other *LBD* genes responsive to root or fungal pathogens, particularly those of class II (this paper), it is of much interest to determine if other LBD members are transcriptional mediators of resistance or susceptibility to pathogen attack and if so how.

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