PAULA RODRIGUES OBLESSUC

"MAPEAMENTO FINO DE LOCOS ASSOCIADOS À RESISTÊNCIA À MANCHA ANGULAR EM FEIJÃO (*PHASEOLUS VULGARIS* L.)"

CAMPINAS
2013

UNIVERSIDADE ESTADUAL DE CAMPINAS

INSTITUTO DE BIOLOGIA

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"MAPEAMENTO FINO DE LOCOS ASSOCIADOS À RESISTÊNCIA À MANCHA ANGULAR EM FEIJÃO (*PHASEOLUS VULGARIS* L.)"

Tese apresentada ao Instituto de Biologia da UNICAMP para obtenção do Título de Doutora em Genética e Biologia Molecular, na área de Genética Vegetal e Melhoramento

Orientadora: Dra. Luciana Lasry Benchimol Reis
Coorientador: Prof. Dr. Luis Eduardo Aranha Camargo

CAMPINAS,
2013

FICHA CATALOGRÁFICA ELABORADA POR
MARA JANAINA DE OLIVEIRA – CRB8/6972
BIBLIOTECA DO INSTITUTO DE BIOLOGIA - UNICAMP

Ob4m

Oblessuc, Paula Rodrigues, 1981-
Mapeamento fino de locos associados à resistência à mancha angular em feijão (*Phaseolus vulgaris L.*) / Luiz Paula Rodrigues Oblessuc. – Campinas, SP: [s.n.], 2013.

Orientador: Luciana Lasry Benchimol Reis.
Coorientador: Luis Eduardo Aranha Camargo.
Tese (doutorado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. *Pseudocercospora griseola*. 2. Mapeamento de QTL. 3. Marcadores genéticos. 4. Genômica. 5. Gene candidato. I. Benchimol, Luciana Lasry. II. Camargo, Luis Eduardo Aranha. III. Universidade Estadual de Campinas. Instituto de Biologia. IV. Título.

Informações para Biblioteca Digital

Título em Inglês: Fine mapping of angular leaf spot resistance loci in common bean (*Phaseolus vulgaris L.*)
Palavras-chave em Inglês:
Pseudocercospora griseola
QTL mapping
Genetic markers
Genomics
Candidate gene
Área de concentração: Genética Vegetal e Melhoramento
Titulação: Doutora em Genética e Biologia Molecular
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Data da defesa: 25-04-2013
Programa de Pós Graduação: Genética e Biologia Molecular

Campinas, 25 de abril de 2013

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Para minha mãe e irmãs queridas e meu maravilhoso marido.

AGRADECIMENTOS

À professora Dra. Luciana Lasry Benchimol Reis pela orientação e pela inestimável contribuição na minha formação na área de marcadores moleculares e mapeamento.

Ao professor Dr. Luis Eduardo Aranha Camargo pela orientação, ensinamentos, e enorme contribuição na discussão dos dados obtidos.

Ao professor Dr. Antonio Augusto Franco Garcia por seus ensinamentos em obtenção de mapas genéticos e mapeamento de QTL.

Aos melhoristas Dr. Fernando Chioratto e Dr. Sérgio Augusto Morais Carbonell pela contribuição nas análises estatísticas e por cederem a população utilizada neste estudo, assim como pelo suporte na área de melhoramento de feijão.

À professora Dra. Maeli Melotto por ter me recebido tão amigavelmente em seu laboratório, contribuindo enormemente para meus conhecimento em fitopatologia molecular.

Aos professores que fizeram parte do exame de qualificação: Profa. Dra. Maria Lúcia Carneiro Vieira e Profa. Dra. Suzete A. Lanza Destéfano, por todas as excelentes contribuições.

Aos membros da banca examinadora pela leitura crítica da tese, sugestões e avaliações.

Aos colegas de laboratório Juliana, Renata, Boris e Denis, que colaboraram diretamente para a realização dos estudos realizados neste trabalho.

Ao meu querido marido Dr. Cleverson Carlos Matiolli pelos momentos de discussão científica, essenciais ao desenvolvimento desta tese.

À minha família pelo apoio e pelas alegrias vidas juntos.

À FAPESP por financiar este projeto e conceder minha bolsa de doutorado.

À CAPES e à UNICAMP pela bolsa de doutorado sanduíche no exterior.

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RESUMO

O feijão comum (*Phaseolus vulgaris* L.) é uma importante fonte de proteínas na dieta humana. A mancha angular (ALS), causada pelo fungo *Pseudocercospora griseola* (Sacc.) Crous & Braun, acarreta grandes prejuízos na produção do feijão. O melhoramento do feijoeiro busca ferramentas que agilizem a transferência de genes de resistência à doenças para cultivares em desenvolvimento. Assim, foi objetivo deste trabalho estudar os mecanismos genéticos e moleculares envolvidos na resposta do feijão à ALS, e com isso contribuir para o melhoramento dessa cultura.

Primeiramente, QTLs (*Quantitative Trait Locus*) de resistência à ALS foram identificados utilizando a população de mapeamento UC (IAC-UNA x CAL 143) a partir do mapa genético previamente desenvolvido com marcadores microssatélites. O estudo quantitativo da severidade da ALS revelou distribuição normal e transgressiva na população UC, com resistência quantitativa observada em CAL 143. Ao todo foram mapeados sete QTLs em cinco diferentes grupos de ligação de feijão. Dentre estes, o loco ALS10.1 mostrou maior efeito (16% - 22%) e estabilidade nos três ambientes analisados: (1) condições naturais de infecção em época chuvosa de plantio; (2) condições naturais de infecção em época seca de plantio; e (3) condições controladas de infecção raça-específica em casa de vegetação.

A região do loco ALS10.1 foi saturada com marcadores microssatélites, SCARs e *Sequence-Tagged Site*-DArTs (STS-DArTs); este último através da técnica de *bulk segregant analysis* (BSA). O intervalo de confiança foi reduzido de 13.4 cM para 3.0 cM após a saturação do loco, que teve seu número de marcadores aumentado de quatro para 10. O estudo do contexto genômico do ALS10.1 através do alinhamento dos marcadores com o rascunho do genoma do feijão possibilitou definir uma região *core* para o QTL na extremidade do cromossomo Pv10, com aproximadamente 5,3 Mb. Análise de *Gene Onthology* (GO) dos 323 genes preditos nesta região do genoma demonstrou que 61,6% destes genes estão envolvidos na resposta à estresse. *Cluster* de genes TIR-NB-ARC (domínios altamente conservado

em genes (R) de Resistência) foi identificado cobrindo aproximadamente 849 Kb na região *core* de ALS10.1; além desta região conter outros genes sabidamente relacionados a imunidade de plantas.

Sete genes presentes na região *core* de ALS10.1 foram selecionados com base na função na resistência à patógenos dos respectivos ortólogos em *Arabidopsis thaliana*, e tiveram sua expressão gênica avaliada na resposta à *P. griseola*. Gene R TIR-NB-ARC (Phvul.010G025700) foi induzido em resposta compatível no genótipo IAC-UNA, com isso deve permitir a proliferação do patógeno, possivelmente através do reconhecimento do Avr (avirulência) do fungo, bloqueando a resposta de defesa. Além disso, genes putativos de regulação negativa da resposta imune pela inativação da via do ácido salicílico (SA) foram reprimidos durante resposta incompatível no genótipo CAL 143. O AS é um hormônio chave para resposta de defesa induzida por patógenos em plantas. Com isso, o reconhecimento do patógeno pelo feijão deve ocorrer através de genes R para sinalização *downstream* da resposta de defesa mediada por AS. Os resultados deste trabalho permitirão que o melhorista manipule a diversidade genética do feijão, seja pela introgressão e piramidação dos genes de resistência através de seleção assistida por marcadores ou transgenia; seja pela identificação de cultivares geneticamente resistentes pela análise de expressão gênica.

Palavras-chave: *Pseudocercospora griseola* (Sacc.) Crous & Braun; mapeamento de QTL; marcadores genéticos; contexto genômico; genes candidatos

ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is an important protein source in human diet. The angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun, leads to great common bean yield losses. The common bean breeding search for tools that improve the transferring of disease resistance genes to developing cultivars. Therefore, the objective of the present work was study the genetic and molecular mechanisms enrolled in the common bean responses to ALS, and with this contribute to this crop breeding.

Initially, ALS resistance QTLs were identified using the UC (IAC-UNA x CAL 143) mapping population based on the genetic map previously developed with microsatellites markers. The quantitative study of the ALS disease severity reveals normal and transgressive distribution on the UC population, with quantitative resistance observed in CAL 143. Seven QTLs were mapped in five different common bean linkage groups. Of these, the ALS10.1 locus showed major effect (16% - 22%) and stability in all three environments analyzed: (1) natural infection conditions in the dry season; (2) natural infection conditions in wet season; and (3) race-specific controlled infection conditions in greenhouse.

The ALS10.1 locos region was saturated with microsatellites, SCARs and *Sequence-Tagged Site*-DArTs (STS-DArTs) markers; the latter using the *bulk segregant analysis* (BSA). The confidence interval was reduced from 13.4 cM to 3.0 cM after the locus saturation, which had the markers number increased from four to 10. The study of the ALS10.1 genomic context through the alignment of the markers to the draft of the common bean genome enabled the identification of the QTL core at the end of the chromosome Pv10, with approximately 3.5 Mb. The Gene Ontology (GO) analyses of the 323 predicted genes for this genomic region demonstrated that 61.6% of the genes are involved in stress responses. A TIR-NB-ARC gene cluster (domains highly conserved in Resistance (R) genes), was observed covering approximately 849 Kb on the ALS10.1 core region; besides this region also presents other genes known to be related to plant immunity.

Seven genes on ALS10.1 core region were selected based on the role in pathogen resistance of the respective *Arabidopsis thaliana* orthologs, and had their gene expression pattern evaluated in response to *P. griseola*. The R gene TIR-NB-ARC was induced during the compatible response of the genotype IAC-UNA; therewith it should enable the pathogen proliferation, probably through the fungus Avr recognition, blocking the defense response. In addition, putative negative regulator genes of immune response through the inactivation of salicylic acid (SA) via were repressed during the incompatible response of the CAL 143. The SA is a key hormone to pathogen induced plant defense response. Therefore, the common bean pathogen recognition should take place through the R genes to the downstream signaling of the SA-mediated defense response. The results of the present work will enable the manipulation of the bean genetic diversity by the breeder either by introgression and pyramiding of resistance genes through marker assisted selection or transgenesis; or by the identification of genetically resistant cultivars through gene expression analysis.

Keywords: *Pseudocercospora griseola* (Sacc.) Crous & Braun; QTL mapping; genetic markers; genomic context; candidate genes

INTRODUÇÃO

O feijoeiro

O feijoeiro comum (*Phaseolus vulgaris* L.) é uma leguminosa diploide que apresenta uma grande diversidade genética comparado a outras espécies autógamas (Gepts & Papa et al., 2010). Através de pequenas diferenças nos padrões eletroforéticos de faseolina e do padrão de marcadores moleculares como *Amplified Fragment Lenght Polymorphism* (AFLP), *Random Amplified Polymorphic DNA* (RAPD) e microssatélites (SSRs) pode-se determinar a existência de dois principais centros de origem e domesticação (Chacón et al., 2005). O primeiro centro é denominado Mesoamericano, se estendendo desde o sudeste dos Estados Unidos até o Panamá, tendo como zonas principais o México e a Guatemala. Nesta região, teriam surgido as variedades de grãos pequenos. O segundo centro é chamado Andino, abrangendo as regiões do Peru, Argentina, Colômbia e Venezuela, onde teriam sido originadas as variedades de sementes grandes. Além destes centros americanos, podem ser identificados centros secundários de domesticação em regiões da Europa, Ásia e África, onde foram introduzidos genótipos americanos (Singh, 2001). O Brasil também se destaca com um importante centro secundário de domesticação e diversidade genética (Burle et al., 2010).

Considerada a mais importante das 50 espécies de *Phaseolus* nativas das Américas, o feijoeiro comum ocupa mais de 85% das áreas semeadas desse gênero em todo do mundo (Singh , 2005), sendo o Brasil o maior produtor e consumidor mundial desse grão, com a safra de 2012/13 estimada em aproximadamente 3 milhões de toneladas (Companhia Nacional de Abastecimento – CONAB). Assim, o feijão se destaca como importante fonte protéica na dieta humana, além de conter grandes quantidades de outros fatores nutricionais como carboidratos complexos, fibras e isoflavonas (Vieira et al., 1998; Anderson et al., 1999); e em menor escala ferro, fósforo, magnésio, manganês, zinco e cálcio (Broughton et al., 2003).

O melhoramento do feijoeiro comum é extremamente importante, não só para aumentar a produção das cultivares, mas também para minimizar o manejo agrícola, agregando características de interesse agro-econômico em um único cultivar, como: qualidade de grão, porte de planta, tolerância à seca e outros estresses abióticos, resistência à doenças, entre outros. Objetiva-se assegurar o desempenho e estabilidade dos cultivares em campo, garantindo a aceitação dos produtores (Xu & Crouch, 2008). Para isso, a compreensão do funcionamento do genoma desta espécie é de grande importância. Através de informações genômicas é possível desenvolver e aplicar ferramentas como seleção assistida e transgenia e com isso melhorar a cultura (Beaver & Osorno, 2009). Desta forma, o desenvolvimento de ferramentas moleculares que auxiliem o melhoramento são de extrema importância.

Marcadores moleculares: SSRs, DArTs e SCARs

Uma ferramenta bastante usada no melhoramento de plantas é o estudo genético baseado em marcadores moleculares, com obtenção de mapas genéticos e mapeamento de locos de interesse (Xu & Crouch, 2008). Dentre os diversos tipos de marcadores moleculares utilizados estão os microssatélites (SSRs; *Simple Cipoense Repeats* - Tautz, 1989) que se destacam por ser multi-alélico e codominante. Os microssatélites são amplamente distribuídos no genoma de plantas, sendo altamente mutáveis pela coexistência e coevolução com elementos transponíveis (Gao et al., 2013). Estes locos se encontram sob pressão seletiva na resposta às mudanças ambientais, atuando na evolução de espécies de plantas. Ainda, as seqüências contendo microssatélites estão associadas com a atividade catalítica e funções de ligação na maioria das espécies de plantas, sendo expressas na membrana e em organelas, com participação em processos metabólicos e de desenvolvimento (Gao et al., 2013). Este tipo de marcador é amplamente aplicado no mapeamento genético do feijão, tanto aqueles desenvolvidos com base em seqüências genômicas (Blair et al., 2006a; Buso et al., 2006; Benchimol et al., 2007; Grisi et al., 2007; Blair et al., 2008; Cardoso et al., 2008; Oblessuc et al., 2009; Campos et al., 2011), como baseados em seqüências expressas (Gaitán-Solís et al., 2002; Caixeta et al., 2005; Hanai et al., 2007; 2010; Blair et al., 2011).

Os DarTs (*Diversity Arrays Tecchnology* - Jaccoud et al., 2001) também são muito utilizados no estudo de plantas cultivadas, principalmente para obtenção de mapa altamente saturados por serem marcadores amplamente distribuídos no genoma. O desenvolvimento deste marcador baseia-se em digestão de um *pool* de material genético representativo de uma espécie com enzimas de restrição e posterior ligação com adaptadores para clonagem de DNA. Os clones obtidos são utilizado como matriz de hibridização de uma amostra em estudo. Um único ensaio de DArT é capaz de genotipar simultaneamente centenas a milhares de SNPs (*Single Nucleotide Polymorphism*) e INDELs (inserções e deleções no DNA) ao longo do genoma de uma espécie (Wenzl et al., 2004). Os DArTs mostraram ser uma ótima alternativa no mapeamento de populações contrastantes (Lezar et al., 2004; Wittenberg et al., 2005; Xie, 2006; Wenzl et al., 2006; Akbari et al., 2006; Bolibok-Bragoszewska et al., 2009; Mace et al., 2009). A plataforma DArT também foi adequada para análise de *Bulk Segregant Analysis* (BSA) (Wenzl et al., 2007). A técnica de DArT-BSA foi testada inicialmente em cevada para mapeamento do gene de resistência a ferrugem *Rph14* (Golegaonkar et al., 2009); e posteriormente, também foi utilizada no mapeamento de características importantes para outras culturas (Tah et al., 2010). Recentemente, plataforma DArT foi desenvolvida para feijão, contendo 15.360 clones representativos da espécie (Briñez et al., 2012).

Assim como os DArTs, os *Sequence Characterized Amplified Regions* (SCARs; Kesseli et al., 1992) também são marcadores difundidos como ferramentas para o melhoramento de plantas por apresentarem associação a locos de interesse tanto via mapeamento como por análise de BSA (Kelly et al., 2003; Namayanja et al., 2006; Gepts et al., 2008). Seu uso em seleção assistida por marcadores também tem sobressaído (Alzate-Marin et al., 2005; Garzón et al., 2008; Beraldo et al., 2009; Ragagnin et al., 2009; Costa et al., 2010). SCARs são marcadores derivados de RAPDs de forma a serem loco específico e não apresentarem problemas de reprodutibilidade como seu precursor. Diversos estudos utilizando este marcador já foram realizados em feijão, inclusive na busca por locos de resistência à doenças, uma vez que a resistência genética em plantas cultivadas é a estratégia mais econômica, segura e eficiente de

controle de doenças, disponível para pequenos e grandes agricultores (Namayanja et al., 2006; Varshney et al., 2009).

Mancha angular

A mancha angular (ALS) é uma importante doença que afeta a cultura do feijoeiro, sendo encontrada em diversos países ao redor do mundo e levando a até 80% de perdas na produção (Stenglein et al., 2003; Singh & Schwartz 2010). No Brasil, a época das águas de cultivado do feijão (de setembro à outubro na região sudeste do Brasil) favorece a infecção de *P. griseola* pela abundância de chuvas na fase adulta das plantas, uma vez que *P. griseola* que se beneficia da umidade para infectar as folhas de feijão, uma vez que em condições naturais de infecção em campo, os tubos de germinação penetram mais facilmente pelos estômatos abertos durante a transpiração foliar (Monda et a., 2001; Allorent & Savary, 2005).

A ALS é causada pelo fungo *Pseudocercospora griseola (Sacc.) Crous & Braun (sin. Phaeoisariopsis griseola (Sacc.) Ferraris)* (Crous et al., 2006), que causa lesões necróticas nas partes aéreas da planta (Fig. 1), diminuindo a produtividade e a qualidade dos grãos. A infecção se dá pela penetração dos conídios tanto através da epiderme como pelos estômatos, cerca de três a sete dias após a inoculação (Monda et al., 2001). É um fungo biotrófico nas fases iniciais da infecção, passando a necrotrófico, quando seu ataque resulta em manchas necróticas angulares, delimitadas pelas nervuras foliares, que são características da doença (Allorent & Savary, 2005).

Figura 1 Sintomas de mancha angular em folha de feijão. As lesões necróticas delimitadas às nervuras foliares são características de infecção por *P. griseola*, sendo observáveis a olho nu.

O fungo *P. griseola* apresenta uma grande variabilidade genética e diversas raças fisiológicas (Pastor-Corrales & Jará, 1995; Pastor-Corrales et al., 1998; Sartorato, 2002; Silva et al., 2008; Abadio et al., 2012) que são definidas de acordo com sistema de valor binário descrito por Pastor-Corrales & Jara (1995) (Figura 2). As raças de *P. griseola* podem ser agrupadas em dois *pools* gênicos principais: Mesoamericano e Andino (Wagara et al., 2004). Isolados do primeiro grupo apresentam maior variabilidade genética (Wagara et al., 2004) e infectam tanto cultivares Mesoamericanas como Andinas, enquanto que isolados do segundo grupo infectam apenas feijoeiros da mesma origem (Pastor-Corrales & Jara, 1995).

Quadro 10 - Cultivares de feijão utilizados como diferenciadores para caracterizar raças de *Phaeoisariopsis griseola*

Cultivar diferenciador	Tamanho da semente[1]	Acervo genético	Valor binário quando suscetível[2]
A. Don Timóteo	G	Andino	1
B. G11796	G	Andino	2
C. Bolón Bayo	G	Andino	4
D. Montcalm	G	Andino	8
E. Amendoim	G	Andino	16
F. G5686	G	Andino	32
G. PAN 72	P	Mesoamericano	1
H. G2858	M	Mesoamericano	2
I. Flor de Mayo	P	Mesoamericano	4
J. México 54	M	Mesoamericano	8
K. BAT 332	P	Mesoamericano	16
L. Cornell 49-242	P	Mesoamericano	32

1/ G= grande, M = médio e P = pequeno.
2/ Valor binário para nomear as raças de *P. griseola*. Por exemplo, se um isolado ataca os cultivares diferenciadores F (grupo andino, valor binário 32), G (grupo mesoamericano, 1) e K (grupo mesoamericano, 16), a raça denomina-se 32.17. Obtém-se o fenótipo de virulência da raça, somando-se os valores binários, primeiro os referentes aos acervos andinos e, posteriormente, aos mesoamericanos com reação de compatibilidade.
Fonte: Pastor-Corrales e Jara (1995) e Pastor-Corrales (informação pessoal).

Figura 2 Lista dos 12 cultivares diferenciadores para identificação das raças fisiológicas de *Pseudocercospora griseola*, com seus respectivos valores, em sistema de notas binário. Fonte: Borém, 1998.

Diversos genótipos de feijoeiro já foram identificados como fontes de resistência à ALS (Pastor-Corrales et al., 1998; Mahuku et al., 2003; Sartorato, 2006; Muthomi et al., 2012). Dentre estes se destaca CAL 143 por apresentar alto nível de resistência contra um grande número de raças, seja em condições de campo ou de casa de vegetação (Aggarwal et al., 2004). Esta linhagem também é resistente a ferrugem, oídio, mancha-de-alternária e antracnose (Vieira et al., 2002) e tolerante a variações de pH e a baixos teores de fósforo e nitrogênio no solo (Kimani et al., 2007). Apesar de sua importância, estudos para determinar os genes responsáveis por tais fenótipos de resistência ainda não foram conduzidos nesta linhagem.

Até o momento apenas dois genes dominantes foram descritos para resistência à ALS. O primeiro, denominado *Phg-1*, foi identificado na variedade AND 277 (Carvalho et al., 1998) e recentemente mapeado no grupo de ligação B01 (Gonçalvez-Vidigal et al., 2011). O segundo, denominado *Phg-2*, foi identificado na variedade México 54 (Sartorato et al., 2000) ligado aos marcadores SCAR OPN02 e RAPD OPE04, este último mapeado no grupo de ligação B08 (Mahuku et al., 2011). Estes dois marcadores (SCAR OPN02 e RAPD OPE04) estão ligados entre si (Nietsche et al., 2000) e também estão ligados a gene de resistência nas variedades Cornell 49-242 (Nietsche et al., 2000), MAR 2 (Ferreira et al., 2000) e BAT 332 (Namayanja et al., 2006). Testes de alelismo demonstraram que o gene *Phg-2* de México 54 é o mesmo que o de BAT 332 (Namayanja et al., 2006). Além destes dois genes, herança monogênica dominante para resistência à ALS também foi descrita na variedade Ouro Negro (Corrêa et al., 2001) e G10474 (Mahuku et al., 2004), mas a relação destes genes com *Phg-1* e *Phg-2* permanece desconhecida. Por fim, há ainda o caso de US Pinto 111, que apresenta resistência monogênica recessiva (Corrêa et al., 2001).

Além de genes de resistência qualitativa, também há relatos de QTLs (*Quantitative Trait Locus*) controlando resistência à ALS. Cinco QTLs foram mapeados no grupo de ligação B04, um no B08, um no B09 e três no B10 (López et al., 2003; Mahuku et al., 2009; Mahuku et al., 2011). Estes estudos revelaram que a resistência à ALS é mais complexa do que a descrita nos trabalhos citados anteriormente, como

pôde ser observado nas variedades G19833, G5686 e G10909. Na verdade, apesar do loco mapeado no grupo B08 em G10909 também estar ligado ao marcador OPE04, testes de alelismo mostraram que este loco é distinto de *Phg-2* (Mahuku et al., 2011). Estes resultados corroboram a teoria de maior complexidade de herança para resistência à ALS observada por Caixeta et al. (2005). Os autores mostraram via testes de alelismo que três outros genes (*Phg-3*, *Phg-4* e *Phg-5*), com pelo menos um segundo alelo cada, estariam contribuindo com a resistência à ALS em quatro variedades de feijão anteriormente caracterizadas como contendo resistência monogênica (AND 277, México 54, MAR 2 e Cornell 49-242).

Resistência à doenças em plantas

A interação entre um patógeno e seu hospedeiro é uma luta entre dois organismos pela própria sobrevivência, em que as células do hospedeiro reagem à penetração do patógeno através de vários mecanismos estruturais e/ou bioquímicos para se defender do ataque (Pascholatti & Leite, 1995). A defesa das plantas é baseada no reconhecimento de moléculas específicas de patógenos e subseqüente indução de uma ampla resposta de defesa. Este reconhecimento evolui germinalmente, de modo que uma planta só pode se defender com o espectro de genes que herdou de seus pais. A diversidade genética entre os indivíduos é, portanto, essencial para a sobrevivência das espécies hospedeiras contra patógenos em rápida evolução. As plantas são resistentes à maioria dos microorganismos (resistência de não-hospedeiro) por meios de constituintes químicos e barreiras físicas, como revestimentos cuticulares, que os fitopatógenos devem atravessar ou contornar (Dangl & Holub, 1997). A imunidade da plantas depende de eventos celulares autônomos, que estão relacionados ao sistema imune inato em animais, no qual as plantas apresentam um repertório de reconhecimento do patógeno muito maior para compensar a falta de um sistema imune adaptativo (Dodds & Rathjen, 2010). Existem dois principais tipos de resistência à doenças em plantas: defesa mediada por genes de resistência (R); e a defesa basal mediada por receptores de reconhecimento de padrões (PRRs), ambos presentes na superfície da célula hospedeira (Jones & Dangl, 2006).

Genes R participam da resposta imune inata de plantas, que atua como barreira inicial de defesa contra patógenos. A resposta mediada por genes R é conhecida como ETI (*Effector-Trigger Immunity*), na qual proteínas do hospedeiro reconhecem a(s) proteína(s) efetora(s) promotora de virulência do patógeno e desencadeiam sinalização *downstream* que leva a ativação de vias metabólicas e expressão de genes que levam a imunidade da planta. Tipicamente, a propensão para o gatilho ETI é estirpe ou raça específica de patógeno e está associada com a morte celular programada da célula do hospedeiro, uma resposta que é referida como a resposta de hipersensibilidade (HR). De acordo com o modelo em ziguezague, além da ETI existe uma segunda via uma resistência basal regida pelo reconhecimento de moléculas microbianas conservadas (*Pathogen-Associated Molecular Patterns*; PAMPs), a PTI (PAMP *Trigger Immunity*). Estas duas fases de imunidade da planta podem ser discriminadas, em que a ETI é mais rápida, mais prolongada e mais robusta do que a PTI (Jones & Dangl, 2006; Tsuda & Katagiri, 2010).

Em ambas as fases de resposta, ETI e PTI, a produção de ROS (*Reactive Oxygen Species*) é importante na ativação de HR e morte celular programada (Thomma et al., 2011). As ROS são produzidas de forma contínua como resultado do metabolismo aeróbico ou em resposta a estresses bióticos e abióticos, sendo também moléculas sinalizadoras envolvidas em vários processos do desenvolvimento de todos os organismos (Nanda et al., 2010). Na resposta à patógenos, a produção de ROS na explosão oxidativa é uma das reações de defesa mais rápidas no local da tentativa de invasão, com a produção de superóxido (O_2^-) e peróxido de hidrogênio (H_2O_2) (Apel & Hirt, 2004). A produção de ROS ocorre ainda de forma mais intensa numa segunda fase de defesa da célula do hospedeiro, na qual atua também como importante molécula de sinalização de resposta imune (Nanda et al., 2010).

Além de moléculas de ROS, proteínas celulares também são responsáveis pela sinalização imediatamente *downstream* ao reconhecimento de patógenos para resposta imune (Dodds & Rathjen, 2010). Proteínas quinase, como *mitogen-activated protein kinase*s (MAPK), são importantes responsáveis pela transdução de sinais em plantas, ativando ou inibindo seus substratos pela fosforilação de resíduos de serina, treonina ou tirosina em sítios específicos (Endicott et al., 2012). Em adição, outros domínios protéicos também

atuam nesta transdução de sinal em plantas, como os domínio TIR (*Toll/interleukin-1 receptor like*) e NB-ARC (*nucleotide-binding adaptor*) implicados na interação proteína-proteína. Investiga-se que provavelmente tais domínios levam a alteração conformacional da proteína interatora pela hidrólise ou troca de nucleotídeo através da ação do sub-domínio ATPase; e com isso leva a transdução do sinal (Tameling et al., 2008).

Assim, após o reconhecimento do patógeno e a transdução deste sinal até o núcleo celular, importantes genes de resposta imune são induzidos ou reprimidos. Dentre os diversos genes finais de resposta à patógenos, os genes PR (*pathogenesis-related*) e as fitoalexinas são extremamente importantes na defesa da planta. Até o momento 11 famílias de PRs (PR1 a PR11) foram identificadas (Datta & Muthukrishnan, 2010), e incluem enzimas hidrolíticas (tais como β-1,3-glucanase e quitinase) e defensinas, que têm atividade antimicrobianas potentes através da hidrólise das paredes das células de agentes patogênicos e a ruptura da membrana do patógeno, respectivamente (Spoel & Dong, 2012). Já as fitoalexinas são metabólitos secundários extremamente diversos produzidos em plantas em resposta a estresses, como a infecção por patógenos. A camalexina de *Brassica*, a glyceolina de soja, a 3-deoxiantocianina de sorgo e o resveratrol de videiras, são alguns exemplos de fitoalexinas com atividade anti-microbiana (Ahuja & Bones, 2012). Assim, a produção de proteínas PRs e fitoalexinas são respostas ativadas na defesa de plantas contra patógenos e exercem um importante papel com efetiva ação antimicrobiana.

Estudos genômicos de resistência à doenças do feijoeiro

Até o momento, o estudo do genoma do feijão tem se baseado principalmente em análises de ligação genética entre marcadores moleculares e locos associados a caracteres de interesse agronômico (Beaver & Osorno, 2009). Para isso, muitos mapas genéticos já foram desenvolvidos, com diferentes populações segregantes (Freyre et al., 1998; Yan et al., 2004; Blair et al., 2006b; Miklas et al., 2006; Tian et al., 2007; Checa et al., 2008; Hanai et al., 2010; Campos et al., 2011). Na realidade, a obtenção de mapas altamente saturados, com boa cobertura do genoma, é uma importante ferramenta na identificação de genes

candidatos, principalemnte em espécies com pouco informação genômica (Varshney et al., 2009), como o feijão.

Estudos de mapeamento posicional que relacionam locos de resistência a doenças com os genes envolvidos na resposta imune são observados em feijão. O primeiro trabalho que buscou mapear e clonar um gene ligado à resistência em feijão foi realizado por Creusot et al. (1999), a partir do loco *Co-2*, associado à resistência a antracnose (*Colletotrichum lindemuthianum*). Duas ORFs (*Open Read Frame*) foram identificadas a partir de uma pequena população RIL (*Recombinant Inbred Lines*) segregante, derivada do cruzamento entre as linhagens EO2 e Corel. O marcador SCH20.1 foi utilizado como sonda para hibridização com biblioteca de cDNA. Porém, foram encontrados apenas supostos genes, sendo que a ORF1 era interrompida por dois códons de terminação e a ORF2 era similar a um pseudogene.

Posteriormente, uma ORF de 1.110 pb foi isolada e nomeada de *COK-4,* localizada dentro do loco *Co-4*, associado também à resistência a antracnose (Melotto & Kelly, 2001). O mapeamento fino do loco *Co-4* foi realizado a partir do SCAR SAS13, proximamente ligado à ele. A estratégia de mapeamento baseou-se na obtenção de uma população inicial de 1018 indivíduos F_2 originada do cruzamento entre a linhagem resistente SEL 1308 e a linhagem suscetível Black Magic. Uma biblioteca BAC (*Bacterial Artificial Chromosome*) construída para a cultivar Sprite (Vanhouten & MacKenzie, 1999) foi utilizada na saturação da região do *Co-4*, juntamente com a fenotipagem de 1350 plantas F_3, com a raça 73 do fungo *C. lindemuthianum*.

Esta biblioteca BAC também foi utilizada por Vallejos et al. (2006) para clonagem do loco *I*, envolvido na resistência ao vírus do mosaico comum do feijoeiro e outros Potyvirus relacionados. Neste trabalho, foram utilizadas inicialmente 96 RILs para o mapeamento de baixa resolução do loco *I*. Já o mapeamento fino foi realizado com uma população F_2 de 3056 plantas agrupadas em *pools* pelo método do *pooled sample mapping* (Churchill et al., 1993) para identificação das recombinações na região do loco em estudo. Os marcadores Bng45 e Phgp foram identificados como intimamente ligados ao loco *I*, à 0,2 cM e

0,1 cM de distância, respectivamente. Estes marcadores foram, então, utilizados na clonagem desta região, por mapeamento físico via alinhamento de clones BAC. Um *cluster* com mais de 425 kb de seqüências TIR–NBS–LRR foi identificado.

Recentemente, trabalho de mapeamento físico do loco de maior efeito na resistência ao crestamento bacteriano comum (*Xanthomonas axonopodis* pv. *phaseoli*) foi realizado em feijão comum (Liu et al., 2010). Os autores realizaram mapeamento do QTL de maior efeito no grupo de ligação B6 através da população de mapeamento HR67 x OAC95-4 de 81 RILs. Quatro marcadores (UBC420, STS333, Pv-tttc001 e STS183) próximos ao QTL foram utilizados na hibridização de *pools* de clones BAC. Dezoito clones foram positivos para estes 4 marcadores, apresentando insertos entre 70 – 150 Kb. O alinhamento das seqüências desses clones resultou em um *contig* de aproximadamente 750 Kb, cobrindo a região deste QTL.

Contudo, tais estudos não fornecem informação específica sobre mecanismos moleculares que estão envolvidos na resistência. Para isso, o estudo estrutural e funcional do genoma do feijão é extremamente importante. Inicialmente, mapa físico foi obtido a partir de biblioteca BAC da linhagem Andina G19833, com 9,45% do genoma coberto (Schlueter et al., 2008). Em continuidade a este trabalho, foi disponibilizado rescentemente um rascunho do genoma do feijão no Phytozome (http://www.phytozome.net/). Da mesma forma, o grupo "Ibero-American Program for Science, Technology and Development" (CYTED) também trabalha na obtenção do genoma do feijão (http://www.cyted.org/), contudo o material genético foi obtido da linhagem Mesoamericana BAT93 para este estudo. Estas duas iniciativas são de grande importância para a cultura do feijoeiro, permitindo estudos funcionais mais aprofundados.

Apesar destes genomas ainda não estarem disponíveis para uso em trabalhos de análise funcional global, uma vez que ainda não foram publicados, estudos de expressão gênica vêem sendo realizados para feijão. Na área de resistência a doenças, estudos baseados em bibliotecas de estas e RNAseq ressaltaram a

importância da ação de genes R na resistência à patógenos, sendo identificados 365 genes R não-redundantes em todo genoma do feijão, dos quais 25 tiveram sua expressão confirmada por RT-PCR (Liu et al., 2012). Genes R em feijão também foram divididos em famílias TIR e não-TIR, cada uma com diferentes agrupamentos. As seqüências TIR foram agrupadas em 14 clados; enquanto as seqüências não-TIR foram agrupadas em sete clados (Garzón et al., 2013).

Recentemente, foi demonstrado ainda que a expressão heteróloga de cDNA *antisense* da peroxidase de feijão em *Arabidopsis thaliana* leva a redução de expressão de duas peroxidases PRX33 e PRX34, bloqueando ROS; além de reduzir o depósito de calose nas folhas, outra comum resposta a PAMPs (Daudi et al., 2012). Em adição, análise geral de transcriptoma de feijão em interação incompatível com *C. lindemuthianum* revelou que HR decorrente da interação do feijão com este patógeno ocorre também pela repressão de genes de PTI como *FLS2-like* (*FLAGELLIN SENSITIVE2-like*) e *MKK5-like* (*MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) KINASE 5-like*) (Oblessuc et al., 2012a). Estes resultados corroboram com a nova visão sobre o modelo ziguezague de ETI/PTI, a qual sugere que na realidade PTI e ETI podem ocorrer concomitantemente durante resposta imune inata de plantas (Thomma et al., 2011).

OBJETIVOS

Tendo em vista a complexidade da resposta imune de plantas e a importância de seu entendimento para o melhoramento de plantas cultivadas, o presente estudo visou identificar mecanismos genéticos e moleculares que fazem parte da resposta do feijão ao fitopatógeno causador da mancha angular, *Pseudocercospora griseola*. Assim,o trabalho pode ser dividido em três pontos principais: (1) a identificação de QTLs de resistência à mancha angular, com avaliação dos efeitos; (2) a saturação da região do QTL de maior efeito na resistência à mancha angular; (3) e a caracterização da estrutura genômica deste loco de maior efeito com a identificação de genes candidatos envolvidos na resistência à mancha angular. Para isso os seguintes objetivos específicos foram alcançados:

1. Avaliação da resistência quantitativa à mancha angular na população de mapeamento segregante IAC-UNA x CAL-143 (UC), composta por 346 linhagens endogâmicas (F_{12}) em diferentes condições experimentais (campo, com infecção natural; e casa de vegetação, com infecção controlada raça-específica), de forma a reduzir os efeitos ambientais.
2. Identificação dos efeitos editivos e quantificação da magnitude dos QTLs mapeados. As possíveis interações genótipo x ambiente dos locos mapeados também foram verificadas por análise conjunta de mapeamento por intervalo composto (*joint*-CIM), através do programa WinQTLCartographer.
3. Saturação da região do QTL de maior efeito identificado (ALS10.1) com marcadores SSRs, SCARs e derivados DArTs; este último utilizando a técnica de BSA. Para isso, *screening* dos marcadores polimórficos foi realizado nos genitores IAC-UNA e CAL 143 para os marcadores SSRs e SCARs; e com adição dos dois *bulks* segregantes (resistente e susceptível) para os DArTs. Todos os marcadores polimórficos foram utilizados na genotipagem das linhagens endogâmicas para análise de ligação utilizando o programa MapMaker.
4. Identificação da região genômica que compõem o QTL de maior efeito por s*creening* de biblioteca BAC derivada do genótipo G2333, composta de aproximadamente 25.000 clones,

utilizando os marcadores ligados ao QTL de maior efeito pela técnica de PCR de colônia. A técnica de *chromosome walking* também foi utilizada para total cobertura da região do QTL, com posterior análise de alinhamento dos marcadores ligados ao loco de maior efeito contra o genoma do feijão (Phytozome).

5. Identificação dos genes que compõe o QTL de maior efeito através do genoma do feijão (Phytozome), com identificação dos possíveis ortólogos em *Arabidopsis thaliana* por filogenia com posterior análise de expressão dos genes de feijão possivelmente envolvidos na resposta à patógenos, para identificação de genes candidatos à resistência à mancha angular e das possíveis vias metabólicas atuantes nesta resposta de defesa.

CAPÍTULO I

Oblessuc PR, et al. Mapping of angular leaf spot resistance QTL in common bean (*Phaseolus vulgaris* L.) under different environments. BMC Genetics 2012, 13:50.

RESUMO

O feijão comum (*Phaseolus vulgaris* L.) é a leguminosa mais importante para a dieta humana em todo o mundo e a mancha angular (ALS) é uma das mais devastadoras doenças desta cultura, levando a perdas de rendimento tão alto quanto 80%. Para obtenção de cultivares resistentes, é importante compreender primeiro o modo de herança da resistência e também desenvolver ferramentas que possam ser utilizadas em seleção assistida. Portanto, o objetivo deste estudo foi identificar locos controladores de características quantitativas (QTL) envolvidos na resistência à ALS, em condições de infecção natural no campo e sob condições de inoculação em casa de vegetação.

Os QTLs foram identificados apartir de três experimentos, dois dos quais foram realizados no campo em diferentes estações do ano e um em casa de vegetação. Análise conjunta de mapeamento por intervalo composto foi realizada, permitindo identificar possíveis interações QTL x ambiente. Ao todo, sete QTLs foram mapeados em cinco grupos de ligação. A maioria deles, com exceção de dois, foram significativas em todos os experimentos. Entre estes, ALS10.1$^{DG,\ UC}$ apresentou maior efeito sobre a resistência (R^2 entre 16% - 22%). Este QTL foi encontrado ligado ao marcador GATS11b no grupo de ligação B10, o qual foi consistentemente amplificado através de um conjunto de linhagens de feijoeiro, sendo associado com a resistência. Quatro novos QTLs também foram identificados. Entre eles o ALS5.2 mostrou um efeito importante (9,4%) em condições inoculadas em casa de vegetação. ALS4.2 foi outro QTL de maior efeito sob infecção natural no campo, explicando 10,8% da variabilidade de reação de resistência. Os outros QTLs mostraram efeitos menores sobre a resistência.

Os resultados deste trabalho indicaram um padrão de herança quantitativa da resistência à ALS oriunda da linhagem de feijoeiro CAL 143. Interações QTL x ambiente foram observadas. Além disso, o QTL de maior efeito identificado no grupo de ligação B10 pode ser importante para o melhoramento do feijoeiro, uma vez que mostrou ser estável em todos os ambientes estudados. Dessa forma, o marcador GATS11b é uma ferramenta potencial para a seleção assistida por marcadores para resistência ALS.

Oblessuc et al. BMC Genetics 2012, 13:50
http://www.biomedcentral.com/1471-2156/13/50

BMC Genetics

RESEARCH ARTICLE Open Access

Mapping of angular leaf spot resistance QTL in common bean (*Phaseolus vulgaris* L.) under different environments

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Abstract

Background: Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for human diet worldwide and the angular leaf spot (ALS) is one of the most devastating diseases of this crop, leading to yield losses as high as 80%. In an attempt to breed resistant cultivars, it is important to first understand the inheritance mode of resistance and to develop tools that could be used in assisted breeding. Therefore, the aim of this study was to identify quantitative trait loci (QTL) controlling resistance to ALS under natural infection conditions in the field and under inoculated conditions in the greenhouse.

Results: QTL analyses were made using phenotypic data from 346 recombinant inbreed lines from the IAC-UNA x CAL 143 cross, gathered in three experiments, two of which were conducted in the field in different seasons and one in the greenhouse. Joint composite interval mapping analysis of QTL x environment interaction was performed. In all, seven QTLs were mapped on five linkage groups. Most of them, with the exception of two, were significant in all experiments. Among these, ALS10.1^{DG,UC} presented major effects (R^2 between 16% - 22%). This QTL was found linked to the GATS11b marker of linkage group B10, which was consistently amplified across a set of common bean lines and was associated with the resistance. Four new QTLs were identified. Between them the ALS5.2 showed an important effect (9.4%) under inoculated conditions in the greenhouse. ALS4.2 was another major QTL, under natural infection in the field, explaining 10.8% of the variability for resistance reaction. The other QTLs showed minor effects on resistance.

Conclusions: The results indicated a quantitative inheritance pattern of ALS resistance in the common bean line CAL 143. QTL x environment interactions were observed. Moreover, the major QTL identified on linkage group B10 could be important for bean breeding, as it was stable in all the environments. Thereby, the GATS11b marker is a potential tool for marker assisted selection for ALS resistance.

Keywords: *Phaseolus vulgaris* L., Angular leaf spot, Joint composite interval mapping, CIM, Resistance QTL

Background

The common bean (*Phaseolus vulgaris* L.) is an important source for human diet of protein, complex carbohydrates, fiber, isoflavones [1] and minerals such as iron and phosphorus [2]. This crop is cultivated in various countries around the world, among which Brazil stands out as the largest producer [3], with over 3,000 t produced in 2010 [4].

Several factors affect bean yield, among which the incidence of diseases is the biggest one. One of the diseases with the greatest impact is the angular leaf spot (ALS) [5,6]. The disease is caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun (sin. *Phaeoisariopsis griseola* (Sacc.) Ferraris) [7], which causes necrotic lesions on the aerial parts of the plant, reducing the productivity and quality of the bean seed. Infection occurs due to conidia that penetrate through both the

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leaf epidermis and stomata, about three to seven days after inoculation [8]. It is a biotrophic fungus in the early stages of infection, which then becomes necrotrophic, when the attack causes the characteristic symptoms of the disease, which are angular necrotic spots limited by the leaf veins [9].

P. griseola presents great genetic variability and several physiological races [10-13] that can be grouped into two gene pools: Mesoamerican and Andean [14]. *P. griseola* isolates from the first group have a higher genetic variability [14] and infect both Andean and Mesoamerican bean cultivars, while isolates from the latter group infect bean plants only from the same origin [10].

Several sources of resistance to ALS have been identified [11,15,16] and among them, CAL 143 stands out due to having a high level of resistance against a large number of *P. griseola* races, whether in the field or the greenhouse [17]. This line is also resistant to rust, powdery mildew, alternaria leaf spot and anthracnose [18] and tolerant to variations of pH and low levels of phosphorus and nitrogen [19].

As the best form of disease control includes using resistant cultivars, the genetic characterization of resistance sources is very important for the genetic improvement of the crop. In the case of ALS, two dominant resistance genes have been described so far. The first, called *Phg-1*, was identified in the AND 277 variety [20] and recently mapped on linkage group B01 linked to markers from soybean [21]. The second, called *Phg-2*, was identified in the Mexico 54 variety [22] linked to SCAR OPN02 and RAPD OPE04 markers. The latter was recently mapped on linkage group B08 [23]. These two markers are linked to each other [24] and are also linked to the ALS resistance gene in the Cornell 49–242 [24], MAR 2 [25] and BAT 332 varieties [26]. Allelism tests showed that the *Phg-2* gene from Mexico 54 is the same as the BAT 332 gene [26]. Apart from these two genes, dominant monogenic inheritance for resistance to ALS has also been described in the Ouro Negro [27] and G10474 varieties [28], but the relationship of these genes with *Phg-1* and *Phg-2* remains unknown. Finally, there is also the case of US. Pinto 111, which presents recessive monogenic resistance [27].

In addition to qualitative resistance genes, there are also reports of QTLs controlling resistance to ALS. Five QTLs were mapped on linkage group B04, one on B08, one on B09 and three on linkage group B10 [23,29,30]. These studies revealed, therefore that resistance to ALS is more complex than described in the papers cited above. Mahuku et al. [23], for example, identified two resistance genes in G10909, where the gene mapped in the B08 group, and despite also being linked to the OPE04 marker, is distinct from *Phg-2*. These results substantiate the more complex inheritance theory towards resistance to ALS observed by Caixeta et al. [31]. These authors showed, via allelism tests, that three other genes (*Phg-3, Phg-4* and *Phg-5*), with two alleles each, control resistance to ALS in four bean varieties that were previously characterized as containing monogenic resistance (AND 277, Mexico 54, MAR 2 and Cornell 49–242).

The objective of this study was to identify QTLs that impart resistant to ALS by means of resistance quantitative analysis of 346 recombinant inbreed lines (RILs) derived from the IAC-UNA x CAL 143 (UC) cross. Line resistance was assessed in three experiments that reflect two distinct infection conditions: evaluation in the field (dry and wet seasons) under natural infection conditions, and evaluation in the greenhouse under controlled inoculation conditions. Linkage analysis between QTL and molecular markers, previously used to construct a genetic map for this same UC population [32], was carried out by means of joint composite interval mapping analysis (joint CIM; [33]).

Results

Statistical analysis of disease severity data

Angular necrotic spots, which are typical of the disease, were seen in the more susceptible RILs 10 days after inoculation on plants growing in the greenhouse and 30 days after sowing, in plants grown in the field. Parent lines presented a contrasting profile for resistance (Figure 1), as expected. The average severity for CAL 143 was 1.23 ± 0.4 considering all experiments, while for IAC-UNA, it was 5.16 ± 0.4 (Table 1). Broad-sense heritability for ALS resistance (Table 1) was high in both wet season experiment on field and in the greenhouse experiment and moderate in dry season experiment. Character showed high heritability (Table 1) in joint analysis.

Severity levels among RILs ranged from 1.0 to 8.9 (Table 1). Severity data values of recombinant inbreed lines (RILs) showed normal distribution judging by skewness (0.0 for all experiments) and kurtosis values (0.32, 0.62 and 1.44 for dry and wet seasons and greenhouse experiments, respectively). Variance analyses (data not shown) confirmed great variability among RILs shown by highly significant values of F tests (F value 3.9, 2.4 and 1.82, $p < 0.0001$ for dry and wet seasons and greenhouse experiments, respectively). Higher or lower resistance levels in relation to the parents were observed for RILs, which showed transgressive segregation.

The genetic and phenotypic correlations were significant in all pairwise combinations between experiments. Pearson's (r_{phe}) values were small, though significant (i.e. values different from zero with p-value ≤ 0.001), with lower values among field and greenhouse experiments (Table 2). The experiments showed no significant environmental correlation. This analysis also revealed a higher correlation between dry and wet seasons in field experiments in relation to the greenhouse experiment.

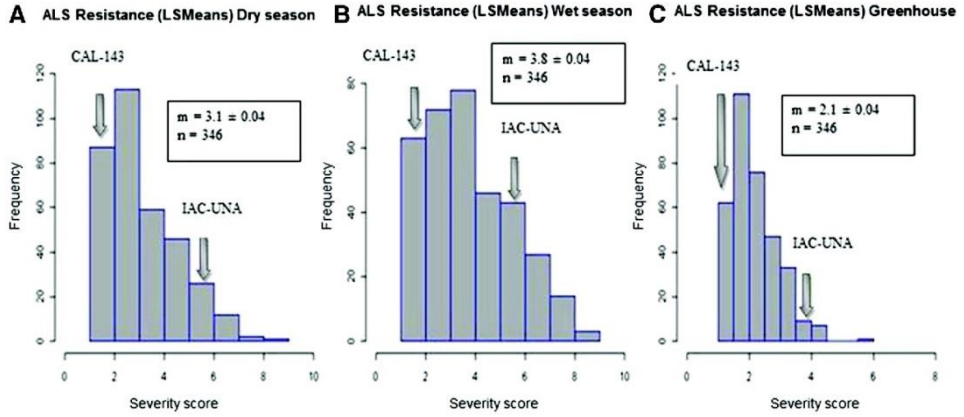

Figure 1 Distribution of angular leaf spot (ALS) severity scores (Least Square Means - LSMeans). A. Scores from RILs evaluated in the field during wet season and **B**. during dry season; **C**. severity scores from greenhouse experiments. Severity values of the parental lines (IAC-UNA and CAL 143) are indicated by arrows.

As each of the three experiments proved to be a different environment, the QTL mapping analyses were not performed separately with the severity values for each experiment, but using a joint model [33]. The joint variance analysis revealed significant interaction between genotype x environment (F value 1.53, $p < 0.0001$).

QTL mapping analysis

QTL mapping through joint composite interval mapping (joint CIM) analysis revealed seven QTLs (Table 3, Figure 2) which were named according to Miklas et al. [34]. Five QTLs were significant in all three experiments ($ALS2.1^{UC}$, $ALS4.1^{GS,UC}$, $ALS4.2^{GS,UC}$, and $ALS5.2^{UC}$ $ALS10.1^{DG,UC}$; Figures 2A, C, E) and only two were not significant in dry season experiment (ALS3.1 and ALS5.1; Figures 2B and 2D, respectively). The ALS10.1 QTL, located on the B10 linkage group, showed the highest LOD and R^2 values of all QTLs and the maximum LOD value was located on the same map position for all the experiments (Figure 2E). Two QTLs that were significant in all experiments showed greater effect only in one of them (Table 3): ALS4.2 had a greater effect in wet season experiment and ALS5.2 in the greenhouse. The remaining QTLs had minor effect on the phenotype.

The percentage of phenotypic variation explained by the combined effects of each QTL was 28% in dry season field experiment, 42% in wet season field experiment and 38% in the greenhouse experiment. As expected, the values of additive effects of each QTL revealed that the alleles from the CAL 143 parent favored resistance in most loci, except in locus ALS5.1, whose favorable allele, in the greenhouse experiment, came from the susceptible parent IAC-UNA (Table 3).

Table 1 Means of disease severity values (LS Means) and heritability for each environment and joint analysis

Genotype	Disease severity			
	Dry season	Wet season	Greenhouse	Joint
CAL-143	1.0 ± 0.4	1.2 ± 0.4	1.5 ± 0.4	1.2 ± 0.4
IAC-UNA	5.6 ± 0.4	5.8 ± 0.4	4.1 ± 0.4	5.2 ± 0.4
RILs	3.1 ± 0.4	3.8 ± 0.4	2.2 ± 0.4	3.0 ± 0.4
range	1.0 – 8.1	1.1 – 8.9	1.0 – 5.6	1.0 – 8.9
H^2	0.51	0.81	0.69	0.80

Standard errors for the parental lines and RILs; range of severity values observed on RILs and broad-sense heritability (H^2) of angular leaf spot resistance, for each environment (dry and wet seasons field experiments and greenhouse) and joint analysis are indicated.

Major QTL validation across bean lines

The marker closest to the maximum LOD value from the major QTL ALS10.1 (GATS11b) was used to

Table 2 Pairwise correlation analysis between experiments: genetic, environmental and phenotypic correlation for the ALS severity values

Environments	r_{gen}*	r_{env}*	Pearson's Correlation (r_{phe}*)
dry season x wet season	0.841**	0.095	0.504**
dry season x greenhouse	0.685**	-0.038	0.334**
wet season x greenhouse	0.535**	0.009	0.339**

* r_{gen} = genetic correlation coefficient; r_{env} = environmental correlation coefficient; r_{phe} = Pearson's correlation.
** significant at 1% based on *t* test (*p*-value ≤ 0.001).

Table 3 Genetic parameters estimated by joint CIM analysis for angular leaf spot resistance

QTL*	Linkage Group	Marker Interval	Environment	LOD	LOD Threshold	R^2 %	Additive effect
ALS2.1^{UC}	**B2**	**IAC134 -IAC18b**	**Joint**	**7.3**	**6.5**		
			dry season	2.8		2.2	-0.292
			wet season	5.8		5.6	-0.466
			gh**	4.5		1.9	-0.188
ALS3.1^{UC}	**B3**	**PVBR21 - FJ19**	**Joint**	**5.8**	**3.4**		
			wet season	3.0		1.3	-0.344
			gh**	4.1		4.3	-0.165
ALS4.1^{GS,UC}	**B4**	**IAC52 - BMd9**	**Joint**	**9.3**	**3.4**		
			dry season	2.5		1.4	-0.362
			wet season	5.5		0.7	-0.586
			gh**	7.0		4.4	-0.280
ALS4.2^{GS,UC}	**B4**	**PVBR92 - Pv-gaat001**	**Joint**	**8.2**	**3.4**		
			dry season	3.3		0.8	-0.225
			wet season	7.3		10.8	-0.629
			gh**	3.4		2.0	-0.170
ALS5.1^{UC}	**B5**	**BMd53 - FJ05**	**Joint**	**5.9**	**4.8**		
			wet season	2.3		2.9	-0.343
			gh**	2.7		0.2	0.149
ALS5.2^{UC}	**B5**	**BM175 - IAC261**	**Joint**	**11.2**	**4.8**		
			dry season	1.9		1.3	-0.147
			wet season	3.3		1.3	-0.164
			gh**	11.2		9.4	-0.272
ALS10.1^{DG,UC}	**B10**	**GATS11b - IAC137**	**Joint**	**25.5**	**3.5**		
			dry season	15.0		22.3	-0.703
			wet season	17.1		21.2	-0.913
			gh**	10.1		15.9	-0.304

*Resistance QTLs were named according to Miklas et al. [34]. ALS corresponding to disease name: Angular Leaf Spot; the first number refers to common bean linkage group; the second one is the QTL number for that linkage group in chronological order of publication; and superscript shorthand is the abbreviation for mapping populations used to discover each QTL, also in chronological order (GS = G5686 x Sprit [30]; GS = G10909 x Sprit [23]; DG = DOR364 x G19833 [29,35]; UC = IAC-UNA x CAL 143 [32]).
**gh = Greenhouse.

genotype resistant and susceptible bean lines. Only in two cases was there discrepancy between line phenotype and its genotype for the locus, where the BAT 332 and Mexico 54 lines, which are resistant to ALS, presented a marker allele from the susceptible parent (IAC-UNA). Thus, all susceptible lines presented the same allele as IAC-UNA, while three of five resistant lines showed the same allele as CAL 143 (Additional file 1).

Discussion

This is the first QTL x environment interaction report for common bean resistance to ALS. The results revealed the existence of seven QTLs with variable magnitudes of phenotypic effects depending on the environments (Table 3), which indicated a complex and quantitative inheritance pattern of this trait on the CAL 143 line. The results contrast with those of other studies that reported dominant monogenic resistance inheritance [20,22,24,25,27,37,38]. One cause for this discrepancy is that these studies have assessed and analyzed the resistance in a qualitative manner rather than in a quantitative one. In fact, when using QTL analysis or when symptom evaluation is made quantitatively, different resistance genes may be observed in the same genotype. López et al. [29] conducted a QTL mapping through the DOR364 x G19833 population and found five ALS resistance QTLs. Mahuku et al. [23,30], on the other hand, used severity assessment on a quantitative scale and found three resistance genes in G5686 and two in G10909. Likewise, allelism tests showed that lines, previously characterized as containing dominant monogenic resistance, actually have different resistance loci to ALS, with allelic variations between lines [26,31]. It was shown, for example, that AND 277 has three other genes

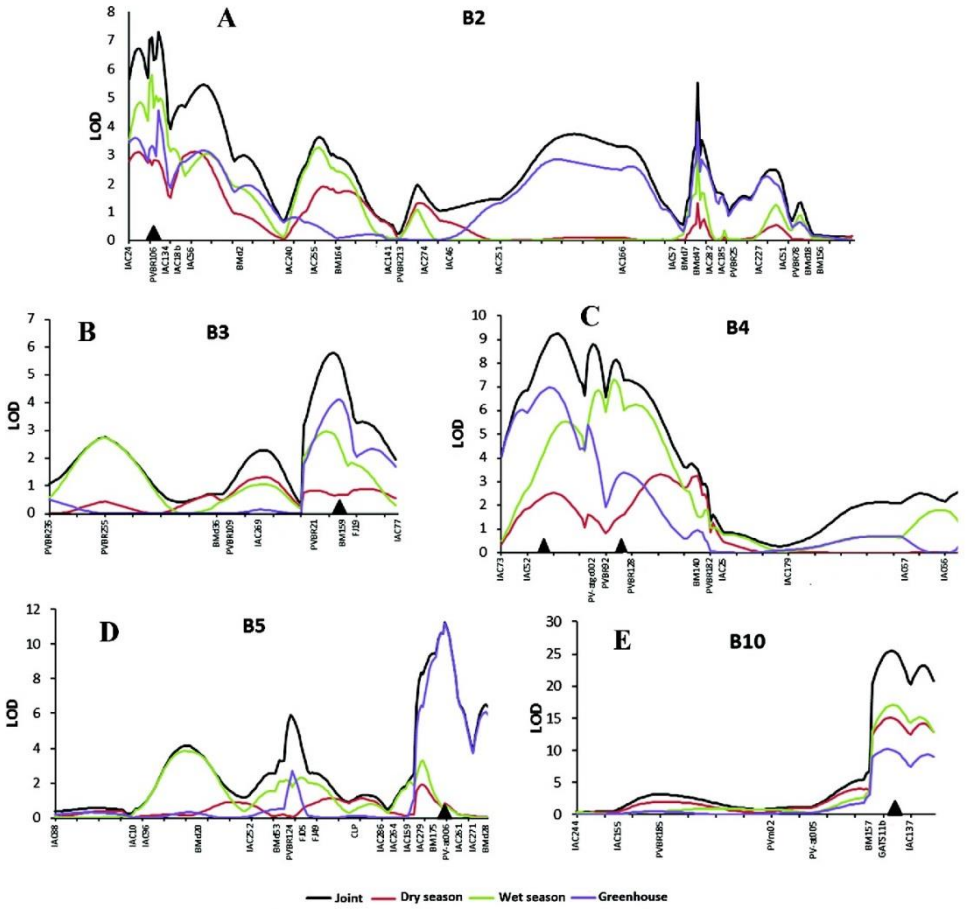

Figure 2 QTL graphs indicating the LOD score values for each marker position. LOD scores obtained by the joint CIM analysis (y) using the molecular makers distances of UC genetic map for each experiment (dry season – red; wet season – green and greenhouse – purple) and for the joint analysis (black). Black triangles indicate the position of maximum LOD values for significant QTLs. The linkage groups 1, 3, 4, 5 and 10 are indicated as B2, B3, B4, B5 and B10, respectively, according to Pedrosa et al. [36].

(*Phg-2*[2] to *Phg-4*[2]) in addition to the previously identified *Phg-1* [31]. As AND 277 is one of CAL 143 parents, then it is possible that these three genes are segregating in the UC mapping population. The quantitative resistance nature to ALS can also be inferred by the presence of transgressive RILs both for resistance and susceptibility, a phenomenon observed both in field experiments as well as in the greenhouse.

A reasonable part of the phenotypic variation was explained by the sum of the effects of QTLs, especially in wet season and the greenhouse experiments. The lowest total of R^2 observed in dry season may have occurred because the experiment was conducted during the dry season when the crop reaches the adult plant stage, since the dry climate discourages the development of the disease [8,9]. This condition did not prevail in wet season, which was carried out in the wet season, similar to the greenhouse, where conditions were controlled, with temperature and humidity favorable to fungal growth. These differences reflect the low correlations between the experiments probably due to genotype x environment interaction. However, the higher correlation

between the field experiments than between them and the greenhouse was expected, since in the field the infection occurred in a natural way, differently of the experiment in the greenhouse.

Nevertheless, a major QTL (ALS10.1) was identified in all three experiments. This QTL is interesting because of its stability and its pronounced effect which explains the high resistance heritability revealed by variance analysis. A high heritability level for this trait was also reported by Amaro et al. [39] in a study of recurrent selection. The ALS10.1 QTL was located on linkage group B10, where López et al. [29] mapped a QTL with a large resistance effect for this same disease in the DOR364 × G19833 population. Due to being close to a resistance gene analog marker (RGA7) and to it also being linked to an anthracnose resistance gene, the authors suggested the existence of an R gene cluster in this genomic region. As RGA7 is linked to GATS11b (approximately 2 cM), it is very likely that the QTL reported by López et al. [29] corresponds to the ALS10.1 identified in this study.

The closest marker to the maximum LOD value in ALS10.1 (GATS11b) was used to validate this QTL in a set of lines that are resistant or susceptible to ALS. There was a correlation between phenotype and genotype marker in most cases. The two resistant genotypes (BAT 332 and Mexico 54) which presented the same marker allele as the susceptible IAC-UNA parent are known sources of Mesoamerican resistance, and due to this must have different resistance genes [31,38] which are not present in the GATS11b locus of ALS10.1. The hypothesis is reinforced by the fact that an allelism test with AND 277, CAL 143 parent, identified different genes in relation to Mexico 54, where the only gene in common (*Phg-2*) revealed a different allelic form [31]. Thus, it is plausible that the BAT 332 and Mexico 54 lines have a different allele for the ALS10.1 locus, taking into consideration that they are from diverging gene pools in relation to CAL 143.

QTLs with minor effects were also identified. Among these, ALS5.2 and ALS4.2 showed an interesting QTL x environment interaction. ALS5.2 revealed a greater resistance effect under greenhouse conditions, but only a small effect in the field experiments. ALS4.2 on the other hand, presented an opposite interaction with a greater resistance effect only under field conditions but not in the greenhouse. The remaining QTLs did not present such a variable effect among the experiments. Therefore, ALS4.2 and ALS5.2 are interesting QTLs for breeding approaches, as in the field the plants are subject to infection by different races of the pathogen, and in the greenhouse, the infection is race-specific, thus, the pyramiding of these two loci tends to result in more resistant cultivars in both conditions.

To date, no QTL has been identified on linkage group B05. The peak LOD score of the ALS5.2 QTL coincided with the position of the Pv-att006 marker in both individual and joint analysis. This is a microsatellite that occurs within a gene related to pathogenesis (PR gene) that codes for an endochitinase [40], which is an enzyme involved in the degradation of fungal cell walls. The co-localization between resistance QTLs and defense genes in plants reported in several pathosystems suggests the existence of a functional relationship between the QTLs and these genes [41]. The co-localization between an endochitinase and a resistance QTL, for example, has been reported in the pathosystem pepper - *Phytophthora capsici* [42].

The ALS4.1 and ALS4.2 QTLs were located on linkage group B04, where López et al. [29] reported resistance gene linkage to ALS with RGA markers. However, as there are no common markers on this linkage group, it was not possible to establish a relationship between resistance QTLs described by López et al. [29] and those mapped in this study. However, it is possible that ALS4.1 and ALS4.2 identified in this study and Phg_{G5686A} identified by Mahuku et al. [30] in the G5686 Andean line, are part of an Andean resistance gene cluster, as in the cross-map information [32,35], it can be noted that the Pv-ag004 marker (0.0 cM of Phg_{G5686A}) is located between BMd 9 and PVBR92, that are close to the maximum LOD values for ALS4.1 (10 cM) and ALSb4.2 (4 cM).

Due to harboring genes that confer resistance to different *P. griseola* gene pool races, this cluster could be interesting to be used in common bean breeding programs, as the Andean resistance genes are most effective when transferred to cultivars of the Mesoamerican *pool* when they are grown in regions in which both Andean and Mesoamerican *P.griseola* isolates predominate [6,10]. Thus, the markers identified in this work in addition to those identified by Mahuku et al. [30] are applicable tools for marker assisted selection to obtain improved cultivars containing this ALS resistance cluster.

Conclusion

The results indicate quantitative resistance control to angular leaf spot in CAL 143. Seven QTLs with variable effects were identified, four of which had never been mapped before. One major QTL of stable effect in the three experiments (ALS10.1) was identified in this study. The presence of this QTL explains the high heritability of the character reported in this study. Alleles at the GATS11b marker locus linked to the major QTL distinguished lines that are resistant and susceptible to ALS. Thus, GATS11b can be an important tool to be used in common bean improvement to carry out marker-assisted selection. The results also point to a significant QTL x environment interaction. ALS4.2 and ALS5.2 are seen as being interesting for application in bean breeding, since their pyramiding can lead towards obtaining

more resistant cultivars under both infection conditions. In addition, the QTLs present on the B04 group could be part of a resistant gene cluster to different *P. griseola* gene pools.

Methods

Plant material

A population of 346 RILs in generation F_{12} from the IAC-UNA x CAL 143 cross [32] were used in the ALS resistance experiments. The IAC-UNA has Mesoamerican origin, is black seeded and susceptible to ALS, while CAL 143 has Andean origin, red striped calima type seeds and is resistant to ALS.

Field evaluation of resistance to ALS

The RILs were evaluated in two experiments at the Experimental Station 'Fazenda Santa Eliza' (Instituto Agronômico in Campinas - SP, Brazil). The first was carried out in February/2009 during the dry season and the second in August/2009, in the wet season. The experimental plot consisted of a row with 10 plants/RIL, spaced 50 cm apart. The experimental design was a completely randomized block design with two replicates. The Carioca Comum cultivar was a susceptible genotype used as borderline. The IAC-UNA and CAL 143 parents and the Carioca Comum cultivar were also included as controls, between treatments.

The symptoms were evaluated approximately 60 days after planting, in the six central plants of the portions, using a diagrammatic scale that classifies the severity levels in grades ranging from 1 (no symptoms) to 9 (30% or more of leaf area with symptoms) [43].

Greenhouse evaluation of resistance to ALS

The seeds were sown in plastic boxes (29.5 cm x 46.5 cm x 12.5 cm) with Dystrophic Red Latosol type soil, fertilized with NPK 04-14-08 (400 kg/ha), each containing 3 RILs sown in rows at a distance of approximately 4 cm, containing six plants each. The experimental design was also made up of completely randomized blocks with four replicates. The IAC-UNA and CAL 143 parents and the Carioca Comum cultivar were included among the treatments as controls.

Plants were inoculated 2 to 3 weeks after planting, when the plants were at the V3 development stage (first expanded trifoliate), by spraying both leaf surfaces with a 10^4 conidia/ml suspension prepared from *P. griseola* monosporic colonies grown in V8 medium [8]. The isolate used (14259–2) was classified as belonging to the 0–39 race based on the reaction of the differential cultivars according to Pastor-Corrales et al. [11]. After inoculation, the RILs were kept for 48 h at room temperature between 22 to 24°C, relative humidity between 95 to 100% and photoperiod of 12 h [8]. After this period, plants were transferred to the greenhouse. The severity evaluation was made 17 days after inoculation, as described above.

Statistical data analysis

Severity data were used for genetic (r_{gen}), environmental (r_{env}) and phenotypic (r_{phe}) correlation analyses between experiments by the Genes software [44]. Spearman's rank correlation as well as Pearson's correlation were performed by the R software (packages "Hmisc" [45]). As the results for all phenotypic correlations were the same (data not shown), it was chosen the Pearson's values to be discussed in the article. The severity data were also used in individual and joint variance analysis [46]. The normality of data distribution was evaluated by skweness and kurtosis values. Broad-sense heritability based on means was calculated for each experiment in joint analysis, using the mean square values of the ANOVAs [47]. Least Square Means (LSMeans) of severity of each RIL for each experiment/environment were used for QTL mapping.

Joint CIM QTL mapping

Previously mapped microsatellite markers by Campos et al. [32], based on segregation data from UC population, were used to identify QTLs. The genetic map comprises 198 markers distributed into eleven bean linkage groups (B01 to B11), with a total length of 1865.9 cM and an average distance between markers of 9.4 cM. The joint composite interval mapping analysis (model 6 - JZmapqtl, [33]) was used to determine possible QTL x environment interactions. The QTL evidence was checked at 1 cM intervals and with a 10 cM window using the likehood ratio test (LRT). LRT values were converted to the LOD scale using formula: LOD = 0.2172 * LRT. The multiple regression (*stepwise*) with a 5% significance level was used to obtain the cofactors used in the CIM analysis, by the QTL Cartographer vs.1.17 software [48].

Due to the performance of multiple tests, the threshold values for QTL detection were determined separately for each linkage group, based on the Σi [(Ti/50) +1] formula, where Ti is the length in cM of the *ith* linkage group, considering adjacent regions every 50 cM as independent [49]. The threshold LOD values were compared to the maximum LOD values of the joint analysis for each linkage group to determine the presence of significant QTLs. The additive effect values were estimated for each experiment individually only for significant QTLs in the joint analysis. The model with significant QTLs for all linkage groups for each experiment was adjusted to determine the phenotypic variation (R^2) explained by each QTL.

Validation of marker linked to major QTL

A marker linked to the major QTL was used in the genotyping of 32 bean lines (Additional file 1). The line

reactions to the 0–39 race were evaluated in the greenhouse as previously described. The symptom evaluation also followed the grade scale from 1–9 [43].

DNA extraction from each plant was performed according to Hoisington et al. [50]. Genotyping was conducted according to Campos et al. [32]. The polymorphic fragments were visualized on denaturing 6% polyacrylamide gels silver stained.

Additional file

Additional file 1: Marker validation. Common bean lines (*Phaseolus vulgaris* L.) used to validate the GATS11b marker, the closest linked marker to the maximum LOD value for the major QTL, ALS10.1. The resistance or susceptibility of each line to angular leaf spot is discriminated.

Abbreviations

ALS: Angular Leaf Spot; ANOVA: Analysis of Variance; Joint CIM: Joint Composite Interval Mapping; LRT: Likehood Ratio Test; LSMeans: Least Square Means; QTL: Quantitative Trait Locus; RIL: Recombinant Inbreed Line; UC: IAC-UNA x CAL 143.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PRO conceived the experimental design; conducted the experiments and data analyses, and drafted the manuscript. RMB contributed with the genotyping and phenotyping, helping also on linkage and QTL analyses. LEAC participated in the initial design of the project, discussions and in the editing of the manuscript. AAFG supported QTL and experimental data. AFC and SAMC provided the mapping population and performed the ANOVA analyses. LLB conceived the project, was responsible for the project coordination, helped with data interpretation, and editing of the manuscript. All authors have read and approved the final version of the manuscript.

Acknowledgements

This work was supported by FAPESP through grants 2005/53819-0, 2010/51673-7. PRO received a fellowship from FAPESP (2006/58332-5, 2009/024112). The authors thank Gliciane Micaele Borges Silva for collaborating in the field and greenhouse experiments and Dr. Margarida Fumiko Ito, from the Fitossanitary Center at Instituto Agronômico (IAC, Campinas, SP, Brazil), for providing isolates of *P. griseola*.

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Received: 26 October 2011 Accepted: 8 June 2012
Published: 27 June 2012

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doi:10.1186/1471-2156-13-50
Cite this article as: Oblessuc *et al.*: Mapping of angular leaf spot resistance QTL in common bean (*Phaseolus vulgaris* L.) under different environments. *BMC Genetics* 2012 **13**:50.

CAPÍTULO II:

Oblessuc PR, et al. Increasing the density of markers around a major QTL controlling resistance to angular leaf spot in common bean.

RESUMO

A mancha-angular (ALS) causa grandes perdas na produção de feijão comum (*Phaseolus vulgaris* L.), uma importante fonte de proteína na dieta humana. O presente estudo descreve a saturação do loco de característica quantitativa (QTL) ALS10.1 com maior efeito sobre a resistência à ALS, localizado no grupo de ligação Pv10, e explora o contexto genômico desta região usando dados disponíveis a partir do genoma de *P. vulgaris*.

Marcadores derivados de DArT (STS-DArT) selecionados por análise de *bulks* segregantes (BSA), SCARs e microssatélites (SSRs) foram usados para aumentar a resolução do QTL, reduzindo o intervalo de confiança da ALS10.1 de 13,4 para 3,0 cM. A posição do SSR ATA220 coincidiu com o valor máximo de LOD no QTL. Além disso, um novo QTL ($ALS10.2^{UC}$) foi identificado no final do grupo de ligação Pv10. Análise da seqüência usando o genoma de *P. vulgaris* localizou dez SSRs e sete STS-DArTs no cromossomo 10 (Pv10). As posições genômica e genéticas foram coincidentes para cinco marcadores, o que permitiu a definição de uma região núcleo do QTL ALS10.1 de 5,3 Mb. Estes marcadores estão ligados a genes putativos relacionados com a resistência à doenças, tais como: glicosil-transferases, *ankyrin repeat-containing*, fosfolipases, e *squamosa-promoter binding*. Análise de sintenia entre os marcadores ligados à ALS10.1 e o genoma de soja sugeriu uma evolução dinâmica deste loco no feijoeiro.

Este estudo resultou na identificação de novos marcadores intimamente ligados ao QTL de maior efeito ALS10.1 e novos genes candidatos de resistência à ALS, que podem ser utilizados em trabalhos de seleção assistida, o mapeamento fino e clonagem posicional.

Increasing the density of markers around a major QTL controlling resistance to angular leaf spot in common bean

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ABSTRACT

Angular leaf spot (ALS) causes major yield losses in the common bean (*Phaseolus vulgaris* L.), an important protein source in the human diet. This study describes the saturation around a major quantitative trait locus (QTL) region, ALS10.1, controlling resistance to ALS located on linkage group Pv10 and explores the genomic context of this region using available data from the *P. vulgaris* genome sequence. DArT-derived markers (STS-DArT) selected by bulk segregant analysis and SCAR and SSR markers were used to increase the resolution of the QTL, reducing the confidence interval of ALS10.1 from 13.4 to 3.0 cM. The position of the SSR ATA220 coincided with the maximum LOD score of the QTL. Moreover, a new QTL (ALS10.2^{UC}) was identified at the end of the same linkage group. Sequence analysis using the *P. vulgaris* genome located ten SSRs and seven STS-DArT on chromosome 10 (Pv10). Coincident linkage and genome positions of five markers enabled the definition of a core region for ALS10.1 spanning 5.3 Mb. These markers are linked to putative genes related to disease resistance such as glycosyl transferase, ankyrin repeat-containing, phospholipase, and squamosa-promoter binding protein. Synteny analysis between ALS10.1 markers and the genome of soybean suggested a dynamic evolution of this locus in the common bean. The present study resulted in the identification of new candidate genes and markers closely linked to a major ALS disease resistance QTL, which can be used in marker-assisted selection, fine mapping and positional QTL cloning.

KEYWORDS – *Pseudocercospora griseola* (Sacc.) Crous & Braun, *Phaseolus vulgaris* L., DArT-BSA, QTL mapping, disease candidate genes, genome location

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) represents an important source of protein in the human diet, especially in developing countries (Broughton et al. 2003; Gepts et al. 2008). Angular leaf spot (ALS), which has been reported in more than 60 countries worldwide, is one of the most devastating diseases of this crop, leading to yield losses of up to 80% (Stenglein et al. 2003; Singh and Schwartz 2010). This disease is caused by the hemibiotrophic fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun (syn. *Phaeoisariopsis griseola* (Sacc.) Ferraris) (Crous et al. 2006), which causes angular necrotic spots on the plant leaves and pods (Allorent and Savary 2005). Infection occurs within three days of inoculation, with conidia penetrating either through the leaf epidermis or stomata, followed by intercellular colonization of the mesophyll, leading to plasmolysis of the host cells (Monda et al. 2001).

The most effective approach to controlling this disease is the use of resistant cultivars. Therefore, it is important to develop molecular markers for plant breeding and to understand the mechanisms controlling host-pathogen interactions (Dodds et al. 2010). In the case of ALS, two dominant resistance genes, known as *Phg-1* and *Phg-2,* have been described (Carvalho et al. 1998; Sietsche et al. 2000; Gonçalves-Vidigal et al. 2011; Mahuku et al. 2011). In addition, dominant monogenic inheritance for resistance to ALS has been reported (Corrêa et al. 2001; Mahuku et al. 2004; Namayanja et al. 2006), but the relationship between the genes underlying this resistance and *Phg-1* and *Phg-2* remains unknown. In fact, resistance to ALS can be controlled by both qualitative resistance genes and quantitative trait loci (QTL) (López et al. 2003; Mahuku et al. 2009; 2011; Oblessuc et al., 2012a), which explains the complex inheritance mode of ALS resistance observed by Caixeta et al. (2005). These authors reported the existence of three additional genes (*Phg-3, Phg-4* and *Phg-5*) with two alleles each controlling resistance to ALS in four bean varieties that were previously characterized as possessing monogenic resistance.

Recently, Oblessuc et al. (2012a) reported the existence of an ALS resistance QTL on linkage group Pv10 ($ALS10.1^{DG,UC}$) that controls 22% and 16% of the resistance under both field and greenhouse infection

conditions, respectively. In addition, the study reported six other ALS resistance QTLs in the IAC-UNA x CAL 143 recombinant inbred line population (UC RIL) developed by Campos et al. (2011). Although these results were important to the understanding of ALS resistance, none of the markers located near ALS10.1 coincided with the maximum LOD score of the QTL; the closest marker was the microsatellite (SSR) GATS11b located 6.0 cM away from the QTL (Oblessuc et al. 2012a).

The increasing of markers density in QTLs region such as the ALS10.1 is very important to further study the QTL genomic context and to facilitate marker-assisted selection. Therefore, the development of new molecular markers is needed. Recently, the diversity array technology (DArT) was developed for beans (Briñez et al. 2012). This technique is based on restriction enzyme digestion and adapter ligation of a representative DNA pool of a species, followed by amplification and cloning of this DNA; the DNA is then used as a target for array hybridization of the query sample (Wenzl et al. 2004). Among other applications, the DArT has been used in QTL mapping of resistance loci (Hickey et al. 2011; Vazquez et al. 2012) and bulked segregant analysis (BSA) in various plant species (Wenzl et al. 2007; Lillemo et al. 2008; Golegaonkar et al. 2009; Tah et al. 2010; Satish et al. 2012).

The present study aimed to increase the marker saturation on the genetic region of the major QTL involved in ALS resistance; the ALS10.1 (Oblessuc et al. 2012). The goal was to develop PCR-based markers linked to the ALS10.1 and therefore, to obtain markers that could be efficiently used by breeders in marker-assisted selection towards ALS resistance. In addition, the genomic region of this QTL was investigated using the common bean genomic data from Phytozome. The results of this study will also facilitate further map-based approaches to cloning resistance genes.

MATERIALS AND METHODS

Plant material and marker-based composition of resistant and susceptible bulks

The recombinant inbred lines (RILs) from the IAC-UNA x CAL 143 cross were developed by advancing the F_2 generation through F_8 adopting the principles of single-pod descent for mapping (Funada et al. 2012). From F_8 till F_{10}, single seed descent method was used, resulting in 380 F_{10} plants that generated the UC map (Campos et al. 2011). In this study, 346 UC RILs in the F_{12} generation were used. For the BSA approach, resistant and susceptible RILs were selected from this population to compose the bulks based on previously available data on their respective responses to ALS (Oblessuc et al. 2012a). Briefly, the RILs were screened for ALS resistance under two different experimental conditions consisting of either exposure to natural infection in the field or inoculation with race 0-39 of *P. griseola* in the greenhouse. Disease severity was evaluated based on disease scores that ranged from one (no symptoms) to nine (30% or more of the leaf area with symptoms). After identifying the most resistant and most susceptible RILs in both the field and greenhouse, 50 lines from each class were selected based on their genotypes for the SSR markers GATS11b and IAC137, which flank the confidence interval of ALS10.1, as reported by Oblessuc et al. (2012a). Therefore, the resistant bulk was composed of RILs which combined resistant marker alleles and phenotypic resistance to *P. griseola*; similarly the susceptible bulk was composed of RILs that combined susceptible genotype and susceptible phenotype.

DArT-BSA analysis and development of STS-DArT markers

DNA from the selected RILs was pooled in equimolar amounts to compose the bulks for DArT analysis. Both the resistant (CAL 143) and the susceptible (IAC-UNA) parents were also included in the array hybridization. The hybridization was performed at the Diversity Arrays Technology Pty Ltd (DArT P/L, Canberra, Australia; http://www.diversityarrays.com/) with all 15,360 clones available for *P. vulgaris*. The bulks and the parents were scored for the presence (1) or absence (0) of hybridization based on fluorescence intensities, and a polymorphism information content of 0.5 was used to assess the

distribution of the marker scores between the two bulks according to the method described by Anderson et al. (1993). Only clones with quality scores of 100% (average reproducibility and call rate) were selected.

The inserts of selected polymorphic DArT clones were sequenced at the DArT P/L facility. The NEBcutter V2.0 software (http://tools.neb.com/NEBcutter2/ - Vincze et al. 2003) was used to identify possible chimeric sequences based on the presence of *Pst*I, *Bst*NI or *Taq*I restriction sites. In addition, DArT sequences were compared pairwise using the BLASTn software from NCBI (http://www.ncbi.nlm.nih.gov/). Clones with sequence identities ≥ 95% and E-values ≤ 1 x 10^{-5} were considered redundant, and the clone with the longest sequence and an adequate PCR band pattern was used for the linkage analyses. Putative functions of the DArTs were inferred by performing tBLASTx searches (E-values ≤ 1 x 10^{-5}) in the non-redundant sequences and the EST databases of NCBI (Altschul et al. 1990).

Insert sequences of selected DArTs were used to design primers and convert them into co-dominant sequence-tagged site (STS-DArT) markers. Primers were designed using the Primer3 v.0.4.0 software (http://frodo.wi.mit.edu/primer3/input.htm - Rozen and Skaletsky 2000) with the following criteria: (1) primer size between 18 – 22 bases, (2) maximum annealing temperature difference of 1°C between forward and reverse primers, and (3) CG content of 40% to 60%. Primers were tested using the DNA of the bulks and of the parents in a PCR reaction containing 30 ng of DNA, 1U of *Taq*-DNA polymerase (Promega®), 1.5 mM of $MgCl_2$, 0.25 mM of each dNTP, and 0.8 pmol /µl of each primer for a final concentration of 1x reaction buffer in a final volume of 15 µl. The amplification followed a touchdown protocol with a hot start of 94°C for 4 min., followed by 30 cycles of 30 sec at 94°C, 45 sec at 60°C (decreasing 0.5°C each cycle), and 45 sec at 72°C, and 30 cycles with the same denaturation and extension conditions but annealing temperature set at 45°C. A final elongation step was performed at 72°C for 7 min. STS-DArTs were resolved in 3% agarose gels, and those sequences that were

polymorphic between bulks and parents were used to genotype the 342 UC RILs in 6% acrylamide gel electrophoresis followed by silver nitrate staining (Creste et al. 2001).

Genotyping of RILs with additional SSR and SCAR markers

In addition to STS-DArT markers, six sequence-characterized amplified markers (SCARs) known to be linked to ALS resistance loci (SN02 - Sietsche et al. 2000; SH13, SAA19, SBA16, and SM02 - Queiroz et al. 2004; $PF5_{330}$ - Mahuku et al. 2004) and SSR markers described in the literature (Gaitán-Solís et al. 2002; Caixeta et al. 2005; Benchimol et al. 2007; Grisi et al. 2007; Hanai et al. 2007; 2010; Blair et al. 2008; Cardoso et al. 2008; Campos et al. 2011) were tested for polymorphisms between the IAC-UNA and CAL 143 parents. The polymorphic markers were genotyped in the 346 RILs of the UC population (Campos et al. 2011). PCR amplification and electrophoresis conditions were the same as described in the respective articles.

Linkage and QTL mapping analyses

Linkage analysis of new STS-DArTs, SCARs and SSRs was performed using the Pv10 framework from Campos et al. (2011). New markers were added using the *try* command of MAPMAKER 3.0b (Lander et al. 1987) with a LOD score of 3.0 and a maximum genetic distance of 37.5 cM calculated by the Kosambi (1944) mapping function as thresholds. The final order of the markers was tested with the *ripple* command with a window of six markers. Finally, multipoint distance estimates were obtained using the *map* command.

Composite interval mapping (CIM) analysis was used to redefine the position of the ALS 10.1 resistance QTL in group Pv10 using the available ALS severity scores of the RILs (Oblessuc et at. 2012a). Analyses were performed with QTL Cartographer v.1.17 (CIM; model 6 – Zmapqtl; Wang et al. 2005); evidence of the QTL was checked at 1.0 cM intervals with a 10.0 cM window using the likelihood ratio test (LRT) and converted to LOD values by the equation LOD = 0.2172*LRT. Stepwise multiple regressions (P = 0.05) were also used to obtain the cofactors for the CIM analysis. The LOD threshold was determined by

computing 1,000 permutations ($p \leq 0.05$) for each experimental condition (field or greenhouse evaluations). The 95% confidence intervals were determined by the two-LOD support interval method (Van Ooijen, 1992; Lynch and Walsh, 1998).

Sequence analyses of markers linked to ALS10.1

To verify the genome location of all markers of linkage group Pv10, marker sequences were aligned to the draft sequences of the bean chromosomes of the Phytozome v1.0 assembly (http://www.phytozome.net/). The criteria used to assign putative regions to the markers included E-values $\leq 1 \times 10^{-10}$ and a minimum identity of 50% between query and database sequences. Transcripts predicted by Phytozome located within 10.0 kb of each marker linked to ALS10.1 were annotated for their putative function, with the goal of analyzing the genomic context of the QTL region. Markers were also aligned to the soybean (*Glycine max*) genome using the PhaseolusGenes website (http://phaseolusgenes.bioinformatics.ucdavis.edu; Gepts and Lin 2011) to assess the synteny between these species. Hits with E-values $\leq 1 \times 10^{-10}$ were considered significant.

RESULTS

BSA-DArT, STS-DArT, SCAR and SSR marker analyses

The BSA-DArT approach was used in an attempt to saturate the ALS10.1 region. Twenty-one DArT clones were found to be polymorphic between the resistant and the susceptible bulks (Supplemental Table S1). After trimming the vector sequence, the average clone insert was 490 bp long (Table 1); only one chimeric insert, inferred from the presence of a *Taq*I restriction enzyme site, was separated into two independent sequences (864174a and 864174b). Five inserts (23.8%) had the *Taq*I restriction site at the end of the insert; of these, only one clone (clone 866317) also had the *Bst*NI site. Pairwise comparisons indicated no significant differences between DArT sequences in 59.1% of the cases (Table 1). Therefore, 13 unique sequences were selected to verify their putative function. Of these, eight had no significant matches in the non-redundant and EST databases, and the remaining showed similarity with either *G. max*

or *P. vulgaris* sequences deposited in the GenBank. Primers were developed for these 13 DArT clones in an attempt to convert them into STS-DArTs markers (Table 2); eight of these clones, displaying well defined fragments in agarose gels and found to be polymorphic between IAC-UNA, CAL 143 and the bulks, were used in the linkage analysis. The primers designed for the remaining five DArTs clones did not generate an amplification product.

Table 1 Redundancy analysis of DArT clones based on pairwise alignments with BLASTn and putative functions inferred by tBLASTx searches.

Redundancy (BLASTn)				**Putative function (tBLASTx)**			
Dart clones	**size (bp)**	**E-value**	**Identities[a]**	**Annotation**	**Organism[a]**	**GI number[a]**	**E-value**
827737 / 864415	507 / 421	1×10^{-120}	99%	No Hit	-	-	$> 1 \times 10^{-5}$
827737 / 880625	507 / 413	1×10^{-120}	99%	No Hit	-	-	$> 1 \times 10^{-5}$
827737 / 880625	507 / 413	1×10^{-120}	99%	No Hit	-	-	$> 1 \times 10^{-5}$
827737 / 883634	507 / 704	0.0	91%	No Hit	-	-	$> 1 \times 10^{-5}$
827887 / 882462	562 / 554	0.0	100%	No Hit	-	-	$> 1 \times 10^{-5}$
882462 / 866012	554 / 600	0.0	99%	No Hit	-	-	$> 1 \times 10^{-5}$
882462 / 883131	554 / 556	0.0	99%	No Hit	-	-	$> 1 \times 10^{-5}$
864174a / 866317	436 / 287	1×10^{-130}	95%	transcription factor bHLH-like	*G. max*	gi|356496190|	5×10^{-43}
864174b	496	No Hit	-	PVUSE1NG-RP-025_M22 mRNA	*P. vulgaris*	gi|312036377|	2×10^{-20}
864614	881	No Hit	-	No Hit	-	-	$> 1 \times 10^{-5}$
865197 / 879964	419 / 447	0.0	99%	No Hit	-	-	$> 1 \times 10^{-5}$
873626 / 883124	491 / 491	0.0	100%	CCA tRNA nucleotidyl transferase	*G. max*	gi|356576136|	1×10^{-13}
880279	389	No Hit	-	No Hit	-	-	$> 1 \times 10^{-5}$
880323	531	No Hit	-	Sprite BAC 78L17	*P. vulgaris*	gi|38194906|	2×10^{-64}
880463	481	No Hit	-	No Hit	-	-	$> 1 \times 10^{-5}$
881808	527	No Hit	-	No Hit	-	-	$> 1 \times 10^{-5}$

882636	712	No Hit	-	PVUSE4NG-RP-010_A08 mRNA	*P. vulgaris*	gi|312044997|	2 x 10^{-6}
883703	360	No Hit	-	No Hit	-	-	> 1 x 10^{-5}

[a] "-" indicates absence of information, since the E-value was not significant (E-value > 10^{-5}).

SCAR and SSR markers were used to increase the number of markers in linkage group Pv10. Of the six SCAR markers analyzed, three were polymorphic between the UC parents (SH13, SBA16, and $PF5_{330}$) and were used to genotype the RILs. The population was also genotyped with 94 polymorphic SSRs, 49 of which were previously screened but not mapped by Campos et al. (2011) and 45 of which were screened in this study (Supplemental Table S2).

Table 2 Primer sequences for PCR amplification of non-redundant DArT clones polymorphic between the resistant and susceptible bulks.

STS-Dart marker	Primer sequences (5'- 3')	Annealing temperature	Predict PCR product (bp)	Polymorphism[a]
D864174a	F- AGCACCTTTTCCACCCTGTA R- TGACCATCAAGTCTGAAGACAG	60°C	436	C+ / U-
D864174b	F- CGAGAGTTTACTCTGACTCGTG R- CCAGAAATTTCGCCCATAG	58°C	496	No amplicon
D864415	F- GGATCCAGTGCAGTAGCACA R- GAACGGTTCTGTTCACAAGCTA	60°C	421	C+ / U-
D864614	F- GCAGACTTATGTCCAATTGTGG R- CAGCATGAAAGTACCAAAACCA	60°C	881	No amplicon
D865197	F- GATTGCATTCAACACAATAGGC R- AGGGGCTGGAGTAGAAAAGG	60°C	419	C+ / U-
D873626	F- ATCCAGTGCAGTTCCAGATG R- TGCAGCTGTTTGAACTAGTGA	58°C	491	C- / U+
D880279	F- TCCAGTGCAGAGTCAGAAAA R- TTGCGTATAATTGCGTACAA	56°C	389	C- / U+
D880323	F- CCAACCATTGGTATCAGAGC R- TGGATCCAGTGCAGTTCAAA	60°C	531	No amplicon
D880463	F- GCTACGAGCTCGGATCACTA R- AGTGCAGTAGCACAATGCAGA	58°C	481	No amplicon
D881808	F- GGATCCAGTGCAGAAGGATA R- TGAAAGCTGCCTTCTTAAGTGT	58°C	527	No amplicon
D882462	F- CAGTGCAGCTTCAAGCAAAA R- AGTGCAGTAGCACAATGCAGA	60°C	554	C+ / U-
D882636	F- CAGTGCAGCTTCAAGCAAAA R- TGCAGTGTTTTGTGTGCCTA	60°C	712	C+ / U-
D883703	F- AGTGCAGTTGTATCCGTTGCT R- CCGCCAGTGTGATGGATATT	60°C	360	C- / U+

[a]The plus and minus signals indicate presence (+) or absence (-) of PCR product in CAL 143 (C) or IAC UNA (U) parental lines.

Addition of new markers to linkage group Pv10 increased the resolution of ALS10.1

Linkage analyses incorporated three of the eight STS-DArTs, one SCAR ($PF5_{330}$), and six SSRs into linkage group Pv10, increasing the total number of markers reported in the previous study (Campos et al. 2011) from nine to 19 (Fig. 1). For all three STS-DArTs (D864415, D865197 and D882462), both the resistant parent CAL 143 and the resistant bulk displayed the presence of amplicons (scored as '1' in supplemental Table S1). The number of markers in the ALS10.1 region increased from four to ten markers: STS-DArT marker D865197 and five new SSRs were incorporated to the region, thus reducing the average distance between markers from 6.0 cM (reported in the previous analysis) to 3.1 cM (Fig. 1). The position of a newly added SSR ATA220 coincided with the peak LOD score of the QTL (Fig. 2), whereas in the previous study, the closest marker (GATS11b) was positioned 6.0 cM away. The addition of new markers reduced the confidence interval of ALS10.1 from 13.4 cM to 3.0 cM and increased the maximum LOD score of the field screening data from 17.7 to 20.0, but decreased the maximum LOD score of the greenhouse data from 10.1 to 9.3. Similarly, the R^2 values of the QTL increased from 21.2 to 22.8 in the field analysis and decreased from 15.9 to 10.2 in the greenhouse analysis.

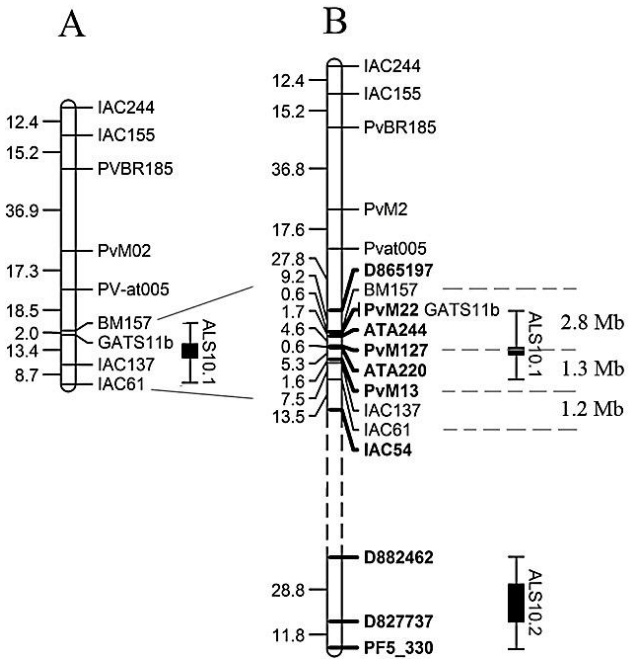

Fig 1 Previous (A) and actual (B) versions of linkage group Pv10 after the addition of six SSRs, three STS-DArTs and one SCAR marker. The confidence intervals of QTLs ALS10.1 and ALS10.2 are indicated by the black boxes and the region with LOD values above the threshold (3.0) are indicated by the whiskers. Physical distances between markers linked to ALS10.1 in the new map are represented by gray dashed lines and were estimated based on alignments with the draft genome sequence of Phytozome v1.0. The black dashed lines on the new Pv10 indicate a distance larger than 37.5 cM.

The graphical genotypes of the ten most resistant and ten most susceptible RILs (Table 3) indicated the absence of recombination among six markers located in the core of ALS10.1 because the genotypes had the same marker alleles as the resistant or the susceptible parental line, respectively (bold markers in Table 3). In addition to increasing the resolution of ALS10.1, CIM analysis identified a new QTL in this group, hereafter referred to as ALS10.2^{uc} (Fig. 1 and 2), which explained 5% and 15% of the phenotypic variance in the field and greenhouse experiments, respectively.

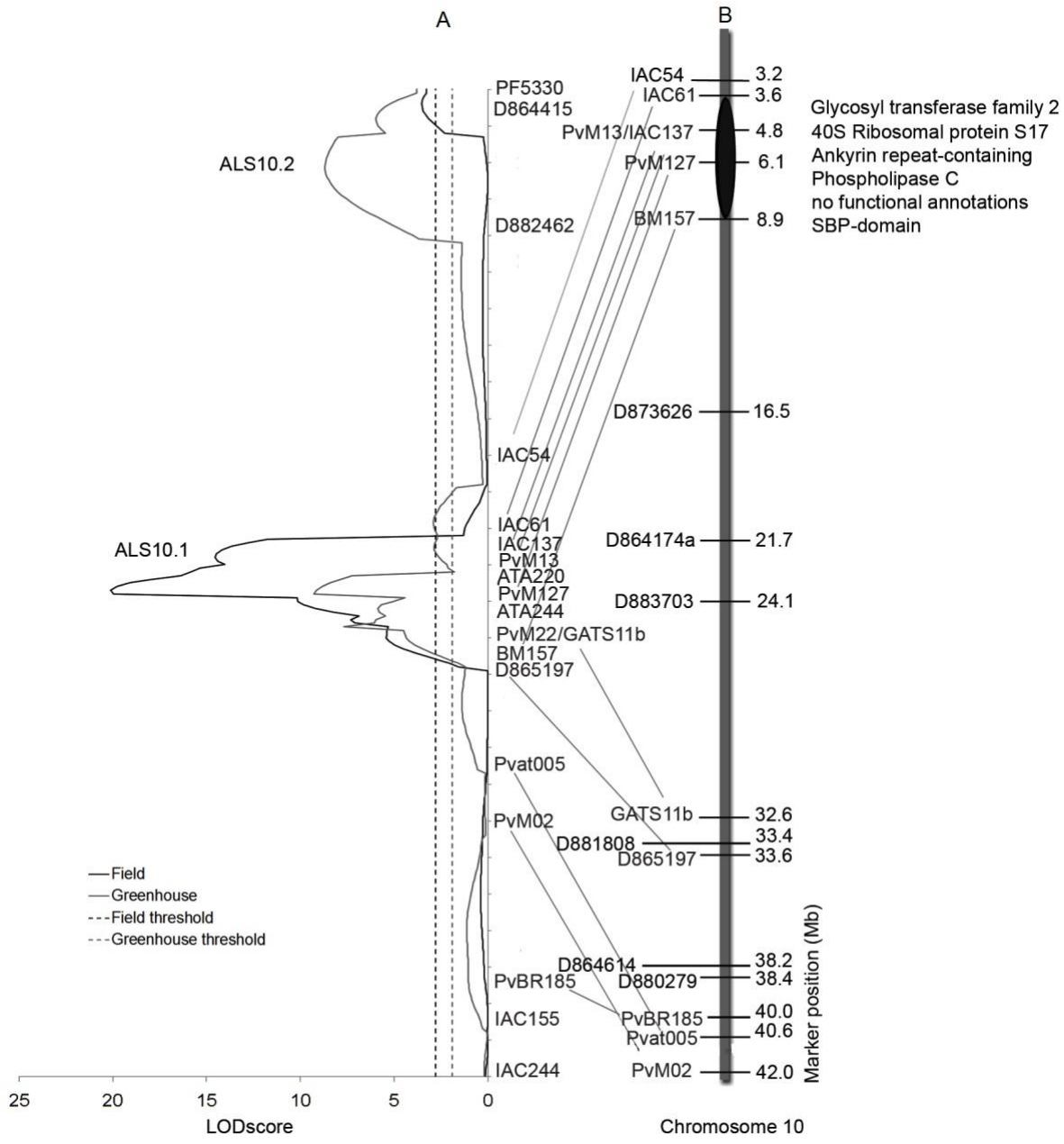

Fig 2 Inferred positions of markers mapped on linkage group Pv10 based on linkage analysis and alignments with the draft sequence of chromosome 10 according to Phytozome v1.0. (A) Composite interval mapping LOD scores of the angular leaf spot resistance QTLs ALS10.1 and ALS10.2 on linkage group Pv10 based on disease screenings of the UC RIL population under natural conditions of infection in the field (black line) and under controlled conditions in the greenhouse (gray line). Traced horizontal lines indicate LOD thresholds. (B) Physical positions on the chromosome are indicated in front each marker. The region highlighted in black represents the core of the QTL ALS10.1, with the linked genes listed alongside the markers positions.

Table 3 Graphical genotypes of markers from linkage group Pv10 and ALS severity scores of the field and greenhouse trials for the ten most resistant and most susceptible UC RILs.

		Resistant RILs[a]										**Susceptible RILs[a]**									
Markers position (cM)	**Markers names**	208	162	100	73	215	64	148	354	353	322	371	375	379	270	26	139	177	27	55	262
0.0	IAC244	■						■	■	■		■	■	■	■		■	■	■	■	■
12.4	IAC155	■				■		■		■					■		■				■
27.6	PvBR185				■			■		■					■		■		■		■
64.4	PvM2			■				■		■		■			■		■		■	■	■
82.0	Pvat005		■	■				■				■	■	■	■			■	■	■	■
109.8	D865197		■	■				■				■		■	■			■	■		■
119.0	BM157							■				■	■	■	■		■	■	■	■	■
119.6	PvM22							■				■	■	■	■		■	■	■	■	■
119.6	**GATS11**											■	■	■	■		■	■	■	■	■
121.3	**ATA244**											■	■	■	■		■	■	■	■	■
125.9	**PVM127**											■	■	■	■		■	■	■	■	■
126.5	**ATA220**											■	■	■	■		■	■	■	■	■
131.8	**PVM13**											■	■	■	■		■	■	■	■	■
133.4	**IAC137**											■	■	■	■		■	■	■	■	■
140.9	IAC61								■			■	■	■	■		■	■	■	■	■
154.4	IAC54			■	■				■			■	■	■	■			■	■	■	■
220.8	D882462		■	■	■	■	■	■	■			■	■	■			■	■	■	■	■
249.6	D864415					■			■			■	■	■	■	■	■	■	■	■	■
261.4	$PF5_{330}$			■		■					■	■	■	■	■	■	■	■	■	■	■
disease severity field		1.06	1.11	1.06	1.00	1.25	1.33	1.79	1.71	1.08	1.50	7.04	6.79	6.75	5.63	5.58	5.50	5.04	5.02	5.00	4.83
disease severity greenhouse		1.00	1.33	1.42	1.53	1.56	1.68	1.22	1.32	1.96	1.67	7.83	6.38	5.58	5.12	5.64	5.16	5.99	5.06	5.89	5.53

[a] Filled squares represent the allele from the susceptible parent IAC-UNA and the empty the allele from the resistant CAL 143 parent. Markers spanning the core of the ALS10.1 are marked in bold

Alignment of ALS10.1 markers with the draft genome sequence of *P. vulgaris* assigned its core to the end portion of Pv10

The genome locations of markers from linkage group Pv10 and from the DArT-BSA that were not mapped were analyzed through the alignment of all available sequences with the draft sequence of the common bean chromosomes from Phytozome v1.0. In total, 17 markers were positioned on chromosome 10 (Pv10), being distributed throughout the chromosome and covering approximately 40 Mb of Pv10, with an average distance of 2.5 Mb (Fig. 2). The sequence alignment of all eight STS-DArTs indicated that five STS-DArTs are located on Pv10, in addition with other two DArTs which were not successfully converted in STS-DArT markers. Interestingly, linkage analysis indicated that only one of these STS-DArTs was linked to QTL ALS10.1 (D865197, Fig. 2). Of the three STS-DArTs that did not align with Pv10, the D882636 marker was assigned to Pv01 (E-value = 2×10^{-39}), D882462 aligned with Pv08 (E-value = 1×10^{-19}), and no similar sequence from this version of the genome assembly was found for D864415 (E-value > 1×10^{-10}), even though D882462 and D864415 mapped to linkage group Pv10 close to the ALS10.2 QTL (Table 4). Moreover, of the DArTs that generated no amplicons in UC parents, two (D864614 and D881808) were located in the 40 Mb stretch of Pv10, with the D881808 marker only 0.2 Mb apart from the D865197 ALS10.1 marker (Fig. 2). Finally, the D864174b marker aligned with Pv05 (E-value = 5×10^{-57}), and no similar sequences in the genome were found for either D880323 or D880463 (E-value > 1×10^{-10}).

Of the 15 SSR markers mapped on linkage group Pv10, 13 were aligned with the genome; the ATA220 and ATA244 markers had no sequences available. Ten of the SSRs were located on Pv10 (Table 4 and Fig. 2). The linkage and chromosomal orders of four markers linked to ALS10.1 were in agreement (from IAC161 to BM157), while the GATS11b marker was positioned outside of the QTL region in the genome sequence (Fig. 2). In addition, the order of PvM02 and PvBR185, which mapped outside of the confidence interval of ALS10.1, was inverted. Interestingly, more than one copy of PvM13 and IAC137 were identified on Pv10, resulting in two clusters 2.7 kb apart from each other, in which the markers were

located in tandem. The two identical copies of PvM13 spanned approximately 14.3 kb, and the three highly similar copies of SSR IAC137 spanned approximately 22.2 kb (Supplemental Fig. S1 and Table S3). Markers IAC155 and IAC244 aligned with Pv09 (E-value = 0.0), and PvM22 was the only marker linked to ALS101.1 that was located on a different chromosome (Pv03; E-value = 0.0) (Table 4).

Taking into consideration the genome and map locations of the markers linked to ALS10.1, the genomic core of this QTL was defined as the region between IAC61 and BM157 spanning 5.3 Mb at one end of the Pv10 assembly (Fig. 2). The marker closest to the QTL LOD peak with an available sequence (PvM127, 0.6 cM; Fig. 1B) was located 6.1 Mb from the end of the chromosome (Fig. 2). Because the core of ALS10.1 spans 21.9 cM, a ratio of 242 Kb/cM can be estimated for this region.

Putative genes linked to markers of the ALS10.1 core indicated enrichment of genes involved in disease resistance

The genomic context of ALS10.1 was analyzed by investigating the function of putative genes located within 10.0 Kb of each marker (Table 4). At the core of the QTL, 13 transcripts were observed (Table 4); interestingly, the majority of the transcripts (60%) were genes known to be related to the immune response in plants, including genes coding for glycosyl transferase, ankyrin repeat-containing protein, phospholipase, and SBP-binding protein. Three genes coding for ribosomal proteins and two with no functional annotation were also detected in this region. In addition, the transcripts linked to other markers of Pv10 were analyzed, and a total of 33 transcripts were identified (Table 4). Disease-related genes, including kinase and nucleotide-binding adaptor (NB-ARC), were observed throughout the entire chromosome.

Table 4 Chromosomal locations of markers from linkage group Pv10 and DArT-BSA clones, with functional annotation of putative genes linked to them based on the Phytozome database.

Marker name[a,b]	Ch[c]	E-value[d]	Chromosome Position	Predicted gene	Distance[e]	Functional annotation
IAC54	10	1×10^{-176}	3,276,703..3,277,072	Phvul.010G022100	0 Kb	ER lumen retaining receptor
				Phvul.010G022200	6 Kb	ER lumen retaining receptor
IAC61	10	1×10^{-153}	3,614,814..3,615,256	Phvul.010G024700.1	0 Kb	Glycosyl transferase family 2
IAC137	10	4×10^{-135}	4,872,149..4,872,394	Phvul.010G033000.1	0 Kb	Ankyrin repeat-containing
			4,888,088..4,888,545	Phvul.010G033100.1	0 Kb	Ankyrin repeat-containing
			4,893,842..4,894,343	Phvul.010G033200.1	3 Kb	40S Ribosomal protein S17
PvM13 (CB543664)	10	0.0	4,897,032..4,897,540	Phvul.010G033200.1	0 Kb	40S Ribosomal protein S17
				Phvul.010G033100.1	8 Kb	Ankyrin repeat-containing
			4,910,779..4,911,290	Phvul.010G033300.1	0 Kb	40S Ribosomal protein S17
				Phvul.010G033400.1	3 Kb	Phospholipase C
PvM127 (CB450175)	10	0.0	6,174,932..6,175,465	Phvul.010G041200.1	0 Kb	no functional annotations
				Phvul.010G041300.1	7 Kb	no functional annotations
BM157 (AF483873)	10	4×10^{-18}	8,999,124..8,999,246	Phvul.010G056000.1	0.5 Kb	Squamosa-promoter binding protein (SBP) domain
D873626	10	0.0	16,505,342..16,505,821	-	-	-
D864174a	10	0.0	21,757,602..21,758,036	Phvul.010G073200.1	0 Kb	Aprataxin-related
D883703	10	3×10^{-64}	24,137,115..24,137,310	Phvul.010G076700.1	0 Kb	Cytochrome P450
GATS11b (AF483840)	10	5×10^{-111}	32,692,053..32,692,354	Phvul.010G087600.1	0 Kb	Glycosyl transferase family 8
				Phvul.010G087700.1	5 Kb	Sin3 associated polypeptide p18 (SAP18)
D881808	10	0.0	33,405,401..33,405,916	Phvul.010G089800.1	5 Kb	no functional annotations
D865197	10	0.0	33,667,342..33,667,758	Phvul.010G091200.1	1 Kb	nucleotide-binding adaptor (NB-ARC) domain
D864614	10	0.0	38,257,070..38,257,940	Phvul.010G115900.1	1 Kb	Yippee putative zinc-binding protein
				Phvul.010G115800.1	3 Kb	Protein tyrosine kinase
D880279	10	0.0	38,464,656..38,467,110	Phvul.010G117200.1	8 Kb	Homeobox associated leucine zipper
PvBR185	10	1×10^{-55}	40,020,362..40,020,544	Phvul.010G130100.1	6 Kb	no functional annotations
				Phvul.010G130200.1	7 Kb	AP2-like factor

Pvat005	10	0.0	40,678,008..40,685,937	Phvul.010G134800.1	0 Kb	Lipoxygenase
PvM02	10	3.3×10^{-128}	42,004,352..42,005,489	Phvul.010G149900.1	0 Kb	no functional annotation
D882636	01	2×10^{-39}	37,702,027..37,702,175	Phvul.001G133400.1	8 Kb	Leucine-rich repeat containing protein 2
				Phvul.001G133500.1	9 Kb	hypothetical protein
PvM22 (CB539406)	03	0.0	42,106,427..42,107,050	Phvul.003G206600.1	6 Kb	Transmembrane amino acid transporter protein
				Phvul.003G206700.1	0.5 Kb	RNA binding protein
				Phvul.003G206800.1	5 Kb	Protein-L-isoaspartate-methyltransferase (PCMT)
D864174b	05	5×10^{-57}	9,387,722..9,387,863	Phvul.005G063900.1	4 Kb	Cytochrome P450
D882462	08	1×10^{-19}	29,659,103..29,659,219	Phvul.008G147500.1	1 Kb	Cytochrome P450
IAC155	09	0.0	12,778,016..12,778,577	Phvul.009G078600.1	1 Kb	Q calmodulin-binding motif
				Phvul.009G078500.1	5 Kb	Mitochondrial carrier protein
IAC244	09	0.0	14,313,568..14,314,142	Phvul.009G092600.1	0 Kb	Clathrin assembly protein

[a] Underlined markers were located at the ALS10.1 genomic core region.

[b] All DArT derived markers were named with the first letter D; all other markers are SSRs.

[c] Chromosome.

[d] E-values threshold $\leq 1 \times 10^{-10}$

[e] The approximated distance between the marker and its linked transcripts, which are around 10 kb from the marker.

Synteny analysis between ALS10.1 markers and the soybean genome suggested the presence of duplicated regions in the QTL

The synteny between the common bean and soybean genomes in the ALS10.1 QTL region was investigated. Markers of the ALS10.1 core were distributed on six different *G. max* chromosomes (Gm) (Table 5). One cluster was composed of markers BM157, PvM22 and GATS11b, located at one end of ALS10.1 and spanning 0.6 cM (Fig. 1). It was identified on chromosomes Gm03 and Gm07. In addition, PvM127 aligned to Gm05 and Gm17, while IAC137 and PvM13 aligned to Gm16 and Gm20, respectively.Table 5 Synteny analysis

between markers in the ALS10.1 locus and the soybean genome.

***Glycine max* chromosome**[a]	***P. vulgaris* markers**	**E-value**[b]
Gm03	BM157	3×10^{-13}
	GATS11b	1×10^{-12}
	PvM22	2×10^{-42}
Gm05	PvM127	2×10^{-27}
Gm07	BM157	9×10^{-11}
	GATS11b	1×10^{-14}
	PvM22	9×10^{-36}
Gm16	IAC137	3×10^{-57}
Gm17	PvM127	8×10^{-18}
Gm20	PvM13	2×10^{-104}
-	D865197	$> 1 \times 10^{-10}$

[a] Gm = Soybean chromosomes, based on PhaseolusGenes website (http://phaseolusgenes.bioinformatics.ucdavis.edu; Gepts and Lin, 2011)

[b] E-values threshold $\leq 1 \times 10^{-10}$

DISCUSSION

In previous work, the same UC RILs used in this study were scored for resistance to ALS under field and greenhouse conditions; the study identified a major QTL on linkage group Pv10 (ALS10.1) that controlled ALS resistance and spanned a large confidence interval with four markers (Oblessuc et al. 2012a). In the present study, STS-DArT, SSR and SCAR markers were added to this linkage group, thus

reducing the confidence interval of the QTL (Fig. 1). This analysis identified an SSR marker (ATA220) with a position that coincided with the peak LOD score of the QTL (Fig. 2); therefore, this co-dominant marker can be used in marker-assisted breeding for resistance to ALS and can aid in positional gene cloning. The study of the genomic context of ALS10.1 identified many genes related to plant immunity in that region, which highlights the importance of this QTL. In addition, a new resistance QTL was identified in this group; this finding could explain the fact that the R^2 value of ALS10.1 in the greenhouse data analysis decreased by 6% compared to the previously reported value because the effects of both QTLs were confounded due to the lack of markers in the previous study.

The STS-DArT markers were developed through the DArT-BSA approach (i.e., the DArT technique combined with marker-oriented BSA) to maximize the chances of finding DArT markers linked to ALS10.1. This is the first report of the use of this new class of marker in linkage analysis in the common bean. Sequence alignment analysis of DArT clones indicated a high redundancy (59.1%) in the bean DArT libraries (Table 1). A similar percentage of redundancy (50%) based on sequence analysis was observed in oats (*Avena sativa* L.) (Tinker et al. 2009). In beans, the large number of repetitive elements inferred from the sequence analysis of BAC inserts (Schlueter et al. 2008; Ribeiro et al. 2011) could explain the similarity between sequences observed in the DArT-BSA results. Nevertheless, it should be considered that the redundancy reported in this study might be overestimated due to the stringent conditions used in the analysis.

Combining DNA pool analysis with selective genotyping is the simplest and cheapest approach to identifying markers for major genes, although some problems have been associated with this method due to the insufficient marker density (i.e., 15–25 cM) and the high level of false positives observed (Xu and Crouch 2008). Indeed, of the eight non-redundant STS-DArTs developed based on the BSA results, three mapped to linkage group Pv10 and only one to the ALS10.1 region, 16.7 cM away from the QTL LOD peak. However, the combination of the BSA and mapping analyses used in this study reinforces the identification of D865197 as a marker flanking the ALS10.1 QTL region; this result was also confirmed

by the sequence analysis which aligned this STS-DArT to Pv10. In addition, the small increase in the number of markers on the ALS10.1 region identified with the BSA approach may result from the absence of recombinant RILs for that region, which could render the linkage mapping of the new markers unfeasible. This lack of recombinant RILs also could explain why five STS-DArTs did not map to linkage group Pv10, but four of these aligned to Pv10, indicating that the BSA method actually identified DArT clones around the ALS10.1 region. The identification of markers by DArT-BSA near ALS10.1 but not within the QTL region may also be a result of the low resolution of the QTL obtained by Oblessuc et al. (2012a), with the bulks for DArT-BSA selected based on phenotype and marker genotypes of the only two SSRs available at the time (GATS11b and IAC137), which were 13.4 cM apart. Indeed, if the chromosomal location of these two SSRs were considered, five DArT sequences were assigned to a major QTL region, which would validate the DArT-BSA results.

SSRs represent an important source of markers in assisted breeding (Collard and Mackill 2008) and were therefore used in addition to the STS-DArT markers to increase the resolution of the ALS10.1. The graphical genotypes of the most resistant and most susceptible RILs illustrate the validity of these markers, with a clear distinction between resistant and susceptible lines (Table 3). In addition to being associated with ALS resistance, some of these markers were reported to be linked to other useful traits, including resistance to halo blight caused by *Pseudomonas syringae pv. phaseolicola* and seed weight. The halo blight resistance gene *Pse*-1 was mapped in the vicinity of BM157 and GATS11b (Miklas et al. 2009; 2011), whereas BM157 was reported to be linked to a QTL that accounts for approximately 19% of the variation in seed weight (Davis et al. 2006). Therewith, the addition of these markers to ALS10.1 could be useful when breeding for ALS resistance and other relevant traits.

Similarly to the SSRs, SCARs are useful markers for marker-assisted selection (Collard and Mackill, 2008), even though only one ($PF5_{330}$) of the three polymorphic SCARs used was mapped on Pv10. It probably is result of the dominant character of the SCAR markers that are less informative than the SSRs, which are co-dominant markers. Nevertheless, the SCAR $PF5_{330}$ was previously linked to ALS resistance

(Mahuku et al. 2004), and here it was linked in coupling phase to the newly identified QTL (Fig. 1B), demonstrating the utility of this marker in ALS QTL mapping. The ALS10.2 QTL was located at the end of the linkage group and spanned two STS-DArTs in addition to the SCAR. The SCAR $PF5_{330}$ was developed from an AFLP marker linked 5.0 cM apart from an ALS resistance locus located on the Pv08 linkage group of the DOR364 x G19833 map (Mahuku et al. 2004). Because $PF5_{330}$ was mapped on linkage group Pv10 in this study, we infer that these groups share a duplicated region and that these two QTLs could be paralogs. A duplication of these regions was also inferred by synteny analysis with soybean because similarities between markers present on the Pv08 and Pv10 groups of the common bean were identified on both Gm07 and Gm08 chromosomes of soybean (McClean et al. 2010). In contrast, the synteny analysis of the STS-DArT markers present on ALS10.2 and the soybean genome did not identify any similarities between these sequences. Therefore, it is important to note that even though the markers linked to ALS10.2 and mapped 66.4 cM away from the other markers of this group were linked to Pv10 based on the BSA evidence, it is possible that this QTL maps to group Pv08, and further studies should investigate this possibility.

As a preliminary version of the common bean genome was recently made available, the physical locations of the Pv10 markers and consequently the ALS10.1 genomic context were investigated along with the linkage analysis. Sequence analyses indicated that most STS-DArT and SSR markers of linkage group 10 were located on chromosome Pv10. Moreover, the order of the markers that comprise the core of ALS10.1 was only in agreement with the inversion observed between the SSR markers BM157 and GATS11b in most cases. Similarly, the marker order for the entire chromosome was the same in the linkage and sequence analyses with the exception of an inversion on the end of Pv10 (Fig. 2). These differences might reflect genomic rearrangements in the divergent bean genotypes (Kwak and Gepts 2009). For instance, different positions of Pv10 markers analyzed using both draft genomes available (CYTED – http://www.cyted.org/ and Phytozome – http://www.phytozome.net/) resulted in different rearrangements of the markers (Supplemental Table 3), most likely due to the divergent *P. vulgaris* gene

pools used (Mesoamerican (BAT93; CYTED) and Andean (G19833; Phytozome)). Such rearrangements are suggested by inversions in marker order observed in different bean linkage maps, such as that of the six SSRs between BM157 and ATA220 (Fig. 1) on ALS10.1, which were mapped in a different order (but maintained as a linked cluster) in different RILs from a cross between DOR364 x G19833 (Blair et al. (2003). The GATS11b and PvM22 markers were similarly mapped to linkage groups Pv08 and Pv09, respectively, instead of Pv10, in a RIL population from a BAT93 x Jalo EEP558 cross (Grisi et al. 2007; Hanai et al. 2010). Nevertheless, it is also important to consider the possibility of these rearrangements be in fact artefacts of the linkage mapping analysis or the genome assembly analysis, in which statistical probabilities are used.

Although inversions in marker order were observed and despite the fact that multiple copies of certain markers of the ALS10.1 core occur both at the ALS10.1 core region and elsewhere in the genome (Supplemental Table S3), the physical location of ALS10.1 predicted in our analysis is further confirmed by fluorescence *in situ* hybridization (FISH) studies. The genetic and genomic position of SSR markers indicated that ALS10.1 is located between clusters of ribosomal DNA (rDNA) on the long arm of Pv10. FISH of BAC clones and plasmids containing 5S and 45S rDNA bean sequences showed that this chromosome contains a large region of 45S rDNA in the end of its long arm and a smaller 5S rDNA region close to the centromere (Fonsêca et al. 2010). Moreover, the BAC probe 173P6 was located in Pv10 between these rDNA clusters (Fonsêca et al. 2010). This clone contains the Bng200 SSR marker that was mapped to linkage group Pv10 near the ALS10.1 core markers PvM127 and PvM13 in the BAT93 x Jalo EEP558 population (Hanai et al. 2010). These two markers were located in the ALS10.1 core in both genomic and linkage analyses, which corroborates this putative physical position of the QTL. In addition, the PvM13 marker was developed from a putative 40S ribosomal EST (Hanai et al. 2010), and curiously, PvM13 and its closest marker, IAC137 (1.6 cM; Fig. 1), were positioned in a region within ankyrin repeat-containing paralogs (Table 4). These genes are composed of repetitive motifs like the rDNA, and play an important role in pathogen resistance and the promotion of reactive oxygen species

(ROS) production (Yang et al. 2012).This points to the presence of pathogen resistance genes in the long arm of Pv10.

The genome location of ALS10.1 allowed us to investigate the genomic context of this region and identify disease resistance-related genes which may play a role in resistance to ALS. One of these genes codes for a phospholipase C located 3.0 Kb away from PvM13; phospholipids are structural components of cell membranes involved in plant signaling, particularly in immune responses (Canonne et al. 2011). When an invading microbe is detected, several phospholipases are activated and contribute to the establishment of an appropriate defense response consisting of the production of oxylipins, jasmonates and the potent second messenger phosphatidic acid, which has been shown to modulate the activity of a variety of proteins involved in defense signaling (Canonne et al. 2011). In *Oryza sativa,* the phospholipase C1 (*OsPLC1*) was shown to be activated by diverse chemical and biological inducers of plant defense pathways and by the incompatible interaction between rice and the pathogenic fungus *Magnaporthe oryzae* (Song and Goodman 2002). In addition, phospholipase C isoforms were required for the hypersensitive response and activation of disease resistance genes; in the tomato (*Solanum lycopersicum*), for example, the *SlPLC4* gene of the *PLC* gene family is needed to produce an immune response against the biotrophic fungus *Cladosporium fulvum* (Vossen et al. 2010).

Another resistance candidate gene in the QTL region was identified as a member of the glycosyl transferase family 2, located 6.0 kb away from IAC61. This gene family is involved in many aspects of plant primary and secondary metabolism, including protein glycosylation and cellulose biosynthesis (Coutinho et al. 2003). Reduction in the synthesis of cellulose leads to changes in the cell wall composition/structure and activates the synthesis of lignin (Caño-Delgado et al. 2003; Hamann et al. 2009) and of defense responses through the production of jasmonic acid (JA) and the activation of ethylene signaling pathways (Ellis et al. 2002; Hernández-Blanco et al. 2007). Studies have shown that glycosylation can also be important in pathogen recognition (Nekrasov et al., 2011). The Phvul.010G056000.1 transcript, located 0.5 Kb away from BM157, is also potentially responsive to

ethylene; this gene encodes for an SBP-binding domain protein, which is a transcription factor that acts upstream of the ethylene-mediated regulation of ripening in tomato (Manning et al. 2006). Plant defense against herbivores and necrotrophic fungi via the JA and ethylene pathways is an established response mechanism (Antico et al. 2012), and transcriptome analysis of the common bean showed a down-regulation of these hormone responses during infection with the hemibiotrophic fungus *Colletotrichum lindemuthianum* (Oblessuc et al. 2012b). Thus, because *P. griseola* is also a hemibiotroph, it is possible that this pathway is also responsive to the attack of this pathogen.

In addition to analyzing the genomic context of ALS10.1, we investigated transcripts linked to DArT sequences that aligned with Pv10 but were outside of the QTL core. The 40 Kb region covered by such markers encompasses almost the entire chromosome, and all identified transcripts with known functions are related to plant disease resistance, indicating that this class of genes is not restricted to the ALS10.1 region but is spread out over the entire chromosome. For instance, genes coding for Yippee-like proteins (He et al. 2005), NB-ARC domain proteins (van Ooijen et al. 2008), aprataxin-related proteins (Nanda et al. 2010; Oblessuc et al. 2012b), cytochrome P450 (Matthes et al. 2011) glycosyl transferase (Vorwerk et al. 2004), protein kinases (Melotto et al. 2004; Schwessinger and Ronald 2012), and homeobox-associated leucine zippers (Vleeshouwers et al. 2011) were observed, which reinforces the importance of studying this chromosome for breeding disease resistance in the common bean.

Conserved sequences between common bean and soybean chromosomes involving Gm03, Gm07, Gm16 and the bean Pv10 were detected by McClean et al. (2010) and Miklas et al. (2011). Synteny analysis of the markers in ALS10.1 and the soybean genome was therefore performed. Complex evolutionary dynamics in ALS10.1, characterized by rearrangements and duplications not found in soybean was observed, suggesting a continuous adaptation of this locus. Previous studies have shown that the soybean genome experienced a whole genome duplication after diverging from the common bean (Lin et al. 2010) in such a way that almost all single-copy sequences of the common bean are present in two copies in the soybean genome (McClean et al. 2010). Indeed, the sequence of one single-copy marker of ALS10.1

(PvM127) aligned with two soybean chromosomes. However, this observation did not always hold true for other markers of this region, and in some cases the opposite situation was observed, i.e., multiple copy markers from the bean (PvM13 and IAC137) aligned as single markers in the soybean, illustrating that independent duplication events may occurred within the *P. vulgaris* genome. In addition, the observation of multiple copies sequences in common bean which are single copies in soybean could also be result of a significant diversion that took place on the soybean paralog sequences.

In conclusion, the incremental increase in resolution of the major QTL for ALS resistance (ALS10.1) from the addition of the newly developed STS-DArT and SSRs available in literature will be useful for molecular breeding, particularly at the ALS10.1 core region spanning the markers IAC61, PvM13, IAC137, PvM127 and BM157. The genomic context analysis of this QTL showed an enrichment of disease-related genes in this region; at the same time, the rearrangements and inversions observed either through comparison of genetic and genomic marker positions or through synteny analysis with the soybean hint at the dynamic evolution of the region. This report represents one of the first steps toward the cloning of ALS resistance genes.

ACKNOWLEDGMENTS

The authors thank to Dr. Phil McClean for allowing the usage of the bean genome sequence assembly data from Phytozome. PRO received a fellowship from São Paulo Research Foundation - FAPESP (2009/02411-2). This work was supported by São Paulo Research Foundation - FAPESP (2010/51673-7).

AUTHOR CONTRIBUTION STATEMENT

PRO conceived and conducted the DArT-BSA experiment and analysis, design the primers for the new STS-DArT markers making the RIL population genotyping with the SSRs, SCARs and STS-DArT; and also performed all the linkage and QTL mapping analyses, as well as the genomic analysis using the Phytozome database; in addition drafted the manuscript. LMKCP helped with the STS-DArT genotyping. RMB helped with the SSR genotyping. JMCM and ROV performed the genomic analysis using the CYTED data base. AFC and SAMC are the associated

bean breeders, participating of the discussions. LEAC participated in the initial design of the project, discussions and in the editing of the manuscript. LLBR conceived the project and coordinated the sponsoring project through FAPESP agency, helped with data interpretation and editing of the manuscript. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests

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SUPPLEMENTAL MATERIAL

Table S1 Information of DArT clones that revealed polymorphisms between the resistant and susceptible bulks.

Clone ID	P[a]	Avarege reproducibility	Call rate	PIC	CAL 143	IAC-UNA	Resistant Bulk	Susceptible Bulk
827737	92.6	100%	100%	0.5	1	0	1	0
827887	91.2	100%	100%	0.5	1	0	1	0
864174	93.8	100%	100%	0.5	1	0	1	0
864415[b]	94.5	100%	100%	0.5	1	0	1	0
864614	96.5	100%	100%	0.5	1	0	1	0
865197[b]	92.3	100%	100%	0.5	1	0	1	0
866012	95.9	100%	100%	0.5	1	0	1	0
866317	96.3	100%	100%	0.5	1	0	1	0
873626	96.9	100%	100%	0.5	0	1	0	1
879964	92.3	100%	100%	0.5	1	0	1	0
880279	90.7	100%	100%	0.5	0	1	0	1
880323	95.7	100%	100%	0.5	1	0	1	0
880463	91.5	100%	100%	0.5	1	0	1	0
880625	94.7	100%	100%	0.5	1	0	1	0
881808	96.4	100%	100%	0.5	0	1	0	1
882462[b]	95.1	100%	100%	0.5	1	0	1	0
882636	94.5	100%	100%	0.5	1	0	1	0
883124	97.1	100%	100%	0.5	0	1	0	1
883131	92.2	100%	100%	0.5	1	0	1	0
883634	94.4	100%	100%	0.5	1	0	1	0
883703	91.6	100%	100%	0.5	0	1	0	1

[a] ANOVA based estimate of marker quality, which reflects how well the two phases (present = 1 vs absent = 0) of the marker are separated in this dataset. Markers with probability $P \geq 90$ were scored as very reliable.

[b] DArT clones converted to STS-DArT markers and mapped on linkage group B10.

Table S2 Microsatellite (SSR) markers scored in the UC RILs mapping population.

SSR marker	Reference	SSR marker	Reference
ATA138	Blair et al., 2008	**IAC236**	Campos et al., 2011
ATA145	Blair et al., 2008	**IAC241**	Campos et al., 2011
ATA150	Blair et al., 2008	**IAC243**	Campos et al., 2011
ATA2	Blair et al., 2008	**IAC246**	Campos et al., 2011
ATA209	Blair et al., 2008	**IAC247**	Campos et al., 2011
ATA22	Blair et al., 2008	**IAC248**	Campos et al., 2011
ATA220	Blair et al., 2008	**IAC250**	Campos et al., 2011
ATA244	Blair et al., 2008	**IAC265**	Campos et al., 2011
ATA26	Blair et al., 2008	**IAC270**	Campos et al., 2011
ATA27	Blair et al., 2008	**IAC271**	Campos et al., 2011
ATA3	Blair et al., 2008	**IAC273**	Campos et al., 2011
ATA32	Blair et al., 2008	**IAC275**	Campos et al., 2011
ATA7	Blair et al., 2008	**IAC278**	Campos et al., 2011
ATA77	Blair et al., 2008	**IAC281**	Campos et al., 2011
BM187	Gaitan-Solis et al., 2002	**IAC283**	Campos et al., 2011
BM188	Gaitan-Solis et al., 2002	**PvBR139**	Grisi et al., 2007
BM189	Gaitan-Solis et al., 2002	**PvBR167**	Grisi et al., 2007
BM197	Gaitan-Solis et al., 2002	**PvBR229**	Grisi et al., 2007
BM200	Gaitan-Solis et al., 2002	**PvBR46**	Grisi et al., 2007
BM205	Gaitan-Solis et al., 2002	**PvBR87**	Grisi et al., 2007
BM210	Gaitan-Solis et al., 2002	**Pvat011**	Caixeta et al., 2005
BM211	Gaitan-Solis et al., 2002	**Pvct002**	Caixeta et al., 2005
BM212	Gaitan-Solis et al., 2002	**Pvggc01**	Caixeta et al., 2005
IAC01	Benchimol et al., 2007	**PvM01**	Hanai et al., 2007
IAC05	Benchimol et al., 2007	**PvM03**	Hanai et al., 2007
IAC07	Benchimol et al., 2007	**PvM11**	Hanai et al., 2010
IAC13	Benchimol et al., 2007	**PvM13**	Hanai et al., 2010

IAC14	Benchimol et al., 2007	**PvM17**	Hanai et al., 2010
IAC19	Benchimol et al., 2007	**PvM22**	Hanai et al., 2010
IAC20	Benchimol et al., 2007	**PvM36**	Hanai et al., 2010
IAC28	Benchimol et al., 2007	**PvM40**	Hanai et al., 2010
IAC32	Benchimol et al., 2007	**PvM52**	Hanai et al., 2010
IAC54	Benchimol et al., 2007	**PvM56**	Hanai et al., 2010
IAC59	Benchimol et al., 2007	**PvM58**	Hanai et al., 2010
IAC68	Benchimol et al., 2007	**PvM66**	Hanai et al., 2010
IAC77	Benchimol et al., 2007	**PvM75**	Hanai et al., 2010
IAC83	Benchimol et al., 2007	**PvM79**	Hanai et al., 2010
IAC129	Cardoso et al., 2008	**PvM93**	Hanai et al., 2010
IAC144	Cardoso et al., 2008	**PvM97**	Hanai et al., 2010
IAC150	Cardoso et al., 2008	**PvM98**	Hanai et al., 2010
IAC224	Campos et al., 2011	**PvM100**	Hanai et al., 2010
IAC225	Campos et al., 2011	**PvM123**	Hanai et al., 2010
IAC225	Campos et al., 2011	**PvM126**	Hanai et al., 2010
IAC229	Campos et al., 2011	**PvM127**	Hanai et al., 2010
IAC230	Campos et al., 2011	**PvM148**	Hanai et al., 2010
IAC231	Campos et al., 2011	**PvM150**	Hanai et al., 2010
IAC233	Campos et al., 2011	**PvM153**	Hanai et al., 2010

Table S3 Physical location of the marker on the ALS10.1 based on the two drafts of common bean genome.

Common bean Scaffolds (CYTED[a])	Makers name	Markers size (bp)	Identity (%)	Alignment size (bp)	Scaffold position	E-value[c]
scaffold00023	PvM22 (CB539406)	539	99.8	476	768,659.. 768,184	0.0
scaffold00034	PvM13 (CB543664)	558	90.8	382	524,694.. 525,066	3×10^{-130}
scaffold00108	IAC61	701	98.4	429	429,149.. 429,576	0.0
scaffold00133	PvM13 (CB543664)	558	91.6	382	7,057.. 7,434	2×10^{-137}
scaffold00136	BM157 (AF483873)	123	100.0	65	316,240.. 316,304	6×10^{-029}
	BM157 (AF483873)	123	100.0	62	316,196.. 316,257	4×10^{-027}
scaffold00153	D865197	419	89.4	426	536,634.. 536,211	6×10^{-134}
scaffold00266	PvM13 (CB543664)	558	98.4	558	332,302.. 331,748	0.0
	PvM13 (CB543664)	558	97.2	526	343,218.. 342,694	0.0
	IAC137	360	97.4	308	329,749.. 329,444	4×10^{-153}
	IAC137	360	92.1	292	314,845.. 314,557	2×10^{-105}
	IAC137	360	93.4	227	296,223.. 295,997	7×10^{-90}
scaffold00313	GATS11b (AF483840)	306	98.3	302	382,264.. 382,562	2×10^{-154}
scaffold00392	PvM127 (CB450175)	524	99.4	524	66,992.. 66,471	0.0
Common bean Scaffolds (Phytozome[b])	**Makers name**	**Markers size (bp)**	**Identity (%)**	**Alignment size (bp)**	**Scaffold position**	**E-value[c]**
scaffold00053	PvM22 (CB539406)	539	99	475	55,916.. 56,391	0.0
scaffold00107	IAC61	701	86	442	53,034.. 53,467	1×10^{-159}
scaffold00123	PvM13 (CB543664)	588	93	393	608,347.. 607,954	3×10^{-172}
scaffold00327	D865197	419	99	416	287,858.. 288,274	0.0
scaffold00341	BM157 (AF483873)	123	98	85	182,520.. 182,605	2×10^{-40}
scaffold00383	PvM13 (CB543664)	588	99	524	136,410.. 136,934	0.0
	PvM13 (CB543664)	588	96	531	151,450.. 151,981	0.0
	IAC137	606	99	501	154,672.. 155,173	0.0
	IAC137	606	89	364	161,029.. 161,393	2×10^{-137}
	IAC137	606	91	245	175,185.. 175,430	2×10^{-103}
scaffold00648	GATS11b (AF483840)	306	99	301	47,396.. 47,095	5×10^{-156}
scaffold00835	PvM13 (CB543664)	588	91	372	32,787..33,159	1×10^{-152}
scaffold00882	PvM127 (CB450175)	524	96	533	70,504.. 69,971	0.0

[a] Ibero-American Programme for Science, Technology and Development (CYTED; http://www.cyted.org/).
[b] *Phaseolus vulgaris* v0.9, DOE-JGI and USDA-NIFA (http:://www.phytozome.net/commonbean).
[c] E-value threshold $\leq 1 \times 10^{-10}$

CAPÍTULO III:

Oblessuc PR, et al. Determining the genomic structure of a QTL linked to angular leaf spot. Bean Improvement Cooperative Annual Report 2012, 55:39 - 40.

RESUMO

O QTL de maior efeito sobre a resistência à mancha angular ALS10.1 foi previamente identificado por nosso grupo de pesquisa no grupo de ligação B10, correspondendo à 22% e 16 % da resistência à esta doença em condições de campo e de casa de vegetação, respectivamente. Para caracterizar este QTL, um mapa físico de sua região genômica esta sendo estabelecido através do *screening* de uma biblioteca de *Bacterial Artificial Chromosome* (BAC) utilizando marcadores moleculares intimamente ligados ao QTL.

A biblioteca BAC utilizada possue 30.720 clones com tamanho médio de insertos de 125 Kb. Ao total, 11 marcadores moleculares foram utilizados no *screening* desta biblioteca através da técnica de PCR de colônia. As colônias positivas tiveram o DNA das BACs isolado e as seqüências dos *BAC-ends* (BES) obtidas. Até o momento, seis clones BAC foram identificados através do PCR de colônia. Quatro clones contêm um único marcador, enquanto os outros dois (P02H22 e P10A12) mostraram a presença de dois marcadores (Pvm13 e IAC137) em seus insertos. O tamanho dos insertos destes clones contendo os mesmos dois marcadores foram estimados por digestão do DNA-BAC com a enzima de restrição *Sac*I, com ambos as insertos contendo cerca de 100 Kb. Com isso, a razão entre distância genética e distância física para esta região do genoma pode ser estimada em 62 Kb/cM.

Os genes contidos no QTL ALS10.1 também foram analisados. Análise de BLASTN das BES revelou a existência de seqüências similares à retrotransposons (RT), assim como seqüências putativas codantes para proteína quitinase e TIR-NBS-LRR também foram observadas. Estas últimas famílias de proteínas desempenham sabidamente papel na resistência de plantas contra patogénicos. Os resultados deste trabalho devem levar a identificação de possívies genes candidatos envolvidos na resistência do feijoeiro contra mancha angular.

DETERMINING THE GENOMIC STRUCTURE OF A QTL LINKED TO ANGULAR LEAF SPOT RESISTANCE

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INTRODUCTION

Angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun *(sin. Phaeoisariopsis griseola* (Sacc.) Ferraris*)* is one of the most devastating diseases of common bean (*Phaseolus vulgaris* L.), leading to yield losses of 80% in bean fields around the world [1]. As disease control is most effective by using resistant varieties, it is important to understand the mechanisms of host-pathogen interaction and develop tools that could be applied on breeding programs. Our group has previously identified a major QTL on the linkage group B10 that explain 22% of the ALS resistance under field and greenhouse conditions [2], using the UC (IAC-UNA x CAL 143) mapping population [3]. To further characterize this QTL, we develop a physical map in that region by screening a bacterial artificial chromosome (BAC) library with tightly linked molecular markers and determining the gene content within the QTL.

MATERIAL AND METHODS

A BAC library that covers 5-7 times the bean genome, with 30,720 clones and an average insert size of 125 Kb [4], was screened with 11 molecular makers using polymerase chain reaction (PCR). Bulks of 32 clones were co-cultured in 500 µl of Luria Bertani (LB) medium (10 g of Bacto tryptone, 5 g of yeast extract, and 5 g of NaCl in 1 liter of distilled water) supplemented with 12.5 µg/ml chloramphenicol. Cultures were incubated in 96 deep well plates with shaking at 240 rpm, at 37°C for 16 hours. Liquid cultures were grown on solid LB medium and used for colony PCR reactions performed in 25 µL reactions with 0.8 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR buffer, and 1 unit of Taq polymerase (GoTaq®, Promega). BAC DNA was isolated using standard lysis protocol [5]. BAC-end sequencing (BES) was performed at the Genome Core Facility at UTA (http://gcf.uta.edu/Core_Facility) using the T7 and SP6 primers that border the insert of the pBeloBAC11 vector. New primers for chromosome walking were designing based on the BES using the PRIMER3 (v. 0.4.0) software (http://frodo.wi.mit.edu/primer3).

RESULTS AND DISCUSSION

Six BAC clones were identified with the colony PCR screening; four clones contain only one marker, while the other two carry two markers (Fig. 1A). These six clones span the entire QTL, resulting in a reasonable coverage of the region (Fig. 1A). The insert size of the clones P02H22 and P10A12 containing the same two markers (Pvm13 and IAC137) were estimated by BAC DNA digestion with the *Sac*I restriction enzyme (Fig. 1B). Both BAC inserts have approximately 100 Kb (Fig. 1B). Considering that the genetic distance between the two markers was estimated to be 1.6 cM [3], the kilobase (Kb) per centimorgan (cM) ratio in this QTL region is approximately 62 Kb/cM and does not coincide with the estimated genome average of 120 Kb/cM [6]. BES analysis revealed that the P10A12 insert lies within the P02H22 clone. BLASTN analysis of these sequences revealed strong similarities with retrotransposon (RT) sequences (E-value < 10^{-30}; Fig. 1A). As RT have repeated conversed regions [7], it is possible that their presence in the QTL could be the cause of increased recombination frequency in this locus that could explain low Kb/cM ratio observed. Sequence analysis also indicated the presence of regions with strong similarities with a chitinase (E-value = $9e^{-120}$) and a TIR-NBS-LRR (E-value = $6e^{-20}$) genes, which are known to play a role in plant resistance against pathogens [8]. Outward chromosome walking is

underway with the goal of closing the gaps between the six BAC identified. We expect to identify candidate genes involved in common bean resistance against ALS for future transgenic technologies and develop robust molecular markers to facilitate the development of resistance cultivars using marker assisted selection.

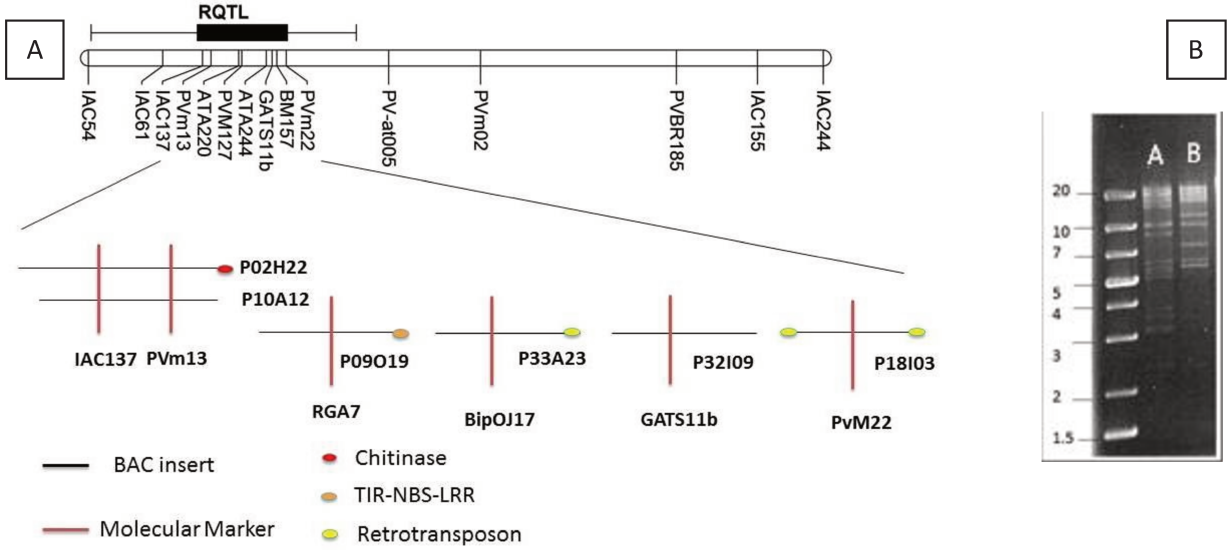

Figure 1. Current genome structure of the ALS resistance QTL. **(A)** Six BAC clones spanning the QTL were identified. P02H22 and P10A12 shared two markers (PvM13 and IAC137) and are overlapping. **(B)** P02H22 (lane A) and P10A12 (lane B) DNA digestion with the *Sac*I restriction enzyme. DNA banding patterns indicates that those clones have differences in DNA sequences. First lane shows DNA Ladder (1Kb Plus, Fermentas®) and the numbers on the left indicate fragment size in Kb.

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CAPÍTULO IV:

Oblessuc PR, et al. Innate immunity-responsive genes in a major QTL controlling angular leaf spot resistance. Bean Improvement Cooperative Annual Report 2013.

RESUMO

A abordagem mais efetiva para o controle de doenças, como a mancha angular que ataca o feijoerio, é a utilização de cultivares resistentes. Com isso, compreender os mecanismos que controlam a interação patógeno-hospedeiro é muito importante. Neste estudo, determinou-se a resposta à patógenos de genes putativos localizados no QTL de resistência à mancha angular ALS10.1.

Os marcadores previamente utilizados no *screening* de bibliotecas BAC para estudar a estrutura do QTL ALS10.1 foram alinhados com as seqüências de genes, disponíveis em bases de dados do NCBI através de TBLASX. Os *primers* para os marcadores atribuídos a regiões codantes de genes foram utilizados em estudos de expressão gênica em resposta à flg22, um padrão molecular associado a patógeno (PAMP) derivado da flagelina capás de elicitar resposta imune em plantas. Para isso foi utilizada a técnica de RT-PCR semi-quantitativo.

O resultados revelaram a expressão diferencial de dois genes putativos contidos em ALS10.1: ribonucleoproteina (PvM22) e TIR-NBS-LRR (RGA07). A ribonucleoproteina analisada através do marcador PvM22 foi induzida durante a resposta do feijão à flg22. Assim, este gene deve possuir papel na resposta PTI (*PAMP-triggered immunity*) do feijão. Já o gene observado pelo marcador RGA07 foi reprimido na presença de flg22. Proteínas TIR-NBS-LRR são conhecidas por sinalizar o reconhecimento de moléculas de virulência de patógenos e com isso desencadear a resposta imune ETI (*Effector-Triggered Immunity*). Portanto, em feijão a proteína TIR-NBS-LRR estudada poderia estar envolvida na ETI, sendo reprimida durante PTI. Os outros três genes (PvM13, PvM127 e P09O19) estudados não foram responsivos à flg22. A descoberta de genes que respondem a PAMP na região ALS10.1 destaca o seu papel na resposta imune do feijão.

INNATE IMMUNITY-RESPONSIVE GENES IN A MAJOR QTL CONTROLING ANGULAR LEAF SPOT RESISTANCE

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INTRODUCTION

Angular leaf spot (ALS) causes great yield losses in common bean (*Phaseolus vulgaris* L.). This disease is caused by the hemibiotrophic fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun. As the most effective approach to control this disease is to use resistant cultivars, understand the mechanisms controlling the host-pathogen interaction and the development of gene-specific molecular markers is important to plant breeding efforts. In this study, we determined whether putative genes located in a major ALS resistance QTL (ALS10.1) on the linkage group B10 [1] were responsive to flg22; a hallmark elicitor of plant innate immune response [2]. Available markers for these genes [3] were used in semi-quantitative RT-PCR (Reverse Transcriptase – Polymerase Chain Reaction) to access their expression levels. Indeed, we observed differential expression of two putative genes reinforcing the importance of the ALS10.1 QTL in bean defense responses. These results will facilitate further studies in cloning ALS resistance genes and the development of tools for marker-assisted breeding.

MATERIAL AND METHODS

The markers used in the BAC library screening for studying the QTL ALS10.1 [3] were aligned to gene sequences available in the NCBI non-redundant and EST databases using the tBLASTX (≥50% identity and E-values ≤ 1 x 10^{-5}). Primers for the markers assigned to gene coding regions were used in RT-PCR. Leaves of the bean line G2333 were immersed in a solution of 10 mM flg22 or water (control) for 30 minutes, and then frozen in liquid nitrogen for total RNA extraction using the Plant RNeasy kit (Qiagen ®) according to the manufacturer's instructions. RT-PCR was carried out with gene-specific primers in a two-step reaction using the Takara ® RNA PCR kit according to the manufacturer's instructions. The *PvACT2* gene (GI: 165882002) was used to normalize the RNA input in the amplification reactions.

RESULTS AND DISCUSSION

Previously, we have characterized the genome structure of the major QTL for ALS resistance ALS10.1 [3]. In the present study, we found five markers as part of putative open reading frames (Table 1), which were chosen to assess gene expression. Two genes were found to be differentially expressed and three showed no response to flg22 treatment (Fig. 1).

Pathogen-associated molecular patterns (PAMPs) are typically essential components of whole classes of pathogens, such as fungal chitin, bacterial flagellin and its derived peptide flg22. Stimulation with PAMPs leads to PAMP-triggered immunity (PTI) in plants [2, 4]. The bean N-like protein containing the RGA07 marker was down-regulated in PTI response. It is a TIR-NBS-LRR protein, which provides a downstream signaling usually after recognition of pathogen virulence molecules [4]. This molecules leads to a second class of pathogen perception called effector-triggered immunity (ETI) [4]. Therefore, this bean TIR-NBS-LRR protein could

be involved in ETI, being down-regulated during PTI [5]. The putative ribonucleoprotein (PvM22 marker) was up-regulated during the bean PTI response. This kind of protein plays a role in many metabolic activities of plants, mainly through the genes involved in translational control [6]. Although the participation of ribonucleoproteins in stress responses was already established [6], its direct link with plant immunity remains elusive.

The three genes that were not responsive to flg22 treatment encoded for a ribosomal 40S protein (PvM13), an unknown protein (PvM127), and a TIR-NBS-LRR protein (P09O19). Ribosomal proteins act in protein synthesis and do not show a direct involvement in plant immune response. Although the P09O19 was assigned to a TIR-NBS-LRR gene that belongs to defense-associated gene families [4], it showed no differentially expression in response to flg22 indicating that gene-specific response may exist. The discovery of PAMP-responsive genes in the ALS10.1 region highlights its role in bean immune response.

Table 1 Markers assigned to gene coding regions of the ALS10.1 QTL.

Marker	Gene Annotation	E-value	Species
RAG07	TMV resistance protein N-like	4×10^{-86}	*Glycine max*
P09O19	TIR-NBS-LRR	6×10^{-20}	*Glycine max*
PvM22	Ribonucleoprotein	9×10^{-65}	*Glycine max*
PvM127	unknown protein	3×10^{-09}	*Arabidopsis thaliana*
PvM13	Ribosomal protein 40S	1×10^{-93}	*Glycine max*

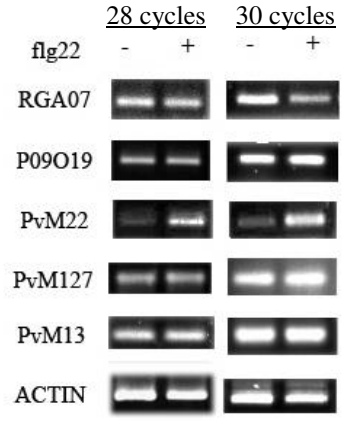

Fig 1 Genes in the QTL ALS10.1 region may play a role in bean innate immunity. RT-PCR was performed with total RNA extracted from bean leafs treated with flg22 (10 µM) or water (control) for 30 minutes. Left column shows amplicons after 28 PCR cycles whereas the second column shows the same amplicons after 30 PCR cycles. The experiment was performed in three biological replicates and the bean actin gene (*PvACT2*) was used as loading control.

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CAPÍTULO V:

Oblessuc PR, et al. *Phaseolus vulgaris* responsive genes to *Pseudocercospora griseola* infection on a major resistance QTL.

RESUMO

O feijão comum (*Phaseolus vulgaris* L.) é uma importante fonte de proteína na dieta humana. A mancha angular (ALS), causada pelo fungo *Pseudocercospora griseola* (Sacc.) Ferraris, leva a grandes perdas de produtividade na cultura do feijoeiro. Portanto, o objetivo do presente trabalho foi estudar os mecanismos moleculares envolvidos na respostas de *P. vulgaris* à *P. griseola*, e com isso contribuir para o desenvolvimento de ferramentas eficazes para o melhoramento de plantas. Para isso, a estrutura do genômica e os genes presentes no loco ALS10.1 de maior efeito na resistência ALS foram estudados.

O *core* de ALS10.1 foi identificado previamente na extremidade do cromossomo Pv10, cobrindo cerca de 5,3 Mb, com base em informação do genoma de *P. vulgaris*. *Screening* de uma biblioteca BAC (*Bacterial Artificial Chromosome*) foi realizado para a determinação da seqüência genômica do QTL. Apenas dois clones BAC tiveram seqüência atribuída ao núcleo de ALS10.1. Em adição, os 323 genes preditos para esta região genômica foram analisados e os resultados da análise de *Gene Ontology* (GO) mostraram que 61,9% dos genes estão envolvidas na resposta à estresse. Um *cluster* de genes TIR-NB-ARC foi observado cobrindo aproximadamente 849 Kb da região núcleo do QTL, além de outros genes que sabidamente estão relacionados com a imunidade em plantas.

O padrão de expressão gênica também foi avaliado na resposta à *P. griseola* para sete genes presentes no *core* de ALS10.1. O gene R TIR-NB-ARC (Phvul.010G025700) foi induzido durante resposta compatível no genótipo IAC-UNA, no qual deve estar permitindo a proliferação do patógeno. Além disso, genes putativos reguladores negativos de resposta imune, através da inativação da via do ácido salicílico (AS), foram reprimidos durante a resposta incompatível em CAL 143. O AS é um hormônio induzido por patógenos fundamental para a resposta de defesa de plantas. Assim, o reconhecimento do patógeno por *P. vulgaris* deve se dar através de genes R que ativam a sinalização da resposta de defesa mediada por AS. Os resultados do presente trabalho permitirão a manipulação da diversidade genética do feijoeiro pela identificação de cultivares geneticamente resistentes.

Phaseolus vulgaris responsive genes to *Pseudocercospora griseola* infection on a major resistance QTL

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Key words: common bean, angular leaf spot, fungal resistance, plant defense, candidate gene.

ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is an important protein source in human diet. The angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola* (Sacc.) Ferraris, leads to great yield losses in common bean. Therefore, the objective of the present work was study the molecular mechanisms enrolled in the *P. vulgaris* responses to *P. griseola*, and through it helped the development of effective tools to this crop breeding. Therewith, the genome structure and the genes on the major ALS resistance locus ALS10.1 were studied. The ALS10.1 core was first identified on the end of the chromosome Pv10, with approximately 5.3 Mb, using the draft of the *P. vulgaris* genome. A BAC library screening to determining the genomic sequence of this region was performed. Only two BAC clones could be assigned to the ALS10.1 core. In addition, the 323 predicted genes for this genomic region were analyzed and the Gene Ontology (GO) analyses demonstrated that 61.9% of the genes are involved in stress responses. A TIR-NB-ARC gene cluster was observed covering approximately 849 Kb, in addition to other genes known to be related to immunity in plants. Gene expression pattern was evaluated in response to *P. griseola* for seven genes on ALS10.1. The R gene TIR-NB-ARC (Phvul.010G025700) was induced during the compatible response of the genotype IAC-UNA, in which should be enabling the pathogen proliferation. In addition, putative negative regulator genes of immune response through the inactivation of salicylic acid (SA) via were repressed during the incompatible response of the CAL 143. The SA is a key hormone to pathogen induced plant defense response. Therefore, the *P. vulgaris* pathogen recognition should take place through the R genes to the downstream signaling of the SA-mediated defense response. The results of the present work will enable the manipulation of the bean genetic diversity by the identification of genetically resistant cultivars.

INTRODUCTION

One of the main objectives of molecular genetics is to identify and isolate genes governing important traits. For this, two major approaches can be used: reverse or forward genetics. For species with genomic sources like genome sequenced and mutant libraries, as *Arabidopsis thaliana* for example, the reverse genetic have been shown to be an important mechanism in gene discovery and function analysis. However, for species with low or none genome resources the direct genetics by positional cloning showed to be efficient in gene discovery, especially those controlling traits of agronomical importance (Xia et al., 2012; Li et al., 2012). *Phaseolus vulgaris* or common bean is one of those species, and although its social and economic importance (Broughton et al., 2003; Gepts et al., 2008) the *P. vulgaris* genome have been sequenced just recently (American Program for Science, Technology and Development (CYTED) - http://www.cyted.org/; and Phytozome - http://www.phytozome.net/).

Thereby, studies have made use of the available genetic maps for *P. vulgaris* to positional cloning genes of interest (Gepts et al., 2008). For instance, Resistance (R) genes, with nucleotide binding – leucine rich repeat (NB-LRR) and/or kinase domains, governing resistance against important agronomical diseases like anthracnose (Creusot et al., 1999; Melotto et al., 2004), bean common mosaic virus (Vallejos et al. 2006) and common bacterial blight (Shi et al., 2011) were identified until now. In addition, a cluster of NB-LRR genes on B4 linkage group of *P. vulgaris* was well studied (Geffroy et al., 2009; David et al., 2009), with the discovery of surrounding genes decoding for formate dehydrogenase, which showed differential expression under biotic and abiotic stress (David et al., 2010).

This kind of study enables the understanding of the mechanisms evolved in plant-pathogens interaction. It is known that upon pathogen recognition, R proteins trigger defense responses that often result in the so-called hypersensitive response (HR); a rapid induction of host cell death localized at the site of invasion of the pathogen (Health, 2000). This plant response is associated with massive cellular ion fluxes,

activation of mitogen-activated protein (MAP) kinase cascades, generation of reactive oxygen species (ROS), and strengthening of cell walls, as well as the induction of the expression of many defense genes, including pathogenesis-related (PR) genes (Dodds and Rathjen, 2010). Among the classical gene-for-gene response (Flor, 1955); a complex interplay between plant hormones such as salicylic acid (SA), ethylene (ET), jasmonic acid (JA) and other gene regulators are necessary for an effective response during innate and systemic immunity (Pieterse et al., 2009).

In general, the innate immune system of plants consists of two layers: (1) called PAMP-triggered immunity (PTI), a basal resistance governed by the recognition of conserved microbial molecules (Pathogen-Associated Molecular Patterns; PAMPs) by host cell surface-localized pattern-recognition receptors (PRRs), which fends plant off most attempted invasions; and (2) effector-triggered immunity (ETI), based on R genes that mediate recognition of effectors (or Avrs – Avirulence molecules), molecules secreted by pathogens to suppress or evade basal resistance. Typically, the tendency to trigger ETI is pathogen strain or race specific and it is associated with programmed cell death, a response which is referred to as the HR, and systemic acquired resistance (SAR) in the host. In accordance with the zigzag model, these two phases of plant immunity can be discriminated, in which ETI is quicker and more prolonged and more robust than PTI (Jones and Dangl, 2006; Tsuda and Katagiri, 2010). However, new insight about this model suggests PTI and ETI could occur concomitantly (Thomma et al., 2011). Beyond PTI and ETI, there are still more to be understood in plant-pathogen interaction, especially in species with fill genomic and genetic resources (*i.e.*, extensive gene annotation and mutant lines) such as *P. vulgaris*.

The angular leaf spot (ALS) is one of the diseases with the greatest impact on common bean yield, leading to loses as high as 80% and found in more than 60 countries around the world (Stenglein et al., 2003; Miklas et al., 2006). Although its importance, the molecular and biochemical responses involved in this host-pathogen interaction were not established yet. A fill its known about the infection process, when the ALS causal agent, *Pseudocercospora griseola* (Sacc.) Crous & U. Braun (Crous et al., 2006), has its spore germinated, with the hypha penetration through epidermis or stomata (Monda et al., 2001). The

information about the fungus hemibiotrophic live cycle is restricted to it hyphae growth intercellularly on the leaf mesophyll during the biotrophic phase, and latter host cell plasmolysis during the fungus necrotrophic phase (Monda et al., 2001). Indeed, the genetic knowledge behind the host response is based only on molecular markers that were linked to different resistance loci (Sartorato et al., 2000; Ferreira et al., 2003; Silva et al., 2003; López et al., 2003; Mahuku et al., 2009; Gonçalves-Vidigal et al., 2011), but no characterization or functional study were conducted to understand the genes present in these regions.

So far, two dominant ALS resistance genes (*Phg-1* and *Phg-2*) have been identified using linkage analysis (Carvalho et al., 1998; Sietsche et al., 2000; Gonçalves-Vidigal et al., 2011; Mahuku et al., 2011). Apart from these, dominant monogenic inheritance for resistance to ALS has also been reported (Corrêa et al., 2001; Mahuku et al., 2004; Namayanja et al., 2006), but the relationship of the genes underlying this resistance with *Phg-1* and *Phg-2* remains unknown. In addition to qualitative resistance genes, resistance to ALS can be controlled by quantitative trait loci (QTL) (López et al., 2003; Mahuku et al., 2009; 2011; Oblessuc et al., 2012a), which explains the more complex inheritance mode of ALS resistance observed by Caixeta et al. (2005).

Many *P. vulgaris* sources of resistance for this disease were described, still the CAL 143 line showed to be an important one, with field resistance to a great number of *P. griseola* isolates (Aggarwal et al., 2004). Studies have also shown that this genotype should carry resistance against rust (*Uromyces appendiculatus*), leaf spot (*Alternaria alternata*) and anthracnose (*Colletotrichum lindemuthianum*) (Vieira et al., 2002); being also tolerant to abiotic stresses like phosphorus and nitrogen soil deficiency, and pH soil variations (Kimani et al., 2007). Thereby, the CAL 143 line was used in the present work, which aimed the identification of candidate genes for resistance against ALS in order to improve the knowledge in the host-pathogen interaction and contribute breeding against *P. griseola*.

The major resistance QTL ALS10.1, mapped using the IAC-UNA x CAL 143 recombinant inbreed line (RIL) population, on linkage group B10 (Oblessuc et al., 2012a), had its genomic core defined by crossing information of the *P. vulgaris* genomic and genetic data (Oblessuc et al., in submission). The

ALS10.1 core spanned six molecular markers in a region of 5.3 Mb on chromosome 10 (Pv10), and the first studies of its genomic context indicated an enrichment for disease related genes on this region (Oblessuc et al., in submission). Thereby, the ALS10.1 was the target for the discovery of responsive genes to *P. griseola* inoculation.

The results of the present study indicated that 61.9% of the genes at the ALS10.1 core are involved in stress responses, which includes a putative R genes cluster, with 57 genes coding for TIR-NB proteins. In addition, down-regulation of genes such as *EDR3-like* (*Enhanced Disease Resistance3-like*) and *BIR1-like* (*BAK1-INTERACTING RECEPTOR KINASE1-like*) during incompatible interaction suggest the involvement of the SA-mediate response during the innate immunity of the *P. vulgaris* against the *P. griseola* infection. In conclusion, the ALS10.1 locus is an important region to be studied in order to improve the knowledge about *P. vulgaris* immunity, with the presence of infection responsive genes, probably involved in both PTI and ETI against pathogens. The identification of the genes associated with the resistance response is an important step to understand and therefore, manipulate the biological mechanisms of resistance, enabling the improvement of the crop.

MATERIAL AND METHODS

Plant material

The resistance source of ALS resistance CAL 143 was crossed with the susceptible *P. vulgaris* cultivar IAC-UNA (Campos et al., 2010). The 346 RILs of the UC population were then used to map QTLs involved in ALS resistance (Oblessuc et al., 2012a). A major QTL for ALS resistance, ALS10.1, was mapped on linkage group B10 of *P. vulgaris* in a confidence interval of 3 cM. ALS10.1 had its genomic context studied, with an indication of plant defense genes present on the QTL region (Oblessuc et al., 2013), and therefore, used in the present study to ALS resistance candidate genes identification.

BAC library screening

The genomic Bacterial Artificial Chromosome (BAC) library obtained with the *P. vulgaris* G2333 genotype, a known anthracnose resistance source, was used in a screening to determine the genomic sequence of QTL ALS10.1. This BAC library covers 5-7 times the bean genome, with 30,720 clones and an average insert size of 125 Kb (Melotto et al., 2003). The screening was performed using 10 molecular makers linked to ALS10.1, in a polymerase chain reaction (PCR) approach, including the microsatellites (SSRs) mapped in the UC map (PvM22, BM157, GATS11b, ATA244, PvM127, ATA220, PvM13 and IAC137); and two markers mapped in different *P. vulgaris* genetic maps, with a high indication of their presence in the ALS10.1 region by the linkage with common markers between maps were also used: BIP-OJ17 (McClean, et al., 2002), RGA7 (López et al., 2003).

Bulks of 32 BAC clones were co-cultured in 500 µl of Luria Bertani (LB) medium (10 g of Bacto tryptone, 5 g of yeast extract, and 5 g of NaCl in 1 liter of distilled water) supplemented with 12.5 µg/ml chloramphenicol. Cultures were incubated in 96 deep well plates with shaking at 240 rpm, at 37^{o}C for 16 hours. Liquid cultures were grown on solid LB medium and used for colony PCR reactions performed in 25 µL reactions with 0.8 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR buffer, and 1 unit of *Taq* polymerase (GoTaq®, Promega). BAC DNA was isolated using standard lysis protocol (Sambrook and Russell, 2001). BAC-end sequencing (BES) was performed at the Genome Core Facility at University of Texas at Arlington (http://gcf.uta.edu/Core_Facility) using the T7 and SP6 primers that border the insert of the pBeloBAC11 vector. New primers for chromosome walking were designing based on the BES using the PRIMER3 (v. 0.4.0) software (http://frodo.wi.mit.edu/primer3 - Rozen and Skaletsky, 2000). Putative functions for the BAC-end sequences were inferred by performing tBLASTx searches (E-values $\leq 1 \times 10^{-5}$) in the non-redundant sequences and the EST databases of NCBI (Altschul et al. 1990).

Whole BAC sequencing and assembly analysis

Each BAC clone was individually cultured overnight in LB medium to BAC plasmid DNA isolation using the ZR BAC DNA Miniprep Kit (Zymo Research®). DNA qualities were verified following the usual Nanodrop 1000 spectrophotometer (Thermo®) parameters. All plasmid DNA samples were sent to the DNA Core facility ate University of Missouri (https://web.rnet.missouri.edu/biotech/dnacore/) for sequencing using Illumina HiSeq 2000, in a multiplex run where a specific library were constructed for each BAC.

The 100 bases resulted reads had the quality measured and the low-quality ends trimmed using the software FASTX-Tollkit (http://hannonlab.cshl.edu/fastx_toolkit/ - Pearson et al., 1997). In addition, the *E. coli* sequences were identified through the BLAST against the *E. coli* genome, being excluded of the following analysis. Therewith, the *P. vulgaris* sequences were pre-assembly using the Velvet (http://www.ebi.ac.uk/~zerbino/velvet/ - Zerbino and Birney, 2008) and the CLC Genomics Workbench (http://www.clcbio.com/products/clc-genomics-workbench/) software. In addition, the *P. vulgaris* genome data were used as framework to assembly the BAC sequences using the CLC Genomics Workbench (http://www.clcbio.com/products/clc-genomics-workbench/).

Gene content of the ALS10.1 QTL

The core region assigned to ALS10.1 QTL on Pv10 (Oblessuc et al., in submission) was used to identify putative responsive genes to *P. griseola*. First, all genes observed in this region, of approximately 5.3 Mb, had their putative functions and closest related ortholog in *A. thaliana* obtained from Phytozome (http://www.phytozome.net/). The AGI (Arabidopsis Genome Initiative) number of the top hit was used as input for assigning Gene Ontology (GO) categories (http://www.geneontology.org/ - Ashburner et al., 2007), performing GO enrichment with the Singular Enrichment Analysis (SEA) of AgriGO (http://bioinfo.cau.edu.cn/agriGO - Du et al., 2010). The AGI numbers were also used to indicate which

genes were responsive to pathogens using the public microarray data of *A. thaliana*, available in the PathoPlant website (http://www.pathoplant.de/ - Bülow et al., 2007). The *A. thaliana* genes which showed differential expression levels (1.5 fold, up- or down-regulated) in response to the biotrophic (*Erysiphe orontii*) and to the necrotrophic (*Botrytis cinerea*) fungus were selected. The *P. vulgaris* genes orthologs to these *A. thaliana* pathogen responsive genes were used in real-time quantitative PCR experiments. The gene orthology inference was conducted through phylogeny analysis when more than one *P. vulgaris* gene was assigned to the same *A. thaliana* ortholog. The both species proteins were aligned by CLUSTALW, and the conserved domains were used in the distance-based analysis through the Maximum Parsimony algorithm in the MEGA5 software (Tamura et al., 2011). Statistical support for distance trees was evaluated using bootstrapping (1,000 replicates).

Gene expression analysis

Nine genes possible related with plant resistance were used to quantitative RT-PCR (qRT-PCR) study. Primers were designed to a single transcript, using the Primer3 v.0.4.0 software (http://frodo.wi.mit.edu/primer3/input.htm - Rozen and Skaletsky 2000) and the *P. vulgaris* data base from Phytozome. Total RNA was isolated using the RNeasy plant mini kit (QIAGEN) from leaves sampled from plants in V3 stage of development after 22h, 40h and 64h of *P. griseola* inoculation and mock inoculation for control, in three biological replicates. Plants were maintained in 22-24^{o}C and photoperiod of 12h, with high humidity conditions in the first 48h post inoculation (hpi). Control plants were maintained in the greenhouse for 17 days to verify disease symptoms. The RNA extraction was followed by DNase treatment (DNAse I Amplification Grade, Invitrogen) to remove contaminating DNA from the extracts. The effectiveness of the DNAse treatment was checked by one run of the treated and untreated RNA of qRT-PCR. Two micrograms of total RNA were converted to cDNA with SuperScript® III First-Strand Synthesis (Invitrogen). To quantify the expression levels of transcripts, qRT-PCR analysis was performed using Platinum® SYBR® Green qPCR (Invitrogen) in optical 96-well plates, including a

passive reference dye (ROX) according to the manufacturer's instructions (Invitrogen). The expression of each gene was assayed in technical duplicates. To verify amplification of one specific target cDNA, a melting-curve analysis was included according to the thermal profile suggested by the manufacturer (Applied biosystems). The amount of plant RNA in each sample was normalized using PvIDE (insulin degrading enzyme) gene (Borges et al., 2011) as internal control and samples collected from control in each time point were selected as calibrator. Statistical analyses were performed using Student's t test (*$p < 0.05$; **$p < 0.01$) and the comparative ΔΔCt method (Livak and Schmittgen 2001). Relative expression was then expressed as $2^{-\Delta\Delta Ct}$ and data are presented as fold change from treated relative to control conditions.

RESULTS

BAC library screening

In order to determine the genomic structure of the major QTL for ALS resistance ALS10.1, a BAC library was screened with the molecular markers linked to the QTL. Of the 10 SSRs mapped on ALS10.1 region, seven have sequence available, from which three (PvM13, IAC137 and PvM22) displayed a sharp and well defined band pattern at agarose gel after colony PCR. These three markers enabled the identification of two BAC clones (Table 1) that were used for chromosome walking based on the BAC-end sequences to primer design for new colony PCR tests. Altogether, the colony PCR technique and the chromosome walking identified eight BAC clones (Table 1), which were sequenced using the Illumina platform. The assembly analyses for each clone were performed using the *P. vulgaris* genome data from Phytozome, showing an average BAC insert size of 188Kb. Two BACs mapped on Pv10, in which the P10A12 showed the best sequence coverage and the P33A23 presented the smaller sequence coverage on the

genome for all BAC alignments (supplemental Fig. S1). Other three BACs were mapped on Pv11; two on Pv04 and one BAC on Pv02 (Table 1).

Table 1 Genomic position of the BAC clones identified by colony PCR molecular marker on the ALS10.1 and chromosome walking.

BAC clone	Molecular marker or BAC-end	Chr[a]	Chr[a] Position	BAC insert size
P09O19	RGA7 (GI: 28190629)	Pv11	33,373,526..33,496,979	123kb
P10A12	PvM13 (GI: 59938335) and IAC137	Pv10	4,831,613..4,993,188	162kb
P10E22	P18I03_T7	Pv04	25,903,769..26,085,182	182kb
P18I03	PvM22 (GI: 59933969)	Pv04	25,903,760..26,068,304	165kb
P28L23	P33A23_SP6	Pv02	20,985,107..21,085,696	100kb
P33A23	BipOJ17 (GI: 150859110)	Pv10	12,963,459..13,033,307	70kb
P31P02	P09019_SP6	Pv11	725,765..949,905	224Kb
P36A15	P09019_T7	Pv11	49,972,701..50,451,149	478Kb

[a]Chr = chromosome

ALS10.1 gene content

To further study the genomic region of the ALS10.1 core, the *P. vulgaris* (Pv) genomic data was used in the identification of the 323 predicted transcripts in the QTL region (supplemental Table S1). As the *A. thaliana* (At) is the plant model with the greatest tools for gene analyses, the higher score and identity At homolog for each Pv protein on the Phytozome was identified. In order to verify the GO categories of the genes on the ALS10.1, the 149 At orthologs (supplemental Table S1) were analyzed through the AgriGO enrichment tool (supplemental Table S2). The majority of the genes showed to be component of organelles (34%), with molecular binding function (43%) and involvement in stress responses (13%) (Fig. 1). In addition, if all categories involved in plant immunity were considered, 25.5% of the genes could be

assigned to this biological process. Indeed, the total of 200 (61.9%) Pv genes putatively related with stress response were found on ALS10.1 core (Table 2).

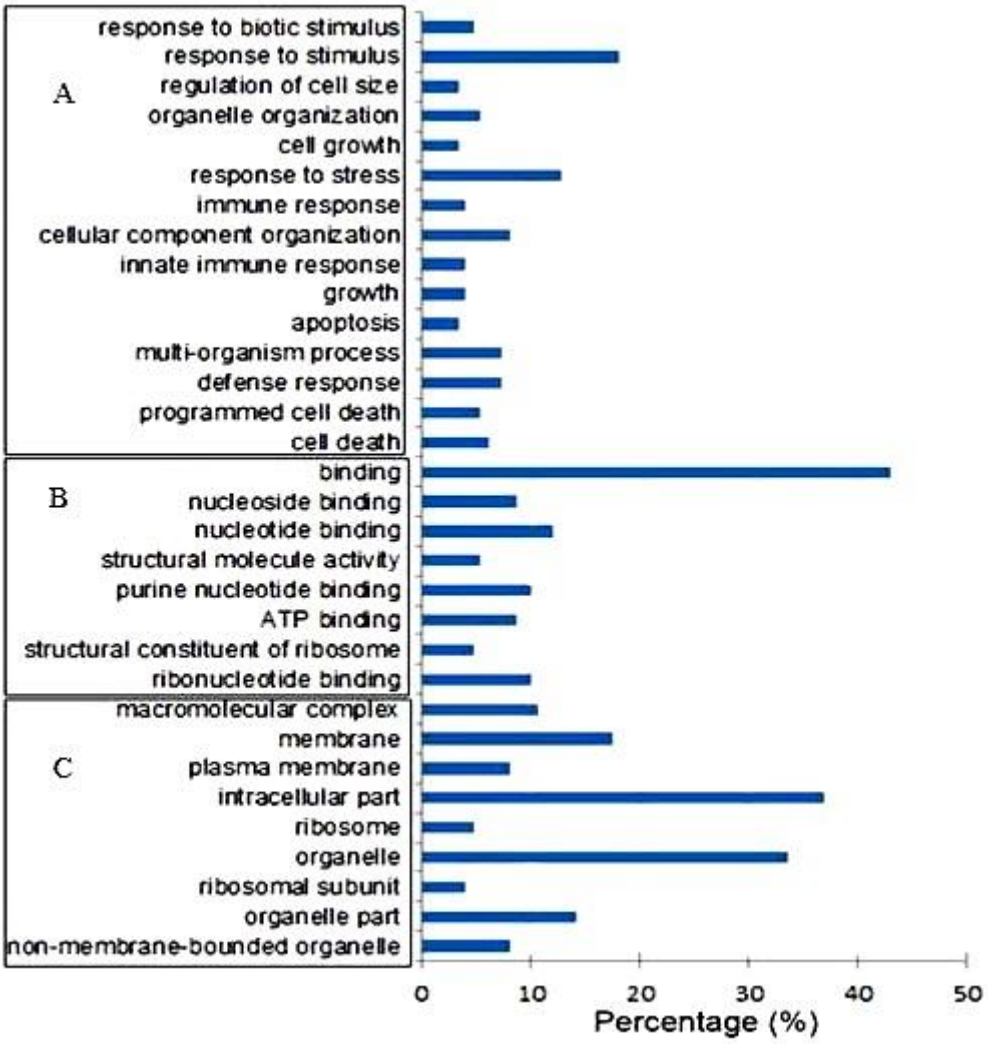

Fig 1 Gene ontology (GO) categories assigned to the genes from the ALS10.1 core. The GO enrichment was performed using the Singular Enrichment Analysis (SEA) of AgriGO, with the AGI numbers (Arabidopsis Genome Initiative) of the *A. thaliana* orthologs for the *P. vulgaris* predicted genes according to the Phytozome assemble. A. biological process; B. molecular function; C. cellular component.

Table 2 Putative function of the 200 genes on the ALS10.1 core related to GO categories of stress responses.

Putative function	# of *P. vulgaris* transcripts	*A. thaliana* ortholog (AGI #)[a]
R gene	52	
Disease resistance protein		AT5G48770
Disease resistance protein		AT1G72890
Disease resistance protein		AT5G36930
Disease resistance protein		AT5G17680
Disease resistance protein		AT4G16950
Disease resistance protein		AT5G44510
Disease resistance protein		AT1G12280
Kinase	16	
Leucine-rich repeat transmembrane protein kinase		AT1G34420
Suppressor of npr1-1 constitutive 4		AT1G66980
Protein kinase family protein		AT2G32800
Phosphoribosyltransferase family protein		AT2G44530
Protein kinase superfamily protein		AT2G44830
Ataurora 3		AT2G45490
AGC (cAMP-dependent, cGMP-dependent) protein		AT3G25250
Nucleoside diphosphate kinase family		AT4G09320
Protein kinase superfamily protein		AT4G16970
Histone H3 K4-specific methyltransferase SET7/9		AT4G17080
P-loop nucleoside triphosphate hydrolases		AT5G26667
Protein kinase superfamily protein		AT5G47070
BAK1-interacting receptor-like kinase 1		AT5G48380
Receptor-like protein kinase 1		AT5G60900
Glycosyl hydrolases family	15	
Pectin lyase-like superfamily protein		AT2G43870
O-Glycosyl hydrolases family 17 protein		AT3G07320
Cellulose-synthase-like C6		AT3G07330
Pectin lyase-like superfamily protein		AT3G15720
Pectin lyase-like superfamily protein		AT3G59850
Pectin lyase-like superfamily protein		AT5G17200
homolog of asparagine-linked glycosylation 12		AT1G02145
homolog of asparagine-linked glycosylation 12		AT1G02145
Transcriptional factor	12	
Basic helix-loop-helix (bHLH) DNA-binding		AT1G06150
Mitochondrial transcription termination factor family		AT1G79220
Basic helix-loop-helix (bHLH) DNA-binding		AT2G22750
AP2/B3-like transcriptional factor family		AT2G22760
Myb-like HTH transcriptional regulator family		AT3G07340
Non-LTR retroelement reverse transcriptase		AT3G18960
Transcriptional factor B3		AT3G60460
Cellular transport	10	
Heavy metal ATPase 5		AT1G63440
Sugar transporter 1		AT1G11260
Lipase class 3 family protein		AT3G07400
Sec23/Sec24 protein transport family protein		AT2G21630
Heavy metal transport/detoxification superfamily		AT1G29000
Multidrug resistance-associated protein 9		AT3G60160
H(+)-ATPase 11		AT5G62670
Sugar transporter 1		AT1G11260

Hormone response	8	
Auxin-responsive family protein		AT4G12980
Ethylene-responsive element binding factor 13		AT2G44840
Auxin efflux carrier family protein		AT1G76520
Ribossome	8	
Ribosomal protein S8e family protein		AT5G59240
Ribosomal protein S6e		AT5G10360
Ribosomal S17 family protein		AT2G05220
Ribosomal protein L16p/L10e family protein		AT1G26910
Zinc-binding ribosomal protein family protein		AT3G10950
Ribosomal protein L4/L1 family		AT2G20060
Ribosomal protein L4		AT1G07320
Other Disease related genes	9	
Cytochrome P450		AT5G24910
Cytochrome P450		AT2G45510
Cytochrome P450		AT1G11680
Ankyrin repeat family protein		AT3G18670
EDR3 (Enhanced Disease Resistance 3)		AT3G25400
Stress up-regulated Nod 19		AT5G61820
Unknown function	70	
WD-40 repeat family protein / beige-related		AT2G45540
stomagen		AT4G12970
SNARE associated Golgi protein family		AT4G12000
Protein of unknown function (DUF581)		AT5G47060
Protein of unknown function (DUF506)		AT3G07350
Protein of unknown function (DUF3550/UPF0682)		AT3G03570
Protein of unknown function (DUF1005)		AT5G17640
Plant protein of unknown function (DUF247)		AT2G44930
MuDR family transposase		AT1G64260
ferredoxin-related		AT1G02180
Domain of unknown function (DUF3598)		AT2G44760
DNA binding; ATP binding		AT3G48770
copper ion transmembrane transporters		AT2G37920
Calcium-binding EF hand family protein		AT1G64850
Auxin-responsive family protein		AT4G12980
AP2/B3-like transcriptional factor family		AT3G18960
5'-3' exonuclease family protein		AT1G34380
Putative membrane lipoprotein		AT5G17590
Plant protein of unknown function (DUF247)		AT2G28580
Homeodomain-like superfamily protein		AT3G53440
No functional annotation		AT1G12650
No functional annotation		AT2G35215
No functional annotation		AT2G44640
No functional annotation		AT3G03560
No functional annotation		AT3G07440
No functional annotation		AT3G15351
No functional annotation		AT4G31980
No functional annotation		AT4G37445
No functional annotation		AT5G17610
No functional annotation		AT5G48500

[a] AGI = Arabidopsis Genome Initiative

The analysis of the putative function of the Pv genes related to immunity, based on the At orthologs annotation, revealed 57 putative disease resistance genes (Table 3).Two clusters of R genes were found on the ALS10.1 core region; the first one between 3,531 Kb and 4,380 Kb; and the second one on the opposite chromosome position, between 8,568 Kb and 8,879 Kb (supplemental Table S1). Of the R genes, the majority (67.2%) showed have the AT5G36930 as the close At ortholog (Table 3), with 37 of those on the initial portion of the QTL genome region, i.e., on the first R gene cluster; and the other two being located on the second R cluster, which also showed the presence of all eight R genes orthologs to the AT5G17680.

Table 3 Putative R genes present on the ALS10.1 genome core region, based on the *A. thaliana* orthologs function, and their Gene Ontology categories.

At ortholog (AGI #)	R gene putative function	# of Pv transcripts	GO category
AT5G36930	TIR-NB-ARC	39	GO:0045087 innate immune response GO:0006915 apoptosis GO:0005524 ATP binding GO:0004888 transmembrane receptor activity GO:0007165 signal transduction GO:0005515 protein binding GO:0031224 intrinsic to membrane
AT5G17680	TIR-NBS-LRR	8	GO:0045087 innate immune response GO:0006915 apoptosis GO:0005524 ATP binding GO:0004888 transmembrane receptor activity GO:0007165 signal transduction GO:0005515 protein binding GO:0031224 intrinsic to membrane
AT5G48770	TIR-NBS-LRR	1	GO:0045087 innate immune response GO:0006915 apoptosis GO:0005524 ATP binding GO:0004888 transmembrane receptor activity GO:0007165 signal transduction GO:0005515 protein binding GO:0031224 intrinsic to membrane
AT1G72890	TIR-NBS	1	GO:0045087 innate immune response GO:0006915 apoptosis GO:0005524 ATP binding GO:0004888 transmembrane receptor activity

			GO:0007165 signal transduction GO:0031224 intrinsic to membrane
AT4G16950	Recognition of Peronospora Parasitica 5 (RPP5)	1	GO:0009817 defense response to fungus, incompatible interaction GO:0000166 nucleotide binding
AT5G44510	Target of AvrB operation 1 (TAO1)	1	GO:0042742 defense response to bacterium
AT1G12280	Suppressor of MKK1 MKK2 2 (SUMM2)	1	GO:0006952 defense response GO:0006915 apoptosis GO:0012505 endomembrane system GO:0005524 ATP binding GO:0005515 protein binding
no At ortholog	TIR-NB	5	-

Gene expression analysis

As a large number of immunity related transcripts were found at the ALS10.1 core, the At orthologs were used to identify the more likely Pv genes involved in ALS host response. For that, the PathoPlant database were used to identify the At genes responsive to a biotrophic and a necrotrophic fungus, with 36 At genes selected. As these genes represent 92 Pv genes (supplemental Table S3), phylogeny analyses to identify the closest Pv gene to the selected At genes were than performed, with 36 Pv genes chosen to qRT-PCR primer design. Phytozome data base was used to check that the primers sequences amplify a unique gene, with 33 pairs of primers selected (supplemental Table S4). These primers were also tested in RT-PCR, which result in the exclusion of 7 genes due the amplification of double bands (Phvul.010G032300, Phvul.010G043100) or absence of amplicon (Phvul.010G047700, Phvul.010G049200, Phvul.010G030300, Phvul.010G047700, Phvul.010G043900). Between the 26 genes remaining, the nine more likely related to plant immunity were chosen to qRT-PCR assays, and of those two (Phvul.010G037200, a glycosyl hydrolase; and Phvul.010G035800, a *BAK1-INTERACTING RECEPTOR KINASE1–like* (*BIR1-like*), showed unspecific dissociation curves and therefore they were not considered in qRT-PCR results.

Five of the seven genes used in the quantitative experiments showed differential expression during ALS response in relation to the control (mock inoculated) (Fig. 2). The Phvul.010G040900 transcript, coding for an ENHANCED DISEASE RESISTANCE3-LIKE (EDR3-like) protein, showed significant difference in expression 22 hpi in both resistant and susceptible genotypes, being repressed (3.2 fold) in CAL 143 and induced (2.8 fold) in IAC-UNA. Likely, the Phvul.010G026300, a TIR-domain transcript, was down-regulated in CAL 143 (2.4 fold) and up-regulated in IAC-UNA (1.3) 22 hpi; but this transcript was also up-regulated in the resistant genotype 40 hpi (2.2 fold). Interestingly, the *BIR1-like* gene, Phvul.010G033800, showed to be induced (2.8 fold) at the first 22 hpi in the susceptible genotype and repressed (1.2 fold) only at 40 hpi in the resistant genotype. By the other hand, the kinase transcript (Phvul.010G031900) was induced during the compatible interaction (2.3 fold) at 40 hpi. The mRNA of the unknown function gene Phvul.010G052900 was the only one that showed expression response 64 hours after *P. griseola* inoculation, being up-regulated (1.4 fold) in the CAL 143. The only transcripts that did showed response to the fungus were a phosphoglycerate mutase (Phvul.010G053300).

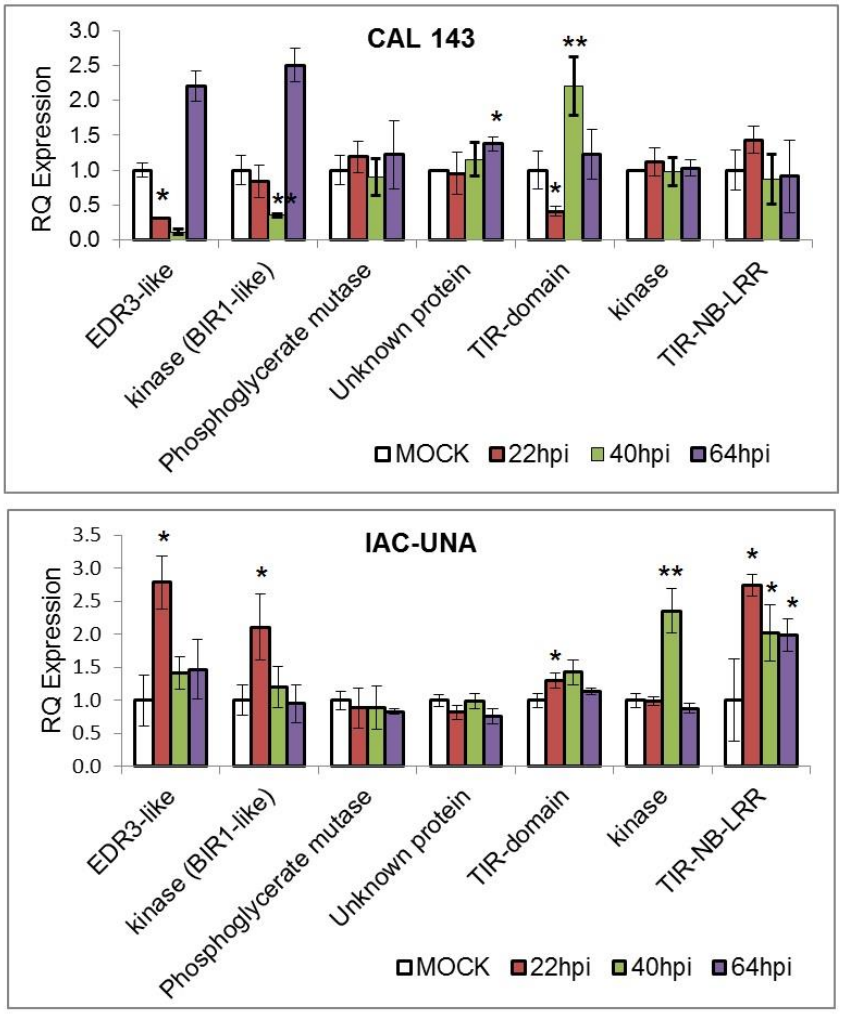

Fig 2 Relative expression of putative immunity related genes of ALS10.1 core. The genes were selected based on their *A. thaliana* orthologs responses to biotrophic (*Erysiphe orontii*) and to necrotrophic (*Botrytis cinerea*) fungus. *P. vulgaris* predicted genes were used to primer design. Total RNA was isolated of leaves with 22 hpi, 40 hpi and 64 hpi of *P. griseola* and mock (control) in biological triplicates. The relative expression of the control was shown for the first time point (22 hpi) for each gene, once it showed no significant variation for the other time points. The qRT-PCR was performed using two technical replicates, and the reference gene used was the PvIDE (insulin degrading enzyme). RQ = Relative Quantification. (*$p \leq 0.05$; **$p \leq 0.01$)

DISCUSSION

The identification of genes enrolled in *P. vulgaris* immunity is an important step to achieve genetic resistance in cultivated genotypes. The first step to identify those genes is to study the genomic regions linked to the resistance. The ALS10.1 is a major locus involved in ALS resistance, a disease which causes great impact in *P. vulgaris* yield. During the BAC screening, the clone P09O19 was identified using the RGA7 marker, a resistance gene analog (RGA) homolog of a TIR-NB-ARC-LRR gene, mapped on linkage group B10 and linked to an ALS resistance QTL (Lopez et al., 2003). The RGA7 (GI: 28190629) BLAST against the *P. vulgaris* genome identify 10 best matches with the Pv10 (E-value $\leq 1 \times 10^{-136}$), all in the region of TIR-NB-ARC genes, but also present best matches with Pv11 (E-value $\leq 5 \times 10^{-153}$) (data not shown). This result indicated the presence of R genes in both chromosomes, explaining the similarity of the P09O19 sequence with Pv11, instead of Pv10. In fact, the R genes NB-LRR compose one of the largest gene family in plants; and in *P. vulgaris* a NB-LRR cluster involved in *C. lindemuthianum* resistance was identified on linkage group B04 (Ferrier-Cana et al., 2003), as well as RGAs were mapped in all linkage groups with exception of B05, B06 and B09, being linked to resistance QTLs for anthracnose, ALS and *bean golden yellow mosaic virus* (López et al., 2003). Altogether, these results reinforce the great distribution of this gene family on *P. vulgaris* genome and although the primers used here in BAC screening were designed for gene specificity, the small sequence of RGA7 (644 bp) could enabled the identification of any of these NB-LRR genes through PCR. Hence, the P31P02 and P36A15 clones were also mapped on Pv11, since they were identified using chromosome walking based on the P09O19 BAC-end sequences.

In a similar way, the P18I03 was mapped on Pv04 using the PvM22 (GI: 59933969) SSR marker. PvM22 is a homolog to a RNA-binding protein of *Glycine max* (GB: ACF22879; E-value = 9×10^{-58}). RNA-binding proteins play a role in many metabolic pathways, with a post-transcriptional control including mRNA and rRNA processing, RNA export, and RNA stability, and therefore is highly abundant domain in plant proteins (Marchler-Bauer et al., 2011); what could explain the positioning of the BAC clone on

Pv04 instead of Pv10. In addition, PvM22 was also aligned with Pv03 (Oblessuc et al., 2013), reinforcing the distribution of this kind of gene though out the genome. As the P10E22 was identified using the BAC-end sequence from P18I03 during chromosome walking, it was also located in Pv04.

At last, the BAC clone P28L23 was mapped on Pv02 even though it was identified using the P33A23 BAC-end sequence; a clone located on Pv10. The P33A23 BAC-end showed high similarity with retrotransposon sequence (GB: FJ402919.1; E-value = 1 x 10^{-33}); another problem observed during the BAC screening, once retrotransposons are highly conserved sequences spread out in plant genomes (Schlueter et al., 2008), and in addition, others BAC-end sequences with homology to retrotransposons sequences were observed. Indeed, these findings demonstrate the high complexity of the *P. vulgaris* genome and the necessity of deeper studies within plant genomic and functional analysis for this species.

In order to address the genomic and functional studies in *P. vulgaris*, and to improve the knowledge in *P. vulgaris* immunity, the genes observed in the previously identified genomic core region of the major ALS resistance QTL ALS10.1 were studied. The disease control is, in fact, most effective by using genetic resistant varieties, and therefore it is important to understand the mechanisms of host-pathogen interaction and develop tools that could be applied on breeding programs, allowing the development of improved cultivars. Most of the knowledge about plant immunity system came from model species as *Arabidopsis thaliana* and *Medicago truncatula*, for instance. Studying these species researches were able to identify a number of genes and hormone molecules involved in plant immune responses, trigger extremely complex pathways following the co-evolution between host and pathogen (Pieterse et al., 2009; Thomma et al., 2011). Therefore, the *P. vulgaris* ALS10.1 core genes had their orthologs identified in *A. thaliana*. Indeed, it enable the identification of At genes mainly with binding function involved in stress responses, what leads to the inference that the Pv genes orthologs showed same functions.

Therewith, a new TIR-NB-LRR gene cluster was discovered in the *P. vulgaris* genome, indicating an important role of this kind of R gene in the *P. vulgaris* response to *P. griseola*. In fact, studies of *P. vulgaris* genome subdivide the NB-LRR genes in TIR and non-TIR classes (Liu et al., 2012; Garzón et

al., 2013), highlighting the important of the TIR domain in the signal transduction in *P. vulgaris*. The Phvul.010G026300 transcript, coding for a TIR-domain protein, should be one of the genes enrolled in the signal transduction downstream the pathogen recognition, once it was 2.2 fold induced in the incompatible response to *P. griseola* 40 hpi, but not showed differential expression during the compatible response.

By the other hand, the Phvul.010G025700 transcript was induced in all time points during the compatible response in the IAC-UNA and showed no alteration in expression on the CAL-143. This transcript encodes for a TIR-NB-ARC protein and its up-regulation may indicate the recognition of the pathogen Avr by this protein, which should be inhibiting the resistance signal transduction. Interestingly, the Phvul.010G025700 is one of the 39 Pv orthologs of the *A. thaliana* AT5G36930, composing the biggest R gene cluster observed in the ALS 10.1 core. The AT5G36930 RNA was shown to be induced when plants were treated with SA in a NON-EXPRESSOR OF PR1 (NPR1)-dependent way (Balnco et al., 2009); an important regulator of SA defense pathways (Blanco et al., 2005). Therewith, in similarity, the Phvul.010G025700 may play a role in the SA-mediated plant defense. Although it is the closest gene to AT5G36930 (Supplemental Fig. S2), further studies may be performed to verify the response of the others Pv orthologs to ensure there is no other important alteration in these genes expression during the immune response.

In addition to AT5G36930 orthologs, others TIR-NB disease resistance genes were identified in the ALS10.1 core, as well as other genes involved in signal transduction. The second major class of genes observed in ALS10.1 region belongs to the kinase family. Kinases are protein also involved in signal transduction, but through the phosphorylation of target proteins (Endicott et al., 2012). Between the many sub-classes of kinases, the receptor-like kinases (RLK) play an important role in signal perception, including pathogen recognition (Gao et al., 2009). The putative *BIR1-like* gene of *P. vulgaris* may be involved not directly in the *P. griseola* recognition, but as its ortholog in *A. thaliana* (At*BIR1*), the *BIR1-like* should repress the immune response. The AtBIR1 interacts with the BRASSINOSTEROID

INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) inhibiting its association with others RLKs involved in direct recognition of PAMPs (Gao et al., 2009), such as the FLAGELLIN-SENSITIVE-2 (FLS2) and the ELONGATION FACTOR TU RECEPTOR (EFR). Interestingly, the At*BIR1* mutants showed an elevated accumulation of SA and an enhanced cell death (Gao et al., 2009), two common responses to biotrophic pathogens.

The Phvul.010G040900 transcript, encoding for a putative EDR3-like, should also be involved in SA-mediated defense. The *EDR-like* have the *AtEDR3* as its closest ortholog, which encodes for a dynamin-related protein 1E (Tang et al., 2006). The *A. thaliana* mutant for this gene (*edr3*) showed increased disease resistance to powdery mildew (*Golovinomyces cichoracearum*), in a SA-dependent, while *edr3* plants displayed enhanced susceptibility to the necrotrophic fungal pathogen, such as *Botrytis cinerea* (Tang et al., 2006). The repression of the *EDR3-like* of *P. vulgaris* indicates that this gene may present similar function in immunity against *P. griseola*.

The *BIR1-like* and *EDR3-like* were repressed 22 hpi and 40 hpi, respectively, in the incompatible interaction between *P. vulgaris* and *P. griseola*; when it is expected the fungus to be in its biotrophic phase of infection; while the TIR-NB-ARC gene was induced in all time points in the compatible interaction. The time course for the gene expression analysis was chosen based on the *Colletotrichum* development during infection. The *Colletotrichum* is another hemibiotrophic important fungus which infects *P. vulgaris*, and it was shown that at 22 hpi this fungus developed the appressoria to penetrate the plant leaves; 40 hpi it is leaving intracelullarily in its biotrophic phase; and after 60 hpi it passes to the necrotrophic phase causing plasmolysis of the host cells (O'Connell et al., 2012). Although there is no similar study to *Pseudocercospora*, it is possible these two fungi showed similar infection cycle, as they both are Ascomycota fungus, with hemibiotrophic life cycle and infect the shoots of the plants. In addition, the expression analyses of the genes discussed above also indicate a biotrophic life pattern in the first 40 hpi for the *P. griseola*, with the observation of SA-mediated response in the host cell. Therewith,

it is hypothesized that the SA may play an important role in *P. vulgaris* immunity against *P. griseola*, in which the resistance is established during the initial phases of infection.

The *P. vulgaris* is an important crop to human diet and increment in yield is a mainly goal of this plant breeding. As the disease incidence is one of the most important problems in crops, it is important to understand the mechanisms controlling the host-pathogen interaction and develop molecular markers for plant breeding (Dodds and Rathjen, 2010). The present study improves the knowledge about *P. vulgaris* x *P. griseola* interaction, point genes and pathways that may play a role not only in the resistance against this fungus, but also in general immune responses of this plant species. Genes such as *BIR1-like* and *EDR3-like* seems to act in the SA-mediated resistance; while the putative R gene Phvul.010G025700 is an interesting target to markers design in order to facilitate *P. vulgaris* assisted selection against the susceptibility to *P. griseola*. All these finds together are important tools to be applied in plant breeding to resistance, as well as to study the molecular mechanisms of *P. vulgaris* immunity.

ACKNOWLEDGMENTS

This work was supported by FAPESP through grant 2010/51673-7 and PRO received a fellowship from FAPESP 2009/024112 and CAPES 6899/10-2. The authors thank to Dr. Alisson F. Chioratto and Dr. Sérgio A. M. Carbonell for support in the inoculation experiment.

AUTHOR CONTRIBUTION STATEMENT

PRO conceived and conducted the inoculation experiments, designed the primers for the quantitative PCR; also performed the Gene Ontology, Phylogeny and BLAST analyses; in addition drafted the manuscript. CCM gave support for the real time experiment, analysis and discussion. RVS conducted the bioinformatics analysis for the BAC clones assembly. LEAC participated in the initial design of the project, discussions and in the editing of the manuscript. LLBR conceived the project, helped with data

interpretation and editing of the manuscript. MM supported part of the project, helped with the discussion and editing of the manuscript. All authors have read and approved the final version of the manuscript.

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Supplemental material

Supplemental Table S1 – The predicted genes for the ALS10.1 region based on the Phytozome (http://www.phytozome.net/) database.

Pv Transcript	Start position	End position	Pv functional annotation	AGI #	At functional annotation
PHVUL.010G031400.1	4603684	4603941	ATP SYNTHASE DELTA/EPSILON CHAIN	ATCG00470	ATP synthase epsilon chain
PHVUL.010G023800.1	3500201	3505911	Glycosyl hydrolases family 17	AT3G07320	O-Glycosyl hydrolases family 17 protein
PHVUL.010G023900.1	3512060	3520083	Cation transport ATPase	AT1G63440	heavy metal atpase 5
PHVUL.010G024000.1	3531133	3533564	LEUCINE-RICH REPEAT-CONTAINING PROTEIN	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G024100.1	3537155	3540753	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G024200.1	3553137	3556750	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G024300.1	3561290	3563172	no functional annotations	-	TIR NB-ARC AAA ATPase
PHVUL.010G024400.1	3571899	3575512	FK506 BINDING PROTEIN	AT5G48580	FK506- and rapamycin-binding protein 15 kD-2
PHVUL.010G024500.1	3584138	3589883	FK506 BINDING PROTEIN	AT3G25230	rotamase FKBP 1
PHVUL.010G024600.1	3593720	3596179	40S RIBOSOMAL PROTEIN S8	AT5G59240	Ribosomal protein S8e family protein
PHVUL.010G024700.1	3599858	3608266	Glycosyl transferase family 2	AT3G07330	Cellulose-synthase-like C6
PHVUL.010G024800.1	3629864	3633299	CENTROMERE-BINDING PROTEIN 1, CBP-1	AT3G07340	basic helix-loop-helix (bHLH) DNA-binding superfamily
PHVUL.010G024900.1	3702389	3707644	METALLOPROTEASE	AT3G02090	Insulinase (Peptidase family M16) protein
PHVUL.010G025000.1	3709306	3716148	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G025100.1	3728084	3733013	NB-ARC domain	AT1G12280	LRR and NB-ARC domains-containing disease resista
PHVUL.010G025200.1	3751033	3754735	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G025300.1	3762416	3764929	LEUCINE-RICH REPEAT-CONTAINING PROTEIN	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G025400.1	3765682	3769652	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G025500.1	3786002	3790114	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G025600.1	3791239	3791493	*no functional annotations*	-	H+-transporting two-sector ATPase,

					alpha/beta
PHVUL.010G025700.1	3811658	3815819	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G025800.1	3827903	3829434	Protein of unknown function (DUF506)	AT3G07350	Protein of unknown function (DUF506)
PHVUL.010G025900.1	3838549	3840001	mTERF	AT1G79220	Mitochondrial transcription termination factor family
PHVUL.010G026000.1	3851464	3856068	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G026100.1	3860208	3864654	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G026200.1	3875269	3877859	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G026300.1	3892972	3895290	TIR domain	AT5G44510	target of AvrB operation1 (TIR-NBS-LRR class)
PHVUL.010G026400.1	3896814	3905599	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G026500.1	3909177	3909987	*no functional annotations*	-	-
PHVUL.010G026600.1	3911568	3914942	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G026700.1	3924009	3927700	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G026800.1	3936918	3938204	*no functional annotations*	-	TIR NB-ARC AAA ATPase
PHVUL.010G026900.1	3938344	3940623	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G027000.1	3960244	3960603	*no functional annotations*	-	Leucine-rich repeat
PHVUL.010G027100.1	3961261	3963877	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G027200.1	4002517	4006320	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G027300.1	4019402	4023362	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G027400.1	4030289	4031111	*no functional annotations*	-	TIR NB-ARC Immunoglobulin/major histocompatibil
PHVUL.010G027500.1	4037819	4041178	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G027600.1	4041283	4043553	*no functional annotations*	-	-
PHVUL.010G027700.1	4069147	4073022	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G027800.1	4073759	4078412	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G027900.1	4080585	4084830	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)

PHVUL.010G028000.1	4090392	4094611	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028100.1	4103114	4106897	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028200.1	4120276	4127007	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028300.1	4139870	4143592	Protein kinase domain	AT1G66980	suppressor of npr1-1 constitutive 4 (receptor like-kinase)
PHVUL.010G028400.1	4150952	4152458	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028500.1	4155918	4158626	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028600.1	4161096	4163176	Receptor protein kinase	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028700.1	4183725	4187624	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028800.1	4194497	4198156	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G028900.1	4245377	4248629	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G029000.1	4249527	4253271	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G029200.1	4286913	4288227	*no functional annotations*	-	TIR NB-ARC AAA ATPase
PHVUL.010G029100.1	4280101	4290566	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G029300.1	4294164	4296012	*no functional annotations*	-	TIR NB-ARC AAA ATPase
PHVUL.010G029400.1	4328553	4334491	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G029500.1	4354588	4356195	ARGININE/SERINE-RICH SPLICING FACTOR	-	Nucleotide-binding, alpha-beta plait
PHVUL.010G029600.1	4365771	4369561	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G029700.1	4375277	4379568	NB-ARC domain	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G029800.1	4380448	4382284	NB-ARC domain	AT5G48770	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G029900.1	4383724	4394180	Chaperone-dependent E3 ubiquitin protein ligase	AT3G07370	carboxyl terminus of HSC70-interacting protein
PHVUL.010G030000.1	4396430	4398061	SUGAR TRANSPORTER	AT1G11260	sugar transporter 1
PHVUL.010G030100.1	4404523	4406792	Protein kinase domain	AT3G25250	AGC (cAMP-dependent, cGMP-dependent and protein
PHVUL.010G030200.1	4414611	4418394	DNA-DIRECTED RNA POLYMERASE II	AT5G09920	RNA polymerase II, Rpb4, core protein

PHVUL.010G030300.1	4419303	4420849	SUGAR TRANSPORTER	AT1G11260	sugar transporter 1
PHVUL.010G030400.1	4424099	4425257	*no functional annotations*	AT1G64260	MuDR family transposase
PHVUL.010G030500.1	4462414	4463696	Protein of unknown function (DUF568)	AT4G12980	Auxin-responsive family protein
PHVUL.010G030600.1	4484154	4497886	ABC TRANSPORTER ATP-BINDING PROTEIN-RELATED	AT3G07400	lipase class 3 family protein
PHVUL.010G030700.1	4499041	4501909	Exocyst complex subunit Sec15-like	AT3G56640	exocyst complex component sec15A
PHVUL.010G030800.1	4504448	4508679	tRNA synthetases class II	AT3G07420	asparaginyl-tRNA synthetase 2
PHVUL.010G030900.1	4509863	4511644	*no functional annotations*	AT3G53440	Homeodomain-like superfamily protein
PHVUL.010G031000.1	4520620	4521637	*no functional annotations*	AT4G12970	stomagen
PHVUL.010G031100.1	4549594	4551721	MazG nucleotide pyrophosphohydrolase domain	AT3G25400	-
PHVUL.010G031200.1	4574988	4578405	*no functional annotations*	AT3G07440	-
PHVUL.010G031300.1	4592006	4595055	*no functional annotations*	AT5G48500	-
PHVUL.010G031500.1	4619574	4628913	Ca2+-binding actin-bundling protein (fimbrin/plastin), EF-Hand protein superfamily	AT5G48460	Actin binding Calponin homology (CH) domain-conta
PHVUL.010G031600.1	4645873	4652231	*no functional annotations*	AT3G48770	DNA binding;ATP binding
PHVUL.010G031700.1	4662159	4671779	GAMMA TUBULIN COMPLEX PROTEIN	AT5G17410	Spc97 / Spc98 family of spindle pole body (SBP) co
PHVUL.010G031800.1	4679721	4686085	*no functional annotations*	AT3G48770	DNA binding;ATP binding
PHVUL.010G031900.1	4689083	4691820	Protein kinase domain	AT5G60900	receptor-like protein kinase 1
PHVUL.010G032000.1	4702117	4704615	Protein kinase domain	AT5G60900	receptor-like protein kinase 1
PHVUL.010G032100.1	4708463	4712331	40S RIBOSOMAL PROTEIN S6	AT5G10360	Ribosomal protein S6e
PHVUL.010G032200.1	4719501	4725242	SERINE PROTEASE FAMILY S10 SERINE CARBOXYPEPTIDASE	AT4G12910	serine carboxypeptidase-like 20
PHVUL.010G032300.1	4727367	4734461	PLAT/LH2 domain	AT3G22400	PLAT/LH2 domain-containing lipoxygenase family
PHVUL.010G032400.1	4752514	4761768	*no functional annotations*	AT3G48770	DNA binding;ATP binding
PHVUL.010G032500.1	4781886	4784027	hAT family dimerisation domain	AT5G33406	hAT dimerisation domain-containing protein / transpos
PHVUL.010G032600.1	4785760	4788667	FAR1 DNA-binding domain	AT2G43280	Far-red impaired responsive (FAR1) family protein
PHVUL.010G032700.1	4790248	4803162	PROTEIN TRANSPORT PROTEIN SEC23	AT2G21630	Sec23/Sec24 protein transport family protein
PHVUL.010G032800.1	4809578	4809961	*no functional annotations*	AT5G17590	Putative membrane lipoprotein
PHVUL.010G032900.1	4849961	4857055	NAD dependent epimerase/dehydratase family	AT4G31530	NAD(P)-binding Rossmann-fold superfamily protein

PHVUL.010G033000.1	4865782	4873066	ANKYRIN REPEAT-CONTAINING	AT3G18670	Ankyrin repeat family protein
PHVUL.010G033100.1	4884563	4888968	ANKYRIN REPEAT-CONTAINING	AT3G18670	Ankyrin repeat family protein
PHVUL.010G033200.1	4896774	4897595	40S RIBOSOMAL PROTEIN S17	AT2G05220	Ribosomal S17 family protein
PHVUL.010G033300.1	4910725	4911375	40S RIBOSOMAL PROTEIN S17	AT2G05220	Ribosomal S17 family protein
PHVUL.010G033400.1	4913572	4916446	phospholipase C	AT3G03530	non-specific phospholipase C4
PHVUL.010G033500.1	4933602	4955706	Enoyl-CoA hydratase/isomerase family	AT5G65940	beta-hydroxyisobutyryl-CoA hydrolase 1
PHVUL.010G033600.1	4967279	4967995	Yippee putative zinc-binding protein	AT4G27745	Yippee family putative zinc-binding protein
PHVUL.010G033700.1	4974258	4976305	OHCU decarboxylase	AT5G58220	transthyretin-like protein
PHVUL.010G033800.1	4976870	4979228	Protein kinase domain	AT1G34420	leucine-rich repeat transmembrane protein kinase
PHVUL.010G033900.1	4982518	4983132	*no functional annotations*	AT1G34380	5'-3' exonuclease family protein
PHVUL.010G034000.1	4990314	4993454	URICASE AND TRANSTHYRETIN-RELATED	AT5G58220	transthyretin-like protein
PHVUL.010G034100.1	5003316	5004487	DNA POLYMERASE I	AT1G34380	5'-3' exonuclease family protein
PHVUL.010G034200.1	5010528	5013147	COPPER TRANSPORT PROTEIN ATOX1-RELATED	AT1G29000	Heavy metal transport/detoxification superfamily
PHVUL.010G034300.1	5020736	5022034	COPPER TRANSPORT PROTEIN ATOX1-RELATED	AT1G29000	Heavy metal transport/detoxification superfamily
PHVUL.010G034400.1	5038474	5044438	Mlo family	AT4G02600	Seven transmembrane MLO family protein
PHVUL.010G034500.1	5053626	5053970	*no functional annotations*	-	-
PHVUL.010G034700.1	5098792	5099784	*no functional annotations*	-	-
PHVUL.010G034600.1	5093161	5103616	CHROMOBOX PROTEIN	AT5G17690	like heterochromatin protein (LHP1)
PHVUL.010G034900.1	5133693	5134582	SNARE associated Golgi protein	AT1G12450	SNARE associated Golgi protein family
PHVUL.010G035000.1	5134677	5135306	*no functional annotations*	AT4G12000	SNARE associated Golgi protein family
PHVUL.010G034800.1	5132712	5165088	*no functional annotations*	AT3G03560	-
PHVUL.010G035100.1	5181654	5187432	MAINTENANCE OF KILLER 16 (MAK16) PROTEIN-RELATED	AT1G23280	MAK16 protein-related
PHVUL.010G035200.1	5211022	5213130	AP2 domain	AT4G27950	cytokinin response factor 4
PHVUL.010G035300.1	5221593	5223130	Stress up-regulated Nod 19	AT5G61820	-
PHVUL.010G035400.1	5230315	5231880	Stress up-regulated Nod 19	AT5G61820	-
PHVUL.010G035500.1	5269519	5270952	*no functional annotations*	-	-
PHVUL.010G035600.1	5273631	5275540	PPR repeat	AT1G06150	basic helix-loop-helix (bHLH) DNA-binding

PHVUL.010G035700.1	5279680	5281244	Stress up-regulated Nod 19	AT5G61820	-
PHVUL.010G035800.1	5285366	5288993	Protein kinase domain	AT5G48380	BAK1-interacting receptor-like kinase 1
PHVUL.010G035900.1	5304495	5309692	Frigida-like protein	AT5G48385	FRIGIDA-like protein
PHVUL.010G036000.1	5322226	5328430	Frigida-like protein	AT5G48385	FRIGIDA-like protein
PHVUL.010G036100.1	5348634	5351369	Iron/ascorbate family oxidoreductases	AT3G13610	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase
PHVUL.010G036200.1	5360493	5362875	hAT family dimerisation domain	AT1G79740	hAT transposon superfamily
PHVUL.010G036300.1	5367851	5371049	AUTOPHAGOCYTOSIS PROTEIN AUT1-RELATED	AT3G07525	autophagocytosis-associated family protein
PHVUL.010G036400.1	5371694	5377616	FtsJ-like methyltransferase	AT3G25470	bacterial hemolysin-related
PHVUL.010G036500.1	5378824	5388076	ALPHA/BETA HYDROLASE RELATED	AT4G12830	alpha/beta-Hydrolases superfamily protein
PHVUL.010G036600.1	5390515	5393522	Glycosyl hydrolases family 28	AT5G17200	Pectin lyase-like superfamily protein
PHVUL.010G036700.1	5397915	5400127	POLY-A BINDING PROTEIN 2	-	Nucleotide-binding, alpha-beta plait
PHVUL.010G036800.1	5415483	5417072	UDP-glucoronosyl and UDP-glucosyl transferase	AT2G22590	UDP-Glycosyltransferase superfamily protein
PHVUL.010G036900.1	5430175	5431625	UDP-glucoronosyl and UDP-glucosyl transferase	AT2G22590	UDP-Glycosyltransferase superfamily protein
PHVUL.010G037000.1	5437642	5439097	UDP-glucoronosyl and UDP-glucosyl transferase	AT2G22590	UDP-Glycosyltransferase superfamily protein
PHVUL.010G037100.1	5442461	5444869	Glycosyl hydrolases family 28	AT3G15720	Pectin lyase-like superfamily protein
PHVUL.010G037200.1	5457216	5463011	Glycosyl hydrolases family 28	AT3G15720	Pectin lyase-like superfamily protein
PHVUL.010G037300.1	5466888	5471446	Glycosyl hydrolases family 28	AT3G15720	Pectin lyase-like superfamily protein
PHVUL.010G037400.1	5478771	5482726	Glycosyl hydrolases family 28	AT3G15720	Pectin lyase-like superfamily protein
PHVUL.010G037500.1	5489427	5489642	*no functional annotations*	-	-
PHVUL.010G037600.1	5490952	5492277	UDP-glucoronosyl and UDP-glucosyl transferase	AT2G22590	UDP-Glycosyltransferase superfamily protein
PHVUL.010G037700.1	5499042	5502715	Glycosyl hydrolases family 28	AT5G17200	Pectin lyase-like superfamily protein
PHVUL.010G037800.1	5505184	5512123	Glycosyl hydrolases family 28	AT5G17200	Pectin lyase-like superfamily protein
PHVUL.010G037900.1	5527825	5532170	Glycosyl hydrolases family 28	AT3G15720	Pectin lyase-like superfamily protein
PHVUL.010G038000.1	5562682	5565487	Glycosyl hydrolases family 28	AT3G15720	Pectin lyase-like superfamily protein
PHVUL.010G038100.1	5594993	5599441	Glycosyl hydrolases family 28	AT3G15720	Pectin lyase-like superfamily protein
PHVUL.010G038200.1	5608626	5612307	Zinc-binding dehydrogenase	AT5G16990	Zinc-binding dehydrogenase family protein
PHVUL.010G038300.1	5637297	5649562	Protein of unknown function (DUF3550/UPF0682)	AT3G03570	Protein of unknown function (DUF3550/UPF0682)

PHVUL.010G038400.1	5651103	5657718	RNA synthetases class I	AT5G64050	glutamate tRNA synthetase
PHVUL.010G038500.1	5666476	5670187	*no functional annotations*	AT5G17610	-
PHVUL.010G038600.1	5672162	5676406	*no functional annotations*	AT1G64850	Calcium-binding EF hand family protein
PHVUL.010G038700.1	5682847	5686192	*no functional annotations*	AT1G64850	Calcium-binding EF hand family protein
PHVUL.010G038800.1	5687068	5689841	Zinc-binding dehydrogenase	AT5G16970	alkenal reductase
PHVUL.010G038900.1	5703841	5704101	Glycosyl hydrolases family 28	AT2G43870	Pectin lyase-like superfamily protein
PHVUL.010G039000.1	5711128	5714114	Glycosyl hydrolases family 28	AT3G59850	Pectin lyase-like superfamily protein
PHVUL.010G039100.1	5720600	5725519	Zinc-binding dehydrogenase	AT1G26320	Zinc-binding dehydrogenase family protein
PHVUL.010G039200.1	5747134	5751690	Zinc-binding dehydrogenase	AT1G26320	Zinc-binding dehydrogenase family protein
PHVUL.010G039300.1	5766668	5772081	Zinc-binding dehydrogenase	AT3G03080	Zinc-binding dehydrogenase family protein
PHVUL.010G039400.1	5775600	5776887	*no functional annotations*	-	-
PHVUL.010G039500.1	5777341	5777799	*no functional annotations*	-	-
PHVUL.010G039600.1	5780897	5785503	Nodulin-like	AT5G45275	Major facilitator superfamily protein
PHVUL.010G039700.1	5789041	5817755	*no functional annotations*	-	-
PHVUL.010G039800.1	5819748	5824669	Protein of unknown function (DUF1005)	AT5G17640	Protein of unknown function (DUF1005)
PHVUL.010G039900.1	5836337	5838237	*no functional annotations*	AT2G37920	copper ion transmembrane transporters
PHVUL.010G040000.1	5840583	5844859	*no functional annotations*	-	-
PHVUL.010G040100.1	5845750	5849748	Putative methyltransferase	AT5G17660	tRNA (guanine-N-7) methyltransferase
PHVUL.010G040200.1	5850105	5854169	NUCLEAR PROTEIN E3-3	AT2G44525	Protein of unknown function (DUF498/DUF598)
PHVUL.010G040300.1	5859119	5870447	RIBOSE-PHOSPHATE PYROPHOSPHOKINASE	AT2G44530	Phosphoribosyltransferase family protein
PHVUL.010G040400.1	5873509	5885268	ABC transporter	AT3G60160	multidrug resistance-associated protein 9
PHVUL.010G040500.1	5958326	5969543	STEROL DESATURASE	AT1G02205	Fatty acid hydroxylase superfamily
PHVUL.010G040600.1	6001122	6011332	STEROL DESATURASE	AT1G02205	Fatty acid hydroxylase superfamily
PHVUL.010G040700.1	6018592	6031430	Rgp1	AT1G50120	-
PHVUL.010G040800.1	6062628	6067816	Adenylate kinase	AT5G26667.3	P-loop containing nucleoside triphosphate hydrolases
PHVUL.010G040900.1	6074155	6081593	DYNAMIN	AT3G60190	DYNAMIN-like 1E
PHVUL.010G041100.1	6145753	6146228	GAG-POL-RELATED RETROTRANSPOSON	-	hypothetical protein

PHVUL.010G041000.1	6098638	6153443	MALIC ENZYME-RELATED	AT2G13560	NAD-dependent malic enzyme 1
PHVUL.010G041200.1	6174673	6176354	*no functional annotations*	-	hypothetical protein
PHVUL.010G041300.1	6180921	6181620	*no functional annotations*	-	-
PHVUL.010G041400.1	6210351	6210915	*no functional annotations*	-	-
PHVUL.010G041500.1	6262945	6263559	Plant invertase/pectin methylesterase inhibitor	AT5G46940	Plant invertase/pectin methylesterase inhibitor super
PHVUL.010G041600.1	6266532	6272276	rotein of unknown function (DUF3769)	AT2G44640	-
PHVUL.010G041700.1	6274189	6277504	GROES CHAPERONIN	AT2G44650	chloroplast chaperonin 10
PHVUL.010G041800.1	6288833	6289909	Predicted E3 ubiquitin ligase	AT3G60220	TOXICOS EN LEVADURA 4
PHVUL.010G041900.1	6319978	6323392	*no functional annotations*	AT1G02180	ferredoxin-related
PHVUL.010G042000.1	6327107	6331155	FE(II)/ ASCORBATE OXIDASE SUPERFAMILY	AT1G06650	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenas
PHVUL.010G042100.1	6347983	6354595	VACUOLAR MEMBRANE PROTEIN RELATED	AT2G05170	vacuolar protein sorting 11
PHVUL.010G042200.1	6363284	6364941	GLUCOSYL/GLUCURONOSYL TRANSFERASES	AT4G01070	UDP-Glycosyltransferase superfamily protein
PHVUL.010G042300.1	6372468	6373889	UDP-glucoronosyl and UDP-glucosyl transferase	AT4G01070	UDP-Glycosyltransferase superfamily protein
PHVUL.010G042400.1	6387428	6388846	UDP-glucoronosyl and UDP-glucosyl transferase	AT4G01070	UDP-Glycosyltransferase superfamily protein
PHVUL.010G042500.1	6399499	6400920	UDP-glucoronosyl and UDP-glucosyl transferase	AT4G01070	UDP-Glycosyltransferase superfamily protein
PHVUL.010G042600.1	6418598	6420240	UDP-glucoronosyl and UDP-glucosyl transferase	AT4G01070	UDP-Glycosyltransferase superfamily protein
PHVUL.010G042700.1	6441162	6445367	Helix-loop-helix DNA-binding domain	AT2G22760	basic helix-loop-helix (bHLH) DNA-binding
PHVUL.010G042800.1	6447693	6456925	Cytosine specific DNA methyltransferase replication foci domain	AT5G55390	ENHANCED DOWNY MILDEW 2
PHVUL.010G042900.1	6464311	6467074	ATP SYNTHASE DELTA/EPSILON CHAIN	AT5G47030	ATPase, F1 complex, delta/epsilon subunit
PHVUL.010G043000.1	6522962	6523849	Protein of unknown function (DUF581)	AT5G47060	Protein of unknown function (DUF581)
PHVUL.010G043100.1	6535734	6540624	Protein kinase domain	AT5G47070	Protein kinase superfamily protein
PHVUL.010G043200.1	6556700	6560256	Helix-loop-helix DNA-binding domain	AT2G22750	basic helix-loop-helix (bHLH) DNA-binding
PHVUL.010G043300.1	6575815	6578663	Helix-loop-helix DNA-binding domain	AT2G22750	basic helix-loop-helix (bHLH) DNA-binding
PHVUL.010G043400.1	6594213	6596171	Helix-loop-helix DNA-binding domain	AT2G22750	basic helix-loop-helix (bHLH) DNA-binding
PHVUL.010G043500.1	6603617	6605812	60S RIBOSOMAL PROTEIN L10	AT1G26910	Ribosomal protein L16p/L10e family protein

PHVUL.010G043600.1	6611080	6613931	Protein kinase domain	AT2G32800	protein kinase family protein
PHVUL.010G043700.1	6616402	6627039	RANSLATION INITIATION FACTOR 4 GAMMA	AT3G60240	eukaryotic translation initiation factor 4G
PHVUL.010G043800.1	6629129	6631112	alpha/beta hydrolase fold	AT3G50440	methyl esterase 10
PHVUL.010G043900.1	6632573	6636796	Cytochrome P450	AT5G24910	cytochrome P450, family 714, subfamily A, polypepti
PHVUL.010G044000.1	6641085	6647335	PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE	AT4G17080	Histone H3 K4-specific methyltransferase SET7/9
PHVUL.010G044100.1	6649974	6651725	60S RIBOSOMAL PROTEIN L37A	AT3G10950	Zinc-binding ribosomal protein family protein
PHVUL.010G044200.1	6661971	6662699	hAT family dimerisation domain	AT5G33406	hAT dimerisation domain-containing protein / transpos
PHVUL.010G044300.1	6665968	6676655	ACTIN	AT1G18450	actin-related protein 4
PHVUL.010G044400.1	6699410	6703979	PENTATRICOPEPTIDE REPEAT-CONTAINING PROTEIN	AT1G28710	Nucleotide-diphospho-sugar transferase family
PHVUL.010G044500.1	6730634	6731138	PENTATRICOPEPTIDE REPEAT-CONTAINING PROTEIN	AT1G28710	Nucleotide-diphospho-sugar transferase family
PHVUL.010G044600.1	6741257	6747296	PENTATRICOPEPTIDE REPEAT-CONTAINING PROTEIN	AT1G28710	Nucleotide-diphospho-sugar transferase family
PHVUL.010G044700.1	6752985	6758675	RNA-BINDING PROTEIN	AT2G44710	RNA-binding (RRM/RBD/RNP motifs) family
PHVUL.010G044800.1	6762848	6765496	Cyclin	AT2G44740	cyclin p4;1
PHVUL.010G044900.1	6774767	6775817	*no functional annotations*	-	-
PHVUL.010G045100.1	6782945	6784306	*no functional annotations*	-	-
PHVUL.010G045000.1	6775858	6785502	*no functional annotations*	-	-
PHVUL.010G045200.1	6824495	6827168	*no functional annotations*	-	-
PHVUL.010G045300.1	6874457	6877771	ENGULFMENT AND CELL MOTILITY	AT2G44770	ELMO/CED-12 family protein
PHVUL.010G045400.1	6884394	6886415	Nucleoside diphosphate kinase	AT4G09320	Nucleoside diphosphate kinase family
PHVUL.010G045500.1	_	6902401	PPR repeat	AT1G02150	Tetratricopeptide repeat (TPR)-like superfamily
PHVUL.010G045600.1	6903806	6925958	GLYCOSYLTRANSFERASE	AT1G02145.3	homolog of asparagine-linked glycosylation 12
PHVUL.010G045700.1	6933195	6935672	PPR repeat	AT1G71460	Pentatricopeptide repeat (PPR-like) superfamily
PHVUL.010G045800.1	6939602	6942032	PPR repeat	AT1G08070	Tetratricopeptide repeat (TPR)-like superfamily
PHVUL.010G045900.1	6943458	6948353	PAX6 NEIGHBOR PROTEIN (PAXNEB)	AT3G11220	Paxneb protein-related
PHVUL.010G046000.1	6949244	6953123	PPR repeat	AT1G15510	Tetratricopeptide repeat (TPR)-like superfamily
PHVUL.010G046100.1	6955267	6961294	PAX6 NEIGHBOR PROTEIN (PAXNEB)	AT3G11220	Paxneb protein-related

PHVUL.010G046200.1	6974805	6978034	PPR	AT1G15510	Tetratricopeptide repeat (TPR)-like superfamily
PHVUL.010G046300.1	6982915	6989703	tRNA synthetases class I	AT3G04600	Nucleotidylyl transferase superfamily protein
PHVUL.010G046400.1	6993568	7009345	tRNA synthetases class II	AT3G13490	Lysyl-tRNA synthetase, class II
PHVUL.010G046500.1	7105698	7106642	*no functional annotations*	-	-
PHVUL.010G046600.1	7146537	7151772	50S RIBOSOMAL PROTEIN L4	AT2G20060	Ribosomal protein L4/L1 family
PHVUL.010G046700.1	7156981	7161474	Domain of unknown function (DUF3598)	AT2G44760	Domain of unknown function (DUF3598)
PHVUL.010G046800.1	7162410	7172384	Protein kinase domain	AT4G16970	Protein kinase superfamily protein
PHVUL.010G046900.1	7174462	7178188	Mago nashi protein	AT1G02140	mago nashi family protein
PHVUL.010G047000.1	7215459	7224703	Patatin-like phospholipase	AT2G26560	phospholipase A 2A
PHVUL.010G047100.1	7242032	7243138	UBIQUITIN-PROTEIN LIGASE	AT1G55860	ubiquitin-protein ligase 1
PHVUL.010G047200.1	7259424	7260140	*no functional annotations*	-	Transcriptional factor B3
PHVUL.010G047300.1	7279187	7281474	TRANSFORMER-2-RELATED	-	Nucleotide-binding, alpha-beta plait
PHVUL.010G047400.1	7314520	7316487	Patatin-like phospholipase	AT2G26560	phospholipase A 2A
PHVUL.010G047500.1	7321338	7321775	*no functional annotations*	-	Non-LTR retroelement reverse transcriptase
PHVUL.010G047600.1	7328315	7328638	*no functional annotations*	-	hypothetical protein
PHVUL.010G047700.1	7343088	7344843	Patatin-like phospholipase	AT2G26560	phospholipase A 2A
PHVUL.010G047800.1	7372206	7373645	Patatin-like phospholipase	AT2G26560	phospholipase A 2A
PHVUL.010G047900.1	7424413	7427704	Patatin-like phospholipase	AT5G43590	Acyl transferase/acyl hydrolase/lysophospholipase
PHVUL.010G048000.1	7462230	7465929	Patatin-like phospholipase	AT2G26560	phospholipase A 2A
PHVUL.010G048100.1	7495886	7499343	50S RIBOSOMAL PROTEIN L4	AT1G07320	ribosomal protein L4
PHVUL.010G048200.1	7505629	7509703	Ras family	AT1G02130	RAS 5
PHVUL.010G048300.1	7521054	7523372	PPR repeat	AT4G32430	Pentatricopeptide repeat (PPR) superfamily
PHVUL.010G048400.1	7529054	7532473	WRC	AT2G45480	growth-regulating factor 9
PHVUL.010G048500.1	7585260	7587935	NADH-UBIQUINONE OXIDOREDUCTASE	AT5G18800	Cox19-like CHCH family protein
PHVUL.010G048600.1	7600494	7604155	Protein kinase domain	AT2G45490	ataurora3
PHVUL.010G048700.1	7607676	7610405	Cytochrome P450	AT2G45510	cytochrome P450, family 704, subfamily A,
PHVUL.010G048800.1	7623367	7627672	Cytochrome P450	AT2G45510	cytochrome P450, family 704, subfamily A,
PHVUL.010G048900.1	7647183	7655782	BEIGE/BEACH-RELATED	AT2G45540	WD-40 repeat family protein / beige-

					related
PHVUL.010G049000.1	7693208	7700576	*no functional annotations*	AT2G45540	WD-40 repeat family protein / beige-related
PHVUL.010G049100.1	7710067	7713646	Domain of unknown function (DUF947)	AT1G12650	-
PHVUL.010G049200.1	7746397	7747593	Plastocyanin-like domain	AT2G44790	uclacyanin 2
PHVUL.010G049300.1	7781808	7784979	HYALURONIC ACID-BINDING PROTEIN 4	AT4G17520	Hyaluronan / mRNA binding family
PHVUL.010G049400.1	7792266	7802399	Glycyl-tRNA synthetase beta subunit	AT3G48110	glycine-tRNA ligases
PHVUL.010G049500.1	7812299	7813159	*no functional annotations*	-	-
PHVUL.010G049600.1	7813319	7826191	Glycyl-tRNA synthetase beta subunit	AT3G48110	glycine-tRNA ligases
PHVUL.010G049700.1	7841761	7848251	E3 ubiquitin-protein ligase RNF25	AT3G60300	RWD domain-containing protein
PHVUL.010G049800.1	7849409	7850671	Lipase (class 3)	AT2G44810	alpha/beta-Hydrolases superfamily protein
PHVUL.010G049900.1	7896187	7907787	GRAM domain	AT1G02120	GRAM domain family protein
PHVUL.010G050000.1	7924816	7929232	Protein kinase domain	AT2G44830	Protein kinase superfamily protein
PHVUL.010G050100.1	7932455	7939248	Protein of unknown function (DUF632)	AT3G60320	Protein of unknown function (DUF630 and DUF632)
PHVUL.010G050200.1	7967523	7969516	*no functional annotations*	-	Zinc finger, U1-type
PHVUL.010G050300.1	7985693	7986424	*no functional annotations*	-	-
PHVUL.010G050400.1	8010126	8010506	GDSL-like Lipase/Acylhydrolase	AT5G33370	GDSL-like Lipase/Acylhydrolase superfamily protein
PHVUL.010G050500.1	8020695	8021348	AP2 domain	AT2G44840	ethylene-responsive element binding factor 13
PHVUL.010G050600.1	8042080	8042795	AP2 domain	AT2G44840	ethylene-responsive element binding factor 13
PHVUL.010G050700.1	8078193	8078828	AP2 domain	AT2G44840	ethylene-responsive element binding factor 13
PHVUL.010G050800.1	8082893	8083593	AP2 domain	AT2G44840	ethylene-responsive element binding factor 13
PHVUL.010G050900.1	8103199	8111936	CATION-TRANSPORTING ATPASE	AT5G62670	H(+)-ATPase 11
PHVUL.010G051000.1	8112692	8118234	Protein prenyltransferase alpha subunit repeat	AT1G10095	Protein prenylyltransferase superfamily protein
PHVUL.010G051100.1	8127789	8132844	Membrane transport protein	AT1G76520	Auxin efflux carrier family protein
PHVUL.010G051200.1	8148079	8154274	Membrane transport protein	AT1G76520	Auxin efflux carrier family protein
PHVUL.010G051300.1	8160870	8162654	Pyridoxal-dependent decarboxylase conserved domain	AT2G20340	Pyridoxal phosphate (PLP)-dependent transferases
PHVUL.010G051400.1	8172978	8173824	SERINE/ARGININE RICH SPLICING FACTOR	-	Nucleotide-binding, alpha-beta plait
PHVUL.010G051500.1	8184680	8190029	Membrane transport protein	AT1G76520	Auxin efflux carrier family protein

PHVUL.010G051600.1	8202425	8203117	*no functional annotations*	AT4G37445	-
PHVUL.010G051700.1	8210591	8211028	BETA 1,3-GALACTOSYLTRANSFERASE-RELATED	AT4G32105	Beta-1,3-N-Acetylglucosaminyltransferase family
PHVUL.010G051800.1	8217449	8217811	*no functional annotations*	AT2G35215	-
PHVUL.010G051900.1	8239030	8241339	*no functional annotations*	-	Putative transmembrane protein
PHVUL.010G052000.1	8242300	8246826	FKBP-type peptidyl-prolyl cis-trans isomerase	AT3G60370	FKBP-like peptidyl-prolyl cis-trans isomerase family
PHVUL.010G052100.1	8248297	8251826	ARP2/3 complex 16 kDa subunit (p16-Arc)	AT4G01710	ARP2/3 complex 16 kDa subunit (p16-Arc)
PHVUL.010G052200.1	8254147	8257046	HOMEOBOX PROTEIN	AT4G16780	homeobox protein 2
PHVUL.010G052300.1	8294380	8297353	Oxygen evolving enhancer protein 3 (PsbQ)	AT4G05180	photosystem II subunit Q-2
PHVUL.010G052400.1	8300017	8301758	Plant protein of unknown function	AT2G28580	Plant protein of unknown function (DUF247)
PHVUL.010G052500.1	8306653	8308038	Plant protein of unknown function	AT2G28580	Plant protein of unknown function (DUF247)
PHVUL.010G052600.1	8309408	8310062	Zinc finger, C3HC4 type (RING finger)	AT3G15070	RING/U-box superfamily protein
PHVUL.010G052700.1	8322513	8325670	Plant protein of unknown function	AT2G44930	Plant protein of unknown function (DUF247)
PHVUL.010G052800.1	8335953	8337338	*no functional annotations*	-	TGF-beta receptor, type I/II extracellular region
PHVUL.010G052900.1	8344966	8348182	Plant protein of unknown function	AT4G31980	-
PHVUL.010G053000.1	8354485	8355819	Plant protein of unknown function	AT2G28580	Plant protein of unknown function (DUF247)
PHVUL.010G053100.1	8356026	8359179	Cytochrome P450	AT1G11680	CYTOCHROME P450 51G1
PHVUL.010G053200.1	8369275	8371339	Myb-like DNA-binding domain	AT3G60460	myb-like HTH transcriptional regulator family
PHVUL.010G053300.1	8373159	8375864	Phosphoglycerate mutase family	AT3G60450	Phosphoglycerate mutase family protein
PHVUL.010G053400.1	8376796	8378368	SUGAR TRANSPORTER	AT1G11260	sugar transporter 1
PHVUL.010G053500.1	8409491	8411309	UDP-glucoronosyl and UDP-glucosyl transferase	AT4G01070	UDP-Glycosyltransferase superfamily protein
PHVUL.010G053600.1	8412714	8430627	CNH DOMAIN CONTAINING	AT1G22860	Vacuolar sorting protein 39
PHVUL.010G053700.1	8452999	8454730	UDP-glucoronosyl and UDP-glucosyl transferase	AT4G01070	UDP-Glycosyltransferase superfamily protein
PHVUL.010G053800.1	8471813	8473044	DNAJ/HSP40	AT5G22060	DNAJ homologue 2
PHVUL.010G053900.1	8473049	8477136	DNAJ/HSP40	AT3G44110	DNAJ homologue 3
PHVUL.010G054000.1	8503366	8504597	AP2 domain	AT2G44940	Integrase-type DNA-binding superfamily protein

PHVUL.010G054100.1	8534006	8545951	Lecithin:cholesterol acyltransferase	AT2G44970	alpha/beta-Hydrolases superfamily protein
PHVUL.010G054200.1	8568461	8574233	Leucine Rich Repeat	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G054300.1	8595794	8596857	MTH538 TIR-like domain (DUF1863)	AT5G36930	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G054400.1	8600367	8602193	NB-ARC domain	AT4G16950	Disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G054500.1	8613084	8616535	NB-ARC domain	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G054600.1	8624322	8626384	NB-ARC domain	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G054700.1	8636338	8640216	NB-ARC domain	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G054800.1	8664723	8668986	TIRAP	AT5G36930	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G054900.1	8676960	8680299	*no functional annotations*	AT3G18960	AP2/B3-like transcriptional factor family
PHVUL.010G055000.1	8682451	8683176	*no functional annotations*	-	Transcriptional factor B3
PHVUL.010G055100.1	8692617	8696318	NB-ARC domain	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G055200.1	8782595	8788498	NB-ARC domain	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G055300.1	8848784	8851898	TIR domain	AT1G72890	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G055400.1	8867168	8869755	NB-ARC domain	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G055500.1	8879057	8883704	NB-ARC domain	AT5G17680	disease resistance protein (TIR-NBS-LRR class)
PHVUL.010G055600.1	8899093	8908102	ENOYL-COA HYDRATASE-RELATED	AT3G60510	ATP-dependent caseinolytic (Clp) protease/crotonase
PHVUL.010G055700.1	8911620	8915538	MALATE DEHYDROGENASE	AT5G43330	malate dehydrogenase
PHVUL.010G055800.1	8936518	8940030	*no functional annotations*	-	-
PHVUL.010G055900.1	8942561	8947857	*no functional annotations*	AT3G15351	-
PHVUL.010G056000.1	8995761	8998939	SBP domain	AT1G02065	squamosa promoter binding protein-like 8

Supplemental Table S2 – Gene Ontology (GO) terms assigned to the *Arabidopsis thaliana* genes, orthologs to the *P. vulgaris* predicted genes from ALS10.1, using AgriGO Singular Enrichment Analysis.

GO #	Term type[a]	GO Term	AGI #
GO:0043232	C	intracellular non-membrane-bounded organelle	// AT5G10360 // AT4G01710 // AT1G07320 // AT2G20060 // AT5G17690 // AT1G26910 // AT5G17410 // AT2G45490 // AT2G44770 // AT3G10950 // AT5G59240 // AT2G05220
GO:0043228	C	non-membrane-bounded organelle	// AT5G10360 // AT4G01710 // AT1G07320 // AT2G20060 // AT5G17690 // AT1G26910 // AT5G17410 // AT2G45490 // AT2G44770 // AT3G10950 // AT5G59240 // AT2G05220
GO:0044422	C	organelle part	// AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT1G07320 // AT2G20060 // AT3G02090 // AT5G17410 // AT3G60370 // AT5G47030 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT5G59240 // AT2G44640 // AT4G31530 // AT2G05170 // AT3G10950 // AT2G05220 // AT5G48580
GO:0044446	C	intracellular organelle part	// AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT1G07320 // AT2G20060 // AT3G02090 // AT5G17410 // AT3G60370 // AT5G47030 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT5G59240 // AT2G44640 // AT4G31530 // AT2G05170 // AT3G10950 // AT2G05220 // AT5G48580
GO:0033279	C	ribosomal subunit	// AT5G10360 // AT1G07320 // AT2G20060 // AT5G59240 // AT3G10950 // AT2G05220
GO:0043229	C	intracellular organelle	// AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT3G07330 // AT2G44530 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G25230 // AT2G44770 // AT3G10950 // AT2G22760 // AT1G11680 // AT1G02150 // AT1G11260 // AT2G20060 // AT2G05220 // AT3G60370 // AT5G61820 // AT5G17410 // AT5G59240 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT3G07340 // AT1G02065 // AT5G64050 // AT4G12830 // AT5G47030 // AT3G60460 // AT2G44640 // AT2G05170 // AT1G07320 // AT3G48770 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT1G55860 // AT1G18450 // AT3G07420 // AT5G48580
GO:0043226	C	organelle	// AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT3G07330 // AT2G44530 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G25230 // AT2G44770 // AT3G10950 // AT2G22760 // AT1G11680 // AT1G02150 // AT1G11260 // AT2G20060 // AT2G05220 // AT3G60370 // AT5G61820 // AT5G17410 // AT5G59240 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT3G07340 // AT1G02065 // AT5G64050 // AT4G12830 // AT5G47030 // AT3G60460 // AT2G44640 // AT2G05170 // AT1G07320 // AT3G48770 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT1G55860 // AT1G18450 // AT3G07420 // AT5G48580
GO:0005840	C	ribosome	// AT5G10360 // AT1G07320 // AT2G20060 // AT1G26910 // AT5G59240 // AT3G10950 // AT2G05220
GO:0044424	C	intracellular part	// AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT3G07330 // AT2G44530 // AT3G07420 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G25230 // AT2G44770 // AT3G10950 // AT2G22760 // AT2G26560 // AT1G02150 // ATCG00470 // AT1G11260 // AT2G20060 // AT1G11680 // AT3G60370 // AT5G61820 // AT3G56640 // AT5G59240 //

GO:0005622	C	intracellular	AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT3G07340 // AT1G02065 // AT5G64050 // AT4G12830 // AT5G47030 // AT3G60460 // AT2G44640 // AT2G05170 // AT1G07320 // AT3G48770 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT3G03570 // AT5G17410 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT1G55860 // AT2G05220 // AT1G18450 // AT5G58220 // AT5G48580 // AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT3G07330 // AT2G44530 // AT3G07420 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G25230 // AT2G44770 // AT3G10950 // AT2G22760 // AT2G26560 // AT1G02150 // ATCG00470 // AT1G11260 // AT2G20060 // AT1G11680 // AT3G60370 // AT5G61820 // AT3G56640 // AT5G59240 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT3G07340 // AT1G02065 // AT5G64050 // AT4G12830 // AT5G47030 // AT3G60460 // AT2G44640 // AT2G05170 // AT1G07320 // AT3G48770 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT3G03570 // AT5G17410 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT1G55860 // AT2G05220 // AT1G18450 // AT5G58220 // AT5G48580
GO:0005886	C	plasma membrane	// AT5G58220 // AT5G43330 // AT3G15720 // AT4G02600 // AT5G62670 // AT5G47070 // AT3G60190 // AT3G03530 // AT5G48380 // AT3G03560 // AT5G16990 // AT2G44640
GO:0016020	C	membrane	// AT4G05180 // AT2G44790 // AT1G72890 // AT3G60190 // AT3G02090 // AT3G03560 // AT2G26560 // ATCG00470 // AT1G11260 // AT5G17680 // AT5G48380 // AT5G59240 // AT4G02600 // AT5G48770 // AT5G47030 // AT5G36930 // AT5G62670 // AT5G47070 // AT3G03530 // AT2G44640 // AT2G05170 // AT5G43330 // AT3G15720 // AT1G26910 // AT5G16990 // AT5G58220
GO:0032991	C	macromolecular complex	// AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT1G07320 // AT2G20060 // AT3G02090 // ATCG00470 // AT5G47030 // AT1G26910 // AT1G02140 // AT3G56640 // AT1G55860 // AT3G10950 // AT5G59240 // AT2G05220
GO:0044444	C	cytoplasmic part	// AT5G10360 // AT3G48770 // AT4G05180 // AT3G07330 // AT2G44530 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G10950 // AT1G11680 // AT1G02150 // AT2G20060 // AT2G05220 // AT3G60370 // AT5G61820 // AT5G17410 // AT5G59240 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT3G07340 // AT5G64050 // AT4G12830 // AT5G47030 // AT2G44640 // AT2G05170 // AT1G34380 // AT5G16970 // AT4G09320 // AT1G07320 // AT3G03570 // AT1G26910 // AT3G56640 // AT1G55860 // AT3G07420 // AT5G48580
GO:0043233	C	organelle lumen	// AT5G09920 // AT4G05180 // AT3G60370 // AT1G02140 // AT3G02090 // AT5G48580
GO:0070013	C	intracellular organelle lumen	// AT5G09920 // AT4G05180 // AT3G60370 // AT1G02140 // AT3G02090 // AT5G48580
GO:0031974	C	membrane-enclosed lumen	// AT5G09920 // AT4G05180 // AT3G60370 // AT1G02140 // AT3G02090 // AT5G48580
GO:0044425	C	membrane part	// AT4G05180 // AT5G48770 // AT2G44790 // ATCG00470 // AT1G72890 // AT5G47030 // AT5G36930 // AT5G17680 // AT3G15720 // AT3G02090 // AT5G58220
GO:0044464	C	cell part	// AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT3G07330 // AT5G48380 // AT2G44530 // AT1G72890 // AT3G07420 // AT5G17200 // AT1G28710 // AT2G43870 // AT5G33406 // AT3G59850 // AT3G60190 // AT2G45490 // AT3G25230 // AT2G44770 // AT1G12280 // AT3G03560 // AT3G10950 // AT2G22760 // AT5G33370 // AT5G17610 // AT1G02150 // ATCG00470 // AT3G07320 // AT1G11260 // AT2G20060 // AT5G17590 //

GO:0005623	C	cell	AT2G26560 // AT3G60370 // AT2G44790 // AT4G02600 // AT5G17680 // AT5G61820 // AT3G56640 // AT5G59240 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT5G16990 // AT3G07340 // AT1G02065 // AT1G02180 // AT5G48770 // AT5G17690 // AT5G64050 // AT4G12830 // AT5G47030 // AT5G36930 // AT5G62670 // AT5G47070 // AT3G60460 // AT3G03530 // AT2G44640 // AT4G12970 // AT2G05170 // AT1G07320 // AT3G48770 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT1G11680 // AT5G43330 // AT5G17410 // AT3G03570 // AT3G15720 // AT1G26910 // AT1G02140 // AT3G02090 // AT1G55860 // AT2G05220 // AT1G18450 // AT5G58220 // AT5G48580 // AT5G09920 // AT5G10360 // AT4G01710 // AT4G05180 // AT3G07330 // AT5G48380 // AT2G44530 // AT1G72890 // AT3G07420 // AT5G17200 // AT1G28710 // AT2G43870 // AT5G33406 // AT3G59850 // AT3G60190 // AT2G45490 // AT3G25230 // AT2G44770 // AT1G12280 // AT3G03560 // AT3G10950 // AT2G22760 // AT5G33370 // AT5G17610 // AT1G02150 // ATCG00470 // AT3G07320 // AT1G11260 // AT2G20060 // AT5G17590 // AT2G26560 // AT3G60370 // AT2G44790 // AT4G02600 // AT5G17680 // AT5G61820 // AT3G56640 // AT5G59240 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT5G16990 // AT3G07340 // AT1G02065 // AT1G02180 // AT5G48770 // AT5G17690 // AT5G64050 // AT4G12830 // AT5G47030 // AT5G36930 // AT5G62670 // AT5G47070 // AT3G60460 // AT3G03530 // AT2G44640 // AT4G12970 // AT2G05170 // AT1G07320 // AT3G48770 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT1G11680 // AT5G43330 // AT5G17410 // AT3G03570 // AT3G15720 // AT1G26910 // AT1G02140 // AT3G02090 // AT1G55860 // AT2G05220 // AT1G18450 // AT5G58220 // AT5G48580
GO:0030529	C	ribonucleoprotein complex	// AT5G10360 // AT1G07320 // AT2G20060 // AT1G26910 // AT5G59240 // AT3G10950 // AT2G05220
GO:0043231	C	intracellular membrane-bounded organelle	// AT5G09920 // AT3G48770 // AT4G05180 // AT3G07330 // AT2G44530 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G25230 // AT2G22760 // AT1G11680 // AT1G02150 // AT1G11260 // AT2G20060 // AT3G60370 // AT5G61820 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT3G07340 // AT1G02065 // AT5G64050 // AT4G12830 // AT5G47030 // AT3G60460 // AT2G44640 // AT2G05170 // AT1G07320 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT1G55860 // AT1G18450 // AT3G07420 // AT5G48580
GO:0043227	C	membrane-bounded organelle	// AT5G09920 // AT3G48770 // AT4G05180 // AT3G07330 // AT2G44530 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G25230 // AT2G22760 // AT1G11680 // AT1G02150 // AT1G11260 // AT2G20060 // AT3G60370 // AT5G61820 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT5G16970 // AT3G07340 // AT1G02065 // AT5G64050 // AT4G12830 // AT5G47030 // AT3G60460 // AT2G44640 // AT2G05170 // AT1G07320 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT4G16780 // AT5G17690 // AT1G26910 // AT1G02140 // AT2G45490 // AT1G55860 // AT1G18450 // AT3G07420 // AT5G48580
GO:0005737	C	cytoplasm	// AT5G10360 // AT3G48770 // AT4G05180 // AT3G07330 // AT2G44530 // AT5G33406 // AT3G60190 // AT3G02090 // AT3G25230 // AT3G10950 // AT1G11680 // AT1G02150 // AT2G20060 // AT2G26560 // AT3G60370 // AT5G61820 // AT5G17410 // AT5G59240 // AT2G45510 // AT4G31530 // AT1G15510 // AT3G25470 // AT3G07340 // AT5G64050 //

			AT4G12830 // AT5G47030 // AT2G44640 // AT2G05170 // AT1G34380 // AT5G16970 // AT4G09320 // AT1G07320 // AT3G03570 // AT1G26910 // AT3G56640 // AT1G55860 // AT2G05220 // AT3G07420 // AT5G48580
GO:0044435	C	plastid part	// AT4G05180 // AT1G07320 // AT4G31530 // AT3G60370 // AT1G26910 // AT2G44640 // AT5G48580
GO:0005739	C	mitochondrion	// AT5G64050 // AT5G47030 // AT5G33406 // AT3G60190 // AT3G02090 // AT1G55860 // AT2G44640 // AT3G25470 // AT3G07420
GO:0005829	C	cytosol	// AT5G10360 // AT3G03570 // AT5G59240 // AT3G10950 // AT1G55860 // AT2G05220 // AT5G16970
GO:0044434	C	chloroplast part	// AT4G05180 // AT4G31530 // AT3G60370 // AT1G26910 // AT2G44640 // AT5G48580
GO:0031975	C	envelope	// AT4G31530 // AT3G02090 // AT5G47030 // AT2G44640 // AT1G26910
GO:0031967	C	organelle envelope	// AT4G31530 // AT3G02090 // AT5G47030 // AT2G44640 // AT1G26910
GO:0009507	C	chloroplast	// AT1G02150 // AT3G07340 // AT4G05180 // AT2G44530 // AT5G64050 // AT4G12830 // AT2G20060 // AT1G26910 // AT3G07420 // AT3G48770 // AT1G15510 // AT2G44640 // AT4G31530 // AT1G34380 // AT3G60370 // AT5G48580
GO:0009536	C	plastid	// AT1G02150 // AT3G07340 // AT4G05180 // AT1G07320 // AT2G44530 // AT5G64050 // AT4G12830 // AT2G20060 // AT1G26910 // AT3G07420 // AT3G48770 // AT1G15510 // AT2G44640 // AT4G31530 // AT1G34380 // AT3G60370 // AT5G48580
GO:0031224	C	intrinsic to membrane	// AT5G48770 // AT2G44790 // AT1G72890 // AT3G15720 // AT5G17680 // AT5G36930
GO:0005634	C	nucleus	// AT5G09920 // AT1G02065 // AT1G06150 // AT4G16780 // AT1G11260 // AT5G17690 // AT1G02140 // AT3G60460 // AT2G45490 // AT3G25230 // AT2G22760 // AT5G16970 // AT1G18450 // AT3G07340 // AT2G22750
GO:0043234	C	protein complex	// AT5G09920 // AT4G01710 // AT4G05180 // AT3G02090 // ATCG00470 // AT5G47030 // AT1G02140 // AT3G56640 // AT1G55860
GO:0012505	C	endomembrane system	// AT5G17200 // AT1G02180 // AT5G17610 // AT5G17590 // AT1G12280 // AT1G28710 // AT2G43870 // AT4G12970 // AT5G33370 // AT3G59850
GO: 0032555	F	purine ribonucleotide binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G02130 // AT5G48770 // AT1G72890 // AT1G12280 // AT5G36930 // AT5G17680 // AT5G47070 // AT3G60190 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G34420
GO:0032553	F	ribonucleotide binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G02130 // AT5G48770 // AT1G72890 // AT1G12280 // AT5G36930 // AT5G17680 // AT5G47070 // AT3G60190 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G34420
GO:0003735	F	structural constituent of ribosome	// AT5G10360 // AT1G07320 // AT2G20060 // AT1G26910 // AT5G59240 // AT3G10950 // AT2G05220
GO:0005524	F	ATP binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G34420 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G12280
GO:0032559	F	adenyl ribonucleotide binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G34420 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G12280
GO:0017076	F	purine nucleotide binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G02130 // AT5G48770 // AT1G72890 // AT1G12280 // AT5G36930 // AT5G17680 // AT5G47070 // AT3G60190 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G34420

GO:0005198	F	structural molecule activity	// AT5G10360 // AT1G07320 // AT2G20060 // AT1G26910 // AT5G59240 // AT3G10950 // AT1G18450 // AT2G05220
GO:0000166	F	nucleotide binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G02130 // AT5G48770 // AT1G72890 // AT1G34420 // AT5G36930 // AT5G17680 // AT1G12280 // AT5G47070 // AT3G60190 // AT2G45490 // AT5G48380 // AT4G16950 // AT2G45540 // AT2G32800 // AT2G44710
GO:0030554	F	adenyl nucleotide binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G34420 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G12280
GO:0001883	F	purine nucleoside binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G34420 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G12280
GO:0001882	F	nucleoside binding	// AT3G48770 // AT4G16970 // AT4G09320 // AT1G34420 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800 // AT1G12280
GO:0005488	F	binding	// AT3G48770 // AT4G01710 // AT2G44530 // AT1G72890 // AT5G22060 // AT3G53440 // AT5G33406 // AT1G26320 // AT3G60190 // AT2G45490 // AT3G25230 // AT1G64260 // AT1G12280 // AT2G22760 // AT5G33370 // AT5G60900 // AT2G44710 // AT1G02150 // AT1G79740 // AT3G07320 // AT1G02130 // AT1G11680 // AT3G60370 // AT2G44790 // AT4G02600 // AT5G17680 // AT5G48380 // AT1G64850 // AT2G45510 // AT4G16950 // AT3G25470 // AT5G16990 // AT3G07340 // AT1G02065 // AT4G16970 // AT5G48770 // AT3G60220 // AT4G31530 // AT3G60320 // AT5G36930 // AT3G60240 // AT5G47070 // AT3G44110 // AT3G60460 // AT5G48580 // AT3G15070 // AT1G07320 // AT3G60300 // AT1G34380 // AT2G22750 // AT4G09320 // AT1G06150 // AT1G34420 // AT5G43330 // AT4G16780 // AT3G18670 // AT5G17690 // AT1G02140 // AT5G24910 // AT1G29000 // AT3G18960 // AT2G45540 // AT3G03080 // AT2G32800
GO:0005515	F	protein binding	// AT4G01710 // AT1G79740 // AT5G48770 // AT5G22060 // AT3G60220 // AT4G16780 // AT3G18670 // AT5G36930 // AT5G17680 // AT1G02140 // AT5G33406 // AT5G48380 // AT3G25230 // AT1G12280 // AT3G44110 // AT3G15070 // AT3G60300 // AT4G02600 // AT5G17690 // AT1G34420
GO:0004553	F	hydrolase activity, hydrolyzing O-glycosyl compounds	// AT5G17200 // AT3G59850 // AT3G15720 // AT3G07320 // AT2G43870
GO:0003824	F	catalytic activity	// AT5G09920 // AT5G17200 // AT3G07330 // AT1G11680 // AT2G44530 // AT2G43870 // AT1G26320 // AT3G60190 // AT2G45490 // AT3G25230 // AT3G22400 // AT3G13610 // AT2G26560 // AT5G60900 // AT3G50440 // AT5G33370 // AT3G60370 // AT5G48380 // AT4G31530 // AT5G16970 // AT5G16990 // AT4G16970 // AT3G07525 // AT3G07320 // AT3G02090 // AT5G64050 // AT4G12830 // AT2G44740 // AT5G62670 // AT5G47070 // AT3G03530 // AT5G48580 // AT3G60160 // AT1G34380 // AT1G06650 // AT1G02205 // AT4G09320 // AT1G34420 // AT3G59850 // AT5G43330 // AT1G63440 // AT3G15720 // AT5G26667 // AT5G65940 // AT3G07400 // AT1G55860 // AT3G03080 // AT3G07420 // AT2G32800 // AT2G22590
GO:0016798	F	hydrolase activity, acting on glycosyl bonds	// AT5G17200 // AT3G59850 // AT3G15720 // AT3G07320 // AT2G43870
GO:0016491	F	oxidoreductase activity	// AT3G03080 // AT5G43330 // AT3G60370 // AT1G26320 // AT1G06650 // AT3G22400 // AT3G13610 // AT1G11680 // AT5G16990 // AT5G16970

GO:0016772	F	transferase activity, transferring phosphorus-containing groups	// AT5G09920 // AT4G16970 // AT4G09320 // AT1G34420 // AT2G44530 // AT2G44740 // AT5G26667 // AT5G47070 // AT2G45490 // AT5G48380 // AT5G60900 // AT2G32800
GO:0004672	F	protein kinase activity	// AT4G16970 // AT1G34420 // AT2G44740 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800
GO:0015075	F	ion transmembrane transporter activity	// ATCG00470 // AT2G37920 // AT1G63440 // AT5G47030 // AT1G11260
GO:0016301	F	kinase activity	// AT4G16970 // AT4G09320 // AT1G34420 // AT2G44740 // AT5G26667 // AT5G47070 // AT2G45490 // AT5G48380 // AT5G60900 // AT2G32800
GO:0043169	F	cation binding	// AT3G03080 // AT3G07320 // AT2G44790 // AT3G60220 // AT1G29000 // AT2G44530 // AT1G26320 // AT1G64850 // AT1G64260 // AT3G15070 // AT3G60300 // AT5G16990
GO:0043167	F	ion binding	// AT3G03080 // AT3G07320 // AT2G44790 // AT3G60220 // AT1G29000 // AT2G44530 // AT1G26320 // AT1G64850 // AT1G64260 // AT3G15070 // AT3G60300 // AT5G16990
GO:0016787	F	hydrolase activity	// AT5G17200 // AT3G50440 // AT2G43870 // AT3G07320 // AT3G02090 // AT2G26560 // AT4G12830 // AT1G63440 // AT3G15720 // AT3G07400 // AT5G62670 // AT5G65940 // AT3G60190 // AT3G03530 // AT1G34380 // AT3G60160 // AT5G33370 // AT3G59850
GO:0046872	F	metal ion binding	// AT3G03080 // AT2G44790 // AT3G60220 // AT1G29000 // AT2G44530 // AT1G26320 // AT1G64850 // AT1G64260 // AT3G15070 // AT3G60300 // AT5G16990
GO:0016773	F	phosphotransferase activity, alcohol group as acceptor	// AT4G16970 // AT1G34420 // AT2G44740 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800
GO:0016788	F	hydrolase activity, acting on ester bonds	// AT3G50440 // AT1G34380 // AT3G07400 // AT5G65940 // AT5G33370 // AT3G03530 // AT2G26560
GO:0005215	F	transporter activity	// ATCG00470 // AT3G07525 // AT1G11260 // AT2G37920 // AT1G63440 // AT5G47030 // AT2G05170 // AT3G60160
GO:0008270	F	zinc ion binding	// AT3G03080 // AT3G60220 // AT1G26320 // AT1G64260 // AT3G15070 // AT3G60300 // AT5G16990
GO:0003677	F	DNA binding	// AT3G07340 // AT1G02065 // AT1G06150 // AT4G16780 // AT3G60320 // AT5G17690 // AT3G53440 // AT3G48770 // AT3G60460 // AT3G18960 // AT2G22760 // AT1G79740 // AT1G34380 // AT2G22750
GO:0022857	F	transmembrane transporter activity	// ATCG00470 // AT1G11260 // AT2G37920 // AT1G63440 // AT5G47030 // AT3G60160
GO:0022891	F	substrate-specific transmembrane transporter activity	// ATCG00470 // AT2G37920 // AT1G63440 // AT5G47030 // AT1G11260
GO:0046914	F	transition metal ion binding	// AT3G03080 // AT2G44790 // AT3G60220 // AT1G26320 // AT1G64260 // AT3G15070 // AT3G60300 // AT5G16990
GO:0022892	F	substrate-specific transporter activity	// ATCG00470 // AT2G37920 // AT1G63440 // AT5G47030 // AT1G11260
GO:0016740	F	transferase activity	// AT5G09920 // AT4G16970 // AT4G09320 // AT3G07330 // AT2G22590 // AT2G44530 // AT2G44740 // AT5G26667 // AT5G47070 // AT2G45490 // AT5G48380 // AT5G60900 // AT2G32800 // AT1G34420
GO:0003676	F	nucleic acid binding	// AT3G07340 // AT1G02065 // AT1G79740 // AT1G06150 // AT4G16780 // AT3G60320 // AT5G17690 // AT3G60240 // AT3G48770 // AT3G60460 // AT3G53440 // AT3G18960 // AT1G07320 // AT2G22760 // AT2G22750 // AT1G34380 // AT3G25470 // AT2G44710
GO:0003700	F	transcription factor activity	// AT3G07340 // AT1G06150 // AT4G16780 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0030528	F	transcription regulator activity	// AT3G07340 // AT1G06150 // AT4G16780 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750

GO:0016265	P	death	// AT1G02120 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT3G13610 // AT2G26560 // AT4G02600 // AT1G12280
GO:0008219	P	cell death	// AT1G02120 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT3G13610 // AT2G26560 // AT4G02600 // AT1G12280
GO:0012501	P	programmed cell death	// AT1G02120 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT3G13610 // AT2G26560 // AT1G12280
GO:0006952	P	defense response	// AT1G02120 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT4G02600 // AT3G60190 // AT4G16950 // AT2G26560 // AT5G44510 // AT1G12280
GO:0051704	P	multi-organism process	// AT1G02120 // AT5G44510 // AT3G60240 // AT1G02140 // AT3G60190 // AT3G56640 // AT4G16950 // AT3G25470 // AT3G60160 // AT2G26560 // AT3G07330
GO:0006915	P	apoptosis	// AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT1G12280
GO:0040007	P	growth	// AT4G01710 // AT4G16780 // AT5G17690 // AT3G56640 // AT3G11220 // AT5G58220
GO:0045087	P	innate immune response	// AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT4G16950 // AT2G26560
GO:0016043	P	cellular component organization	// AT4G01710 // AT4G16780 // AT5G64050 // AT3G60370 // AT5G17410 // AT5G17690 // AT3G56640 // AT2G45490 // AT2G44770 // AT2G05170 // AT1G15510 // AT1G18450
GO:0006955	P	immune response	// AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT4G16950 // AT2G26560
GO:0002376	P	immune system process	// AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT4G16950 // AT2G26560
GO:0006950	P	response to stress	// AT1G02120 // AT5G48770 // AT1G72890 // AT5G44510 // AT5G47030 // AT5G36930 // AT5G17680 // AT4G02600 // AT5G65940 // AT1G26320 // AT3G60190 // AT3G25230 // AT4G12000 // AT1G12280 // AT4G16950 // AT5G17690 // AT2G26560 // AT5G16990 // AT5G16970
GO:0016049	P	cell growth	// AT5G17690 // AT4G01710 // AT3G56640 // AT5G58220 // AT4G16780
GO:0006996	P	organelle organization	// AT4G01710 // AT5G64050 // AT5G17410 // AT5G17690 // AT2G45490 // AT1G15510 // AT2G05170 // AT1G18450
GO:0008361	P	regulation of cell size	// AT5G17690 // AT4G01710 // AT3G56640 // AT5G58220 // AT4G16780
GO:0032535	P	regulation of cellular component size	// AT5G17690 // AT4G01710 // AT3G56640 // AT5G58220 // AT4G16780
GO:0090066	P	regulation of anatomical structure size	// AT5G17690 // AT4G01710 // AT3G56640 // AT5G58220 // AT4G16780
GO:0050896	P	response to stimulus	// AT1G72890 // AT1G26320 // AT3G60190 // AT3G25230 // AT4G12000 // AT2G26560 // AT5G44510 // AT5G17680 // AT4G16950 // AT5G16970 // AT4G02600 // AT5G48770 // AT1G12280 // AT5G47030 // AT5G36930 // AT3G60240 // AT2G43280 // AT3G60160 // AT5G16990 // AT4G16780 // AT1G02120 // AT1G63440 // AT5G17690 // AT1G02140 // AT5G65940 // AT1G18450 // AT5G58220
GO:0051707	P	response to other organism	// AT1G02120 // AT5G44510 // AT3G60240 // AT3G60190 // AT4G16950 // AT3G60160 // AT2G26560
GO:0009607	P	response to biotic stimulus	// AT1G02120 // AT5G44510 // AT3G60240 // AT3G60190 // AT4G16950 // AT3G60160 // AT2G26560
GO:0048869	P	cellular developmental process	// AT1G02065 // AT4G01710 // AT4G16780 // AT3G60460 // AT3G56640 // AT1G18450
GO:0007275	P	multicellular organismal development	// AT5G10360 // AT1G02065 // AT4G01710 // AT3G07330 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT3G60460 // AT3G56640 // AT3G22400 // AT1G02205 // AT1G11680 // AT1G18450 // AT4G16780

GO:0022414	P	reproductive process	// AT5G10360 // AT3G07330 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT3G60460 // AT3G56640 // AT1G18450 // AT1G11680
GO:0006629	P	lipid metabolic process	// AT3G07400 // AT1G02205 // AT2G26560 // AT5G43590 // AT5G65940 // AT5G33370 // AT3G03530 // AT1G11680
GO:0000003	P	reproduction	// AT5G10360 // AT3G07330 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT3G60460 // AT3G56640 // AT1G18450 // AT1G11680
GO:0009791	P	post-embryonic development	// AT5G10360 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT1G18450 // AT1G11680
GO:0032501	P	multicellular organismal process	// AT5G10360 // AT1G02065 // AT4G01710 // AT3G07330 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT3G60460 // AT3G56640 // AT3G22400 // AT1G02205 // AT1G11680 // AT1G18450 // AT4G16780
GO:0048856	P	anatomical structure development	// AT5G10360 // AT4G01710 // AT3G07330 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT3G56640 // AT3G22400 // AT1G02205 // AT1G11680 // AT1G18450 // AT4G16780
GO:0003006	P	reproductive developmental process	// AT5G10360 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT3G56640 // AT1G18450 // AT1G11680
GO:0032502	P	developmental process	// AT5G10360 // AT1G02065 // AT4G01710 // AT3G07330 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT3G60460 // AT3G56640 // AT3G22400 // AT1G02205 // AT1G11680 // AT1G18450 // AT4G16780
GO:0065008	P	regulation of biological quality	// AT4G01710 // AT4G16780 // AT5G17690 // AT3G56640 // AT3G25470 // AT5G58220
GO:0044238	P	primary metabolic process	// AT5G09920 // AT5G10360 // AT5G17200 // AT5G43590 // AT2G44530 // AT5G22060 // AT2G43870 // AT3G02090 // AT3G10950 // AT2G22760 // AT1G11680 // ATCG00470 // AT2G20060 // AT2G26560 // AT5G48380 // AT2G45490 // AT3G44110 // AT5G59240 // AT3G59850 // AT3G07340 // AT4G16970 // AT3G07320 // AT5G17690 // AT5G64050 // AT3G15720 // AT5G47070 // AT5G33370 // AT3G03530 // AT1G07320 // AT1G02205 // AT2G05220 // AT1G15510 // AT2G22750 // AT1G06150 // AT1G34420 // AT5G43330 // AT4G16780 // AT3G60460 // AT1G26910 // AT5G26667 // AT5G65940 // AT3G07400 // AT1G55860 // AT3G18960 // AT3G13610 // AT3G07420 // AT2G32800
GO:0009987	P	cellular process	// AT5G09920 // AT5G10360 // AT4G01710 // AT2G44530 // AT1G72890 // AT3G07420 // AT5G22060 // AT3G60190 // AT2G45490 // AT2G44770 // AT3G10950 // AT2G22760 // AT2G26560 // ATCG00470 // AT1G02130 // AT2G20060 // AT3G60370 // AT1G34420 // AT5G17680 // AT5G48380 // AT5G59240 // AT3G44110 // AT1G15510 // AT4G02600 // AT3G07340 // AT1G02065 // AT4G16970 // AT3G07525 // AT5G48770 // AT5G64050 // AT1G12280 // AT2G44740 // AT5G36930 // AT5G47070 // AT3G11220 // AT3G60460 // AT3G03530 // AT2G05170 // AT1G07320 // AT1G02205 // AT2G05220 // AT2G22750 // AT1G06150 // AT1G02120 // AT5G43330 // AT5G17410 // AT4G16780 // AT5G17690 // AT1G26910 // AT5G26667 // AT5G65940 // AT3G56640 // AT1G55860 // AT3G18960 // AT1G18450 // AT3G13610 // AT5G58220 // AT2G32800
GO:0008152	P	metabolic process	// AT5G09920 // AT5G10360 // AT5G17200 // AT5G43590 // AT2G44530 // AT5G22060 // AT2G43870 // AT3G02090 // AT3G10950 // AT2G22760 // AT1G11680 // ATCG00470 // AT2G20060 // AT2G26560 // AT3G60370 // AT5G48380 // AT2G45490 // AT4G31530 // AT3G44110 // AT5G59240 // AT3G59850 // AT3G07340 // AT4G16970 // AT3G07525 // AT3G07320 // AT5G17690 // AT5G64050 // AT3G15720 // AT5G47070 // AT5G33370 // AT3G03530 // AT2G22590 // AT1G07320 // AT1G02205 // AT2G05220 // AT1G15510 //

			AT2G22750 // AT3G13610 // AT1G06150 // AT1G34420 // AT5G43330 // AT4G16780 // AT3G60460 // AT1G26910 // AT5G26667 // AT5G65940 // AT3G07400 // AT1G55860 // AT3G18960 // AT3G03080 // AT3G07420 // AT2G32800
GO:0048608	P	reproductive structure development	// AT5G10360 // AT5G64050 // AT2G37920 // AT5G17690 // AT1G02140 // AT1G18450 // AT1G11680
GO:0043436	P	oxoacid metabolic process	// AT1G02205 // AT5G43330 // AT5G64050 // AT5G65940 // AT2G26560 // AT3G07420
GO:0006082	P	organic acid metabolic process	// AT1G02205 // AT5G43330 // AT5G64050 // AT5G65940 // AT2G26560 // AT3G07420
GO:0019752	P	carboxylic acid metabolic process	// AT1G02205 // AT5G43330 // AT5G64050 // AT5G65940 // AT2G26560 // AT3G07420
GO:0006412	P	translation	// AT5G10360 // AT1G07320 // AT2G20060 // AT5G64050 // AT1G26910 // AT5G59240 // AT3G10950 // AT2G05220 // AT3G07420
GO:0016310	P	phosphorylation	// AT4G16970 // ATCG00470 // AT1G34420 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800
GO:0005975	P	carbohydrate metabolic process	// AT5G17200 // AT3G07320 // AT5G43330 // AT3G15720 // AT2G43870 // AT3G59850
GO:0042180	P	cellular ketone metabolic process	// AT1G02205 // AT5G43330 // AT5G64050 // AT5G65940 // AT2G26560 // AT3G07420
GO:0044267	P	cellular protein metabolic process	// AT5G10360 // AT5G22060 // AT1G07320 // AT1G34420 // AT2G20060 // AT5G64050 // AT4G16970 // AT1G26910 // AT5G47070 // AT2G45490 // AT1G55860 // AT5G48380 // AT3G10950 // AT3G44110 // AT5G59240 // AT2G05220 // AT3G07420 // AT2G32800
GO:0044248	P	cellular catabolic process	// AT5G65940 // AT3G03530 // AT1G55860 // AT3G07525 // AT5G43330
GO:0006468	P	protein amino acid phosphorylation	// AT4G16970 // AT1G34420 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800
GO:0006796	P	phosphate metabolic process	// AT4G16970 // ATCG00470 // AT1G34420 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800
GO:0006793	P	phosphorus metabolic process	// AT4G16970 // ATCG00470 // AT1G34420 // AT5G47070 // AT2G45490 // AT5G48380 // AT2G32800
GO:0034645	P	cellular macromolecule biosynthetic process	// AT5G09920 // AT3G07340 // AT1G06150 // AT4G16780 // AT2G20060 // AT5G64050 // AT1G07320 // AT1G26910 // AT5G17690 // AT3G60460 // AT5G10360 // AT5G59240 // AT3G18960 // AT3G10950 // AT2G22760 // AT2G05220 // AT3G07420 // AT2G22750
GO:0043687	P	post-translational protein modification	// AT4G16970 // AT1G34420 // AT5G47070 // AT2G45490 // AT1G55860 // AT5G48380 // AT2G32800
GO:0009059	P	macromolecule biosynthetic process	// AT5G09920 // AT3G07340 // AT1G06150 // AT4G16780 // AT2G20060 // AT5G64050 // AT1G07320 // AT1G26910 // AT5G17690 // AT3G60460 // AT5G10360 // AT5G59240 // AT3G18960 // AT3G10950 // AT2G22760 // AT2G05220 // AT3G07420 // AT2G22750
GO:0048513	P	organ development	// AT5G64050 // AT4G01710 // AT4G16780 // AT3G22400 // AT5G17690
GO:0048731	P	system development	// AT5G64050 // AT4G01710 // AT4G16780 // AT3G22400 // AT5G17690
GO:0009058	P	biosynthetic process	// AT5G09920 // AT5G10360 // AT2G44530 // AT3G10950 // AT2G22760 // AT1G11680 // ATCG00470 // AT2G20060 // AT2G26560 // AT5G59240 // AT2G22750 // AT3G07340 // AT5G64050 // AT3G60460 // AT1G07320 // AT2G05220 // AT1G02205 // AT1G06150 // AT4G16780 // AT5G17690 // AT1G26910 // AT3G18960 // AT3G13610 // AT3G07420
GO:0019538	P	protein metabolic process	// AT5G10360 // AT5G22060 // AT1G07320 // AT1G34420 // AT2G20060 // AT3G02090 // AT5G64050 // AT4G16970 // AT1G26910 // AT5G47070 // AT2G45490 // AT1G55860 // AT5G48380 // AT3G10950 // AT3G44110 // AT5G59240 // AT2G05220 // AT3G07420 //

			AT2G32800
GO:0044249	P	cellular biosynthetic process	// AT5G09920 // AT5G10360 // AT2G44530 // AT3G10950 // AT2G22760 // AT2G26560 // ATCG00470 // AT2G20060 // AT5G59240 // AT2G22750 // AT3G07340 // AT5G64050 // AT3G60460 // AT1G07320 // AT2G05220 // AT1G02205 // AT1G06150 // AT4G16780 // AT5G17690 // AT1G26910 // AT3G18960 // AT3G13610 // AT3G07420
GO:0044237	P	cellular metabolic process	// AT5G09920 // AT5G10360 // AT5G22060 // AT2G44530 // AT2G45490 // AT3G10950 // AT2G22760 // AT2G26560 // ATCG00470 // AT2G20060 // AT3G60370 // AT5G48380 // AT5G59240 // AT3G44110 // AT1G15510 // AT3G07340 // AT4G16970 // AT3G07525 // AT5G64050 // AT5G47070 // AT3G60460 // AT3G03530 // AT1G07320 // AT1G02205 // AT2G05220 // AT2G22750 // AT1G06150 // AT1G34420 // AT5G43330 // AT4G16780 // AT5G17690 // AT1G26910 // AT5G26667 // AT5G65940 // AT1G55860 // AT3G18960 // AT3G13610 // AT3G07420 // AT2G32800
GO:0044260	P	cellular macromolecule metabolic process	// AT5G09920 // AT5G10360 // AT5G22060 // AT2G45490 // AT3G10950 // AT2G22760 // AT2G20060 // AT5G48380 // AT5G59240 // AT3G44110 // AT1G15510 // AT2G22750 // AT3G07340 // AT4G16970 // AT5G64050 // AT5G47070 // AT3G60460 // AT1G07320 // AT2G05220 // AT4G16780 // AT1G06150 // AT1G34420 // AT5G43330 // AT5G17690 // AT1G26910 // AT1G55860 // AT3G18960 // AT3G07420 // AT2G32800
GO:0006139	P	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	// AT5G09920 // AT3G07340 // ATCG00470 // AT1G06150 // AT4G16780 // AT2G44530 // AT5G64050 // AT5G17690 // AT5G26667 // AT3G60460 // AT1G15510 // AT3G18960 // AT2G22760 // AT3G07420 // AT2G22750
GO:0010467	P	gene expression	// AT5G09920 // AT3G07340 // AT1G06150 // AT4G16780 // AT2G20060 // AT5G64050 // AT1G07320 // AT1G26910 // AT5G17690 // AT3G60460 // AT5G10360 // AT5G59240 // AT3G18960 // AT3G10950 // AT2G22760 // AT2G05220 // AT3G07420 // AT2G22750
GO:0043412	P	macromolecule modification	// AT4G16970 // AT1G34420 // AT5G47070 // AT2G45490 // AT1G55860 // AT5G48380 // AT1G15510 // AT2G32800
GO:0016070	P	RNA metabolic process	// AT5G09920 // AT4G16780 // AT5G64050 // AT5G17690 // AT3G60460 // AT1G15510 // AT3G18960 // AT3G07420
GO:0007165	P	signal transduction	// AT4G16780 // AT5G48770 // AT1G72890 // AT5G36930 // AT5G17680 // AT5G58220
GO:0006350	P	transcription	// AT5G09920 // AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0050794	P	regulation of cellular process	// AT3G07340 // AT1G06150 // AT1G02120 // AT5G48770 // AT1G72890 // AT4G16780 // AT2G44740 // AT5G36930 // AT5G17680 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT5G58220 // AT2G22750
GO:0009628	P	response to abiotic stimulus	// AT4G16780 // AT5G47030 // AT5G17690 // AT5G65940 // AT2G43280 // AT3G25230 // AT1G18450
GO:0006464	P	protein modification process	// AT4G16970 // AT1G34420 // AT5G47070 // AT2G45490 // AT1G55860 // AT5G48380 // AT2G32800
GO:0065007	P	biological regulation	// AT3G07340 // AT4G01710 // AT1G06150 // AT1G02120 // AT5G48770 // AT1G72890 // AT4G16780 // AT2G44740 // AT5G36930 // AT5G17680 // AT5G17690 // AT3G60460 // AT3G56640 // AT3G18960 // AT2G22760 // AT3G25470 // AT5G58220 // AT2G22750
GO:0006351	P	transcription, DNA-dependent	// AT5G09920 // AT3G18960 // AT3G60460 // AT4G16780 // AT5G17690
GO:0032774	P	RNA biosynthetic process	// AT5G09920 // AT3G18960 // AT3G60460 // AT4G16780 // AT5G17690

GO:0043170	P	macromolecule metabolic process	// AT5G09920 // AT5G10360 // AT5G22060 // AT3G02090 // AT3G10950 // AT2G22760 // AT2G20060 // AT5G48380 // AT5G59240 // AT3G44110 // AT1G15510 // AT3G07340 // AT4G16970 // AT5G64050 // AT5G47070 // AT3G60460 // AT1G07320 // AT2G05220 // AT2G22750 // AT1G06150 // AT1G34420 // AT5G43330 // AT4G16780 // AT5G17690 // AT1G26910 // AT2G45490 // AT1G55860 // AT3G18960 // AT3G07420 // AT2G32800
GO:0045449	P	regulation of transcription	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0006807	P	nitrogen compound metabolic process	// AT5G09920 // AT3G07340 // ATCG00470 // AT1G06150 // AT4G16780 // AT2G44530 // AT5G64050 // AT5G17690 // AT5G26667 // AT5G65940 // AT3G60460 // AT1G15510 // AT3G18960 // AT2G22760 // AT3G07420 // AT2G22750
GO:0010556	P	regulation of macromolecule biosynthetic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0042221	P	response to chemical stimulus	// AT4G16780 // AT1G63440 // AT4G12000 // AT5G16990 // AT5G65940 // AT1G26320 // AT1G02140 // AT5G58220 // AT5G16970
GO:0009889	P	regulation of biosynthetic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0031326	P	regulation of cellular biosynthetic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0051171	P	regulation of nitrogen compound metabolic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0050789	P	regulation of biological process	// AT3G07340 // AT1G06150 // AT1G02120 // AT5G48770 // AT1G72890 // AT4G16780 // AT2G44740 // AT5G36930 // AT5G17680 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT5G58220 // AT2G22750
GO:0080090	P	regulation of primary metabolic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0010468	P	regulation of gene expression	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0031323	P	regulation of cellular metabolic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0060255	P	regulation of macromolecule metabolic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0009056	P	catabolic process	// AT5G65940 // AT3G03530 // AT1G55860 // AT3G07525 // AT5G43330
GO:0019222	P	regulation of metabolic process	// AT3G07340 // AT1G06150 // AT4G16780 // AT5G17690 // AT3G60460 // AT3G18960 // AT2G22760 // AT2G22750
GO:0006810	P	transport	// AT1G29000 // AT3G60190 // ATCG00470 // AT2G44770 // AT1G02130
GO:0051234	P	establishment of localization	// AT1G29000 // AT3G60190 // ATCG00470 // AT2G44770 // AT1G02130
GO:0051179	P	localization	// AT1G29000 // AT3G60190 // ATCG00470 // AT2G44770 // AT1G02130

[a] C = cellular component; F = molecular function; P = biological process

Supplemental Table S3 Putative genes involved in bean resistance to ALS based on the At orthologs microarray data from PathoPalnt (http://www.pathoplant.de/).

P. vulgaris gene[a]	AGI #	E-value	Identity	Gene annotation	Description[b]
Phvul.010G040600	AT1G02205	0.0	63%	Sterol desaturase	Aldehyde catabolic process, development of cuticle, defense response to bacteria and fungus, oxidation-reduction process, response to drought
Phvul.010G047700	AT2G26560	4×10^{-79}	40%	Patatin-like fosfolipase	MAPK cascade, cell death, cell membrane fusion, defense response to fungi and viruses, detection of biotic stimulus, JA signaling pathway
Phvul.010G049200	AT2G44790	6×10^{-38}	44%	Blue copper protein	Response to nitrate and injury, cell death, oxidative burst
Phvul.010G044100	AT3G10950	2×10^{-57}	93%	Ribosomal protein 60S	Ribosomal biogenesis, translation
Phvul.010G032100	AT5G10360	4×10^{-163}	90%	Ribosomal protein 40S	Cytosolic ribosomal subunit, embryogenic development
Phvul.010G053400 Phvul.010G030000 **Phvul.010G030300**	AT1G11260	0.0 0.0 0.0	64% 58% 68%	Sugar transporter	Regulation of defense response, systemic acquired resistance, transmembrane transport
Phvul.010G049100	AT1G12650	4×10^{-72}	60%	Unknown protein	unknown
Phvul.010G035100	AT1G23280	4×10^{-111}	63%	MAK16 related	RNA Methylation
Phvul.010G044400 Phvul.010G044500 **Phvul.010G044600**	AT1G28710	3×10^{-118} 4×10^{-40} 3×10^{-120}	55% 48% 56%	Diphospho-transferase	unknown
Phvul.010G034100 Phvul.010G033900	AT1G34380	9×10^{-99} 4×10^{-19}	72% 71%	DNA polimerase I	exonuclease activity
Phvul.010G033800	AT1G34420	2×10^{-167}	53%	Protein kinase	Innate immune response, a negative regulator of defense response, phosphorylation of proteins signaling pathway salicylic acid, systemic resistance adiquirida
Phvul.010G043600	AT2G32800	0.0	56%	Protein kinase	protein phosphorylation
Phvul.010G044800	AT2G44740	1×10^{-97}	71%	ciclin	regulation of cell cycle progression strain stomatal
Phvul.010G054100	AT2G44970	0.0	68%	α/β hydrolase	Intracellular transport of proteins
Phvul.010G034800	AT3G03560	0.0	53%	Unknown protein	unknown
Phvul.010G030800	AT3G07420	0.0	51%	tRNA synthetase	Synthesis of aminoacyl tRNA

Phvul.010G036100	AT3G13610	8×10^{-177}	65%	Iron/ ascorbate oxidoreductase	Redox, cell death via hydrogen peroxide, response to injury, secondary metabolic process, catabolism of toxins
Phvul.010G037100		6×10^{-105}	51%		
Phvul.010G037200		4×10^{-95}	44%		
Phvul.010G037300		9×10^{-94}	42%		
Phvul.010G037400	AT3G15720	8×10^{-91}	42%	Glycosyl transferase family 28	Carbohydrate metabolism, response to cyclopentanes
Phvul.010G037900		8×10^{-103}	45%		
Phvul.010G038100		4×10^{-95}	47%		
Phvul.010G038000		9×10^{-40}	62%		
Phvul.010G032300	AT3G22400	0.0	46%	Lipoxygenase 5 (LOX5)	Defense response to injury and insects, JA biosynthesis, root development, lipid oxidation
Phvul.010G031100	AT3G25400	2×10^{-59}	71%	Pirophosphohydrolase	desconhecido
Phvul.010G040900	AT3G60190	0.0	81%	Dynamin related	EDR3 (Enhanced Disease Resistance 3), MAPK cascade, defense response to bacteria and fungus, the JA signaling pathway, hypersensitive response, salicylic acid biosynthesis, negative regulator of defense response
Phvul.010G053300	AT3G60450	7×10^{-103}	56%	Phosphoglycerate mutase	Endoplasmic reticulum stress response, systemic acquired resistance
Phvul.010G045400	AT4G09320	3×10^{-95}	88%	Nucleoside diphosphate kinase	GTP, CTP e UTP biosynthesis, cadmium response, salt stress response
Phvul.010G052900	AT4G31980	1×10^{-51}	29%	Unknown protein	Transport amino acid
Phvul.010G051600	AT4G37445	2×10^{-28}	50%	Family of calcium binding	unknown
Phvul.010G038800	AT5G16970	6×10^{-179}	70%	Alkene reductase	Amino acid transport, response to cadmium and cyclopentane, oxidative stress response
Phvul.010G053800	AT5G22060	3×10^{-20}	58%	Heat shock protein	Methylation of proteins, protein folding, response to heat and high light intensity, response to hydrogen peroxide
Phvul.010G043900	AT5G24910	1×10^{-163}	47%	Cytochrome P450	Brassinosteroid biosynthesis, oxidation-reduction process
Phvul.010G026300	AT5G44510	3×10^{-38}	42%	TIR-NB-LRR	Defense response to bacteria, signal transduction
Phvul.010G043000	AT5G47060	4×10^{-29}	59%	Unknown protein	unknown
Phvul.010G043100	AT5G47070	1×10^{-152}	59%	Protein Kinase	Phosphorylation of protein, the biosynthesis of salicylic acid, systemic acquired resistance
Phvul.010G035800	AT5G48380	0.0	63%	Protein Kinase	BIR1 (BAK-1 interactor), defense response to bacteria, cell death signaling via

					salicylic acid pathway, systemic acquired resistance
Phvul.010G031900	AT5G60900	0.0	46%	Protein Kinase	MAPK cascade, JA signaling, hydrogen peroxidase metabolism, hipersenssível response, response to bacteria, systemic acquired resistance
Phvul.010G032000		0.0	44%		
Phvul.010G035300		2×10^{-37}	48%		
Phvul.010G035400	AT5G61820	8×10^{-88}	42%	Unknown protein	Abscisic acid signaling pathway, response to symbiotic fungus
Phvul.010G035700		4×10^{-114}	43%		
Phvul.010G024000		1×10^{-79}	39%		
Phvul.010G024200		1×10^{-124}	37%		
Phvul.010G025200		1×10^{-134}	38%		
Phvul.010G026000		3×10^{-177}	36%		
Phvul.010G026100		0.0	44%		
Phvul.010G026600		3×10^{-123}	37%		
Phvul.010G027800		2×10^{-113}	37%		
Phvul.010G027900		6×10^{-163}	42%		
Phvul.010G028100		4×10^{-158}	40%		
Phvul.010G026200		2×10^{-171}	44%		
Phvul.010G054800		5×10^{-44}	45%		
Phvul.010G026900		5×10^{-81}	37%		
Phvul.010G028800		9×10^{-137}	38%		
Phvul.010G028900		1×10^{-158}	41%		
Phvul.010G029700		9×10^{-122}	37%		
Phvul.010G025000		0.0	45%		
Phvul.010G025500		2×10^{-145}	40%		
Phvul.010G026700		2×10^{-129}	38%		
Phvul.010G028600		1×10^{-50}	45%		
Phvul.010G028700	AT5G36930	3×10^{-136}	38%	TIR-NBS-LRR	Innate immune response, cell death, signal transduction
Phvul.010G029000		2×10^{-114}	38%		
Phvul.010G029100		2×10^{-134}	38%		
Phvul.010G029400		5×10^{-44}	31%		
Phvul.010G029600		3×10^{-98}	41%		
Phvul.010G024100		7×10^{-91}	37%		
Phvul.010G025300		2×10^{-52}	39%		
Phvul.010G025400		4×10^{-141}	34%		
Phvul.010G025700		0.0	45%		
Phvul.010G026400		9×10^{-153}	37%		
Phvul.010G027100		2×10^{-132}	38%		
Phvul.010G027200		3×10^{-91}	37%		
Phvul.010G027300		2×10^{-134}	37%		
Phvul.010G027500		5×10^{-96}	38%		
Phvul.010G027700		5×10^{-109}	32%		
Phvul.010G028000		1×10^{-177}	42%		
Phvul.010G028200		0.0	44%		
Phvul.010G028400		2×10^{-75}	36%		
Phvul.010G028500		8×10^{-172}	42%		
Phvul.010G054300		1×10^{-45}	47%		

[a] Bold gene names were chosen to primer design through phylogeny analysis

[b] The gene descriptions were based on the *Arabidopsis thaliana* database for each At ortholog

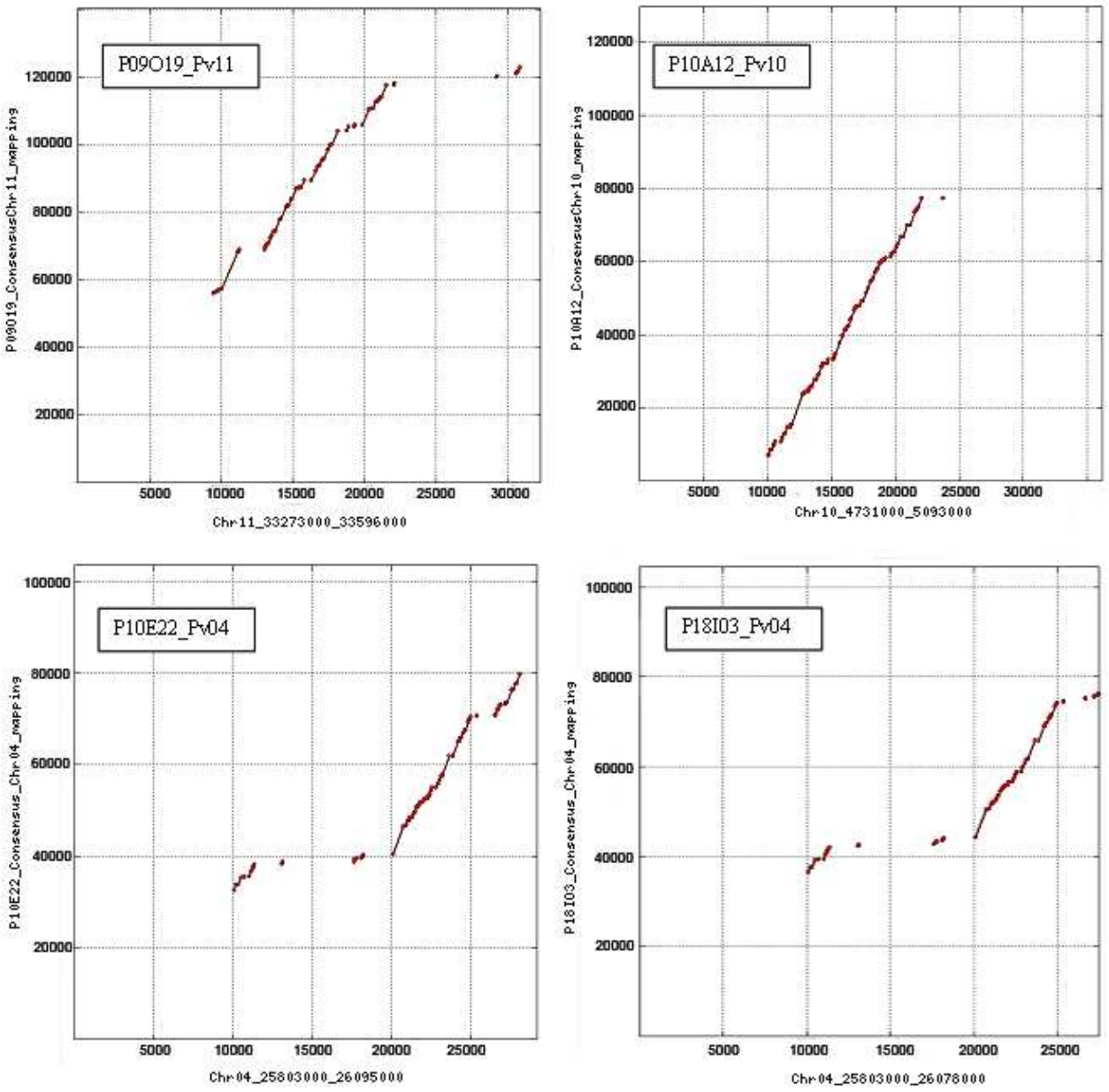
Supplemental Table S4 Primer sequences of selected genes for quantitative PCR analysis.

Pv Gene	Primer sequence (5' – 3')
Phvul.008G011000-ACT11_F	CAGAGCGGGAAATTGTAAGG
Phvul.008G011000-ACT11_R	AACTGGTCCTGGCTGTCTC
Phvul.001G133200-IDE_F	GAGAGACTATGAGGTTGAAGC
Phvul.001G133200-IDE_R	CCATGAACTCGTACACTTAAAG
Phvul.010G040600_F	CATGCCCTAGAAGGTTGGAA
Phvul.010G040600_R	CATGGTGAAGACTTGCTTGC
Phvul.010G047700_F	CGATGGGGAGCCTTTACATA
Phvul.010G047700_R	GCTTTCGTGTCTTGATGTCG
Phvul.010G049200_F	CAACACCACCTCACCATCAG
Phvul.010G049200_R	CTAGCCCATGAAACCAAGAAC
Phvul.010G044100_F	TGAAGGCTGGAGGTGCTTAC
Phvul.010G044100_R	TAACTCTCAGTCTGCTCCCTC
Phvul.010G032100_F	AACTTCTTGCCTCCAGATTG
Phvul.010G032100_R	AGGCTGCTTAGAAACACTGG
Phvul.010G030300_F	GCTATCGTGGTTGTGTGTGG
Phvul.010G030300_R	TCTCACTTGGAACCAACCATC
Phvul.010G044600_F	GGATTTTAGGGCAGTCGTCA
Phvul.010G044600_R	CTTTCTGTTGAGTTTGCTTCC
Phvul.010G034100_F	GAGTGGTTGGTTAAGAGAGAC
Phvul.010G034100_R	GGTCTGCCATTGTAAGTGAGC
Phvul.010G033800_F	ATTGGTCAAGTGGGTTTTGC
Phvul.010G033800_R	GCAAGCATCTGGTTTCTGAC
Phvul.010G043600_F	CCCGTTCTGACCCAAAACTG
Phvul.010G043600_R	CTGCTCTCCATGTTCTCCAC
Phvul.010G044800_F	GTTTTGCAGAACATTCCAGC
Phvul.010G044800_R	CTGTTGCTGCTTTTGATGAG
Phvul.010G054100_F	TTCCTGGATCTGTTGTGGTG
Phvul.010G054100_R	TCAAGAGAGCCTCGCACAC
Phvul.010G034800_F	CATCTGGTCTCTCGGTGC
Phvul.010G034800_R	CTCTGGAAGAATCGCATGG
Phvul.010G030800_F	TGGGAGTCAAAACGAGGAAC
Phvul.010G030800_R	TCAGGGTGAAGCCAGAGTTC
Phvul.010G036100_F	CTAGACCTTCAGATGTGATTG
Phvul.010G036100_R	TCAACTGTCATCTTCCCATC
Phvul.010G032300_F	GAGATTCAAGCAAGGATCATG
Phvul.010G032300_R	ATTCCCCTTCCTGTGATTCC
Phvul.010G031100_F	TGTGTGGTGTTGATCTTGGG
Phvul.010G031100_R	ATCTTTGGTGGTGCTTGAAG
Phvul.010G040900_F	TCCAGCTACTACCCCAAATG
Phvul.010G040900_R	GGATAGTGTTCCTGAGAGTG

Phvul.010G053300_F	GGTGGCGTTTTCTTCATTCC
Phvul.010G053300_R	AAATTGGGCGTCTGAGTTGG
Phvul.010G045400_F	TATCTGCCAAGCCTTTCTTC
Phvul.010G045400_R	CCCAATTATCTTTCTCCCAG
Phvul.010G052900_F	GTTGGGGAGTGATAAGGAAG
Phvul.010G052900_R	GTTCTGGTAATGCTTGTTGAG
Phvul.010G051600_F	CTCAGTGTCATTAAAGCCATC
Phvul.010G051600_R	GGAAGGGATATTCTTGAAGG
Phvul.010G037200_F	GAGTTAATGAGTGAAGGAGG
Phvul.010G037200_R	CTGGTAGAGAAGATGTAATGC
Phvul.010G038800_F	CAGTACAATCTCATAGAGCC
Phvul.010G038800_R	AGGCAGGACGAACTCCAAG
Phvul.010G043900_F	GGGTCCTGATGCTCACAAG
Phvul.010G043900_R	GTCATGGCTAAGTGCTGTCC
Phvul.010G043100_F	CAACGATGAGTCAGGTGGTG
Phvul.010G043100_R	GCCTAACTGATTTGCCTTCAC
Phvul.010G035800_F	TGTTCTGCCAACTCCAAAGG
Phvul.010G035800_R	ATCAGCATCGCCATTATCAG
Phvul.010G031900_F	ATTGCCCTTTGGTGTGTGC
Phvul.010G031900_R	GCACCTCCACTTCAACAAC
Phvul.010G026300_F	CGTCGCATGTTCGTAATCAG
Phvul.010G026300_R	TCATCGTTCAACCCTTGAGG
Phvul.010G025700_F	ATCAACCCCTGAAAACATGG
Phvul.010G025700_R	ACACCCTGCCAATCTTCATC
Phvul.010G043000_F	GAGGGAACACACCATTTTGC
Phvul.010G043000_R	TCTCACACCCCTTTTGGAAG

[a] All primers were designing with the annealing temperature between 58°C to 60°C; and PCR products of 80 pb to 130 pb.

Supplemental Fig S1 BAC clones alignment with the *P. vulgaris* genome.



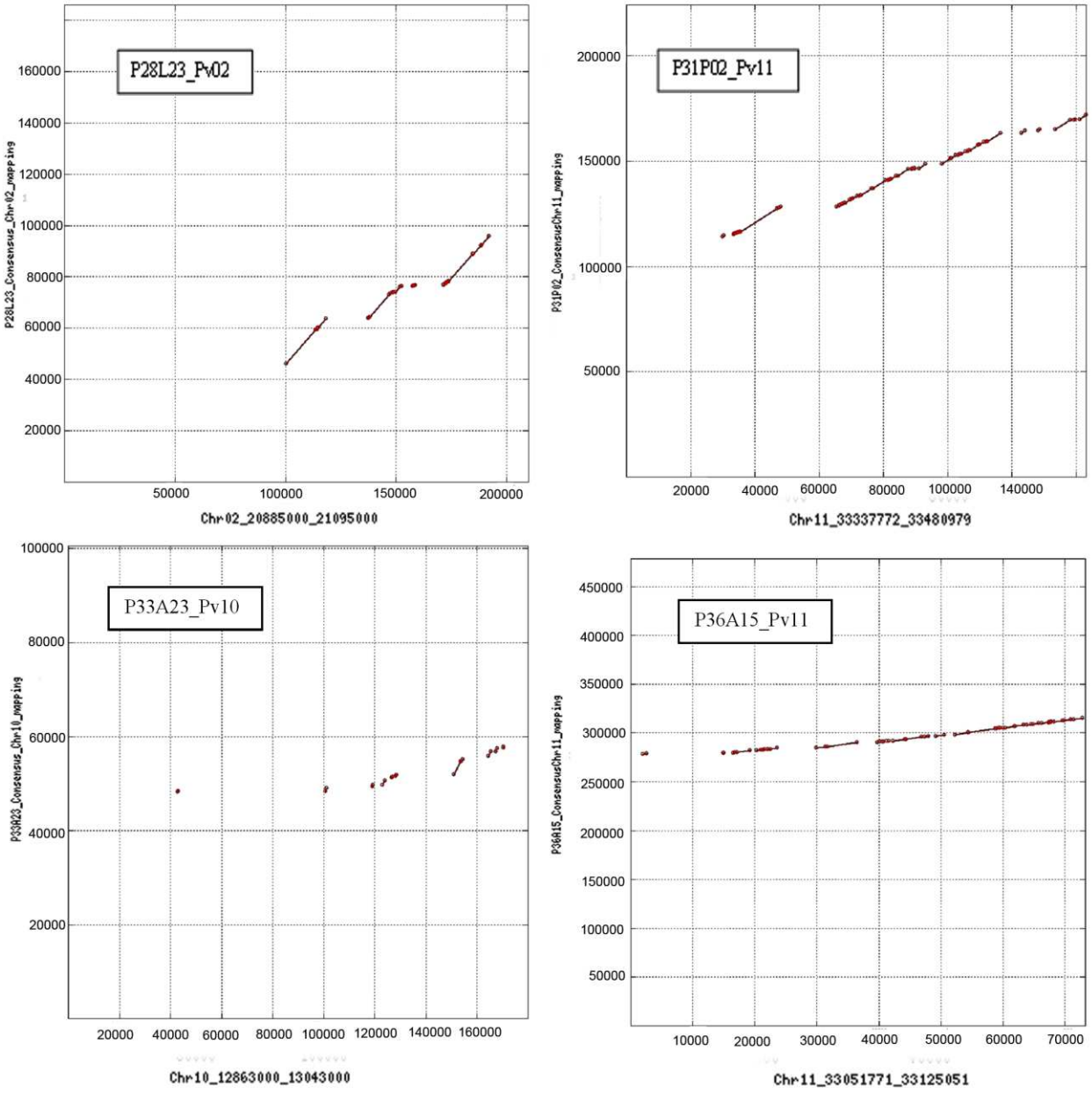


Fig S1 Aligment results for all eight BAC clones using the Phytozome assemble for the *P. vulgaris* genome. Each graph represents the coverage of the BAC contigs in each *P. vulgaris* chromosome. The analysis were performed using the CLC Genomics Workbench (http://www.clcbio.com/products/clc-genomics-workbench/).

Supplemental Figure S2 – Phylogeny analysis of the *P. vulgaris* orthologs of the AT5G36930.

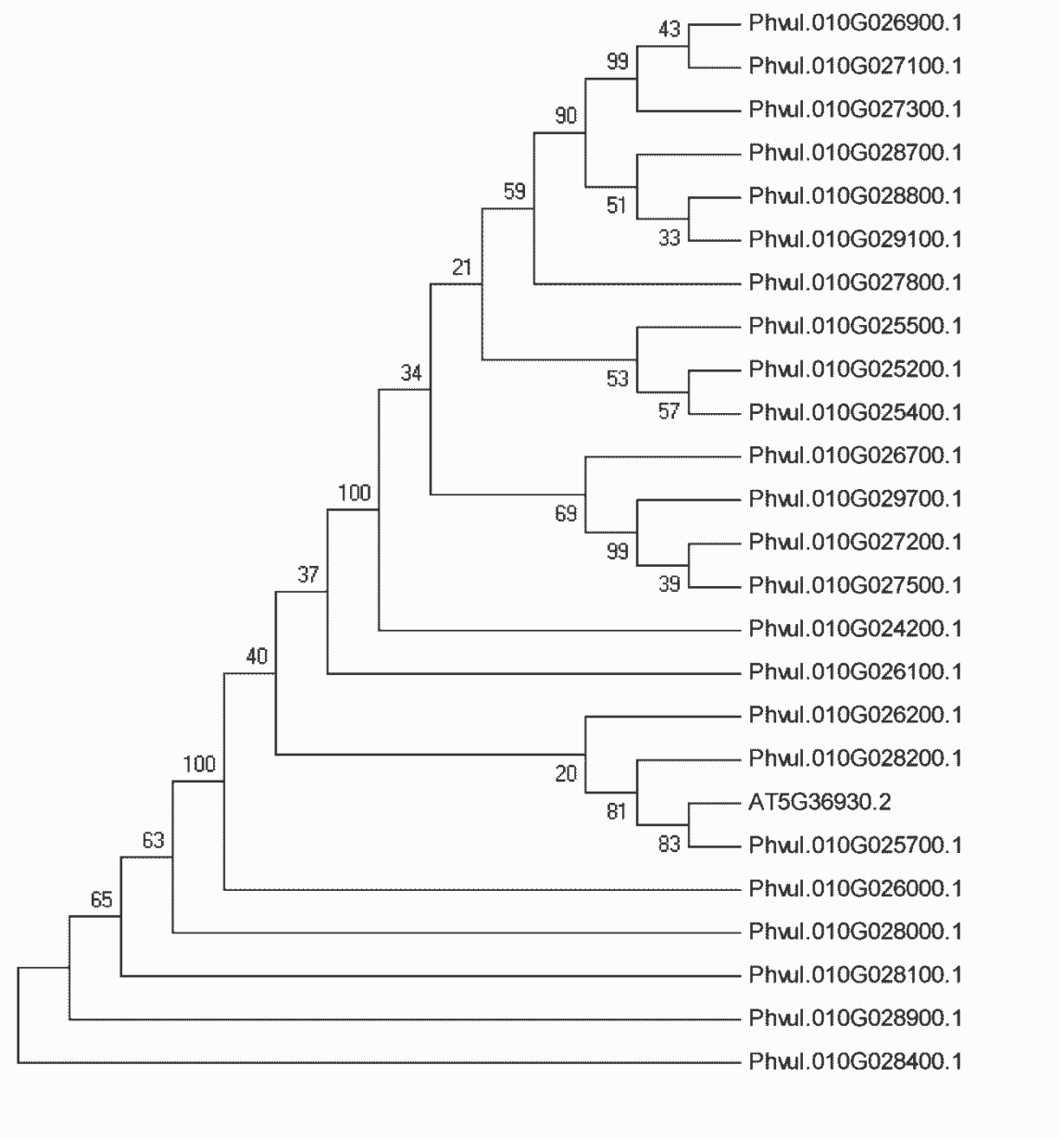


Fig 2 The closest ortholog of the *A. thaliana* AT5G36930 in *P. vulgaris* ALS10.1 core region is the Phvul.010G025700. The *P. vulgaris* proteins of all homologs of the *A. thaliana* AT5G36930 were fisrt aligned using CLUSTALW. The conserved domains were then used in the distance-based analysis through the Maximum Parsimony algorithm in the MEGA5 softwere (Tamura et al., 2011). Statistical support for distance trees was evaluated using bootstrapping (1,000 replicates) expressed in percentages.

RESULTADOS E DISCUSSÕES GERAIS

Na busca por ampliar o entendimento das respostas atuantes na interação *P. vulgaris – P. griseola*, o presente projeto buscou dar continuidade aos esforços do Programa de Melhoramento do Feijoeiro do IAC (PMF - IAC) que tem interesse em incorporar resistência às raças fisiológicas de *P. griseola* em cultivares de interesse econômico. O primeiro passo foi o desenvolvimento de um novo mapa genético para feijão utilizando marcadores microssatélites. Para isso uma nova população segregante de 380 linhagens endogâmicas foi desenvolvida pelo PMF - IAC. A população foi gerada pelo cruzamento entre a variedade IAC-UNA (Mesoamericana/suscetível à ALS) e a linhagem CAL 143 (Andina/resistente à ALS). O mapa UC (IAC-UNA x CAL 143) foi gerado usando LOD mínimo de 3,0 e fração de recombinação máxima de 0,40, que resultou em 198 microssatélites ligados e distribuídos nos onze grupos de ligação de feijão. O comprimento total de mapa encontrado foi de 1.864,2 cM (Campos et al., 2011).

Em continuidade, QTLs de resistência à ALS foram identificados e publicados em artigo científico na revista *BMC Genetics* (Oblessuc et al., 2012b – CAPÍTULO I). Neste trabalho, duas condições de infecção por *P. griseola* foram utilizadas (natural em campo; e raça-específica em casa de vegetação), sendo analisados três ambientes: (1) campo em época chuvosa; (2) campo em época de seca; (3) e casa de vegetação (infecção com a raça 0-39). Em todos os ambientes estudados foi observada distribuição normal e transgressiva dos sintomas de severidade da doença na população de mapeamento UC, o que levou a indicação de controle quantitativo de resistência a ALS em CAL 143. No total foram identificados sete QTLs, mapeados em cinco grupos de ligação. A maioria dos QTLs foram estáveis, com exceção de dois, apresentando LODscore significativo para os três ambientes analisados. Dentre estes o QTL ALS10.1, mapeado no grupo de ligação B10, mostrou possuir efeito maior na resistência (R^2 entre 16% - 22%). A presença deste QTL explica a alta herdabilidade do caráter encontrada neste estudo. Trabalhos

anteriores também mostraram resistência quantitativa em outra linhagens de feijão (López et al., 2003; Mahuku et al., 2009; Mahuku et al., 2011), contudo padrão de herança monogênico ainda é aferido à resistência à ALS (Mahuku et al., 2004; Namayanja et al., 2006; Mahuku et al., 2011). Desta forma, a caracterização da resistência em CAL 143 deve acrescentar informação à estes estudos, colaborando com o melhoramento do feijão.

Como ALS10.1 mostrou-se um interessante loco de resistência, este passou a ser o alvo para o trabalho de saturação do QTL e análise de seu contexto genômico. Assim, o grupo de ligação B10 foi saturado com marcadores SCARs, DArTs e novos SSRs para aumentar o número de marcadores na região do QTL (Oblessuc et al., em submissão – CAPÍTULO II). Inicialmente foram identificados 3 SCARs (SH13, SBA16 - Queiroz et al., 2004, e $PF5_{330}$ - Mahuku et al., 2004) e 100 SSRs (Gaitán-Solís et al., 2002; Caixeta et al., 2005; Benchimol et al., 2007; Grisi et al., 2007; Hanai et al. 2007; 2010; Blair et al., 2008; Cardoso et al. 2008; Campos et al., 2011) polimórficos para os genitores IAC-UNA e CAL 143. Vinte e um clones DArTs também foram polimórficos para os genitores e *bulks* resistente e susceptível; contudo apenas 13 seqüências únicas foram identificadas por análise de BLAST e utilizadas no desenvolvimento dos marcadores *Sequence Tag Site* – DArT (STS-DarTs). Análises de ligação resultaram na adição de três STS-DArT, um SCAR ($PF5_{330}$) e seis SSRs no grupo B10. Com isso, o intervalo de confiança de ALS10.1 foi reduzido de 13.5 cM para 3 cM, uma vez que houve um incremento de 6 marcadores na região do ALS10.1, com o SSR ATA220 coincidindo com o valor máximo de LOD do QTL. Ainda foi possível observar que sete dos 10 marcadores ligados ao QTL apresentaram alelo oriundos do genitor resistente em todas as 10 RILs mais resistentes. Além disso, novo QTL foi identificado neste mesmo grupo de ligação, $ALS10.2^{UC}$, abrangendo dois STS-DArTs e o SCAR.

Em adição ao estudo genético, análises de alinhamento das seqüências dos marcadores ligados no grupo de ligação B10 com o rascunho do genoma do feijão (http://www.phytozome.net/) também foram realizadas. A localização física de 10 SSRs e sete STS-DArTs no cromossomo Pv10 de feijão foi determinada, o que possibilitou a identificação de uma região genômica *core* para o QTL ALS10.1,

contendo seis SSRs dos 10 marcadores associados ao QTL. Através deste posicionamento, genes putativamente ligados a resistência à ALS puderam ser identificados, tais como: glicosil-transferase, *ankyrin repeat-containing*, fosfolipase, *yippee-like*, NB-ARC, *aprataxin-related*, citocromo P450, e quinases. Tais genes sabidamente participam da resposta imune de plantas, o que foi mais um indicativo da importância deste loco na resistência do feijoeiro à ALS.

Contudo, esta etapa do trabalho só pode ser realizada com a liberação das informações genômicas do feijão, que ocorreu apenas recentemente. Assim, o aprofundamento dos estudos do loco ALS10.1 se iniciou com a identificação de clones BAC contendo os marcadores ligados à ele. Interessantemente, dois clones BAC foram identificados contendo os mesmo dois marcadores (PvM13 e IAC137), o que indicou uma razão de 62 Kb/cM para esta região do genoma (Oblessuc et al., 2012c – CAPÍTULO III). Assim, foram selecionados oito clones BAC para seqüênciamento em plataforma Illumina®. Apenas dois foram mapeados no cromossomo 10, e dentre estes um clone (P10A12) foi localizado na região *core* do ALS10.1, baseado no rascunho do genoma do feijão utilizado. Concomitantemente ao estudo da estrutura genômica do ALS10.1, trabalho preliminar de expressão dos genes putativamente presentes neste loco foi realizado. Os marcadores ligados ao QTL presentes em regiões codantes de genes foram amplificados via PCR-semiquantitativo a partir de cDNA obtido da linhagem de feijão G2333, sob condições de interação com água (controle) e flg22 (Oblessuc et al., 2013 – CAPÍTULO IV). Alteração na expressão dos genes contendo RGA07 e PvM22 foi observada, indicando que este loco efetivamente atua na resposta inata de feijão, uma vez que flg22 é um peptídeo de 22 aminoácidos da PAMP flagelina, capaz de elicitar PTI em plantas.

Em seqüência, o rascunho do genoma foi utilizado na identificação dos genes contidos na região *core* do ALS10.1, para um estudo mais aprofundado deste loco (Oblessuc et al., em preparação – CAPÍTULO V). Os 323 genes obtidos tiveram seus homólogos em *Arabidopsis thaliana* identificados, os quais foram utilizados em estudo de *Gene Ontology* (GO). Grande parte dos genes de feijão presentes no ALS10.1 *core* (61,6%) mostraram ser putativos de resposta à estresse em plantas. Interessantemente, estes genes

apresentam principalmente atividade *binding*, com um *cluster* de 52 genes R (TIR-NB-ARC) identificado, de aproximadamente 849 Kb. Em adição, outras famílias gênicas também foram observadas na região *core* do QTL, como: quinases, glicosil hidrolases, citocromo, *ankyrin repeat* e fatores de transcrição; além de genes de transporte celular, resposta à hormônios, ribossomais e de função desconhecida. Dentre estes, sete foram analisados via PCR quantitativo (qPCR), o que não apenas confirmou a existência genes envolvidos na resistência em ALS10.1, mas também permitiu a identificação de possíveis vias metabólicas atuantes neste resposta.

Dentre os genes testados via qPCR, o gene codante para proteína TIR-NB-ARC foi induzido durante resposta compatível entre IAC-UNA e *P. griseola*. Sendo estes domínios característicos de genes R é provável que o TIR-NB-ARC testado esteja atuando no reconhecimento do Avr (gene de avirulência) do fungo e desta forma impedindo a ativação das vias de resposta ETI, em similaridade ao AvrPtoB de *Pseudomonas syringae* (Zhang & Zhou, 2010). Desta forma, este é um interessante alvo para seleção negativa de genótipos susceptíveis à ALS. Outros genes do ALS10.1 *core* também foram diferencialmente expressos durante resposta à *P. griseola*. O transcrito *EDR3-like* de feijão, o qual tem como ortólogo o regulador negativo da resposta imune de *A. thaliana* (At) *AtEDR3* (*Enhanced Disease Resistance* 3) (Tang et al., 2006), foi reprimido em CAL143 durante interação incompatível com *P. griseola*. *AtEDR3* atua inibindo a resposta PTI mediada pela via ácido salicílico (AS), assim *EDR3-like* deve possuir mesmo papel na resposta resistente do feijão. De forma similar o *BIR1-like* também foi reprimido em resposta incompatível. *BIR1-like* é ortólogo do *AtBIR1* (*BAK1-INTERACTING RECEPTOR KINASE1*), outro repressor da resposta imune com inibição da via do AS (Gao et al., 2009). Com isso, os resultados indicam que genes observados na região do QTL ALS10.1 participam tanto de resposta ETI como PTI, principalmente pela via do AS.

CONCLUSÕES GERAIS

Os resultados deste estudo são de grande utilidade no melhoramento do feijoeiro, uma vez que fornecem novas informações de marcadores moleculares que podem ser aplicados em trabalhos de piramidação por seleção assistida por marcadores para resistência à ALS. A observação de um padrão de herança quantitativo de resistência à ALS na linhagem CAL143, tanto em campo quanto em casa de vegetação, permitirá um maior direcionamento nos trabalhos de melhoramento do feijão. Além disso, a identificação da composição gênica do loco ALS10.1 fornece um importante fonte de genes candidatos à resistência à ALS, sendo observados genes tanto de resposta imune basal (PTI) de plantas como possíveis genes de resistência raça-específica, ditos genes R (ETI). Inclusive o gene R analisado mostrou ser um alvo para seleção negativa de genótipo susceptíveis, uma vez que foi induzido de forma consistente na resposta compatível. Diferenças na expressão entre os genótipos susceptível e resistente de alguns dos genes no ALS10.1, somado a suas funções putativas relacionadas aos homólogos mais próximos em *Arabidopsis thaliana*, também indicaram a via de resposta imune do ácido salicílico como uma importante via na defesa do feijão à ALS. Apesar deste estudo representar um avanço no entendimento dos mecanismos de resposta do feijão à ALS, estudos mais aprofundados devem ser conduzidos para o maior detalhamento das vias metabólicas que atuam na resistência, assim como para a confirmação de genes alvo a serem utilizados no desenvolvimento de marcadores gene-específicos para uso no melhoramento da cultura do feijoeiro.

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APÊNDICE I:

Campos T, et al. Inheritance of growth habit detected by genetic linkage analysis using microsatellites in the common bean (*Phaseolus vulgaris* L.). Mol Breeding 2011, 27:549-560.

Mol Breeding (2011) 27:549–560
DOI 10.1007/s11032-010-9453-x

Inheritance of growth habit detected by genetic linkage analysis using microsatellites in the common bean (*Phaseolus vulgaris* L.)

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Received: 29 January 2010 / Accepted: 26 April 2010 / Published online: 20 May 2010

Abstract The genetic linkage map for the common bean (*Phaseolus vulgaris* L.) is a valuable tool for breeding programs. Breeders provide new cultivars that meet the requirements of farmers and consumers, such as seed color, seed size, maturity, and growth habit. A genetic study was conducted to examine the genetics behind certain qualitative traits. Growth habit is usually described as a recessive trait inherited by a single gene, and there is no consensus about the position of the locus. The aim of this study was to develop a new genetic linkage map using genic and genomic microsatellite markers and three morphological traits: growth habit, flower color, and pod tip shape. A mapping population consisting of 380 recombinant F10 lines was generated from IAC-UNA × CAL143. A total of 871 microsatellites were screened for polymorphisms among the parents, and a linkage map was obtained with 198 mapped microsatellites. The total map length was 1865.9 cM, and the average distance between markers was 9.4 cM. Flower color and pod tip shape were mapped and segregated at Mendelian ratios, as expected. The segregation ratio and linkage data analyses indicated that the determinacy growth habit was inherited as two independent and dominant genes, and a genetic model is proposed for this trait.

Keywords SSR markers · Qualitative traits · Phenotypic markers · Molecular mapping · *Phaseolus vulgaris* L.

Abbreviations

AFLP	Amplified fragment length polymorphism
CTAB	Cetyltrimethylammonium bromide
CNL	Coiled-coil nucleotide-binding site leucine-rich repeat
EST	Expressed sequence tag
IAC	Agronomic Institute of Campinas
PCR	Polymerase chain reaction

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RAPD	Random amplified polymorphic DNA
RIL	Recombinant inbred line
RFLP	Restriction fragment length polymorphism
SSR	Single sequence repeat
UC	IAC-UNA × CAL143

Introduction

The common bean (*Phaseolus vulgaris* L.) is a major staple food crop in Africa and Latin America, and is valued as one of the primary protein sources in the diet of low-income populations in developing countries. Moreover, it is a legume that is favorable to genetic studies because it has certain model plant characteristics, such as a small genome (637–675 Mb per haploid genome; Arumuganathan and Earle 1991; Guo et al. 2007), diploidy ($2n = 2x = 22$), and a highly self-pollinated breeding system.

The cultivated bean is morphologically diverse and has variations in many traits, such as growth habit, phenology, pod pigmentation, and seed pigmentation (Singh et al. 1991). Determinacy is an important agronomic trait in the common bean. Indeterminate growth habit was first reported to be controlled by a single dominant gene by Lamprecht (1935). It is considered a simple trait, and there is no consensus about its genomic location or the number of loci involved (McClean et al. 2002).

Genetic maps provide useful information for many studies, such as the localization of genomic regions that control phenotypic traits, synteny studies, and gene cloning. Moreover, they can be used to study the genetic architecture of quantitative traits. Morphological traits can be used as markers when qualitative variation and discrete phenotypic segregation classes are present.

Microsatellites or simple sequence repeats (SSRs) (Tautz and Renz 1984) are one of the most popular markers used to study polymorphisms between DNA sequences. Variations in these repetitive sequences within loci are detected using polymerase chain reaction (PCR). Microsatellites present a high level of polymorphism, co-dominance, multi-allelism, and a Mendelian pattern. They have been used in genetic analyses in mammals (Moore et al. 1998), insects (Harper et al. 2003), birds (Primmer et al. 1997), fishes (Rico et al. 1993) and plants (Condit and Hubbell 1991; Yang et al. 1994; Yu et al. 2000).

As a result of research efforts from many groups aiming to increase knowledge of the common bean, microsatellites have recently been described (Yu et al. 1999; Gaitán-Solís et al. 2002; Métais et al. 2002; Blair et al. 2003, 2008; Buso et al. 2006; Hanai et al. 2007; Benchimol et al. 2007; Campos et al. 2007; Cardoso et al. 2008). When microsatellites are derived from ESTs (expressed sequence tags), they can be associated with genes of known function and can be used as functional markers, tagging genes of interest in a more efficient manner (Hackuf and Wehling 2002; Oliveira et al. 2009).

Genetic mapping of the common bean began with the use of morphological markers and isozymes (Basset 1991). The maps developed by Vallejos et al. (1992), Gepts et al. (1993), Nodari et al. (1993) and Adam-Blondom et al. (1994) were first created using restriction fragment length polymorphism (RFLP)-based markers. A core map was established in the common bean that was based on a recombinant inbred population BAT 93 × Jalo EEP558 (Freyre et al. 1998). Random amplified polymorphic DNA (RAPD) (Adam-Blondom et al. 1994; Bai et al. 1997; Rodríguez-Suárez et al. 2006) and amplified fragment length polymorphism (AFLP) (Tar'an et al. 2002) were also used to construct linkage maps for the crop.

Microsatellites have been used in diversity analyses (Blair et al. 2006a, 2009; Zhang et al. 2008) and genetic maps (Yu et al. 2000; Blair et al. 2003, 2006b; Grisi et al. 2007), but some linkage groups that exist have low saturation and gaps. A central effort of the research on the common bean genome is to integrate physical and genetic maps. Schlueter et al. (2008) recently published a draft physical map for the common bean genome. Our primary objective was to characterize new microsatellites and develop a linkage map based on genic and genomic microsatellite markers. Another major objective was to map phenotypic markers and to study the genetic control of the growth habit trait.

Materials and methods

Mapping population

A recombinant inbred line (RIL) population was obtained from a cross between the CAL143 and IAC-UNA inbred lines. CAL143 is an Andean line with

cream coat color, red stringed seeds, and calima type. IAC-UNA is a black seeded variety developed by the Agronomic Institute of Campinas (IAC) and is from the Mesoamerican gene pool. The two parents are divergent for many phenotypic traits and have contrasting responses to many common bean pathogens. This mapping population was advanced by inbreeding to the F10 generation and 380 lines were obtained.

Microsatellite amplification and analysis

Total genomic DNA for all recombinant inbred lines was isolated from bulked young leaves of ten plants per line, using the CTAB extraction method as described in Hoisington et al. (1994).

A total of 871 microsatellites were tested for polymorphisms among the CAL143 and IAC-UNA lines. Of these, 567 have been published previously. There were 44 markers from the PV series (Yu et al. 2000; Caixeta et al. 2005); 46 markers from the BM, AG, and GAT series (Gaitán-Solís et al. 2002); 2 markers from the PVbng series (Murray et al. 2002); 49 markers from the Bmd series (Blair et al. 2003); 15 markers from the M series (Yaish and Pérez de la Vega 2003); 18 markers from Guerra-Sanz (2004); 71 markers from the PVBR series (Buso et al. 2006; Grisi et al. 2007); 80 markers from the PvM and FJ series (Hanai et al. 2007); 222 markers from SSR-IAC series (Benchimol et al. 2007; Cardoso et al. 2008); and 20 markers from the FJ series (Campos et al. 2007). The gene-based microsatellites used were from the PvM series that was developed from ESTs, Bmd, and PV from Yu et al. (2000) and the markers from Guerra-Sanz (2004) that were developed from GenBank gene searches. The remaining 304 tested markers were developed in this work according to Campos et al. (2007). The polymorphic markers developed are further described in Table 1.

Microsatellite fragments were amplified using PCR, with each reaction containing 40 ng of template DNA, 0.8 μM each of forward and reverse primer, 150 μM of each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris–HCl, 50 mM KCl, and 0.5 U Taq DNA polymerase. Reactions were performed using the following cycling conditions: 1 min at 94°C; followed by 30 cycles of 94°C for 1 min, the specific annealing temperature for 1 min and 72°C for 1 min; and a final step of 72°C for 5 min. Some microsatellites were mapped using cycles of touchdown (TD) PCR (Table 1), as described by Hanai et al. (2007). PCR products were separated using electrophoresis in denaturing polyacrylamide gels (6% w/v) and visualized using silver staining (Creste et al. 2001). The molecular size of the fragments was estimated using a 10-bp ladder (Invitrogen). Fluorescent primers were used for 39 loci as described by Schuelke (2000), and these loci were genotyped using an ABI 3730 automatic sequencer.

Morphological markers

Three morphological markers were evaluated in the mapping population: flower color, growth habit, and pod tip shape. Flower color is controlled by the *V* locus (Lamprecht 1939) and segregates as purple (IAC-UNA) and pink (CAL143) in the mapping population. Growth habit segregates as determinate type I, present in the Andean parent, and indeterminate type III, present in the Mesoamerican parent. Determinacy has been described as a qualitative trait controlled by the *fin* locus (Lamprecht 1935; Rudorf 1958). The pod tip shape can be curved (*Ct*; Al-Mukhtar and Coyne 1981), present in IAC-UNA, or straight (*ct*), present in CAL143.

Segregation and linkage analyses

The chi-square (χ^2) test for 1:1 segregation ratios was performed for all polymorphic markers. The expected segregation ratios were tested based on *P*-values after performing Bonferroni corrections (Lynch and Walsh 1998). The linkage map was constructed using the software MAPMAKER 3.0b (Lander et al. 1987). The threshold for considering markers to be linked was a LOD score of 3.0 and a maximum genetic distance of 37.5 cM using the Kosambi (1944) map function.

To order the markers within groups, markers with known positions in other published maps were anchored in the corresponding group. Markers whose position could not be anchored with high precision were verified based on their LOD score.

Ordering analyses of markers within groups were performed following the guidelines suggested by Mollinari et al. (2009). First, a subset of nine informative markers was chosen based on missing data and segregation distortion. For each group, all possible

Table 1 Description of the polymorphic microsatellites for CAL143 and IAC-UNA that were developed, including primer sequences, repeat motifs, allele sizes and annealing temperatures (T_a)

	Locus/GenBank accession no.	Primer sequence (5′–3′)	Motif	Size (bp)	T_a (°C)
1	SSR-IAC223/FJ529449	GTGGCACCTGGAATAAGACAAC ACAAATGCTCACTCACCAAATC	(AG)15	214–220	TD*
2	SSR-IAC224/FJ529450	ATTATGGATTTAGGGTAGGTGA GCTTGACAATTGGAAGTGG	(TC)13	116–120	TD
3	SSR-IAC226/FJ529452	TTTTTCTCGTTTTACTTTTATCTG TACTGATTTATTTCTTCCACTTC	(TG)8	150–155	60
4	SSR-IAC227/FJ529453	GGATCTGTGCCTTCTCTGTG TTCCATATCCCCAAAACTT	(AG)18	135–148	45
5	SSR-IAC228/FJ529454	CAAATTTTTGCTTGAACTGAT TTCTCTCCTCAAATGTAACTAAC	(CA)6	203	60
6	SSR-IAC229/FJ529455	CCTAAGGATGAACCACTCTAATA ACCCTTGCATGTGTTGTTT	(CA)7	198	60
7	SSR-IAC230/FJ529456	GGATTCGGCATTTGATAGAC AGAGAAGAAATTAGGGAGAACAG	(CT)6	188–190	60
8	SSR-IAC231/FJ529457	TGCATTTTGAAAGGACAGAT ACCAGGCATAGGTAGGACA	(TA)7 A (AG)16	246	60
9	SSR-IAC232/FJ529458	GGTTTGCATTCTTCATTTATTT ACCCATTCCCAGACTTCC	(TG)6(TA)5	241–245	60
10	SSR-IAC233/FJ529459	GGCAACTCTAAGGCAATCC CGAAGAATGTCAGAGAAATAAAC	(GT)9(TA)7	271	60
11	SSR-IAC234/FJ529460	GACCGTTAAAATGGAATCAGTT TGCCTATGTTTATGTGCTTGTT	(TC)5	258	60
12	SSR-IAC235/FJ529461	TTCAGCATGCATATTCAAGTGT GGCTCCCAGGCATAGTTCT	(TA)10	253	60
13	SSR-IAC236/FJ529462	TCTTCCTCTTCCTCTTCCTCTA TCTGATGTGGCAACGATAAAAT	(CT)12	170	60
14	SSR-IAC237/FJ529463	TGAAAGGCCACAACGACAAGT GCACCCAATCTCCCACAAA	(GT)2 AT (TG)5	155–160	60
15	SSR-IAC238/FJ529464	GAGATAGATCATAGACCCCAA CTTTCAATTTTAACTCATCCTC	(AG)18	233	60
16	SSR-IAC239/FJ529465	AAAGAAAAAGTGGGGAAAGA GCTCATGCAAAAATAGACTCA	(AG)15	247	60
17	SSR-IAC240/FJ529466	TGAATGTATGAGATTTCGTAGTTA ATTTTATTAGTGGCATTGTCCT	(CT)10	213–228	60
18	SSR-IAC241/FJ529467	AATGTGGCTATGATGGAAGAGG AAGAGAATGGGGGAAAAGGAG	(TC)8	151	60
19	SSR-IAC242/FJ529468	ATGAGATGCGAGGATTTTTTAT GGATGGGTAAGAAGGCTGAA	(CA)5	242–280	60
20	SSR-IAC243/FJ529469	GCTGGTGAGGCTTGTTGA AATTGACCTTGGATCTTCTTAG	(AT)2 (GT)3	145	60
21	SSR-IAC244/FJ529470	CGCGTTGTATTGCAGTAAAGAA AACTCCGTGGAACCCTGTGAT	(TC)9	192	60

Table 1 continued

	Locus/GenBank accession no.	Primer sequence (5′–3′)	Motif	Size (bp)	T_a (°C)
22	SSR-IAC245/FJ5294471	TAGGCCATCATCATTCAGTTTG GGTTGCCCTCATAGTCATCAC	(AT)2 (GT)3	185–192	60
23	SSR-IAC246/FJ529472	TTCCCTTCATGGTCAACA CAGTAACTACAGATCAGCACAC	(AG)10	225–228	60
24	SSR-IAC247/FJ529473	TGGATGAGACGGCTTTT CTAGATACATTCTTGTCTTCAGT	(AT)3 (GT)3	210–214	TD
25	SSR-IAC248/FJ529474	TTATAAGTTTACATCAGTTTGGTT AGAATCAGGCCTTGCTTGT	(GT)2 GC (GT)4	286–288	TD
26	SSR-IAC249/FJ529475	TTTATGAGTGGTCGAAGTGAAC AAGGGGAGGGTATTAGACAGT	(AG)9	227–231	60
27	SSR-IAC250/FJ529476	TATCTAGATCTTTGCCCTTGAA CATTGCCTGTATTGTTTGAA	(GT)4 AT (GT)3	238	60
28	SSR-IAC251/FJ529477	AAGTCTTGCCCTTTTTGTTTAT TTTTTGGCTAAGTTGGTTCC	(AC)11 (AT)12	278	45
29	SSR-IAC252/FJ529478	AATGAATTCGCAACTGTC GTGGCTAAGATGAAGAACAT	(GA)5	303–305	60
30	SSR-IAC253/FJ529479	GTTCGGTCGGGGATGGA TTAGACGGCTACTGGCAAAGAG	(GA)10	218–220	60
31	SSR-IAC254/FJ529480	TTGGGTTTTATGTTTTCTGTGA CCATATCTTGTTGTGCCTTGAC	(TG)6	245–249	60
32	SSR-IAC255/FJ529481	TTACAATTCACAACAACAGAGA TTAAGAGAAAATGAAGGATGAG	(AG)23	148–168	60
33	SSR-IAC256/FJ529482	TATTTTATTAGTGGCATTGTCC TGAGAGATCCTGTAGAAGTAGTAGT	(GA)9	156–160	60
34	SSR-IAC257/FJ529483	GCAACTGAAAGGCTAAGATT TATTGGAAAATATGGGAGAA	(GT)8	300–302	60
35	SSR-IAC258/FJ529484	CATTGTCGGTGTCGGAGAAGTC CCCACGCTCTTGTTGCTGTC	(GT)7	175–178	60
36	SSR-IAC259/FJ529485	TATATGCCTGCACCACTGTAAC GAAAATCCGGAAACTCAAGAA	(TC)11	241–247	45
37	SSR-IAC260/FJ529486	TGAACAGTGCAGCAGTAACAA CACCAGACACCAATCATCAA	(AG)9	119–129	60
38	SSR-IAC261/FJ529487	TTCCCAAACACCACACCTAAGT TCACCGCGCACGAGATAA	(AC)8	260–270	60
39	SSR-IAC262/FJ529488	ATATCGTTTGATATCCTTACACA CAAACACTGGTTCACATCTCAC	(GT)9	243–245	60
40	SSR-IAC263/FJ529489	TGCAATTGACAAAAAGTTCGTA TGTATGATAGGCCTCCACCA	(AC)9	156–160	60
41	SSR-IAC264/FJ529490	TGGGATCTGTGCCTTCTC TTCCATATCCCCAAAACTT	(GA)15	125–135	45
42	SSR-IAC265/FJ529491	GTAGGTTTGTGTGCGTGC GGAAAGAAAGTTAAGATTGAGT	(TG)5	245–247	60
43	SSR-IAC266/FJ529492	TTGAGGATGTAGATTATTTTGTT CATCATTTGTGCAGTTACCAG	(TA)5 (TG)8	269–273	60

Table 1 continued

	Locus/GenBank accession no.	Primer sequence (5′–3′)	Motif	Size (bp)	T_a (°C)
44	SSR-IAC267/FJ529493	TGAGTGAACCAGCATAATCTAA CACCCGGTTGAAAATACA	(CT)15	139–162	51.4
45	SSR-IAC268/FJ529494	ATGTAGATCTGGTAAGGAGTGAA AATTAGATTAGTCAAGAAACAAAAC	(TC)9	190–193	60
46	SSR-IAC269/FJ529495	TGCGCCACTGTTTCGTATT ACCCAACCCAGGACTTCAC	(AC)6	145–147	60
47	SSR-IAC270/FJ529496	ATGTGTTTAGCAAGGATGGTCT GCTGATTTGCTGTAACGAAC	(TG)6	214–216	60
48	SSR-IAC271/FJ529497	GATTTTCTTTTCTTCCCTCAA TGCTTCCATTCCATCAA	(CA)7	227–228	49
49	SSR-IAC272/FJ529498	TAACTGAACAGGCTAAAAGAAC TGATGAGACTAAAAATGACACTT	(CA)6	210–215	60
50	SSR-IAC274/FJ529500	TCTACACATAACTGCCCTGAAC AACACTGCCACAACTAAAGAAA	(TG)7	200–205	60
51	SSR-IAC275/FJ809021	AGCAACTCATAGCACTTCTG TGAGTAATTCATTGTCTGTCCT	(TA)3G(AC)6	255	48.6
52	SSR-IAC276/FJ809022	AAGCCCAAGCCCAAACCAG CACCACCAAGACCCGATTCAG	(GAA)4(GT)7	249	52.8
53	SSR-IAC277/FJ809023	ATGGAAGGCTGCAATACATCAG CAGCGACGGTGCTTACTGG	(AC)6	286	52.1
54	SSR-IAC278/FJ809024	TCTGTCAGTTTAGCTTCGTCAC CCGTTGGAGGGTCGTTAC	(CT)10	191	51.9
55	SSR-IAC279/FJ809025	CAATACCACAAAACGCAA CATTATGATTAAATTCTGGTGT	(TG)3GG(TG)2	220	45.4
56	SSR-IAC280/FJ809026	ACTCCTGGCAAAAATCTCG GCAAAACCTCCATGAAGACAG	(TTGA)3	141	49.0
57	SSR-IAC281/FJ809027	AGTCCAGGCAGAGCAAGCAAG CGCATTCATCTCATCCATTATCC	(GT)9	189	52.7
58	SSR-IAC282/FJ809028	CTCTTGGTTACGCGTGGACTAC CGCCTATGTTGGGTTGAGATG	(CAA)2T(CA)3	234	54.8
59	SSR-IAC283/FJ809029	GAACTCATTCCCCTCTCC GCAGCTCCTCCATTCTAC	(TTA)7	208	47.9
60	SSR-IAC284/FJ809030	AAGGAGAAAATAAAATCACAGTC GTTTCGAGGTTATTGGGGA	(CT)13	240	50.7
61	SSR-IAC285/FJ809031	ACCTAGATGGATTTGTGACC GTCTAGTTTTACGCATTCATTC	(AT)11(TG)11(TA)6(AG)7	204	48.1
62	SSR-IAC286/FJ809032	TTGTTCTGAGGACTGGATGTAT TCGGTTGATTTTTCGTTTTA	(TC)18	181	49.1
63	SSR-IAC287/FJ809033	ACAACGTTAGAAAGGGAAGG GAAAAAAGCGAGAAAAAGAGT	(GA)13	179	51.4
64	SSR-IAC288/FJ809034	TCCCGTGAGTTACAAATAG ATCACCACTCCTCTCCAG	(ATTT)4	182	46.6

TD: Touchdown PCR described in Hanai et al. 2007

orders of the nine markers were compared based on their log-likelihoods. Additional markers from each group were added using the 'TRY' command, starting with more informative markers. The final order was verified using the 'RIPPLE' command, with a window with six markers. Finally, multipoint estimates of distance were obtained using the 'MAP' command.

Results

Microsatellite polymorphisms tests

The conditions for amplification of the 871 microsatellites were optimized and the polymorphisms were tested for the parents of the IAC-UNA × CAL143 population. A total of 248 (28.5%) microsatellites were polymorphic and presented adequate visualization patterns with no stutter or non-specific bands.

There were eight (38.1%) polymorphic markers from the PV series described by Caixeta et al. (2005), 19 (41.3%) from the BM, AG, and GAT series, one (50.0%) from the PVbng series, 38 (53.5%) from the PVBR series, 11 (26.3%) from the FJ series described by Hanai et al. (2007), 69 (31.1%) from the SSR-IAC series, and five (25.0%) from the FJ series described by Campos et al. (2007). All the 15 M series markers were monomorphic. Among the gene-based microsatellites, there were five (21.8%) described by Yu et al. (2000), 15 (32.7%) from the Bmd series, three (16.7%) described by Guerra-Sanz (2004), and 10 (25.0%) from the PvM series that were polymorphic. In total, there were 33 (25.4%) polymorphic markers from 130 gene-based markers tested and 232 (31.3%) polymorphic markers from 741 genomic-based markers. The 64 new IAC series microsatellites, described in Table 1, were also polymorphic.

Genetic map

A total of 198 microsatellites and three phenotypic markers were mapped, with distributions in all 11 linkage groups (Fig. 1). The total map length was 1865.9 cM with an average linkage group length of 170.5 cM. The average distance between markers was 9.4 cM, with an average of 18 markers in each group. The number of markers in each group varied from five (B11 group) to 37 (B2 group).

The B2, B3, B4, B5, B7, B8, and B9 linkage groups had gaps that require more saturation. The B2, B4, B8, and B9 groups have markers with genetic distances greater than 37.5 cM; in these cases, sub-groups were discriminated by segmented lines (Fig. 1). The B8 group was split into A and B sub-groups, as it was not possible to determine the correct sense order between them. The 8A subgroup was formed by ten markers with previous mapping position unknown in the literature, not allowing further comparison. While there was statistical evidence for the PVBR251 marker being linked to the B1 group, its distance from the adjacent marker (IAC235) was greater than 37.5 cM. The same phenomenon occurred in the B9 group with marker IAC242.

The Bonferroni correction was used, but even markers with segregation distortion were included in the map. Distortion was observed for 92 (37.3%) microsatellite loci, and 76 of these were positioned on the map (Fig. 1). As expected, these markers clustered in certain chromosomal regions.

Phenotypic markers

All three phenotypic markers were mapped. The *V* locus was detected in the B6 group and was closely linked to the microsatellites IAC268 (0.2 cM) and IAC183 (0.6 cM) at the edge of the B6 group. The *fin* and *Ct* loci were mapped, but their position in the linkage groups could not be estimated with sufficient accuracy; therefore they are indicated by arrows in Fig. 1. The pod tip shape trait, determined by the *Ct* locus, was linked to the B9 group and was close to the molecular markers FJ17 and PVBR60.

Phenotypic segregation (Table 2) was tested. The expected Mendelian segregation ratio (1:1) was observed for flower color and pod tip shape. However, the growth habit trait showed significant deviation from this value ($P < 10^{-5}$), which could be the result of two loci controlling this phenotype. A ratio of 259:106, which is a good fit to the ratio 3:1 ($\chi^2 = 3.62$, $P = 0.06$), was observed for growth habit, confirming this hypothesis. In addition, the F1 phenotype was 100% determinate growth habit. We propose a novel genetic model wherein two recessive loci control the phenotype of the indeterminate growth habit. For example, consider the cross *finfinfin′fin′* (indeterminate growth) × *FinFinFin′Fin′*

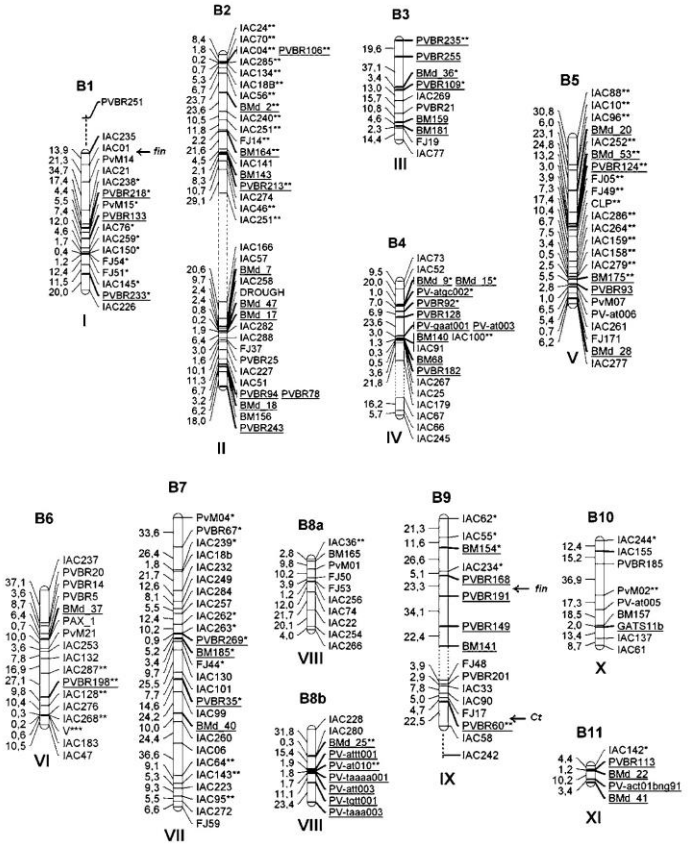

Fig. 1 Genetic map for IAC-UNA × CAL143 (UC). B1 to B11 refer to linkage groups, and I to XI are the corresponding chromosomes (Pedrosa et al. 2008). The underlined microsatellites were anchored to form the linkage groups. (*) Markers with segregation distortion to CAL143 and to (**) IAC-UNA. (***) Phenotypic marker *V* linked to group B6. *Arrows* indicate possible position of the *fin* and *Ct* loci, and dotted lines indicate distances larger than 37.5 cM

(determinate growth). Four homozygous genotypes with equal proportions are expected in a RIL population: *FinFinFin′Fin′*, *FinFinfin′fin′*, *finfinFin′Fin′* and *finfinfin′fin′*. If only the homozygous genotype with two recessive alleles (*finfinfin′fin′*) expressed indeterminate growth, a segregation of 3:1 would be expected. The growth habit trait (*fin*) was mapped in the B1 (LOD = 3.76) and B9 (LOD = 3.95) groups, supporting the hypothesis that two loci could be involved in the genetic control of this trait.

Table 2 Segregation of three qualitative traits from an F10 population of the common bean cross IAC-UNA × CAL143

Morphological marker	Segregation ratio 1:1 (CAL143:IAC-UNA)	χ^2	*P*	Map location
Pod tip shape	165:195	2.50	0.11	B9
Flower color	161:197	3.17	0.07	B6
Growth habit	259:106	64.14	$<10^{-5}$	B1 and B9

Discussion

Mapping population and polymorphism tests

The new mapping population IAC-UNA × CAL143 (UC) established in this study represents the largest population currently developed with regard to the number of recombinant inbred lines ($n = 380$) and generations of self-breeding. The UC population is able to detect recombinant events because of the high number of recombinant lines. For this reason, a well-saturated map with precise estimates of distance and marker order is expected.

The parents were chosen from different gene pools (Mesoamerican and Andean) and have contrasting features for several agronomical traits. Segregation for disease resistance, such as resistance against anthracnose (caused by *Colletotrichum lindemuthianum*), angular leaf spot (caused by *Pseudocercospora griseola*) and rust (caused by *Uromyces phaseoli*), was observed. These parents define an interesting mapping population, as several traits can be mapped.

A moderate polymorphism rate (28.5%) was found between microsatellite markers. Low diversity is expected for a cross between two cultivated genotypes. The observed polymorphism rate is similar to results reported in other studies, such as 45.7% (Blair et al. 2006b) and 42.8% (Grisi et al. 2007). If the parents were from the same gene pool, they would most likely have a narrower genetic base and less genetic variability.

One probable reason for the moderate polymorphism rate found is the extensive number of microsatellites developed with small motif repeats, considered from five units in enriched library screening (Benchimol et al. 2007; Campos et al. 2007; Hanai et al. 2007). For example, the PVBR series contains larger repeated motifs and was the most polymorphic microsatellite series (53.5%) in polymorphism tests. In this work, we described 64 new microsatellite markers that presented parental UC polymorphisms, which will be useful for other genetic studies in the common bean.

Genetic map

The final UC genetic map covered all 11 linkage groups of the common bean. The total length (1865.9 cM) was consistent with other genetic mapping studies (Blair et al. 2003; Grisi et al. 2007). Some linkage groups presented a highly dense coverage distribution of markers (e.g., B5 and B7), while others contained gaps (e.g., B2 and B8) or presented few markers (e.g., B11, with only five markers). The microsatellite-based genetic map presented by Grisi et al. (2007) also found a small number of markers in the B11 group (only four markers were detected). The Co-2 cluster, containing one of the R genes involved in anthracnose disease resistance in the common bean, was found by molecular analysis in the subtelomeric region of the B11 group (Creusot et al. 1999). R genes contain leucine-rich regions called coiled-coil nucleotide-binding site leucine-rich repeat (CNL), and can contain heterochromatic knobs. There is evidence that the Co-2 cluster, through an ectopic recombination, gives rise to the Pv B4-CNL sequences in the B4 group (David et al. 2009). The difficulty in mapping the B4 linkage group could be due to the presence of two knobs detected in the CNL subtelomeric region (Geffroy et al. 2009).

A total of 201 markers have been positioned in the UC map to date, including 131 microsatellite markers that were not mapped previously. The use of anchor markers was efficient to form the linkage groups and to order the markers within them. Among the gene-based microsatellites, in the B4 linkage group the loci *BMd9*, *Bmd15* and *PVatgc002* were mapped very close together and were derived from different phytohemagglutinin gene sequences, suggesting a single location of a gene family cluster in this region. It confirms the results of Blair et al. (2003), who found other markers (*Bmd16* and *PVatgc004*) related to phytohemagglutinin gene sequences also in the B4 group.

In the UC map, a concentration of markers with distortion to Andean alleles was detected in the B1 group, and a concentration of markers with distortion to Mesoamerican alleles in the B2 and B5 groups. In the B2 linkage group only a small region presented markers with segregation distortion. For the B5 group, the region with distortion is larger, and in the B1 group, Andean alleles predominated (Fig. 1). Blair et al. (2003) found the same segregation distortion to Mesoamerican alleles in regions of the B5 group. Freyre et al. (1998) also found the same distortion for the B1 and B2 groups for the same parental gene pools.

The phenomenon of markers with segregation distortion in the common bean has been extensively described (Freyre et al. 1998; Blair et al. 2003; Grisi et al. 2007; Hougaard et al. 2008). This phenomenon was observed not only for microsatellite markers, but also for legume anchor markers (Hougaard et al. 2008) and RFLP markers (Freyre et al. 1998). The reasons for this segregation distortion could be associated with genomic regions related to adaptive advantages that were selected during each self-breeding cycle of the population mapping development. These distortion genomic regions did not present mixture between the Andean and Mesoamerican pools, supporting an incompatible genomic region hypothesis. The distortion of Mendelian ratios associated with these markers clustering inside the linkage groups represents a trend towards retaining the ancestral pool origin.

The segregation distortion observed for some markers did not exclude them from being potentially informative for recombinant detection. For this reason, they were incorporated into the linkage analysis. The selection effect cannot be ignored, however, even in a controlled cross in a population mapping experiment. According to Shizhong (2008), the use of markers with segregation distortion could actually increase the mapping saturation and statistical power of quantitative trait locus mapping.

The phenotypic mapping of the *V* locus in the B6 group is consistent with other genetic maps, as it was also obtained by Nodari et al. (1993) and McClean et al. (2002). The *Ct* locus was previously identified by Al-Mukhtar and Coyne (1981), but it was never mapped. Flower color is related to seed color, an important trait selected by the consumer market. The markers that are tightly linked to the *V* and *Ct* loci can be used for future cloning experiments. The use of markers for simply inherited traits in marker-assisted backcrossing and introgression across Andean and Mesoamerican gene pools is also suggested.

The *fin* and *fin′* loci were mapped in two distinct groups (B1 and B9), with almost the same LOD score for each. It is necessary to obtain more markers for these groups to saturate the regions in order to estimate the positions of these loci. Koinange et al. (1996) and Blair et al. (2006b) also mapped the *fin* locus to the B1 group. Kwak et al. (2008) found a candidate gene for the *fin* locus, *PvTFL1y*, and they also mapped it to the B1 linkage group. Ta'an et al. (2001) and Tar'an et al. (2002) mapped the growth habit gene to the B9 linkage group.

The existence of only one locus to define growth habit in the common bean has been previously questioned, and it is probable that multiple genes controlling growth habit exist in the common bean (McClean et al. 2002). We propose a genetic model based on two loci (*fin* and *fin′*), according to Mendelian segregation ratios and linkage mapping results. This genetic model approach related the phenotypic and genotypic analyses of segregating progeny. The single cross UC confirmed that the two growth habit loci probably resulted from the huge effective size of the mapping population, with the segregation and linkage data analyzed together.

The source of determinacy of CAL143 may be an example of a dominant determinate genotype derived from the Andean region that has been domesticated without maize as a physical support for the climbing bean in traditional agriculture, as proposed by Koinange et al. (1996). The F1 cross UC also revealed the dominant inheritance of the determinate growth habit trait, which was usually inherited recessively. Determinant genotypes must represent different sources of genetic control of determinacy, and the frequent recessive state cannot be an exclusive state for the common bean growth habit. This is in agreement with Kornegay et al. (1992), whose results also suggested a genotype (G13624) with two dominant determinate growth habit genes.

The use of qualitative traits such as morphological markers allows the study of genetic models and represents basic genetic knowledge about any species. We propose a new genetic model regarding growth habit, a characteristic of simple inheritance that is important in an agronomic context. Further studies, such as fine-structure mapping in these regions, other crosses and segregating progeny could be performed to study both loci. It is evident that advances can be achieved in genetic breeding of the common bean with the development of genetic mapping. The presented map gives an overview of the genomic organization and provides anchor points for future maps.

Acknowledgments This work was supported by grants from State of São Paulo Research Foundation (FAPESP). The authors are also grateful Brazilian National Council for Scientific and Technological Development (CNPq) for a graduate fellowship to C.T. and grants to A.P.S.; FAPESP

for graduate fellowship to P.R.O., J.M.K.C., R.M.B., A.C.B.S. and an undergraduate fellowship to D.A.S.

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APÊNDICE II:

Oblessuc PR, et al. Dissecting *Phaseolus vulgaris* Innate Immune System against *Colletotrichum lindemuthianum* Infection. PLoS ONE 2012, 7(8):e43161.

OPEN ACCESS Freely available online

PLOS ONE

Dissecting *Phaseolus vulgaris* Innate Immune System against *Colletotrichum lindemuthianum* Infection

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Abstract

Background: The genus *Colletotrichum* is one of the most economically important plant pathogens, causing anthracnose on a wide range of crops including common beans (*Phaseolus vulgaris* L.). Crop yield can be dramatically decreased depending on the plant cultivar used and the environmental conditions. This study aimed to identify potential genetic components of the bean immune system to provide environmentally friendly control measures against this fungus.

Methodology and Principal Findings: As the common bean is not amenable to reverse genetics to explore functionality and its genome is not fully curated, we used putative Arabidopsis orthologs of bean expressed sequence tag (EST) to perform bioinformatic analysis and experimental validation of gene expression to identify common bean genes regulated during the incompatible interaction with *C. lindemuthianum*. Similar to model pathosystems, Gene Ontology (GO) analysis indicated that hormone biosynthesis and signaling in common beans seem to be modulated by fungus infection. For instance, cytokinin and ethylene responses were up-regulated and jasmonic acid, gibberellin, and abscisic acid responses were down-regulated, indicating that these hormones may play a central role in this pathosystem. Importantly, we have identified putative bean gene orthologs of Arabidopsis genes involved in the plant immune system. Based on experimental validation of gene expression, we propose that hypersensitive reaction as part of effector-triggered immunity may operate, at least in part, by down-regulating genes, such as *FLS2-like* and *MKK5-like*, putative orthologs of the Arabidopsis genes involved in pathogen perception and downstream signaling.

Conclusions/Significance: We have identified specific bean genes and uncovered metabolic processes and pathways that may be involved in the immune response against pathogens. Our transcriptome database is a rich resource for mining novel defense-related genes, which enabled us to develop a model of the molecular components of the bean innate immune system regulated upon pathogen attack.

Citation: Oblessuc PR, Borges A, Chowdhury B, Caldas DGG, Tsai SM, et al. (2012) Dissecting *Phaseolus vulgaris* Innate Immune System against *Colletotrichum lindemuthianum* Infection. PLoS ONE 7(8): e43161. doi:10.1371/journal.pone.0043161

Editor: Els J M. van Damme, Ghent University, Belgium

Received May 31, 2012; **Accepted** July 17, 2012; **Published** August 17, 2012

Funding: This study was supported in part by The University of Texas at Arlington startup funds to MM. No additional external funding received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important staple foods in developing countries and it has been suggested as a model species for studying legume crops [1,2]. Dry bean production can be drastically reduced by fungal pathogens. For instance, anthracnose caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, can result in total yield loss depending on the cultivar and environmental conditions [3,4]. In fact, anthracnose has been rated within the top 10 most important fungus-caused disease in plants based on its scientific and economic relevance [5]. Development of genetically resistant plants can minimize the occurrence of this disease and maximize crop production. To this end, it is crucial to understand the mechanisms by which plants recognize the presence of a pathogen as well as the molecular changes that occur in the host cell upon pathogen infection. Studies in model pathosystems have revealed that virulence factors interact with host proteins triggering defense responses in an incompatible reaction [6]. The same virulence factors may act as suppressors of defense responses leading to disease development in a compatible reaction [7,8].

The function of the resistance and avirulence genes involved in the *P. vulgaris–C. lindemuthianum* interaction and the kinetics of plant defense have not been fully described yet, hindering research aimed to understanding this specific pathosystem. Genetic studies indirectly indicate that this pathosystem operates in a gene-for-gene manner [9]. Different anthracnose resistance genes/locus can be found clustered in the bean genome. For instance, resistance genes against *C. lindemuthianum* and other diverse pathogens such as *Uromyces appendiculatus* (causative agent of bean rust) and *Pseudomonas syringae pv. phaseolicola* (causative agent of halo blight), were identified at the end of the linkage group B4 [10]. The

Table 1. Differentially expressed ESTs identified in both libraries based on their enzyme codes.

Up-regulated genes						
Enzyme Code[a]	**Enzyme name**	**Cellular localization**	**No. ESTs Control tissue**	**No. ESTs Inoculated tissue**	**p-value**	**RE[b]**
EC:5.3.1.6	ribose-5-phosphate isomerase	chloroplast	0	3	0.09	1.06
EC:3.5.1.1	asparaginase	unknown	0	3	0.09	1.06
EC:2.3.1.0	unknown	membrane	0	3	0.09	1.06
EC:1.1.1.95	D-3-phosphoglycerate:NAD+ oxidoreductase	chloroplast, nucleus, cytosol, mitochondria	0	3	0.09	1.06
EC:3.2.1.39	endo-1,3- β -glucanase; callase	vacuole, endomembrane system	1	5	0.09	1.02
Down-regulated genes						
Enzyme Code[a]	**Enzyme name**	**Cellular localization**	**No. ESTs Control tissue**	**No. ESTs Inoculated tissue**	**p-value**	**RE[b]**
EC:4.1.1.39	ribulose-bisphosphate carboxylase	chloroplast	144	75	0.002	−2.66
EC:2.7.11.25	MAPKKK; MEKK	cytoplasm, plastid, plasma membrane	8	1	0.05	−1.30
EC:2.1.2.1	Serine and threonine aldolase	cytosolic ribosome, membrane, apoplast	5	0	0.07	−1.15

The enzyme codes were established using KEGG as part of the Blast2GO suite. Statistical significance was calculated with the Fisher's exact test ($p \leq 0.1$).
[a]The ESTs names corresponding to enzyme code are described in Table S1.
[b]RE = relative expression values were obtained by -Log_{10} of p-values for the up-regulated transcripts and by Log_{10} of p-values for the down-regulated genes, according to Zhou *et al.* [79].
doi:10.1371/journal.pone.0043161.t001

anthracnose resistance genes, named *Co*, are single dominant genes in the host and specific race/cultivar interaction exists [11]. Among these, *Co-4* located on a gene cluster at the linkage group B8 [12], confers the broadest-base resistance to anthracnose [13] making it very attractive for genomic studies with direct application to agricultural problems. For instance, the bean breeding line SEL 1308 carries the *Co-4*^{2} gene for anthracnose resistance [12] and when inoculated with 34 selected races of C. lindemuthianum chosen to represent a diverse sample of the pathogen population, SEL 1308 demonstrated a resistance index (RI) of 97% [13]. An incompatible interaction between a bean cultivar carrying a *Co* gene and an avirulent race of *C. lindemuthianum* leads to the formation of necrotic spots in the host tissue typical of hypersensitive reaction (HR) and localized host-cell death [14]. Hypersensitive reaction is characterized by an oxidative burst that occurs by the generation of reactive oxygen species (ROS) resulting in programmed cell death [15]. This process is an early response in many pathosystems and in common bean it appears to be dependent on three components: an exocellular peroxidase, an extracellular alkalinization that occurs due to calcium influx and potassium efflux, and release of a substrate [16,17].

The *P. vulgaris–C. lindemuthianum* pathosystem has been genetically studied in attempts to clone resistance genes using map-based approach [18,19,20], to isolate resistance gene analogs [10,21,22], and to assess the expression of resistance gene candidates [12,23,24,25]. The infection process and establishment of compatible interaction have also been well characterized at the cytological level [26,27,28]. Differential accumulation of specific defense-related transcripts such as mRNA for polygalacturonase-inhibiting protein (PGIP) and pathogen related (PR) proteins, during compatible or incompatible interaction between common bean and *C. lindemuthianum* has also been reported [29,30]. More recently, genes encoding glutamine synthetase (GS1α), formate dehydrogenase, and EF-hand calcium-binding have also been implicated in immune response against *C. lindemuthianum* [31,32,33]. A few gene expression studies using northern blot were also performed, showing the up-regulation of genes during the incompatible interaction [34,35].

As genomic and genetic resources (*e.g.*, extensive gene annotation and mutant lines) are scarce for common beans, transcriptome analyses may be a fast and cost-effective way to find differentially regulated genes under stress ultimately leading to the characterization of key steps in the defense response. Expressed sequence tag (EST) libraries are useful resources for mapping of expressed genes [36,37] as well as for providing data for comparisons with related species such as soybean [38] or other plant model organisms such as Arabidopsis and rice [24]. Some EST libraries were developed to identify bean genes related to response to biotic and abiotic stresses such as bean rust [39], drought, low soil phosphorus, and high soil aluminum toxicities [40,41,42], as well as genes expressed during the development of pods, leaves [42], and seeds [43].

In this study, we used EST libraries developed from seedling shoots inoculated or not with *C. lindemuthianum* [24] to examine overall changes in gene expression during the incompatible interaction between the resistant bean breeding line SEL 1308, which carries the *Co-4*^{2} anthracnose resistance gene, and the avirulent race 73 [13]. We uncovered metabolic processes and pathways that may be involved in the common bean innate immune response against pathogens and develop a model representing key components associated in this interaction. In addition, we identified known and novel specific bean genes associated with immune response and experimentally validated gene expression inferred by bioinformatic analysis. Our result should provide insights for the developments of molecular tools (*e.g.* marker for differentially regulated genes) to be used in bean breeding programs as well as basic genetic information for functional annotation of the bean genome.

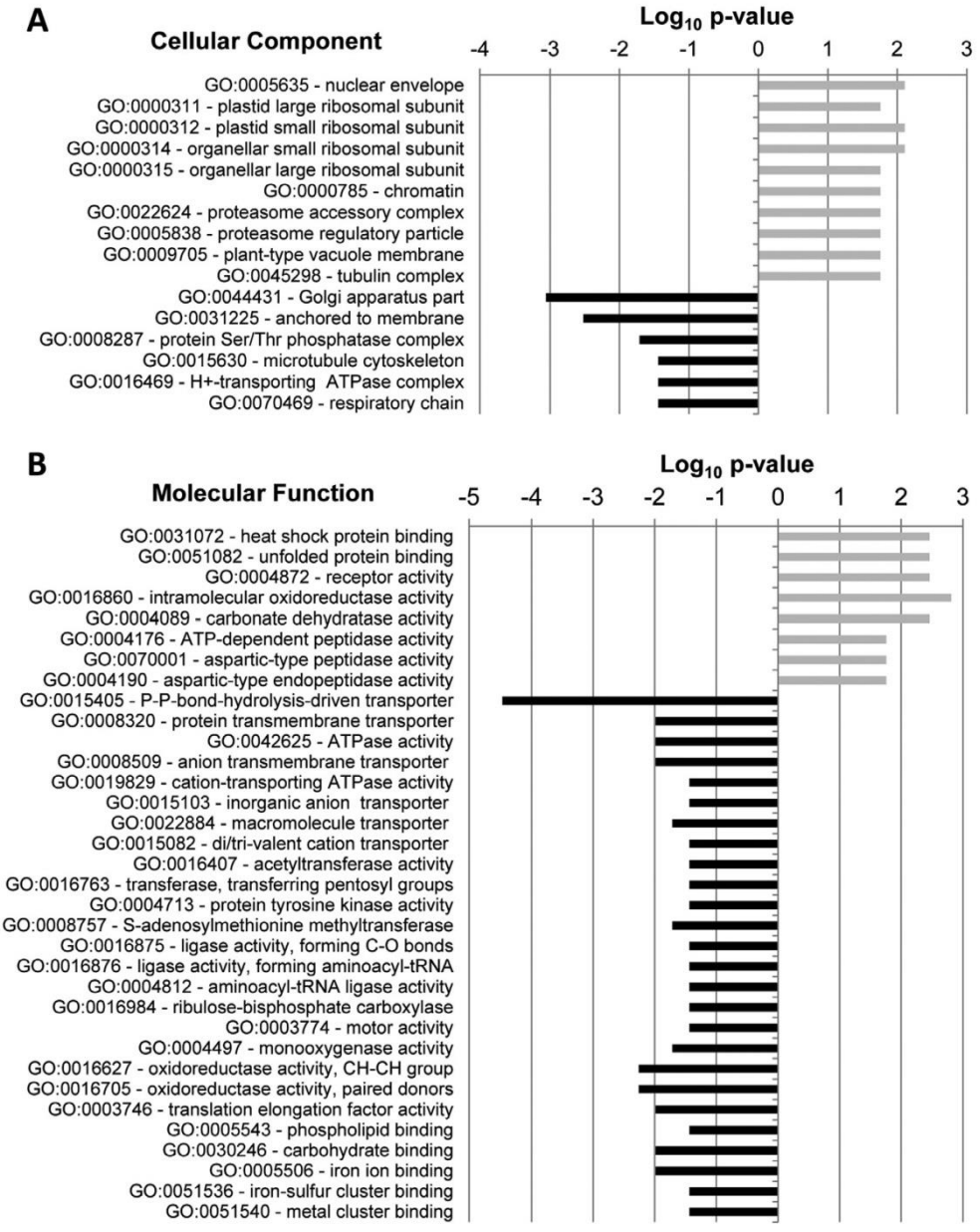

Figure 1. Gene Ontology categories uniquely identified in either EST collections using the AgriGO Singular Enrichment Analysis (SEA). Processes identified only in fungus-inoculated tissue are considered up-regulated by fungal infection (grey bars) and processes identified only in mock-inoculated tissue are considered down-regulated (black bars). Statistical significance was detected with the Fisher's exact test (p-value ≤ 0.05) and data points are indicated as Log_{10} of the p-value. (A) GO terms in the cellular component category. (B) GO terms in the cellular function category. GO terms in the biological processes category are indicated in Fig. S1.
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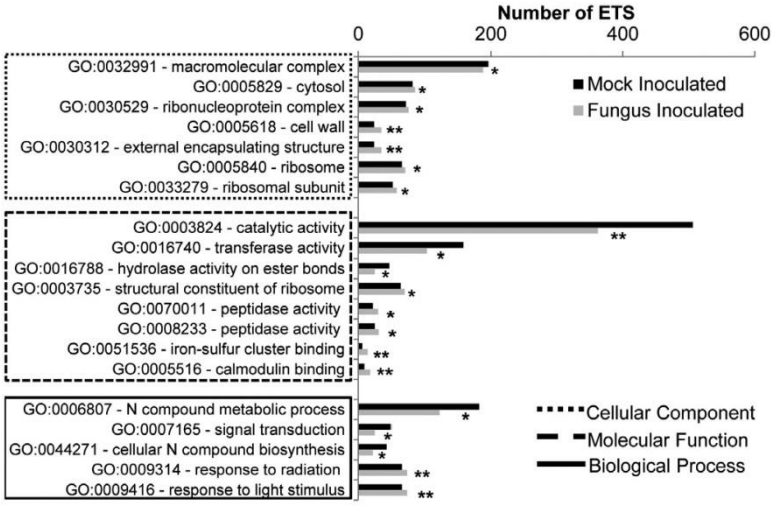

Figure 2. Gene Ontology categories identified in both EST collections using the AgriGO Singular Enrichment Analysis (SEA). Data points represent the number of ESTs in each collection that was placed in each GO category. Statistical significance of the relative abundance of ESTs in each collection was detected with the Fisher's exact test (* = p≤0.1, **p≤0.05).
doi:10.1371/journal.pone.0043161.g002

Results and Discussion

Gene Ontology Analysis of *Phaseolus vulgaris* Transcripts

To assess the overall changes in the transcriptional profile during the incompatible interaction between *P. vulgaris* and *C. lindemuthianum*, we compared two collections of ESTs; one collection obtained from two libraries (PVEPLE1 and PVEPSE2) constructed with control, mock-inoculated seedling shoots and another one obtained from a library (PVEPSE3) constructed with fungus-inoculated seedling shoots [24]. Fungal penetration in the host cell occurs within 54 hours post inoculation (hpi) and the proportion of affected cells increases over time [44]. Previous studies have shown that necrotic cells appear in bean leaves 72–96 hpi with an incompatible race of the fungus [44], when the cytoplasm of epidermal cells beneath appressoria appeared granular and pale brown in color [27] and the fungus complete its biotophic phase around 72 hpi [14,44,45]. Therefore, we collected plant tissue 65 hpi to construct the EST libraries, when the majority of host cells may be infected by the fungus and the HR is still ongoing.

Initially, each EST was aligned (tBLASTX) against the non-redundant database using the Blast2GO suite [46] to assess the overall enzymatic activity affected by fungus infection (Table S1). Interestingly, 20% of ESTs from inoculated tissue and 13% of EST from mock-inoculated tissue had no hit to any sequence. We identified five enzyme categories that were more significantly abundant in the EST collection from fungus-inoculated tissue and three that were more significantly abundant (p≤0.5) in the mock-inoculated tissue (Table 1). Enzymes in two of these categories, 1,3 β-D-glucanase and MAPKKK, are well known to play important roles in plant defense against pathogens [47,48]. Intriguingly, the gene encoding for a 1,3 β-D-glucanase was up-regulated while MAPKKK-encoding gene was down-regulated in fungus-inoculated tissue.

As *Arabidopsis thaliana* (L.) Heynh has one of the most comprehensively annotated and manually curated genome, and in many cases supported by extensive experimental data, individual bean ESTs were aligned (tBLASTX) with Arabidopsis transcript sequences using the TAIR10 database (http://arabidopsis.org/) to enable functional comparison between our EST libraries. Out of 2,923 ESTs from mock-inoculated tissue and 2,232 ESTs from inoculated tissue, 2,489 (85%) and 1,843 (83%) showed significant similarities (E-value≤1×10^{-4}) with Arabidopsis transcripts, respectively (Table S2). Putative Arabidopsis gene orthologs (AGI numbers) were used to identify GO numbers (Table S2) so that: 1) the overall changes in metabolic processes occurring in inoculated tissues could be identified through Singular Enrichment Analysis (SEA) using AgriGO [49]; 2) the relative expression of transcripts could be assessed in each EST collection to identify specific bean genes regulated upon inoculation; 3) regulation of specific genes by different stimuli, their location in the cell and their involvement in specific biochemical activities could be assessed; and 4) specific bean genes could be chosen to validate bioinformatics analysis using qPCR.

Metabolic Processes in *Phaseolus vulgaris* Regulated upon *C. lindemuthianum* Infection

To identify metabolic processes that are specifically modulated by fungus infection, we annotated the bean transcripts using GO terms and identified both EST library-specific and differentially represented GOs. Overall, 842 GO terms were identified, out of which 134, 195, and 513 GO terms were within Cellular Component (GO:0005575), Molecular Function (GO:0003674), and Biological Process (GO:0008150), respectively (Table S3). Out of these, 136 GOs were found to be library-specific and significantly regulated (p≤0.05) indicating substantial metabolic changes in response to fungus (Fig. 1 and S1).

Table 2. Differentially expressed transcripts represented by ESTs identified in both libraries.

AGI number[a]	Annotation	Cellular localization	No. ESTs Control tissue	No. ESTs Inoculated tissue	p value	RE[d]
Up-regulated transcripts						
ATCG00470	ATP synthase epsilon chain (ATPE)	mitochondria	3	11	0.01	1.89
AT2G39730	Rubisco activase	nucleus, chloroplast	22	31	0.05	1.29
Down-regulated transcripts						
AGI number	**Annotation**	**Cellular localization**	**No. ESTs Control tissue**	**No. ESTs Control tissue**	**p value**	**RE[d]**
AT2G34430	Type I chlorophyll a/b-binding protein of Photosystem II	chloroplast thylakoid	83	14	<0.001	−9.50
AT5G54270	Type III chlorophyll a/b-protein complex of Photosystem II	chloroplast thylakoid	33	8	<0.001	−3.00
AT5G38430	Ribulose bisphosphate carboxylase	chloroplast thylakoid	55	20	<0.001	−2.70
AT2G05100	Type II chlorophyll a/b-protein complex of Photosystem II	chloroplast thylakoid	22	5	0.01	−2.22
AT3G61470	Type II chlorophyll a/b-protein complex of Photosystem I	chloroplast thylakoid	26	8	0.02	−1.82
AT1G61520	Type III chlorophyll a/b-binding protein of Photosystem I	chloroplast thylakoid	14	3	0.03	−1.55
AT1G67090	Ribulose bisphosphate carboxylase	chloroplast thylakoid	91	51	0.04	−1.40
AT1G15820	Light harvesting complex of photosystem II (LHCB6)	chloroplast: envelope, thylakoid, and thylakoid membrane; plastoglobule	20	7	0.08	−1.10
AT2G13360	Alanine glyoxylate aminotransferase (AGT1)	chloroplast stroma, peroxisome, plasma membrane	7	1	0.08	−1.08
AT1G08380	Photosystem I subunit O (PSAO)	chloroplast thylakoid	14	4	0.09	−1.03

The AGI number indicates putative Arabidopsis orthologs of bean transcripts identified with tBLASTX search against the TAIR10 database (E-value $\leq 1\times10^{-4}$ was considered as significant). Statistical significance was calculated with the Fisher exact test ($p\leq0.1$).

[a]The bean EST orthologs of each AGI number are listed in Table S2.

[b]RE = relative expression values obtained by $-\text{Log}_{10}$ of p-values for the up-regulated transcripts and by Log_{10} of p-values for the down-regulated genes, according to procedures described by Zhou *et al.* [79].

doi:10.1371/journal.pone.0043161.t002

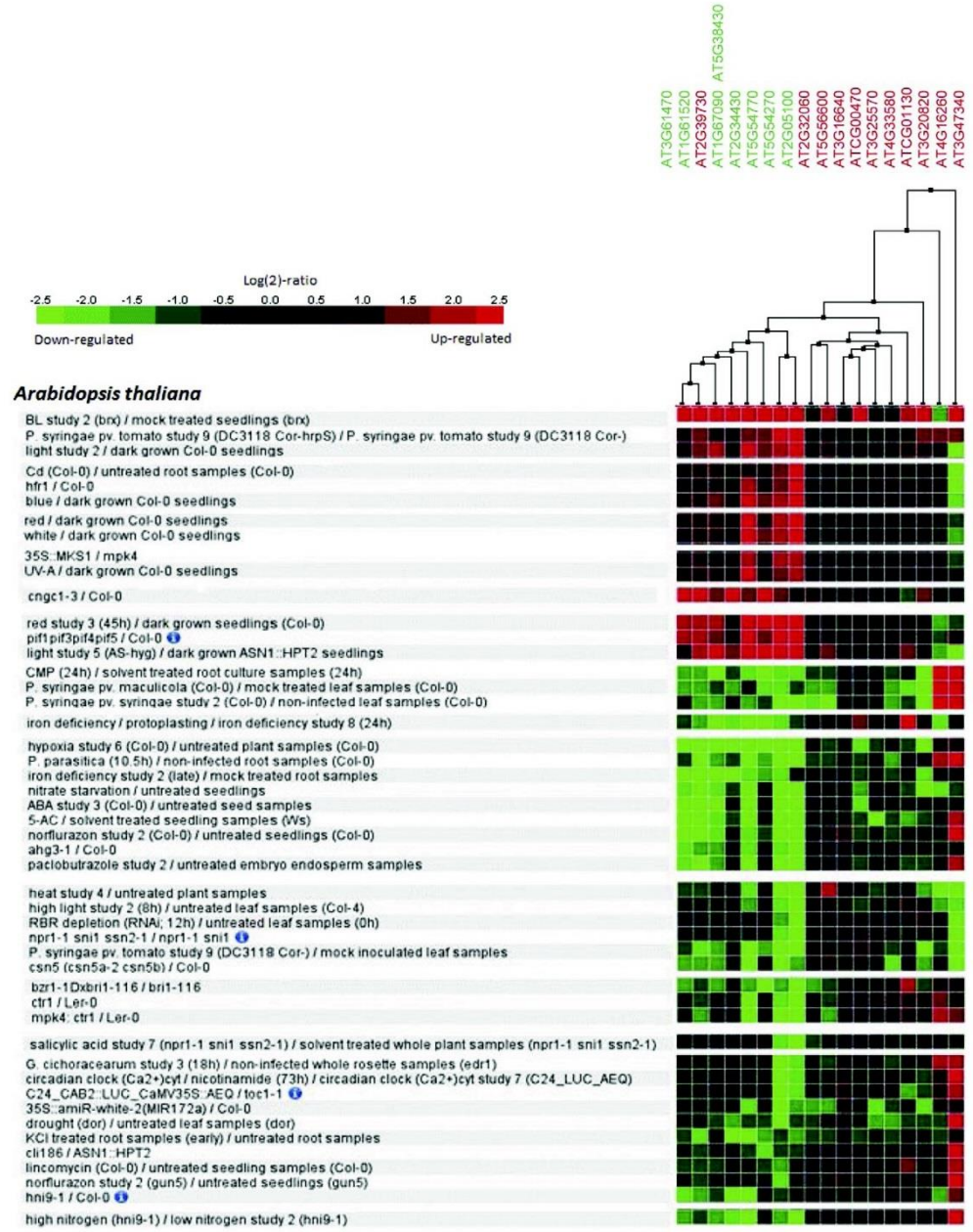

Figure 3. Patterns of expression of differentially expressed genes in fungus-inoculated tissue (p≤0.05). Expression of these genes in response to several other stresses was assessed through the publicly available microarray database, TAIR. Putative Arabidopsis orthologs of those bean genes were used to create a heat map obtained with the clustering tool of Genevestigator. Gene numbers in green and red colors indicate their down- or up-regulation in our study, respectively.
doi:10.1371/journal.pone.0043161.g003

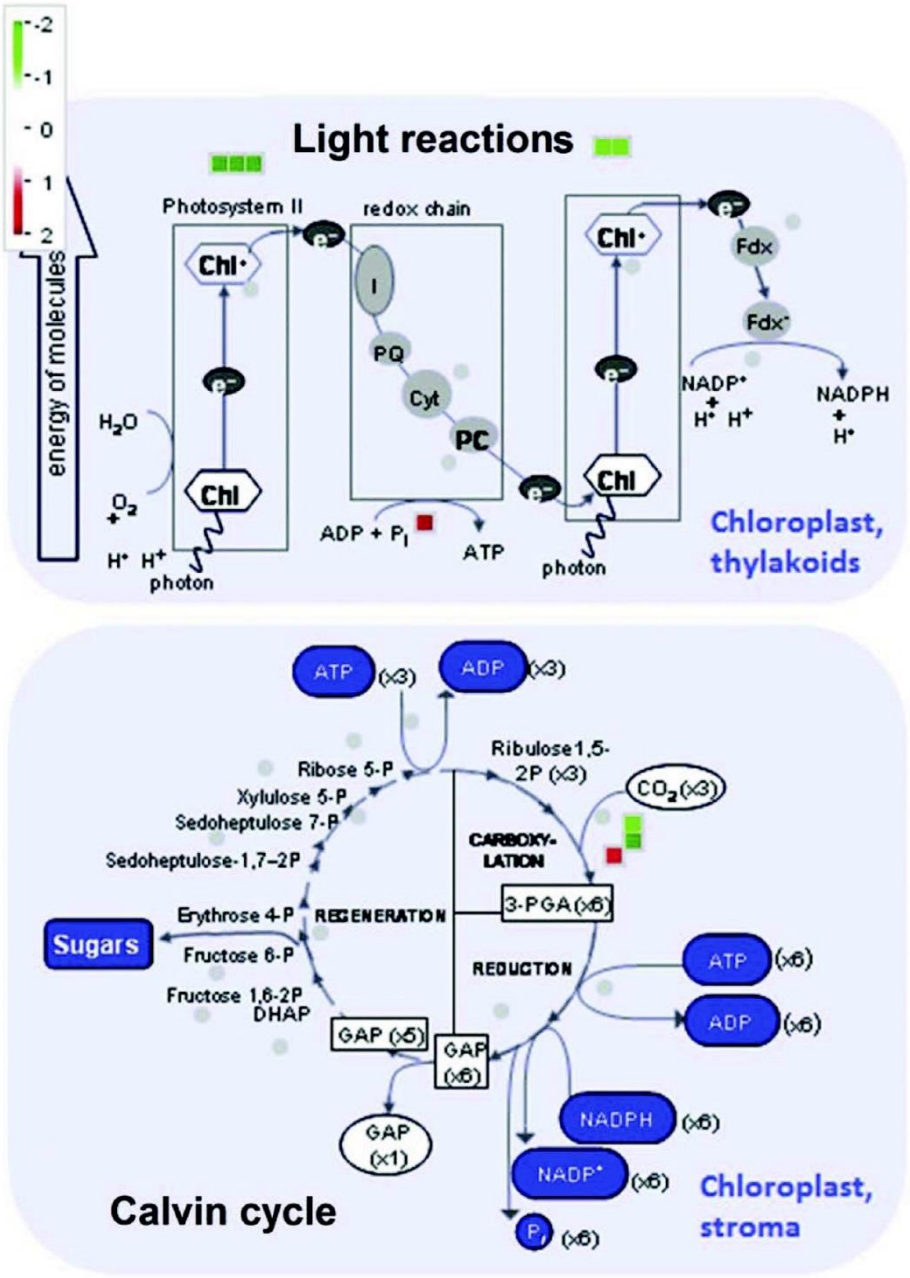

Figure 4. Cellular function overview of proteins encoded by photosynthesis-related genes differentially expressed upon fungus attack. Putative Arabidopsis orthologs of these genes ($p \leq 0.05$, Table 2) were used as input for Mapman analysis.
doi:10.1371/journal.pone.0043161.g004

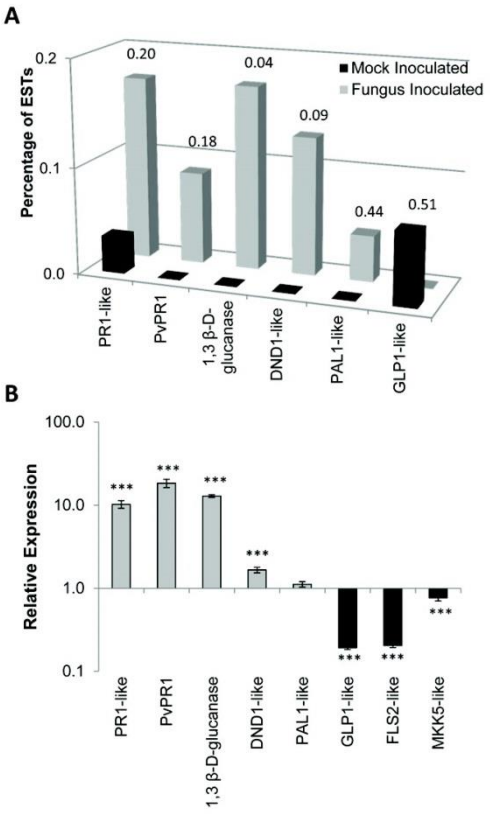

Figure 5. Expression level of selected gene used to validate the bioinformatic analysis of the bean EST collection. (A) Percentage of ESTs encoding the indicated protein in each EST collection obtained from mock- or fungus-inoculated tissue. Numbers on top of the bars indicate the p-value calculated with Fisher's exact test. (B) Relative expression of the same genes assessed by RT-qPCR. Statistical significance was detected with the Student's t-test in (*** = $p<0.001$).
doi:10.1371/journal.pone.0043161.g005

We have identified both well-known and novel metabolic processes involved in plant response and HR to fungal infection indicating that information from model plants such as Arabidopsis can be used to study less understood pathosystems. For instance, metabolic processes known to be involved in plant-pathogen interactions, such as the defense response to fungus (GO:0050832), regulation of defense response (GO:0031347), regulation of response to stress (GO:0080134), and stomatal movement (GO:0010118), were up-regulated in fungus inoculated tissue (Fig. S1). Likewise, up-regulation of response to cytokinin stimulus (GO:0009735) and ethylene mediated signaling pathway (GO:0009873) indicate that these hormones may also play an important role in common bean defense against *C. lindemuthianum*. Interestingly, jasmonic acid biosynthetic (GO:0031408) and metabolic (GO:0009694) processes, as well as response to gibberellin stimulus (GO:0009739) and abscisic acid mediated signaling pathway (GO:0009738) were down-regulated upon fungal infection (Fig. S1) highlighting the importance of hormonal control and cross-talk (antagonist or synergistic) in response to pathogen attack. Specific hormones may be required for resistance in some pathosystems and for susceptibility in others, which may be related to the pathogen life style (*i.e.* necrotroph or biotroph). Jasmonates (JA), for instance, are required for disease susceptibility of Arabidopsis and tomato plants to the biotroph bacterium *Pseudomonas syringae* [50,51]. *Colletotrichum lindemuthianum* is a hemi-biotroph pathogen; thus it is not surprising that JA signaling is down-regulated in an incompatible reaction with the bean plant. Furthermore, components of the proteasome accessory complex (GO:0022624) and proteasome regulatory particle (GO:0005838) are over-represented in inoculated tissue. Plant hormone signaling is tightly regulated post-transcriptionally by the ubiquitin-proteasome system [52]; however, it is not clear how these two processes are connected in the bean-*C. lindemuthianum* pathosystem.

Oxygen and ROS metabolic process (GO:0006800) was also up-regulated suggesting that the bean plant modulates its metabolism for detoxification from ROS burst that occurs during HR [15]. Even though specific gene transcripts related to photosynthesis were down-regulated in fungus inoculated tissue, we observed an increase in gene products located in the plastid ribosome (GO:0000311, GO:0000312, GO:0000314, GO:0000315; Fig. 1A). The strong activity in the plastids could be explained by their involvement in ROS production.

Changes in transcription profile seem to be a key component occurring in plants under pathogen attack [39,53] and it is known that this involves negative regulation of genes related to plant development in order to reallocate the resources to defense responses. This seems to be the case of common bean infected by *C. lindemuthianum*, as the abundance of transcripts encoding proteins associated with organelle fission (GO:0048285), cell cycle process (GO:0022402), pattern specification process (GO:0007389), post-embryonic morphogenesis (GO:0009886), and regulation of post-embryonic development (GO:0048580) were reduced in fungus-infected seedling shoots, while the abundance of transcripts encoding protein involved in positive regulation of biosynthetic process (GO:0009891) was significantly increased (Fig. S1). Furthermore, resources seems to be reallocated, at least in part, from up-regulation of catabolic process of lipids (GO:0016042), fatty acids (GO:0009062), glycine (GO:0006546), and serine family amino acids (GO:0009071).

We extended the GO enrichment analysis to identify quantitative differences between mock- and fungus-inoculated tissues (Fig. 2). Twenty GO categories were differentially represented in the two EST collections and supported by strong statistical significance. The high number of transcripts encoding calmodulin binding (GO:0005516) proteins observed in inoculated tissue agrees with the production of ROS during the cell immunity response as they are involved in Ca^{+2} signaling and redox homeostasis of the cell [54]. Furthermore, we observed an increase in transcripts encoding proteins located in the cell wall of inoculated tissue suggesting that activities at the cell wall may be an important component of bean defense against *C. lindemuthianum* as it has been reported in the *Populus* × *Melampsora* pathosystem [55]. These activities may include modification of cell wall material for resistance against penetration, pathogen recognition, and transport and secretion of defense compounds [56].

Differential Expression of Specific Genes

To identify specific genes that were differentially expressed in fungus-infected tissue, we searched for library-specific ESTs. Each EST was aligned with Arabidopsis transcripts (TAIR10) using tBLASTX with a cut-off E-value of 1×10^{-4} (Table S2) and the

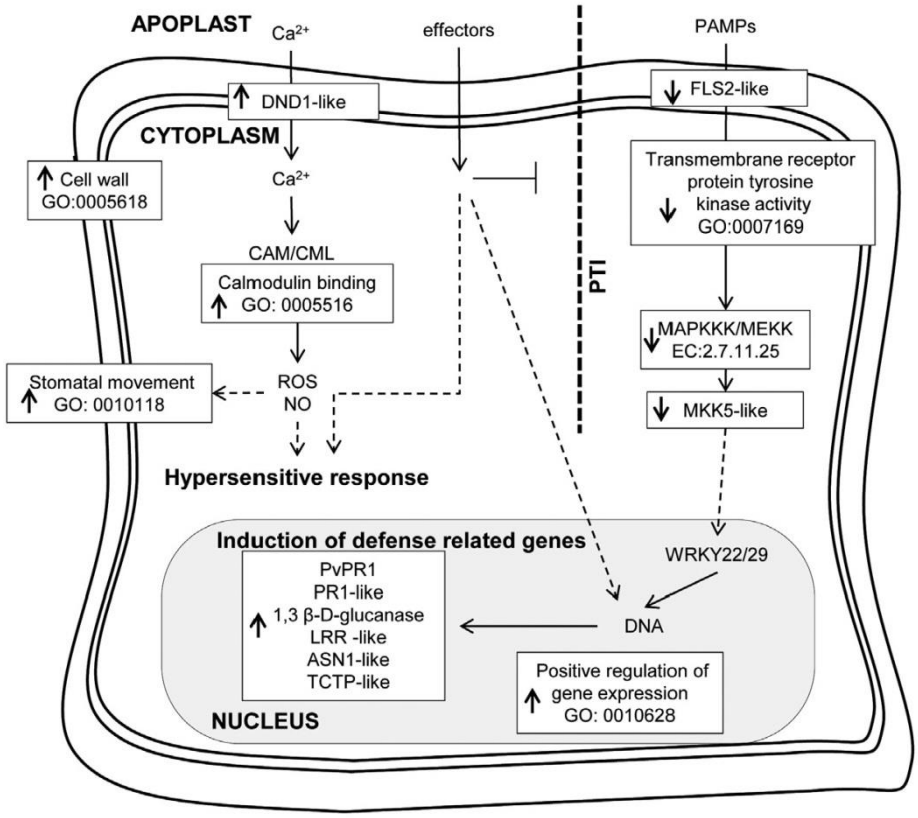

Figure 6. A model of the bean innate immune system. The proposed model represents key molecular components and metabolic processes known to be involved in plant-pathogen interactions based on KEGG, for which bean orthologs have been identified in our EST collection with significant statistical support ($p \leq 0.05$). Gene Ontology (GO) categories and Enzyme Codes (EC) inside boxes are differentially represented in the bean EST libraries (arrowheads pointing down represent down-regulation and arrowheads pointing up represent up-regulation). Continuous arrows represent established relationship between components of the pathway and intermittent arrows represent undirected relationship. Components of PAMP-triggered immunity (PTI) are depicted to the right of the diagram.
doi:10.1371/journal.pone.0043161.g006

number of ESTs in each collection that aligned to the same Arabidopsis gene transcript was determined (Table S4). Many ESTs aligned with the same Arabidopsis gene and were used to infer the level of expression of each bean transcript. The EST library constructed with inoculated tissue had 25 unique transcripts represented by three or more ESTs, whereas the library constructed with control, mock-inoculated tissue had 28 unique transcripts (Table S5). No function could be assigned to two gene transcripts found only in the EST collection from fungus-inoculated tissue and five gene transcripts observed only in the mock-inoculated tissue (Table S5). Interestingly, all but one was predicted to locate at the chloroplast. This fact points out that these seven genes potentially play a role in incompatible interactions, thus opening doors for the characterization of novel defense genes in common bean. Furthermore, transcripts identified only in fungus-inoculated tissue seem to be involved in response to abiotic stress, such as cold, starvation, and drought, as well as biotic stress, such as response to pathogenic fungus or bacteria. Genes involved in ubiquitination (E3 ligase), response to hydrogen peroxide and ion transport also appear to be up-regulated during incompatible interaction (Table S5).

We further identified genes with significant differential expression ($p \leq 0.1$) between the mock-inoculated and fungus-inoculated tissue (Table 2). Interestingly, transcripts encoding for ribulose bisphosphate carboxylase and chlorophyll a/b-binding proteins were down-regulated during fungal infection; however, the bean plant seems to compensate with up-regulation of rubisco activase (Table 2) and gene transcripts encoding proteins related to carbon utilization (GO:0015946, Fig. S1). Decrease in photosynthetic rates due to anthracnose infection has also been observed by gas-exchange analysis [57]. Similar changes have been demonstrated in different pathosystems. For instance, tomato plants inoculated with *P. syringae* pv. *tomato* also showed down-regulation of photosynthesis and chloroplast related genes within four hours of

incubation, whereas transcription factors were up-regulated and protein turnover through the ubiquitation pathway and proteases increased [58].

To verify the possibility that differentially expressed genes between our EST collections are co-regulated by other types of stresses, we used their putative Arabidopsis orthologs to create a heat map of relative expression using public microarray data available at Genevestigator (Fig. 3). The 19 differentially expressed genes with a p-value ≤0.05 (Tables 2 and S5) have corresponding array probes. Consistent with our observation that ethylene mediated signaling may be up-regulated (Fig. S1), these genes are also regulated by ethylene in a similar manner as indicated by the microarray analysis of *ctr1-1* mutant plants [59]. Surprisingly, these genes are also regulated in the pathosystem Arabidopsis - *P. syringae* pv. *syringae* and *maculicola*, which represents a compatible interaction. This may be explained by the fact that most of these genes are involved in photosynthesis (Table 2) which is generally negatively affected by pathogens [57]. A complete list of treatments with similar and opposite gene expression patterns is depicted in Fig. 3.

As several transcripts differentially represented in our EST collections encode proteins possibly involved in photosynthetic pathways (Tables 2 and S5), we used their putative Arabidopsis orthologs to visualize the specific reactions these proteins catalyze and evaluate the overall metabolic shift in cells under attack by *C. lindemuthianum* (Fig. 4). The light-dependent reactions of photosynthesis seem to be down-regulated, suggesting that the cell could be depleted from ATP synthesized through the redox chain; however the cells seem to compensate by up-regulating the synthesis of ATP. Similarly, the abundance of the enzyme RUBISCO decreased in fungus-inoculated tissue, however the enzyme RUBISCO activase was up-regulated probably to restore the normal function of the carboxylation step of the light-independent reactions (Calvin cycle).

Validation of Gene Expression using Quantitative PCR

Relative expression of specific bean genes determined by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) agreed with the gene expression patterns predicted by the bioinformatic analysis of the ESTs libraries (Fig. 5). Four up-regulated genes encoding for PR1-like, PvPR1, 1,3 β-D-glucanase, and DND1-like proteins (Fig. 5A), were annotated as being involved with defense response based on the function of their putative Arabidopsis orthologs (Table S6). Genes coding for PR1 proteins are usually used as markers for systemic acquired resistance (SAR), but their functional role has remained elusive [60]. *PvPR1* transcript has been originally identified in a cDNA library obtained from bean cell suspensions treated with *C. lindemuthianum* mycelium cell wall fractions [61] and is a putative ortholog of the MPL-like gene of Arabidopsis also implicated in plant defense. Endoglucanases, such as the 1,3 β-D-glucanase, can also contribute to defense response directly and indirectly, which can result in pathogen cell wall degradation and liberation of oligosaccharides that may act as endogenous signaling agents [47]. A transcript that encodes for a putative cyclic nucleotide regulated ion channel (DND1-like) was found only in the inoculated tissue. This type of proteins allows the passage of cations through the plasma membrane, such as the DND1 of Arabidopsis seems to be essential for triggering HR in a gene-for-gene dependent manner [62]. However, ATPase-coupled transmembrane ion transport activity, represented by GO:0016469 (Fig. 1A), GO:0042625 and GO:0019829 (Fig. 1B), seems to be down-regulated by fungal-inoculation.

The enzyme PAL (phenylalanine ammonium lyase) is known to be involved in plant defense against pathogens [63,64,65]. *PAL* mRNA was also induced in response to *C. lindemuthianum* during incompatible and compatible interactions, being stronger and faster during the incompatible interaction, with a maximum expression at 60 hpi [34]. However, we identified only one EST from fungus-inoculated tissue (65 hpi) that was annotated as encoding for a PAL1-like protein. Because this *PAL1-like* transcript sequence does not align perfectly with the one reported previously (accession number M11939) [66], it is possible that it belongs to a paralogous gene in the bean genome which is not involved in plant defense [65]. The low abundance of *PAL1-like* transcripts (gi|59938140) identified in our EST collection indicates that this particular gene is not regulated by fungus infection. This result was supported by RT-qPCR analysis (Fig. 5B) indicating that our EST collections are representative of the gene expression patterns identified by bioinformatics analysis.

GERMIN and GERMIN-like protein (GLP) have been implicated in defense against abiotic and biotic stress in plants [67]. Although GLP has an oxalate oxidase activity in monocotyledons, degrading oxalate to CO_2 and H_2O_2, which plays an important role in plant immunity, GLP does not appear to have oxalate oxidase activity in dicotyledons [68]. GLP was classified as pathogenesis related (PR) protein specifically induced during hot pepper defense response against viral infection [69]. *GERMIN-like* (*GLP1-like*) transcript was only found in the control, mock-inoculated bean tissue (two ESTs) suggesting down-regulation by fungus infection. Although the calculated p-value is 0.51 (Fig. 5A), RT-qPCR analysis confirmed the down-regulated of this gene (Fig. 5B). We therefore reasoned that the low statistical significance of EST abundance may be due to the fact that our EST collection does not represent the bean transcriptome to the saturation level and the identification of library-specific transcript (Table S5) represents a good source of gene candidates involved in defense against *C. lindemuthianum*.

A Working Model for the Bean Innate Immune Response

The bioinformatic analysis of common bean ESTs and experimental validation of gene expression revealed the transcriptional changes and underlying metabolic processes that occur during an incompatible interaction between *P. vulgaris* and *C. lindemuthianum*. In addition to identifying genes that were specifically expressed or repressed under the stress imposed by this fungus we were able to identify overall cellular activities modulated by this fungus. As this pathosystem represents an incompatible reaction resulting in hypersensitive response, we searched for specific molecular components involved in plant innate immunity, both PTI (PAMP-Triggered Immunity) and ETI (Effector–Triggered Immunity) [70]. In fact, we found the common bean counterparts of several steps of the Arabidopsis immune system against the bacterial pathogen *P. syringae* pv. *tomato* (Fig. 6). As suggested for model pathosystems [70], it seems that ETI, also characterized by HR can negatively regulate PTI in bean plants as we observed overall down-regulation of processes involved in PTI. For instance, the GO enrichment analysis revealed that transcripts classified as transmembrane receptor protein tyrosine kinase activity (GO:0007169) were significantly down-regulated by fungus-inoculation (Fig. S1). Furthermore, transcripts annotated as MAPKKK/MEKK (EC:2.7.11.25) were also under-represented in fungus-inoculated tissue (Table 1). To validate this hypothesis, we searched for bean ESTs with significant similarity to Arabidopsis genes that belonged to those groups (GO:0007169 and EC:2.7.11.25) and known to be involved in PTI. From this selective analysis, we found putative orthologs of the trans-

membrane receptor *FLS2* (*FLAGELLIN SENSING 2*) and *MKK5* (*MITOGEN-ACTIVATED PROTEIN KINASE KINASE 5*). Owing to their conserved transmembrane LRR (leucine-rich repeat) and STK (serine threonine kinase) domains, several bean ESTs were highly similar to these two Arabidopsis genes (Table S7). We therefore chose the EST with highest similarity to these genes to assess their expression by RT-qPCR. As expected, both *FLS2-like* and *MKK5-like* genes were significantly down-regulated by fungus infection (Fig. 5B).

Although our EST collection was estimated to represent about 25% of the common bean transcriptome [24], it has been valuable in identifying robust responses to the fungal pathogen *C. lindemuthianum* and can be used as reference for comparison among different pathosystems. As more common bean sequences are available (*e.g.*, RNA-seq), they can be utilized for a broader analysis of the bean transcriptome allowing for future studies on those common bean genes that have impact on disease resistance and defense against anthracnose.

Materials and Methods

EST Library Construction

The construction and annotation of the bean EST database used in this study have been reported previously [24]. Briefly, the bean breeding line SEL 1308 that carries the *Co-4*^{2} gene [71] was grown in controlled environment (22°C, 80% relative humidity, and 16h of daily light). and used as source of mRNA to construct EST libraries. Mock-inoculated 10-day old seedling shoots or leaves were used to construct the PVEPSE2 and PVEPLE1 libraries, respectively. Some of the seedlings of the same batch were spray-inoculated with race 73 of *C. lindemuthianum*, which is avirulent on bean plants carrying the *Co4*^{2} gene [71] and used to construct the PVEPSE3 library. Inoculum preparation and inoculation methods were conducted as described by Melotto and Kelly [20].Development of HR in the bean/*C. lindemuthianum* pathosystem varies according to the bean cultivar and the fungus race [34,35]; therefore we collected bean tissue 65 hpi when HR, characterized by minute and limited lesions, was observed on the leaf. As a control, susceptible plants (cultivar Black Magic) were included in the inoculation experiment and these plants showed characteristic anthracnose symptoms starting at five days after inoculation. Inoculation procedure and disease phenotyping were conducted as described elsewhere [72]. ESTs from the PVEPLE1 and PVEPSE2 libraries were analyzed together as they both were constructed with mock-inoculated, control tissue. All sequences reported here are deposited in the NCBI EST database (dbEST) under the accession numbers CB280466 through CB280717, CB539100 through CB543715, CB544073 through CB544239, and CB555925 through CB556132.

Enzyme Codes and Gene Ontology

Enzyme codes (EC) were attributed to each ESTs based on tBLASTX results against the non-redundant database using the KEGG function of the Blast2GO suite [46,73]. The two EST collections were analyzed separately. The number of ESTs belonging to each EC was used to assess the relative abundance in each EST collection and calculate statistical significance as described below. Furthermore, each EST sequence was searched against Arabidopsis transcript sequences available at The Arabidopsis Information Resource (TAIR10 database; http://www.arabidopsis.org/) using tBLASTX [74] with a minimum threshold E-value$\leq 1\times10^{-4}$. The AGI (Arabidopsis Genome Initiative) number of the top hit was used as input for assigning Gene Ontology (GO) categories (http://www.geneontology.org/), performing GO enrichment using the Singular Enrichment Analysis (SEA) through AgriGO (http://bioinfo.cau.edu.cn/agriGO/) [49], and assess the representation of GO categories within each EST libraries.

Gene Expression Analysis and Metabolic Pathways Reconstructions

In order to infer relative expression of genes between mock- and fungus-inoculated tissues, ESTs from each library with significant similarity (E-value$\leq 1\times10^{-4}$) to a single Arabidopsis transcript were clustered, representing the number of observations of a single gene in each library. Library-specific genes that were represented by at least three ESTs, as well as differentially represented genes in each library were identified to infer up- or down-regulation upon fungal infection. Putative Arabidopsis orthologs of significantly regulated bean genes ($p\leq0.05$) were used as input to compare their expression patterns under different stress conditions using Genevestigator (https://www.genevestigator.com/gv/) clustering analysis [75] and to reconstruct metabolic pathways using the Mapman 3.5.1R2 software [76] and KEGG mapping [77].

Statistical Analysis

Statistical significance of bioinformatic analyses was calculated with the Fisher's exact test [78]. The Log_{10} of the p-value was used to infer the relative abundance of ECs, GOs, and transcripts identified in EST collections from either mock- or fungus-inoculated tissue [79].

Reverse Transcriptase – Quantitative PCR (RT-qPCR)

Common bean genotype SEL1308 seedlings growth and fungal inoculations were performed under controlled environmental condition as previously described [24]. Total RNA from control (water-sprayed plants) and inoculated plants was extracted at 65 h post treatment using TRIzol® Reagent Kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Eight genes were selected (Table S6) for validation of bioinformatic analyses. Two reference genes, *ACT2* and *UNK2*, were used as internal control according to Borges *et al.* [80]. Three biological replicates and three technical replicates were performed for each selected gene.

The amplification reactions were carried out using 100 ng of total RNA, 250 µM of each primer (Table S6), and reagents of the 2X Sensimix™ SYBR & ROX one-step kit (PEQLAB, London, UK). The reaction conditions were set as follows: one cycle for 10 min at 42°C for cDNA synthesis and 10 min at 95°C for reverse transcriptase inactivation and *Taq* DNA polymerase activation, followed by 40 cycles of 15 sec at 95°C, 30 sec at 60°C, and 30 sec at 72°C. A final step of 15 sec at 95°C and 1 min at 60°C was included to obtain the melting curve (0.7°C variation from 60°C to 95°C).

Baseline correction and linear regression analysis of each amplification curve was performed with the LinRegPCR software [81]. The optimal set of data point (Window-of-Linearity) was defined to obtain the threshold and quantification cycle (C_q) values. The efficiency ($E = 10^{slope}$) was calculated based on the slope line, considering an ideal value range ($1.8\leq E\leq2$) and correlation ($R\geq0.995$). The relative expression of target genes and p-values were obtained based on average efficiency and Cq values of target and reference genes using the REST software [82].

Supporting Information

Figure S1 Gene Ontology terms in the Biological Processes category uniquely identified in either EST collections using the AgriGO Singular Enrichment Analysis

(SEA). Processes identified only in fungus-inoculated tissue are considered up-regulated by fungal infection (grey bars) and processes identified only in mock-inoculated tissue are considered down-regulated (black bars). Statistical significance was detected with the Fisher's exact test (p-value≤0.05) and data points are indicated as Log_{10} of the p-value.
(TIF)

Table S1 GO terms using Blast2GO. Gene ontology terms assigned to individual ESTs from two collections using Blast2GO. One collection from two libraries constructed with control, mock-inoculated seedling shoots (PVEPLE1 and PVEPSE2) and another collection constructed with fungus-inoculated seedling shoots (PVEPSE3).
(XLSX)

Table S2 tBLASTX analysis of bean ESTs against the Arabidopsis TAIR10 database. AGI number of each bean EST ortholog was identified and used for grouping orthologs into GO categories using AgriGO Singular Enrichment Analysis.
(XLSX)

Table S3 GO categories identified in each EST collection and number of transcripts assigned to each GO using their putative Arabidopsis orthologs as input for the AgriGO tool.
(XLSX)

Table S4 Relative expression of ESTs and their putative Arabidopsis orthologs. List of bean ESTs and their putative Arabidopsis orthologs. Relative abundance of bean transcripts in each EST collection was determined by counting the number of ESTs with significant similarity (E-value≤1×10^{-4}) to the same Arabidopsis transcript model (AGI number). Statistical significance of EST abundance in each library was detected by Fisher's exact test to infer relative gene expression values (Log_{10} of p-value).
(XLSX)

Table S5 Library-specific ESTs identified three or more times in a single library. The AGI number indicates putative Arabidopsis orthologs of bean transcripts identified with tBLASTX search against the TAIR10 database (E-value≤1×10^{-4} was considered as significant). Statistical significance of the library-specific ortholog abundance was detected with the Fisher's exact test to obtain p-values. Expression of genes encoding glycosyl hydrolase and DND1 has been validated with qPCR (italic letters).
(DOCX)

Table S6 Bean transcripts and primers for validation of bioinformatics analysis of the EST libraries using RT-qPCR analysis. Putative gene functions were based on the best hit of tBLASTX against the non-redundant database available at NCBI. Primers were designed using the *P. vulgaris* EST sequences. Actin and Unknown genes were used for expression normalization according to procedures described by Borges *et al.* [80].
(DOCX)

Table S7 Bean ESTs with significant similarity to the Arabidopsis genes *FLS2* and *MKK5*.
(DOCX)

Acknowledgments

The authors thank Dr. R. Jones for bioinformatic technical support, Dr. A. Wendland for kindly providing *C. lindemuthianum* race 73 as a gift, and Drs. M. Fumiko Ito, A.F. Chioratto, and S.A.M. Carbonell for helping with the identification of the fungus race.

Author Contributions

Conceived and designed the experiments: PRO AB DGGC MM. Performed the experiments: PRO AB BC DGGC MM. Analyzed the data: PRO AB BC DGGC LEAC MM. Contributed reagents/materials/analysis tools: SMT LEAC MM. Wrote the paper: PRO AB DGGC LEAC MM.

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