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Transcriptome Profiling of the Shoot and Root Tips of *S562L*, a Soybean *GmCLAVATA1A* Mutant

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Abstract

Plant shoot apical meristems (SAM) and root apical meristems (RAM) contain stem cells that form overall-plant architecture. Mechanisms acting in these regions keep a balance between the stem cell population and differentiation. These mechanisms are well-studied in *Arabidopsis*, but little is known in the legume soybean (*Glycine max* (L.) Merr.). In *Arabidopsis*, the Leucine-rich repeat (LRR) Receptor kinase CLAVATA1 (CLV1) is a crucial regulator of this process in the SAM. In soybean, the receptor most similar to ATCLV1 is GmNARK, which is involved in nodulation control. In contrast, the homeologous partner of *GmNARK* in soybean, called *GmCLV1A*, appears to have no function in 'Autoregulation of nodulation' (AON) a role in regulating shoot architecture in the SAM. Here, the transcriptome of the shoot and root tip areas of a chemically induced and TILLING-selected GmCLV1A missense mutant, *S562L*, and its wild type, cultivar Forrest, were analysed to identify genes which are affected by impaired function of *GmCLV1A*. Among the differentially expressed genes identified, many were categorised as having a role in receptor kinase activity, transcription or defense/stress-response. Molecular categories over-represented in the shoot tip of the mutant include those involved in hormone biosynthesis/activity and secondary metabolism, signalling, photosynthesis, and transport. Functional categories including those involved in polyamine metabolism, nucleotide metabolism, RNA regulation, protein targeting and protein degradation were under-represented in the shoot tip of the mutant. In the root tip, categories associated with signalling, transport, protein synthesis and metabolism were over-represented in the mutant, while categories associated with cell wall degradation, stress, RNA regulation, protein degradation and targeting were under-represented in the mutant. Factors similar to Arabidopsis regulatory components are most likely functioning in specialised shoot structures in legumes. Furthermore, *GmCLV1A* may have an unexpected role in the regulation of flavonoid biosynthesis in soybean.

Keywords: *Glycine max*, legume, plant development, RAM, receptor kinase, RNAseq, SAM, symbiosis.

Introduction

Soybean (*Glycine max* (L.) Merr.), garden pea (*Pisum sativum*), common bean (*Phaseolus vulgaris*) and alfalfa/lucerne (*Medicago sativa*) are some of the important crops belonging to the legume family, which are second to the grasses in providing food for the world's population. One-third of all dietary protein and one-third of processed vegetable oil for human consumption are provided by grain legumes (Gepts et al., 2005; Graham and Vance, 2003).

Nitrogen is the most required nutrient of plants and enters into many biological molecules such as amino acids, proteins and nucleic acids. Although dinitrogen gas (N_2) forms a main part of the earth's atmospheric gas (78.1%), it cannot be used by most of the plants, generating a global need for nitrogen-containing fertiliser. Leguminous plants, however, are able to use dinitrogen gas through a symbiotic association with soil bacteria, collec-

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tively named rhizobia (Ferguson et al., 2003).

Apical meristems including those of the shoot (SAM) and root (RAM) are responsible for the aerial and underground organs of the plant, respectively (Stahl and Simon, 2010; Traas and Hamant, 2009). SAM and RAM are specialised regions containing stem cells, which allow plants to grow continuously throughout their life through the control of a pool of stem cells. Similar to animal stem cells, plant stem cells are capable of generating diverse tissues and renewing the stem cell population (Sharma et al., 2003).

Therefore, the major function of the SAM is to keep a dynamic balance between maintenance of the pluripotent stem cell population and the formation of new organs (leaves and flowers) and enables plants to grow and reproduce (Fletcher, 2002). Some pathways and regulatory mechanisms in this process have been identified through studies using the model plant *Arabidopsis*, a crucifer. Of particular significance is the role of the CLAVATA (CLV) signalling network to regulate the size of the stem cell reservoir in the SAM. Mutations in genes (like *CLV1*, *CLV2* and *CLV3*) acting in the CLV network lead to the proliferation of undifferentiated cells in the SAM and the development of an abnormal apical meristem (Clark et al., 1993; 1997). Within this gene network, *CLV1* encodes a leucine-rich repeat receptor kinase (LRR-RK) and plays a critical role in this pathway.

AtCLV1-like genes in soybean are *GmNARK* and *GmCLV1A*. *GmNARK* regulates nodulation through a mechanism called Autoregulation Of Nodulation (AON) (Searle et al., 2003) and *GmCLV1A* appears to have a function in the SAM (Mirzaei et al., 2014; submitted). However, to date, little is known about the 'CLV' network of soybean. Recently, through TILLING, an EMS-induced missense mutation in a putative S-glycosylation site (*S562L*) in *GmCLV1A* was isolated (Batley et al., 2014; submitted). The mutant shows an alternative function from *GmNARK* and behaves as a loss-of-function allele. *GmCLV1A* lacks any measurable effect on nodulation despite sharing over 90% DNA sequence with *GmNARK*. The S562L mutation leads to severe nodal identity alterations in the basal parts of the emergent plant such as branching, as well as flower and pod abnormalities (Mirzaei et al., 2014; submitted).

Several methods are available to study gene expression, such as qRT-PCR, microarrays and high-throughput RNA sequencing (RNA-seq) (Ozsolak et al., 2009). qRT-PCR is utilised for studying a small number of genes and samples, microarrays are used for large scale gene expression studies (e.g., whole transcriptome), and RNA-seq also evaluates the whole transcriptome, but with less danger of confusing data caused by cross-hybridisation of related genes. In recent years, RNA-seq has been widely used for transcript profiling and gene discoveries in plant species including legumes, such as soybean (Hayashi et al., 2012; Libault et al., 2010; Reid et al., 2012).

In this study, we compared the transcriptome of the shoot and root tip of the *S562L*, *Gmclv1a* mutant, and its wild type parent (cultivar Forrest) using RNA-seq. The CLC genomics workbench program was subsequently used to map the RNA-seq data to the soybean reference genome and determine the relative transcript abundance. The results provide further evidence to aid the understanding of meristem maintenance in soybean.

Materials and Methods

Plant Growth Conditions

Soybean (*Glycine max* (L. Merr.) wild type cv. Forrest and the EMS-induced and TILLING-selected missense mutant S562L were used for this experiment. For RNA-seq, seeds were surface-sterilised by immersion in 70% ethanol for 30 s, then rinsed 5 times with sterile water, and were put between filter paper in sterile Petri dish and kept in a growth chamber at 25°C in dark conditions.

Tissue Harvest

Shoot tips (1 mm) and root tips (2 mm) were harvested after 48 hours using a sterile scalpel and immediately frozen in liquid nitrogen. Shoot tips of plants 48-hours old were collected under the dissecting microscope after opening the cotyledon and removing the emerging leaves.

RNA Sample Preparation and Library Construction

For RNA-seq, total RNA was extracted from dissected shoot and root tips using the Qiagen RNeasy Minikit with on-column DNAse digestion according to the manufacturer's instructions (Qiagen, Maryland, USA). The Australian Genome Research Facility (AGRF) subsequently conducted cDNA library construction and RNA sequencing. cDNA libraries for plant transcriptome sequencing were constructed using the Illumina Truseq RNA kit according to Illumina protocols and RNA sequencing was performed using the Illumina HiSeq 2000 platform, with four multiplexed samples run on one flowcell lane generating 100 bp single-end reads.

For qRT-PCR experiment, RNA was converted to cDNA in a 20-µl reaction mixture containing 0.5 mM deoxynucleoside triphosphates (dNTPs), 1 µl of 50 µm oligo(dT) primers, 40 unit of RNaseOUT (Invitrogen), 0.5 µg of DNA-free RNA, 1x first-strand buffer (Invitrogen), 5 mM dithiothreitol (DTT) and 100 units of SuperScript III reverse transcriptase (Invitrogen) at 50°C for 60 min. Finally, cDNA was confirmed using *GmCons6* primers (Libault et al., 2008) (*Glyma12g05510*; F box protein family) and PCR.

Quantitative Real Time PCR

Primers used for quantitative real-time PCR were designed using the online primer design program, Primer 3 version 0.4.0 (available at http://frodo.wi.mit.edu). Sequences from the soybean genome (Phytozome version 8.0; the United States Department of Energy Joint Genome Institute and Centre for Integrative Genomics; available at http://www.phytozome.net) were used for primer design. The sequences for forward and reverse primers for each gene are shown in Table 3. To ensure that the primers were specific and produced only a single band, normal PCR was run using Forrest cDNA. All primer pairs were found to amplify a single product of the correct size.

The relative transcript abundance was detected using SYBR

Green PCR Master Mix (Applied Biosystems) on an ABI 7900HT cycler (Applied Biosystems) in a 384-well plate. The 384-well plates were set up using an Eppendorf epMotion 5075 Robotic system and contained no template (water) control and reverse transcription negative (RT-) controls to verify genomic DNA contamination of the samples. All reactions were carried out in duplicate of one biological replicate. The qRT-PCR conditions used were as follows: initial denaturation of 95°C for 10 min, then 45 cycles of 95°C for 15 sec and 60°C for 1 min followed by a dissociation stage of 95°C for 2 mins to assess the specificity of the PCR. The expression level of the genes was normalised to the mRNA expression level of soybean *GmCons6* (Libault et al., 2008) amplified by forward primer 5'-AAAGGTGAAATTGCCTCTTCC-3' and reverse primer 5'-CCCAAAGATCTGCCAAATGTA-3'. PCR efficiency for each sample was calculated using the LinRegPCR 7.5 program (Ramakers et al., 2003).

Bioinformatics and Data Analysis of Sequencing Output

The read quality score was determined using the FastX tool kit. Shoot and root read sequence data were mapped separately against the soybean genome (available at Phytozome; http://www.phytozome.net/) using the CLC Genomics Workbench with the RNA-seq function and the mapping setting: minimum length fraction 0.9, and minimum similarity fraction 0.8. Relative transcript abundance was yielded in "Read Per Kilobase" of exon model Per Million mapped reads (RPKM) values. This value only uses the mapped reads and relative size of transcripts to determine expression level.

Differentially expressed genes were identified by comparing expression values between samples and using Kal's test (Kal et al., 1999) which considers proportions, rather than raw data. Genes were determined to be differentially expressed using thresholds of fold change >=2 with Kal's Z test p-value <0.05.

Results

Transcriptome Sequencing (RNA-seq) Outputs

To help understand how *GmCLV1A* affects the gene expression network in soybean, transcript profiles of the shoot and root tip of *S562L Gmclv1a* mutant and wild type (Forrest) were compared at germination. One hundred (100) bp single-end sequence reads generated using an Illumina Hi-seq 2000 platform had a good quality (Phred quality score ≥ 32; Figure 1). Results of mapping reads against the soybean reference genome (available at Phytozome; http://www.phytozome.net/) are summarised in Table 1. Overall a total of 84-87% of the reads from each sample uniquely mapped to the soybean genome, whereas ~4% were non-specifically mapped.

Transcriptomes of Wild Type Forrest and Mutant S562L Shoot Tips

By having at least one read match, a total of 40,229 genes were expressed in the wild type shoot tip compared with 41,172 genes in the *S562L* shoot tip. Comparison of wild type and *S562L* shoot tip genes expression value (RPKM) indicated that 631 genes had a differential transcript abundance (Kal's Z test; $P \leq 0.05$). Around 71% of differentially expressed genes (448 genes) had significantly higher expression in the *S562L* shoot tip relative to the wild type, and 29% of differentially expressed genes (183 genes) genes had less expression. Of these, 277 (~62%) of the more-highly expressed genes had a fold change of two or greater, whereas 62 (~34%) lower-expressed genes had a fold change of two or greater. A subset of these differentially expressed genes, which were predicted to encode either protein kinases, transcription factors, protein binding, defense, stress response or catalytic activity, is presented in Table S1 [see additional file].

Transcriptomes of wild type Forrest and Mutant S562L Root Tips

A total of 40,460 genes were expressed in the wild type root tip compared with 40,714 genes in the *S562L* root tip. Comparison of gene expression values in the wild type and *S562L* root tips identified 1,204 genes that had differential transcript abundance ($P \leq 0.05$). Around 64.5% of differentially expressed genes (777 genes) increased in expression in the *S562L* root tip relative to wild type and 35.5% of differentially expressed genes (427 genes) decreased. Four hundred and forty six (446: ~58%) of these more-highly expressed genes had a fold change of two or greater and 140 (~33%) lower-expressed genes had a fold change of two or greater. A subset of these differentially expressed genes, with activities such as protein kinase and signalling activity, transcription factor activity, protein binding, defence and stress response, and catalytic activity are presented in Table S2 [see additional file].

Table 1. Mapping RNA sequencing reads to the *Glycine max* genome.

	Wild type shoot tip	*S562L* shoot tip	Wild type root tip	*S562L* root tip
Total reads	39,284,610	44,789,650	34,169,345	33,856,295
Uniquely mapped reads	34,063,809	38,886,356	28,999,578	28,343,248
	87%	87%	85%	84%
Non-specifically mapped reads	1,639,245	1,879,153	1,450,399	1,400,816
	4%	4%	4%	4%
Un-mapped reads	3,581,556	4,024,141	3,719,369	4,112,231
	9%	9%	11%	12%

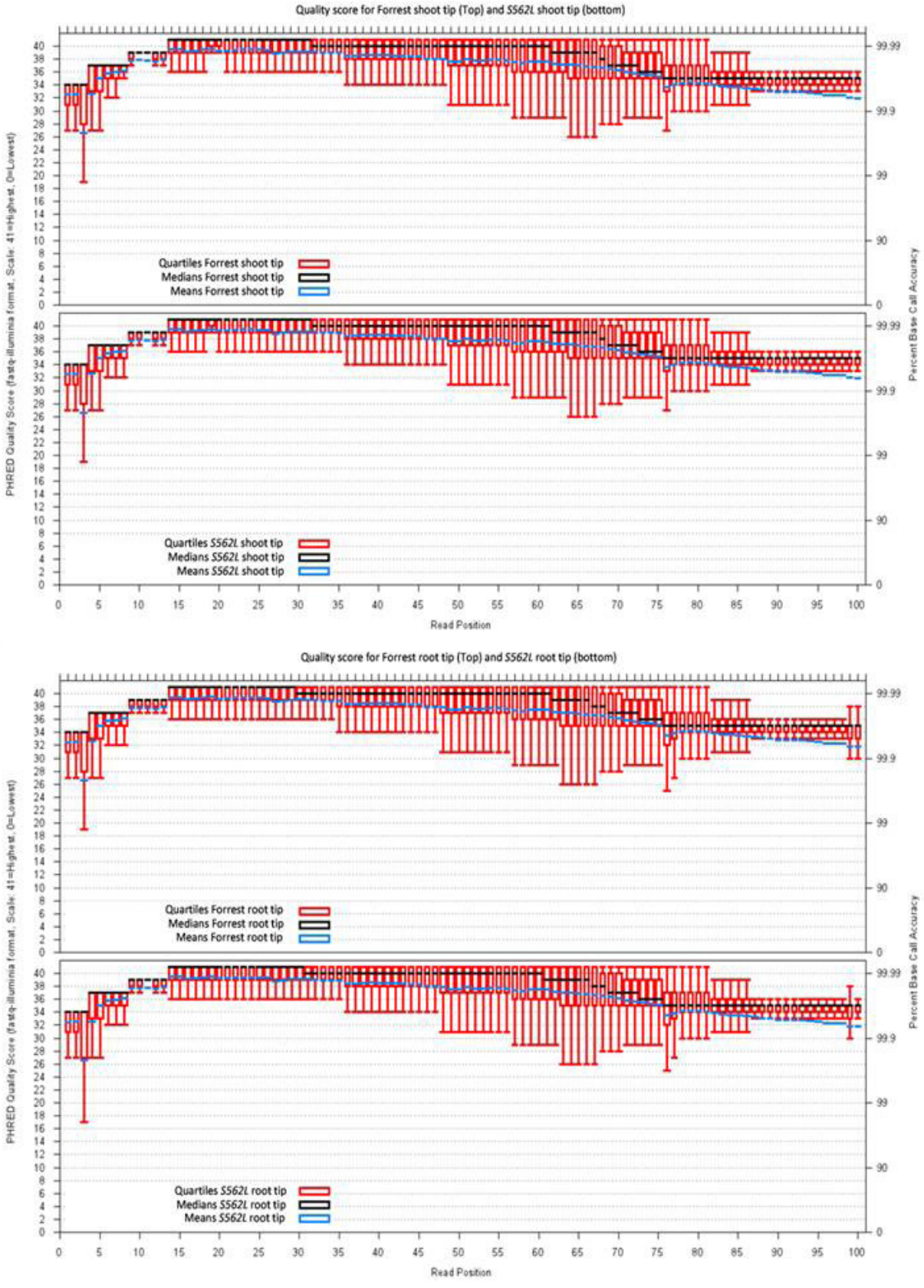

Figure 1. Phred quality scores of RNA sequencing reads. The percentage of base calling accuracy for each base position of the read. The X axis shows read position and the Y axis on the left shows the phred score and on the right shows the percentage of base call accuracy.

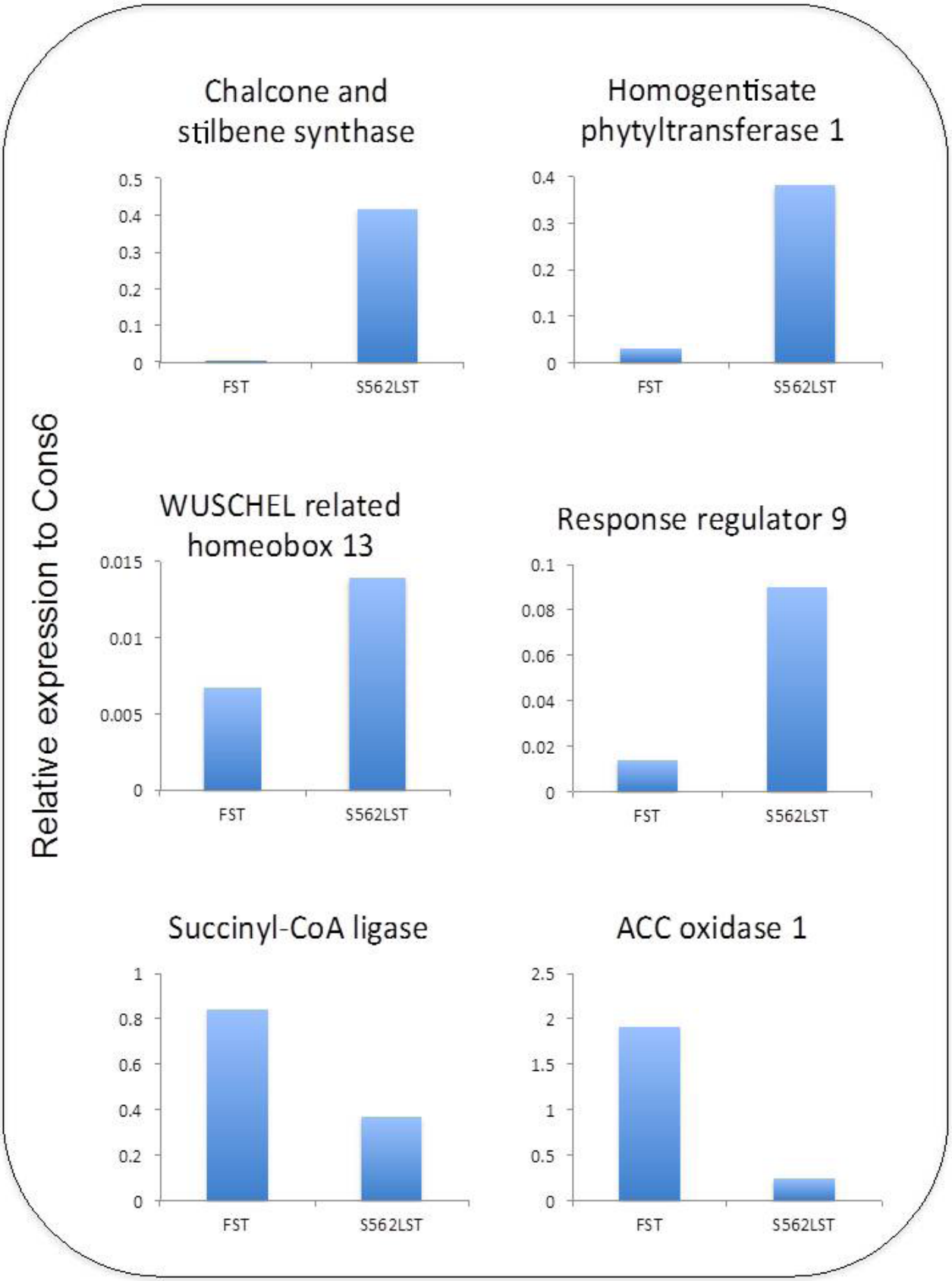

Figure 2. qRT-PCR analysis of six differentially expressed genes (based on RNA-seq) in the shoot samples. This analysis was done in the same shoot samples used for high-throughput RNA sequencing. FST: Forrest shoot tip, S562LST: S562L shoot tip.

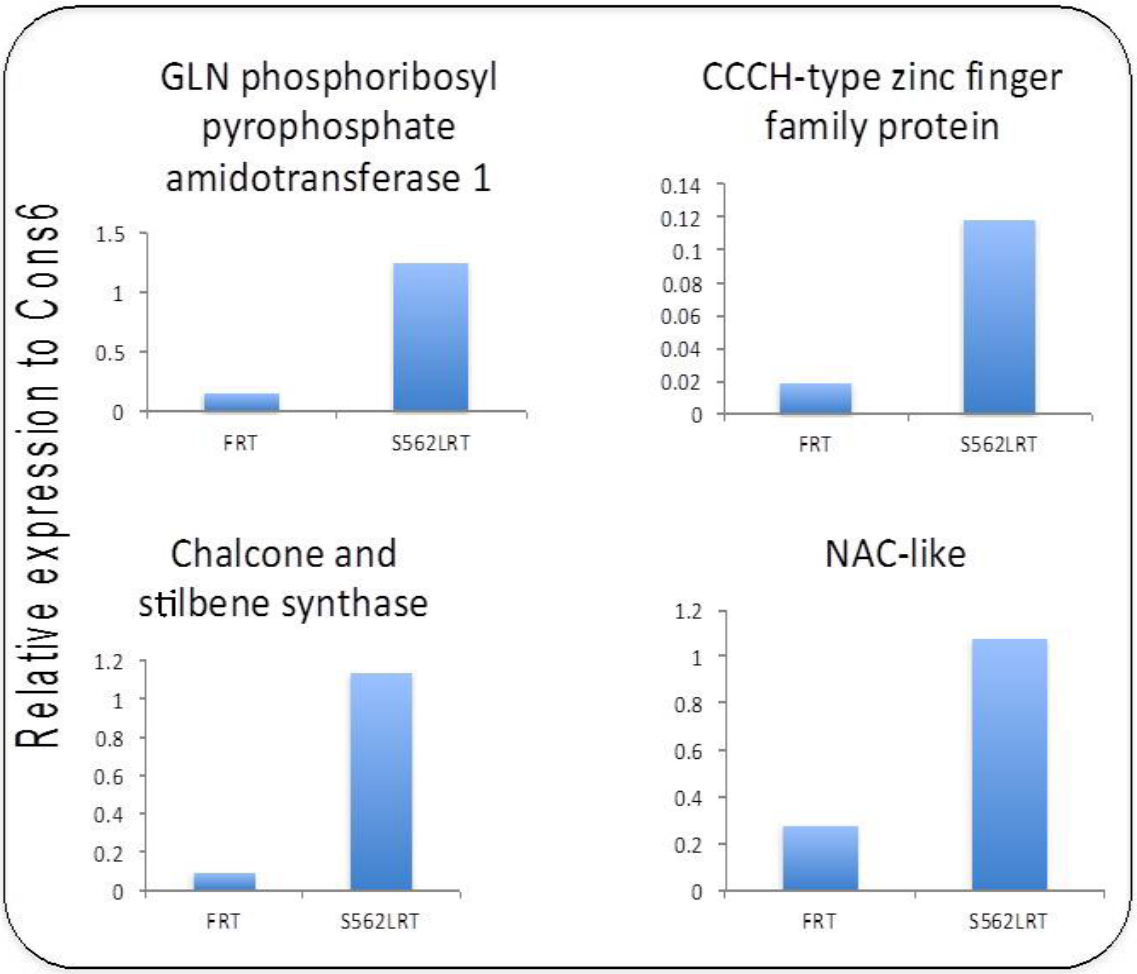

Figure 3. qRT-PCR analysis of four differentially expressed genes (based on RNA-seq) in the root samples. This analysis was done in the same root samples used for high-throughput RNA sequencing. FRT: Forrest root tip, S562LRT: *S562L* root tip.

Confirming the Expression of Some Differentially-Expressed Genes at the Shoot Tip

From differentially-expressed genes, ten candidate genes (eight had over-expression and two had reduced-expression in the shoot or root tip of *S562L*) were selected based on homology to *Arabidopsis* genes that could influence the *CLV* pathway or were acting in developmental pathways (Table 2). The expression of the selected genes was confirmed by qRT-PCR. qRT-PCR was carried out on the exact RNA samples that were used for deep sequencing. The result showed that the expression of all selected genes was consistent with the deep sequencing results (Figures 2 and 3).

Soybean Functional Categories Regulated in the Shoot Tip

Functional categories of genes that were differentially expressed in the *S562L* shoot tip compared to the wild type shoot tip were analysed using Mapman (Thimm et al., 2004) and Pageman, an integrated program in Mapman. This analysis, applying the Wilcoxon test with Benjamini-Hochberg correction, revealed 141 functional pathways (bins and sub-bins) to be statistically different from the other pathways ($p<0.05$; Table S3; see additional file). These pathways are distributed into 22 bins out of 37 bins. Categories over-represented in the shoot tip of S562L included photosynthesis, cell wall, secondary metabolism, hormone metabolism, redox regulation, signalling and transport bins and sub-bins. Under-represented functional categories included polyamine metabolism, nucleotide metabolism, RNA, DNA and protein bins and sub-bins.

Soybean Functional Categories Regulated in the Root Tip

A comparison of the RNA-seq root tip outputs of *S562L* and its wild type found a total of 71 biological functional pathways to be statistically different from all other Bins ($p<0.05$; Table S4; see additional file). Over-represented categories included photosynthesis, lipid metabolism, amino acid metabolism, secondary metabolism hormone metabolism, redox regulation, nucleotide metabolism, DNA, signalling, development and transport and under-represented functional categories included fermentation, cell wall, biosynthesis, stress, RNA, protein and cell biology.

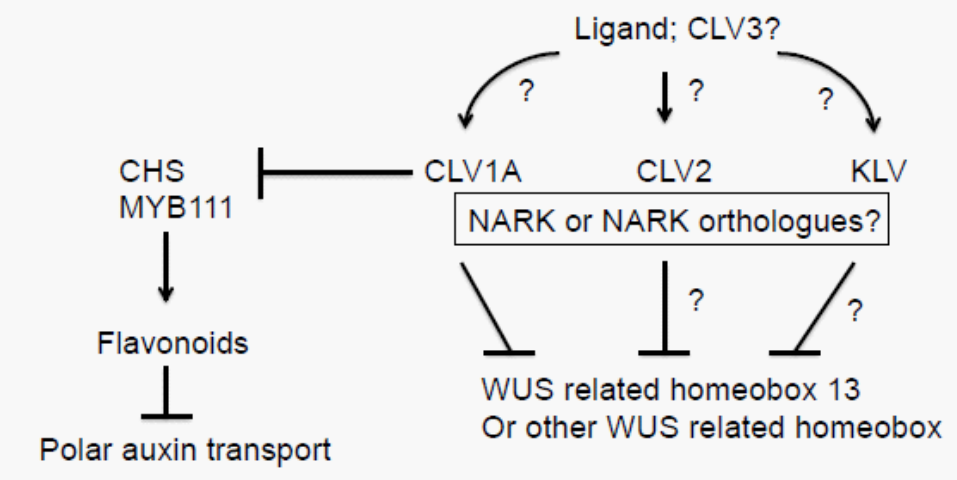

Figure 4. Predicted model of the regulatory network of factors acting in the legume SAM. In the model, it is proposed that GmCLV1A, CLV2 and KLV perceive a ligand probably similar to CLV3 in Arabidopsis and then negatively regulate a WUS-related protein. There is possibility that AON genes such as NARK also interact with GmCLV1A in some aspect of plant development. GmCLV1A also negatively regulates flavonoids biosynthesis. Arrows indicate positive regulation and barred lines indicate negative regulation. '?' implies 'unknown'.

Table 2. Expression fold changes and putative annotation of genes selected for qRT-PCR.

Gene ID	Putative annotation	Proportion fold change
Shoot Samples		
Glyma08g11620	Chalcone and stilbene synthase	39.30
Glyma10g44170	Homogentisate phytyltransferase 1	9.73
Glyma06g01940	WUSCHEL related homeobox 13	5.38
Glyma04g29250	Response regulator 9	8.19
Glyma16g33870	Succinyl-CoA ligase	-18.38
Glyma05g36310	ACC oxidase 1	-5.29
Root Samples		
Glyma04g00930	GLN phosphoribosyl pyrophosphate amidotransferase 1	7.99
Glyma06g05300	CCCH-type zinc finger family protein	7.15
Glyma08g11620	Chalcone and stilbene synthase	6.91
Glyma07g35630	NAC-like, activated by AP3/PI	4.58

Discussion

RNA-seq analysis provided a broad view of the gene expression in the *S562L* shoot and root tip regions compared with the wild type. Our data revealed genes involved in signalling, transcription, metabolism and defense and stress response are over-represented in the *S562L* shoot and root tip. Moreover, genes that belong to the receptor protein kinase and transcription factor families, which are important in signalling and plant development, were also shown to have higher transcript abundance in the shoot and root tip of *S562L* compared to the wild type.

WUS encodes a homeodomain transcription factor and is expressed in the organising centre at the SAM (Schoof et al., 2000). WUS regulates the meristem size through cytokinin signaling via repression of several type-A *Arabidopsis response regulators* (*ARRs*) (*ARR5, ARR6, ARR7* and *ARR15*) as well as activation of CLV3 transcription by binding to its regulatory region (Leibfried et al., 2005; Yadave et al., 2011). Our RNA-seq data and qRT-PCR revealed that Glyma06g01940 (putative orthologue of AT4G35550; WUSCHEL related homeobox 13) was transcribed higher in the *S562L* shoot tip, which is reminiscent of the finding in *Arabidopsis* where the *WUS* expression domain expanded in the *clv* SAM (Schoof et al., 2000). This indicates that GmCLV1A of soybean is acting through a similar component as the CLV network in *Arabidopsis*.

Receptor kinases are key elements in ligand-receptor systems to communicate signals in multicellular organism (*i.e.*, plants) and are involved in diverse pathways in growth and development (Searle et al., 2003; Dievart et al., 2004; Shiu et al., 2004). In

Table 3. Primer sequences used for qRT-PCR.

Gene ID	Forward primer (5'-3')	Reverse primer (5'-3')
Glyma08g11620	TCCACCCCCATCATCATATC	TTGCGCCTTACGAATCTCTT
Glyma10g44170	TTCACGACACAAAAGGGAAAC	AGCATGAGCTTCAAAACCAA
Glyma06g01940	TCAAACGCTGGTGGTATTATTG	GACAGATGGTGGCATAGACAGA
Glyma04g29250	CTCAGAGAATGTCCCAGCAAG	TTTCAACAAATGTGGCCTCAG
Glyma16g33870	TGACATATGAAGCGGTTTTCC	TCTGAAGGCAGTCAACGAAGT
Glyma05g36310	ACCTTCCAAGAACAATGCCAT	TCCAATGGGGTTATAGAAGGTG
Glyma04g00930	GGTGTACCCGGGTGAAGTTAT	ACCTCCCGAAAACAACAGAGT
Glyma06g05300	AACAACTCCGCCTCGTAGTAAC	ATTTTAACATTCCGCGTTGAGT
Glyma07g35630	CCTCCTGGCTTTAGGTTTCAC	GCAATTCCCAAGGATCAAACT

Gene ID is according to the Phytozome database (http://www.phytozome.net).

Arabidopsis thaliana, the CLV family [19], ERECTA family (Torii et al., 1996; Uchida et al., 2013), BAM family (DeYoung et al., 2006; DeYoung and Clark, 2008), RPK2 (Kinoshita et al., 2010), RPK1 (receptor like protein kinase1) and ACR4 (*Arabidopsis* Crinkly4) (De Smet et al., 2008; 2009) are all receptor kinases and function in regulating cell division and differentiation in shoot and root meristems. LRR RKs, differentially regulated in the *S562L* shoot tip and root tip, are presented in Table S1 and Table S2 [see additional file]. None of these LRR RKs are paralogues of the above mentioned LRR RKs, indicating that they represent new candidates that function in signalling at the shoot and root tip and may play a similar role — controlling cell division and differentiation — as the above mentioned receptor kinases.

Transcription factors play critical roles in plant development by regulating positively or negatively the expression level of relevant genes. Analysis of differentially expressed genes revealed their presence in the *S562L* shoot and root tip (Table S1, 2; see additional file).

Transcripts that are related to AP2 (APETALA2) and a group of AP2, ethylene responsive element binding proteins, EREBPs, were also found to be differentially expressed in the shoot and root tip of *S562L* (Table S1, 2; see additional file). AP2/EREBP transcription factors are expressed in different tissues including: flower, leaves, inflorescence stem and root (Okamuro et al., 1997) and they are involved in several developmental processes that include: seed development, stem cell identity, floral organ identity, plant growth, nodulation and defense response (Agrawal et al., 2011; Andriankaja et al., 2007; Aoyama et al., 2012; Jofuku et al., 1994; Krishnaswamy et al., 2011; Yant et al., 2010). AP2-related transcripts are under-represented in the shoot while they are over- and under-represented in the root tip of *S562L* (Table S1, 2; see additional file). This suggests that the defect in *GmCLV1A* function, which is involved in plant development of soybean, might associate with over- and under-represented transcripts of this group of transcription factors which function in developmental processes such as stem cell and thus nodal identity.

Some transcripts correspond to the NAC (NAM, ATAF1/2 and CUC2) domain containing proteins, are also over-represented in the shoot and root tip of S562L (Table S1, 2). NAC domain containing proteins are another group of transcription factors that are involved in a wide range of biological processes, including embryogenesis, flower development, SAM development, wood formation and shoot branching (Aida et al., 1997; Hu et al., 2010; Mao et al., 2007; Ohtani et al., 2011; Xie et al., 2000). Interestingly, Glyma02g26480 is a putative orthologue of ATAF1 (AT1G01720) was over-represented in the shoot tip of S562L. ATAF1 is a homologue of the *NAM* (No Apical Meristem) gene in Petunia (Souer et al., 1996). In Petunia, *nam* mutants fail to develop a SAM (Souer et al., 1996). This indicates that the over-expression of this gene might be due to impaired GmCLV1A function in the *S562L* mutant. However, it has been shown that ATAF1 is involved in stress responses (Hu et al., 2010; Wang et al., 2009). Furthermore, Glyma13g35550, over-represented in the root tip data set, is also an *ATAF1*-like gene.

There are genes highly expressed in the root and shoot tip of *S562L* that correspond to those of the WRKY transcription factors family (Table S1, 2; see additional file). WRKY transcription factors act as activators or repressors and control many plant biological processes including germination, senescence, biotic and abiotic responses and development. Moreover, WRKY factors have a key role in the innate immune system of plants (Rushton et al., 2010).

Flavonoids play a pivotal role in plant biology. They protect plants against UV irradiation, attract pollinators and symbionts, and contribute to plant hormone signalling (Dixon and Pasinetti, 2010; Stracke et al., 2007). MYB transcription factors also act in various plant biological processes and impact development, biotic and abiotic stresses and metabolism (Dubos et al., 2010). There is a member of the MYB transcription factors among the differentially regulated genes in the *S562L* shoot tip, which is involved in flavonol biosynthesis. *Glyma16g02570*, a putative orthologue of *MYB111*, was over-represented in the

transcriptome data base of the *S562L* shoot tip. In *Arabidopsis*, *MYB111* controls flavonol biosynthesis and is mainly active in cotyledons (Stracke et al., 2007). As flavonol accumulation regulates polar auxin transport (Kuhn et al., 2011), it is likely that some phenotypes of *S562L*, such as increased branching, are the result of flavonol accumulation due to higher expression of *Glyma16g02570*. Members of this group were also over-represented in the root tip data of *S562L*. Of interest is *Glyma03g34110*, a putative orthologue for *AtMYB68*. It is specifically expressed in the root and responds to environmental conditions, temperature in particular (Feng et al., 2004). There is a possibility that over-expression of this gene causes a stronger phenotype of *S562L* under cold conditions; however, *AtMYB68* expression is elevated at high temperature (Feng et al., 2004).

Putative orthologues of *Arabidopsis* chalcone synthase (CHS) including *Glyma08g11620*, *Glyma08g11520*, *Glyma0811650* and *Glyma02g14450*, were found to have higher expression in the *S562L* shoot and root tips compared to wild type shoot and root tips. CHS is a key enzyme in flavonoids biosynthesis (Winkel-Shirley, 2001). This suggests a correlation between flavonoid biosynthesis and CLV signalling which may cause developmental outcomes.

Overall, the existence of numerous transcription factors among the differentially expressed genes identified in this study is consistent with recent studies which show a wide range of transcription factors are active in the SAM and RAM of soybean (Haerizadeh et al., 2009; 2011). Furthermore, over-representation of transcripts for MYB and WRKY transcription factors which are involved in a wide range of plant processes and mainly function in abiotic and biotic stresses, could be explained by SAM and RAM immunity systems, which may be affected by impaired function of *GmCLV1A*. A recent study demonstrates that CLV3, a main regulator of stem cell homeostasis, can also activate innate immunity (Lee et al., 2011). Moreover, Mathesius et al. (2011) by comparison of root tip and differentiated root, highlighted the importance of stress, defense response and flavonoid metabolism in the root apex.

Conclusions and Future Work

Past studies using the model plant *Arabidopsis* revealed regulatory pathways in the SAM and RAM that sustain stem cells in both shoot and root meristems and exhibited some similarities between molecules and mechanisms. In legumes, it seems there is a divergence in *CLV1* function as *CLV1* orthologues in legumes, except for *GmCLV1A* in soybean, are involved in nodulation control. *GmCLV1A* (a paralogue of GmNARK, which is a key component in the regulation of nodule formation), acts in shoot architecture, leaf and pod development (Mirzaei et al., 2014; submitted). Investigation of the shoot tip transcriptome of S562L indicated that *GmCLV1A* suppresses the expression of *Glyma06g01940* (WUSCHEL related homeobox 13) reminiscent of *CLV1* function in *Arabidopsis*. This finding along with the evidence of the function of *LjCLV2, PsCLV2* (Krusell et al., 2011) and *LjKLV* (*Lotus japonicus KLAVIER*) (Miyazawa et al., 2010) in the SAM indicates that components similar to *Arabidopsis* regulatory elements are most likely acting in specialised shoot structures in legumes (Figure 4). Furthermore, it seems that *GmCLV1A* negatively controls the flavonoids biosynthesis through the chalcone synthase and the *MYB111* transcription factor. As auxin polar transport is regulated by flavonoids (Kuhn et al., 2011; Falcone et al., 2012), there is a possibility that they also have a function in regulating legume shoot structure.

Further research is required revealing how pathways regulating flavonoids biosynthesis and pathways regulating plant development are connected together in the legume family. Moreover, more studies are required to identify other components (known or unknown in *Arabidopsis*) regulating the SAM of legumes. As mutations in *LjCLV2/PsCLV2* and *LjKLV* lead to hyper-nodulation as well as stem fasciation (Krusell et al., 2011; Miyazawa et al., 2010), such lines of research may aid in understanding not only the molecular mechanisms underlying SAM regulation in legumes, but also pathways acting in nodulation.

Abbreviations

SAM: Shoot Apical Meristems
RAM: Root Apical Meristems
CLV: CLAVATA
LRR-RK: Leucine-Rich Repeat Receptor Kinase
AON: Autoregulation Of Nodulation
RPKM: Read Per Kilobase of exon model per Million mapped reads
ARRs: *Arabidopsis* Response Regulators
RPK1: Receptor like Protein Kinase1
ACR4: *Arabidopsis* Crinkly4
AP2: APETALA2
EREBPs: Ethylene Responsive Element Binding Proteins
KLV: KLAVIER
dNTPs: deoxynucleoside triphosphates
DTT: dithiothreitol

Competing Interest

The authors declare that they have no competing interests.

Author Contribution

SM conducted experiments, evaluated results and wrote the manuscript. JB, BJF and PMG contributed to the conception, interpretation and supervision of the research and editing of the manuscript.

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Table S1. A representative list of the genes that were differentially transcribed in the shoot tip of *S562L* compared with those of the wild type. Light green and light pink represent the most up-regulated and down-regulated gene in each category respectively. Grey shows genes that were differentially-expressed in both the shoot and root tip.

Gene Name	Proportion fold change	P-value	Putative annotation
Protein kinase			
Glyma13g09870	6.95	0.02	Protein kinase superfamily protein
Glyma13g09810	6.08	0.015	Protein kinase superfamily protein
Glyma11g00320	4.55	0.041	Leucine-rich repeat (LRR) family protein
Glyma20g27480	3.76	0.0009794	Cysteine-rich RLK (RECEPTOR-like protein kinase) 29
Transcription factor			
Glyma05g31800	271.42	0.004925	WRKY DNA-binding protein 51
Glyma08g15050	41.48	0.002085	WRKY DNA-binding protein 51
Glyma15g00570	9.28	0.0004699	WRKY DNA-binding protein 40
Glyma18g44030	9.02	0.029	WRKY DNA-binding protein 33
Glyma05g36970	5.07	0.016	WRKY family transcription factor
Glyma09g00820	3.66	0.005466	WRKY family transcription factor
Glyma09g41050	3.45	0.001657	WRKY DNA-binding protein 70
Glyma08g23380	3.35	0.041	WRKY DNA-binding protein 40
Glyma19g43420	4.11	0.049	bZIP transcription factor family protein
Glyma03g40730	3.14	0.039	bZIP transcription factor family protein
Glyma11g11790	-2.08	0.032	Basic-leucine zipper (bZIP) transcription factor family protein
Glyma05g35050	19.91	0.043	myb domain protein 116
Glyma10g00930	18.05	0.014	myb domain protein 15
Glyma16g02570	8.04	0.00002565	myb domain protein 111
Glyma10g32410	7.53	0.003432	myb domain protein 15
Glyma02g00820	5.33	0.025	myb domain protein 15
Glyma20g35180	3.08	0.01	myb domain protein 15
Glyma16g04740	6.30	0.038	NAC domain containing protein 47
Glyma02g26480	2.10	0.027	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein
Glyma08g47240	-13.65	0.029	AP2/B3-like transcriptional factor family protein
Glyma18g38490	-4.65	0.033	AP2/B3-like transcriptional factor family protein
Glyma18g43750	-2.28	0.022	Integrase-type DNA-binding superfamily protein
Glyma13g31010	-2.05	0.0002355	ERF domain protein 12
Glyma12g12600	5.20	0.018	RING membrane-anchor 1

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Table S1. Continued.

Protein binding			
Glyma14g07410	73.64	0.007283	Calcium-dependent lipid-binding (CaLB domain) family protein
Glyma06g44870	30.02	0.019	Ankyrin repeat family protein
Glyma02g41540	22.40	0.044	Calcium-dependent lipid-binding (CaLB domain) family protein
Glyma03g16600	6.20	0.0008464	Glutathione S-transferase TAU 8
Glyma11g00320	4.55	0.041	Leucine-rich repeat (LRR) family protein
Glyma16g25080	3.68	0.037	Disease resistance protein (TIR-NBS-LRR class), putative
Glyma08g08360	3.24	0.045	Polygalacturonase inhibiting protein 1
Glyma10g33650	3.13	0.0008941	Glutathione S-transferase TAU 15
Glyma01g26220	2.66	0.0000691	Glutathione S-transferase TAU 8
Glyma15g40190	2.33	0.029	Glutathione S-transferase TAU 19
Glyma06g40710	2.22	0.047	Disease resistance protein (TIR-NBS-LRR class), putative
Glyma11g33760	2.18	0.043	Calcium-dependent lipid-binding (CaLB domain) family protein
Glyma15g03160	-2.06	0.035	Alfin-like 5
Glyma08g44960	-3.39	0.006228	Proteasome activating protein 200
Glyma07g04820	-3.75	0.002134	Chaperone DnaJ-domain superfamily protein
Defense and stress response			
Glyma09g04520	46.20	9.26E-09	Pathogenesis-related protein Bet v I family
Glyma07g37280	33.05	0.033	MLP-like protein 423
Glyma15g15600	28.99	0.006306	Pathogenesis-related protein Bet v I family
Glyma15g15590	15.76	3.558E-10	MLP-like protein 423
Glyma17g03340	10.33	3.268E-11	MLP-like protein 423
Glyma07g37270	8.05	1.355E-09	MLP-like protein 423
Glyma09g04510	4.41	4.124E-10	MLP-like protein 423
Glyma07g37240	4.08	0	MLP-like protein 423
Glyma17g03350	3.87	0.000003783	MLP-like protein 423
Glyma03g41390	6.36	0.006478	Senescence-associated gene 21
Glyma20g23090	3.86	0.00249	Adenine nucleotide alpha hydrolases-like superfamily protein
Glyma10g02210	3.01	0.006941	Senescence-associated gene 21
Glyma04g01130	-2.36	0.000005269	Cold-regulated 47

Table S1. Continued.

Catalytic activity			
Glyma11g31310	7.52	0.019	AMP-dependent synthetase and ligase family protein
Glyma09g40580	7.20	0.034	NAD(P)-binding Rossmann-fold superfamily protein
Glyma08g37670	6.66	0.013	Deoxyxylulose-5-phosphate synthase
Glyma01g44270	6.39	0.002263	4-coumarate:CoA ligase 3
Glyma04g11250	5.96	0.047	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
Glyma01g43460	5.38	0.033	Highly ABA-induced PP2C gene 3
Glyma04g13880	4.97	0.004299	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
Glyma18g45260	4.29	0.00007771	NAD(P)-binding Rossmann-fold superfamily protein
Glyma06g05280	3.25	0.013	Branched-chain amino acid transaminase 2
Glyma03g19810	2.81	0.032	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
Glyma08g42070	2.23	0.029	ATP-dependent caseinolytic (Clp) protease/crotonase family protein
Glyma13g44950	2.22	0.049	4-coumarate:CoA ligase 2
Glyma07g36770	-2.95	0.039	Enoyl-CoA hydratase/isomerase A

Table S2. A representative list of genes that were differentially transcribed in the root tip of *S562L* compared with that of the wild type. Light green and light pink represent the most up-regulated and down-regulated gene in each category respectively. Grey indicates genes that were differentially-expressed in both the shoot and root tip.

Gene Name	Proportions fold change	P-value	Putative annotation
Receptor kinase & signalling			
Glyma20g23890	7.67	0.00000151	ACT-like protein tyrosine kinase family protein
Glyma08g23340	4.64	0.018	CBL-interacting protein kinase 5
Glyma09g33650	4.57	0	Phosphoenolpyruvate carboxykinase 1
Glyma08g25600	4.41	0.023	Leucine-rich repeat transmembrane protein kinase
Glyma02g46670	3.24	0.021	Protein kinase superfamily protein
Glyma01g02330	3.04	0.0000316	Phosphoenolpyruvate carboxykinase 1
Glyma13g38170	2.93	0.022	Receptor-like protein kinase-related family protein
Glyma17g05660	2.69	0.037	Protein kinase superfamily protein
Glyma09g41340	2.64	0.0005498	SOS3-interacting protein 1
Glyma01g37100	2.45	0.05	Calcium-dependent protein kinase 28
Glyma12g07770	2.31	0.025	Mitogen-activated protein kinase 3
Glyma02g02790	2.17	0.004924	Disease resistance protein (TIR-NBS-LRR class) family
Glyma16g06940	2.07	0.044	Leucine-rich repeat receptor-like protein kinase family protein
Glyma10g30710	-2.15	0.003837	Leucine-rich receptor-like protein kinase family protein
Glyma06g06550	-2.23	0.013	CBL-interacting protein kinase 25
Glyma11g00320	2.48	0.046	Leucine-rich repeat (LRR) family protein
Glyma08g04390	-2.12	0.00277	Leucine-rich repeat (LRR) family protein
Glyma15g26790	-2.72	0.0002545	polygalacturonase inhibiting protein 1

Table S2. Continued.

Transcription factor			
Glyma19g40470	6.61	0.028	WRKY DNA-binding protein 35
Glyma01g31920	6.40	0.012	WRKY DNA-binding protein 33
Glyma01g43420	6.11	0.00856	WRKY family transcription factor
Glyma08g02580	5.14	0.004004	WRKY family transcription factor
Glyma03g05220	4.90	0.007609	WRKY DNA-binding protein 33
Glyma05g36970	3.37	0.009669	WRKY family transcription factor
Glyma15g00570	3.33	0.002048	WRKY DNA-binding protein 40
Glyma17g15480	2.96	0.023	Ethylene responsive element binding factor 1
Glyma10g34760	2.65	0.036	AP2/B3 transcription factor family protein
Glyma17g15460	2.07	0.007356	Ethylene responsive element binding factor 5
Glyma14g09320	2.06	0.032	Related to AP2 1
Glyma15g16260	-2.26	0.025	Ethylene-responsive element binding protein
Glyma17g33060	-2.89	0.001162	Integrase-type DNA-binding superfamily protein
Glyma19g27790	-2.89	0.0004498	Integrase-type DNA-binding superfamily protein
Glyma03g26450	-4.71	0.02	Ethylene-responsive element binding factor 13
Glyma19g43420	5.27	0.0000127	bZIP transcription factor family protein
Glyma03g40730	2.46	0.002065	bZIP transcription factor family protein
Glyma06g38410	8.29	0.038	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein
Glyma07g35630	4.58	0.00009966	NAC-like, activated by AP3/PI
Glyma01g06150	4.20	0.024	NAC-like, activated by AP3/PI
Glyma16g04740	4.06	0.004638	NAC domain containing protein 47
Glyma13g35550	3.87	0.0002846	NAC domain containing protein 3
Glyma17g23740	3.42	0.044	NAC domain containing protein 83
Glyma03g34110	7.83	0.045	myb domain protein 68
Glyma09g03690	5.65	0.022	myb domain protein 78
Glyma02g00820	3.45	0.02	myb domain protein 15
Glyma09g37340	-2.27	0.009459	myb domain protein 30
Glyma06g05300	7.16	0.009363	CCCH-type zinc finger family protein
Glyma04g05290	6.90	0.0000799	CCCH-type zinc finger family protein
Glyma12g12600	3.11	1.516E-07	RING membrane-anchor 1
Glyma10g29750	3.09	0.004816	RING/U-box superfamily protein
Glyma03g37740	3.07	0.039	RING/FYVE/PHD zinc finger superfamily protein
Glyma05g36110	3.00	0.039	CCCH-type zinc finger family protein
Glyma08g03540	2.97	0.036	CCCH-type zinc finger family protein
Glyma11g36040	2.39	0.03	RING-H2 finger A2A
Glyma07g24480	8.51	0.007142	GDP dissociation inhibitor family protein / Rab GTPase activator family protein
Glyma01g39260	3.29	0.008994	Heat shock factor 4
Glyma20g32050	-2.02	0.00006938	GATA transcription factor 9
Glyma02g06560	5.44	0.032	Homeobox protein 40

Table S2. Continued.

Protein binding			
Glyma10g43670	9.06	0.034	F-box family protein
Glyma16g32230	7.46	0.028	Phloem protein 2-A13
Glyma02g04420	4.95	0.028	Octicosapeptide/Phox/Bem1p family protein
Glyma06g17910	4.56	0.000001083	SNF1-related protein kinase regulatory subunit gamma 1
Glyma13g44260	4.03	0.019	Cystathionine beta-synthase (CBS) protein
Glyma11g33760	3.33	0.0002613	Calcium-dependent lipid-binding (CaLB domain) family protein
Glyma01g03150	3.29	0.01	Octicosapeptide/Phox/Bem1p family protein
Glyma05g28820	2.95	0.04	Galactose oxidase/kelch repeat superfamily protein
Glyma01g42420	2.57	0.031	Phospholipase D beta 1
Glyma13g09290	2.53	0.008898	RNI-like superfamily protein
Glyma08g10890	2.38	0.018	Galactose oxidase/kelch repeat superfamily protein
Glyma15g01610	2.38	0.002209	Galactose oxidase/kelch repeat superfamily protein
Glyma08g23540	2.26	0.001665	Chaperone DnaJ-domain superfamily protein
Glyma10g33650	2.17	0.00002115	Glutathione S-transferase TAU 15
Glyma18g01140	2.15	0.008673	Galactose oxidase/kelch repeat superfamily protein
Glyma04g37140	2.04	0.033	SNF1-related protein kinase regulatory subunit gamma 1
Glyma08g04390	-2.12	0.00277	Leucine-rich repeat (LRR) family protein
Glyma19g24640	-2.39	0.011	Galactose oxidase/kelch repeat superfamily protein
Glyma09g15600	-2.39	0.05	CCCH-type zinc finger protein with ARM repeat domain
Glyma16g06690	-2.81	0.002079	Galactose oxidase/kelch repeat superfamily protein
Glyma08g44960	-2.82	0.0002941	Proteasome activating protein 200
Defense and stress response			
Glyma02g03680	7.10	0.00001028	MLP-like protein 423
Glyma15g15590	5.84	0.021	MLP-like protein 423
Glyma07g37280	5.28	0.00628	MLP-like protein 423
Glyma17g03330	4.55	0.026	MLP-like protein 423
Glyma20g23090	2.34	0.02	Adenine nucleotide alpha hydrolases-like superfamily protein
Glyma06g16810	2.23	0.00000118	Scorpion toxin-like knottin superfamily protein
Glyma15g13870	2.04	0.019	
Catalytic activity			
Glyma06g45950	31.05	0.00003716	Isocitrate lyase
Glyma06g05280	4.69	1.221E-14	Branched-chain amino acid transaminase 2
Glyma04g05190	4.34	1.416E-08	Branched-chain amino acid transaminase 2
Glyma03g40720	3.05	0.004779	GDP-D-mannose 3\',5\'-epimerase
Glyma06g41520	3.03	0.017	NAD(P)-binding Rossmann-fold superfamily protein
Glyma20g28320	2.90	4.441E-16	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
Glyma13g01420	2.89	0.04	Trehalose phosphatase/synthase 11
Glyma06g19590	2.70	0.018	Trehalose-phosphatase/synthase 9
Glyma18g12660	2.41	0.009343	Rhamnose biosynthesis 1
Glyma11g31310	2.35	0.003131	AMP-dependent synthetase and ligase family protein
Glyma03g19810	2.11	0.002617	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
Glyma09g05590	-2.01	0.009678	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
Glyma15g16850	-2.35	0.015	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein

Table S3. Biological categories that are regulated in the *S562L* shoot tip in comparison to wild type shoot tip as analysed by Mapman (Benjamini Hochberg-corrected; $P < 0.05$).

Bin	Name	Elements	p-value
Photosynthesis			
1	PS	317	0
1.1	PS.light reaction	220	0
1.1.1	PS.light reaction.photosystem II	98	0
1.1.1.1	PS.light reaction.photosystem II.LHC-II	38	1.46223E-10
1.1.1.2	PS.light reaction.photosystem II.PSII polypeptide subunits	60	1.28332E-06
1.1.2	PS.light reaction.photosystem I	39	1.82784E-07
1.1.2.2	PS.light reaction.photosystem I.PSI polypeptide subunits	31	9.16001E-08
1.1.5	PS.light reaction.other electron carrier (ox/red)	33	0.042731782
Major CHO metabolism			
2.1.2	major CHO metabolism.synthesis.starch	56	0.019806396
2.1.2.1	major CHO metabolism.synthesis.starch.AGPase	17	0.011872219
2.1.2.2	major CHO metabolism.synthesis.starch.starch synthase	15	0.015657959
2.2	major CHO metabolism.degradation	147	0.033712098
2.2.1.3	major CHO metabolism.degradation.sucrose.invertases	37	0.028318178
2.2.1.3.2	major CHO metabolism.degradation.sucrose.invertases.cell wall	22	0.000328236
2.2.1.3.3	major CHO metabolism.degradation.sucrose.invertases.vacuolar	7	0.007550217
2.2.2	major CHO metabolism.degradation.starch	58	0.043862952
2.2.2.1	major CHO metabolism.degradation.starch.starch cleavage	26	0.021405036
Minor CHO metabolism			
3.1	minor CHO metabolism.raffinose family	19	0.014279496
3.1.2	minor CHO metabolism.raffinose family.raffinose synthases	12	0.003851643
3.1.2.2	minor CHO metabolism.raffinose family.raffinose synthases.putative	12	0.003851643
3.2.2	minor CHO metabolism.trehalose.TPP	22	0.033634009
3.4.3	minor CHO metabolism.myo-inositol.InsP Synthases	4	0.040258978
Cell wall			
10	cell wall	875	9.90407E-06
10.5.3	cell wall.cell wall proteins.LRR	22	0.020416261
10.7	cell wall.modification	153	0.009841828
10.8	cell wall.pectin*esterases	153	0.006763777
10.8.1	cell wall.pectin*esterases.PME	130	0.027926432
Lipid metabolism			
11.9.3.4	lipid metabolism.lipid degradation.lysophospholipases.phospholipase A2	6	0.021694885
Amino acid metabolism			
13.1.3.6	amino acid metabolism.synthesis.aspartate family.misc	11	0.015036573
13.1.3.6.1	amino acid metabolism.synthesis.aspartate family.misc.homoserine	11	0.015036573
13.1.3.6.1.1	amino acid metabolism.synthesis.aspartate family.misc.homoserine.aspartate kinase	8	0.015473113
S- assimilation			
14.2	S-assimilation.APR	3	0.048354292

Table S3. Continued.

Secondary metabolism			
16	secondary metabolism	761	2.10308E-09
16.1	secondary metabolism.isoprenoids	192	0.009275191
16.1.3	secondary metabolism.isoprenoids.tocopherol biosynthesis	24	0.000171833
16.1.3.2	secondary metabolism.isoprenoids.tocopherol biosynthesis.homogentisate phytyltransferase	12	0.003851643
16.2	secondary metabolism.phenylpropanoids	261	6.13238E-05
16.2.1	secondary metabolism.phenylpropanoids.lignin biosynthesis	118	5.45265E-07
16.2.1.1	secondary metabolism.phenylpropanoids.lignin biosynthesis.PAL	8	0.000396601
16.2.1.3	secondary metabolism.phenylpropanoids.lignin biosynthesis.4CL	31	0.011872219
16.2.1.6	secondary metabolism.phenylpropanoids.lignin biosynthesis.CCoAOMT	15	0.036064324
16.8	secondary metabolism.flavonoids	155	6.45017E-07
16.8.2	secondary metabolism.flavonoids.chalcones	26	2.09935E-08
16.8.5	secondary metabolism.flavonoids.isoflavonols	22	0.000638941
Hormone metabolism			
17	hormone metabolism	1302	2.80432E-08
17.5	hormone metabolism.ethylene	430	1.19416E-05
17.5.2	hormone metabolism.ethylene.signal transduction	178	0.000622743
17.6	hormone metabolism.gibberelin	101	0.009275191
17.7	hormone metabolism.jasmonate	72	0.00068077
17.7.1	hormone metabolism.jasmonate.synthesis-degradation	70	0.000507643
17.7.1.2	hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase	45	0.009861992
17.7.1.5	hormone metabolism.jasmonate.synthesis-degradation.12-Oxo-PDA-reductase	12	0.014129403
Stress			
20	stress	1776	7.21114E-07
20.1	stress.biotic	979	0
20.1.7	stress.biotic.PR-proteins	711	0
20.2	stress.abiotic	775	0.013788265
20.2.1	stress.abiotic.heat	375	5.46072E-10
Redox regulation			
21	redox.regulation	405	0.005916683
21.4	redox.glutaredoxins	89	0.029935211
Polyamine metabolism			
22.2	polyamine metabolism.degradation	4	0.021694885
22.2.1	polyamine metabolism.degradation.polyamin oxidase	4	0.021694885
Nucleotide metabolism			
23	nucleotide metabolism	269	0.007504987
23.1	nucleotide metabolism.synthesis	66	0.000192645
23.1.1	nucleotide metabolism.synthesis.pyrimidine	26	0.009342322
23.1.1.1	nucleotide metabolism.synthesis.pyrimidine.carbamoyl phosphate synthetase	7	0.041272522
23.1.2	nucleotide metabolism.synthesis.purine	28	0.025728984
Biodegradation of Xenobiotics			
24.2	Biodegradation of Xenobiotics.lactoylglutathione lyase	27	0.048107793

Table S3. Continued.

RNA			
27	RNA	6310	1.69051E-36
27.1	RNA.processing	582	4.64734E-33
27.1.1	RNA.processing.splicing	101	5.37497E-06
27.1.2	RNA.processing.RNA helicase	73	3.60128E-12
27.2	RNA.transcription	232	0.00022923
27.3	RNA.regulation of transcription	5289	9.85113E-13
27.3.25	RNA.regulation of transcription.MYB domain transcription factor family	667	1.55764E-11
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	174	0
27.3.44	RNA.regulation of transcription.Chromatin Remodeling Factors	102	3.61765E-06
27.3.50	RNA.regulation of transcription.General Transcription	60	8.02228E-05
27.3.52	RNA.regulation of transcription.Global transcription factor group	29	0.015473113
27.3.54	RNA.regulation of transcription.Histone acetyltransferases	24	0.013788265
27.3.57	RNA.regulation of transcription.JUMONJI family	32	0.014129403
27.3.63	RNA.regulation of transcription.PHD finger transcription factor	39	0.009861992
27.3.64	RNA.regulation of transcription.PHOR1	39	1.17597E-06
27.3.67	RNA.regulation of transcription.putative transcription regulator	397	1.95682E-11
27.3.69	RNA.regulation of transcription.SET-domain transcriptional regulator family	77	0.033634009
27.3.99	RNA.regulation of transcription. unclassified	621	2.00383E-06
27.4	RNA.RNA binding	267	1.14682E-12
DNA			
28	DNA	939	4.19444E-18
28.1	DNA.synthesis/chromatin structure	567	8.43108E-16
28.1.3	DNA.synthesis/chromatin structure. histone	71	4.61178E-08
28.2	DNA repair	116	0.000367641
Protein			
29	protein	6420	2.50503E-26
29.1	protein.aa activation	135	2.80432E-08
29.1.30	protein.aa activation.pseudouridylate synthase	24	0.022243842
29.2	protein.synthesis	976	3.46656E-17
29.2.1	protein.synthesis.ribosomal protein	670	0.001970238
29.2.1.1.1.1	protein.synthesis.ribosomal protein.prokaryotic.chloroplast.30S subunit	31	0.009275191
29.2.1.2	protein.synthesis.ribosomal protein.eukaryotic	428	0.000668567
29.2.1.2.1	protein.synthesis.ribosomal protein.eukaryotic.40S subunit	163	0.006166941
29.2.2	protein.synthesis.misc ribososomal protein	22	0.007251695
29.2.2.50	protein.synthesis.misc ribososomal protein.BRIX	11	0.007550217
29.2.3	protein.synthesis.initiation	184	7.66744E-16
29.2.4	protein.synthesis.elongation	66	0.004180482
29.2.99	protein.synthesis.misc	12	0.037356552
29.3	protein.targeting	490	1.60382E-08
29.3.1	protein.targeting.nucleus	98	1.60564E-08
29.3.3	protein.targeting.chloroplast	66	0.022901299
29.3.4.2	protein.targeting.secretory pathway.golgi	24	0.019980876
29.4.1	protein.postranslational modification.kinase	550	1.31382E-07
29.4.1.57	protein.postranslational modification.kinase.receptor like cytoplasmatic kinase VII	515	2.97425E-07
29.5	protein.degradation	2751	0.000556946
29.5.11	protein.degradation.ubiquitin	1824	0.000367641
29.5.11.5	protein.degradation.ubiquitin.ubiquitin protease	72	4.92242E-08
29.5.11.20	protein.degradation.ubiquitin.proteasom	151	2.00383E-06

Table S3. Continued.

Signalling			
30	signalling	2836	0
30.1	signalling.in sugar and nutrient physiology	98	0.037356552
30.2	signalling.receptor kinases	1307	0
30.2.11	signalling.receptor kinases.leucine rich repeat XI	365	2.49818E-07
30.2.17	signalling.receptor kinases.DUF 26	554	0
30.2.24	signalling.receptor kinases.S-locus glycoprotein like	66	0.000142501
30.2.25	signalling.receptor kinases.wall associated kinase	39	0.028132661
30.2.99	signalling.receptor kinases.misc	219	7.58628E-06
30.3	signalling.calcium	496	1.02576E-07
30.5	signalling.G-proteins	482	0.004180482
Cell			
31.1	cell.organisation	843	0.008218999
31.2	cell.division	161	0.001000067
Development			
33.1	development.storage proteins	89	1.26332E-05
Transport			
34.3	transport.amino acids	180	0.002846344
34.9	transport.metabolite transporters at the mitochondrial membrane	141	0.015657959
34.19	transport.Major Intrinsic Proteins	96	9.49911E-05
34.19.2	transport.Major Intrinsic Proteins.TIP	37	0.000233643
34.22	transport.cyclic nucleotide or calcium regulated channels	41	0.003764466
34.99	transport.misc	639	1.5495E-18

Table S4. Biological categories that are regulated in the *S562L* root tip in comparison to wild type root tip as analysed by Mapman (Benjamini Hochberg-corrected; $P < 0.05$).

Bin	name	elements	p-value
Photosynthesis			
1	PS	317	2.23E-06
1.1	PS.light reaction	220	1.56E-08
1.1.1	PS.light reaction.photosystem II	98	1.88E-06
1.1.1.1	PS.light reaction.photosystem II.LHC-II	38	4.79E-04
1.1.1.2	PS.light reaction.photosystem II.PSII polypeptide subunits	60	0.006735025
1.1.2	PS.light reaction.photosystem I	39	7.10E-04
1.1.2.2	PS.light reaction.photosystem I.PSI polypeptide subunits	31	5.16E-05
Fermentation			
5.2	fermentation. PDC	8	0.01995843
Cell wall			
10	cell wall	875	0.001131608
10.6	cell wall degradation	254	0.004153439
Lipid metabolism			
11.9	lipid metabolism.l ipid degradation	271	0.0015716
11.9.2	lipid metabolism.lipid degradation.l ipases	113	0.00294316
11.9.2.1	lipid metabolism.lipid degradation.lipases.triacylglycerol lipase	89	0.038454121
Amino acid metabolism			
13.2	amino acid metabolism.degradation	156	0.018542453
13.2.3	amino acid metabolism.degradation.aspartate family	45	0.046371554
13.2.4	amino acid metabolism.degradation.branched chain group	31	0.002102358
Secondary metabolism			
16	secondary metabolism	761	0.034592476
16.2.1	secondary metabolism.phenylpropanoids.lignin biosynthesis	118	0.005204386
16.2.1.1	secondary metabolism.phenylpropanoids.lignin biosynthesis.PAL	8	0.014817383
16.2.1.3	secondary metabolism.phenylpropanoids.lignin biosynthesis.4CL	31	0.014331695
16.8	secondary metabolism.flavonoids	155	0.011867805
16.8.2	secondary metabolism.flavonoids.chalcones	26	0.001215041
Hormone metabolism			
17	hormone metabolism	1302	0.004747464
17.2.2	hormone metabolism.auxin.signal transduction	54	0.021272135
17.5.2	hormone metabolism.ethylene.signal transduction	178	0.04073287
17.7	hormone metabolism.jasmonate	72	0.033698515
Stress			
20.1	stress.biotic	979	0.025578528
20.1.7.6	stress.biotic.PR-proteins.proteinase inhibitors	34	0.002102358
20.2	stress.abiotic	775	0.002668161
20.2.1	stress.abiotic.heat	375	5.16E-05
20.2.3	stress.abiotic.drought/salt	129	0.033698515

Table S4. Continued.

Redox regulation			
21.4	redox.glutaredoxins	89	0.033698515
Nucleotide metabolism			
23.1.2	nucleotide metabolism.synthesis.purine nucleotide	28	0.034892781
23.1.2.1	metabolism.synthesis.purine.amidophosphoribosyltransferase	5	0.021645967
Misc			
26.2	misc.UDP glucosyl and glucoronyl transferases	502	0.011867805
26.1	misc.cytochrome P450	398	0.007547013
26.12	misc.peroxidases	185	2.37E-05
RNA			
27	RNA	6310	7.58E-05
27.3	RNA.regulation of transcription	5289	0.014817383
27.3.4	RNA.regulation of transcription.ARF, Auxin Response Factor family	57	0.002008989
27.3.14	RNA.regulation of transcription.CCAAT box binding factor family, HAP2	21	0.008713324
27.3.30	RNA.regulation of transcription.Trihelix, Triple-Helix transcription factor family	64	0.008713324
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	174	4.18E-11
27.3.64	RNA.regulation of transcription.PHOR1	39	1.05E-04
27.3.80	RNA.regulation of transcription.zf-HD	43	0.002668161
27.3.99	RNA.regulation of transcription.unclassified	621	3.38E-07
DNA			
28.1.3	DNA.synthesis/chromatin structure.histone	71	2.24E-08
Protein			
29.2	protein.synthesis	976	2.44E-09
29.2.1	protein.synthesis.ribosomal protein	670	0
29.2.1.2	protein.synthesis.ribosomal protein.eukaryotic	428	0
29.2.1.2.1	protein.synthesis.ribosomal protein.eukaryotic.40S subunit	163	1.76E-08
29.2.1.2.2	protein.synthesis.ribosomal protein.eukaryotic.60S subunit	265	3.25E-13
29.2.3	protein.synthesis.initiation	184	0.007187207
29.3	protein.targeting	490	0.00294316
29.3.4	protein.targeting.secretory pathway	237	2.13E-06
29.3.4.2	protein.targeting.secretory pathway.golgi	24	0.001431831
29.5	protein.degradation	2751	0.014770075
29.5.11	protein.degradation.ubiquitin	1824	0.034568824
29.5.11.5	protein.degradation.ubiquitin.ubiquitin protease	72	0.003802264
29.5.11.20	protein.degradation.ubiquitin.proteasom	151	5.16E-05
29.7	protein.glycosylation	58	0.029399721
Signalling			
30	signalling	2836	0.008713324
30.2	signalling.receptor kinases	1307	2.24E-08
30.2.17	signalling.receptor kinases.DUF 26	554	2.26E-12
30.2.24	signalling.receptor kinases.S-locus glycoprotein like	66	0.010926445
30.2.25	signalling.receptor kinases.wall associated kinase	39	0.007347366
30.2.99	signalling.receptor kinases.misc	219	0.018034925
Cell			
31	cell	1584	6.23E-05
31.4	cell.vesicle transport	336	2.13E-06
Development			
33.1	development.storage proteins	89	1.06E-04
Transport			
34.19	transport.Major Intrinsic Proteins	96	0.002528114