



Identification of SNPs in RNA-seq data of two cultivars of *Glycine max* (soybean) differing in drought resistance

Ramon Oliveira Vidal^{1,2}, Leandro Costa do Nascimento¹, Jorge Maurício Costa Mondego³,
Gonçalo Amarante Guimarães Pereira¹ and Marcelo Falsarella Carazzolle^{1,4}

¹Laboratório de Genômica e Expressão, Universidade Estadual de Campinas, Campinas, SP, Brazil.

²Laboratório Nacional de Biociências/Associação Brasileira de Tecnologia de Luz Síncrotron, Campinas, SP, Brazil.

³Centro de Pesquisa e Desenvolvimento em Recursos Genéticos Vegetais, Instituto Agrônomo de Campinas, Campinas, SP, Brazil.

⁴Centro Nacional de Processamento de Alto Desempenho, Universidade Estadual de Campinas, Campinas, SP, Brazil.

Abstract

The legume *Glycine max* (soybean) plays an important economic role in the international commodities market, with a world production of almost 260 million tons for the 2009/2010 harvest. The increase in drought events in the last decade has caused production losses in recent harvests. This fact compels us to understand the drought tolerance mechanisms in soybean, taking into account its variability among commercial and developing cultivars. In order to identify single nucleotide polymorphisms (SNPs) in genes up-regulated during drought stress, we evaluated suppression subtractive libraries (SSH) from two contrasting cultivars upon water deprivation: sensitive (BR 16) and tolerant (Embrapa 48). A total of 2,222 soybean genes were up-regulated in both cultivars. Our method identified more than 6,000 SNPs in tolerant and sensitive Brazilian cultivars in those drought stress related genes. Among these SNPs, 165 (in 127 genes) are positioned at soybean chromosome ends, including transcription factors (MYB, WRKY) related to tolerance to abiotic stress.

Key words: single nucleotide polymorphisms, deep sequencing, drought resistance.

Introduction

Soybean (*Glycine max*) is a legume crop that plays an important economic role in the international market, with a world production of almost 260 million tons for the 2009/2010 harvest. Brazil ranks as the world's second largest producer and exporter, with about 25% of the production.

Soybean production is influenced by weather oscillations, especially long periods of drought. In the Brazilian soybean culture, the frequency of drought events has increased in recent decades, probably associated with the weather changes in the world (Stokstad, 2004; Schiermeier, 2006). For example, in the states of the south of Brazil, responsible for 40% of domestic production, losses have been as much as 25% of production in recent harvests. During this time some drought tolerant and sensitive cultivars were isolated from these regions. An understanding of the molecular mechanisms governing such contrasting pheno-

types upon water deprivation could provide insights for the creation of new cultivars and help in assisted selection.

Single nucleotide polymorphisms (SNPs) are single base differences between DNA sequences of individuals or lines. They can be assayed and exploited as high-throughput molecular markers. SNP markers have the potential for use in association genetics approaches (Cardon and Bell, 2001; Flint-Garcia *et al.*, 2003). The recent availability of high-throughput DNA sequencing data has enabled studies based on highly informative SNPs. The evaluation of SNPs in large EST sequence data sets from agricultural crops has been employed to generate high-density genetic maps and identify variable genomic regions (Du *et al.*, 2003; Choi *et al.*, 2007; Novaes *et al.*, 2009; Pindo *et al.*, 2008; Duran *et al.*, 2009). The scalability and availability of SNPs in highly automated genotyping assays has made this molecular marker the first choice in genetic linkage and association studies in a variety of species.

High quality reference genome sequences and resources used to perform low coverage resequencing by novel sequencing technologies such as 454 Life Sciences

(Barbazuk *et al.*, 2007), Illumina Solexa (Van Tassel *et al.*, 2008), and SOLiD (Melum *et al.*, 2010) on different individuals, cultivars or even species are prerequisites for the traditional method of whole genome SNP discovery. In this context, genomic sequences of different individuals are aligned to a reference genome and nucleotide variation is detected.

This study used high-throughput mRNA sequencing (RNA-seq) reads derived from suppression subtractive libraries (SSH) to identify SNPs in up-regulated genes from tolerant (Embrapa 48) and sensitive (BR 16) cultivars submitted to drought stress. Such SNPs can be useful for assisted selection of soybean varieties with higher drought tolerance.

Material and Methods

Construction of cDNA libraries and sequencing

Soybean genotype Embrapa 48 (tolerant) and BR 16 (sensitive) were analyzed under two experimental conditions: drought stress and normal irrigation (for further details see Rodrigues *et al.*, 2012, this issue). Leaves and roots from stressed and control plants were collected at three different times (25-50, 75-100, 125-150 min). RNA was isolated from tissue samples and used to construct suppression subtractive hybridization (SSH) cDNA libraries (Rodrigues *et al.*, 2012). These three libraries, enriched in genes up-regulated during drought stress, were sequenced using Illumina/Solexa technology. The reads corresponding to genes enriched in such libraries were used in SNP mapping (see below).

Gene identification from RNA-seq data

The reference transcriptome assembly was constructed using 1,276,813 soybean ESTs available at NCBI. The btrimmer software (Baudet and Dias, 2007) was used to exclude ribosomal, vector, low quality and short (less than 100 bp) sequences (using default parameters). The remaining sequences were assembled with the CAP3 program (Huang and Madan, 1999) using default parameters, generating 60,747 unigenes (30,809 contigs and 29,938 singlets) (Nascimento *et al.*, 2012, this issue).

The RNA-seq reads from SSH libraries from tolerant and sensitive cultivars were submitted to quality filtration considering bases greater than Q20 and merged in one single file. The information about the cultivars was included on the read ID to facilitate SNP genotyping. Bowtie software (Langmead *et al.*, 2009), considering default parameters (maximum of 2 mismatches), was used to map the reads against the reference transcriptome and the output file was saved in SAM format (Figure 1).

SNP detection

SNP detection was performed with the SAMtools pileup program (Li *et al.*, 2009), which found the variations

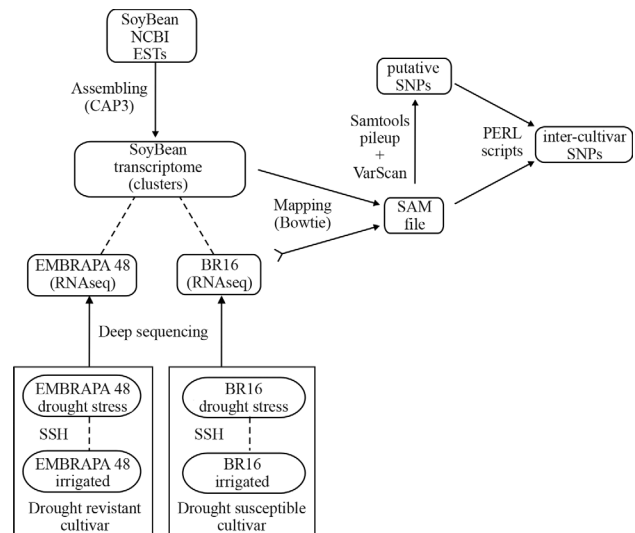


Figure 1 - Pipeline description of inter-cultivar SNP detection from suppression subtractive libraries (SSH) of drought stress against irrigated conditions on tolerant and sensitive cultivars.

in the SAM file, followed by VarScan (Koboldt *et al.*, 2009), which identifies and filters variants based on read counts, base quality and allele frequency (Figure 1).

We developed a Perl script to compare the filtered SNP lists generated by the pipeline described above for the two datasets. Putative SNPs were tagged if the reads involved were mapped unambiguously on the reference transcriptome and the minor allele appeared at least 10 times. The SNPs were discarded if the depth was less than 20 and the frequency of one allele was more than 80%. For each candidate SNP, the algorithm accessed the reads over that position in the SAM file, and observed if all variations occurred only between the two cultivars.

The unigenes identified through mapping of RNA-seq reads that presented polymorphic sites between both cultivars were annotated and grouped into Gene Ontology classification (GO terms) using blast2go software (Conesa and Götze, 2008). In order to identify the chromosome position of polymorphic genes, the unigenes were mapped to the soybean genome assembly (Schmutz *et al.*, 2010) using Exonerate software (Slater and Birney, 2005).

Results and Discussion

Alignment of RNA-seq reads to reference transcriptome

The soybean data available for bioinformatics analysis comprise the reference genome (Williams 82 cultivar; Schmutz *et al.*, 2010), gene models and the EST assembly described in Material and Methods. We chose this assembly as reference to the mapping in order to avoid (1) splicing alignment problems and (2) the absence of the untranslated regions.

After evaluating the SSH libraries, a total of 12,285,871 reads of 45 bp and 30,326,963 reads of 76 bp were obtained for sensitive and tolerant cultivars, respectively. For the sensitive cultivar, 6,317,010 reads were aligned to 7,039 contigs and 2,659 singlets, providing an average depth of coverage of 651 reads by reference sequence. For the tolerant cultivar, 6,120,258 reads were aligned to 15,667 contigs and 6,284 singlets, providing an average depth of coverage of 279 reads by reference sequence. We searched for SNPs in contigs that mapped into both cultivars. After mapping, 7,897 contigs have reads assigned to both cultivars, and were used in SNP identification.

Polymorphism detection

The identification of sequence polymorphisms was performed using SAMtools pileup and VarScan software. We identified a total of 44,510 variations (in 11,000 unigenes) that come from SNPs within each cultivar, SNPs between the cultivars and reference assembly, and SNPs between the cultivars.

To identify inter-cultivar SNPs in the soybean reference sequences and to identify the SNPs between both cultivars (i.e. non-allelic SNPs), we used an in-house SNP filter developed to identify the positions of robust candidate sequence polymorphisms relative to the aligned Solexa reads from each cultivar. We applied another filter requiring at least 10 reads on each cultivar and a maximum of 80% difference between the major and minor allele.

The 6,698 putative polymorphisms were identified in over 2,222 transcripts in tolerant and sensitive cultivars (~3 SNPs/gene). The majority of these polymorphisms represent allelic SNPs (intra-cultivar SNPs) and are not useful as molecular markers for soybean breeding. Nevertheless, we found 165 putative SNPs between tolerant and sensitive cultivars in a total of 127 unigenes (Supplementary Material Table S1). Figure 2 summarizes the GO annotation. As expected, the GO analysis of these 127 genes revealed that many are related to stress response and other ontologies possibly related to stress. Among such genes are a series of

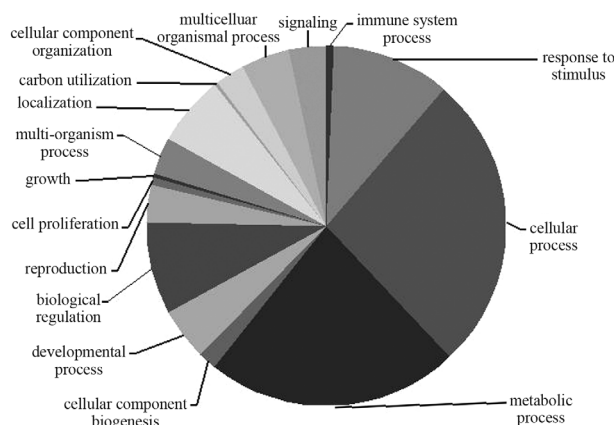


Figure 2 - GO terms of the 127 genes with intra-cultivar SNPs.

signal transducers (calmodulin, ankyrin, GTP binding proteins) and transcription factors (i.e., WRKY, MYB, Zinc Finger, Homeodomain-ZIP), all of which were cataloged in a set of soybean transcription factors (TFs) database focusing on abiotic stress responsive transcription factors (Mochida *et al.*, 2009). Among these soybean TFs, the WRKY and MYB genes were experimentally evaluated. Zhou *et al.* (2008) verified that soybean WRKY genes provide tolerance to abiotic stresses in transgenic *Arabidopsis* plants. Soybean MYB genes were considered part of the stress tolerance apparatus based on salt and freezing stress assays in transgenic plants (Liao *et al.*, 2008). The presence of variability in genes that likely coordinate the first steps of stress signaling, thus controlling a series of protective proteins against drought effects, denote such genes as possible markers for assisted selection. We consider all of these polymorphisms as strong candidates for molecular markers for selective breeding.

Mapping SNPs in soybean chromosomes

Putative SNPs detected in uniquely mapped reference sequence unigenes were plotted along the soybean chromosomes. Alignment with the soybean genome showed that the genes with these identified putative SNPs were not distributed uniformly across the genome. Instead, they are more prominent at the chromosome ends (Figure 3). Wu *et al.* (2010) detected that many SNPs were clustered in gene-rich, high-recombination euchromatic regions in soybean chromosomes. This positional trait of SNPs may be a consequence of intense recombination/mutation events that are crucial for increased variability in autogamous species such as soybean.

Conclusion

The identification of SNPs in contrasting cultivars for drought stress may be useful to breeders in Marker Assisted Selection (MAS) or even in Genome Wide Selection

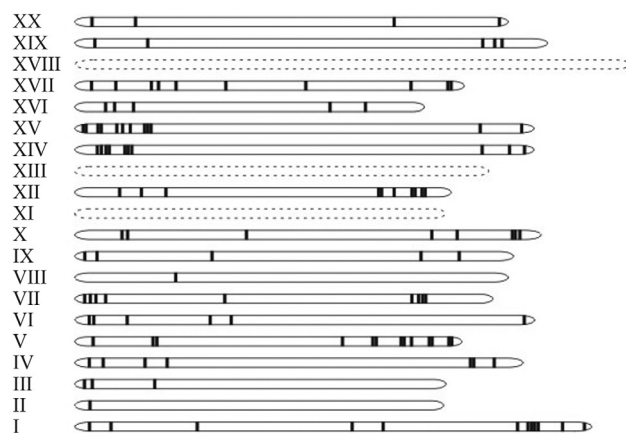


Figure 3 - Positions of inter-cultivar (EMBRAPA 48 and BR 16) SNPs on soybean chromosomes. As these genes come from the aforementioned suppression subtractive libraries, they should be up-regulated in drought stress condition. Dashed lines represent the chromosomes that do not carry any of the discovered SNPs.

(GWS). They can be added to the upcoming markers derived from high-throughput gene sequencing. We believe that the presence of SNPs in transcription factors is outstanding. Such proteins could be controlling a series of genes responsive to stress, making them good candidates for transgenesis and as starting points to understand drought tolerance mechanisms in soybean.

Acknowledgments

The authors would like to thank all researchers of the Brazilian Soybean Genome Consortium (GENOSOJA) involved in the generation of data used in this paper, and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brazil) for financial support.

References

- Barbazuk WB, Emrich SJ, Chen HD, Li L and Schnable PS (2007) SNP discovery via 454 transcriptome sequencing. *Plant J* 51:910-918.
- Baudet C and Dias Z (2007) New EST trimming procedure applied to SUCEST sequences. In: *Proceedings of the Second Brazilian Conference on Advances in Bioinformatics and Computational Biology*. Springer-Verlag, Berlin, pp 57-68.
- Cardon LR and Bell JI (2001) Association study designs for complex diseases. *Nat Rev Genet* 2:91-99.
- Choi IY, Hyten DL, Matukumalli LK, Song Q, Chaky JM, Quigley CV, Chase K, Lark KG, Reiter RS, Yoon MS *et al.* (2007) A soybean transcript map: Gene distribution, haplotype and single-nucleotide polymorphism analysis. *Genetics* 176:685-96.
- Conesa A and Götts S. (2008) Blast2GO: A comprehensive suite for functional analysis in plant genomics. *Int J Plant Genomics* 2008:e619832.
- Du CF, Liu HM, Li RZ, Li PB and Ren ZQ (2003) Application of single nucleotide polymorphism in crop genetics and improvement. *Yi Chuan* 25:735-739.
- Duran C, Appleby N, Clark T, Wood D, Imelfort M, Batley J and Edwards D (2009) AutoSNPdb: An annotated single nucleotide polymorphism database for crop plants. *Nucleic Acids Res* 37:D951-D953.
- Flint-Garcia SA, Thornsberry JM and Buckler ES (2003) Structure of linkage disequilibria in plants. *Annu Rev Plant Biol* 54:357-374.
- Huang X and Madan A (1999) CAP3: A DNA sequence assembly program. *Genome Res* 9:868-877.
- Koboldt DC, Chen K, Wylie T, Larson DE, McLellan MD, Marth ER, Weinstock GM, Wilson RK and Ding L (2009) VarScan: Variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics* 25:2283-2285.
- Langmead B, Trapnel C, Pop M and Salzberg S (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10:R25.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R and 1000 Genome Project Data Processing Subgroup (2009) The Sequence alignment/map (SAM) format and SAMtools. *Bioinformatics* 25:2078-2079.
- Liao Y, Zou HF, Wang HW, Zhang WK, Ma B, Zhang JS and Chen SY (2008) Soybean GmMYB76, GmMYB92, and GmMYB177 genes confer stress tolerance in transgenic Arabidopsis plants. *Cell Res* 18:1047-1060.
- Melum E, May S, Schilhabel MB, Thomsen I, Karlsen TH, Rosentiel P, Schreiber S and Franke A (2010) SNP discovery performance of two second-generation sequencing platforms in the NOD2 gene region. *Hum Mutat* 31:875-885.
- Mochida K, Yoshida T, Sakurai T, Yamaguchi-Shinozaki K, Shinozaki K and Tran LS (2009) *In silico* analysis of transcription factor repertoire and prediction of stress responsive transcription factors in soybean. *DNA Res* 16:353-369.
- Nascimento LC, Costa GGL, Binneck E, Pereira GAG and Carazzolle MF (2012) A web-based bioinformatics interface applied to Genosoma Project: Databases and pipelines. *Genet Mol Biol* 35(suppl 1):203-211.
- Novaes E, Drost DR, Farmerie WG, Pappas Jr GJ, Grattapaglia D, Sederoff RR and Kirst M (2009) High-throughput gene and SNP discovery in *Eucalyptus grandis*, an uncharacterized genome. *BMC Genomics* 9:e312.
- Pindo M, Vezzulli S, Coppola G, Cartwright DA, Zharkikh A, Velasco R and Troglio M (2008) SNP high-throughput screening in grapevine using the SNPlex genotyping system. *BMC Plant Biol* 8:e12.
- Schiermeier Q (2006) The costs of global warming. *Nature* 439:374-375.
- Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J *et al.* (2010) Genome sequence of the palaeopolyploid soybean. *Nature* 463:178-183.
- Slater G and Birney E (2005) Automated generation of heuristics for biological sequence comparison. *BMC Bioinformatics* 6:e31.
- Stokstad E (2004) Global survey documents puzzling decline of amphibians. *Science* 306:391.
- Van Tassel CP, Smith TP, Matukumalli LK, Taylor JF, Schnabel RD, Lawley CT, Haudenschild CD, Moore SS, Warren WC and Sonstegard TS (2008) SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nat Meth* 5:247-252.
- Wu X, Ren C, Joshi T, Vuong T, Xu D and Nguyen HT (2010) SNP discovery by high-throughput sequencing in soybean. *BMC Genomics* 11:469.
- Zhou QY, Tian AG, Zou HF, Xie ZM, Lei G, Huang J, Wang CM, Wang HW, Zhang JS and Chen SY (2008) Soybean WRKY-type transcription factor genes, GmWRKY13, GmWRKY21, and GmWRKY54 confer differential tolerance to abiotic stresses in transgenic Arabidopsis plants. *Plant Biotechnol J* 6:486-503.

Internet Resources

Soybean transcription factors database, <http://soybeantfdb.psc.riken.jp> (October 10, 2011).

Supplementary Material

The following online material is available for this article:
Table S1 - All genes with intra-cultivar SNP annotations.

This material is available as part of the online article from <http://www.scielo.br/gmb>.

Table S1. All genes with SNPs intra-cultivars annotations.

Seq. Name	Seq. Description	Seq. Length	min. eValue	GOs	Enzyme Codes
Contig11685	3-dehydroquinase synthase	1152	3.92E-166	P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity; C:plastid	EC:4.2.3.4
Contig123	40s ribosomal protein s11	819	3.31E-77	C:ribosome; F:structural molecule activity; C:plastid; P:translation; F:RNA binding	EC:3.6.5.3
Contig11958	40s ribosomal protein s15a	740	2.03E-64	F:structural molecule activity; C:plasma membrane; C:cell wall; C:membrane; C:vacuole; C:ribosome; C:cytosol; P:translation; C:mitochondrion	EC:3.6.5.3
Contig23481	40s ribosomal protein s29	613	6.92E-29	C:ribosome; F:structural molecule activity; P:translation	EC:3.6.5.3
SJ07-E1-S10-274-C02-UC.F	60s ribosomal protein l19	604	8.15E-11	C:mitochondrion	
Contig5872	60s ribosomal protein l44	795	8.09E-57	C:ribosome; F:structural molecule activity; P:translation; C:mitochondrion	EC:3.6.5.3
Contig22380	acyl acp-thioesterase	686	6.17E-46	P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:hydrolase activity; F:transferase activity; C:plastid	EC:3.1.2.14
SJ01-E1-L06-022-G03-UC.F	acyl- oxidase	555	1.42E-98	F:catalytic activity; F:transporter activity; C:peroxisome; P:response to biotic stimulus; P:response to external stimulus; P:response to stress; P:biosynthetic process; P:cellular process; P:lipid metabolic process; P:catabolic process; P:multicellular organismal development; F:nucleotide binding; P:biological_process	EC:1.3.99.3; EC:1.9.3.1; EC:1.3.3.6
Contig19038	af149277_1peroxidase 1 precursor	1085	4.58E-121	F:binding; F:catalytic activity	-
Contig14738	akin gamma	1877	0	F:kinase activity; C:cell wall; P:metabolic process; P:cellular process	-
SJ17-E1-R04-027-H06-UC.F	al-induced protein	479	3.57E-51	P:response to endogenous stimulus; C:plasma membrane; P:biological_process	-
Contig985	allene oxide synthase	786	5.05E-104	F:catalytic activity; F:binding; F:molecular_function	EC:4.2.1.92
Contig23356	alpha tubulin 1	1759	0	C:cytoskeleton; P:cellular component organization; P:cellular process; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; P:catabolic process; F:hydrolase activity;	EC:3.6.5.1; EC:3.6.5.2; EC:3.6.5.3;

				F:structural molecule activity; C:cytoplasm; F:carbohydrate binding; F:nucleotide binding	EC:3.6.5.4
Contig27771	alpha tubulin 1	1661	0	C:cytoskeleton; P:cellular component organization; P:cellular process; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; P:catabolic process; F:hydrolase activity; F:structural molecule activity; C:cytoplasm; F:carbohydrate binding; F:nucleotide binding	EC:3.6.5.1; EC:3.6.5.2; EC:3.6.5.3; EC:3.6.5.4
Contig1153	aluminum-induced protein	980	1.64E-111	P:response to endogenous stimulus; C:plasma membrane; P:biological_process	-
Contig25238	ankyrin repeat-containing	1060	4.81E-59	C:membrane; P:response to stress; P:response to abiotic stimulus; C:plastid	-
Contig22377	annexin 2	875	7.41E-110	P:response to stress; P:response to abiotic stimulus; C:cell; P:transport; F:binding; C:thylakoid; C:nucleus; C:cytosol; F:lipid binding	-
Contig3332	aquaporin-like transmembrane channel protein	1335	1.79E-152	C:membrane; P:response to stress; C:plasma membrane; P:transport; P:cellular process; F:transporter activity	-
Contig1476	at1g19180 t29m8_5	846	2.06E-132	P:response to stress; P:response to biotic stimulus; P:multicellular organismal development; P:response to external stimulus; P:response to endogenous stimulus; P:signal transduction; F:protein binding; P:flower development; C:nucleus	-
Contig7639	at1g47330 t3f24_2	763	5.05E-106	C:cytoplasm; C:plasma membrane	-
Contig4713	at5g18670 t1a4_50	2035	0	P:carbohydrate metabolic process; P:catabolic process; F:hydrolase activity; F:binding	EC:3.2.1.2
Contig19095	auxin efflux carrier component	1844	0	P:transport; P:cellular process; P:anatomical structure morphogenesis; P:multicellular organismal development; C:plasma membrane; C:membrane; P:post-embryonic development; P:response to abiotic stimulus; P:flower development; P:reproduction; F:protein binding; F:transporter activity; P:tropism; P:embryonic development; C:cytoplasm; C:cell	-
Contig13059	auxin response factor 9	1160	5.28E-86	F:binding; P:response to endogenous stimulus; P:transcription	-
Contig4535	beta-galactosidase	263	4.16E-47	P:carbohydrate metabolic process; C:cell wall; C:plastid;	EC:3.2.1.23

	precursor			F:protein binding; F:hydrolase activity; F:carbohydrate binding; F:binding	
Contig16059	calcium-dependent protein	1079	1.16E-100	P:protein modification process; F:binding; F:nucleotide binding; F:protein binding; P:response to endogenous stimulus; P:signal transduction; C:plastid; F:kinase activity; C:plasma membrane	EC:2.7.11.17
SJ10-E1-R05-029-D10-UC.F	calcium-dependent protein kinase	578	1.74E-89	P:protein modification process; F:binding; F:nucleotide binding; F:protein binding; P:response to endogenous stimulus; P:signal transduction; C:plastid; F:kinase activity; C:plasma membrane	EC:2.7.11.17
Contig6964	callose synthase 10	1067	5.93E-174	F:transferase activity; P:biosynthetic process; P:carbohydrate metabolic process; P:cellular process; C:plasma membrane	EC:2.4.1.34
Contig2415	calmodulin	899	1.21E-78	P:metabolic process; F:binding; P:post-embryonic development; P:response to abiotic stimulus; F:catalytic activity; P:biological_process; C:membrane; C:vacuole; F:protein binding; P:pollination; P:cellular process; P:protein metabolic process; P:catabolic process; P:signal transduction; C:plasma membrane	EC:1.3.1.74
Contig22225	calmodulin-binding transcription activator	764	1.18E-70	-	
Contig23083	carbonic anhydrase	873	3.59E-72	F:binding; C:plastid; F:catalytic activity; P:metabolic process	EC:4.2.1.1
Contig2904	catalase	1811	0	P:response to stress; P:catabolic process; P:cellular process; F:binding; C:peroxisome; F:catalytic activity; P:metabolic process	EC:1.11.1.6
Contig15505	chloroplast protease	1222	3.38E-179	F:protein binding; F:hydrolase activity; C:thylakoid; P:cellular process; C:membrane; F:binding; P:protein metabolic process; P:catabolic process; P:generation of precursor metabolites and energy; P:photosynthesis; C:plastid; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; F:nucleotide binding; P:cell cycle; P:response to abiotic stimulus	EC:3.4.24.0
Contig22316	chromatin remodeling complex subunit	1092	3.29E-42	F:binding; P:cellular component organization; P:cellular process; F:DNA binding; F:nucleotide binding; F:transferase activity; C:intracellular; F:hydrolase activity; F:chromatin binding; C:nucleus	-
Contig7885	coatomer gamma	994	2.35E-131	F:protein binding; P:transport; P:cellular process; C:plastid; C:membrane; C:Golgi apparatus; F:structural molecule activity	-
Contig3263	cysteine proteinase inhibitor	803	6.87E-96	P:biological_process; F:enzyme regulator activity	-

Contig10810	cysteine synthase	668	4.42E-46	P:pollination; P:multicellular organismal development; F:binding; C:mitochondrion; C:plastid; F:nucleotide binding; F:transferase activity; F:protein binding; P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity	EC:2.5.1.47
Contig3637	double wrky type transfactor	1556	4.24E-180	P:transcription; F:transcription factor activity; F:DNA binding	-
Contig13855	ferredoxin-nadp+ reductase	1492	0	P:transport; C:thylakoid; F:RNA binding; P:generation of precursor metabolites and energy; P:photosynthesis; F:catalytic activity; F:molecular_function; P:response to stress; P:response to biotic stimulus; C:plastid; F:protein binding; C:membrane; F:nucleotide binding; C:extracellular region	EC:1.6.99.1; EC:1.18.1.2
Contig29868	gcpe protein	2290	0	P:metabolic process; P:signal transduction; P:response to stress; P:response to biotic stimulus; P:biosynthetic process; P:carbohydrate metabolic process; P:cellular process; P:lipid metabolic process; C:plastid; F:binding; P:secondary metabolic process; F:catalytic activity	EC:1.17.4.3
Contig22053	gdp dissociation inhibitor	249	1.83E-31	P:biological_process; P:transport; F:enzyme regulator activity	-
Contig14301	germin-like protein	688	1.97E-116	-	-
Contig4559	global transcription factor group	1876	1.51E-89	F:protein binding; P:biological_process; F:DNA binding; F:transferase activity	-
Contig19241	glycerol 3-phosphate permease	988	4.64E-154	F:transporter activity; P:transport; P:cellular process	-
Contig28897	gtp-binding protein	962	6.66E-118	F:nucleotide binding; C:plasma membrane; F:hydrolase activity; P:signal transduction; P:transport	-
Contig12976	high affinity potassium transporter	537	3.85E-71	F:transporter activity; C:plasma membrane	-
Contig11277	isoflavone synthase 1	1773	0	F:binding; F:catalytic activity; F:molecular_function; P:metabolic process	-
Contig29114	light-harvesting complex ii protein lhcb3	1157	2.40E-147	P:generation of precursor metabolites and energy; P:photosynthesis; C:intracellular; C:membrane; C:plastid; C:thylakoid; F:structural molecule activity	-
Contig10765	lipoygenase	2939	0	C:cytoplasm; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:binding; F:catalytic activity; P:metabolic process	EC:1.13.11.1 2

Contig11027	lipoxygenase	1103	6.35E-126	C:cytoplasm; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:binding; F:catalytic activity; P:metabolic process	EC:1.13.11.1 2
Contig5611	lipoxygenase I-5	2187	0	C:cytoplasm; P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:binding; F:catalytic activity; P:metabolic process	EC:1.13.11.1 2
Contig1867	Irr receptor protein	1027	1.26E-117	F:receptor activity; C:cell wall; C:plasma membrane; P:protein modification process; F:nucleotide binding; C:membrane; F:kinase activity; P:signal transduction; P:metabolic process; F:catalytic activity	EC:2.7.11.0; EC:1.3.1.74
Contig10747	mee14 (maternal effect embryo arrest 14)	1209	3.18E-113	P:reproduction; P:post-embryonic development; P:embryonic development; P:response to stress; P:response to biotic stimulus; C:plastid	-
Contig11066	myo-inisitol oxygenase	1308	5.41E-154	C:cytoplasm; F:binding; P:carbohydrate metabolic process; P:cellular process; P:catabolic process; P:anatomical structure morphogenesis; F:catalytic activity; P:metabolic process	EC:1.13.99.1
Contig7836	n chain improved model of plant photosystem i	965	1.67E-76	C:plastid; C:thylakoid; P:generation of precursor metabolites and energy; P:photosynthesis; C:membrane; F:protein binding	-
Contig26849	na+ h+ antiporter	541	1.64E-85	F:transporter activity; P:transport; P:cellular process; P:biological_process; C:membrane	-
Contig22303	nucleobase ascorbate transporter	1980	0	C:membrane; C:vacuole; C:cell wall; P:transport; P:cellular process; F:transporter activity	-
Contig21879	nucleosome chromatin assembly factor group	1839	0	C:intracellular; P:anatomical structure morphogenesis; P:cell differentiation; P:multicellular organismal development; P:cell cycle; P:flower development; P:regulation of gene expression, epigenetic; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; C:nucleus; P:reproduction; P:post-embryonic development; P:cellular component organization; P:cellular process; F:protein binding; F:transferase activity; C:plastid; P:embryonic development	EC:2.3.1.48
Contig20668	omega-3 fatty acid desaturase	1766	0	P:biosynthetic process; P:cellular process; P:lipid metabolic process; F:catalytic activity; C:membrane; C:plastid; P:metabolic process	-
Contig3844	organic anion transporter	1609	1.88E-162	F:transporter activity; C:membrane	-

Contig8110	peptide methionine sulfoxide reductase	857	1.11E-102	C:cytosol; F:catalytic activity; P:metabolic process; P:protein modification process; P:response to stress	EC:1.8.4.11
Contig26213	phosphate transporter	755	2.46E-81	P:transport; C:plasma membrane; P:cellular process; F:transporter activity; C:membrane	-
Contig11742	phosphoribulokinase precursor	1871	0	P:biosynthetic process; P:carbohydrate metabolic process; P:photosynthesis; P:response to stress; P:response to biotic stimulus; P:response to abiotic stimulus; C:plastid; F:nucleotide binding; F:protein binding; F:kinase activity; C:membrane; C:thylakoid; P:metabolic process; P:cellular process; C:extracellular region	EC:2.7.1.19
Contig27945	plasma membrane intrinsic protein	1146	2.45E-144	C:membrane; C:plasma membrane; P:transport; P:cellular process; F:transporter activity	-
SJ18-P1-S12-007-U51-UC.F	PREDICTED: hypothetical protein [Vitis vinifera]	243	2.59E-09	-	-
Contig1857	Ubiquinone biosynthesis	1271	7.11E-95	C:plasma membrane; C:endoplasmic reticulum	-
Contig3159	WD-repeat protein	803	6.40E-110	F:nucleotide binding; F:protein binding	-
Contig3879	putative phi-1-like phosphate-induced protein	1211	1.07E-177	C:cell wall; P:response to endogenous stimulus	-
Contig5355	NADH-ubiquinone oxidoreductase 24 kD subunit	1092	9.30E-146	P:response to stress; F:nucleotide binding; F:binding; F:catalytic activity; C:membrane; C:mitochondrion; P:generation of precursor metabolites and energy	EC:1.6.5.3
Contig5389	kelch repeat-containing F-box family protein	761	9.51E-81	F:molecular_function; P:biological_process	-
Contig6081	-	1027	4.22E-97	-	-
Contig6275	class III HD-Zip protein 8	2215	0	P:multicellular organismal development; P:growth; P:transcription; P:anatomical structure morphogenesis; F:DNA binding; F:transcription factor activity; P:flower development; C:nucleus	-
Contig7590	heat shock protein binding protein	692	1.34E-88	F:binding; P:cellular homeostasis; P:response to stress; P:response to abiotic stimulus; P:response to endogenous stimulus; P:signal transduction	-
Contig8634	Clp protease 2 proteolytic subunit	369	1.11E-15	C:plastid; F:hydrolase activity; C:thylakoid; P:protein metabolic process; P:catabolic process	EC:3.4.21.0

Contig9422	-	945	1.62E-68	F:molecular_function; P:biological_process	
Contig9779	ubiquitin-protein ligase	756	8.57E-66	P:response to stress; P:response to abiotic stimulus; P:protein modification process; P:biological_process; P:metabolic process; F:catalytic activity; C:cytosol	EC:6.3.2.19
Contig10455	-	621	9.79E-63	P:protein metabolic process; P:cellular process; F:protein binding; C:membrane; C:plastid; C:thylakoid	-
Contig10755	aluminum induced protein with YGL and LRDR motifs	832	5.78E-101	C:cytosol; C:nucleus; C:plasma membrane	-
Contig11725	epsilon-adaptin	1079	1.07E-170	F:protein binding; P:transport; P:cellular process; C:membrane; C:cytoplasm	-
Contig12204	Phosphatidic acid phosphatase (PAP2) family protein	821	7.59E-98	P:metabolic process; F:catalytic activity; C:membrane; P:biological_process; C:chloroplast	
Contig12307	aldehyde dehydrogenase	1434	5.19E-153	F:catalytic activity; C:cytoplasm; P:metabolic process; P:cellular process	EC:1.2.1.3
Contig14052	-	712	4.18E-48	C:mitochondrion; F:binding	-
Contig17122	protein kinase	829	1.68E-84	F:kinase activity; P:protein modification process; F:nucleotide binding; C:plastid	-
Contig19813	-	924	9.04E-133	-	
Contig20875	phosphoribosylanthranilate transferase	1370	2.40E-160	F:molecular_function; P:biological_process; C:cellular_component	
Contig21517	-	574	2.69E-34	C:membrane; C:vacuole; C:mitochondrion	-
Contig26353	SWAP (Suppressor-of-White-APricot)/surp domain-containing protein / ubiquitin family protein	932	7.21E-94	F:RNA binding; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	-
Contig28841	MYB transcription factor MYB122	590	1.65E-82	F:DNA binding; P:transcription; C:nucleus	-
Contig29674	Avr9/Cf-9 rapidly elicited protein 20	465	2.52E-52	P:protein metabolic process; P:cellular process; F:binding; F:protein binding; P:response to endogenous stimulus; C:nucleus; C:cytosol	-
Contig29829	plastid transcriptionally	1875	3.93E-130	C:plastid; F:binding; P:metabolic process; F:catalytic activity;	-

SJ18-P1-S12-232-U15-UC.F	active 16 aconitate hydratase, cytoplasmic / citrate hydro-lyase/	247	5.73E-25	C:membrane; C:thylakoid P:response to stress; C:cytosol; F:binding; C:cell wall; C:mitochondrion; P:response to abiotic stimulus; C:membrane; C:vacuole; F:nucleotide binding; P:response to endogenous stimulus; F:catalytic activity; P:metabolic process; P:cellular process; C:plastid; P:biological_process	EC:4.2.1.3
SJ18-P1-S12-286-X20-UC.F	coatomer gamma subunit	245	7.49E-33	F:protein binding; P:transport; P:cellular process; C:plastid; C:membrane; C:Golgi apparatus; F:structural molecule activity	-
SJ01-E1-F05-036-F07-UC.F	Cell division protein kinase	544	4.75E-56	C:plasma membrane; P:cellular process; F:kinase activity	EC:2.7.11.0
SJ07-E1-S10-199-A01-UC.F	-	311	9.81E-09	-	
Contig27355	protein binding	1401	0	F:zinc ion binding	
Contig832	protein kinase	924	2.72E-12	F:protein kinase activity; F:ATP binding; F:transferase activity; P:protein amino acid phosphorylation; F:RNA polymerase II carboxy-terminal domain kinase activity; F:protein serine/threonine kinase activity; F:kinase activity; C:cytosol; F:protein binding	
SJ08-E1-S11-008-F11-UC.F	protein phosphatase 2c	417	8.79E-21	P:protein modification process; F:binding; F:hydrolase activity; C:cell	-
Contig3754	psbp2_tobac ame: full=oxygen-evolving enhancer protein 2- chloroplastic short=oe2 ame: full=23 kda subunit of oxygen evolving system of photosystem ii ame: full=23 kda thylakoid membrane protein ame: full=oec 23 kda subunit flags: precursor	817	3.93E-38	C:membrane; F:binding; P:photosynthesis; C:plastid; C:thylakoid	-
Contig1650	receptor serine threonine	1209	5.86E-86	F:receptor activity; F:kinase activity; P:protein modification process; F:nucleotide binding	EC:2.7.10.2; EC:2.7.11.0
Contig3679	receptor-like protein	813	7.74E-95	C:cell; P:protein modification process; F:nucleotide binding;	EC:2.7.11.0

Contig14231	kinase ribosomal protein	1178	5.87E-149	F:kinase activity; P:signal transduction P:translation; P:multicellular organismal development; F:binding; C:nucleolus; F:structural molecule activity; P:anatomical structure morphogenesis; P:transport; P:cellular process; P:cellular component organization; C:plastid; C:vacuole; C:ribosome; C:cytosol; C:plasma membrane; F:RNA binding	EC:3.6.5.3
Contig4137	ribosomal protein s14	842	5.06E-60	C:ribosome; F:structural molecule activity; P:translation	EC:3.6.5.3
Contig3398	rubisco activase	1844	0	F:nucleotide binding; C:plastid	-
Contig11808	s-adenosylmethionine decarboxylase	1953	0	P:biosynthetic process; P:cellular amino acid and derivative metabolic process; F:catalytic activity	EC:4.1.1.50; EC:4.1.1.19
Contig24388	serine threonine protein phosphatase 2a regulatory subunit	846	1.41E-134	F:enzyme regulator activity; F:binding; C:plasma membrane; C:intracellular; C:cell wall; P:metabolic process; P:cellular process	-
Contig24411	serine-threonine protein plant-	1761	2.90E-95	C:plastid	-
Contig8686	sin3 histone deacetylase complex	1236	2.85E-109	C:nucleus; P:transcription	-
Contig24008	small gtp-binding protein	1132	1.06E-99	F:nucleotide binding; C:plasma membrane; F:hydrolase activity; P:signal transduction; P:transport	-
Contig5904	stem-specific protein	655	1.11E-50	C:cytosol; C:nucleus; C:plasma membrane	-
Contig12813	succinate dehydrogenase	2287	0	C:cell wall; C:membrane; C:mitochondrion; F:binding; F:molecular_function; P:generation of precursor metabolites and energy; P:catabolic process; F:nucleotide binding; F:catalytic activity	EC:1.3.5.1
Contig14015	sucrose transport protein suc2	2027	0	P:pollination; P:cellular process; P:transport; P:response to biotic stimulus; C:vacuole; C:plasma membrane; F:transporter activity; C:membrane; P:reproduction; P:post-embryonic development	-
Contig27892	transcription initiation factor iib	1279	4.12E-167	C:cytoplasm; P:translation; F:transcription regulator activity; P:cellular component organization; P:transcription; F:protein binding; F:translation factor activity, nucleic acid binding; C:nucleus; F:binding	-
Contig25975	transitional endoplasmic reticulum	259	6.70E-29	P:cell cycle; F:hydrolase activity; F:nucleotide binding; C:plasma membrane	EC:3.6.4.3
Contig9825	triosephosphate	1249	3.61E-160	P:reproduction; P:biosynthetic process; P:carbohydrate	EC:5.3.1.1

	isomerase			metabolic process; P:photosynthesis; P:multicellular organismal development; C:mitochondrion; P:cellular process; P:lipid metabolic process; C:plastid; P:catabolic process; P:cellular component organization; C:thylakoid; F:catalytic activity; F:protein binding; C:extracellular region	
Contig14497	tubulin alpha-3	949	3.69E-121	C:cytosol; C:cell wall; C:cytoskeleton; P:cellular component organization; P:cellular process; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; P:catabolic process; P:response to abiotic stimulus; F:hydrolase activity; F:structural molecule activity; P:biological_process; F:nucleotide binding; C:plasma membrane	EC:3.6.5.1; EC:3.6.5.2; EC:3.6.5.3; EC:3.6.5.4
Contig416	ubiquitin-conjugating enzyme e2 i	930	1.09E-93	P:protein modification process; F:catalytic activity	EC:6.3.2.19
Contig28259	udp-d-apiose udp-d-xylose synthase 1	1769	0	F:nucleotide binding; C:cytoplasm; C:extracellular region; F:catalytic activity; P:biosynthetic process; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	EC:4.1.1.35
SJ06-E1-SO1-008-D01-UC.F	udp-glucuronic acid decarboxylase 2	502	4.91E-85	C:membrane; C:Golgi apparatus; P:biosynthetic process; P:carbohydrate metabolic process; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; F:catalytic activity; P:cellular process; C:plasma membrane; C:vacuole; F:binding	EC:4.1.1.35; EC:4.2.1.46
Contig22609	uncharacterized protein	635	1.12E-24	F:molecular_function; P:biological_process; C:cellular_component	
Contig21502	vacuolar atp synthase subunit	1215	1.06E-124	P:transport; P:biosynthetic process; P:generation of precursor metabolites and energy; P:nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; F:transporter activity; F:nucleotide binding; F:hydrolase activity; C:membrane	EC:3.6.3.14
Contig28734	vacuolar sorting protein 4b	636	1.49E-101	C:endosome; P:anatomical structure morphogenesis; P:cellular component organization; P:cell differentiation; P:multicellular organismal development; P:cellular process; P:transport; F:nucleotide binding; F:hydrolase activity; C:nucleus	EC:3.6.4.3
Contig21850	virion binding	719	1.62E-47	F:binding	-
Contig23300	wd40-repeat protein	1309	8.76E-104	C:intracellular; F:nucleotide binding	-
Contig5209	wrky transcription	870	1.77E-77	P:transcription, DNA-dependent; F:sequence-specific DNA binding; P:regulation of transcription; F:transcription factor activity	

Contig18831	wrky transcription	1909	0	F:DNA binding; P:biological_process; F:protein binding; F:transcription factor activity; P:transcription; P:response to extracellular stimulus; P:cell communication; P:response to stress	-
Contig21742	yth domain-containing	3028	0	C:cytoplasm; C:nucleus; F:protein binding	-
Contig23318	zinc finger protein	1360	4.58E-119	P:flower development; P:transcription; F:DNA binding; F:protein binding; C:nucleus	-
