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Genotypic variation of gene expression during the soybean innate immunity response

Oswaldo Valdés-López[1,2]*, Saad M. Khan[3], Robert J. Schmitz[4,5,6], Shiqi Cui[7], Jing Qiu[7], Trupti Joshi[3,8], Dong Xu[3,8], Brian Diers[9], Joseph R. Ecker[4,5,10] and Gary Stacey[1]*

[1]*Divisions of Biochemistry and Plant Sciences, National Center for Soybean Biotechnology, C.S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA,* [2]*Laboratorio de Bioquímica, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla, Estado de México C.P. 54090, Mexico,* [3]*Informatics Institute, University of Missouri, Columbia, MO 65211, USA,* [4]*Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA,* [5]*Genomic Analysis Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA,* [6]*Department of Genetics, Division of Life Sciences, University of Georgia, 120 East Green Street, Athens, GA 30602, USA,* [7]*Department of Statistics, University of Missouri, Columbia, MO 65211, USA,* [8]*Department of Computer Science, National Center for Soybean Biotechnology, C.S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA,* [9]*Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA and* [10]*Howard Hughes Medical Institute, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA*

Abstract

Microbe-associated molecular pattern (MAMP)-triggered immunity (MTI) is an important component of the plant innate immunity response to invading pathogens. Although several MTI responses can be measured in different plant species, their magnitude is probably plant species specific and even cultivar specific. In this study, a genome-wide transcriptome analysis of two soybean parental lines and two progeny lines treated for 30 min with the MAMPs flg22 and chitin was carried out. This analysis revealed a clear variation in gene expression, under both untreated and flg22+chitin-treated conditions. In addition, genes with potential additive and non-additive effects were identified in the two progeny lines, with several of these genes having a potential function in the control of innate immunity. The data presented herein represent the basis for further functional analysis that can lead to a better understanding of the soybean innate immunity response.

Keywords: gene expression; innate immunity; microbe-associated molecular pattern; mRNA-seq soybean

Introduction

Plants have developed a sophisticated, multilayered system to detect pathogens and trigger a strong defence response (Segonzac and Zipfel, 2011). For example, plants can recognize the invading pathogen through the detection of conserved structural motifs, termed microbe-associated molecular patterns (MAMPs). Well-studied examples of MAMPs include chitin, flagellin and elongation factor Tu (Monaghan and Zipfel, 2012). Once MAMPs are detected by specific pattern recognition receptors, which are localized at the plant cell surface,

* Corresponding authors. Email: oswaldo_valdesl@yahoo.com.mx; staceyg@missouri.edu

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a defence response, termed MAMP-triggered immunity (MTI), is mounted that leads to disease resistance (Monaghan and Zipfel, 2012).

MTI is characterized by a variety of plant responses, which include changes in ion flux across the membrane, changes in intracellular calcium levels, production of reactive oxygen species and deposition of callose as well as modification of the expression of several genes (Zipfel, 2009). Although several of these responses can be measured in different plant species, their magnitude is probably plant species specific and even cultivar specific (Valdés-López *et al.*, 2011; Veter *et al.*, 2012). For example, we have recently demonstrated a significant genotypic variation in the MTI response of four different soybean [*Glycine max* (L.) Merr] genotypes (Valdés-López *et al.*, 2011). Additionally, based on the expression of 25 MAMP-responsive genes, we observed that the progeny inherited the variation in expression demonstrated by the parental lines. These data suggested a genetic basis for the strength of the MTI response that could potentially be exploited to improve soybean agronomic performance. However, these observations were based on a limited number of genes and may not reflect the whole transcriptome response to MAMP elicitation. Therefore, to expand upon the previous study, in the present study, we conducted a genome-wide transcriptome analysis of the parental lines LD00-2817P and LDX01-1-65 (hereafter called LD and LDX, respectively) and two F_4 recombinant inbred lines.

Materials and methods

Plant material and MAMP treatment

The soybean genotypes LD and LDX and two of their F_4 lines, LD08-11 272 and LD08-11 268 (hereafter called R72 and R68, respectively), were used to contrast their response to 1 μM flg22+50 μg chitin (hereafter called MAMP) elicitation.

High-throughput sequencing and processing of mRNA-seq reads

Twenty-four non-strand-specific mRNA-seq libraries [four genotypes, two treatments (MAMP or Mock) and three replicates] were processed and analysed as described by Schmitz *et al.* (2013).

Identification of differentially expressed genes

Low-count reads with a total sum <10 were filtered out before a Poisson linear mixed-effects model (Blekhman *et al.*, 2010) was applied to the raw read counts separately for each gene with the library size as the offset value. Likelihood ratio tests were conducted to identify differentially expressed genes in the treatment and control groups for each of the four genotypes, as well as in genotypes for either the control or the treatment group. *P* values for the likelihood ratio tests were obtained and then transformed into *q* values (Storey and Tibshirani, 2003) to produce lists of differentially expressed genes with an estimated false discovery rate of 5% and with a fold change >2.

Results and discussion

Transcriptional variation between parental and F_4 lines in the absence of MAMP treatment

In a previous study, we had demonstrated variations in the oxidative burst triggered by the application of flg22 or chitin between the genotypes LD and LDX (Valdés-López *et al.*, 2011). We had also observed that several F_4 lines, from a cross between the genotypes LD and LDX, exhibited an oxidative burst that was similar to, higher than or lower than that exhibited by the parental lines. In particular, the F_4 lines R72 and R68 exhibited a response that was very similar to that exhibited by either of the parental lines (see online supplementary Fig. S1). Given the differences observed in the oxidative burst response of the parental and progeny lines, we expanded our study by conducting a transcriptome analysis (using mRNA-seq) to compare and contrast the two parental lines and the two F_4 lines.

Genotypic variation among different cultivars is the basis for plant breeding and has been successfully used to develop well-adapted, high-yielding crops. Additionally, it has been reported that heterosis affects the mode of gene action by either increasing or decreasing the expression in the progeny (Baranwal *et al.*, 2012; Richards *et al.*, 2012). In the case of the four soybean genotypes under study, we first compared variation in basal expression in the absence of any treatment. Although most of the 43,600 expressed genes in this mRNA-seq analysis exhibited similar expression levels in all the four genotypes, a pairwise analysis of the parental lines and F_4 lines (i.e. LD vs. R72) did identify several differentially regulated genes (Fig. 1; see online supplementary Table S1). For example, the comparison between LD and LDX genotypes revealed 1258 genes that were differentially regulated in the two parental lines (Fig. 1(A); see online supplementary Table S1). A similar trend was observed in the pairwise comparisons between the parental line LD and the F_4 line R72 (1012 differentially regulated genes; Fig. 1(B)) as well as between the parental line LDX and the F_4 line R68 (865 differentially regulated genes; Fig. 1(E)). In contrast to these

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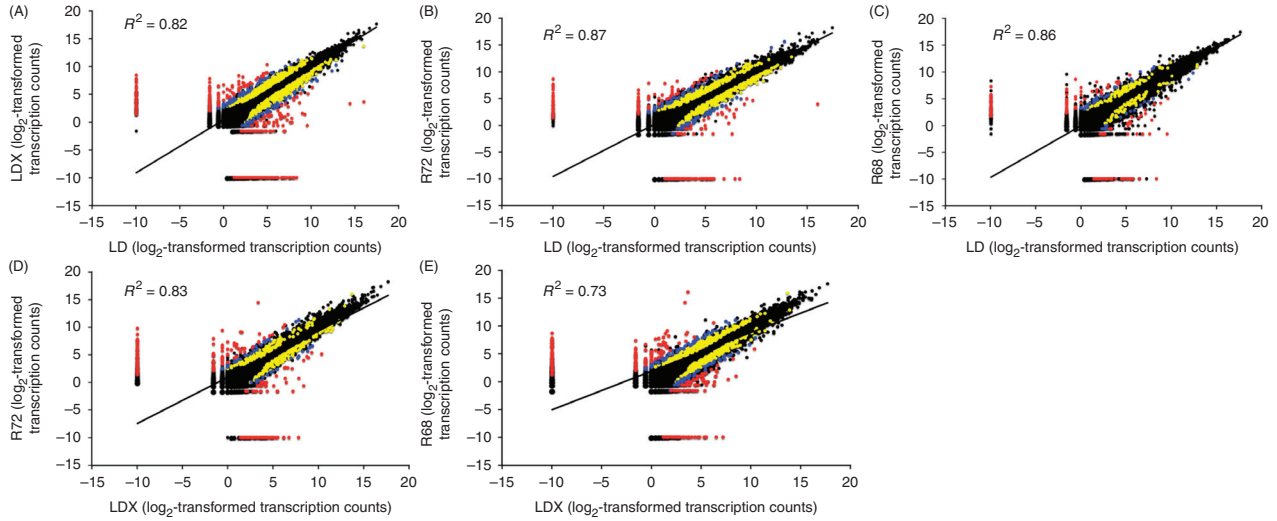


Fig. 1. Pairwise comparisons of transcript abundance between parental lines [LD00-2817P (LD) and LDX01-1-65LD (LDX)] and F_4 soybean lines (R72 and R68) under untreated conditions: (A) LD versus LDX; (B) LD versus R72; (C) LD versus R68; (D) LDX versus R72; and (E) LDX versus R68. Black dots indicate 43,600 genes detected in this study; yellow, blue and red dots indicate differentially regulated genes with 2-, 3- or >3-fold change, respectively. Results are the average of three biological replicates. Statistical parameters are given in online supplementary Table S1.

comparisons, the comparisons of LD versus R68 and LDX versus R72 revealed relatively few differences in basal gene expression (Fig. 1(C) and (D); see online supplementary Table S1). A gene functional enrichment analysis using Gene Ontology terms (Du *et al.*, 2010) revealed that programmed cell death, defence response, innate immunity response and response to bacterial infection were the most enriched biological processes identified in either of the pairwise comparisons (see online supplementary Fig. S2 and Table S2). These results indicate a clear genotypic variation in the gene expression levels among the parental and F_4 lines.

Transcriptome analysis of parental and F_4 lines in response to MAMP treatment

Consistent with previous results, the two parental lines exhibited a number of differentially expressed genes when comparing the untreated and MAMP-treated tissues (see online supplementary Figs S3 and S4 and Table S3). Likewise, similar differences in the two F_4 lines were detected when comparing their transcriptomes in a pairwise fashion (R72 vs. LD, R72 vs. LDX, R68 vs. LD, R68 vs. LDX and R68 vs. R72). Across all the four genotypes, a total of 7803 differentially regulated genes were identified. A comparison across the four genotypes revealed that 1525 genes were differentially up-regulated in all the genotypes. Of these 1525 common differentially regulated genes, 1027 were regulated in all the four genotypes. In addition, 169, 168, 298 and 28 genes were specifically up-regulated in the genotypes LD, LDX, R72 and R68, respectively (see online supplementary Fig. S4 and Table S4). Furthermore, to assess whether the two F_4 lines exhibited a response that was similar to or different from that exhibited by the parental lines, a pairwise comparison of the fold ratios of the 1725 (including up- and down-regulated) commonly regulated genes was made (Table 1). This comparison revealed that 57–72%

Table 1. Pairwise comparison of the fold ratios (microbe-associated molecular pattern/Mock) of the parental lines [LD00-2817P (LD) and LDX01-1-65 (LDX)] and F_4 lines (R72 and R68)[a]

	Similar response		Higher response in the parental lines		Higher response in the F_4 lines	
Pairwise comparison	No. of genes	%	No. of genes	%	No. of genes	%
LD vs. R72	848	63	332	25	162	12
LD vs. R68	750	60	407	33	87	7
LDX vs. R72	700	57	286	23	246	20
LDX vs. R68	813	69	245	21	120	10

[a] The percentage in each category represents the genes that exhibited similar or different responses in the parental and F_4 lines.

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of these genes in the F_4 lines exhibited a response that was very similar to that exhibited by the parental lines, whereas only 21–33% exhibited smaller changes and 3–20% larger changes in responses when compared with the two parental lines (Table 1). Collectively, these data indicate that approximately 70% of the differentially regulated genes are commonly regulated when comparing the four genotypes.

In conclusion, the results of this study demonstrate a clear variation in gene expression, under both untreated and MAMP-treated conditions, across the parental and progeny lines. Similarly, the heritability of the MTI response suggests that specific selection for a strong MTI response, in conjunction with extensive field testing, could be one avenue for developing soybean cultivars with broader and more sustainable disease resistance.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000197

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Sequence data can be downloaded from the National Center for Biotechnology Information Gene Expression Omnibus (accession GSE43463 for treated RNA-seq data and GSE41753 for untreated RNA-seq data).

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