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"Type A" response regulators are involved in the plant-microbe interaction

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Abstract

Plant-microbe interaction can be established as either symbiotic or pathogenic association. Regardless of any type of interaction interplayed, common strategy can be seen in the partners. Recent advances in bioinformatics and availability of abundant transcriptomics data have provided new tools for comparative analysis and achieving significant insights into underlying regulatory background of symbiosis and pathogenesis. In the present study, to find the genes which are involved in both pathogenic and symbiotic interactions, we used the microarray data pertaining bacterial interactions in *M. truncatula*. Those data which were publically available in NCBI analyzed using Expression Console and FlexyArray. In order to interpret gene expression patterns and investigate the relationship between co-over expressed genes, a literature survey was performed using the tools provided by Pathway Studio v9 (Elsevier). Our data analysis identified type-A response regulators (RRs) as genes that potentially respond to pathogenic and symbiotic interactions. Type-A RRs act as negative regulators of cytokinins. This study speculates that plants do not recognize bacterial pathogens from symbionts at early stage of plant-microbe interactions. Pathway analysis revealed the involvement of WUSCHEL (WUS) and SIAMES (SIM) in cross-talk between pathogen and type-A of RRs. Considering the central role of WUS and SIM in cell division, we suggest that the reduction of growth by repression of cell division is one of the adaptive responses in plants to bacterial interaction. In this way, plants try to preserve the limited energy of the mother cell and to avoid heritable damage.

Keywords: Cytokinine, Microarray analysis, Pathway analysis, SIAMES, Response Regulators
Abbreviations: RR_Response regulator; SIM_SIAMES; WUS_ WUSCHEL; CK_Cytokinine; HD_Histone Deacetylase; CDKs_Cyclin_dependent protein kinases; HUB1_histone monoubiquitination1; HKs_Histidine Protein Kinases; HPs_Histidine Phosphotransfer Proteins; NSP_Nodulation signaling pathway; NIN_nodule inception.

Introduction

Higher organisms are constantly challenged by insects, viruses, bacteria, and fungi. In the course of evolution, the interaction has been evolved either to the benefit of partners (symbiosis) or to a battle for survival (pathogenic interaction) (Baron and Zambryski, 1995). Although the outcome appears to be completely different, common molecular strategies that mediate communication between the interacting partners seem to be involved (Soto et al., 2006). Ausubel and Bisseling (1999) suggested that there are many common aspects of symbiotic and pathogenic interactions, which are the consequences of similar molecular mechanisms. Common features may include at the initial processes by which the host and symbiont/pathogen recognize each other to determine whether the interaction is 'compatible', establish intra- or inter-cellular infection, and form the specialized cells/structures to facilitate the exchange/transfer of nutrients. These common aspects of plant-microbe interaction are the 'coin' that unifies the field (Ausubel and Bisseling, 1999). In 2006, Soto and co-workers suggested that the success of invasion and survival of microbes within the host also requires that symbionts and pathogens suppress and/or overcome plant defense responses triggered after microbial recognition. An ideal host and comprehensive data are prerequisites to find common aspects of plant-microbe interactions. A legume host seems to be appropriate since it provides unique opportunities for study of vital phenomena such as symbiotic nitrogen fixation, and legume–pathogen interactions. *Medicago truncatula* is a long-established model for studying the biology of legumes with particular attention in symbiotic nitrogen fixing community. Several comprehensive genomic/transcriptomic tools and omics data are already available for *M. truncatula*. Using these data, computational studies have characterized regulatory genes, which have key roles in plant adaptation to various stresses in *M. truncatula* (Zahaf et al., 2012; Godiard et al., 2011; Middletone et al., 2007). The present study was conducted to shed light on common aspects of the relationship between plant-pathogen, and plant-symbiont using publicly available microarray data sets. We focused on transcription factors as important regulatory elements in cells to investigate the role of co-over expressed transcription factors (TFs) which are common in *M. truncatula*-bacterium interactions. Finally, a literature survey was performed to interpret gene expression patterns and investigate the relationship between co-over expressed genes, different proteins, and different molecules.

Results

Analysis of microarray data

Analysis of GSE18473 series of transcriptome data regarding *M. truncatula-R. solanacearum* interaction showed that 596 probe sets out of 61000 probes co-over expressed in both time points 12h and 72h versus control. A total of 444 out of 596 probe sets imported to PLEXdb, had homologous genes in the *Arabidopsis* genome (TAIR annotation). Among the annotated probe sets, 44 transcripts were classified as "DNA binding group" (supplementary Table 2). The most abundant DNA binding proteins which over-expressed during early establishment of *M. truncatula-R. solanacearum* were "basic helix-loop-helix (bHLH) DNA-binding super family protein (bHLH), "WRKY DNA-bindings", "zinc fingers", RING/U-box super family proteins, and "response regulators". Antioxidant genes, cytochrome P450, heat shock proteins, chitinase, chalcone and stilbene synthase family proteins, and O-methyltransferase were also among the most dominant over expressed genes found in the analyzed GSE18473 data (data were not shown). In symbiosis experiment, GSE33636 dataset, microarray analysis data in Expression Console and FlexyArray revealed that 313 probe sets were up-regulated in both treatment time series: 6h versus its control, and 24h versus its relevant control. A total of 254 out of 313 probe sets were assigned to their specific TAIR annotations using PLEXdb analysis tools. Eighteen transcripts out of 254 were grouped as "DNA binding" proteins and listed in supplementary Table 2. The most common DNA binding proteins that co-over expressed in the interaction between *M. truncatula* and *S. meliloti* were "ethylene response transcription factors" and "response regulators". Interstingly, antioxidant genes, cytochrome P450, O-methyltransferase, chalcone and stilberne synthase family proteins, and expansins were also prevailed. Analysis of microarray data showed that "response regulators" 5, 7, 8, and 9 belonging to type-A RRs were common DNA binding proteins that up-regulated in both: *M. truncatula-R. solanacearum* and *M. truncatula-S.meliloti* experiments. Ethylene-responsive genes, basic helix-loop-helix (bHLH), and MYB are another TFs that revealed co-overexpression in the plant-bacterial interaction (supplementary Table 2). In this paper, we focused on the role of the type-A RRs and CKs signaling in plant-microbe interaction

Pathway analysis

In order to get an overview of the complex network of interactions and regulation of type-A RRs in plants, text mining analysis was performed covering papers published over 15 year, period (1995-2010). Using this tool it is possible to identify proteins, genes, or metabolites (small molecules) that are strongly associated with type-A RRs (Figure 1). In addition to currently known interactions, our analysis showed that ARR5 and ARR7, the central genes in pathway, are associated with a new protein known as "WUSCHEL" (WUS). WUS, a positive regulator of stem cells (Laux et al.,1996), plays a central role in the maintenance of stem cell populations. By focusing on the pathway, it is obvious that "Histone Deacetylase1" (HD1), a basal transcription repressor, mediates the relationship between pathogen and WUS (Figure 1). The cyclin-dependant protein kinases (CDKs) inhibitor, known as SIAMESE (SIM), is another gene connecting pathogen to ARRs. SIM plays a key role in the mitosis-to-endo re-duplication transition. Accordingly, SIM overproduction results in a strong inhibition of cell

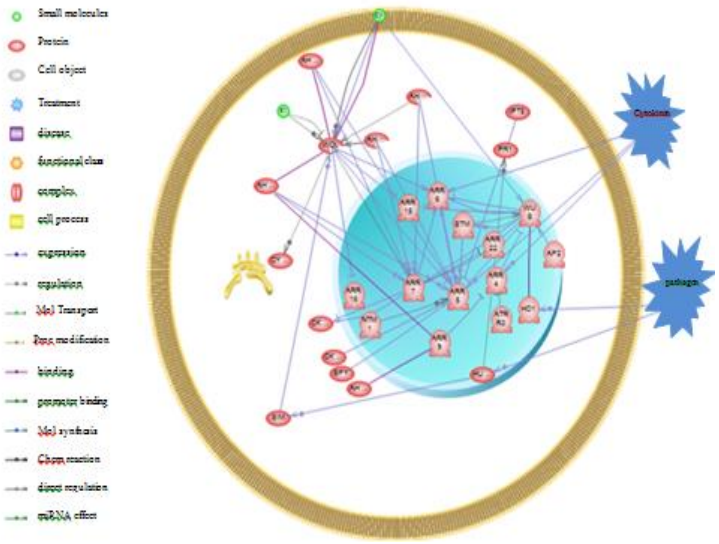

Fig 1. Pathway analysis shows the central role of ARR5 and ARR7 in CK signaling. WUS and SIM mediate the relation between pathogen and ARRs. The involvement of the "Histone Deacetylase1" (HD1), a basal transcription repressor activity, in the connection between pathogen and WUS is observed. Also, SIM can be connected to pathogen treatment via intermediate HUB1. Overexpression of ARRs, which are negatively affected by AHKs and AHP in plant-microbe interaction is one of plant defense responses which mediated by inhibition of plant cell division.

division activity (Peres et al., 2007). As presented in Fig 1, SIM was connected to pathogen treatment mediating through protein "histoe monoubiquitination1" (HUB1).

Discussion

Needless to say legumes are continuously exposed to numerous bacterial interactions. As a result, plants develop an immediate immune response which is accomplished by the action of a multitude of transcriptional regulators. The activity of these transcriptional regulators is orchestrated by a blend of signaling hormones (Moore et al., 2011). In our present study, microarray analysis showed an overexpression of type-A RRs, in both *M. truncatula-R. solanacearum* and *M. truncatula-S. meliloti* interactions. There are ten type-A RRs (ARR3–ARR9, and ARR15–ARR17) in Arabidopsis containing short C-terminal extensions that act as negative regulators of CKs (Muller and Sheen, 2007). CKs *per se* are hormones that regulate many aspects of plant growth and development. According to Muller and Sheen (2007), the CK phosphorylation pathway consists of four signaling components: Histidine Protein Kinases (HKs), Histidine Phosphotransfer Proteins (HPs), and two types of RRs (type A and B). Upon activation, HK phosphorylates HP. Subsequently, the HP migrates to the nucleus and modulate the activation of RRs.

The results of the present study revealed the involvement of CK signaling in either symbiotic or pathogenic interaction. It is not known whether exogenous CKs secreted by bacteria are involved in CK signaling pathway, or plant-originated CKs are themselves responsible in activation of CK pathway

and up-regulation of type-A RRs. CKs play significant role in the formation of root nodules, which are unique plant organs that provide optimal conditions for *Rhizobium* spp. to fix nitrogen (Saur et al., 2011). Activation of nodulation signaling pathway (NSP1, NSP2) and nodule inception (NIN) are necessary for nodule initiation and formation (Crespi and Frugier, 2008). CK signaling leads to activation of NSP1⁄2 and NIN expression and involves ARRs (Saur et al., 2011). A variety of type-A RRs act in *M. truncatula-Rhizobium* symbiosis (Gonzalez-Rizzo et al., 2006; Vernie et al., 2008; Op den Camp et al., 2011). Our study indicated that *MtRR5, MtRR7, MtRR8* and *MtRR9* up regulated in *M. truncatula*- S. *meliloti* interaction. In line with our finding, Rik et al. (2011) demonstrated that *MtRR9* and *MtRR11* in *M. truncatula* and their orthologs in *Lotus japonicus* are rapidly induced upon rhizobial epidermal infection. In plant-pathogenic bacteria interaction, there are also strong evidence that support a link between CKs and pathogenicity (Clarke et al., 1998; Maksimov et al., 2002; Argueso et al., 2009). Research needs to be done to ascertain the origin of CK secreted in the plant-microbe interaction. Nevertheless, application of exogenous CK to tomato roots has shown to induce several type-A RRs (Gupta et al., 2013). It can be proposed that induction of these genes probably occurred to dampen the increased exogenous cytokinin levels added to the roots. Analysis of microarray data reported herein indicated that "expansin" genes were also overexpressed in either pathogenic or symbiotic interactions (Supplementary Table 3). Expansin are non-enzymatic proteins found in the plant, where cell wall loosening occurs. In general, the loosening cell wall may facilitate pathogen entry and also cause nutrient leakage (Huckelhoven, 2007). Co-over expression of expansins in *M. truncatul*a-*R. solanacearum*, and *M. truncatul*a-*S. meliloti* suggestes bacterial pathogens and symbionts prepare a desirable environment in the host for survival. Our pahway analysis showed how type-A RRs, CK, and biotic treatment are connected together. Meta-analysis using Pathway Studio showed that ARR5 and ARR7 at the focus of the pathway are associated with WUS, which plays a central role in the maintenance of stem cell populations (Laux et al.,1996). Type-A RR genes have been reported to be direct targets of WUS. In Arabidopsis, WUS directly represses the transcription of ARR5, ARR6, ARR7 and ARR15 genes (Leibfried et al., 2005). Further quest revealed the involvement of WUS in the "Histone Deacetylase1" (HD1), a basal transcription repressor in the connection between pathogen and WUS. Deacetylation of histones is correlated with transcriptional repression. SIM, an inhibitor of CDK also connects pathogen to type-A RRs. It is required for coordinating cell division and cell differentiation. It plays a key role in the mitosis-to-endo re-duplication transition and its overproduction results in a strong inhibition of cell division (Peres et al., 2007). The coordination of cell division with environmental conditions is important for plant survival. The SIM family of genes was found to be regulated in response to biotic and abiotic stresses revealing a critical role of this family in coordinating cell cycle regulation with stress responsive pathways (Peres et al., 2007). One of the adaptive responses in plants to stresses is the reduction of growth by repression of cell division. In this way, plants try to preserve the limited energy of the mother cell and to avoid heritable damage. As presented in Figure 1, SIM can be connected to pathogen treatment via intermediate protein "HUB1". HUB1 was identified as the potential target of BIK1 (*Botrytis cinerea* Induced Kinase1), a protein kinase required for resistance to necrotrophic pathogens. Loss-of-function alleles of HUB1 in Arabidopsis showed increased susceptibility to the necrotrophic fungal pathogens *B. cinerea* and *A. brassicicola*, whereas HUB1 overexpression conferred resistance to *B. cinerea* (Dhawan et al., 2009).

Materials and Methods

Selecting an internet accessible data and analysis

In the present study, two sets of microarray data including: GSE18473, and GSE33636, related to *M. truncatula*, were downloaded from NCBI. GSE18473 series, prepared by Balzergue et al. (2009), include data from transcriptome analysis of the interaction between *M. truncatula* and *Ralstonia solanacearum*, the causal agent of the devastating bacterial wilt disease. Balzergue et al. (2009) studied *Ralostonia*'s infection process with an in vitro inoculation procedure on intact roots of *M. truncatula*. The pathosystem involved susceptible A17 and resistant F83005.5 *M. truncatula* lines which infected with the pathogenic strain GMI1000. A mutant A17 line, Sickle, which showed a resistant phenotype was also part of the experiment. Balzergue et al. (2009) extracted RNA from roots extremities at time 0, 12h and 72h post inoculation. GSE33636 is gene expression data from *M. truncatula*, cv. A17 in the context of nodulation obtained by Czaja et al. (2012). To trigger root nodule formation, host roots secrete flavonoids and other compounds that attract rhizobia to colonize the rhizosphere and root surfaces, inducing nodulation (nod) genes. The induction of nodulation (nod) genes results in the synthesis and secretion of lipochitin oligosaccharides called Nod factors (Saur et al., 2011). Czaja et al. (2012) studied transcriptional responses towards diffusible signals from symbiotic microbes, treated plantlet roots with symbiotic lipochitooligosaccharides (LCOs). After 6 h of incubation in the climate chamber, Czaja et al. harvested 10 plantlets per batch from the treatment (Nod-LCOs) or control solutions, while the other 10 remained in the respective solutions for a total of 24 h. We selected some parts of this series which were specified for *Sinorhizobium meliloti*, a bacterial symbiont (supplementary data, Table 1).

Analysis of microarray data

Raw data were normalized by RMA normalization method using Expression Console software and the results were exported to FlexyArray (version 1.6.1) software for further analysis. In both pathogenic and symbiosis experiments, "the time series after inoculation" were considered as treatment. Following application of Baysian t-test analysis on the normalized data, over expressed probe sets showing symmetric fold change greater than 2 and significant P value less than 0.05 were selected. Over expressed probe sets were exported to Microsoft Excel® and co-over expressed entries were obtained. To find relevant TAIR annotation (homolog genes in *Arabidopsis*) for these genes, the probe sets were analyzed using Plant Expression database (www.plexdb.org/mod- ules/glSuite/gl_main.php; Dash et al., 2012).

Pathway analysis

To interpret gene expression patterns and investigate the relationship between co-over expressed DNA binding proteins, a literature survey (from 1995 to February 2010) was performed using the tools provided by PathwayStudio v9 (Elsevier). Pathway Studio is an analysis tool supplied with RESNET, the database of Biological Association Networks.

The software collects latest information from deposited literature in PubMed and other public sources including KEGG (metabolic database; http://www.genome.jp/kegg/), BIND (protein interaction database; http://www.bind.ca), and GO (Gene Ontology) (Nikitin et al., 2003). RESNET product includes database of relations for mammalians and plants as well as molecular network databases for model organisms such as plant model, *A. thaliana*. Plant RESNET contains protein-protein relations which include protein-protein binding, expression regulation, promoter binding, protein modification, effects of different proteins and environmental conditions on small molecule and metabolite synthesis, molecular synthesis relations, regulation of proteins by plant hormones, and unknown regulation from small molecule to a protein. In this study, analysis of PathwayStudio was carried out for the interpretation of gene expression and providing comprehensive information on what may happen in the plant-microbe interactions. Further, since a network is composed of sub networks (Ebrahimie et al., 2014; Hosseinpour et al., 2012), we extracted small sub-networks of some specific proteins to find the possible relations with other sub-networks.

Conclusion

Microarray analysis suggested the involvement of type-A RRs and expansins in pathogenic and symbiotic interactions in *M. truncatula*. Both type of interactions exploit the same signaling pathway during early stages of plant-microbe establishment. In fact, at the early stage of interaction, plants may not be able to recognize bacterial pathogen from symbionts and respond to them as invaders.

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