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Improving Soybean Immunity Through Exploring The Ubiquitination System

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Ubiquitination has emerged in recent years as a key regulatory mechanism underlying plant immunity against many different pathogens. To elucidate the possible role and mechanistic basis underlying the regulation of host immunity by soybean UBS, we in this project first identified core members of the soybean ubiquitin system (UBS) using an array of bioinformatics tools. We then investigate the involvement of soybean UBS in the regulation of host immunity to soybean cyst nematode (SCN). In total, we identified 1451 genes which encode putative core soybean ubiquitin system (UBS) members, including four genes encoding E1s, 71 genes that encode the E2s, and 1356 genes encoding the E3-related components. Among the E3-encoding genes, 760 genes encode RING-type E3s, 124 genes encode U-box domain-containing E3s, and 472 genes encode F-box proteins. By in vitro thioester assays, proteins encoded by the soybean E1 gene GmUBA1 and the majority of selected E2 genes are shown to be active E1 or E2 enzymes. Meanwhile, most of the purified RING and U-box domain-containing proteins displayed E3 activity in the in vitro ubiquitination assay. Furthermore, we found that nearly 100 of the identified soybean UBS-related genes are likely involved in soybean cyst nematode (SCN).

Taking advantage of the ThiF motif that is typical for ubiquitin E1 protein, we identified 37 transcripts from 20 genes encoding ThiF motif-containing proteins. Among them, four proteins (Glyma.02G229700, Glyma.11G166100, Glyma.14G196800 and Glyma.18G058900) with a molecular weight around 120 kD were more closely related to human ubiquitin E1s UBE1 and UBA6, and cluster in the same clade with the Arabidopsis AtUBA1 and AtUBC2. Also, we found that the four proteins contain all typical domains of a ubiquitin E1 enzyme, and thus we conclude the soybean genome possesses four genes encoding putative ubiquitin E1 proteins.

To pinpoint soybean genes that encode ubiquitin E2 and E3, the hidden Markov model profiles of interesting domains from the Pfam database were used as queries to search against the soybean proteome database by employing the HMMER 3.1 program. The complete protein sequences were extracted from Soybase based on the HMMER search results, and then submitted to the Pfam and NCBI CDD databases to validate the presence of domains of interest. To finally determine these predicted proteins, we processed manual validation based on alignment of the sequence of domains of interest in candidate proteins and their corresponding consensus sequences that are downloaded from CDD database. Those proteins that lack the highly conserved key amino acids or secondary structures were excluded from the final dataset. Finally, 71 ubiquitin E2s were found to be encoded by the

soybean genome, and 760 RING, 124 U-box and 472 F-box genes in soybean genome were identified with high confidence.

To determine whether the identified soybean E1 and E2 genes encode active ubiquitin-activating and ubiquitin-conjugating enzymes, we cloned an E1 gene, *Glyma.14G196800*, and four E2 genes, *Glyma.17G098000*, *Glyma.09G273100*, *Glyma.12G021800* and *Glyma.04G081200* and expressed their recombinant proteins in *Escherichia coli* (*E. coli*). In the thioester assay, the E1 gene was found to encodes an active ubiquitin E1 enzyme, and *Glyma.17G098000*, *Glyma.09G273100* and *Glyma.12G021800* encode ubiquitin E2 proteins possess ubiquitin-conjugating activity. To determine if the putative soybean RING and U-box proteins are capable of catalyzing protein ubiquitination, we performed *in vitro* ubiquitination assays. To this end, four RING protein-coding genes, *Glyma.04G235700*, *Glyma.17G094000*, *Glyma.15G001100* and *Glyma.10G24100*, and four U-box protein-coding genes, *Glyma.20G013200*, *Glyma.11G140100*, *Glyma.19G199300*, and *Glyma.04G179300* were randomly selected and cloned and their recombinant proteins were expressed and purified from *E. coli*. The *in vitro* ubiquitination results demonstrated that the majority of the RING and U-box genes we examined displayed E3 ubiquitin ligase activity, which validates the algorithms and protocols we used for the identification of core components of soybean UBS at the genome scale.

Finally, we explored publicly-available RNA-seq datasets to examine the transcriptional profiles of the soybean UBS genes in response to soybean cyst nematode (SCN) treatments. Using the cutoff of 2-fold in RPKM, we found the transcription level of 180 soybean UBS genes were significantly altered after inoculation with SCN, accounting for approximately 12.6% of UBS genes in soybean. To confirm the reliability of the RNA-seq results, we randomly selected ten genes out of the 180 soybean UBS genes and further examined their expression after SCN treatment using real time quantitative PCR (real time qPCR) analysis. The expression level of six of ten soybean UBS genes were significantly altered after SCN treatment as detected by real time qPCR. Based on these results, we postulate that the expression levels of approximately 100 soybean UBS gene (approximately 60% of the 180 genes) may be significantly altered during the soybean-SCN interactions.

Our findings indicate the presence of a large and diverse number of UBS proteins in soybean genome, which suggests that target-specific modification by ubiquitin is a complex and important part of cellular and physiological regulation in soybean. We revealed certain members of the soybean UBS may be involved in immunity against soybean cyst nematode (SCN). Our study sets up an essential foundation for further functional characterization of the soybean UBS in many physiological processes, including host immunity against SCN. We submitted recently a manuscript of findings from this project to the journal BMC Plant Biology, which is currently under review.