Comparative analysis of RNA-Binding Proteomes under *Arabidopsis thaliana-Pst* DC3000-PAMP interaction by Orthogonal Organic Phase Separation

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Abstract

RNA-binding proteins (RBPs) are pivotal participants in post-transcriptional gene regulation. They interact with RNA directly to perform several post-transcriptional RNA regulatory functions or direct metabolic processes. Despite the essential importance, the understanding of plant RBPs is elementary, which derives mainly from other kingdoms via bioinformatic extrapolation or mRNA-binding proteins captured through UV crosslinked method. Recently, orthogonal organic phase separation (OOPS) method for RBP identification has been used in mammals and *Escherichia coli*. And plentiful RBPs were enriched without molecular tagging or capture of polyadenylated RNA in an unbiased way. In our study, OOPS was conducted on *Arabidopsis* and 468 RBPs were discovered including 244 putative RBPs. There were 17 peroxidases in 232 RBPs with enzymatic activities. In addition, *Arabidopsis thaliana-Pst* DC3000-chitinpentaose interaction system was chosen to explore whether OOPS can be used to dig specific RBPs under special physiological conditions. Eighty-four differential RBPs in this system were found and some of them involved in reactive oxygen species (ROS) metabolic pathway. These results showed OOPS can be applied to plants successfully and would be a useful method to identify RBPomes and specific RBPs.

Key words RNA-binding proteins; Orthogonal organic phase separation; *Arabidopsis thaliana*;

1. Introduction

RNA-binding proteins (RBPs) are the most important regulators of mRNA stability and translation in eukaryotic cells [1,2]. The study of RBPs helps us further understand the physiological activities of organisms. In mammals, several important RBPs were identified. For example, extracellular cold-inducible RNA binding protein (CIRP), as a novel proinflammatory molecule, induces inflammatory responses leading tissue injury [3,4]. The mammal RBPs have been identified to regulate maturation of mRNA and various small non-coding RNAs (ncRNAs) like miRNAs [5,6]. In addition, a large number of studies on RBPs in mammalian and bacterial systems [5,7,8] have been published. Even though more and more RBPs have been discovered and studied, it is far from enough. Therefore, the study of RNA-binding proteome (RBPome) has received unprecedented attention. It is particularly important to dig up the RBPomes from different species, which can make us understand the growth and development of organisms more comprehensively.

RBPs are very important to plants as it is to mammal with roles in pathogen defense [9,10] and abiotic stress responses [11-15]. Some RBP mutants suffer from severe phenotypes or lethality [16]. Until then, knowledge of RBPs in plants came mainly from targeted studies on individual proteins or from bioinformatic predictions based on sequence homology with canonical RNA binding domains (RBDs) identified in other kingdoms [17]. Recent studies have emphasized that development and improvement of high-throughput approaches to mapping and quantifying protein-RNA interactions promoted the decipherment of large-scale transcriptome and translational

group data in plants [17-20].

Several approaches are available to characterize RNA-RBP interactions to help us enrich RBPomes. Protein-bound RNAs (PBRs) can be purified by immunoprecipitating a specific protein and sequencing its RNA cargo [21]. And individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) identifies protein–RNA crosslink sites on a genome-wide scale [22]. In addition, the cellular repertoire of polyadenylated RNA-binding proteins can be recovered by UV cross-linking RNA-RBP complexes, capturing RNA with oligo(dT) and subsequently identifying bound proteins [14,23-28]. Although mRNA capture technology has been proved to be successful in providing large repertoires of RBPs, drawbacks including the need for sizable amounts of material and complex workflows are apparent.

To identify RBPs comprehensively, methods based on protease digestion [29,30], modified nucleotides [31-33] and organic phase separation of protein-crosslinked RNAs [34] have been developed and applied to mammalian and human cells in recent years, of which organic phase separation was used to define the first bacterial protein-RNA interactome [35].

The development of many other methods [36,37] increased the number of RBPs in mammalian. However, the detection of RBPome in plants is rarely promoted and only a UV cross-linking RNA-RBP complexes method has been used to recover the polyadenylated RBPs in *Arabidopsis thaliana* [14,26-28]. The previous mRNA interactome method represent the first profound system-wide analysis of mRNA interacting proteins and these studies pave the way for the overall analysis of RBP

[14,26-29,38]. But the requirement for poly(A) tails makes it difficult to apply oligo(dT)-based methods to bacterial systems or eukaryotic non-polyadenylated RNAs. The comprehensive and specific identification of RBPs as well as the discovery of RNA-associated protein functions remain major challenges in plant RNA biology.

Orthogonal organic phase separation (OOPS) is a new method based on acidic guanidiniumthiocyanate-phenol-chloroform (AGPC) phase partition. OOPS collects RBPs through the physical properties of RBPs, which remains at the interface between the water phase and the organic phase and does not require molecular tagging or capture of polyadenylated RNA. Through quantitative and sequencing analysis of the enriched RNA, it is proved that all cross-linked RNA were recovered without any systematic bias [34]. The results show that OOPS can overcome the drawbacks of mRNA extraction technology, which could not enrich RBPs that interacting with non-polyA RNAs and recover all cross-linked protein-RNA and free protein, or protein-bound RNA and free RNA in an unbiased way [34,39,40]. In addition, it is simpler to operate and it succeed in mammal cells and *Escherichia coli*. Therefore, OOPS may be an effective method to extend the RBPome in plants.

In this study, we tried to apply OOPS in plants and further emphasized its importance in plant physiology research. Plant-pathogen interaction and a typical PAMP were adopted to formulate the biological functions of RBPs. *Arabidopsis thaliana-Pst* DC3000, a typical plant-disease research model, was employed. The potent PAMP chitinpentaose was utilized as a conventional elicitor in our study.

2. Materials and methods

2.1. Plant Materials and Growth Conditions.

Arabidopsis was grown in an environmentally controlled chamber under a 12-h-day/12-h-night cycle. Four-week-old plants were applied to carry out experiment. chitinpentaose and water were uniformly sprayed on the surface of plant leaves three days before injecting *Pst* DC3000 (OD= 0.002) and 10 mM $MgSO_4$ was injected as control. After three days, four group samples, H_2O+$MgSO_4$ (HM), H_2O+*Pst* (HP), chitinpentaose+$MgSO_4$ (CM), chitinpentaose+*Pst* (CP) were collected. The disease index and colony count were counted as described previously [41]. The bacterial dose infiltrated into the leaves was 1×10^5 CFU per square centimeter of leaf area (equivalent to an optical density at 600 nm = 0.002). According to the proportion (S) of necrotic lesion area on leaves, the symptoms of infected leaves were divided into four levels: 1 ($0 < S < 0.25$), 2 ($0.25 < S < 0.5$), 3 ($0.5 < S < 0.75$) and 4 ($0.75 < S < 1$). The symptom data were obtained using the following formula: Disease index (%) = (+(level × leaves in the level) ÷ total leaves ÷ the highest level) × 100. We collected the whole plants as the next sample.

2.2. OOPS in *Arabidopsis thaliana*.

The whole plant was placed on ice and UV cross-linking was performed by UV irradiation at 254 nm for 60 seconds (CL-1000 Ultraviolet Crosslinker; UVP). After grinding Arabidopsis into powder in liquid nitrogen, it was immediately added to acidic guanidinium-thiocyanate-phenol (Trizol) for lysed cells and incubated at room

temperature (RT) for 5 min to dissociate unstable RNA-protein interactions. For biphasic extraction, chloroform (Trizol: chloroform= 5:1(v/v)) were added and phases were vortexed. The sample was centrifuged for 15 min at 11000 rpm at 4℃. Interface (containing the protein-RNA adducts) was subjected to extra 2 times AGPC phase separation cycles, cryogenic overnight precipitated with 9 volumes of methanol at - 20℃ and pelleted by centrifugation at 12000 rpm, 4℃ for 15 min.

For RNA-binding protein analyses, the precipitated interface was resuspended in 2 mL of 100 mM triethylammonium bicarbonate (TEAB), 1 mM $MgCl_2$, 1% SDS, incubated at 95°C for 20 min, cooled and digested with 20 µL RNase A, T1 mix (2 mg/mL of RNase A and 5,000 U/mL of RNase T1, Thermo Fisher Scientific) for 2–3 h at 37°C. Another 20 µL of RNase mix was added and incubated overnight at 37°C, after which a final cycle of AGPC phase partitioning was performed and released proteins recovered from the organic phase by methanol precipitation.

2.3. Proteomic sample preparation.

Protein sediment was dissolved in 100 mM Tris-HCl supplemented with 6 M guanidine hydrochloride (pH 8.0). Protein reduced by adding Dithiothreitol (DTT) to a final concentration of 20 mM and incubating for 2 h at 37ºC to break the disulfide bond. Iodoacetamide, as an alkylating agent, was then added to a final concentration of 40 mM to protect the sulfhydryl group from disulfide bond formation. The mixture was incubated for 60 min in dark. The proteins were then diluted 8-folds using 100 mM Tris-HCl (pH 8.0) and digested with trypsin (20:1) for 20 h at 37ºC. Peptides were

acidified with 10% trifluoroacetic acid (TFA) (adjust pH to 2-3) and desalted on an HLB SPE cartridge (Waters, 60 mg). The desalted peptides were collected and dried by using Speed Vacuum dryer (Thermo Fisher).

2.4. MS spectra processing and peptide and protein identification.

Liquid chromatography (LC)-Mass spectrum/Mass spectrum (MS/MS) was performed on an LTQ Orbitrap Elite mass spectrometer equipped with an UltiMate 3000 RSLC nano system (Thermo Fisher Scientific, USA). The peptides were loaded into the trap column (200 μm i.d., 4 cm long, C18 AQ beads (5 μm, 120 Å)) at the flow rate of 3.0 μL/min using 0.1% formic acid (Buffer A) in water for 10 min, and then separated at a 15-cm capillary analytical column (150 μm i.d., C18 AQ beads (1.9 μm, 120 Å)). The nano-LC gradient was set as followed at the flow rate of 300 nL/min: 2% Buffer B (80% Acetonitrile /0.1% formic acid) from 0 to 8 min, 10% solvent B at 10 min, 45% solvent B at 100 min, 100% solvent B from 110 to 120 min and 2% solvent B from 125 to 130 min. The normalized collision energy was set as 35 for the peptide's analysis (Collision-Induced Dissociation (CID) mode). The resolution of full mass was set to 60,000 and the resolution of MS/MS was set to 15,000. Survey full scan MS was acquired from m/z 350 to 1800 and 20 most intense ions (charge 2-7) with the intensity threshold of 5×10^3 were selected for MS/MS detection.

The raw data was analyzed using MaxQuant (version 1.5.8.3). Default parameters: an initial mass tolerance of ± 20 ppm and a final mass tolerance of 6 ppm for precursor ions, a mass tolerance of 0.5 Da for CIDion trap fragment ions, a minimum peptide

length of seven amino acids, enzyme specificity set as trypsin with two missed cleavages allowed. The false discovery rate for peptides, proteins was set as 1%.

2.5. Proteomics bioinformatics and data analysis.

Among the three replicates at different processing samples, only proteins present in at least two replicates were retained. For quantitative experiments, peptide-level quantification was obtained by summing the quantification values for all peptides with the same sequence but different modifications. Gene Ontology (GO) terms, Interpro protein domains and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations were obtained using Uniprot (https://www.uniprot.org/) and Interpro (http://www.ebi.ac.uk/interpro/).

3. Results and Discussion

3.1. 468 RBPs were identified by OOPS in Arabidopsis

Four hundred and sixty-eight RBPs were identified by OOPS in *Arabidopsis* (Fig. 1A) including 64 proteins that have been annotated as RBPs in GO database and 160 proteins overlap with previously published RBPome dataset (Fig. 1B) [14,26-28]. Many classical RBDs have been identified in our RBPome, such as RNA recognition motif domains, K homology domains, Like-Sm domains (Fig. 1C) [17]. Heat shock-related protein domains have been also identified in the proteome, which are noncanonical but reported as RNA-binding domains [27,28]. Alongside heat shock domain, we identified multiple nicotinamide adenine dinucleotide (NAD)-binding

194 domains, some of which have not been reported previously to interact with RNA. They
195 included two sites within the NAD-binding pocket of glyceraldehyde 3-phosphate
196 dehydrogenase (GAPDH) [42], which confirmed previous RNA-binding site
197 predictions based on in *vitro* experiments [43]. The existence of GAPDH has been
198 confirmed in the previous work of RBPome, both in plants[14] and mammals[34]. Not
199 surprisingly, four peroxiredoxin proteins (three published RBPs and one candidate
200 RBPs) were enriched and the post-transcriptional regulation of snoRNA expression by
201 peroxiredoxin 1 has been reported recently [44]. All the above evidences showed the
202 reliability of the method.

203 On this basis, more than 48% of the proteomes obtained via OOPS have been
204 identified as RBPs, which greatly improves the reliability of applications of this method
205 in plants. However, a large number of proteins (52%) were newly candidate RBPs in
206 our results. Apart from experimental evidence, most RBPs were predicted and the most
207 important prediction method was to capture mRNA by UV cross-linking. The further
208 expansion of RBPs predicted by OOPS showed that the new method had the potential
209 to break through the limitations of UV crosslinking. It will supplement and improve the
210 existing results. Therefore, a considerable number of other RBPs, including long
211 noncoding RNA, pre-mRNAs and so on, could be effectively and indiscriminately
212 enriched by OOPS. Many factors, such as the growth period of plant materials, the time
213 of ultraviolet irradiation and the selection of separation times, determined the amount
214 of RBP enriched [28,34].

215 In *Arabidopsis* RBPome extracted by OOPS, subcellular localization covers all

organelles (Fig.1D). Chloroplast is a unique and important organelle in plants, which contains many special RBPs. The research on RBPs in chloroplast is a very important part of plant RBPs. The RBP proteome enriched by OOPS contained 8 ribosomal proteins that only existed in chloroplasts (30S/50S) (Table S1). Polyadenylation plays important roles in regulating the function and metabolism of RNAs [45] and it is also an important label of RBP enrichment method. However, polyadenylation in chloroplasts generally leads to RNA degradation, consistent with the bacterial origin of this organelle [46,47]. The previous methods should be improved in RBPs unique to chloroplasts. Therefore, the methods used previously in plants cannot enrich proteins without a poly(A) tail. Ribosomes from prokaryotes, eukaryotes, mitochondria and chloroplasts had characteristically distinct ribosomal proteins [48,49], which were mainly responsible for intracellular translation processes and were very important RBPs. The results showed that the method we adopted could extract ribosomal proteins from mitochondria and chloroplasts effectively, which did not harbor a poly(A) tail [45]. Obviously, OOPS can enrich the rare RBPs from chloroplast, which provides an effective enrichment method and more possibilities for future research of RBPs in plants.

We successfully applied the OOPS to the detection of RBPome in *Arabidopsis*, and expanded the existing database, which provides technology and data support for more extensive research of RBPs in plants.

3.2. RBPs have multitudinous function

In the early stage, the relationship between metabolism and RNA-based regulation of gene expression was formally proposed through the RNA, enzyme, and metabolism (REM) hypothesis [50]. Catalogues of mRNA interactors now suggest a more global functional relevance of enzymes moonlighting as RBPs[2]. In addition to enzymes controlling the fate of bound mRNA, the RNAs could in turn serve as regulators of enzymatic activity, for example through competition or allosteric activation/repression, or by acting as scaffold for the assembly of enzyme complexes [51]. Elucidating such phenomenon may lead to the discovery of novel RNA-based regulatory mechanisms [23,24,27,52,53]. There were also relevant research results in plants. ETHYLENE INSENSITIVE 2 (EIN2) previously used as a candidate RBP [27] had shown to act as a transcription factor in response to ethylene [54]. Furthermore, EIN2 has recently shown to regulate the translation of ethylene-responsive mRNAs through binding to their 3'-untranslated regions [2,55,56].

Among the accumulated proteomes, two hundred and thirty-two proteins had activities. They covered not only the catalytic reactions of various enzymes, including oxidoreductase, transferase, hydrolase, lyase, isomerase and ligase, but also a variety of functional proteins that were not enzymes, such as activator, inhibitor, transporter, and protein homologation, etc. And some of proteins even were bifunctional or trifunctional. It demonstrated that RBP was involved in a range of responses and the functional cognition has been greatly expanded and further illustrates the importance of RBP for plants (Fig. 2A, Table S2). Recent mRNA interactome data in eukaryotes revealed the conspicuous aspect that many enzymes had RNA binding functions.

It was worth mentioning that 17 peroxidases were enriched, including thioredoxin peroxidase, glutathione peroxidase and L-ascorbate peroxidase. Ascorbate peroxidase is a hydrogen peroxide-scavenging enzyme that is specific to plants and algae. It is indispensable to protect chloroplasts and other cell constituents from damage by hydrogen peroxide and hydroxyl radicals produced from it [57,58]. In previous studies, although it has been reported in the RBPomes of human [25] and *Arabidopsis* [27], only a few peroxidases have been found to bind RNA, such as PkTPx-1, which was cloned and characterized thioredoxin peroxidase 1 from *P. knowlesi* and was able to bind to double-strand DNA and RNA and it had RNA chaperone activity in a nucleic acid melting assay [59]. The enrichment results and the RNA binding activity of the above non-plant-derived peroxidases undoubtedly suggested that peroxidase was a kind of probable RBPs. The 17 peroxidases identified by OOPS in *Arabidopsis* laid a substantial foundation for further study on the relationship between peroxidases and RNA.

The functions of RBPs stemmed from their ability to bind specific collections of RNA molecules. Once bound to their target RNAs, RBPs can perform or direct several post-transcriptional RNA regulatory or metabolic processes such as splicing, RNA chemical modification, decay, transport and RNA editing [60-62]. The fact that so many RBPs had activity suggested that a large number of significant RNAs had not yet been discovered. How the interaction between RNA and RBPs affects activities of RBPs may be the focus of future research. And the richness of RBP function will further emphasize its important role in plant research.

3.3. Identification of differential abundant RBPs under plant-pathogen interaction

Arabidopsis thaliana - *Pst* DC3000 - chitinpentaose was selected as a PAMP-induced plant-pathogens interaction system to explore whether OOPS can be used for the detection of differential RPBs under physiological conditions. The resistance induced by chitinpentaose to *Pst* DC3000 was evaluated. The degree of leaf wilting and yellowing area were significantly reduced after chitinpentaose treatment (Fig. 2B). The disease index in chitinpentaose group was 37.71%, which was much lower than water treatment 73.15% (Fig. 2C). In order to further confirm the chitinpentaose - induced resistance, the number of bacterial colonies was analyzed. It reached up to 410 cfu×10^5/cm^2 in water treatment group and in chitinpentaose treatment it was 217 cfu×10^5/cm^2 (Fig. 2D).

In order to analyze differential abundant RBPs after chitinpentaose and *Pst* DC3000 treatment, we compared RBPs in the two treatments and divided them into H_2O + $MgSO_4$ and H_2O + *Pst* DC3000 (HM-HP), chitinpentaose + $MgSO_4$ and chitinpentaose + *Pst* DC3000 (CM-CP), H_2O + $MgSO_4$ and chitinpentaose + $MgSO_4$ (HM-CM), as well as H_2O + *Pst* DC3000 and chitinpentaose + *Pst* DC3000 (HP-CP). The differential abundant RBPs can be divided into two categories, namely the difference in quality and quantity. For qualitative analysis, we drew the Venn plot (Fig. S1). As to quantitative analysis, based on threshold screening ($P < 0.05$), different abundant RBPs in 4 groups (HM-HP, CM-CP, HM-CM and HP-CP) were identified (Table S3). In HM-CM group, twenty-six differential abundant RBPs were identified

after chitinpentaose treatment, sixteen of which were up-accumulated and ten were down-accumulated. What's more, forty-five differentially abundant RBPs were identified and quantified in HM-HP, almost two times than HM-CM. The results showed *Pst* DC3000 caused more RBPs compared chitinpentaose and it as a pathogen with destructive virulence may trigger stronger and more complex responses. In CM-CP, fifty-one RBPs were only accumulated in CM, forty RBPs were accumulated in CP and 314 proteins were in common. Among these 314 proteins, thirty-six RBPs were differentially accumulated among which 13 were up-accumulated and 23 were down-accumulated (Table S3). There were 94 were not detected and 18 appeared after *Pst* DC3000 treatment in HP-CP (Fig. S1). The following analysis of individual treatments was carried out through analysis of HM-HP and CM-CP for *Pst* DC3000, HM-CM and HP-CP for chitinpentaose, respectively.

3.4. Differential abundant RBPs influenced by *Pst* DC3000

Differential abundant RBPs including those that appeared after treatment (according to Fig. S1) and had significant changes in quantity were analyzed under chitinpentaose and *Pst* DC3000 treatment. Gene ontology (GO) analysis ($P < 0.05$) was carried out to show biological process differential abundant RBPs participated in *Pst* DC3000 treatment (Table S4). Compared to HM, the differential RBPs mainly involved in response process, such as stimulus, metal ion, stress and hormone in HP group. *Pst* DC3000, as a biotic stimulus, triggers immune response in plant [63] (Fig. 3B).

To further identify the significant difference of the pathway between HM – HP and

CM-CP, we next drew Volcano map to clear accumulated RBPs with significant differences (more than twice fold changes and P value < 0.05) (Fig. 4) After *Pst* DC3000 treatment, several RBPs such as lactate/malate dehydrogenase family protein involving in atabolism of carbohydrate were up-regulated in HM-HP group. In CM-CP group, key RBPs involved in primary cell wall synthesis (AT3G53430) [64] and Antioxidant Protein1 (AT1G66240) related to heavy metal stress were up-regulated [65].

KEGG pathway analysis was performed to understand specific biological function (Fig. 3). Interestingly, we found "Peroxisome" pathway was enriched in HM-HP group (Fig. 3C). Non-enzymatic cell redox buffer systems can occur in chloroplasts, cytoplasm and mitochondria and recent studies have shown that they can also occur in peroxisomes [66]. The accumulation of reactive oxygen species was increased after *Pst* DC3000 injection and there are high expression of peroxisome-associated genes regulating changes in reactive oxygen species [67]. Peroxisomes regulate cellular responses under stress conditions [68].

3.5. Differential abundant RBPs influenced by chitinpentaose

GO analysis was also applied to the chitinpentaose treatment (Table S5). In HM-CM group, the chitinpentaose activated the enrichment of RBPs with response activity, such as to stress, abiotic stimulus and inorganic substance (Table. S5).The chitinpentaose as the main component of the fungal cell wall, is received by the plant signaling system to induce plants to develop disease resistance [69]. Plants apply induced defense mechanisms to combat the invasion of pathogens. After signal

recognition, multiple defense responses were detected in plants, including reactive oxygen species burst, increased transmembrane ion flux and response to other component [70-72].

Volcano map was drawn to understand the significant differential RBPs in HM-CM and HP-CP group. PDIL1-1 involved in drought stress [73] and AIG2-like (avirulence induced gene) family protein participating in early elicitor signaling [74] were induced by chitinpentaose in HM-CM group.

KEGG pathway analysis was performed to understand specific biological function involved in chitinpentaose-induced process. In HM-CM group, the metabolism pathways, such as fatty acid, pyrimidine and pyruvate metabolism, were up-regulated (Fig 3A). The "Photosynthesis" was exactly augmented in HP - CP group. Pathogen infection of plant results in modification of photosynthesis and defense mechanisms (Fig 3B)[75]. "Peroxisome" pathway was also up-regulated in HM-HP group, down-regulated in CM-CP group and no significance in HP-CP group. The results showed that both chitinpentaose and *Pst* DC3000 could activate "Peroxisome" pathway. The pathway was induced strongly by chitinpentaose before *Pst* injection (CM-CP) and returned to normal levels to HP after *Pst* injection (HP-CP). The chitinpentaose may induce "Peroxisome" pathway to prevent *Arabidopsis thaliana* from diseases.

4. Conclusions

In summary, we have successfully extracted a large number of RBPs from *Arabidopsis thaliana* using OOPS and many of them have not been noticed before. The

results show that the OOPS method can complement the previous UV crosslinking method further expanding the number of RBP groups in Arabidopsis and it provides a broader platform for future research. Moreover, due to the difference of mass spectrometry detection methods and parameter settings, more enrichment methods have a great contribution to standardize the detection of RBP and improve the accuracy of the results. Based on the quantitative analysis of these proteins, we found enriched different RBPs involving in chitinpentaose - *Arabidopsis* - *Pst* DC3000 interaction system tend to associate with stress response and immune process. These results further revealed the indispensable role of RBPs in the immune defense system and it provides a possible target for the study of plant immune defense mechanism. All of these possibilities emphasize that OOPS also has a very wide application value in plants.

CRediT author statement

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Data availability statement

The datasets generated for this study can be found in the PRIDE repository accession PXD018398.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Figure Legends

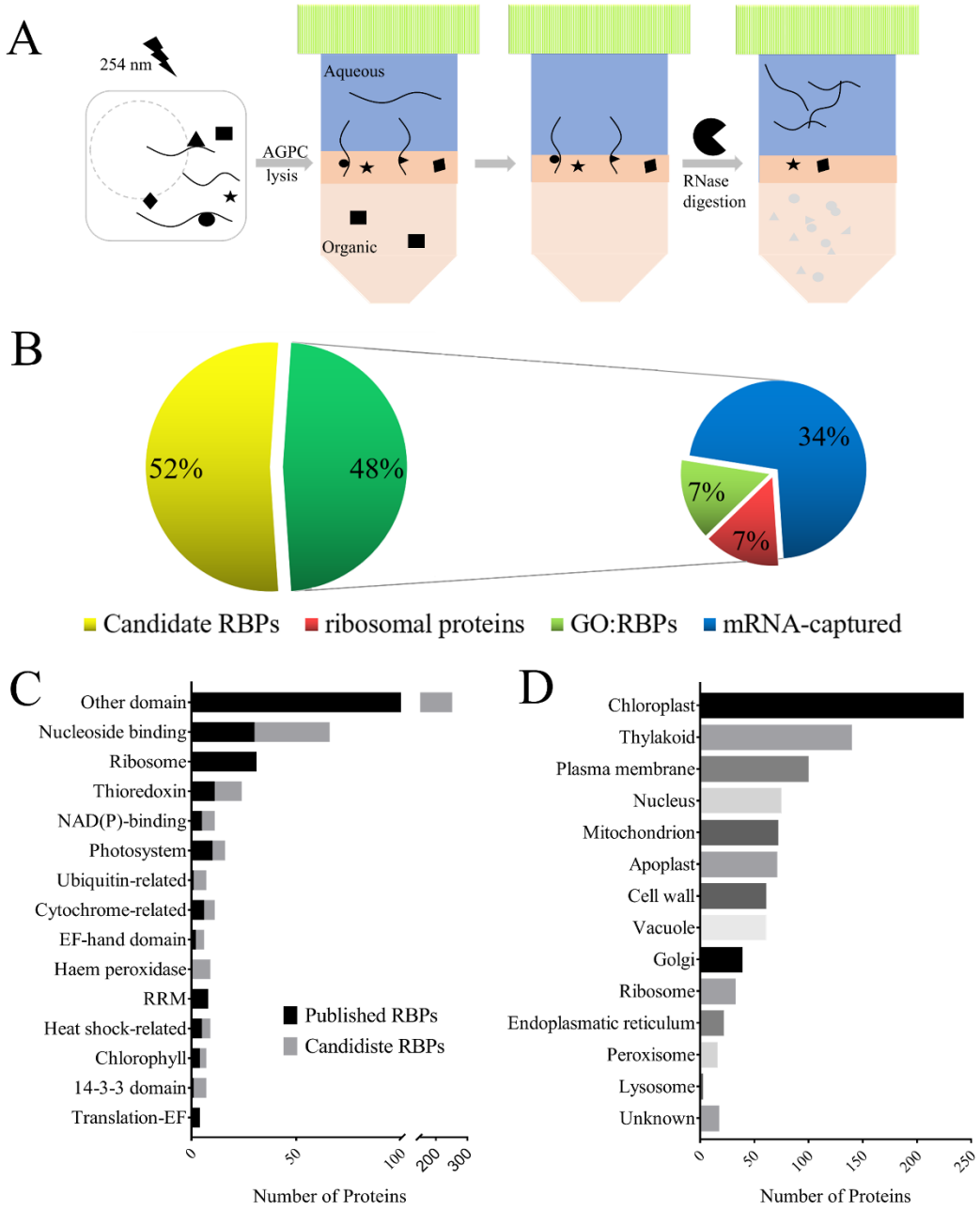
Fig 1 OOPS in *Arabidopsis thaliana.* A), Schematic representation of the OOPS method to extract PBR. Cells are cross-linked to induce RNA–protein adducts, which are drawn simultaneously to the organic and aqueous phases in AGPC and thus remain at the interface. RNase digestion and a further AGPC separation yields protein in the organic phase. B), Classification and proportion of proteome based on identification. ribosomal protein, annotated as ribosomal protein in Uniprot and Interpro; Go: RBPs, annotated as RBPs in the GO database and except for those labeled as ribosomal proteins; mRNA-captured, reported by previous RNA-binding proteomic literature in *Arabidopsis thaliana*[14,26-28]. C), Specific domain of each protein in the RBPome. D), Subcellular localization in the RBPome. OOPS, Orthogonal Organic Phase Separation; AGPC, acidic guanidiniumthiocyanate-phenol-chloroform.

Fig. 2. The effect of COS in induced resistance. A) Classification of protein functions of *Arabidopsis* RBPome. B) Phenotypic symptoms of *Arabidopsis* leaves. C) The disease index which evaluated the disease symptoms on *Pst* DC3000 infected eaves. D) Bacterial growth assay of *Pst* DC3000 in per cm^2 of leaf tissues. HM, H_2O+$MgSO_4$; HP, H_2O+*Pst*; CM, chitinpentaose+$MgSO_4$; CP, chitinpentaose+*Pst*. Values are as the means ± SD in three independent measurements. Asterisks indicate significant differences (**P < 0.01; ***P < 0.001).

Fig. 3. KEGG pathway analysis for the differential abundant RBPs. A) Up-

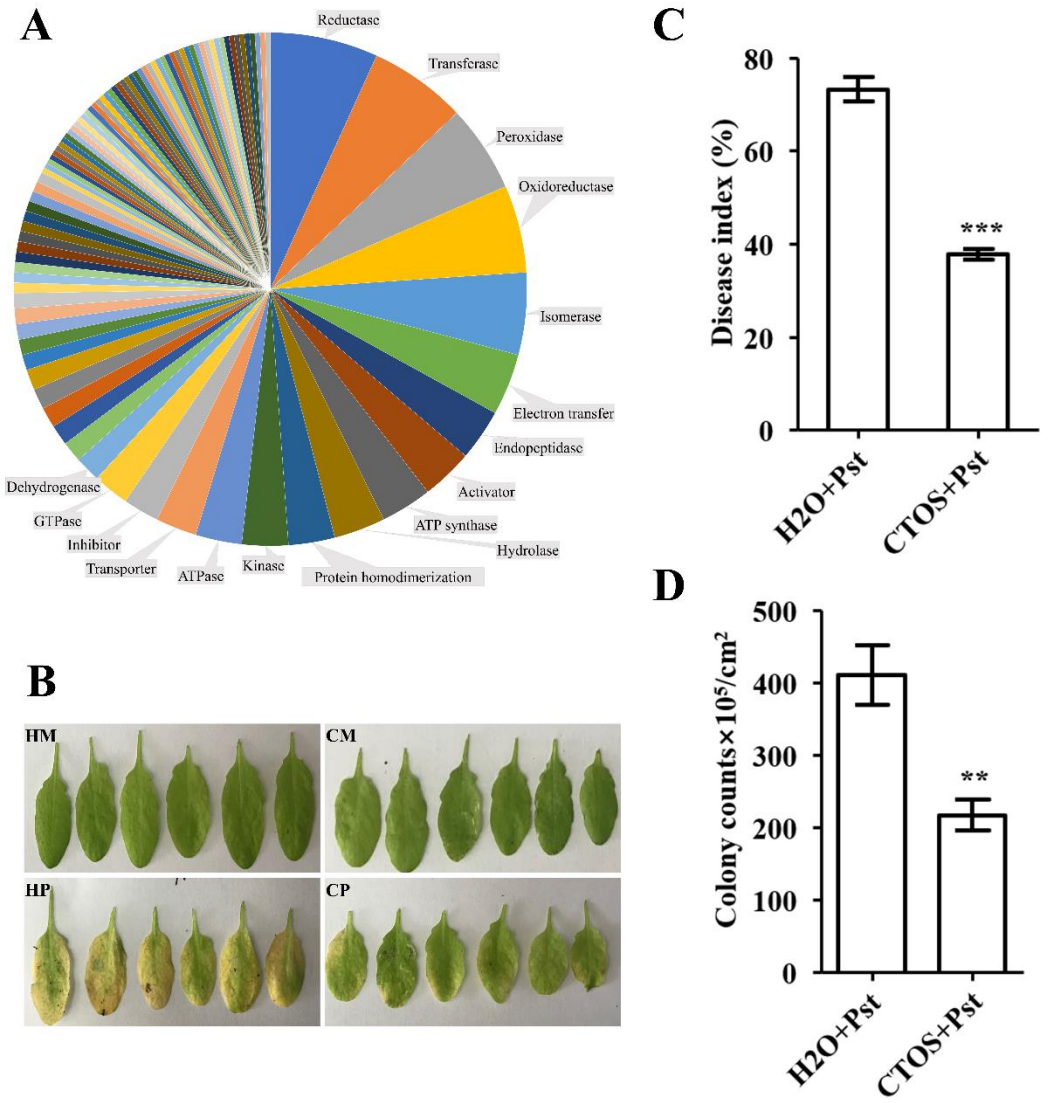
regulated RBPs in HM-CM group. B) Up-regulated RBPs in HP-CP group. C) Up-regulated RBPs in HM-HP group. D) Up-regulated RBPs in CM-CP group.

Fig. 4. Volcano map of four groups. A), significant differential abundant RBPs in HM-HP. B), significant differential abundant RBPs in CM-CP. C), significant differential abundant RBPs in CM-HM. D), significant differential abundant RBPs in HP-CP. FC means “fold changes after treatment”; The red dot and green dot mean up- regulated and down-regulated protein.


A
254 nm
AGPC
lysis
Aqueous
Organic
RNase
digestion
B
52%
48%
34%
7%
7%
Candidate RBPs
ribosomal proteins
GO:RBPs
mRNA-captured
C
Other domain
Nucleoside binding
Ribosome
Thioredoxin
NAD(P)-binding
Photosystem
Ubiquitin-related
Cytochrome-related
EF-hand domain
Haem peroxidase
RRM
Heat shock-related
Chlorophyll
14-3-3 domain
Translation-EF
Published RBPs
Candidiste RBPs
0
50
100
200
300
Number of Proteins
D
Chloroplast
Thylakoid
Plasma membrane
Nucleus
Mitochondrion
Apoplast
Cell wall
Vacuole
Golgi
Ribosome
Endoplasmatic reticulum
Peroxisome
Lysosome
Unknown
0
50
100
150
200
250
Number of Proteins

603

604 **Fig. 1**


A
Reductase
Transferase
Peroxidase
Oxidoreductase
Isomerase
Electron transfer
Endopeptidase
Activator
ATP synthase
Hydrolase
Protein homodimerization
Kinase
ATPase
Transporter
Inhibitor
GTPase
Dehydrogenase
B
HM
CM
HP
CP
C
Disease index (%)
80
60
40
20
0
***
H2O+Pst
CTOS+Pst
D
Colony counts×10⁵/cm²
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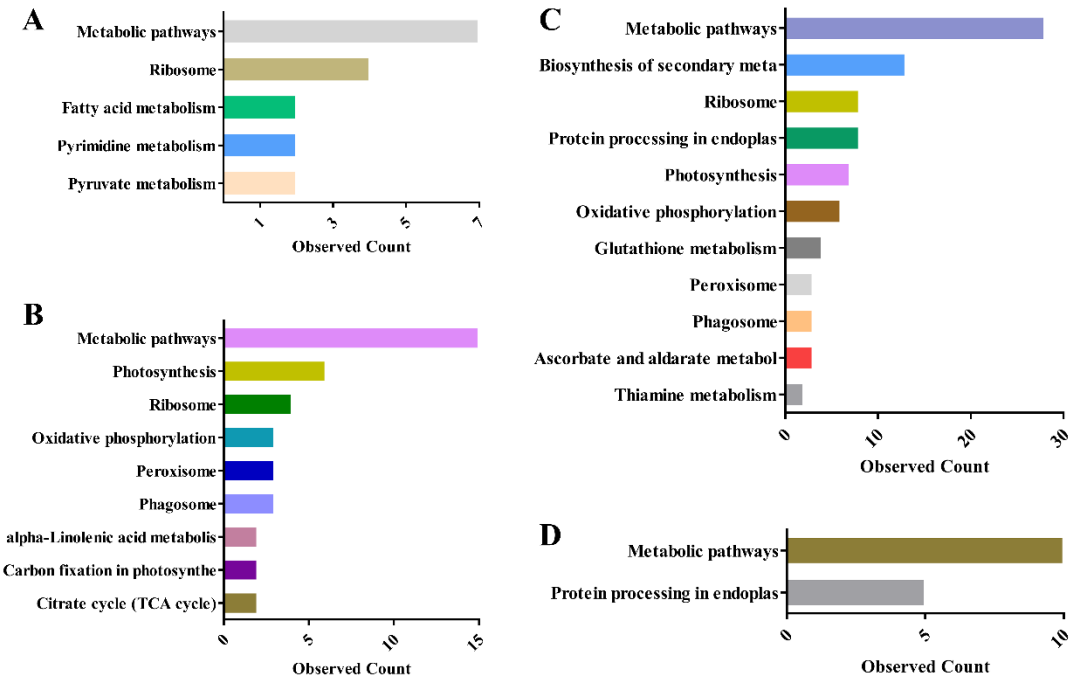
H2O+Pst
CTOS+Pst

605

606 **Fig. 2**



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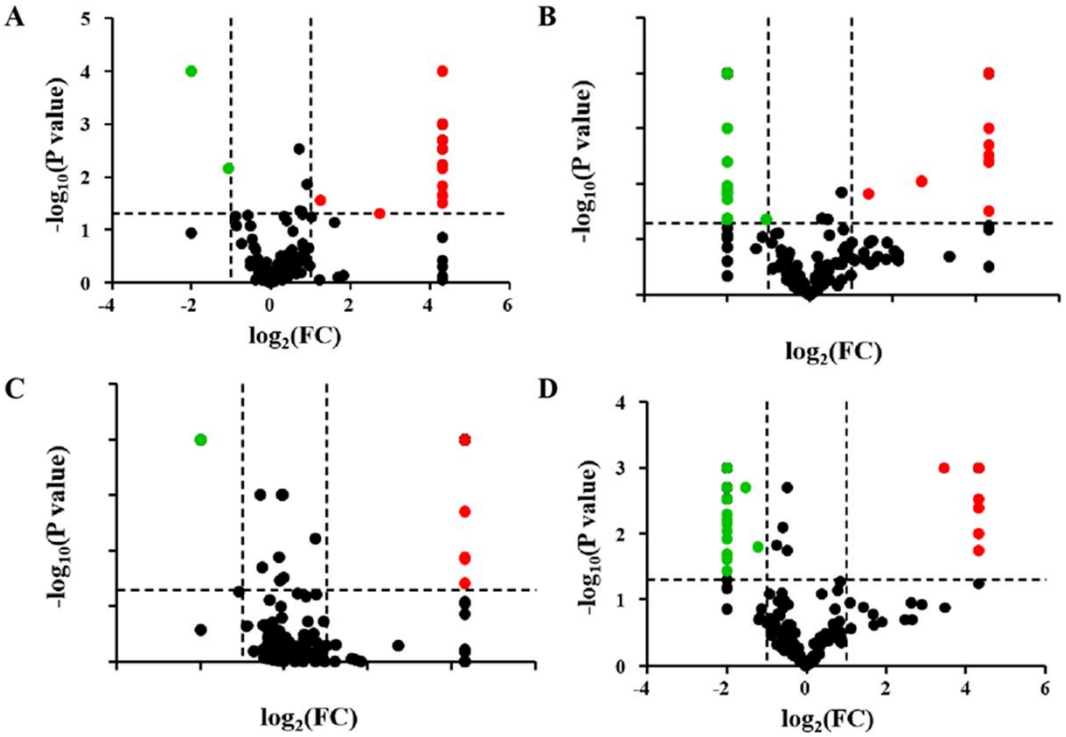
608 **Fig. 3**



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610 **Fig. 4**

Supplementary material

Supplemental Table 1. Ribosomal proteins localized only in chloroplasts.

Supplemental Table 2. Statistics and analysis of RBPs activity in *Arabidopsis.*

Supplemental Table 3. Differentially RBPs in CM-CP, HM-HP, HM-CM and HP-CP.

Supplemental Table 4. GO analysis of up-regulated RBPs after *Pst* DC3000 treatment

Supplemental Table 5. GO analysis of up-regulated RBPs after chitinpentaose treatment

Supplemental Figure 1. Venn plot of RBPs in different treatment group.

Supplemental Excel 1. RBPome Data.

Table S1 Ribosomal proteins localized only in chloroplasts.

Locus	Entry name	Protein name
AT4G34620	RS16A_ARATH	30S ribosomal protein S16-1
AT1G79850	RR17_ARATH	30S ribosomal protein S17
AT3G52150	PSRP2_ARATH	30S ribosomal protein 2
AT5G24490	Q94K97_ARATH	30S ribosomal protein
AT3G63490	RK1_ARATH	50S ribosomal protein L1
AT3G27850	RK123_ARATH	50S ribosomal protein L12-3
AT1G75350	RK31_ARATH	50S ribosomal protein L31
AT1G35680	RK21_ARATH	50S ribosomal protein L21

624 **Table S2** Statistics and analysis of RBPs activity in *Arabidopsis*.

Activity	Number	Protein
Reductase	21	AT4G27440; AT4G03520; AT3G15360; AT5G42980; AT5G40370; AT1G76160; AT4G03280; AT1G23740; AT1G06820; AT2G19940; AT1G03680; AT1G45145; AT3G02730; AT5G16400; AT1G50320; AT5G20500; AT3G54900; AT2G20270; AT5G23395; AT4G37925; AT1G51980
Transferase	21	AT4G02520; AT2G30860; AT1G16880; AT1G62780; AT5G17920; AT5G54770; AT1G19570; AT5G16710; AT1G78870; AT2G33150; AT3G28930; AT3G28940; AT1G10095; AT4G34050; AT1G23320; AT1G27450; AT1G42480; AT4G34180; AT4G39640; AT3G53900; AT2G32520
Peroxidase	17	AT3G26060; AT3G06050; AT4G02520; AT2G30860; AT3G11630; AT1G35720; AT1G07890; AT2G37130; AT2G38380; AT3G28200; AT3G49110; AT3G49120; AT3G32980; AT5G05340; AT5G64120; AT5G20500; AT2G25080
Oxidoreductase	17	AT4G03520; AT3G15360; AT5G42980; AT5G40370; AT1G76160; AT1G06820; AT1G03680; AT1G45145; AT3G02730; AT5G16400; AT1G50320; AT5G20500; AT3G54900; AT2G20270; AT5G23395; AT4G37925; AT1G51980
Isomerase	16	AT1G75690; AT1G21750; AT3G55440; AT2G16600; AT4G34870; AT3G04790; AT1G06820; AT2G47470; AT4G38740; AT3G56070; AT3G01480; AT2G43560; AT3G25220; AT4G39710; AT5G16440; AT3G02780
Electron transfer	12	AT5G40370; ATCG01060; ATCG00340; ATCG00580; ATCG00540; AT5G20500; AT3G54900; AT2G20270; AT1G76100; AT4G27520; AT4G14890; AT2G27510
Endopeptidase	10	AT2G30950; AT1G47128; AT3G14290; AT1G12410; AT4G21650; AT1G11910; AT1G09750; AT1G79330; AT3G27925; AT1G51980
Activator	10	AT4G00810; AT5G47700; AT1G01100; AT4G03520; AT3G15360; AT2G39730; AT1G03680; AT3G02730; AT5G16400; AT1G50320
ATP synthase	10	AT5G08690; AT3G01390; ATCG00130; AT4G04640; AT5G47030; AT4G09650; AT5G13450; ATCG00470; ATCG00480; ATCG00120

Protein homodimerization	10	AT1G26110； AT4G38710； AT3G26650； AT1G35720； AT1G32060； AT4G25530； AT5G54770； AT5G17710； AT4G26780； AT1G03475
Hydrolase	10	AT4G32260； AT3G52300； AT1G29660； AT1G29670； AT3G14210； AT5G16440； AT3G02780； AT4G39640； AT2G32520； AT2G21620
Kinase	10	AT5G21222； AT3G12780； AT1G79550； AT1G32060； AT5G63400； AT5G50370； AT5G26667； AT4G09320； AT4G36080； AT2G17530
ATPase	9	AT1G12840； AT4G23710； AT5G42020； AT3G09440； AT4G24190； AT3G01390； AT4G11150； AT3G47520； AT1G09170
Transporter	8	AT4G32260； AT3G52300； AT5G43970； AT5G62810； AT5G28750； AT5G58070； AT1G57943； AT4G15545
Inhibitor	8	AT3G15360； AT1G03680； AT3G02730； AT5G12140； AT3G14310； AT1G44980； AT5G56260； AT1G71950
GTPase	7	AT5G60390； AT4G20360； AT4G20890； AT1G20010； AT4G14960； AT4G33650； AT4G02510
Dehydrogenase	5	AT2G20360； AT3G15020； AT1G04410； AT3G47520； AT1G16700
Nucleotide exchange factor	5	AT1G30230； AT5G19510； AT5G17710； AT4G26780； AT1G51160
Superoxide dismutase	4	AT2G28190； AT1G08830； AT3G10920； AT5G51100
Translation elongation factor	4	AT5G60390； AT5G19510； AT1G30230； AT4G20360
Ubiquitin conjugating enzyme	4	AT1G78870； AT1G16890； AT3G52560； AT2G36060
Nutrient reservoir	4	AT5G24780； AT1G72610； AT5G20630； AT5G58070
Glucosidase	4	AT1G52400； AT5G24540； AT1G62660； AT3G57260
ATP-dependent peptidase	3	AT2G30950； AT5G50920； AT1G12410
DNA binding transcription factor	3	AT4G19520； AT2G33880； AT4G25530
Lyase	3	AT1G08110； AT1G23320； AT5G57040
Protein dimerization	3	AT3G28857； AT2G19940； AT4G22670
Protein heterodimerization	3	AT5G54640； AT5G02570； AT5G59970
Photosynthesis	3	ATCG00280； AT4G21280； AT4G05180
Translation initiation factor	3	AT4G38710； AT2G39990； AT2G24060
3-isopropylmalate dehydratase	2	AT2G43100； AT2G43090
Monooxygenase	2	AT5G38410； ATCG00490
Oxygen evolving	2	AT5G66570； AT3G50820

Glutathione dehydrogenase (ascorbate)	2	AT1G19570；AT5G16710
Pectinesterase	2	AT3G14310；AT1G44980
Catalase	2	AT1G20620；AT4G39730
Ribulose-bisphosphate carboxylase	2	AT5G38410；ATCG00490
Enzyme regulator	2	AT4G04640；AT2G39730
Acid phosphatase	2	AT5G24780；AT1G54780
Carbonate dehydratase	2	AT3G01500；AT5G14740
Aspartyl esterase	2	AT3G14310；AT1G44980
Cytochrome c oxidase	2	AT3G15640；AT1G80230
Structural molecule	1	AT2G40060
Oxygen carrier	1	AT3G10520
2-alkenal reductase (NADP+)	1	AT1G23740
4-hydroxy-4-methyl-2-oxoglutarate aldolase	1	AT5G56260
DNA polymerase processivity factor	1	AT2G29570
DNA primer / DNA polymerase	1	AT5G67100
G protein-coupled receptor	1	AT4G02510
NAD(P)+ transhydrogenase	1	AT3G47520
NAD(P)H dehydrogenase (quinone)	1	AT5G54500
SNAP receptor	1	AT3G11820
UDP-glucose 4-epimerase	1	AT1G09340
Alpha-L-arabinofuranosidase	1	AT5G49360
Cysteine-type peptidase	1	AT1G47128
Pyruvate dehydrogenase (acetyl-transferring)	1	AT5G50850
Primary amine oxidase	1	AT4G12290
Methionine synthase	1	AT5G17920
Protein tyrosine phosphatase	1	AT3G44620
Porin/voltage-gated anion channel	1	AT5G67500
Toxin	1	AT1G66100
Translation release factor	1	AT5G47880

Antiporter	1	AT3G54900
Ubiquitin protein ligase	1	AT5G42940
Arylformamidase	1	AT4G34180
Coproporphyrinogen oxidase	1	AT1G03475
Glycerophosphodiester phosphodiesterase	1	AT1G66970
Sedoheptulose-bisphosphatase	1	AT3G55800
Glutamate-ammonia ligase	1	AT5G35630
Glutaminyl-tRNA synthase (glutamine-hydrolyzing)	1	AT4G32915
Pectin acetylesterase	1	AT4G19410
Fructose-bisphosphate aldolase	1	AT2G21330
Fructose 1,6-bisphosphate 1-phosphatase	1	AT3G55800
Ribonuclease	1	AT3G14290
Ribulose-1,5-bisphosphate carboxylase/oxygenase activator	1	AT2G39730
Metallopeptidase	1	AT2G30950
Arginase	1	AT4G08870
Methyl indole-3-acetate esterase	1	AT2G23570
Xylan 1,4-beta-xylosidase	1	AT5G49360
Ferroxidase	1	AT5G01600
Deaminase	1	AT3G20390
Abscisic acid glucose ester beta-glucosidase	1	AT1G52400
Microtubule motor	1	AT1G09170
Inorganic diphosphatase	1	AT5G09650
Cellulase	1	AT3G57260
Acyl carrier	1	AT1G65290
Adenosylhomocysteinase	1	AT4G13940
Nitrite reductase [NAD(P)H]	1	AT1G71500
Linoleate 13S-lipoxygenase	1	AT3G45140
Acetyl-CoA carboxylase	1	AT5G16390
Nitrilase	1	AT3G44310

Lipoate synthase	1	AT5G23440
Transcription corepressor	1	AT4G15802
Transketolase	1	AT3G60750

625

626 **Table S3.** Differentially expressed RBPs in CM-CP, HM-HP, HM-CM and HP-CP.

Group		Number	ID	Fold	Group		Number	ID	Fold
CM-CP	Down-regulated proteins	24	AT1G21750	-	HM-CM	Down-regulated proteins	10	AT4G15545	-
			AT3G52150	-				AT5G03850	-
			AT4G24770	-				AT5G28750	-
			AT5G47030	-				AT3G53430	-
			AT2G25970	-				AT4G11150	-
			AT1G03680	-				AT4G32915	-
			AT4G02520	-				AT5G59970	1.39
			AT3G54900	-				AT3G01500	1.46
			ATCG01060	-				AT3G05900	-
			AT5G59970	-				AT1G55490	-
			AT3G50360	-		Up-regulated proteins	16	AT3G54900	0
			AT1G20440	-				AT4G24770	0
			ATCG00540	-				AT4G34120	0
			AT3G55800	-				AT3G47070	0
			AT2G24020	-				AT5G15970	0
			AT2G35370	-				AT1G72610	0
			AT5G35630	-				AT3G28940	0
			AT4G23890	-				AT4G04020	0
			AT4G02530	-				AT5G35630	0
			AT5G15970	-				AT4G23890	0
			AT2G43560	-				AT2G24020	0
			AT4G13340	-				AT1G04410	0
			AT4G34120	-				AT1G21750	0
			AT2G39730	2.07				AT5G39570	0
	Up-regulated proteins	12	AT4G21650	0				AT3G50360	0
			AT4G38740	0				AT4G02520	0
			AT2G44920	0	HP-CP	Down-regulated proteins	50	AT3G01390	2.32
			AT5G22580	0				AT3G52150	-
			AT1G73230	0				AT3G23400	-
			AT4G04020	0.81				AT4G14890	-
			AT3G47070	0.74				AT5G59310	-
			AT3G16140	0				AT4G15545	-
			AT1G66240	0				AT4G34120	-
			AT1G42970	0.37				AT4G13340	-
			AT2G37270	0				AT4G04020	1.41
			AT3G53430	0				AT2G01520	-
HM-HP	Down-regulated proteins	3	AT5G13450	-				AT2G43560	-
			AT4G11150	-				AT4G02530	-
			AT1G29660	2.12				AT1G55490	-
		42	AT3G54900	0				AT1G24020	2.87
			AT5G03850	0.6				AT5G62810	-

	Up-regulated proteins	AT1G72610	0			AT5G54500	-
		AT3G23400	0			AT3G46560	-
		AT4G14890	0			AT3G05900	-
		AT4G38740	0			AT2G24020	-
		AT4G34120	0			AT3G55800	-
		AT2G01520	0			ATCG00540	-
		AT4G04020	0			AT1G65260	-
		AT1G24020	0.53			AT5G26667	-
		AT5G59310	0			AT1G20440	-
		AT5G54500	0			AT1G20450	-
		AT5G62810	0			AT1G20340	-
		AT2G24020	0			AT5G03850	-
		AT3G46560	0			AT3G55280	-
		AT1G66240	0			ATCG00130	-
		AT1G79330	0.59			AT3G28500	1.4
		AT1G73230	0			AT3G50820	-
		AT5G26667	0			AT5G47030	-
		AT3G45140	0			AT5G60390	-
		AT4G02520	0			AT5G59970	-
		AT2G47470	0.15			AT3G54900	-
		AT1G21750	0			AT3G50360	-
		AT1G07890	0			AT3G01500	1.68
		AT3G32980	0			AT4G39730	-
		AT5G52840	0			AT1G54780	-
		AT1G20340	0			AT3G10920	-
		AT1G20450	0			AT4G02520	-
		AT3G50360	0			AT1G03680	-
		AT3G10920	0			AT1G21750	-
		AT4G28660	0			AT3G32980	-
		AT3G16140	0			AT1G07890	-
		AT1G04410	0			AT1G04410	-
		AT1G20010	0			AT4G32915	-
		AT4G23710	0.42			AT2G25970	-
		ATCG00130	0			AT1G20010	-
		AT5G39570	0			AT3G45140	-
		AT4G16830	0		**Up-regulated proteins**	AT4G21650	0
		AT5G60390	0			AT2G37270	0.09
		AT3G50820	0			AT4G14960	0
		AT3G28500	0.61		6	AT3G47070	0
		AT3G55280	0			AT5G22580	0
						AT2G44920	0

627 "-" Represents the latter expression is 0 or not detected

628 "0" represents the former expression is not detected or 0 in the table.

629

630 **Table S4.** GO analysis of up-regulated RBPs after *Pst* DC3000 treatment.

GO ID	Description	observed count	P-value
GO:0010038	response to metal ion	21	2.41E-12
GO:0042221	response to chemical	46	2.95E-12
GO:0050896	response to stimulus	64	2.95E-12
GO:0010035	response to inorganic substance	26	4.88E-12
GO:0046686	response to cadmium ion	17	2.30E-11
GO:0006950	response to stress	46	3.57E-11
GO:0009725	response to hormone	31	9.34E-10
GO:0098869	cellular oxidant detoxification	13	1.19E-09
GO:0055114	oxidation-reduction process	29	1.23E-09
GO:0009735	response to cytokinin	13	5.37E-09
GO:0009628	response to abiotic stimulus	29	1.69E-07
GO:0009651	response to salt stress	16	1.88E-07
GO:0033554	cellular response to stress	19	7.64E-07
GO:0009409	response to cold	13	1.02E-06
GO:0009266	response to temperature stimulus	15	1.47E-06
GO:0010043	response to zinc ion	6	4.66E-06
GO:0051716	cellular response to stimulus	32	6.25E-06
GO:0009987	cellular process	81	6.43E-06
GO:0006979	response to oxidative stress	12	2.12E-05
GO:0070887	cellular response to chemical stimulus	21	2.51E-05
GO:0006457	protein folding	7	0.00013
GO:0072593	reactive oxygen species metabolic process	7	0.00013
GO:0006518	peptide metabolic process	12	0.00028
GO:0034599	cellular response to oxidative stress	6	0.00028
GO:1901566	organonitrogen compound biosynthetic process	19	0.00028
GO:0043603	cellular amide metabolic process	13	0.00037
GO:0051186	cofactor metabolic process	12	0.00037
GO:0008152	metabolic process	71	0.0004
GO:0045454	cell redox homeostasis	6	0.001
GO:1901700	response to oxygen-containing compound	19	0.0013
GO:0006301	postreplication repair	3	0.0014
GO:0006091	generation of precursor metabolites and energy	9	0.0019
GO:0042744	hydrogen peroxide catabolic process	5	0.0021
GO:0051704	multi-organism process	19	0.0023
GO:0000302	response to reactive oxygen species	6	0.0024
GO:1901564	organonitrogen compound metabolic process	37	0.0024
GO:0042743	hydrogen peroxide metabolic process	5	0.0026
GO:0065003	protein-containing complex assembly	9	0.0027
GO:0044237	cellular metabolic process	61	0.0036
GO:0043604	amide biosynthetic process	10	0.0037
GO:0016999	antibiotic metabolic process	6	0.0041

GO:0034976	response to endoplasmic reticulum stress	4	0.0041
GO:0034622	cellular protein-containing complex assembly	8	0.0046
GO:0051707	response to other organism	15	0.0047
GO:0006412	Translation	9	0.0051
GO:0022618	ribonucleoprotein complex assembly	5	0.006
GO:0006952	defense response	16	0.0077
GO:0009605	response to external stimulus	17	0.0091
GO:0010188	response to microbial phytotoxin	2	0.0092
GO:0010731	protein glutathionylation	2	0.0092
GO:0009617	response to bacterium	8	0.0093
GO:0070925	organelle assembly	5	0.0095
GO:0042255	ribosome assembly	4	0.0097
GO:0065008	regulation of biological quality	14	0.0114
GO:0000028	ribosomal small subunit assembly	3	0.0117
GO:0044248	cellular catabolic process	16	0.0117
GO:0044281	small molecule metabolic process	17	0.0136
GO:0022900	electron transport chain	5	0.0157
GO:0070534	protein K63-linked ubiquitination	2	0.0157
GO:0009631	cold acclimation	3	0.0175
GO:0009793	embryo development ending in seed dormancy	7	0.0175
GO:0006996	organelle organization	15	0.0183
GO:0034614	cellular response to reactive oxygen species	3	0.0183
GO:0009056	catabolic process	17	0.0219
GO:0001101	response to acid chemical	13	0.0228
GO:0019430	removal of superoxide radicals	2	0.0228
GO:0006974	cellular response to DNA damage stimulus	6	0.0291
GO:0048316	seed development	8	0.0291
GO:0098542	defense response to other organism	11	0.0307
GO:0016043	cellular component organization	21	0.0326
GO:0022613	ribonucleoprotein complex biogenesis	6	0.0329
GO:0046940	nucleoside monophosphate phosphorylation	2	0.033
GO:0009737	response to abscisic acid	8	0.0366
GO:0017144	drug metabolic process	9	0.0366
GO:0042592	homeostatic process	8	0.0366
GO:0071840	cellular component organization or biogenesis	22	0.0367
GO:0006749	glutathione metabolic process	3	0.039
GO:0015979	Photosynthesis	5	0.039
GO:0006525	arginine metabolic process	2	0.0401

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632 **Table S5.** GO analysis of up-regulated RBPs after chitinpentaose treatment.

GO ID	Description	observed count	P-value
GO:0006950	response to stress	27	3.51E-07
GO:0009628	response to abiotic stimulus	21	3.51E-07
GO:0050896	response to stimulus	34	8.34E-07
GO:0055114	oxidation-reduction process	18	8.34E-07
GO:0010035	response to inorganic substance	14	1.27E-06
GO:0042221	response to chemical	24	1.27E-06
GO:0009409	response to cold	10	1.76E-06
GO:0006091	generation of precursor metabolites and energy	10	2.03E-06
GO:0009987	cellular process	49	2.03E-06
GO:0009266	response to temperature stimulus	11	3.67E-06
GO:0010043	response to zinc ion	5	7.03E-06
GO:0006970	response to osmotic stress	11	9.09E-06
GO:0009651	response to salt stress	10	2.09E-05
GO:0098869	cellular oxidant detoxification	7	2.09E-05
GO:0009735	response to cytokinin	7	4.79E-05
GO:0015979	Photosynthesis	7	4.95E-05
GO:0009617	response to bacterium	8	0.00019
GO:0010033	response to organic substance	16	0.00019
GO:0019684	photosynthesis, light reaction	5	0.00019
GO:0010038	response to metal ion	8	0.00028
GO:0006996	organelle organization	13	0.00043
GO:0042742	defense response to bacterium	7	0.00043
GO:0009607	response to biotic stimulus	12	0.00045
GO:0009725	response to hormone	14	0.00045
GO:0045454	cell redox homeostasis	5	0.00045
GO:0009642	response to light intensity	5	0.00072
GO:0009644	response to high light intensity	4	0.001
GO:0006979	response to oxidative stress	7	0.0012
GO:0022900	electron transport chain	5	0.0012
GO:0007031	peroxisome organization	3	0.0018
GO:0034599	cellular response to oxidative stress	4	0.0021
GO:0043603	cellular amide metabolic process	8	0.003
GO:1901700	response to oxygen-containing compound	12	0.003
GO:0043604	amide biosynthetic process	7	0.0044
GO:0098542	defense response to other organism	9	0.0044
GO:0006518	peptide metabolic process	7	0.005
GO:0006952	defense response	11	0.005
GO:0044281	small molecule metabolic process	12	0.005
GO:0051707	response to other organism	10	0.0051
GO:0065008	regulation of biological quality	10	0.0051
GO:0016043	cellular component organization	15	0.0057

GO:0008152	metabolic process	38	0.006
GO:0006605	protein targeting	4	0.0067
GO:0055086	nucleobase-containing small molecule metabolic process	6	0.0083
GO:0065002	intracellular protein transmembrane transport	3	0.0091
GO:0010205	Photoinhibition	2	0.0093
GO:0044237	cellular metabolic process	34	0.0093
GO:0006412	Translation	6	0.0095
GO:0010206	photosystem II repair	2	0.0098
GO:0009414	response to water deprivation	5	0.01
GO:0009161	ribonucleoside monophosphate metabolic process	4	0.0102
GO:0009695	jasmonic acid biosynthetic process	2	0.0103
GO:0017144	drug metabolic process	7	0.0103
GO:1901566	organonitrogen compound biosynthetic process	10	0.0103
GO:0072594	establishment of protein localization to organelle	4	0.0112
GO:0001101	response to acid chemical	9	0.0121
GO:0006886	intracellular protein transport	5	0.0128
GO:0009117	nucleotide metabolic process	5	0.0128
GO:0006754	ATP biosynthetic process	3	0.0144
GO:0009793	embryo development ending in seed dormancy	5	0.0144
GO:0009694	jasmonic acid metabolic process	2	0.0155
GO:0031408	oxylipin biosynthetic process	2	0.0155
GO:0042592	homeostatic process	6	0.0155
GO:0031407	oxylipin metabolic process	2	0.0159
GO:0009657	plastid organization	4	0.0163
GO:0051186	cofactor metabolic process	6	0.0169
GO:0009165	nucleotide biosynthetic process	4	0.0183
GO:0019637	organophosphate metabolic process	6	0.0183
GO:0006662	glycerol ether metabolic process	2	0.0193
GO:0017038	protein import	3	0.0194
GO:0007005	mitochondrion organization	3	0.0195
GO:0009168	purine ribonucleoside monophosphate biosynthetic process	3	0.0195
GO:0072521	purine-containing compound metabolic process	4	0.0195
GO:0015986	ATP synthesis coupled proton transport	2	0.0203
GO:0051187	cofactor catabolic process	3	0.0206
GO:0009416	response to light stimulus	6	0.0218
GO:0046034	ATP metabolic process	3	0.0244
GO:0006457	protein folding	3	0.0248
GO:0072593	reactive oxygen species metabolic process	3	0.0248
GO:0071826	ribonucleoprotein complex subunit organization	3	0.0255
GO:0006626	protein targeting to mitochondrion	2	0.0272
GO:0009767	photosynthetic electron transport chain	2	0.0273

GO:0044743	protein transmembrane import into intracellular organelle	2	0.029
GO:0009631	cold acclimation	2	0.0298
GO:0046686	response to cadmium ion	4	0.0304
GO:0034440	lipid oxidation	2	0.0312
GO:0000302	response to reactive oxygen species	3	0.0326
GO:0009167	purine ribonucleoside monophosphate metabolic process	3	0.0326
GO:0055085	transmembrane transport	8	0.0363
GO:0009737	response to abscisic acid	5	0.0436
GO:0016999	antibiotic metabolic process	3	0.0443
GO:1901564	organonitrogen compound metabolic process	18	0.0477

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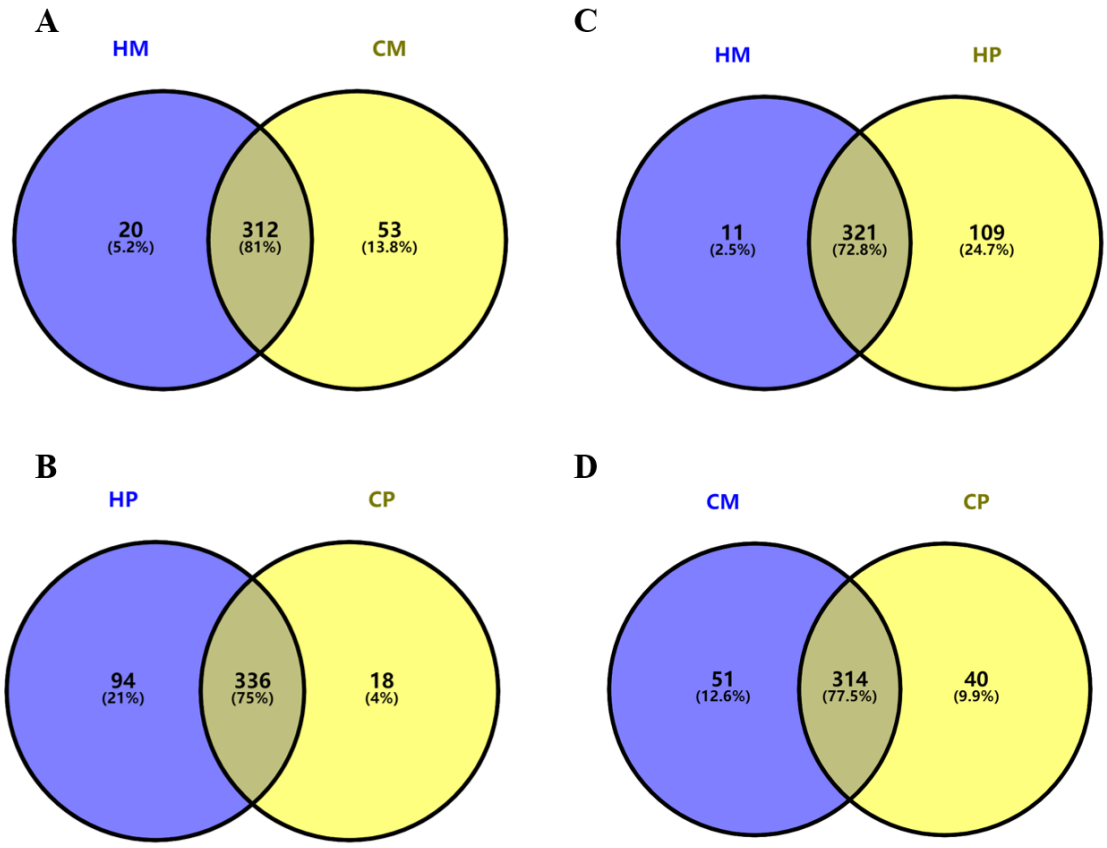

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636 **Fig S1**