Chen *et al. Chem. Biol. Technol. Agric.* (2024) 11:43
https://doi.org/10.1186/s40538-024-00571-3

Chemical and Biological Technologies in Agriculture

RESEARCH

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Genome-wide characterization and expression of *Oryza sativa* AP2 transcription factor genes associated with the metabolism of mesotrione

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Abstract

Background The APETALA2 (AP2) transcription factor (TF) superfamily, one of the largest gene families in plants, plays an essential role in regulating plant growth and their stress responses. However, the role of AP2 in rice under pesticide stress remains unclear. To investigate the characteristics and functions of the rice AP2 gene family under pesticide stress, the expression of 105 AP2-coding genes and 26 AP2 differentially expressed genes (DEGs) were identified in mesotrione (MTR)-treated rice transcriptome datasets.

Results Three subfamilies of the AP2 gene family (AP2/ERF, RAV, and soloists) were identified using sequence alignment and phylogenetic analysis. Chromosome location analysis revealed that the 26 rice AP2 DEGs were unevenly distributed on 10 of the 12 rice chromosomes, and segmental duplication contributed to the expansion of *Oryza sativa* AP2 (OsAP2) gene family. Collinearity analyses demonstrated that rice AP2 genes displayed 16 orthologous gene pairs, and 12 and 26 orthologous gene pairs were shared of *Arabidopsis* and soybean, respectively. In addition, rice AP2 genes featured various gene structures, cis-elements, motif compositions, and conserved domains that allowed them to encode genes that elicit biotic and abiotic stress responses. An analysis of docking between MTR and six AP2 DEGs revealed amino acid residues involved in MTR binding. Quantitative reverse transcription–polymerase chain reaction verified that several AP2 genes were preferentially expressed during MTR-induced stress. The roles of OsAP2 proteins in MTR metabolism were further supported by protein–protein interaction network analysis, which illustrated how these proteins interact with target proteins.

Conclusion The initial findings of this study define the features of the OsAP2 superfamily and offer important tools for functional analyses of OsAP2 genes implicated in the metabolism of MTR.

Keywords AP2 transcription factor, Mesotrione, Rice, Bioinformatics analysis, Metabolism

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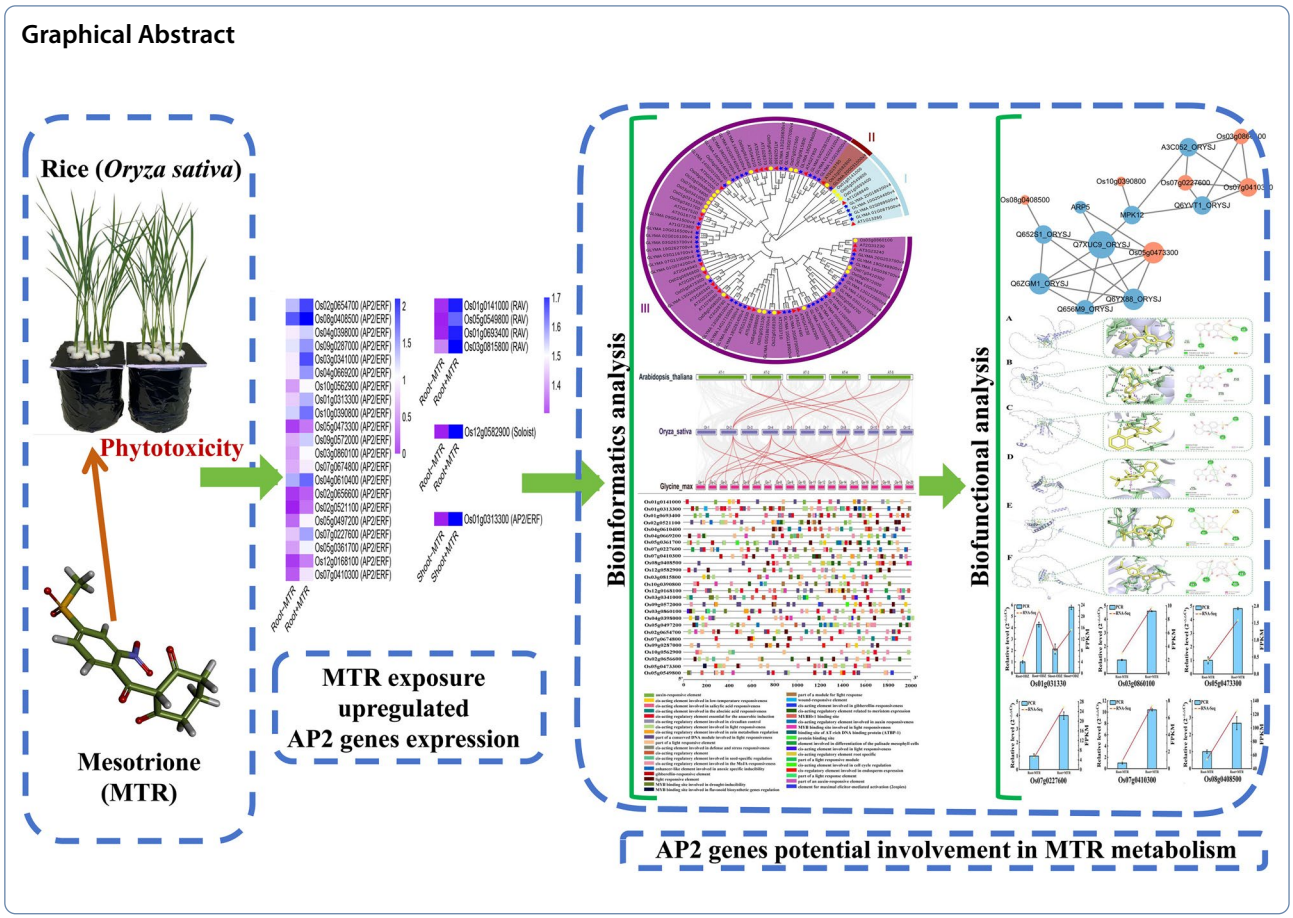

Background

The extensive use of agrochemicals in agricultural production to shield crops from weeds and insects has greatly increased food availability for humans. Agrochemicals include herbicides, which are a vital component of contemporary agriculture and have the greatest total global production [1]. Because of their frequent and wide use, residues of mesotrione (MTR) and other pesticides have been widely detected in recent studies, the majority of which focused on cultivated soil [2–6]. Residual herbicides in the environment can lead to permanent health hazards, such as immune system dysfunction, neurological system impairment, and food poisoning. Chronic illnesses, including cancer and heart disease, can also result from long-term herbicide consumption [6, 7]. To reduce the negative impacts of herbicide residues, the detoxification process for pesticides in plants is crucial. Plants can degrade herbicides via sequestration and degradation enzymes [8]. The metabolic pathway of pesticides in plants is mainly divided into three phases. Phase I metabolism mainly includes reactions such as oxidation, reduction, and hydrolysis, which often convert pesticides into less toxic metabolites. Phase II metabolism mainly involves the conjugation of pesticides and their degradation products to endogenous substances such as glutathione, amino acids, and glucosides through enzyme catalysis, resulting in increased water solubility and reduced toxicity of these pesticides conjugates compared with those of the parent of pesticide. Phase III metabolism mainly involves the removal of metabolites formed in Phase I and conjugates formed in Phase II from cellular fluids or organelles through formation of insoluble binding residues as part of the cell wall or removal from the cell [8, 9]. For example, the overexpression of the Phase I enzymes cytochrome P450 and carboxylesterase, Phase II enzymes glycosyltransferases and acetyltransferases, and Phase III ABC transporters promotes the detoxification and metabolism of pesticides and the reduction of their accumulation in plants [8–11]. However, the detoxification and metabolism of pesticides in plants are mediated by multiphase metabolic enzymes. We previously reported that the formation of MTR metabolites in rice is mediated by metabolic enzymes such as cytochrome P450, glycosyltransferase, glutathione-S-transferase, and acetyltransferase [12, 13]. Therefore, controlling the degradation of residual pesticides in plants by targeting

metabolic enzymes in a single phase is inefficient. If multiple metabolic enzymes can be simultaneously regulated, the ability of plants to detoxify and metabolize pesticides will be greatly improved. However, current research is relatively sparse.

MTR has recently become a popular herbicide for weeding rice, corn, and sugarcane fields. Because MTR has a lower spray dosage and can effectively weed, it is used in more than 50 nations, and its sales market has been gradually expanding since 2013 [2]. MTR has greatly increased agricultural productivity, but its continuous addition to soils can jeopardize crop safety and yields, resulting in environmental concerns [13]. Records indicate that MTR significantly decreases the genetic diversity and structure of the photosynthetic microbial flora in soil. In particular, its metabolite 2-amino-4-methylsulfonyl benzoic acid is more harmful to microbes than the parent compound [2]. DNA of earthworms and *Cyprinus carpio* is disrupted by ambient remnants of MTR [14, 15]. The ecotoxic consequences of residual MTR include increased risks of breast cancer and other chronic diseases [2]. Considering environmental concerns, it is critical to develop a highly effective MTR degradation process to decrease pollution and minimize its contamination of crops.

Transcription factors (TFs) can selectively bind to cis-acting regions found in the promoter regions of eukaryotic genes. The expression of plant TF genes is induced under various stresses such as low temperature, drought, and salinity, thereby enhancing tolerance to these stresses [16]. In addition, rice TF genes are significantly upregulated under pesticide stress. For example, genes encoding the rice TFs such as MYB, APETALA2 (AP2), HalZ, and NAC are significantly induced under MTR or fomesafen exposure [12, 17].

AP2 TFs comprise a diverse group of genes primarily found in plants. They are categorized into four major subfamilies: dehydration responsive element-binding protein (DREB), ethylene-responsive element-binding protein (ERF), AP2 and related to ABI3/VP (RAV), and soloists (a few unclassified factors) [18, 19]. Because of their essential involvement in numerous biological processes, including plant growth and reactions to biotic and abiotic stressors, AP2 TFs have recently received substantial attention [20, 21]. At present, few studies have demonstrated that AP2 can effectively promote the metabolism and degradation of pesticides in plants, thereby increasing their tolerance. It remains unknown whether rice AP2 genes are involved in the metabolism and detoxification of pesticides in rice. We examined the genome-wide transcripts of MTR-responsive AP2 genes to examine their potential molecular and biochemical roles in the detoxification and metabolism of pesticides. We also conducted an extensive analysis of the chromosomal locations, collinearity, structures, cis-elements, motif compositions, and conserved domains of these genes. Quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was used to confirm the responses of these genes to MTR. These analyses fulfilled the objective of this study to develop a useful screening technique to facilitate the screening of MTR-responsive rice AP2 genes to modify toxicological reactions and MTR resistance in both crops and environments.

Methods

Preparation of plant materials

The surface of wild rice seeds (*Oryza sativa*, Nipponbare) was disinfected with 3% H_2O_2 and sprouted at 30 ℃ for 72 h. The sprouted seeds were cultured in 50% Hoagland nutrient solution for 10 days, and rice seedlings with consistent growth trends were selected and transplanted into fresh nutrient solution with or without (control) 0.1 mg L^{-1} MTR [12]. Seedlings were cultivated at a constant temperature in an incubator under 75% relative humidity during the day (30 ℃, 14 h) and night (25 ℃, 10 h), and the daytime light intensity was 200 μmol m^{-2} s^{-1}. The culture solution was changed every 2 days.

Transcriptome library construction and high-throughput RNA sequencing (RNA-seq)

Samples of rice seedling shoots and roots were collected after 2, 4, and 6 days of exposure to MTR. Samples were combined after each time interval. All rice seedling root and shoot samples from different time points were combined for quantitative analysis. Through this approach, four libraries of rice seedling roots and shoots were produced, namely Shoot-MTR (control), Root-MTR (control), Shoot + MTR (+ MTR), and Root + MTR (+ MTR), representing the without MTR (control) and treatment of 0.1 mg L^{-1} MTR (+ MTR). Total RNA for each library was isolated through Trizol (Thermo Fisher Scientific, USA) treatment. DNA was removed using DNase I (Takara, Shiga, Japan). An Illumina HiSeqTM2500 unit (Illumina, San Diego, CA, USA) was used to sequence RNA from each of the four libraries with three biological replicates (3 × 4). Clean bases and reads were mapped to the rice genome after removing poor-quality bases (http://rice.plantbiology.msu.edu/index.shtml) [12, 17]. There were three biological replicates for each treatment.

qRT-PCR analysis of differentially expressed rice AP2 TF genes

Ten-day-old rice seedlings were treated with 0.1 mg L^{-1} MTR for 2, 4, and 6 days. All root or shoot samples of rice plants from different time points were combined in the quantitative analysis. Comprehensive RNA extraction

from rice tissue was performed using the technique described by Chen et al. [12]. Shoot and root tissues were ground to powder in liquid nitrogen and extracted with 1 mL Trizol. Standing for 5 min then centrifuged at 12,000×*g* and 4 ℃ for 10 min. The 800 μL supernatant was transferred to a 1.5-mL centrifuge tube with 400 μL chloroform and centrifuged for 10 min. Equal volume of isopropyl alcohol was added to 400 μL supernatant, mixed and last for more than 0.5 h at − 20 ℃. Centrifuge again for 10 min, discard the supernatant and add 70% ethanol solution to wash the precipitation repeatedly, then place it under sterile wind until it is nearly dry. An appropriate amount of 0.1%DEPC water after autoclaving treatment was dissolved and precipitated, and an appropriate amount was taken for quality detection by ultramicrospectrophotometer (NanoPhotometer N60, Germany). The extracted RNA was processed using the 2 × Q3 SYBR qPCR Master Mix (Universal) kit (Tolo Biotechnology, Shanghai, China). The primers listed in Additional file 1: Table S1 were used to initiate the reaction under the conditions of one cycle of denaturation at 95 °C for 30 s and 40 cycles of annealing (95 °C for 5 s) and extension (60 °C for 30 s). Light Cycler® 96 RealTime PCR System (Roche, Basel, Switzerland) was used to determine the expression levels of 9 AP2 genes in rice. Relative gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method.

Evolutionary analysis of the AP2 TF family in rice

Using the Rice Genome Annotation Project, 26 rice AP2 differentially expressed gene (DEG) sequences were acquired (https://rice.uga.edu/) and employed as query sequences. The BLAST website (https://blast.ncbi.nlm.nih.gov/) was used to search for protein sequences in *Arabidopsis* and soybean that were similar to the rice AP2 gene sequences in the database with a sequence similarity criterion of no less than 40%. Finally, the neighbor-joining method with Poisson correction model parameters was used in Molecular Evolutionary Genetics Analysis (MEGA) 7.0 to construct the phylogenetic tree, which can be used to understand the evolutionary relationships among distinct species [22]. Then, EvolView (https://evolgenius.info//evolview-v2/#login) was used to visualize the phylogenetic tree.

Chromosomal localization and collinearity analysis of the rice AP2 genes

We used Ensembl Plants (https://plants.ensembl.org/index.html) to download genome annotation files for rice, *Arabidopsis*, and soybean. The protein and nucleic acid sequences of 26 rice AP2 DEGs were downloaded from the China Rice Data Center (https://www.ricedata.cn/gene/). Tbtools 1.12 software (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to visualize the distribution of the four rice HAD genes on chromosomes [23]. Multiple collinear scanning toolkits (MCScanX) and the parameters specified by Wang et al. were used to conduct a syntenic study of AP2 genes across rice and among rice, *Arabidopsis*, and soybean [24]. Finally, collinearity analysis of the genes was performed using TBtools.

Structural analysis of rice AP2 TFs

Using the computepl/MW program, the molecular weight, instability index, theoretical isoelectric point, quantity of amino acids, and grand average of hydropathicity of each of the 26 rice AP2 DEGs was predicted. (Expasy: https://web.expasy.org/protparam/). Prediction of the three-dimensional structure of each AP was performed using the SWISS-MODEL workspace (https://swissmodel.expasy.org/interactive). An exon–intron structure diagram was created for the AP2 gene family using the US National Center for Biotechnology Information (NCBI) conserved domain database website (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi), and conservative motif analysis was performed using MEME (https://meme-suite.org/meme/) online software [25]. The exon–intron and domain structure diagram, motif analysis chart was obtained by tbtools.

Cis-acting promoter elements and domain structure analysis of rice AP2 TF DEGs

PlantCARE was used to perform cis-acting element prediction analysis on a 2000-bp nucleotide sequence upstream of the start codon for each of the 26 rice AP2 DEGs obtained from TBtools. Using the NCBI CD-search Tool (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi), the domain structure for each of the 26 rice AP2 DEGs was determined. Tbtools was then used to export each result.

Network analysis of protein–protein interactions among rice AP2 family genes

We searched for analogous O. sativa AP2 (OsAP2) genes in Arabidopsis (https://www.arabidopsis.org/) based on the rice genome (https://www.ricedata.cn/gene/) to study protein–protein interactions by String (http://stringdb.org/). Cytoscape (https://cytoscape.org/) was used to visualize the protein–protein interaction network.

Molecular docking

Using molecular docking, the binding patterns of six AP2 proteins involved in protein interactions with MTR were predicted. The MTR structure files were acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and prepared in ChemBio3DUltra140. The crystal structures of AP2 proteins (UniProt accession numbers:

Q0DLK1, Q65WX1, A0A0P0X444, C7J502, A3BT06, and A0A0P0XTN0) were obtained from the UniProt Knowledgebase (https://www.uniprot.org/). Molecular docking analysis was carried out using AutoDock Vina 1.5.7. Discovery Studio 2019 was used prepare pdb files, and generate the two-dimensional structures for the interactions [26]. Finally, Pymol 2.5 was used to enhance the appearance of ligands and proteins and present the molecular docking interactions between ligands and residues.

Statistical analysis

Each finding in this study resulted from an average of three biological replicates. Analysis of variance ($p < 0.05$) was used to identify significant differences. All statistical analyses were performed using SPSS 19.0 (IBM, Armonk, NY, USA). Data analysis and mapping were performed using Origin 2021 and R 4.2.2software.

Results

Identification of MTR-responsive AP2 TF genes in rice

To identify the rice AP2 TF genes that respond to MTR, we employed RNA-seq and applied the following criteria: false-discovery rate < 0.001 and $\log_2$fold change > 1. Pairwise comparisons of Root-MTR and Root + MTR revealed 95 AP2-encoding genes that exhibited different responses to MTR, whereas pairwise comparisons of Shoot-MTR and Shoot + MTR revealed 80 AP2-encoding genes with divergent responses (Fig. 1A and Additional file 1: Table S2). Based on these datasets, 1 (*Os01g0313300*) and 26 genes were found to be

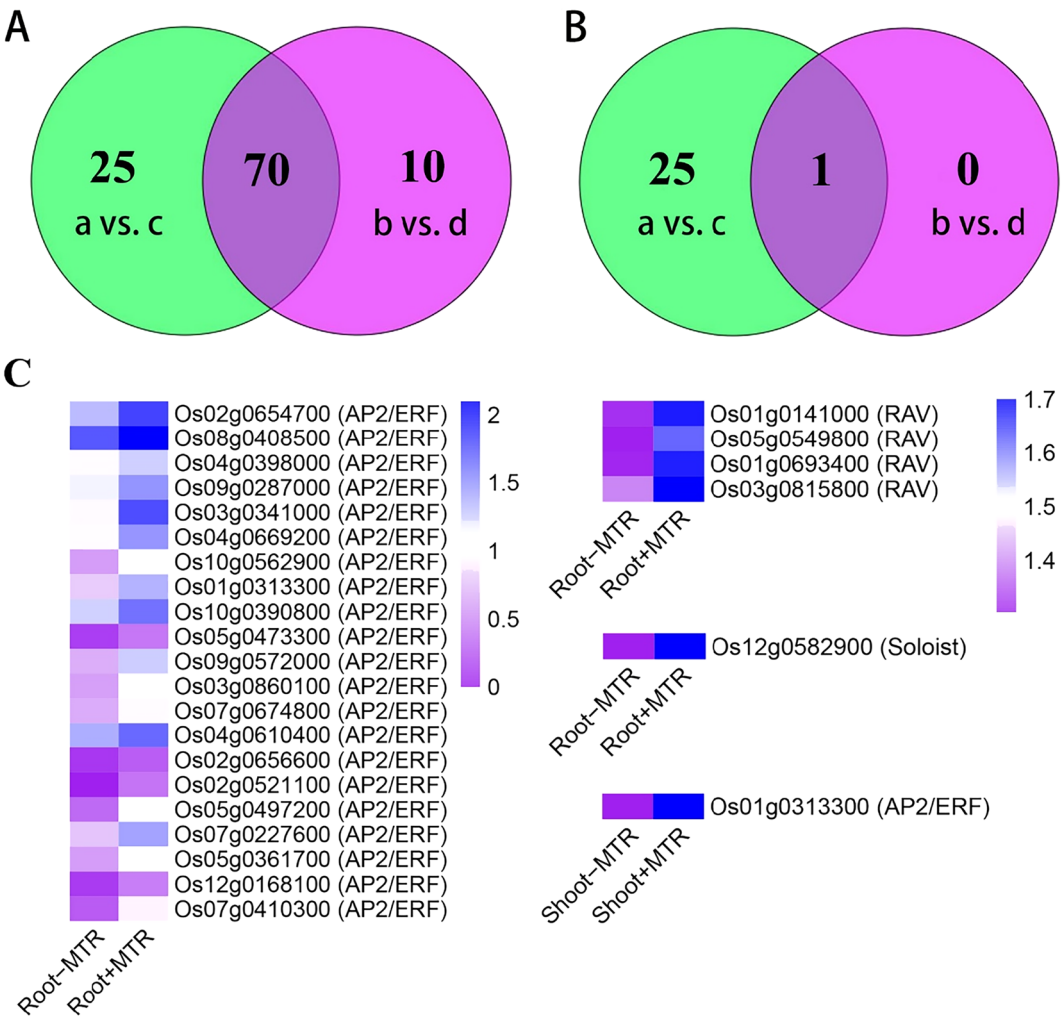

Fig. 1 Venn diagrams of the total number of AP2s gene expression (**A**) and specific upregulation (**B**) in rice is unique and common in different paired comparisons exposed to MTR. Threshold values FC ≥ 2, FDR < 0.001. a, Root-MTR (root control, MTR-free); b, Shoot-MTR (shoot control, MTR-free); c, Root + MTR (root treated with MTR); d, Shoot + MTR (shoot treated with MTR). Heat maps of differentially expressed genes of rice AP2s from roots and shoots exposed to MTR (**C**)

upregulated in rice seedling shoots and roots in response to MTR exposure And the number of genes with expression levels upregulated in roots for more than twofold was 10, but in shoots was 0 after treatment with MTR (Additional file 1: Table S3). In addition, a single gene exhibited simultaneous upregulation in both the roots and shoots of rice seedlings in response to MTR stress was also found (Fig. 1B and Additional file 1: Table S3). These results indicated that MTR exposure upregulated AP2 gene expression, which is potentially linked to abiotic stress responses [20, 21, 27]. Furthermore, the expression of AP2 genes varied across organs, suggesting that certain AP2 genes are unique to particular tissues.

Phylogenetic analysis of the rice AP2 gene family

We constructed an intraspecific phylogenetic tree using the amino acid sequences of 26 rice AP2 TF genes to evaluate their evolutionary relationships. We also constructed an interspecific phylogenetic tree based on the amino acid sequences of 26 AP2 TFs to examine the relationships among rice, *A. thaliana*, and soybean AP2 genes that had > 40% similarity using MEGA 5.0 (Fig. 2B and Additional file 1: Table S4). Three branches were identified in the phylogeny of both intra- and interspecies relationships. The number of AP2 domains and the presence of B3 domains revealed that branches I, II, and III of the rice intraspecific phylogenetic tree corresponded to the RAV, soloist, and ERF subfamilies, respectively (Fig. 2A). Interspecific phylogenetic analysis revealed that branch I contained three rice, two *Arabidopsis*, and four soybean genes. Branch II contained one rice, *Arabidopsis*, and soybean gene each. Branch III contained 22 rice, 23 *Arabidopsis*, and 39 soybean genes (Fig. 2B). Genes belonging to the same branch exhibited homology. The number of genes varied across branches, and phylogenetic tree analysis demonstrated the presence of both intra- and interspecific homology in rice, *A. thaliana*, and soybean, indicating that the AP2 families of rice, soybean, and *A. thaliana* have a conserved evolutionary connection.

Chromosomal location and duplication events of the rice AP2 genes

As presented in Additional file 1: Fig. S1, 26 AP2 DEGs were unevenly distributed on 12 chromosomes. Chr05 contained the highest number of AP2 genes, whereas three AP2 genes were located on Chr01, Chr02, Chr03, Chr04, and Chr7, indicating a difference in the roles of various rice AP2 genes (Additional file 1: Table S5). Gene families originated from tandem and segment duplications in genes during biological evolution [28]. We investigated AP2 gene duplication events to determine whether the AP2 gene family had also experienced duplication-based expansion. In this study, 16 duplicated gene pairs were discovered on 11 chromosomes (Fig. 3A and Additional file 1: Table S6). To

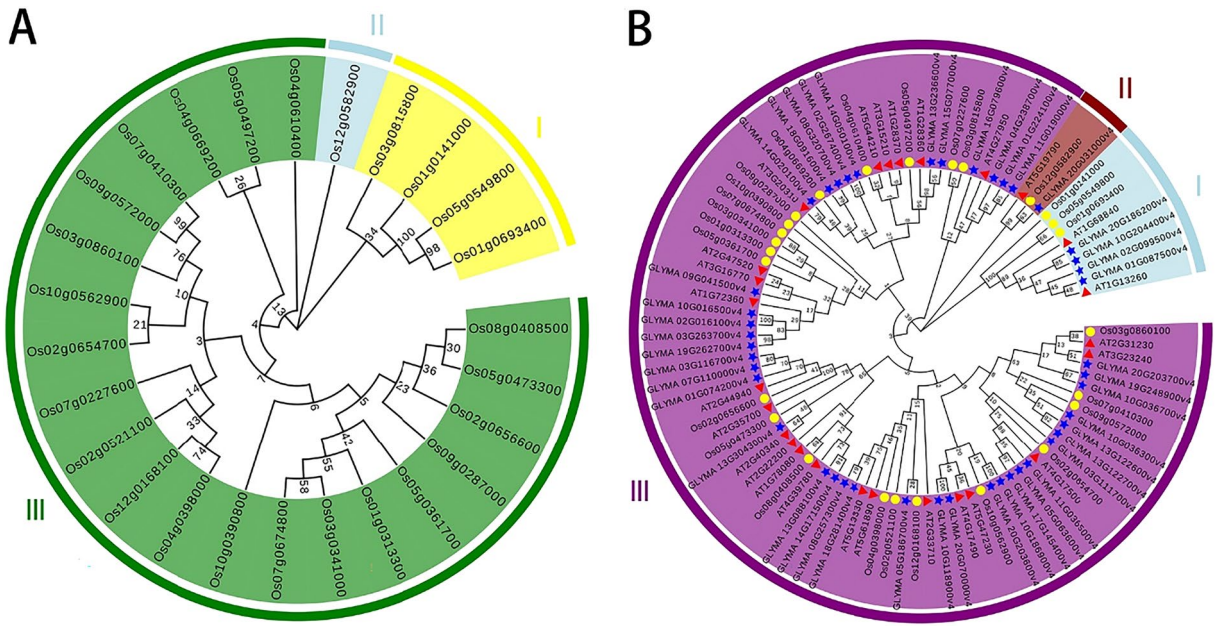

Fig. 2 **A** Phylogenetic analysis of AP2 family genes in rice. The tree split into 3 groups that harbored I, II, and III groups were identified as RAV, soloists, and ERF subfamilies. **B** Phylogenetic relationships of AP2 members in rice, Arabidopsis, and soybean. Rice, Arabidopsis, and soybean AP2 are marked with yellow circles, red triangles, and blue pentagrams, respectively

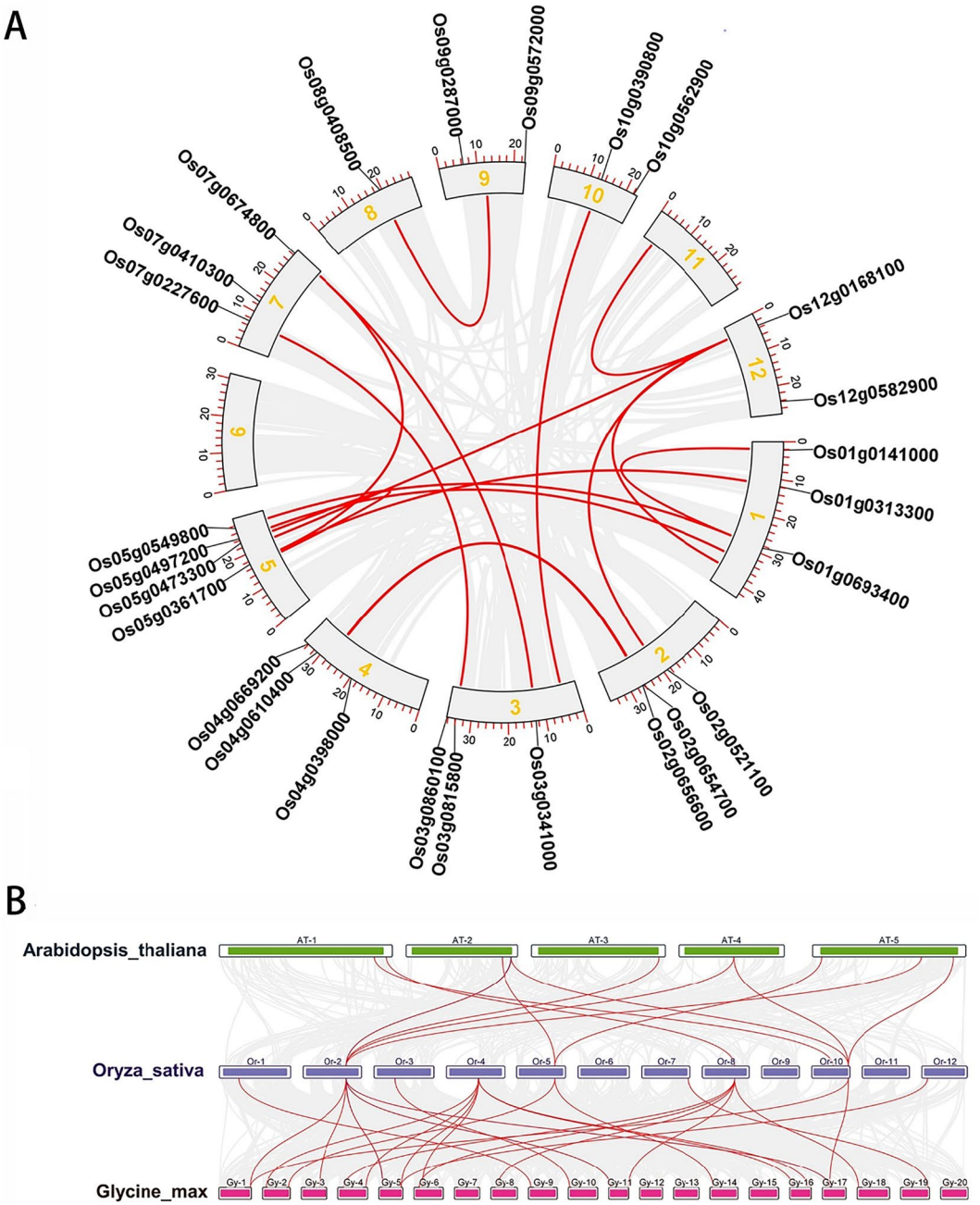

Fig. 3 **A** Schematic representations of inter chromosomal relationships of rice AP2 genes. Gray lines suggest all synteny blocks in rice genome, and the red lines indicate segmental duplicated AP2 gene pairs. **B** Gene duplication and synteny analysis of AP2 genes between rice and two other plant species. Gray lines in the background indicate the collinear blocks within rice, Arabidopsis and soybean genome, while the red lines highlight the syntenic AP2 gene pairs

unravel the potential mechanisms of the development of the rice AP2 family, two comparative collinear maps of rice, *A. thaliana*, and soybean were established (Fig. 3B and Additional file 1: Table S7). The result revealed higher number of orthologous events between rice and soybean than between rice and *Arabidopsis*. Between rice and *Arabidopsis thaliana*, there were 12 pairs of direct homologs, whereas those between rice and soybean were 26. Duplication events served as the primary catalyst for AP2 gene development.

Gene structure and motif sequence analysis of rice AP2 genes

The conserved domains of 26 AP2 DEGs were identified using NCBI-CDD. All 26 genes exhibited typical AP2 domains (Fig. 4 and Additional file 1: Table S8). To gain additional insight into the structural variation of AP2, the composition of conserved motifs and arrangement of exons and intros were examined (Fig. 4). This examination demonstrated that rice AP2 genes in the same branch of the gene evolution tree had different structures. In the EFR subfamily, 10 genes did not have an intron and 11 genes had one. No introns were detected in RAV and soloist genes. In addition, among the three branches, 22 genes possessed both 3′ and 5′ untranslated regions (UTRs), whereas *Os07g0227600* lacked a UTR sequence. The 5′UTR might provide an advantage to the downstream open reading-frame, maintaining uninterrupted and enhanced translation and achieving translation through more ribosomal loading [29]. Plant evolution is related to the number of introns [30]. Genes with fewer introns may be quickly transcribed to respond to environmental changes with high efficiency. Members of the soloist and RAV subfamilies lack introns, indicating that these genes might quickly respond to external challenges. The high intron density of the AP2/EFR subfamily genes raised the possibility of alternative splicing. Differential splicing impart various functions to genes [31]. Thus, the AP2/

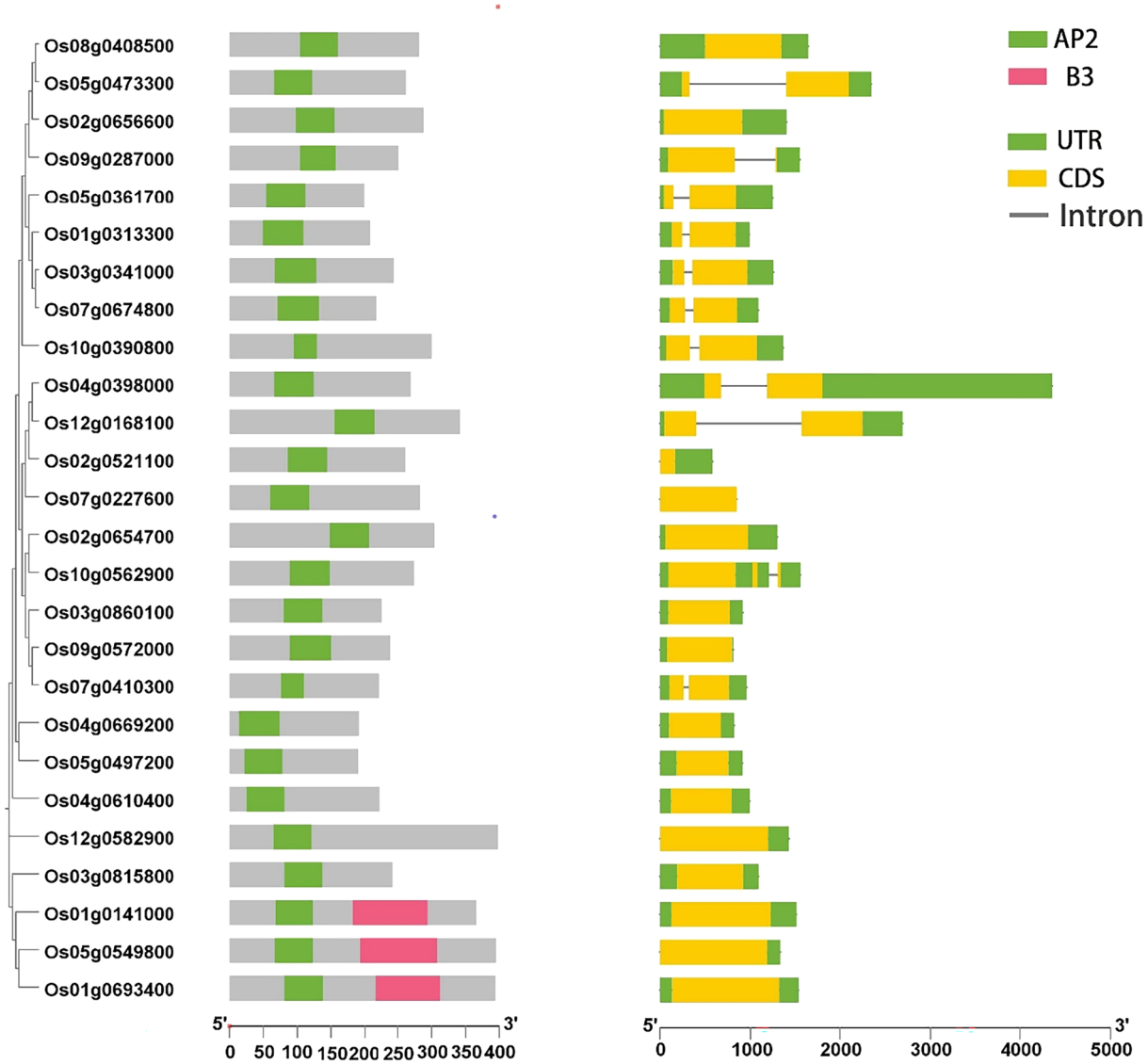

Fig. 4 Phylogenetic relationship, protein structure and gene structure of AP2 genes in rice

EFR subfamily genes could have multiple functions in rice.

To gain more insight into the evolutional and structural diversity of AP2 genes, the conserved motifs in AP2 proteins were analyzed. Six distinct and highly conserved motifs were captured (Fig. 5). All AP2 genes have major motifs 1 and 2, which are primarily composed of the conserved domain AP2. Three genes in branch I contained motifs 3, 5, and 6, and six genes in branch III contained motif 4. Generally, motifs can be unique to a family,

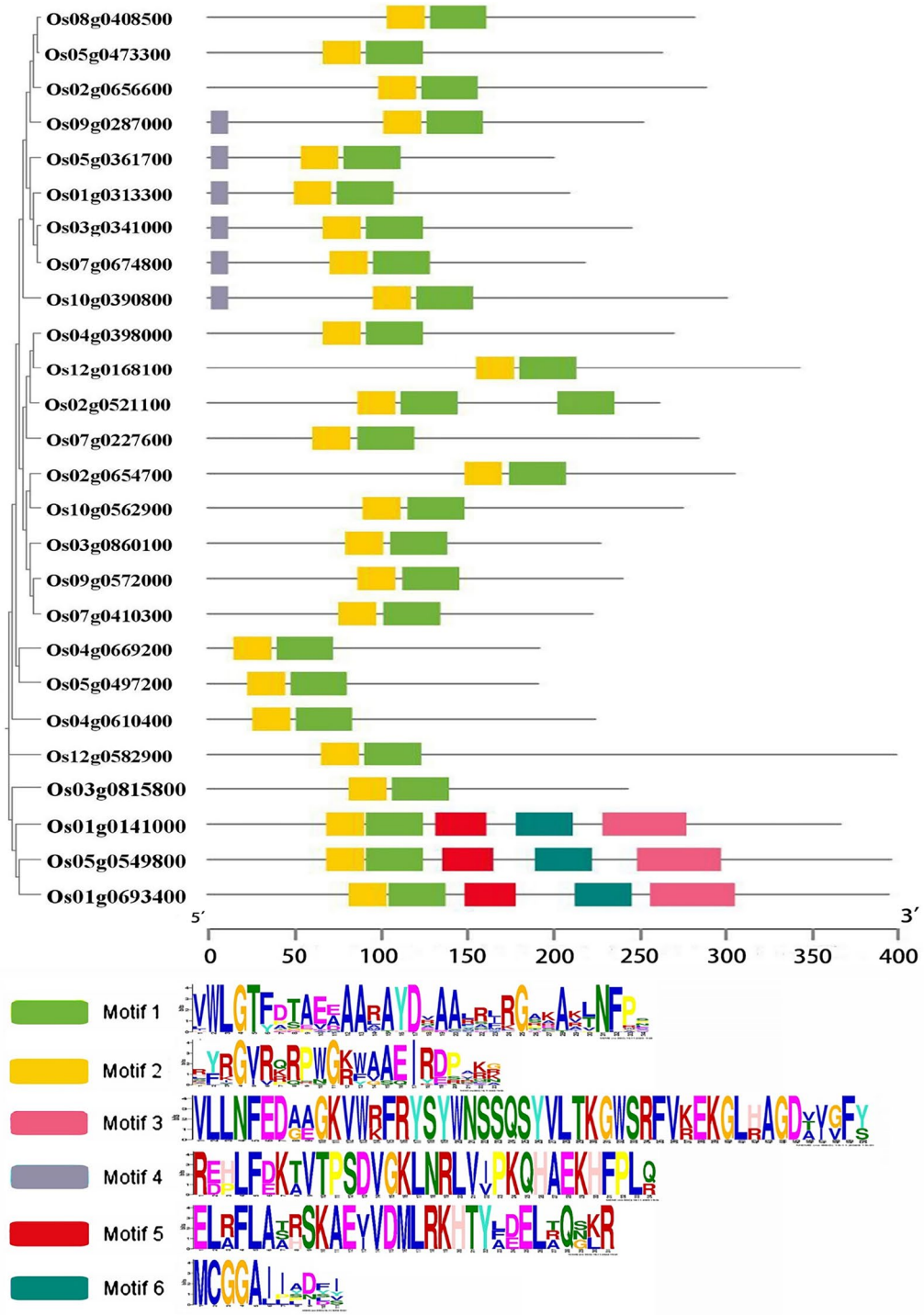

Fig. 5 Distribution of conserved motifs in AP2. The 6 motifs are labeled with various colors

clade, or group [32], and comparable biological roles are shared by genes with comparable motif compositions.

Analysis of rice AP2 *cis*-acting promoter elements

A series of noncoding DNA molecules known as "cis regulatory elements" modify the transcription of nearby genes to control gene expression at various developmental stages [33]. Cis-acting elements were detected using PlantCARE, and the results demonstrated that the AP2 genes contain various promoter elements. Based on their suspected roles in controlling plant growth, responding to abiotic stress, and binding to MYB, these elements were categorized into three primary groups (Fig. 6 and Additional file 1: Table S9). Moreover, these components were able to respond to gibberellin (GA), auxin, salicylic acid (SA), abscisic acid (ABA), and methyl jasmonate

Fig. 6 Analysis of *cis*-acting elements in the promoter regions of the AP2s family. Different *cis*-acting elements are displayed in different colors

(MeJA). Numerous stress-related response components, such as those involved in low-temperature responses, anaerobic induction, and defense and stress responses, were also examined. These findings implied that the rice AP2 family is involved in the response to environmental stress and hormone signaling transduction.

Network of interactions between proteins and analysis of homology models

The AP2 protein family members were analyzed via a three-dimensional (3D) model developed using the SWISS-MODEL online program (Additional file 1: Fig. S2). The prediction model was based on the reported template, which was in turn based on the greatest sequence fragment coverage, sequence identity, and believability score of the test sequence. The predicted AP2 protein structure was deemed reasonably reliable, as evidenced by > 60% structural coverage between the proteins and appropriate model sequence (Additional file 1: Tables S10 and S11). Analysis of the 3D model revealed that these AP2 proteins may have originated from the same ancestral sequence or remained stable during long-term domestication following initial differentiation under the influence of purification selection. These AP2 proteins have similar tertiary structures [20].

AP2 proteins interact with other proteins to create complexes that have biological functions [34]. Therefore, the AP2 protein interaction network was examined to predict possible interactions (Fig. 7 and Additional file 1: Table S12). STRING software was used to create an interaction network. The orthologous proteins with the highest bit scores were classified as STRING proteins. The interaction map demonstrated the relationships between several AP2 genes and target proteins, including ethylene-insensitive protein (EIN3/EILs) (A3C052_ORYSJ, Q6YVT1_ORYSJ), actin-related protein, mitogen-activated protein, fructose/tagatose bisphosphate aldolase (Q652S1_ORYSJ), and RNA polymerase II (Q6YX88_ORYSJ). Os03g0860100, Os07g0227600 and Os07g0410300 were associated with A3C052_ORYSJ and Q6YVT1_ORYSJ. Os05g0473300 was associated with Q6ZGM1_ORYSJ, ARP5, Q7XUC9_ORYSJ, Q656M9_ORYSJ and Q6YX88_ORYSJ. Os08g0408500 was associated with Q652S1_ORYSJ. Os10g0390800 was associated with MPK12. EIN3/EILs promote photosystem II protection during cold stress through two different SA response mechanisms, which can play positive or negative role in plant responses to biotic and abiotic stresses, and they may also play a role in photoprotection [35]. In addition, many AP2 gene members interacted with one another through protein–protein interactions, such as *Os03g0860100*, *Os07g0227600*, and *Os07g0410300*, implying possible functional cooperation among AP2 proteins. Based on the connections of AP2 proteins with target proteins, this protein–protein interaction network analysis offered additional proof that these proteins are involved in plant responses to abiotic stress.

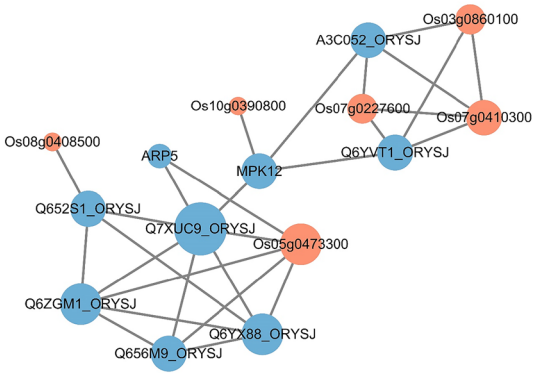

Fig. 7 Protein interactions network diagram of AP2 genes in rice. The pink dots are AP2 genes; the blue dots are other genes added based on the string database. The size of the dots represents the magnitude of the degree

AP2 gene expression under MTR treatment

To further understand the response of AP2 genes to MTR, we selected nine genes with abiotic stress-induced high transcriptional expression levels based on RNA-seq data for qRT-PCR (Fig. 1B). In the shoots, *Os01g0313300* expression changed significantly after MTR treatment. Of the genes positively regulated by MTR, *Os07g0410300* was the most obviously induced (approximately tenfold), followed by *Os12g0582900* (ninefold), *Os05g0473300* (fivefold), Os03g0860100 (4.6-fold), *Os10g390800* (4.5-fold), Os01g0313300 (Root for 4.3-fold, Shoot for 2.7-fold), Os07g0227600 (fourfold), Os09g0572000 (fourfold) and Os08g0408500 (2.7-fold), suggesting that these genes play major roles in the response to MTR stress (Additional file 1: Fig. S3). These findings demonstrated that abiotic stress can induce significant changes in these genes. The expression profiles generated using RNA-seq and qRT-PCR were comparable (Fig. 1B and Additional file 1: S3), demonstrating the reliability of the RNA-seq data and the possible role of the examined genes in rice development and abiotic stress responses.

Molecular docking analysis

We screened six AP2 proteins involved in protein interactions and used Libdock methods to examine their interactions with MTR to better investigate the relationship between AP2 proteins and pesticides (Additional file 1: Table S13). Protein activity can be affected by structural modifications caused by ligand binding [36]. MTR was embedded in the active pocket of each rice

AP2 protein via a combination of hydrogen bonding and hydrophobic interactions (Fig. 8 and Additional file 1: Table S14). The minimum binding energy of MTR for *Os03g0860100* was − 6.1 kcal/mol (Fig. 8A). MTR formed conventional hydrogen bonds with ARG-81, ARG-96 and ARG-128 of *Os03g0860100* and interacted with ILE-95

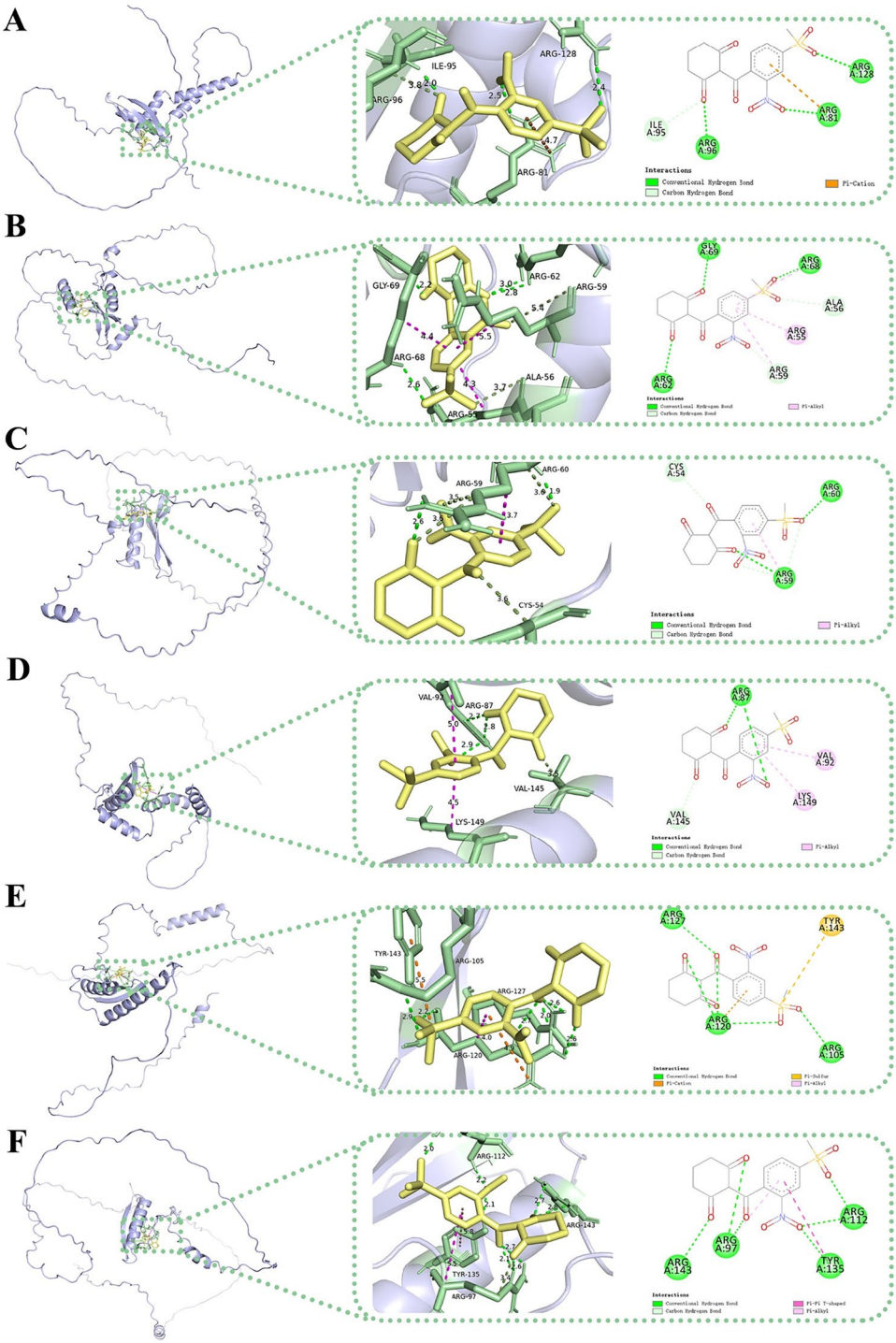

Fig. 8 The receptor–ligand interaction of pesticides with the AP2 protein active site. A-F Were depicted using PyMol soft. Molecular docking of MTR with Os03g0860100 (**A**); molecular docking of MTR with Os05g0473300 (**B**); molecular docking of MTR with Os07g0227600 (**C**); molecular docking of MTR with Os07g0410300 (**D**); molecular docking of MTR with Os08g0408500 (**E**); molecular docking of MTR with Os10g0390800 (**F**)

with carbon hydrogen bonds, the aromatic ring of MTR formed pi–cation stacks with ARG-81. The minimum binding energy of MTR with *Os05g0473300* was − 7.0 kcal/mol (Fig. 8B), which confirmed the strong binding interaction between them. MTR formed conventional hydrogen bonds with ARG-62, ARG-68, and GLY-69; carbon hydrogen bonds with ARG-59 and ALA-56; ARG-55, ARG-59 and ARG-68 residues formed pi–alkyl stacks with the aromatic ring of MTR. The interaction between MTR and Os07g0227600 had a minimum binding energy of − 6.1 kcal/mol (Fig. 8C). Conventional hydrogen bonds were formed between MTR and ARG-59, ARG-60. Carbon hydrogen bonds were formed between MTR and ARG-59, CYS-54. In addition, ARG-59 residues formed pi–alkyl stacks with an aromatic MTR ring. Molecular docking of MTR with *Os07g0410300* resulted in a minimum binding energy of − 6.7 kcal/mol (Fig. 8D). MTR formed conventional hydrogen bonds with ARG-87. Interactions between residues ARG-87 and LYS-149 produced carbon hydrogen bonds. LYS-149 and VAL-92 residues formed pi–alkyl stacks with an aromatic MTR ring. The minimum binding energy of MTR with *Os08g0408500* was − 6.2 kcal/mol (Fig. 8E). MTR formed conventional hydrogen bonds with ARG-120, ARG-105 and ARG-127. S atoms on MTR formed pi–sulfur stacks with TYR-143. Furthermore, the aromatic ring of MTR formed pi–alkyl stacks and pi–cation stacks with ARG-120. The minimum binding energy of MTR with Os*10g0390800* was − 6.1 kcal/mol (Fig. 8F). MTR formed conventional hydrogen bonds with ARG-97, ARG-112, ARG-143 and TYR-135 of Os10g0390800. The aromatic ring of MTR formed pi–pi T-shaped interactions with TYR-135, and pi–alkyl stacks with ARG-97. MTR formed conventional hydrogen bonds, carbon hydrogen bonds, pi–pi T-shaped interactions, pi–alkyl stacks, pi–cation stacks, and pi–sulfur stacks with amino acid residues, which enhanced protein activity. Based on these findings, the potential role of the upregulated AP2 genes in rice detoxification and pesticide metabolism was postulated.

Discussion

The AP2 superfamily is one of the largest families of plant-specific TFs and plays important roles in various biological processes. Many studies aimed to identify the responses of AP2 superfamily proteins in plants to various abiotic stresses, such as low temperature, salinity, and drought [37]. Nevertheless, limited studies have examined this gene family in rice under pesticide exposure. Therefore, we thoroughly examined the responses of the rice AP2 gene family to pesticide exposure using bioinformatics. In total, 105 OsAP2 genes and 26 upregulated OsAP2 genes were identified in this study (Fig. 1). Next, we identified the structures, cis-elements, motif compositions, conserved domains, transcriptional expression, and quantitative expression of the upregulated genes and conducted phylogenetic, chromosome location, collinearity, protein–protein interaction networks, and molecular docking analyses.

Several studies showed that gene duplication is necessary for the evolution of genomes and genetic systems. Thus, tandem and segmental duplications may result in the generation of gene families [37], and plants can quickly respond to environmental stress through these processes [38]. In the current study, 26 OsAP2 genes were involved in segmental duplication events, indicating an unequal distribution of segmental duplication. This suggested that the evolution of rice AP2 genes and their ability to adapt to stress were both significantly influenced by segmental duplication. OsAP2 genes may be involved in the MTR stress response, as demonstrated by the same expression patterns of these aforementioned 26 OsAP2 genes under MTR stress.

To explore the homologous relationships among these upregulated AP2 genes, we further analyzed the intra- and inter-species collinearity of these AP2 genes with those of *A. thaliana* and soybean. The results revealed that these 26 OsAP2 genes were genomically collinear. The 26 rice AP2 genes also exhibited significant collinearity with those of *A. thaliana* and soybean. We infer that the scope of AP2 gene evolution encompassed multiple species through amplification and replication.

According to our evolutionary analysis of OsAP2 among plant species, different AP2 genes are present in plants. To understand the evolutionary relationships of these AP2 genes within rice and among rice, *A. thaliana*, and soybean, a phylogenetic tree was constructed using AP2 genes from rice, *A. thaliana*, and soybean (Fig. 2 and Additional file 1: Tables S4). The 26 rice AP2 genes were classified into three categories according to their evolutionary relationships. In, total, 1, 4, and 21 genes were classified into the RAV, soloist, and AP/ERF gene families, respectively. We found close evolutionary relationships within rice AP2 genes, such as *Os05g0549800* and *Os01g0693400*, between the rice AP2 gene Os08g0408500 and the *A. thaliana* AP2 gene AT1G78080, and between the rice AP2 gene *Os12g0582900* and soybean AP2 gene GLYMA 20G031000v4. These close relationships were also confirmed using a map of intra- and inter-species collinearity among rice, *A. thaliana*, and soybean. According to this investigation, *Arabidopsis* and soybean AP2 genes were less similar. In particular, rice and soybean shared 26 orthologous gene pairs, whereas *Arabidopsis* and rice shared 12 homologous gene pairs. Furthermore, these results were consistent with the results of the phylogenetic study of rice, soybean, and *Arabidopsis*, which revealed that the conserved motifs,

functional domains, and gene structure of these OsAP2 genes were shared by AP2 genes from the same evolutionary subgroup. When paired with earlier research on AP2 TFs in various species, including *Arabidopsis*, rice, barley, and maize, we found that monocot–dicot plants, which are important for responding to various stresses, share comparatively conserved gene family structures, evolution, and function [19]. Thus, we reasonably speculate that these upregulated AP2 genes are involved in MTR stress response. However, the observed substantial variation across the various groups suggests that functional distinctions among rice AP2 proteins were gradually established during evolution. This interpretation is in line with the findings of Li et al. [19], which showed that AP2 genes have a lengthy evolutionary history, originating and beginning to differentiate as early as the evolutionary origin of monocotyledons and dicotyledons.

Furthermore, crucial conserved domains and motifs are always in TFs for their regulatory activities [39]. In this study, six motifs were associated with the AP2 domain in rice. The most conserved regions of the AP2 domain were found in motifs 1 and 2, and all OsAP2 genes had at least one of the six domains, demonstrating the highly conserved nature of the AP2 domain in the rice genome. According to previous studies, the AP2 domain is also highly conserved in other plant species [20, 21, 40]. Notably, TFs belonging to the same group shared one or more motifs outside the AP2 domain region. These motifs are assumed to contain domains related to transcriptional control, nuclear localization, or functional factors [39]. These conserved amino acid residues probably indicate the critical roles that AP2 family genes play in many types of physical DNA binding.

Gene structural analysis revealed that most AP2 family members have no introns. Notably, only members with introns appear in the AP2/ERF subfamily. When plants experience environmental stress, the presence of few introns in the AP2 family genes leads to faster and higher expression, which was confirmed by Jeffares et al. [41], who found that a gene with a compact structure might be expressed more quickly and respond to external and internal stimuli. These small numbers of introns indicate that these upregulated OsAP2 genes can react swiftly to harsh environmental conditions, such as pesticide stress. Overall, these findings indicate that although most motifs in the genes belonging to the AP2 family are well conserved, nonevolving motifs might be linked to unexpected roles in plants, necessitating further research.

Domain analysis of these OsAP2 genes revealed that they contained AP2 and B3 domains (Fig. 4 and Additional file 1: Table S8). The SA-, ABA-, and MeJA-responsive functional domains might also be extremely important to plants. Indeed, the abiotic stress mitigation function of AP2 genes has been demonstrated [18, 19, 37, 39]. AP2/ERF superfamily is characterized by one or two AP2 domains that contain 60–70 amino acid residues. These transcription factors are also closely related to plant growth and development and stress responses [37, 42]. Therefore, we consider it is plausible that these domains are involved in the detoxification and metabolism of pesticides.

In addition to dictating how genes are expressed in different tissues, cis-acting elements are closely linked to stress tolerance [43]. The data for 26 OsAP2 genes revealed that abscisic acid responsive elements (ABREs) have the greatest variation in sequence types among the elements (Fig. 6). In total, 24 OsAP2 genes were upstream of the rice ABREs, which were widely distributed, and 21 appeared more than once. Given the high frequency and broad range of ABRE elements, it is possible that these elements play a role in imparting the ability to plants to produce GA, SA, MeJA, and ABA, which allow plants to withstand abiotic stimuli such as salinity, low temperature, drought, and pesticides [8, 44]. ABA effectively increases the tolerance of rice to abiotic stress [45], whereas SA and MeJA are crucial for the metabolism and detoxification of pesticides [8]. For example, SA enhances isoproturon degradation and alleviates isoproturon phytotoxicity in *Arabidopsis* [46]. MeJA may also enhance the detoxification or degradation of isoproturon and increase isoproturon resistance in wheat [47]. Our previous research demonstrated that the cis-acting element of acetyltransferase responds to ABA and gibberellin under MTR stress [48], and we ultimately demonstrated that this gene promotes MTR degradation and metabolism in rice plants [13]. Thus, it is expected that OsAP2 might participate in responses to multiple biotic and abiotic stresses and possibly participate in MTR degradation.

To understand the role of AP2 in rice, a comparative examination of its sequences and expression in model plants could be helpful [39]. The positive regulatory effect of EIN3 on salt stress tolerance is at least partially mediated by AP2/ERF TF ethylene and salt inducible type 1 (ESE1), which regulate downstream stress response genes. ERF1 is another AP2/ERF TF that acts downstream of EIN3. ERF1 activates different stress genomes in response to different stress responses. For example, in the process of adapting to salinity, ERF1 selectively activates salt-tolerant genes by binding to DRE boxes in the promoters of these genes [49, 50]. Therefore, the corresponding proteins of rice, namely Os03g0860100, Os07g0410300, and Os07g0227600 interact with EIN3, play similar roles. The interaction network demonstrated that OsAP2/ERF genes are essential for responding to both biotic and abiotic stressors, such as pesticides.

To determine how OsAP2 responds to pesticides, qRT-PCR was performed to assess gene expression. According to the results, the expression of nine OsAP2 genes was obviously enhanced under MTR compared with that of the control. The results suggested that the OsAP2 gene family is involved in MTR stress, and the nine selected OsAP2 genes may play similar roles in MTR stress responses, which agrees with the results of the protein interaction analysis.

The interactions between pesticides and their receptor ligands should be further elucidated to better understand the molecular roles of AP2 domains in pesticide metabolism and degradation [10]. As described by Qiao et al., the likelihood of a detoxifying enzyme participating in the detoxification metabolism of a pesticide increases with the strength of the observed interaction between a target protein and a pesticide molecule. In this study, we discovered that the proteins encoded by six AP2 genes, especially *Os10g0390800*, exhibited substantial hydrogen bonds with the MTR molecules (Fig. 8). This finding is crucial for determining the roles of the AP2 gene family in pesticide metabolism in plants.

Conclusions

The OsAP2 gene family was thoroughly analyzed in this study, and 105 OsAP2 genes were found in the rice genome. Based on their structural and functional characteristics, the 26 OsAP2 genes upregulated during MTR stress formed three separate subfamilies (RAV, AP2/ERF, and soloists). Meanwhile, the upstream promoter regions of these upregulated genes featured multispecific cis-elements, suggesting that the stress response of plants to MTR was linked to the transcriptional and translational activation of AP2 family genes. To adapt to various environmental circumstances, most AP2 proteins have distinctive domains or motifs that can interact with various substrates. Classification, chromosomal distribution, and collinearity analyses revealed that OsAP2 genes are dispersed across 10 chromosomes in three groups, with four genes exhibiting a collinearity link with *Arabidopsis* and soybean. Moreover, segmental duplication was found in the genome-wide replication of OsAP2, suggesting that the evolution of the OsAP2 gene family involved one or more large-scale replication events. Docking studies demonstrated that the binding of MTR to AP2 proteins is mediated by various amino acid residues. Additionally, qRT-PCR indicated that OsAP2 might play a key role in defense against MTR stressors. These findings provide a valuable resource for investigating the physiological and molecular mechanisms underlying the action of the AP2 TF family in rice, as well as new research opportunities regarding the involvement of OsAP2 in the regulation of metabolism and detoxifying mechanisms of MTR in rice.

Abbreviations

DEG	Differentially expressed gene
DREB	Dehydration responsive element-binding
MeJA	Methyl jasmonate
NCBI	National Center for Biotechnology Information
SA	Salicylic acid
TF	Transcription factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00571-3.

Additional file 1: Table S1. Primers for genes used for validating gene expression in rice tissues. **Table S2.** The expression abundance of genes coding for AP2s from roots and shoots of rice (Oryza sativa) in the presence of mesotrione (+MTR) and absence of mesotrione (-MTR). The log_2fold change > 0, log_2fold change < 0 and –- means expression of the genes was stimulated and not changed comparing with controls, respectively. **Table S3.** The expression abundance of genes coding for AP2s from roots and shoots of rice (*Oryza sativa*) in the presence of mesotrione (+MTR) and absence of mesotrione (-MTR). The "Up" means expression of the genes that changed more than twofold compared with controls, respectively, with false-discovery rates < 0.001. **Table S4.** Collinearity analysis results of rice AP2 genes (intraspecific). **Table S5.** Collinearity analysis results of rice AP2 genes (interspecific). **Table S6.** List of the AP2 genes identified in this study. **Table S7.** Mapping of rice AP2 genes to those of Arabidopsis/Glycine max. **Table S8.** Prediction of domain of AP2s. **Table S9.** Prediction of cis-regulatory elements in promoter regions of AP2s. **Table S10.** Prediction biophysical properties of AP2 DEGs induced by MTR. **Table S11.** Prediction of three-dimensional (3D) model of AP2 proteins. **Table S12.** Analysis of protein–protein interactions among rice AP2 family genes. **Table S13.** Prediction of crystal structures of AP2 proteins. **Table S14.** Molecular docking analysis of AP2 proteins and MTR. **Fig. S1.** Chromosomal location of AP2 genes from rice response to MTR. The scale on the left is in megabases (Mb). The different colors in chromosomes represent gene density. **Fig. S2.** Predicted 3D models of rice AP2 proteins. **Fig. S3.** The relative expression levels of 9 AP2 genes in response to MTR stress treatment.

Acknowledgements

The authors acknowledge the financial support of the National Natural Science Foundation of China (21976092), Jiangsu Funding Program for Excellent Postdoctoral Talent (2022ZB342) and Post doctoral innovation talent support program (BX20220153).

Author contributions

Zhao Jie Chen and Xu Zhen Shi conducted the RNA-seq and RT-PCR analysis of rice exposed to MTR. Zhi Hai He and Ya Nan Qu carried out the biochemical properties analysis of AP2 transcription factor DEGs. Gan Ai and Yan Hui Wang carried out protein interaction analysis of AP2 DEGs. Yi Zhuo Wang carried out the data analysis. Zhao Jie Chen and Hong Yang conceived and designed the study, drafted, edited and proofread the manuscript.

Funding

National Natural Science Foundation of China (21976092), Jiangsu Funding Program for Excellent Postdoctoral Talent (2022ZB342) and Post doctoral innovation talent support program (BX20220153).

Data availability

Data will be made available on request.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received: 3 February 2024 Accepted: 20 March 2024
Published online: 27 March 2024

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