A member of wheat class III peroxidase gene family, named *TaPRX-2A*, enhanced the tolerance of salt stress

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Abstract

Background: Abiotic stresses including salt stress are environment stresses of limiting the crop growth and yield. It was reported that peroxidases (PRX) were involved in various abiotic stress responses. However, few wheat PRXs were characterized in the mechanism of abiotic stresses.

Results: In this study, a novel wheat PRX gene named TaPRX-2A, a member of wheat class III peroxidase gene family, was cloned and characterized in salt stress response. According to the identification of class III PRXs in 12 different plants, we proposed an evolutionary model that this TaPRX-2A may have experienced some exon fusion events during evolution. The results of expression pattern showed that TaPRX-2A exhibited relatively high expression levels in root tissue, but low in stem and leaf tissues by using qRT-PCR. This TaPRX-2A was also induced by some stress and hormone treatments including PEG6000, NaCl, H 2 O 2 , SA, JA, and ABA. The result of overexpressing transgenic wheat showed that this TaPRX-2A enhanced the tolerance of salt comparing the wild-type wheat (WT). We also studied the molecular mechanism of TaPRX-2A mediating the salt stress response. Physiological experiments indicated that TaPRX-2A -overexpressing transgenic wheat possessed a higher survival rate, higher relative water content, and longer shoot length than WT, but remained the same in the root length under salt stress. Further experiments indicated that TaPRX-2A -overexpressing transgenic lines enhanced abiotic tolerance by enhancing oxidative stress tolerance, such as higher antioxidant activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzymes, reduction of reactive oxygen species (ROS) accumulation, and lower levels of MDA content. Moreover, the transcript levels of stress-related genes were up-regulated by overexpression of TaPRX-2A.

Conclusions: The results showed that TaPRX-2A play a positive factor in response to salt stress by scavenging ROS and regulating stress-related genes.

Background

Abiotic stresses(high salinity and drought)restrain plant development and production, resulting in significant reductions worldwide [1]. In order to adapt to various stresses, plants have developed complex mechanisms at both physiological and biochemical levels during the course of evolution [2, 3]. In the cells of higher plant, reactive oxygen species (ROS) exist in many forms, including hydrogen peroxide (H_2O_2),superoxide radicals (O_2^-) and hydroxyl radicals (OH^-). ROS are generated under abiotic conditions and cause rapid cell damage by damaging membrane lipids, nucleic acids and so on [4]. Plants have established a complex system to scavenge ROS for maintaining the steady-state level of ROS by activating the antioxidant system. The antioxidant system refers mainly to free-radical scavenging by several endogenous antioxidant enzymes, including glutathione peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) [5, 6, 7]. Peroxidases (PRX) protect cells against ROS by catalyzing oxidoreduction [8].

PRXs exist in many species, such as microorganisms, animals and plants [9, 10]. PRXs has different structures and catalytic properties, so peroxidases are divided into three superfamilies. The first peroxidase superfamily includes animal enzymes, for example, eosinophill peroxidase and glutathione peroxidase. The second peroxidase superfamily is widely distributed in many species (bacteria, animals, fungi, plants, and yeast). The third peroxidases superfamily have functions in plants, bacteria and fungi [10]. According to differences in primary structure, peroxidases are divided into three classes, including class I peroxidases distributed in intracellular, Class II peroxidases distributed in extracellular and Class III peroxidases comprised large multigene families [11]. class I peroxidases play key roles in scavenging excess H_2O_2 [11, 12, 13]. Class II peroxidases are from fungi involved in the degradation of soil debris [11, 14]. In many plants, plant-specific peroxidases belong to Class III peroxidases [15]. More than 110 Class III Prx genes have been found in allohexaploid wheat [16]. Oryza sativa comprises 138 class III peroxidase genes [17]. Seventy-three sequences encode class III peroxidase genes of Arabidopsis thaliana, and 119 class III peroxidase genes have been identified in maize [18]. Populus trichocarpa contains 93 class III peroxidase genes [9, 19].

Class III peroxidases play various functions in plant development processes, including cell wall hardening, crosslinking of cell wall components, defense against pathogen infection, H_2O_2 removal, wounding [20, 9, 11]. In A. thaliana, a large number of PRX genes have been studied and proved the function. AtPRX72 plays an important role in lignification [21]. AtPRX33 and AtPRX34 were identified to play a function in cell elongation [11]. Some studies have demonstrated that AtPRX21, AtPRX62 and AtPRX71 were response to wounding and fungal stresses [22, 23]. Gossypium hirsutum GhPOX1 may cause cotton fiber cell elongation through reactive oxygen species production [24]. Some PRXs play a central role in host plant defenses against necrotrophic or biotrophic pathogens by coordinating SA, JA, or ET [15]. In wheat, TaPRX111, TaPRX112 and TaPRX113 are involved in plant response to nematode infection [25]. In rice, expression patterns of the 21 peroxidases genes revealed important diversity, especially in response to stresses [26]. Several reports also showed that the PRX genes have an important functions in response to abiotic stresses. The Zea mays PRX genes, ZmPRX26, -42, -71, -75 and − 78 respond to various abiotic stress conditions [9]. In addition, glutathione peroxidases (GPXs) are members of peroxidases family and play an important function in plant [27]. The A. thaliana GPX gene AtGPX3 acts as a general scavenger and signal transducer under drought stress and ABA signaling (Miao et al. 2006). Six CsGPXs (Cucumis sativus) were identified to respond to ABA treatments and abiotic stress. Moreover, 5 rice GPX genes were identified to play a role in H_2O_2 and cold stress [28]. Several peroxidase genes were identified to regulate in drought-resistant wheat cultivars by a microarray experiment [29]. The two wheat GPX genes, W69 and W106 could improve the salt tolerance in Arabidopsis [4].

Wheat is an important crop in the world, and its yield is often constrained by abiotic stresses [30, 31]. The roles of some PRXs in salinity stress tolerance have been reported before. However, the molecular mechanisms of PRXs in salt response are still not fully understood in wheat. In this report, we cloned a peroxidase gene from wheat named TaPRX-2A. Gene expression pattern analysis demonstrated that TaPRX-2A was up-regulated by drought, salinity, H_2O_2 and ABA treatments. We further investigated the

salinity stress tolerance conferred by TaPRX-2A in transgenic wheat. Ultimately, our results showed that TaPRX-2A improved the wheat salinity tolerance by improving antioxidative stress ability and regulating stress-related genes. This will give the researchers new insights into the mechanisms underlying TaPRX-2A function in abiotic stress tolerance.

Results

Isolation and evolution of TaPRX-2A

In order to obtain further insights into class III peroxidase (PRX) evolution, we performed identification, classification and gene structures of class III PRXs. We searched 12 plants (S. moellendorffii,Z. mays, B. distachyon, T. aestivum, Ae. tauschii, T. dicoccoides, V. vinifera, T. urartu, O. sativa, A. thaliana, P. patens and C. reinhardtii) by HMMER 3.1 and Pfam 32.0 in batch mode with the PRX domain (Additional file 1: Table S1, and Additional file 2: Table S2). We excluded the atypical PRXs of these 12 plants with less than 50% alignment of PRX domain in the following analysis (Additional file 3: Table S3). The classification of these PRXs was based on two methods, HMM scan and NJ phylogenetic tree (Additional file 2: Table S2 and Additional file 4: Figure S1). The exon-intron structures within the PRX domain were also diagrammed in 12 investigated plants (Additional file 5: Figure S2).

Among them, we cloned one member of the TaPRXs in the wheat cultivar “Sumai 3”, and named it TaPRX-2A. The predicted TaPRX-2A ORF is 1026 bp, and the deduced TaPRX-2A protein comprises 342 amino acid residues. We studied this clone in NCBI by BLAST and found that it was a T. aestivum PRX gene (GenBank: AJ878510.1) in cultivar “Cheyenne”. We studied this clone in our identified T. aestivum class III PRX gene family by our local BLAST, and found one sequence (TraesCS2A02G573900.1.cds1) from subfamily VI PRXs with 100% sequence similarity. In order to investigate the evolution of this clone, we conducted a phylogenetic tree and gene structures of subfamily VI PRXs in 12 plants (Fig. 1a). As shown, this clone (TraesCS2A02G573900.1.cds1) in T. aestivum contained only one exon, while four other wheat and Ae. Tauschii homologous PRXs (Tdi_TRIDC2AG080470.2, Ata_AET2Gv21275100.1, Tae_TraesCS2B02G613900.1.cds1, Tdi_TRIDC2BG088710.2) in this clan of the NJ tree also contained only one exon, hinting that this one exon structure of these five PRXs might have formed before the Triticum-Aegilops split in evolution (Fig. 1b). Based on the analysis of exon-intron gene structure diagrams (Additional file 5: Figure S2), we inferred an evolutionary model of this T. aestivum PRX (TraesCS2A02G573900.1.cds1) about exon fusion (Fig. 2a). As shown, P. patens PRX (Pp3c19_20780V3.3) contained the conserved exon-intron structure within the “001” exon phases in the PRX domain. The “001” exon-intron structures could also be found in most subfamilies V-XVIII. Two S. moellendorffii PRXs (Smo_EFJ32905 and Smo_EFJ15769) retained this “001” exon-intron structure. However, the last two exons might have experienced the exon fusion event before the monocot-eudicot split. Indeed, subfamily VI PRX of the two investigated eudicots (A. thaliana and V. vinifera) contained only one member (Ath_AT1G71695.1 and Vvi_VIT_18s0072g00160.t01), and their exon-intron structures changed into “00”. Subfamily VI PRX of monocots also contained this “00” structure. For instance, rice PRX (Osa_Os04t0688200 – 01) contained this “00” structure. These three exons might have fused into

one exon before the Triticum-Aegilops split in evolution to become the ancestor of TraesCS2A02G573900.1.cds1. Interestingly, the alignment and exon phases of these PRXs supported our evolutionary model about exon fusion events (Fig. 2b).

Expression patterns of TaPRX-2A in various tissues and with hormone treatments

To detect the expression patterns of TaPRX-2A we perfomed the qRT-PCR in different tissues and with various hormone treatments. The results showed that this clone exhibited expressions in roots, stems and leaves, and significantly high expression levels in root tissue comparing with leaf and stem tissues (Fig. 3a). In order to determine the stress effects on the expression of this TaPRX-2A clone, the qRT-PCR with various hormone treatments were performed (Fig. 3). The results showed that the expressions of TaPRX-2A were induced by drought (PEG6000), NaCl and H_2O_2 treatments, and the expression levels reached the perk in 6 h (hour) after treatments (Fig. 3b,c and d). We also detected the expression patterns of TaPRX-2A in four phytohormones (salicylic acid (SA), methyljasmonic acid (MeJA), indole-3-acetic acid (IAA) and abscisic acid (ABA) treatments (Fig. 3e-h). As shown in Fig. 3e, this TaPRX-2A clone exhibited approximately 2.5-fold upregulation at 1 h after SA treatment (Fig. 3e). Similarly, the expression levels of TaPRX-2A reached the peak at 3 h and 6 h after JA and ABA treatment, respectively (Fig. 3f, h). However, the expression levels of TaPRX-2A remained almost the same at 0–6 h after IAA treatment, but exhibited about 1.5-fold upregulation at 12 h (Fig. 3g). These results showed that this TaPRX-2A clone was involved in various abiotic stress responses and plays important role in plant stress responses.

Subcellular localization of the TaPRX-2A protein

To characterize the function of TaPRX-2A, the ORF of TaPRX-2A was fused to pBIN35S-eGFP vector with the CaMV 35S promoter (Additional file 6: Figure S3). The pBIN35S:eGFP and recombinant construct pBIN35S:TaPRX-2A:eGFP vector were transformed into tobacco leaf cells by Agrobacterium infection. We observed the epidermal cells of injected N. benthamiana leaves by confocal microscope and found that TaPRX-2A gene was localized mainly in the nuclei. In addition, the pBIN35S-TaPRX-2A-eGFP and pBIN35S-eGFP vector were transformed into onion epidermal cells. Consistent with the location results of tobacco epidermal cells, the TaPRX-2A gene was also located on in the nuclei. In summary, we showed that TaPRX-2A is a nuclear protein.

TaPRX-2A enhanced the salt tolerance in transgenic wheat

To further confirm the function of TaPRX-2A in salinity stress responses, TaPRX-2A-overexpressing wheats were constructed. Firstly, we tested the salinity tolerance between TaPRX-2A-overexpressing wheat lines (TaOE1, TaOE2 and TaOE3) and wild-type (WT) wheats. The result showed that there were no obvious phenotypical differences between the WT and TaPRX-2A-overexpressing wheat lines under normal conditions. However, the TaPRX-2A-overexpressing wheat lines showed better growth under salinity conditions comparing with the WT. Moreover, the leaves of WT wheats began to turn yellow and wilt comparing with the TaPRX-2A-overexpressing transgenic lines under salinity stress (Fig. 4a). We found that the survival rate of WT plants was only 40% after salt treatment, whereas the survival rates of

TaPRX-2A-overexpressing transgenic lines TaOE1, TaOE2 and TaOE3 were 63.6%, 57.6% and 63%, respectively, (Fig. 4b). Moreover, we also compared the shoot lengths, relative water content (RWC) and root lengths between WT plants and TaPRX-2A-overexpressing transgenic lines under salt treatment (Fig. 4c, d and e). The results showed that TaPRX-2A-overexpressing transgenic lines contained longer shoot length and more RWCs than WT plants. However, the root lengths between them were almost the same. Take together, these results indicated that TaPRX-2A dramatically enhanced the salt tolerance in wheat.

To explore further mechanism of the TaPRX-2A involving in the salt stress response, some imperative physiological-biochemical indices were measured between transgenic wheats and WT wheats (Fig. 5a-d). Under salt treatment, we found that TaPRX-2A-overexpressing transgenic lines contained significantly lower MDA contents than WT, but more soluble sugar, proline and soluble protein contents. Moreover, the proline contents of transgenic lines are approximately 2-fold than WT (Fig. 5c). These results suggested that TaPRX-2A-overexpressing transgenic lines increased the osmotic and oxidative stress tolerance, resulting in improved the tolerances to salt.

TaPRX-2A regulates ROS scavenging and the expression of stress-related genes in transgenic wheat

Tolerance to oxidative stress was associated with tolerance to abiotic stresses [30, 40, 41]. The function of TaPRX-2A was confirmed in reducing ROS levels in transgenic lines under salinity stress [4]. As major indicators of the ROS level, O_2^- and H_2O_2 contents were assayed in this study. Subsequently, we detected the accumulation of O_2^- and endogenous H_2O_2 between transgenic plants and WT lines with NBT (Nitroblue Tetrazolium) and 3-diaminobenzidine (DAB) staining, respectively. Under salt treatment, O_2^- (stained by blue with NBT) and the H_2O_2 (stained by brown with DAB) levels were significantly lower in TaPRX-2A-overexpressing transgenic lines than WT (Fig. 6a–d). In addition, the activities of SOD, POD, and CAT antioxidant enzymes were also measured between TaPRX-2A-overexpressing transgenic lines and WT plants. The results indicated that the transgenic plants contained significantly higher activities of these three antioxidant enzymes than the WT plants (Fig. 6e–g).

To determine whether the expressions of stress-responsive genes were involved in the function of TaPRX-2A under salt stresses, some stress-related genes (including dehydration-responsive proteins such as RD22; thaumatin-like proteins such as TLP4; ABA-inducing gene ABAI; germin-like proteins such as GLP4; glutathione S-transferases such as GST22; and the genes encoding ROS-scavenging enzymes (FeSOD, CuSOD, CAT, APX) were selected to detect their expressions by using qRT-PCR (Fig. 7). The results showed that almost all these stress-related genes in TaPRX-2A-overexpressing lines contained more expression levels than WT under salinity stresses. However, the expression levels of the CuSOD gene showed almost the same between WT and transgenic lines under salinity stresses. In addition, we found that the expressions of some stress-related genes, such as RD22, ABAI, CAT and APX, were lower in the WT plants than in TaPRX-overexpressing transgenic lines under normal conditions. In conclusion, these results indicated that TaPRX-2A may improve the salt tolerances by enhancing the stress-responsive genes transcription levels.

Discussion

The evolution of T. aestivum TaPRX-2A

Salinity stress can reduce crop yields [42]. In this study, a wheat peroxidase gene named TaPRX-2A was isolated and characterized. We noticed that the difference between our isolated TaPRX-2A and sequence from Ensembl (TraesCS2A02G573900.1.cds1) was only one amino at 172 site (our PRX “A” and TraesCS2A02G573900.1.cds1 “S”). Based on the classification of NJ phylogenetic tree and HMM scan, TraesCS2A02G573900.1.cds1 belongs to subfamily VI PRXs. Subfamily VI PRXs can be found in S. moellendorffii, but not in P. patens, suggesting that subfamily VI PRXs might have appeared in fern-resembling ancestors. Subfamily VI PRXs contain only one member in two investigated eudicots (A. thaliana and V. vinifera). However, subfamily VI PRXs contain various members in investigated monocots, suggesting that subfamily VI might have experienced monocot-specific duplication events after monocot-eudicot split.

Based on the analysis of exon-intron diagrams of 12 investigated plants, we inferred an evolutionary model of our focused T. aestivum PRX (TraesCS2A02G573900.1.cds1) (Fig. 2). An exon fusion event might have appeared before the Triticum-Aegilops split and then formed the ancestor of TraesCS2A02G573900.1.cds1. It was reported that A sub-genome and B sub-genome diverged 6.5 Mya (million years ago), and then hybridization occurred between A and B sub-genomes 5.5 Mya and formed D sub-genome [43]. We noticed that the chromosome locations of these five PRXs in one NJ clan were 2A (Tae_TraesCS2A02G573900.1.cds1 and Tdi_TRIDC2AG080470.2), 2B (Tae_TraesCS2B02G613900.1.cds1 and Tdi_TRIDC2BG088710.2), and 2D (Ata_AET2Gv21275100.1), suggesting that this exon fusion event might have happened before the A and B sub-genomes split 6.5 Mya. The possible mechanism of this exon fusion might be “retroposition”, which was reported in the origination of the gene jingwei, and ATP synthase PGAM3 [44]. For instance, PGAM1 contains three introns, while PGAM3 is intron-less.

TaPRX-2A plays important roles in salinity stress responses

In our study, we constructed the TaPRX-2A-overexpressing transgenic wheat to investigate the potential function. The results indicated that TaPRX-2A could improve salinity tolerance in transgenic wheat comparing with the control lines (Fig. 4). In higher plants, class III PRXs were a large multigenic gene family and involved in plant development. In addition, some members of class III PRXs were also reported to respond to abiotic stress [11]. Two wheat GPX genes were involved in controlling abiotic stress responses by scavenging H_2O_2 [4]. In rice, OsAPX1 and OsAPX2 respond to oxidative and abiotic stress [45]. The barley gene HvAPX1 have been shown to have an important function in plant salt tolerance [46]. In A. thaliana, there are many peroxidase genes involving in stress. It has been reported that AtPRX03 respond to cold stress and AtPRX22 responds to potassium deficiency [47, 48]. AtPRX21, AtPRX62, and AtPRX71 are involved in wounding stress [22, 23]. In wheat, some PRX genes (TaPRX01, TaPRX03, TaPRX04, TaPRX19, TaPRX107, TaPRX109) have been identified to play important roles in osmotic stress with different transcript profiles [16]. In this study, we examined the expression levels of TaPRX-2A in

three tissues (stems, roots and leaves), and found that the transcript level was significantly higher in roots than in stems and leaves (Fig. 3). This suggested that TaPRX-2A could improve root antioxidant capacity and maintain osmotic balance directly or indirectly, then improving the plant abiotic tolerance. In addition, TaPRX-2A was induced by PEG6000, NaCl, H_2O_2 and ABA treatments. These results suggest that TaPRX-2A improve abiotic stress tolerance by regulating multiple target genes.

TaPRX-2A enhanced the salinity tolerance by the ABA-dependent pathway

It was reported that ABA mediated the expressions of stress-responsive genes. In plants, ABA, the primary signal, was involved in plant abiotic stress by regulating stress-responsive gene [49, 50]. Dehydrin genes and TLP genes play crucial roles in response to abiotic stresses, and may be induced by abiotic stress-related signal molecules such as ABA [51, 52, 53]. In Arabidopsis, ABA could mediate the expression of dehydration-responsive gene RD22 [50]. GLPs have been widely studied, and GLP expression is significantly induced by salt and drought stresses [54]. In addition, GSTs have been reported to play a function in abiotic tolerance [55]. It was reported that peroxidases play roles in abiotic tolerance by mediating the ABA signaling pathway. In Arabidopsis, oxidative signal transducer, AtGPX3, regulated ABA-responsive genes to response drought stress and ABA signal [27]. Previous reports showed that two wheat glutathione peroxidase genes improved the wheat tolerance in salt and ABA signaling [4]. In our study, the expression of TaPRX-2A was highly up-regulated not only by PEG6000 and NaCl treatments, but also by ABA treatment (Fig. 3). Moreover, the transcript levels of the stress-related genes (RD22, TLP4, ABAI, GLP4 and GST22) were up-regulated in TaPRX-2A-overexpressing transgenic lines under salt treatment (Fig. 7). This suggest that TaPRX-2A might be involved in the ABA signaling pathways, and activate the stress-related genes associated with ABA signaling pathways, resulting in improved the salt tolerance in wheat.

TaPRX-2A enhanced the antioxidative stress ability, resulting in improved the salt tolerance in wheat

ROS is involved in plant growth processes and responses to abiotic stress [56]. Oxidative damage is the common trait under abiotic stresses. Once plants suffer from stresses, excessive ROS productions (particularly O_2^-, H_2O_2, and high concentrations of AOS) were accumulated, leading to the phytotoxicity which can damage the cell membrane permeability, integrity, and cell compartmentation [56, 57, 58, 36]. Class III PRX have been identified to regulate the ROS balance in plants [25, 59]. Some PRX genes were reported to involve in ROS scavenging under multiple stresses. The 13 glutathione peroxidase genes were involved in oxidative stress response in Gossypium hirsutum [59]. The wheat PRXs play leading roles in ROS formation under pathogen infection [60]. In our study, we evaluated ROS-mediated injury by measuring MDA contents between transgenic and WT plants under salt stresses. The results showed that MDA content of TaPRX-2A-overexpressing transgenic lines was lower significantly than WT plants. Furthermore, TaPRX-2A-overexpression plants also accumulated less O_2^- and H_2O_2 under abiotic stresses (Fig. 6a–d). The results suggest that TaPRX-2A-overexpression plants were less cell injury and ROS accumulation comparing with WT plants. To maintain ROS balance, plants have evolved a complex antioxidative system to protect cells from damage caused by stress. Several major antioxidant enzymes

participate in this process in scavenging ROS [38, 61]. Our results showed that the activities of SOD, CAT, and POD antioxidant enzymes were significantly higher in TaPRX-2A-overexpressing lines comparing with WT plants (Fig. 7e, f and g). Moreover, the transcript abundances of these antioxidant enzyme genes, such as FeSOD, CuSOD, CAT, and APX, were obviously elevated in TaPRX-2A-overexpressing lines comparing with WT plants (Fig. 7). Thus, together with the higher enzyme activities and up-regulated transcript abundances of the antioxidant genes, TaPRX-2A could enhance salt tolerance by enhanced activities of the antioxidant enzymes regulating these antioxidant enzyme genes.

Conclusions

Taken together, our results clearly demonstrated that TaPRX-2A had an important function in enhancing salinity tolerance in transgenic wheat plants. TaPRX-2A activated antioxidant enzymes to scavenge ROS, and mediated ABA pathway to improve the salt tolerance by regulating the antioxidant enzyme genes. TaPRX-2A-overexpressing lines can provide a transgenic tool for improving salinity tolerance in wheat. Our study will provide novel insights into the complex mechanisms of peroxidase in response to environmental stresses.

Methods

Isolation and cloning of the TaPRX-2A gene and transformation

The leaves of harvest wheat cultivar “Sumai 3” were used to extract the total RNA by using TRIzol reagent (Transgen). cDNA was synthesized to amplify the TaPRX-2A. BLAST was performed in NCBI (https://www.ncbi.nlm.nih.gov/) by using the sequence of the gene TaPRX-2A. We found that the gene TaPRX-2A was located on A chromosome and its Genbank No. was AJ878510.1. The TaPRX-2A cDNA was ligated into the PC186 vector and then transformed into KN199 using particle gun-mediated gene transformation [32].

Plant materials and abiotic treatments

Bread wheat (T. aestivum cultivar “KN199” and "Sumai 3") seedlings were sourced from ourselves laboratory (State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University). The TaPRX-2A-overexpressing transgenic wheat lines and wild type “KN199” were grown at 20 °C-25 °C under photoperiod of 16 h/8 h. When grew to a period of one leaf and one heart, the transgenic plants and “KN199” were treated with 20% (w/v) PEG 6000 treatment and 200 mM NaCl treatment. For drought treatment, the “KN199” and transgenic seedlings were treated with natural drought for two weeks, and then rewatered for one week. For salt treatment, the control and transgenic seedlings were cultured in water for 7–10 days.

Identification and classification of class III peroxidases in wheat, Ae. tauschii, and other plants

The genomes and proteomes of 12 plants including S. moellendorffii,Z. mays, B. distachyon, T. aestivum, Ae. tauschii, T. dicoccoides, V. vinifera, T. urartu, O. sativa, A. thaliana, P. patens and C. reinhardtii, were downloaded from the website (http://plants.ensembl.org/) and analyzed. To identify the PRXs, we scanned all the proteomes of 12 plants in batch mode by our own local server. Hmmer 3.1 (pfam profile f00141.21, oxydase.hmm, PRX domain) and website pfam32.0 (http://pfam.xfam.org/) with an E value of 0.01 were performed. Typical PRXs with the PRX domain with more than 50% alignment were retained and analyzed. Others with less than 50% PRX domain aligment were consider as atypical PRXs and excluded in the following analysis. PRX alignment of truncated sequences in the PRX domain was performed by ClustalW v2.0 [33]. We used the software MEGA-CC 7.0 to construct the NJ phylogenetic tree [34]. The PRX subfamilies classification was performed by HMMER3.1, and the models were generated based on the maize PRX alignments [9].

Domain and intron–exon structure diagram of PRXs

We used our Perl and R scripts to generate the PRXs intron–exon structures and domain of these 12 plants based on the corresponding GFF file information from Ensembl Plants (http://plants.ensembl.org/). The domain information of PRXs was batched from pfam 32.0 (http://pfam.xfam.org/).

Expression pattern of TaPRX-2A induced by different abiotic stress treatments

The wheat leaf tissues of three-leaf stage seedlings were harvested at 0 h, 6 h, 12 h, 24 h, 48 h and 72 h after 20% (w/v) PEG 6000 treatment and 200 mM NaCl treatment. For 10 mM H_2O_2 treatment, the wheat leaf tissues were harvested at 0 h, 2 h, 6 h, 12 h, 24 h, 48 h and 72 h. We harvested the wheat leaf tissues at 0 h, 1 h, 3 h, 6 h, 12 h, 24 h and 48 h after 2 mM salicylic acid (SA), 100 μM methyljasmonic acid (MeJA), 100 μM indole-3-acetic acid (IAA), and 100 mM abscisic acid (ABA) treatments. All harvested samples were extracted the total RNA by TRIzol reagent. cDNA was synthesized and used to qRT-PCR analysis. We selected the gene 18SrRNA as an endogenous control. Each treatment had three independent biological repeats. We calculated the gene relative expression by the cycle threshold (Ct) values using formula $2^{-\Delta\Delta CT}$. We used Primer Premier 7.0 to design all primers in experiment and check it in Additional file 7: Table S4.

Subcellular localization of the TaPRX-2A protein

According to the ORF of the gene TaPRX-2A, we cloned the gene that deleted the termination codon and constructed it into pBIN35S-eGFP vector with the CaMV 35S promoter. Subsequently, the pBIN35S-TaPRX-2A-eGFP and pBIN35S-eGFP was transformed into Agrobacterium EHA105. pBIN35S-eGFP was the control. The Agrobacterium EHA105 was resuspended in the suspension (10 mM $MgCl_2$, 10 mM 4-morpholineethane -sulfonic acid hydrate (MES) (pH 5.6), 200 mM acetosyringone). The Agrobacterium suspension was adjusted about OD600 of 0.6, then injected into tobacco leaves and cultured for 3 days. The epidermal cells of the injected tobacco leaves were observed by confocal microscope. In addition, we also transformed the pBIN35S-TaPRX-2A-eGFP and pBIN35S-eGFP vector into onion epidermal cells by

gene gun mediated transformation [35]. The transformed epidermal cells were cultured in darkness at 28℃ for 8–12 hours and observed by confocal microscope (Zeiss LSM880 Meta Confocal Microscope).

Measurements of physiological–biochemical parameters

We collected leaves of TaPRX-2A-overexpression and KN199 plants after salinity treatment for 10 days for measurement purposes. We used the formula RWC = (FW-DW) / (TW-DW) × 100% (Fresh weight (FW), turgid fresh weight (TW), dry weight (DW) to measure the leaf RWC [36]. we used the thiobarbituric acid method to measure the MDA content and the ninhydrin reaction method to measure the proline content [37]. The soluble total sugars were determined by the anthrone method. The ROS assay was performed using the method described. We used nitroblue tetrazolium (NBT) and 3, 3’-diaminobenzidine (DAB) to visualize O_2^- and H_2O_2. The H_2O_2 and O_2^- levels were measured according to methods reported previously [38]. We also detected antioxidant enzymes activities (SOD, POD, CAT) using the method [39].

Abbreviations

WT, wild type; PRX, peroxidases; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; ROS, reactive oxygen species; GPX, glutathione peroxidase; APX, ascorbate peroxidase; SA, salicylic acid; MeJA, methyljasmonic acid; IAA, indole-3-acetic acid; ABA, abscisic acid; RWC, relative water content; NBT, Nitroblue Tetrazolium; DAB, 3-diaminobenzidine; GFP, green fluorescent protein; qRT-PCR, quantitative real-time PCR; PEG6000, polyethylene glycol 6000.

Declarations

Ethics approval and consent to participate

The wheat materials (cultivar “KN199” and "Sumai 3") used in this study were obtained from the State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Tai'an, Shandong, PR China and are publicly available for non-commercial purposes.

Consent for publication

Not applicable.

Availability of data and materials

The genomes and proteomes of investigated plants are available in Ensembl Plants (http://plants.ensembl.org/). The accession numbers of investigated plants are *T. aestivum* (IWGSC), *Ae. tauschii* (Aet_v4.0), *A. thaliana* (TAIR10), *B. distachyon* (v3.0), *C. reinhardtii* (v5.5), *O. sativa* (IRGSP-1.0), *P. patens* (Phypa_V3), *S. moellendorffii* (v1.0), *T. dicoccoides* (WEWSeq_v.1.0), *T. urartu* (ASM34745v1), *V. vinifera* (12X), and *Z. mays* (B73_RefGen_v4). The nucleic acid and amino acid sequence of T*aPRX-2A* is available in NCBI with accession number AJ878510.1. (https://www.ncbi.nlm.nih.gov/). The identification and exon-intron structures of PRXs in investigated plants are provided in supplementary files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PSS, HWW and LRK conceived and designed the experiments; PSS performed most of the experiments; JY performed the identification and evolution analysis of *TaPRX-2A*, and revised the manuscript; LW, JXZ and WL performed the subcellular location and the plant transformation; AFL contributed plant materials; PSS wrote and revised the manuscript. All authors have read and approved the final manuscript.

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Additional Files

Additional file 1: Table S1. The number of class III peroxidase gene family in 12 plants.

Additional file 2: Table S2. Subfamily classification of class III peroxidases in the investigated plant genomes.

Additional file 3: Table S3. List of atypical class III peroxidase in investigated plant genomes.

Additional file 4: Figure S1. Class III peroxidase phylogenetic tree. (a) Subfamily VI of class III peroxidases; (b) All subfamilies.

Additional file 5: Figure S2. Domain and exon–intron structure diagrams of class III peroxidase in *A. thaliana, V. vinifera, T. aestivum, P. patens, T. dicoccoides, T. urartu, Ae. tauschii, B. distachyon, C. reinhardtii, Z. mays, O. sativa* and *S. moellendorffii*. Filled boxes: red represents the PRX domain; white boxes represent the other exon regions; black boxes represent the untranslated regions (UTRs); lines

represent the PRX introns; numbers 0, 1, and 2 represent the exon phases. The long introns are shortened by "//".

Additional file 6: Figure S3. Localization of *TaPRX-2A* was mainly in nucleus. (a) Vector construction diagrams of *pBIN35S:eGFP* and *pBIN35S:TaPRX-2A:eGFP*. (b_1–d_2) Subcellular localization of the *pBIN35S:TaPRX-2A:eGFP* fusion protein and *pBIN35S:eGFP* protein in tobacco epidermal cells. (b_3–d_4) Subcellular localization of the *pBIN35S:TaPRX-2A:eGFP* fusion protein and *pBIN35S:eGFP* protein in onion epidermal cells (b_1–b_4) Green fluorescent images; (c_1–c_4) Merged images of bright, green fluorescence; (d_1–d_4) Bright field images. Bars, 20 μm.

Additional file 7: Table S4. Primers used for analysis.

Figures

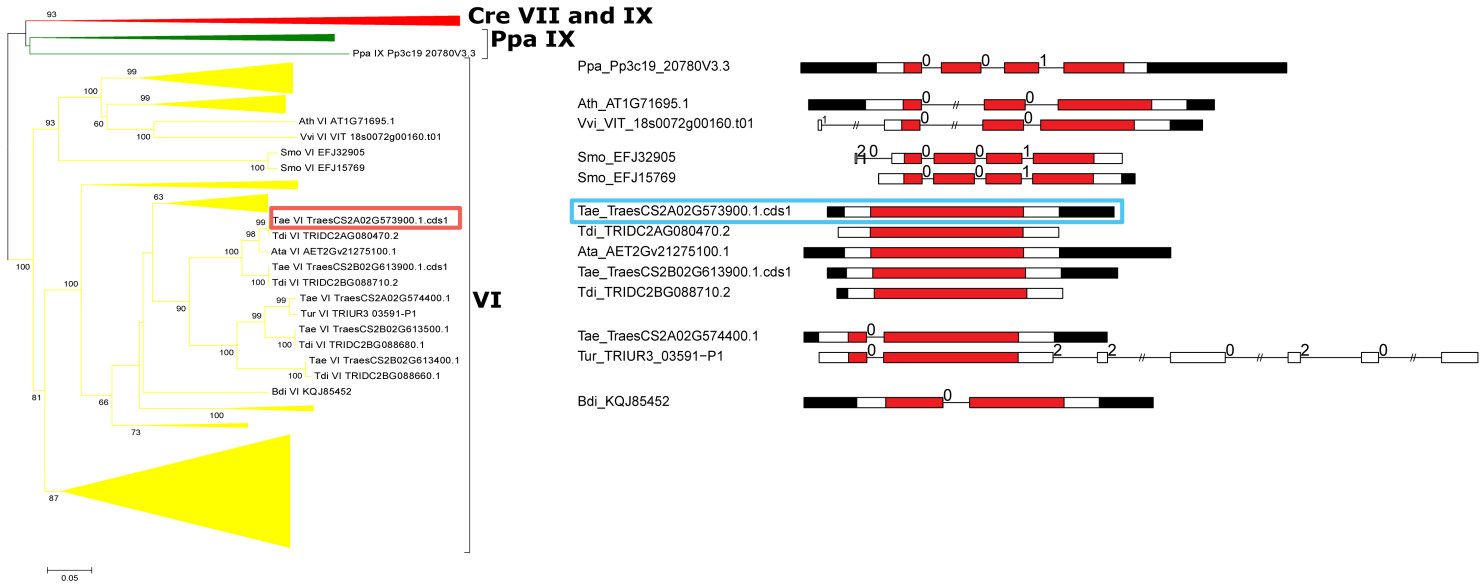

Figure 1

Phylogenetic tree and gene structures of TaPRX-2A and related PRXs in wheat, Ae. Tauschii, and other plants. (a) The diagram indicates the neighbor-joining tree. The amino acid sequences of the PRX domain were used to construct the neighbor-joining tree by using a software MEGA-CC 7.0 with the p-distance model. Most sequences belong to subfamily VI of class III peroxidases, and some branches are compressed. Detailed information is showed in Additional file 4: Figure S1. (b) The diagram indicated the exon–intron structures of some PRXs. Filled boxes: red boxes represent the PRX domain; white boxes represent the other exon regions; black boxes represent the untranslated regions (UTRs); lines represent the PRX introns; numbers 0, 1, and 2 represent the exon phases. The long introns are shortened by "//". Our investigated PRX (TraesCS2A02G573900.1.cds1) in T. aestivum was circled by a red or cyan box.

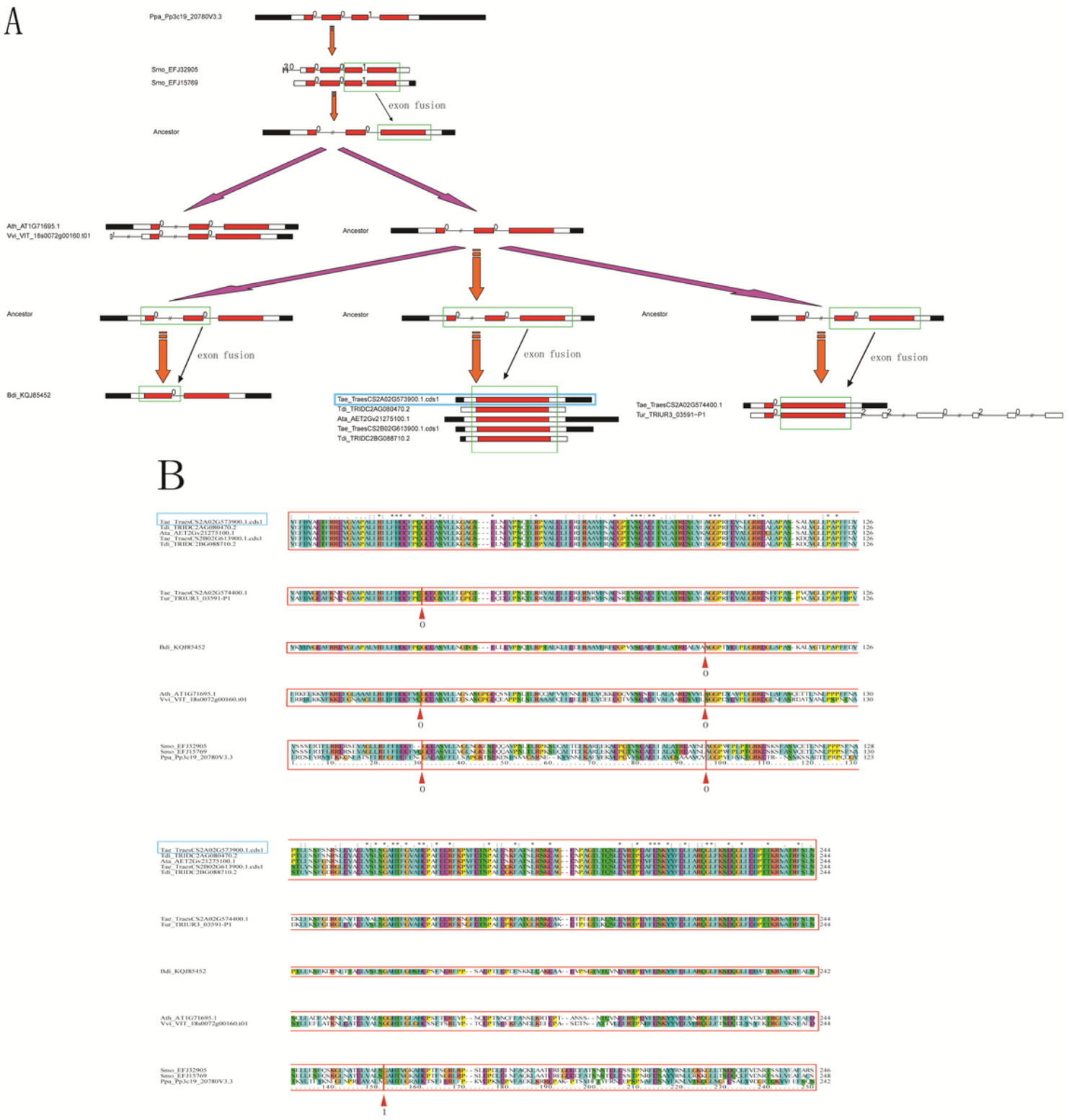

Figure 2

The evolutionary model of some subfamily VI PRXs including TaPRX-2A. (a) The exon fusions of some subfamily VI PRXs in evolution. (b) The alignment of some subfamily VI PRXs. The exon phases were circled by red boxes and arrows. Our investigated PRX (TraesCS2A02G573900.1.cds1) in T. aestivum was circled by cyan box.

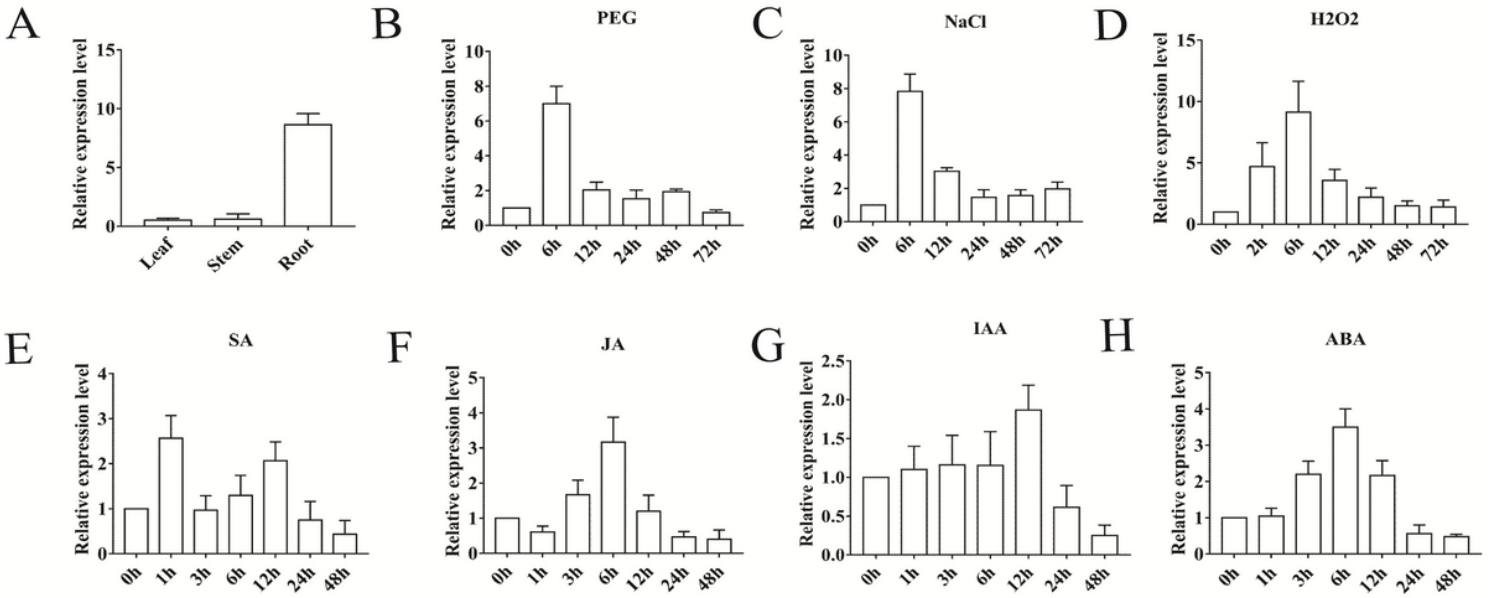

Figure 3

The transcript analysis of TaPRX-2A. (a) The TaPRX-2A expression levels in the leaves, roots, and stems. The TaPRX-2A expression was performed under different stress treatments, 20% (w/v) PEG 6000 (b), 200 mM NaCl (c), 10 mM H2O2 (d), 2 mM salicylic acid (SA) (e), 100 µM methyljasmonic acid (MeJA) (f), 100 µM indole-3-acetic acid (IAA) (g), and 100 mM abscisic acid (ABA) (h). The gene 18SrRNA was as an endogenous control. The gene relative expression was calculated by the cycle threshold (Ct) values using formula 2−ΔΔCT. The data are means ± SD calculated from three technical replicates.

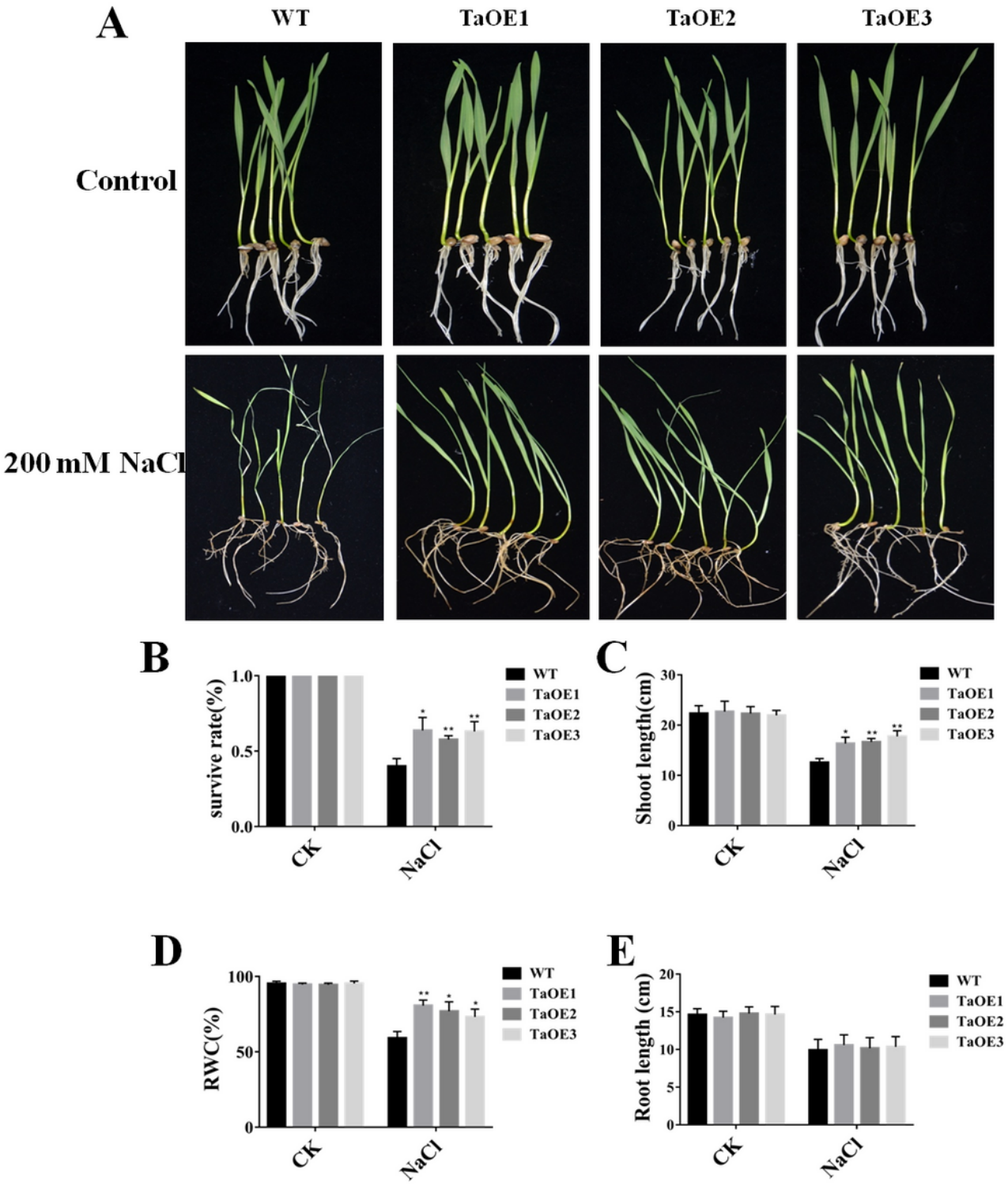

Figure 4

TaPRX-2A enhanced the salt tolerance of wheat. (a) Tolerance responses of the TaPRX-2A-overexpressing lines and wild-type wheat (the cultivar KN199) to salt stress. (b) The survival rates. (c) The shoot length and (d) the root length of TaPRX-2A-overexpressing and WT plants. (e) The relative water content of TaPRX-2A-overexpressing and WT plants. The data are means ± SD calculated from three technical

replicates. Asterisks, * and **, above each column indicate significant difference compared with WT plants (*P <0.05; **P < 0.01).

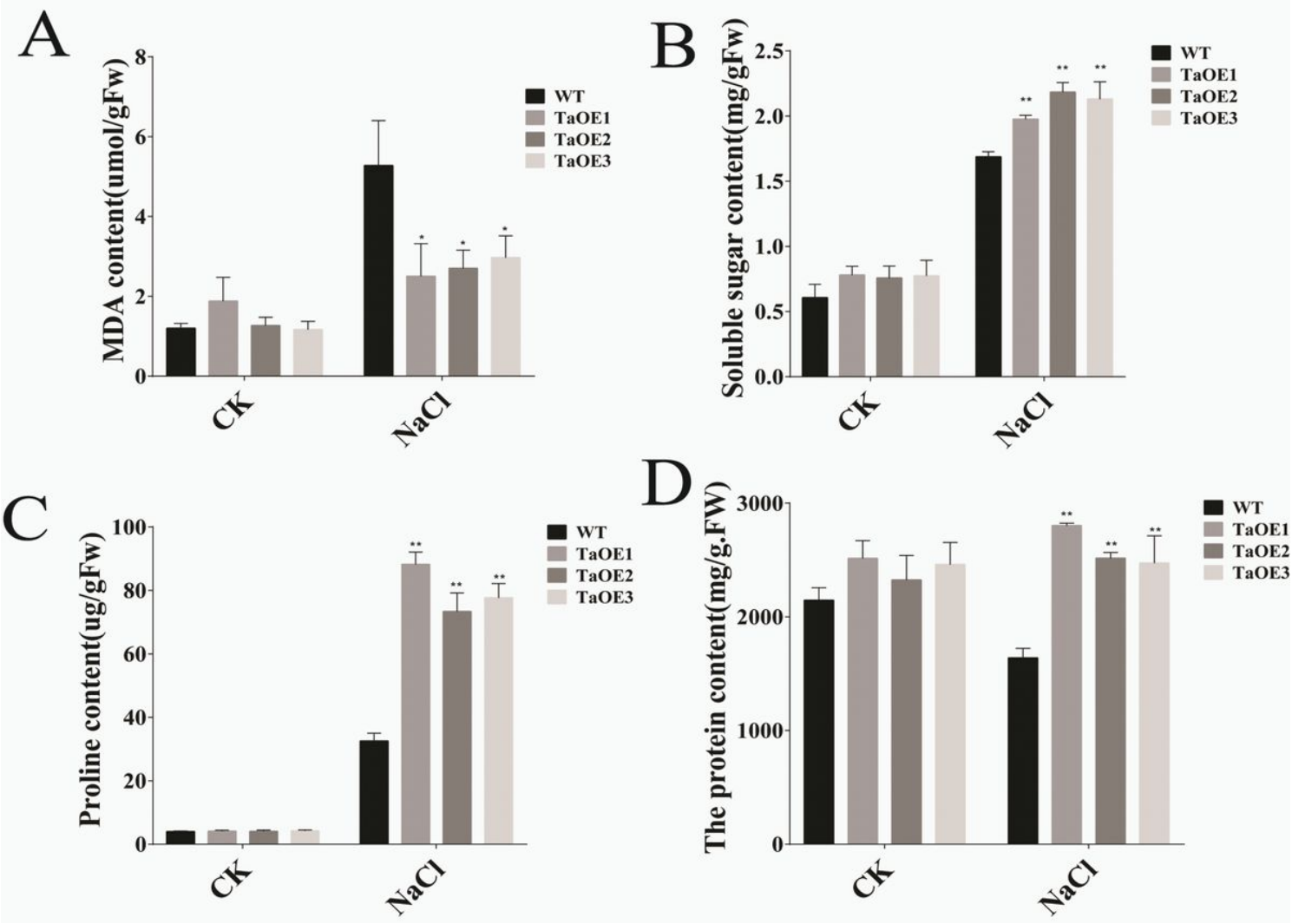

Figure 5

Physiological-biochemical indices between TaPRX-2A-overexpressing transgenic lines and WT plants. (a) MDA content. (b) Soluble sugar content. (c) Proline content. (d) The soluble protein content. The data are means ± SD calculated from three technical replicates. Asterisks, * and **, above each column indicate significant difference compared with WT plants (*P <0.05; **P < 0.01).

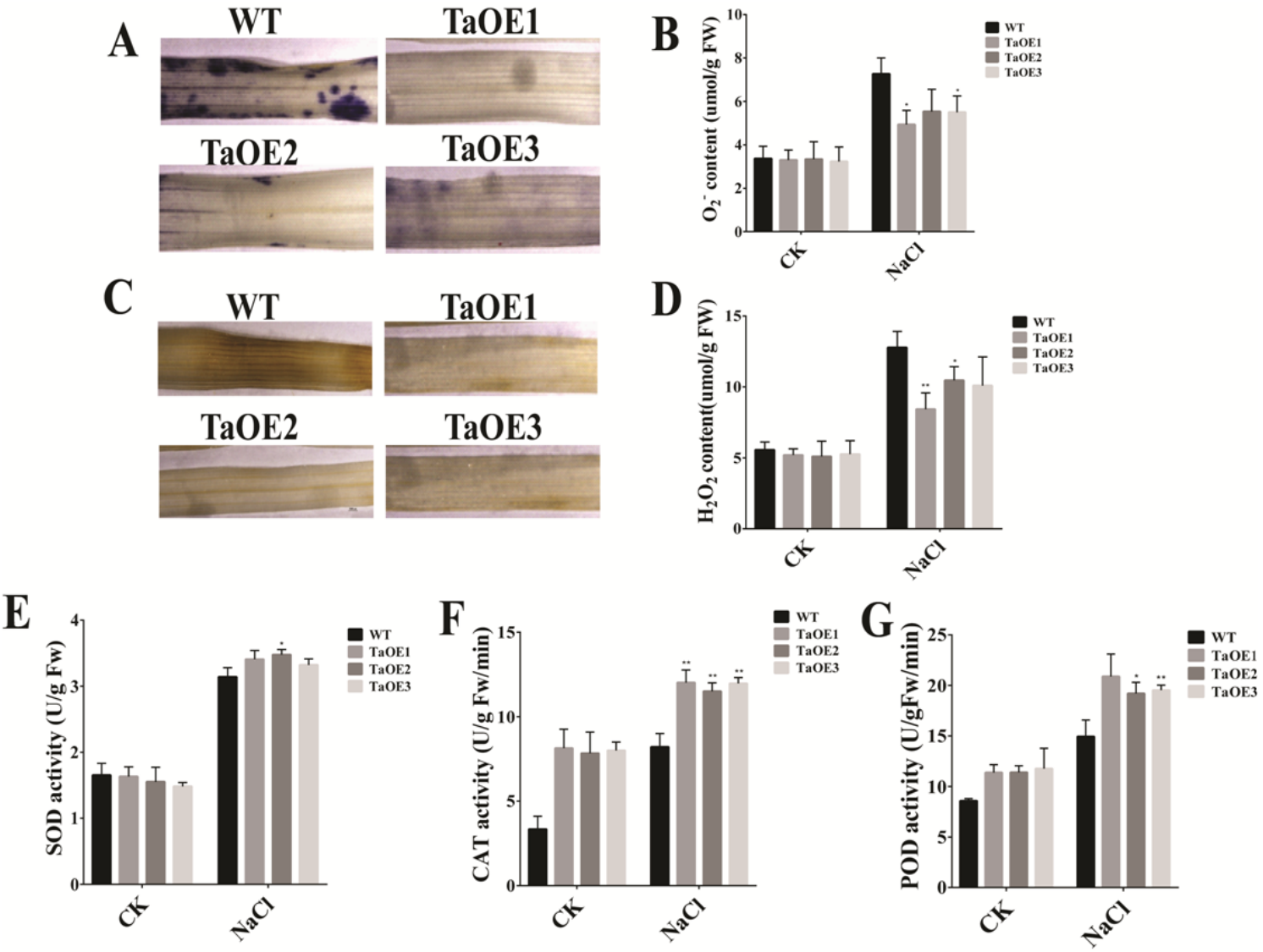

Figure 6

TaPRX-2A confers ROS-scavenging capacity by improving antioxidant enzymes activities. (a) Tissue localization of O2- accumulation. (b) O2- content. (c) Tissue localization of H2O2 generation. (d) H2O2 content. (e) SOD activity. (f) CAT activity. (g) POD activity. The data are means ± SD calculated from three technical replicates. Asterisks, * and **, above each column indicate significant difference compared with WT plants (*P <0.05; **P < 0.01).

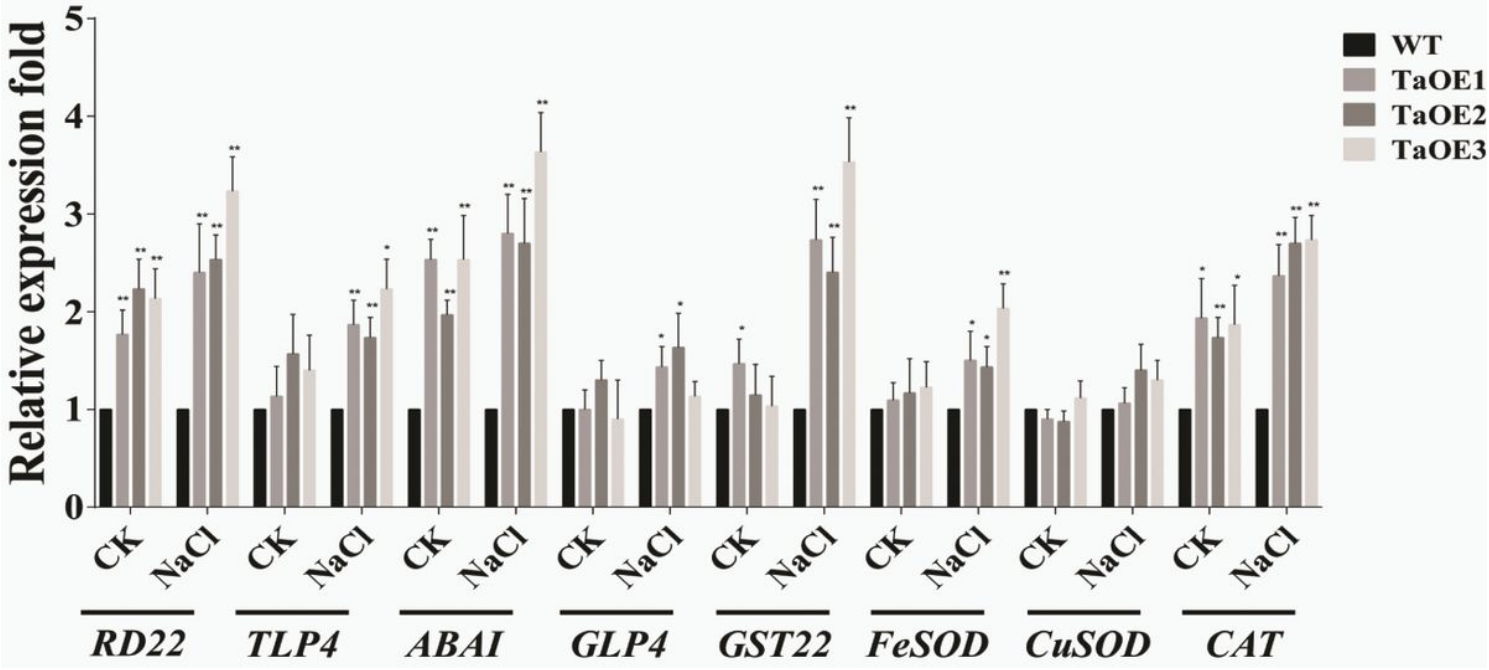

Figure 7

Analysis of transcript profile of some stress-related genes. (a–i) The stress-related genes expression level in TaPRX-2A-overexpressing transgenic lines and WT plants under salt stress by qRT-PCR. The gene 18SrRNA was as an endogenous control. Each treatment had three independent biological repeats. The gene relative expression was calculated by the cycle threshold (Ct) values using formula $2-\Delta\Delta CT$. The data are means ± SD calculated from three technical replicates. Asterisks, * and **, above each column indicate significant difference compared with WT plants (*P <0.05; **P < 0.01).

Supplementary Files

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