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Growth Hormone-like Activities of Organophosphorus Flame Retardants (OPFRs) in growth of Rice (*Oryza sativa* L.)

Qian Bai (s20190899@xs.ustb.edu.cn)
USTB: University of Science and Technology Beijing https://orcid.org/0000-0002-0113-7725
Haiou Wang
USTB: University of Science and Technology Beijing
Wenxiao Zhang
USTB: University of Science and Technology Beijing
Bokai Jiang
USTB: University of Science and Technology Beijing
Yuqing Ma
USTB: University of Science and Technology Beijing
Yuetong Li
USTB: University of Science and Technology Beijing
Hanbo Shi
USTB: University of Science and Technology Beijing

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Abstract

To identify the toxic effect of Organophosphorus Flame Retardants (OPFRs) on plants, six different OPFRs involved in three concentrations (50 μg/L, 100 μg/L and 200 μg/L) were selected to estimate their toxicity to rice growth. The seed germination, seedling growth, OPFRs accumulation and antioxidative defense system were investigated in rice. The results showed that all of OPFRs inhibited the germination of seeds. OPFRs were accumulated in roots and shoots, and the accumulations of OPFRs in roots were higher than in shoots. However, the hormone-like effect of all six OPFRs was found on plants. The growth of seedlings was irrigated by 50 μg/L, 100 μg/L and 200 μg/L OPFRs in our experiment according to fresh weights and lengths of seedlings. And then malondialdehyde (MDA) and antioxidative defense system were impressed after OPFRs exposed 21 d. According to gene expressions of seven antioxidative enzymes at 14 d, most of enzyme expressions were updated to alleviated reactive oxygen species (ROS) by OPFRs exposure. Tris (1, 3-dichloro-2-propyl) phosphate (TDCPP) showed the strongest oxidative toxicity to plants among all of OPFRs. During the early 14 d period, the antioxidant enzymes could play important role in detoxification process. And after 21 d, antioxidants ascorbate (AsA) in roots and glutathione (GSH) in shoots could take over antioxidant enzymes against to OPFRs toxicity.

1. Introduction

Organophosphorus Flame Retardants (OPFRs) were used, among their other uses as plasticizers or lubricants, as a replacement for brominated flame retardants to maintain fire safety standards after their phase-out (Chupeau et al. 2020). The total annual global consumption of OPFRs has continued increasing in recent years. In Europe, the total consumption of flame retardants in 2015 was 498,000 metric tons, of which 18% were phosphorus flame retardants, representing 89,640 metric tons (Chupeau et al. 2020). OPFRs can be released into the environment through various processes, such as production, use, and improper disposal (Wei et al. 2015). OPFRs have been detected in various environments and organism, such as indoor dust, air, water, soil, sediment, algae animals and plants (Chen et al. 2020b; Hou et al. 2016; Lee et al. 2018; Ma et al. 2021; Wang et al. 2018). The widespread existence of OPFRs has led to concerns regarding the biological potential for toxicity and risks to ecosystem.

Some researchers found that OPFRs had many adverse effects on organism, mainly endocrine oxidative toxicity, interference, genotoxicity, immunotoxicity, cytotoxicity, reproductive toxicity, developmental toxicity, neurotoxicity and carcinogenicity (Chandra Yadav and Devi 2019; Chen et al. 2020a; Chen et al. 2019; Lee et al. 2020; Ren et al. 2019; Yu et al. 2019). But the more toxicity effect of OPFRs on organisms is still an issue that deserves much attention, especially in plants. Tris (1, 3-dichloro-2-propyl) phosphate (TDCPP) caused morphological damage and growth inhibition of *Phaeodactylum tricornutum* and a decline in pigments and photosynthetic activity was observed, indicating the occurrence of photosynthesis inhibition (Liu et al. 2020). However, few studies have investigated toxicity of OPFRs in plants. Thus, it becomes a necessity to understand how crop plants cope with OPFRs.

Plant growth, development, and response to environmental stress require the judicious balance of reactive oxygen species (ROS) (Wu et al. 2017). In animal cells, OPFRs exposure has been shown to cause oxidative damage (Chen et al. 2015; Chen et al. 2019; Li et al. 2017). It was still unknown whether or not oxidative stress induced by OPFRs could occur in plants as well as animals. Plants are stressed by the external environment, and they can generate ROS causing oxidative damages to plant development. ROS are transiently produced, they act as signal molecules, which are key mediators of plant defense responses. Plants can defend themselves against oxidative damage induced by stress through an efficient non-enzymatic and enzymatic antioxidant defense system to scavenge overproduced ROS. Non-enzymatic antioxidants include glutathione (GSH), ascorbate (AsA), carotenoids, or tocopherols. Enzymatic scavenging mechanisms include ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT) and so on (Zhang et al. 2020).

Ascorbate-glutathione (AsA-GSH) cycle is an important system for removing hydrogen peroxide (H_2O_2). It consists of the enzymes APX, dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR) and GR (Yao et al. 2021). AsA participates in the regulation of programmed cell death, flower senescence, and response against pathogen attack, and in the protection of plants exposed to UV, heat, high light intensity and pollutants (Zhang et al. 2020). APX reduces H_2O_2 to water through the oxidation of AsA, which is subsequently reduced by sequential reactions carried out by the enzymes MDHR and DHAR using GSH. GSH is involved in various biological process such as detoxification of xenobiotics and heavy metals, storage and transport of reduced sulfur, regulation of nuclear and plastid gene expression, and pathogen resistance, among others. Finally, GR regenerates GSH from its oxidized form, glutathione disulfide (GSSG) allowing the cycle to continue (Cheng et al. 2020). GSH can protect the protein thiol groups of irreversible inactivation by oxidation and can also regulate the activity in a positive or negative way (Wu et al. 2017).

Therefore, the aim of the present study is to reveal the effects of OPFRs on the growth and detoxification mechanism of plants through the response of non-enzymatic and enzymatic antioxidant defense system.

2. Materials And Methods

2.1. Chemicals

Tri-n-butyl phosphate (TBP), Tris (2-butoxyethyl) phosphate (TBEP), Tris-phenyl phosphate (TPHP), p-Cresyl diphenyl phosphate (CDP), Tris (2-chloroethyl) phosphate (TCEP) and Tris (1, 3-dichloro-2-propyl) phosphate (TDCPP) were used for the plant culture were of analytical grade. All chemicals used for gas chromatography-mass spectrometry (GC-MS) were of chromatographic grade. Characteristics of OPFRs were partly presented in Table 1.

2.2. Growth of plants

Indica Rice (*Oryza sativa L.*) varieties of seeds were rinsed with 5% sodium hypochlorite (20 min) and deionized water, and then cultured for 4 d in the dark at 28°C incubator to germinate. The rate of

germination was recorded. Transfer to a Petri dish with 2 layers of filter paper, and obtain a seedling by dark culture in a constant temperature incubator at 28°C for 7 d.

Rice seedlings with similar growth status were selected, and no significant difference between seedlings was observed before OPFRs exposed ($P > 0.05$). The rice seedlings were divided into four groups and cultured in Hoagland's nutrient solution with 0 (CK)), 50 μg/L, 100 μg/L, and 200 μg/L of six compounds including to TDCPP, TCEP, TBP, TBEP, TPHP, CDP, respectively. The 6 different exposure solutions were made by adding 6 different OPFRs standard solutions dissolved in methanol to Hoagland's nutrient solution, the volume of methanol was less than 1‰ (v/v). The factual concentrations of six OPFRs exposed solution were measured by GC-MS, respectively. The factual concentrations of six OPFRs exposed solution and scalar recovery were presented in Table S2 and S3. Chromatogram and calibration curve of GC-MS detect results were presented in Fig.S1. Thus, 50 μg/L, 100 μg/L, and 200 μg/L concentrations for all OPFRs were used in all figures, as considering the convenience of drawing and readability of the figures. After OPFRs exposure for 21 days, length, fresh weight, OPFRs contents, and antioxidative system in shoots and roots were measured. Furthermore, gene transcript of 14 d seedlings' shoots and roots were determined under 100 μg/L six compounds, respectively.

2.3. Analysis of OPFRs contents by GC-MS

Rice seedlings were separated into roots and shoots, weighed, and finely chopped, 5 ml of ethyl acetate was added into plant tissues and OPFRs was extracted by ultrasound for 20 min, shacked for 10 min, centrifuged at 4000 r/min for 5 min. Repeated and extract 3 times with ethyl acetate, and combined all extracted supernatant to obtain 15 mL. Then samples were purified by QuEChERS method (Liu et al. 2021). Add 75 mg Florisil to 15 mL extract, vortex for 3 min, then centrifuged at 10,000 r/min for 3 min, spin-evaporate the supernatant to dryness, reconstitute it with a small amount of ethyl acetate, The solution was syringe filtered through a 0.22 μm nylon, and the volume was adjusted to 1mL.

The concentrations of OPFRs were analyzed by Shimadzu GC-MS (Shimadzu, Japan). OPFRs were separated with an SH-Rxi-1ms column (30 m × 0.25 mm × 0.25 μm) (Shimadzu) and analyzed by GC-MS 2010 Plus (Shimadzu). The MS system was operated in selected ion monitoring (SIM) mode under EI ionization for quantitation. Helium was used as the carrier gas at a flow rate of 1.58 mL/min. The oven temperature program was as follows: 0 min at 60°C, first ramp at 25°C/min to 190°C, second ramp at 10°C/ min to 300°C (8 min hold). The injected volume was 1 μL, and the injection-port temperature was 290°C in the splitless mode. The electron energy was set at 70 eV, and the ion source temperature was 230°C.

2.4. Effects of OPFRs on soluble protein content and malondialdehyde (MDA) content

The soluble protein content was determined by Coomassie blue staining (Jędrzejuk et al. 2018).

2 mL of the extract was added to 2 mL of 0.5 % thiobarbituric acid, mixed in a water bath at 100°C for 20 min, centrifuged at 3000 r/min for 5 min, and the supernatant was taken for the absorption values under

450 nm, 532 nm, and 600 nm, and calculated according to the formula.

c (μmol / L) = 6.45 × (OD532 - OD600) − 0.56 × OD450

MDA content = (c × V1 × V2) / (Vs × m × 1000)

2.5. Assays of antioxidative system

Fresh shoots or roots material (1 g) was homogenized in a pre-cooled mortar with a pestle in the presence of 50 mM Na-phosphate (pH 7.8) buffer containing 1 % (w/v) soluble polyvinylpyrrolidone. Then, the homogenate was centrifuged at 10,000 r/min for 15 min at 4°C. The aliquot was used for the activity assay of enzymes.

For the enzymatic assays related to ascorbate-glutathione-tocopherol triad enzymes such as ascorbate peroxidase (APX) (EC 1.11.1.11), dehydroascorbate reductase (DHAR) (EC 1.8.5.1), monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4) and glutathione reductase (GR) (EC 1.6.4.2) were measured by methods as described earlier (Wang et al. 2013). Catalase (CAT) (EC 1.11.1.6) activity was determined by measuring the disappearance of hydrogen peroxide (H_2O_2), at 240 nm for 60 s. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was appraised by determining the ability of SOD to inhibit the photochemical inhibition of nitroblue tetrazolium (NBT) and it was expressed per unit basis and denoted as the amount of enzyme required to reduce the quantity of cytochrome c by 50%. The peroxidase (POD) (EC 1.11.1.7) activity was determined by the rise in optical density at 470 nm due to guaiacol oxidation by H_2O_2 (Jędrzejuk et al. 2018).

Ascorbate (AsA), dehydroascorbate (DHA) and total ascorbate (AsA + DHA) were measured using L-AsA as standard. For (AsA + DHA) analysis, tissue was grounded in trichloroacetic acid (TCA) (5% w/v) and suitable aliquots of supernatant were added to 0.05 M potassium phosphate buffer (pH 7.4) containing 3.0 mM ethylene diamine tetraacetic acid (EDTA) and 1.0 mM dithiothreitol (DTT). N-ethylmaleimide (NEM) (0.1 ml), 0.61 M TCA, 0.8 M orthophosphoric acid and α, α'-bipyridyl were added after 10 min incubation. The assay mixture was incubated for 1 h at 40°C and absorbance was recorded at 525 nm after addition of ferric chloride ($FeCl_3$) (Wang et al. 2013).

2.6. Transcript analysis of enzymatic antioxidants

The quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed for *OsAPX*, *OsDHAR*, Os*MDHAR*, *OsGR*, *OsCAT*, *OsSOD* and Os*POD* with their specific primers (in Table S1). Total ribonucleic acid (RNA) was isolated from treated rice callus, using kit (Biomega, China) following the manufacturer’s protocol. 10 μL of total RNA was used for reverse transcription with 5000 U of Moloney murine leukemia virus reverse transcriptase (M-MLV RT) (Takara, China) in the presence of oligo (dT) 18 in a 60 μL reaction mixture. First-strand complementary deoxyribonucleic acid (cDNA) was synthesized from 1 μg of total RNA, using the PrimeScript RT reagent Kit with gDNA Eraser (Takara, China).

Diluted cDNAs were used as template in 20 μl PCR reactions containing 2 μL of cDNA, 0.6 μL forward and reverse primers (in supplement Table 1), and 10 μL SYBR Premix Ex Taq (Takara, China). Real-time quantitative PCR was conducted in MiniOption Real-Time PCR System (Takara, China) following the manufacturer's instructions. Three technical trails were performed in each experiment. Parallel reactions to amplify actin were used to normalize the amount of template. the relative expression was calculated with 2-ΔΔCt (Hao et al. 2020).

2.7. Statistical analysis

The results were expressed as mean ± standard deviation (SD). Statistical analyses were performed with Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 7.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Germination rate of rice seeds under OPFRs' exposure

Seed germination rates and hydrogen ion concentration (pH) in culture media were shown by OPFRs (TBP, TBEP, TPHP, CDP, TCEP and TDCPP) exposed in Fig. 1. The different control groups were grown in different media including to no OPFRs (CK), three concentrations (50 μg/L, 100 μg/L and 200 μg/L) of potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4), respectively. Slight differences were observed between CK and other groups. Our study indicated that the type and dose of OPFRs compounds could affect complicatedly the germination of rice seeds. Compared with CK, all six kinds of OPFRs inhibited the germination of rice seeds. However, the effects of OPFRs' doses on the germination rate exhibited no regular changes. In general, the strongest inhibited rate was observed in middle concentration OPFRs treatments. In detail, the turn of inhibited rate was shown: in the lowest does (50 μg/L), TBP > TPHP > CDP > TDCPP > TBEP > TCEP; in the middle does (100 μg/L), TBEP > TBP > TCEP > TDCPP > TPHP > CDP; in the highest does (200 μg/L), TBP > CDP > TDCPP > TCEP > TBEP > TPHP. pH of TBP showed significantly negative effect on germination rate among all compound. TBP media's pH values were the highest than other OPFRs compounds and CK (6.47) media, and even high than 8.0 (50 μg/L). The changes of KH_2PO_4 and K_2HPO_4 treated groups were shown similar with OPFRs.

3.2. Growth of rice seedlings under OPFRs' exposure

The growth of rice seedlings was illustrated in Fig. 2 and Fig. S3. In general, the growth of seedlings was improved by OPFRs exposure, except the growth of seedling shoots. After 21 d of OPFRs exposure, root length, shoot length, root weight, shoot weight and total weight of rice under the stress of six pollutants were increased more rapidly than CK. In detail, compared with CK, the length of seedlings' roots was provoked from 6.25–160.71% by OPFRs exposure, except no obvious increase of those seedlings in addition to TBP (50 μg/L), CDP (50 μg/L, 200 μg/L), TCEP (50 μg/L, 100 μg/L), TDCPP (50 μg/L, 200 μg/L). Similar changes of shoots length were observed under OPFRs exposure, except TBP (100 μg/L), TBEP (100 μg/L), TPHP (200 μg/L), CDP (50 μg/L), TCEP (100 μg/L). Furthermore, the longest length of

roots and shoots were found at TDCPP (50 μg/L) and CDP (200 μg/L) exposure, respectively. The fresh weight of roots in seedlings irritated from 11.74–142.3% by OPFRs ($P > 0.05$), except for no significance changes in those groups in addition to TBP (200 μg/L), TPHP (200 μg/L), CDP (50 μg/L, 200 μg/L), TCEP (100 μg/L). In particular TBP (50 μg/L), CDP (50 μg/L, 200 μg/L), TCEP (50 μg/L), the fresh weight of shoots in seedlings were also increased significantly from 21.30–103.15% ($P > 0.05$). Among six compounds, the effect of CDP exposure was strongest on the fresh weight of roots and shoots.

3.3. Determination of OPFRs concentrations in rice seedlings under OPFRs' exposure

In the present experiment, the OPFRs' contents of roots and shoots in rice seedlings were measured by GC-MS after 21 d cultivation (Fig. 3). TBP, TBEP, TPHP, CDP, TCEP and TDCPP contents in roots and shoots increased with their dose of media. In detail, the turn of increased in roots were shown: in the lowest does (50 μg/L), TDCPP > TCEP > TBEP > TPHP > CDP > TBP; in the middle does (100 μg/L), TCEP > TDCPP > TBEP > CDP > TPHP > TBP; in the highest does (200 μg/L), TDCPP > TCEP > CDP > TBEP > TPHP > TBP. The turn of increased in shoots were shown: in the lowest does (50 μg/L), TCEP > TBEP > TDCPP > TPHP > CDP > TBP; in the middle does (100 μg/L), TCEP > TBEP > TDCPP > CDP > TPHP > TBP; in the highest does (200 μg/L), TCEP > TBEP > TDCPP > CDP > TBP > TPHP. The OPFRs concentrations under OPFRs' exposure were detected from 0.042 (μg/g) to 1.873 (μg/g) in roots and 0.011 (μg/g) to 5.375 (μg/g) in shoots by OPFRs exposure. Furthermore, the highest concentration was found at TCEP (50 μg/L, 100 μg/L) and TDCPP (200 μg/L) exposure in roots and TCEP (50 μg/L, 100 μg/L, 200 μg/L) in shoots. In addition, the accumulations of three concentrations of TBP, TPHP, CDP and TDCPP in roots were more than that in shoots. The accumulations of TBEP and TCEP in shoots were higher in roots.

3.4. Contents of malondialdehyde (MDA) and soluble protein under OPFRs' exposure

From the contents of MDA of rice roots and shoots in Fig. 4a and b, compared with CK, the contents of MDA were decreased by OPFRs' exposure, no including those groups in addition to TBEP (50 μg/L), TDCPP (200 μg/L). And MDA contents of roots in the group with TDCPP (100 μg/L) treatment were increased. TBP (50 μg/L) induced the decrease of MDA contents in rice roots to the lowest level. In shoots, except TBP (100 μg/L), the effects of OPFRs on the contents of MDA were pressed compared with CK. And CDP (50 μg/L) treatment reduced strongest the contents of MDA in shoots.

The contents of soluble protein in roots and shoots were illustrated with or without OPFRs exposure in Fig. 4c and d. After 21 d of treatments, the curve of soluble protein content was similar with MDA. The contents of soluble protein were reduced by OPFRs' exposure compared with CK, expect TPHP (50 μg/L). CDP (200 μg/L) exposure reduced the contents of soluble protein in rice roots to the lowest level. The negative effects of OPFRs on the contents of soluble protein in rice shoots were also observed. TCEP (50 μg/L) exposure restrained the contents of soluble protein in rice shoots to the lowest level.

3.5. Antioxidative enzyme system against oxidative stress induced by OPFRs

To detect oxidative stress induced by OPERs, the responses of ascorbate-glutathione (AsA-GSH) cycle and the antioxidative enzymes were investigated in Fig. 5 and Fig. S5. After 21 d of OPFRs' exposure, the activities of ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR) in rice roots and shoots were illustrated in Fig. 5. Compared with CK, the activities of APX and DHAR of both roots and shoots in rice seedlings were reduced under OPFRs' exposure, except roots under TPHP (50 μg/L) exposure. The response of MDHAR and GR activities were shown difference, depending on the different OPFRs and organs. In detail, the effects of OPFRs on the activities of APX in both roots and shoots were significant difference, compared with CK. The lowest activities of APX in rice roots and shoots was recorded at TCEP (200 μg/L) and CDP (200 μg/L) exposed groups, respectively. Compared with CK, the effects of OPFRs on the activities of DHAR in both roots and shoots were reduced. The lowest activities of DHAR in rice roots and shoots were recorded at CDP (200 μg/L) exposed groups. The effects of OPFRs on the activities of MDHAR in both roots and shoots were also significant difference, compared with CK. The activities of MDHAR in most groups' roots exposed OPFRs were restrained, except TBP and TBEP (100 μg/L). The lowest activities of DHAR in rice roots were recorded at CDP (200 μg/L) exposed groups. The activities of MDHAR in rice shoots were reduced by OPFRs, except TBEP (50 μg/L). The lowest activities of DHAR in rice shoots were recorded at TDCPP (50 μg/L) exposed groups. The effects of OPFRs on the activities of GR in both roots and shoots were also significant difference, compared with CK. The activities of GR in rice roots were inhibited by all six OPFRs. The lowest activities of GR in rice roots were recorded at TDCPP (200 μg/L) exposed groups. The activities of GR in most groups' shoots were increased, except the decrease of TPHP (100 μg/L), TCEP (50 and 200 μg/L) and TDCPP (200 μg/L).

After 21 d OPFRs' exposure, the activities of catalase (CAT) and superoxide dismutase (SOD) in rice roots and shoots were shown in Fig. S5. The effects of OPFRs on the activities of CAT in rice roots were significant impressed, except those in roots exposed by TBEP (200 μg/L) and TDCPP (100 μg/L). The lowest activities of CAT in rice roots were observed at TPHP (200 μg/L) exposed group. The effects of OPFRs on the activities of CAT in rice shoots were significant suppressed, except the roots exposed by TBP (100 μg/L). The lowest activities of CAT in rice shoots were observed at TPHP (100 μg/L) exposed group.

The activities of SOD in rice roots and shoots were affected differently by OPFRs' exposure. Compared with CK, the activities of SOD in rice roots under six pollutants were reduced. The lowest activities of SOD in rice roots and shoots were recorded at CDP (200 μg/L) and TCEP (50 μg/L) exposed groups, respectively. POD activity could not be detected in our experiment.

3.6. Contents of glutathione (GSH), AsA, dehydroascorbate (DHA) and AsA/DHA(A/D) and total ascorbate in rice under OPFRs' exposure

Contents of GSH, AsA, DHA and A/D and total ascorbate in rice roots and shoots were shown in Fig. 6 and Fig. S4. Contents of GSH, AsA, DHA and total ascorbate and the values of A/D in roots and shoots were inhibited by OPFRs exposure. The lowest contents of GSH in rice roots and shoots were recorded at CDP (200 μg/L) exposed groups. The lowest contents of AsA in rice roots and shoots were recorded at TCEP (100 μg/L) and CDP (50 μg/L) exposed groups, respectively. The changes of DHA were similar with AsA. The lowest contents of total ascorbate in rice roots and shoots were recorded at CDP (200 μg/L) exposed groups. The lowest values of A/D were recorded at TBEP (100 μg/L) in rice roots and TBEP (200 μg/L) in shoots exposed groups, respectively.

3.7. Transcript of six of antioxidative enzymes

It was very interesting that the most enzyme activities of AsA-GSH cycle were impressed after 21 d OPFRs exposed. The middle concentration of OPFRs group were chose as representatives to deeply observe their effect on antioxidative enzymes gene expression level in Fig. 7. Moreover, the treated period also was shorted to 14 d, to reveal the regulation response of enzymes to OPFRs during exposure.

Transcript of seven of antioxidative enzymes in roots and shoots exposed by six of OPFRs (100 μg/L) was illustrated (Fig. 7). Between roots and shoots, the 7 of gene expressions were difference. *OsAPX* expressions in shoots were stronger than in roots. *OsAPX* expressions in roots were irritated most strongly by TDCPP exposure, and *OsAPX* expressions in shoots were irritated most strongly by TBP exposure. In shoots, *OsDHAR* expressions were irritated by six OPFRs compounds. In roots, *OsDHAR* expressions were irritated by TBP, TBEP, CDP, TDCPP. *OsDHAR* expressions in roots among all enzymes were irritated most strongly by CDP exposure, and *OsDHAR* expressions in shoots among all enzymes were irritated most strongly by TBEP exposure. Both roots and shoots, *OsMDHAR* expressions were irritated by six OPFRs compounds. However, *OsMDHAR* expressions were stronger in shoots than roots. *OsMDHAR* expressions in roots and shoots among all enzymes were irritated most strongly by CDP exposure. *OsGR* expressions were stronger in roots than shoots. *OsGR* expressions in roots were irritated most strongly by TPHP exposure, and *OsGR* expressions in shoots were irritated most strongly by TDCPP exposure. *OsCAT* expressions were stronger in roots than shoots. *OsCAT* expressions in roots and shoots were irritated most strongly by CDP exposure. *OsSOD* expressions in roots and shoots were upregulated by other OPFRs, except for TDCPP exposure in roots and TPHP exposure in shoots. *OsSOD* expressions in roots were irritated most strongly by TPHP exposure, and *OsSOD* expressions in shoots were irritated most strongly by CDP exposure. Both roots and shoots, *OsPOD* expressions were irritated. *OsPOD* expression in roots were irritated most strongly by TPHP exposure, and *OsPOD* expressions in shoots were irritated most strongly by CDP exposure.

4. Discussion

4.1. Germination rate of rice seeds under OPFRs

Seed germination and seedling establishment are the most sensitive stages in plant life cycle (Kabir et al. 2018). We aimed to find the toxicological impacts of six OPFRs compounds in seed germination rates of

rice. The results indicated that OPFRs compounds could slightly inhibited the germination of rice seeds. In Yun et al.' (2019) report, the germination rates of barley were also inhibited with increasing concentrations of organic pollutant Nitro-PAHs. The dose of OPFRs were not relative to the germination rates ($P < 0.05$). The coats of rice seeds could protect seeds from OPFRs toxicity due to OPFRs defuncting by coats barrier (Yun et al. 2019). It was very interesting that the middle concentrations (100 μg/L) of OPFRs were found more significantly negative effect on germination, except for CDP exposure. Seeds' germination is not so sensitive as an indicator of organic contamination due to protect role of seed coat, although it is an important index for estimating the physiological toxicity of plant. TBP was observed that more significant inhibition of seed germination than other compounds. pH values of three TBP treatments were the highest among all compounds. pH value could be an important reason that the germination rates were reduced. Rice likes to grow under weakly acidic (pH 5.2–6.8) conditions, which is helpful to plant growth. This reaction of seed germinations to pH variations could be due to the inhibitory effect of acidity and basicity on the catalysts involved in the germination process. Acid pH could dissolve the seed coat and have effects (Laghmouchi et al. 2017). Although pH of K_2HPO_4 treatments also higher than 7.0, the seedlings under K_2HPO_4 treated were similar to CK. It meant that inhibition of germinations might depend on not only pH but also OPFRs toxicity.

4.2. Growth of rice seeds under OPFRs and OPFRs concentrations in rice seedlings

Our results showed that all six pollutants promoted the length, the fresh weight of roots and shoots, and total weight of rice seedlings. In 1982, Stebbing (1982) presented a "Hormesis" that the stimulating effect of pollutants on organisms after low doses. "Hormesis" considered that Low-dose pollutants stimulated plant growth. A reason was that low doses pollutants motivated nicotinamide adenine dinucleotide (NADH) oxidase activity and then promoted the rate of cell enlargement (Morré 2000). In our experiment, the used doses of six compounds were 50 μg/L, 100 μg/L, 200 μg/L belong to lower concentrations (Chen and Ma 2021), respectively, which could irritate the growth of plants attributed to hormesis effect. In Liu et al.'s report (2012), all OPFRs increased sex hormones and related major steroidogenic gene transcriptions in H295R cells, especially from 100 μg/L concentrations of OPFRs. This "Hormesis" phenomenon was the most obvious at 50 μg/L of six OPFRs compounds in rice's root length in our experiment.

From OPFRs concentrations in rice seedlings after 21 d exposure (Fig. 3), We could know that the accumulation of six OPFRs compounds increased with increasing dose. The concentrations of all OPFRs compounds could be detected in shoots of rice indicated that OPFRs taken up by roots were subsequently translocated to shoots. TBEP and TCEP accumulated more in the shoots due to TBEP and TCEP were more hydrophobic (Chen and Ma 2021). OPFRs with higher hydrophobicity were more easily taken up by roots, and OPFRs with lower hydrophobicity were more prone to be translocated (Wang et al. 2019). In Wan et al.'s report (2016), TBEP and TCEP were present in higher proportions in the aboveground tissues (mean 45% and 36%, respectively) than in roots (mean 34% and 25%, respectively).

The accumulation of OPFRs in rice roots and shoots lead to oxidative stress in rice, which made AsA-GSH cycle of rice to work.

4.3. Effect of OPFRs exposure on the response of antioxidant defense system

When exposed to environmental pollutants, the balance between endogenous and exogenous oxygen species (ROS) in organisms may be interfered, and the increased levels of ROS can subsequently induce oxidative stress and result in oxidative damage to organisms (Chen et al. 2018). Studying the changes of antioxidant defense system can assess toxicity of oxidative stress effectively.

Various environmental factors cause changes in plants metabolism. Prolonged or increased exposure to stress factor results in an imbalance between the generation of ROS and its antioxidant abilities, what in consequences can lead to plants death (Sharma et al. 2012). Malondialdehyde (MDA) is a good indicator of oxidative stress in plant. MDA induces changes in the structure of the cell membrane leading to its disintegration and uncoupling of phosphorylation in the mitochondria (Islam et al. 2009). Its concentration depends on the level of ROS in tissues. Usually, the higher concentration of MDA were occurred with the greater production of free radicals (Alicja Auriga 2018).

Under stress conditions, MDA was measured as a lipid peroxidation parameter, and its content was often used to explain the degree of peroxidation (Han et al. 2013). However, our result displayed that the contents of MDA in rice roots and shoots were decreased by the most of OPFRs' exposure. The decrease of MDA contents in OPFRs' treated plants except for TDCPP treatment, indicating that the oxidative degree of cell membrane damage was reduced under six pollutants exposure. The data of MDA was different with some reports in which OFPRs could induced the oxidative stress of cell membrane. The reasonable explain could be the different dose of exposure OPFRs between our experiment and other researchers. In our experiment, the dose of OPFRs were lower than those dose of compounds in other study (Chen et al. 2015). The oxidative stress induce by TCEP were found in Chen et al.'s(2015) work in that the exposed concentration were 100 and 300 mg/kg. In our experiment, TDCPP in low doses also could induce oxidative stress, in consistent with Chen et al.' work (2018). TDCPP exposure altered the ROS level and antioxidant defense system in zebrafish at 45.81 μg/L and 229.05 μg/L.

A reduction in the values of oxidative stress parameters MDA under OPFRs exposures as a result of the application of low dose's OPFRs may be a confirmation of the protective properties. The low dose of pollutants could stimulate the growth of plants and induced their resistance and the process of photosynthesis as the role of hormone - melatonin in plants (Kaya et al. 2019), which was supported by data of the plant growth in our experiments. Melatonin treated to plants were found to increase plant growth attributes, but reduce MDA. Functional implications of Melatonin in plants are suggested that could be involved in the coordination of photoperiodic responses and regulation of plant growth, its participation as a free radical scavenging agent and up regulator of certain protective enzymes in the senescent process. In our experiment, the hormone-like effect was also observed in OPFRs treated plants. Although OPFRs is a well-known like-hormone in animals (Liu et al. 2019), its role in the plant kingdom is

not clear enough. To understand fully to the effect of OPFRs, antioxidant synthesis, activation of associated enzymes in plants were investigated.

Ascorbate-glutathione (ASA-GSH) cycle system is one of the most abundant intracellular thiols in living aerobic cells and can protect cells against oxidative damage (Yao et al. 2021). Six pollutants exposure induced the decrease in glutathione (GSH) and ascorbate (AsA) content in rice roots and shoots. Based on the previous reports, the present study showed that OPFRs improved antioxidant defense system thereby consuming antioxidants ASA and GSH, wherein it may play a role in sustaining membrane stability by scavenging hydrogen peroxide (H_2O_2) and MDA. However, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) (in roots and shoots) and glutathione reductase (GR) (in roots) were depressed by OPFRs exposures compare to CK, indicating that the regeneration synthesis of AsA and GSH was deactivated. Dehydroascorbate (DHA), the oxidation product of AsA, was degraded due to the low activity of the AsA regeneration system. The decrease of AsA + DHA, DHA and AsA/DHA also maintained at a low level, indicating that AsA has been consumed in a large amount.

The activity of GR in shoots were irritated by OPFRs to response the decrease of GSH in shoots, indicating that thiols could reduce ROS toxic and eliminate lipid oxidization. AsA and GSH is necessary for AsA-GSH cycle system to maintain its activity and as substrate to clear ROS (Yao et al. 2021). The effect might be partly attributable to the increase in ROS production caused by six pollutants stress, which consumes large amounts of GSH and AsA. The effects of OPFRs on the AsA-GSH cycle were different between roots and shoots. Although AsA and GSH were reacted to OPFRs exposure in roots and shoots, their roles were different in different plant organs. AsA to roots and GSH to shoots could be a more important antioxidant, respectively. MDHAR showed relatively active among antioxidative enzymes in roots, while GR showed relatively active in shoots rather than roots. We also found that OPFRs pressed the activities of catalase (CAT) and superoxide dismutase (SOD). Thus, in the present study, OPFRs could induced a few of ROS production, only antioxidants could remove effectively ROS and kept cell membrane integrity (Fig. S5).

After applied OPFRs, oxidative stress was significantly decreased in plants as could be evidenced from depressed MDA level and lowered antioxidative enzymes activities. The present findings showed that low dose of OPFRs (50 µg/L, 100 µg/L and 200 µg/L) might act as melatonin to plants' antioxidative defense system in Kaya et al.' report (2019). Melatonin was known as a biopromoter because of its obvious physiological roles including inhibition of shoot senescence, improved root and shoot growth, improved mineral nutrition (Zhang et al. 2021). Furthermore, melatonin was found as a scavenger of hydrogen peroxide in reversing the overproduction of hydrogen peroxide in plants exposed to Cd stress (Tan et al. 2000; Tousi et al. 2020).

As well known, transcript of enzymes was earlier than protein translation. To elucidate the negative effect of OPFRs on 21 d's antioxidize defense system, the gene expressions of seven antioxidative enzymes were investigated at 14 d. And judging from the up-regulation of the seven enzymes gene expression, two

enzymatic systems including to ASA-GSH and antioxidant enzymes were activated during 14 d to 21 d of exposure to pollutants, resulting into the reduce of oxidative stress in plants. Therefore, the MDA contents in OPFRs exposure plants were lower than CK at 21 d, indicating that this oxidative damage by OPFRs has been alleviated, and then these enzyme activities were decreased to avoid imbalance of ROS. Compare to two enzymatic systems, the expressions of enzyme gene involved in AsA and GSH cycle in shoots were stronger, suggesting they played mainly role in reducing oxidative damage, especially AsA. Only GR enzyme in shoots activity was more active since 21 d (Fig. 5). In roots, both two enzymatic systems were activated to remove oxidative stress from 14 d to 21 d. Results showed that the control of oxidative damage in plants could be phased from Fig. 5 to Fig. 7. Before 21 d, all enzymes system involved in alleviate of oxidative stress induced by OPFRs. AsA in roots and GSH in shoots charged detoxification process since 21 d.

Compare to other five OPFRs compounds, TDCPP showed stronger oxidative stress as could be evidenced by the highest MDA and DHA level and the elevation of activity of DHAR. Moreover, the treatment of 200 μg/L TDCPP caused significant decrease in APX, GR activities in rice. This phenomenon can be explained as that high dose TDCPP induced too much ROS production which exceeded the capacity of the cellular antioxidant system in rice and causing damage to the enzyme. And in the expression of enzyme-related genes, all enzymes were expressed under TDCPP stress except SOD in roots and CAT in shoots. The increase in the activities of antioxidant enzymes observed in the 100 μg/L TDCPP exposure might contribute to the elimination of ROS induced by TDCPP.

5. Conclusions

This study systematically studied the effects of six OPFRs on seed germination, growth and antioxidative defense system in rice seedlings. The germination rate of rice seeds was inhibited after six OPFRs exposed with 3 different concentrations (50 μg/L, 100 μg/L, 200 μg/L). However, the three-low dose of OPFRs in our experiment promoted the increase of the biomass and the decrease of oxidative stress evidenced from the impress of MDA and antioxidative defense system. These phenomena suggested the response of hormone-like of six pollutants was exhibited in plants. The expressions of related seven enzyme genes also proved their hormone effect, which was the result of endogenous regulation. These responses could stimulate the growth of plants compared to the CK. Meanwhile, in the present study, the accumulation of OPFRs in plants was low and did not cause damage to plant growth. This hormone-like effect of OPFRs to plant was firstly found in our experiment, although it should be further investigated in the future. Our results provided a better understanding of the potential toxic effects of OPFRs to plants.

Declarations

Acknowledgement

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this manuscript, and manuscript is approved by all authors for publication.

Declaration of interest statement

The authors report no conflict of interest.

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Tables

Table 1 Information of the 6 kinds of OPFRs

Name	Abbreviation	CAS no.	Formulae
Tri-n-butyl phosphate	TBP	126-73-8	$C_{12}H_{27}O_4P$
Tris (2-butoxyethyl) phosphate	TBEP	78-51-3	$C_{18}H_{39}O_7P$
Tris-phenyl phosphate	TPHP	115-86-6	$C_{18}H_{15}O_4P$
p-Cresyl diphenyl phosphate	CDP	78-31-9	$C_{19}H_{17}O_4P$
Tris(2-chloroethyl) phosphate	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$
Tris (1,3-dichloro-2-propyl) phosphate	TDCPP	13674-87-8	$C_9H_{15}Cl_6O_4P$

(Hong et al. 2019; Pantelaki and Voutsa 2019; Zhang et al. 2020)

Figures

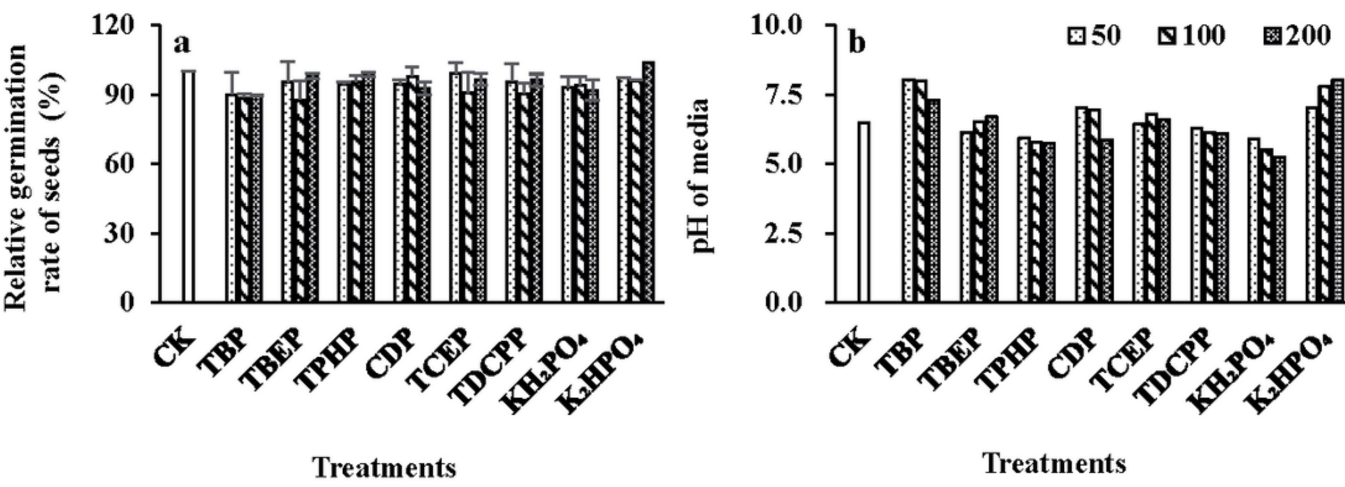

Figure 1

The relative germination rate of rice seeds after 4 days under different OPFRs treatments (a), The pH of different treated solutions (b)

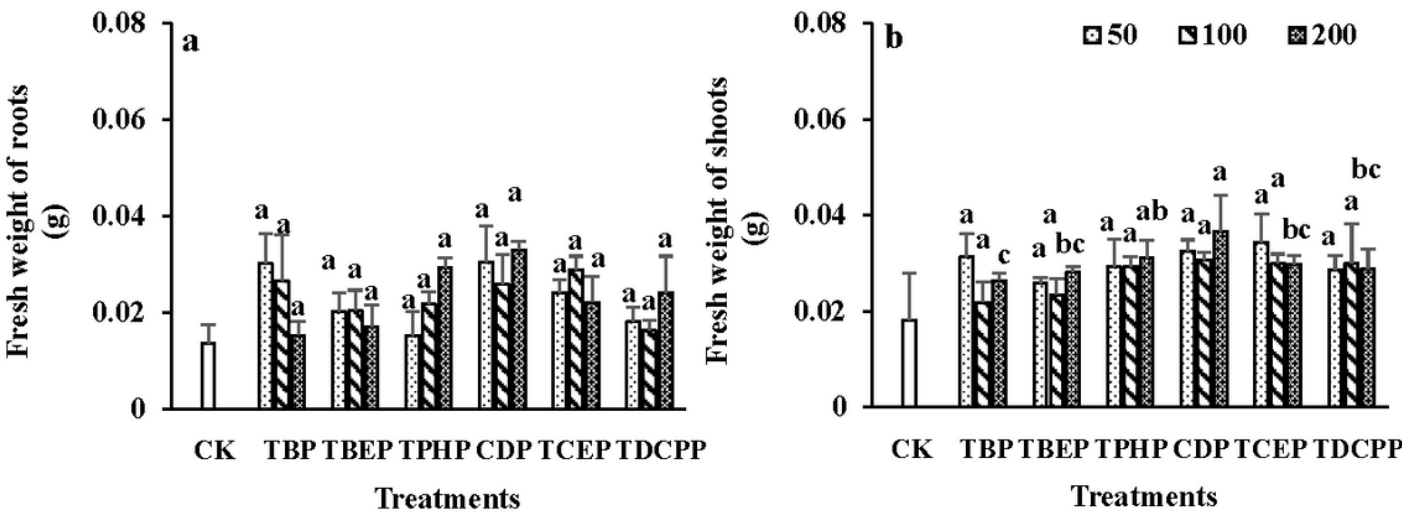

Figure 2

The growth of rice seedlings after 21 days under different OPFRs treatments after 21 days OPFRs exposure: Fresh weight of roots (a), Fresh weight of shoots (b)

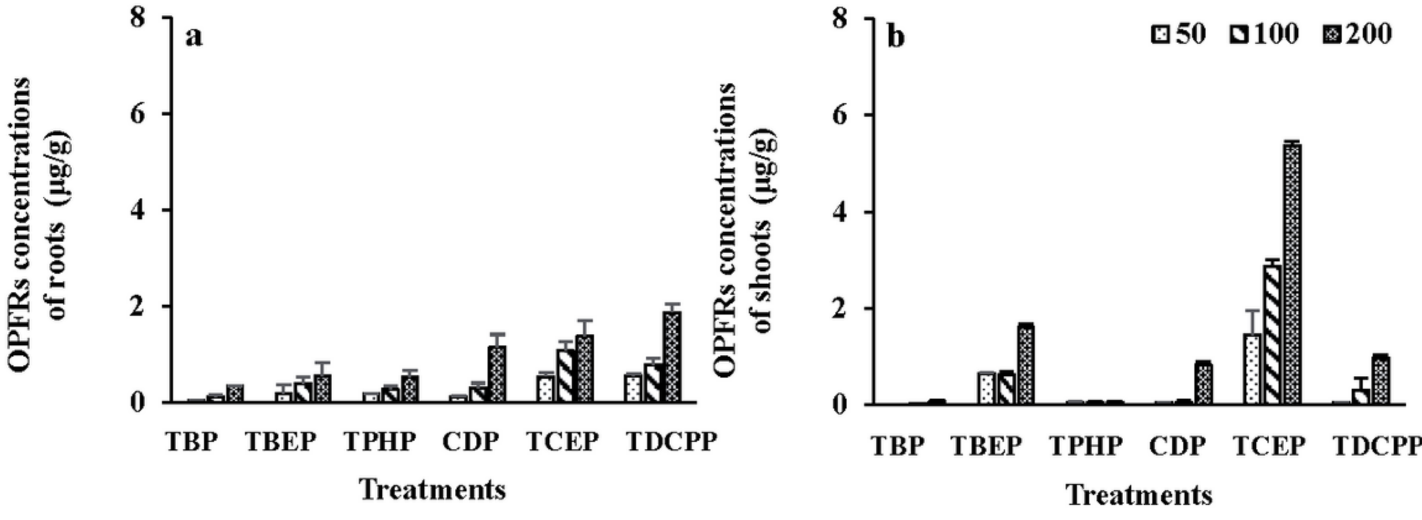

Figure 3

OPFRs concentrations in rice roots and shoots after 21 days under different OPFRs treatments: roots (a), shoots (b)

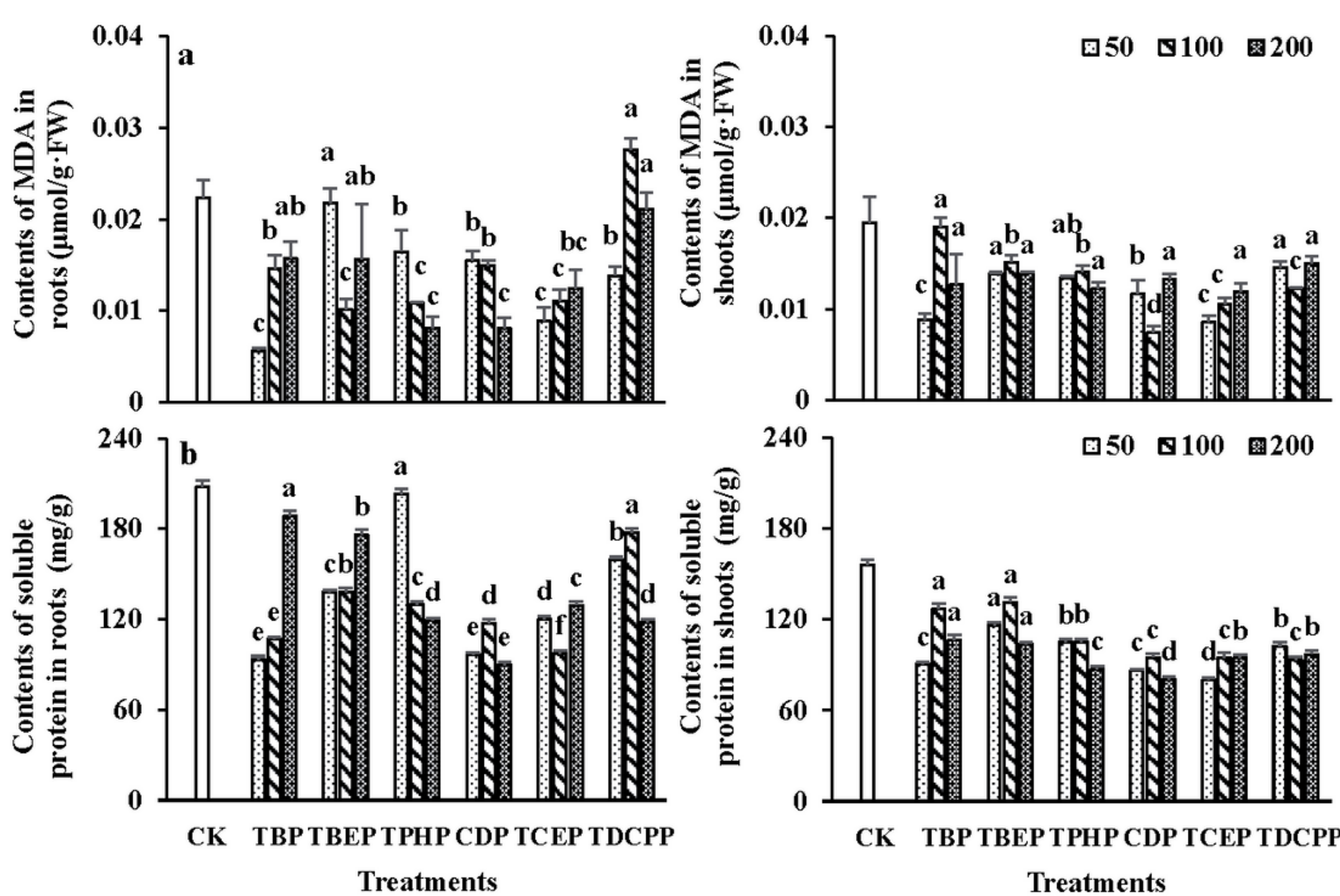

Figure 4

Contents of MDA (a) and soluble protein (b) in rice roots and shoots after 21 days under different OPFRs treatments

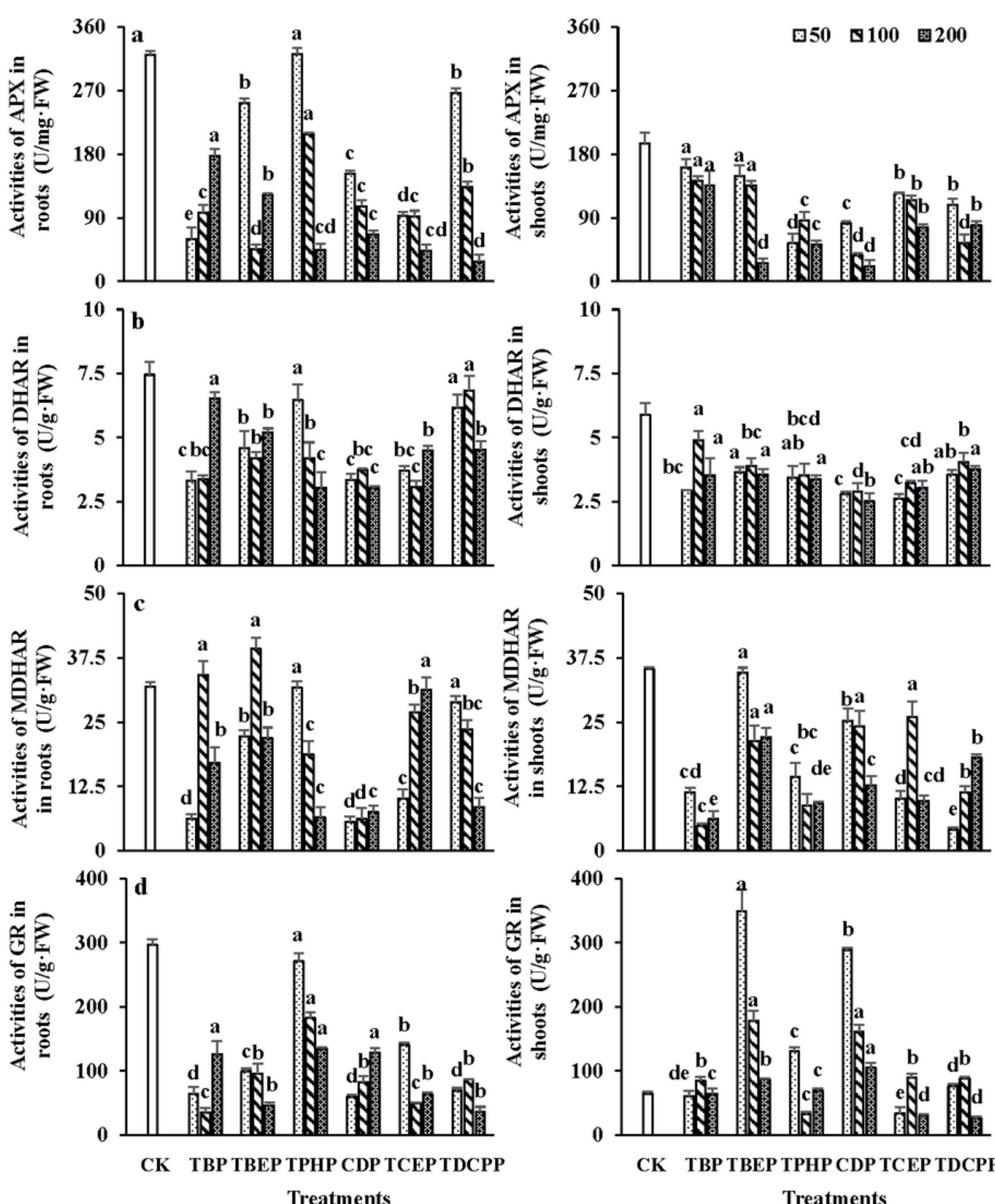

Figure 5

Activities of antioxidative enzymes in rice roots and shoots after 21 days under different OPFRs treatments: APX (a), DHAR (b), MDHAR (c), GR (d)

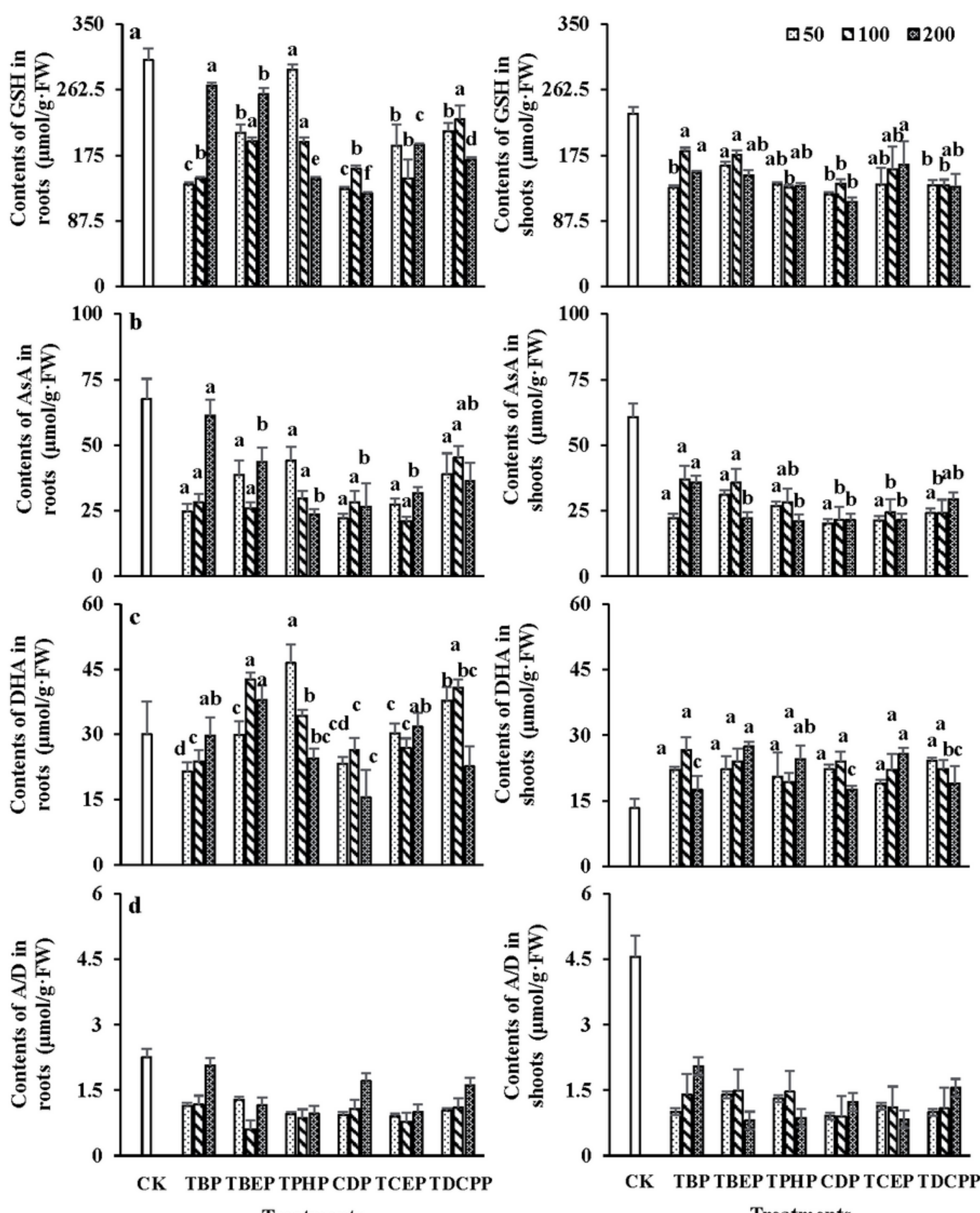

Figure 6

Contents of antioxidants in rice roots and shoots after 21 days under different OPFRs treatments: GSH (a), AsA (b), DHA (c), A/D (d)

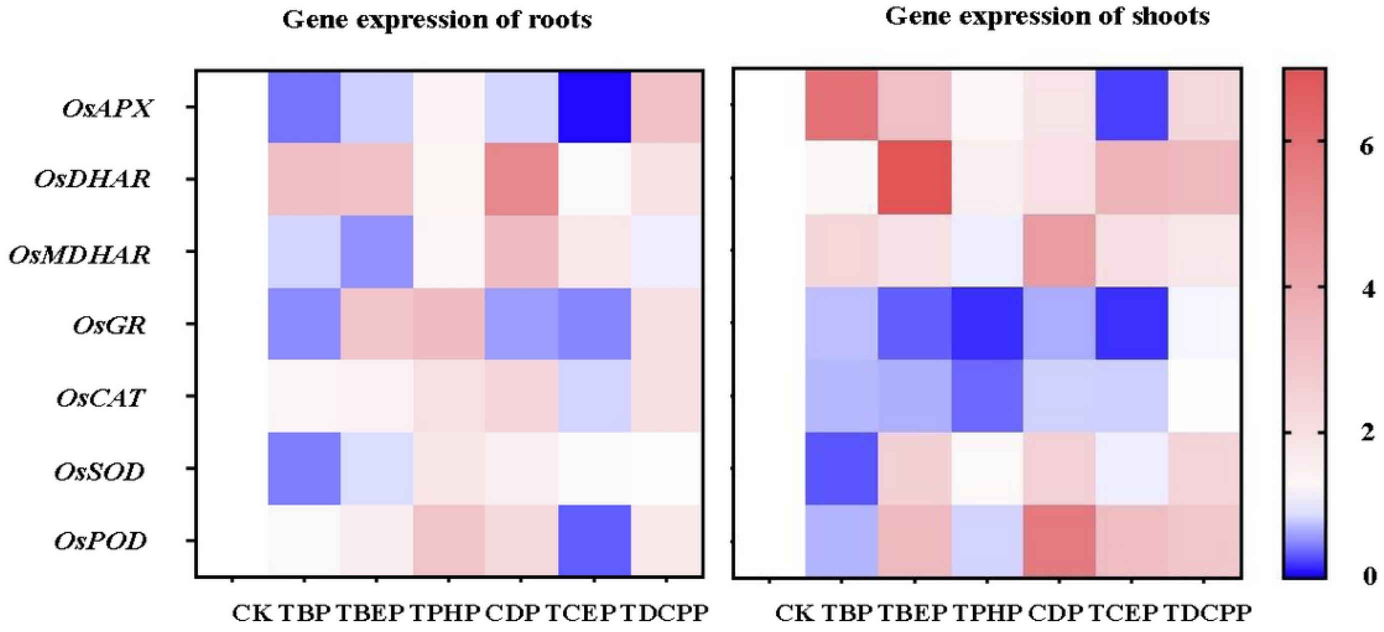

Figure 7

Heatmap of antioxidative enzymes in roots and shoots under different OPFRs treatments

Supplementary Files

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