Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Organophosphate esters uptake, translocation and accumulation in rice (*Oryza sativa* L.): Impacts of lipid transporters and chemical properties

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Fig. S5 OPEs concentrations of (A) OPEs-untreated and (B) OPEs-treated of rice seedlings in shoots and roots (μg/g) ($n \geq 3$).

Fig. S6 Apoplastic and symplast distributions of OPEs in rice roots ($n \geq 3$).

Experimental set up

Plant culture and exposure experiments

Nipponbare (*Oryza sativa* L.) rice seeds were obtained from the Hubei Academy of Agricultural Sciences. Prior to germination, seeds of similar size full grains were surface-sterilized in 5 % (v/v) sodium hypochlorite for 15 min, rinsed thoroughly with deionized water at least three times. Seeds germinated on petri dishes with 2 layers of moist filter paper by dark culture in a constant temperature incubator at 28 ℃ for 4 d. Then, each batch of 30 uniformly sized seedlings were transferred into glass beakers with 50.0 mL the ten-strength Hoagland nutrient solution for 4 d, and glass beakers were wrapped with aluminium foil. The seedlings were cultured in a growth chamber at a light level of 7.5 klux and a photoperiod of 14 h/d. All the filter paper and deionized water were autoclaved and all glassware was cleaned with methanol and ethyl acetate successively to prevent cross-contamination.

Standard solutions of the above 7 different OPEs dissolved in methanol were appended to the ten-strength Hoagland nutrient solution to make exposure solutions, respectively. The volume of methanol was less than 1‰ (v/v) and the OPEs' concentrations were 100.0 μg/L, which exceeded their environmentally relevant concentration. The seedlings were exposed to 50.0 mL the ten-strength Hoagland nutrient solution with 100.0 μg/L OPE exposure solution for 7 d, respectively, and change exposure solution daily. The seedlings were cultured in a growth chamber at a light level of 7.5 klux and a photoperiod of 14 h/d. The containers were positioned randomly and rerandomized every day. An untreated plant control without OPEs in the nutrient solution (CK) was included to controls to monitor possible cross-contamination and any possible volatilization or abiotic degradation of OPEs. All experiments were replicated at least in triplicate.

Extraction and sample preparation

Extracting and purifying OPEs from rice tissues. Rice seedlings were separated into roots and shoots and thoroughly washed with deionized water and wrapped by filter paper after exposure. Then, samples were ground with liquid nitrogen after precisely weighted, and placed into a 15 mL centrifuge tube to freeze-dry. Next, 5.0 mL of ethyl acetate was added to the tube and vortex mixing for 3 min.

After, the mixture in the tube was extracted for 30 min by ultrasonic and then placed on a horizontal oscillator for 10 min before centrifuged at 5,500 r/min for 10 min. The extraction process was repeated three, then mixed all the extracted supernatant and adjusted to 15.0 mL. The all extracts were added 65.0 mg Florisil, and vortexed for 3 min before centrifuged for 10 min at 5,500 r/min. The supernatant was concentrated by spin evaporation. Finally, the residue was dissolved in 1.0 mL of ethyl acetate and the samples were filtered through a 0.22 μm nylon filter membrane for GC-MS analysis.

Extracting and purifying OPEs from nutrient solution. Sodium chloride was added to adjust the ionic strength of nutrient solution sample to 0.086 mol/L. Then, 2 mL of dichloromethane were added and shaken horizontal oscillator for 10 min before leaving it to stand until the organic phase completely separated from the aqueous phase. The organic phase was then transferred to another 50 mL glass tube and dehydrated with anhydrous sodium sulfate. The extracts were added 87.0 mg Florisil, and vortexed for 3 min before centrifuged for 10 min at 5,500 r/min. The supernatant was concentrated by spin evaporation. Finally, the residue was dissolved in 1.0 mL of ethyl acetate and the samples were filtered through a 0.22 μm nylon filter membrane for GC-MS analysis.

Fractionation of OPEs in the Apoplast. It was extracted using the vacuum-infiltration−centrifugation method. Rice seedlings were separated into roots and thoroughly washed with deionized water and wrapped by filter paper after exposure, and then put roots/shoot into a brown glass bottle containing infiltration solution. Root weight : infiltration solution = 1 g : 40 mL. The configuration of the infiltration solution is: 0.6 mol/L NaCl, 50 mmol/L Tris-HCl and 0.1% (V/V) β-mercaptoethanol. The brown glass bottle was vacuum infiltrated at 70 KPa for 10 min and then wiped with filter paper. Put the dried root into a 10 mL syringe, then put it into a 50 mL centrifuge tube and centrifuge at 1000 g for 15 min at 4 °C. Repeat three times to obtain the apoplast sap and stored at −20 °C before extraction. After removal of the apoplastic sap, the OPEs in the symplast were present in the remaining roots.

Recoveries were obtained by the addition of OPE standards individually to the untreated plant controls and nutrient solutions before extraction and analysis. In detail, 25 mL, 10 mL and 5 mL 100.0 μg/L OPEs expose solution were spiked into the freeze-dried plant materials or as solution then equilibrated for 12 h for solvent volatilization. The scalar recoveries of OPEs methods were replicated

at least in triplicate. The method detection limits (MDL) were defined as three times the signal-to-noise ratio (S/N). The concentrations less than the MDLs were labeled as not detectable (ND).

GC-MS analysis of OPEs

The concentrations of OPEs were quantified by Shimadzu GC-MS (QP2020, Shimadzu, Japan). An SH-Rxi-1ms column (30 m × 0.25 mm × 0.25 μm) (Shimadzu) was used to separate OPEs, with helium (> 99.999%) as the carrier gas at a flow rate of 1.58 mL/min. The MS system was operated in selected ion monitoring (SIM) mode under electron-impact (EI) ionization with ionization energy of 70 eV for quantitation. The temperature program for the oven was as follows: 0 min at 60 ℃, first ramp at 25 ℃/min to 190 ℃, second ramp at 10 ℃/min to 300 ℃ (8 min hold). The injector and ion source temperatures were maintained at 290 ℃ and 230 ℃, respectively. Sample injection at 3.0 μL was in spitless mode. The time for solvent delay was set to 3 min.

Table S1 Type, Names, structures, formula and properties of the OPEs in the present study

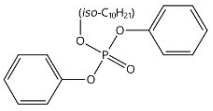
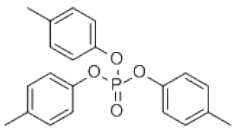
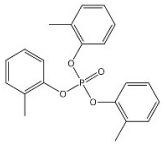
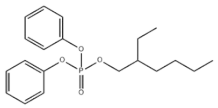
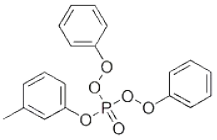
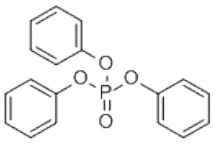
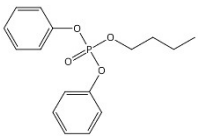
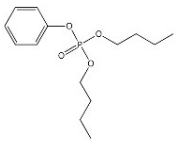
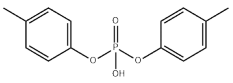
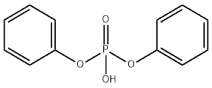
Abbreviation	Name	CAS NO.	Structural Formula	MF[a]	log K_{ow}[b]	MW[c]
Aryl-OPEs						
IDDP	isodecyl diphenyl phosphate	29761–21-5		$C_{22}H_{31}O_4P$	7.28	390.5
TMPP/TCrP	tritolyl phosphate	78-32-0		$C_{21}H_{21}O_4P$	6.34	368.4
TOCP	tri-o-tolyl phosphate	78-30-8		$C_{21}H_{21}O_4P$	6.34	368.4
EHDPP	2-ethylhexyl diphenyl phosphate	1241-94-7		$C_{20}H_{27}O_4P$	5.73	362.4
CDP/CDPP	(3-methylphenyl) diphenyl phosphate	26444-49-5		$C_{19}H_{17}O_4P$	5.3	340.3
TPHP/TPhP	triphenyl phosphate	115-86-6		$C_{18}H_{15}O_4P$	4.59	326.3
BDPHP	butyl diphenyl phosphate	2752-95-6		$C_{16}H_{19}O_4P$	4.41	306.3
DBPHP	dibutyl phenyl phosphate	2528-36-1		$C_{14}H_{23}O_4P$	4.11	286.3
BMPP	phosphoric acid bis(p-tolyl) ester	843–24-3		$C_{14}H_{15}O_4P$	3.97	278.2
DPHP	diphenyl phosphate	838-85-7		$C_{12}H_{11}O_4P$	2.88	250.2

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Table S1 (continued)

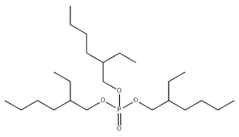
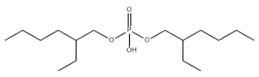
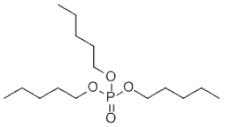
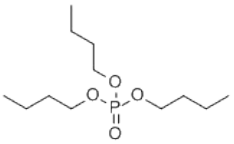
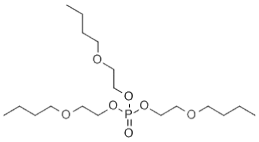
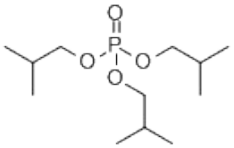
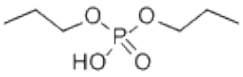
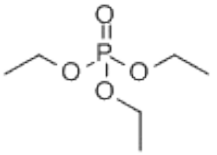
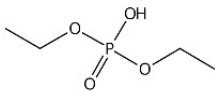
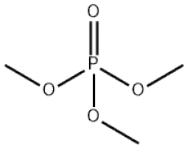
Abbreviation	Name	CAS NO.	Structural Formula	MF[a]	log K_{ow}[b]	MW[c]
Alkyl-OPEs						
TEHP	tris(2-ethylhexyl) phosphate	78-42-2		$C_{24}H_{51}O_4P$	9.49	434.7
BEHP	bis(2-ethylhexyl) hydrogen phosphate	298-07-7		$C_{16}H_{35}O_4P$	6.07	322.4
TrMP	tripentyl phosphate	2528-38-3		$C_{15}H_{33}O_4P$	5.29	308.4
TBP/TnBP	tri-n-butyl phosphate	126-73-8		$C_{12}H_{27}O_4P$	4	266.3
TBEP	tris(2-butoxyethyl) phosphate	78-51-3		$C_{18}H_{39}O_7P$	3.75	398.5
TiBP	tris(2-methylpropyl) phosphate	126-71-6		$C_{12}H_{27}O_4P$	3.6	266.3
DPrP	dipropyl hydrogen phosphate	1804-93-9		$C_6H_{15}O_4P$	1.31	182.2
TEP	triethyl phosphate	78-40-0		$C_6H_{15}O_4P$	0.87	182.2
DEP	diethyl phosphate	598–02-7		$C_4H_{11}O_4P$	0.32	154.1
TMP	trimethyl phosphate	512-56-1		$C_3H_9O_4P$	-0.6	140.1

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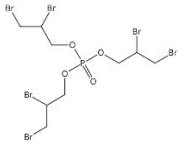
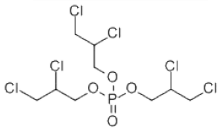
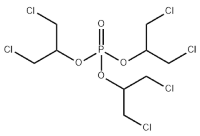
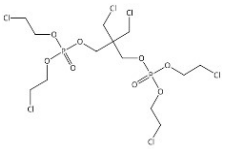
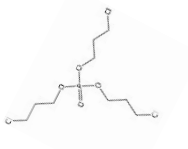
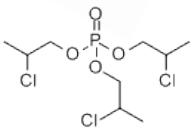
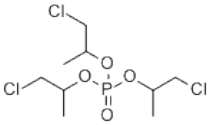
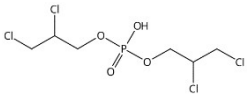
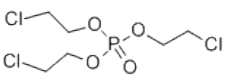
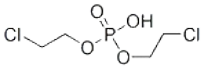
Table S1 (continued)

Abbreviation	Name	CAS NO.	Structural Formula	MF[a]	log K_{ow}[b]	MW[c]
Halogenated alky-OPEs						
TDBPP	tris(2,3-dibromopropyl) phosphate	126-72-7		$C_9H_{15}Br_6O_4P$	4.19	697.6
TDCP	tris(2,3-dichloropropyl) phosphate	78-43-3		$C_9H_{15}Cl_6O_4P$	3.65	430.9
TDCPP	tris(1,3-dichloropropan-2-yl) phosphate	13674-87-8		$C_9H_{15}Cl_6O_4P$	3.65	430.9
V6	2,2-bis(chloromethyl) trimethylene	38051–10-4		$C_{13}H_{24}Cl_6O_8P_2$	3.31	583
T3CIPP	tris(3-chloropropyl) phosphate	1067-98-7		$C_9H_{18}Cl_3O_4P$	3.11	327.6
TCIPP	tris(2-chloropropyl) phosphate	6145-73-9		$C_9H_{18}Cl_3O_4P$	2.89	327.6
TCPP	tris(1-chloropropan-2-yl) phosphate	13674-84-5		$C_9H_{18}Cl_3O_4P$	2.89	327.6
BDCIPP	bis(2,3-dichloropropyl) phosphate	37509-70-9		$C_6H_{11}Cl_4O_4P$	2.18	319.9
TCEP	tris(2-chloroethyl) phosphate	115-96-8		$C_6H_{12}Cl_3O_4P$	1.63	285.5
BCEP	bis(2-chloroethyl) hydrogen phosphate	3040-56-0		$C_4H_9Cl_2O_4P$	0.83	223

NOTE:

All the values of properties are generated using the US Environmental Protection Agency's EPISuite™ (https://www.epa.gov/tsca-screening tools).

a: MF=Molecular formula; b: K_{OW}=Octanol-water partition coefficient, c: MW=Molecular weight.

Table S2 Types, Abbreviation, Name and Accession of the lipid transporter in the present study

Type	Abbreviation	Name	Accession
lipocalins	OsTIL	temperature-induced lipocalin-1	XP_015627280.1
	OsCHL	chloroplastic lipocalin	XP_015635937.1
	OsVDE	violaxanthin de-epoxidase	XP_015636342.1
	OsUN	uncharacterized LOC4325583	XP_015621660.1
	OsZEP1	zeaxanthin epoxidase	NP_001406295.1
	OsZEP2	monooxygenase 2	XP_025880216.1
nsLTPs	OsLTP1	non-specific lipid-transfer protein 1-like	NP_001391564.1
	OsLTP2	non-specific lipid-transfer protein 2-like	NP_001404088.1
	OsLTP2A	non-specific lipid-transfer protein 2	NP_001391721.1
	OsLTP2B	non-specific lipid-transfer protein 2B-like	NP_001391563.1
	OsLTP3	non-specific lipid-transfer protein 3-like	NP_001391371.1
	OsLTP4	non-specific lipid-transfer protein 4-like	XP_015618920.1
	OsLTPC4	non-specific lipid-transfer protein C4-like	NP_001391821.1
	OsLTPC6	non-specific lipid-transfer protein C6-like	XP_015615738.1
	OsLTPL1	non-specific lipid transfer protein-like 1	NP_001389094.1
apoproteins	OsPsaA	PSI P700 apoprotein A1	NP_039383.1
	OsPsaB	PSI P700 apoprotein A2	NP_039382.1
	OsPsbB	photosystem II P680 chlorophyll A apoprotein	NP_039411.1
PR-10s	OsMLP	MLP-like protein 423	XP_015634232.1
	OsPR-10	pathogenesis-related protein 10	XP_015620489.1

Table S3 Subcellular localization prediction of lipid transporters

protein	GPI[a]	TargetP[b]	Plant-mPLoc[c]
OsCHL	0	Thylakoid luminal transfer peptide (0.7188); Chloroplast transfer peptide (0.2269)	Cell membrane
OsTIL	0	Mitochondrial transfer peptide (0.0004); Signal peptide (0.0003)	Cell membrane; Cell wall; Chloroplast; Cytoplasm; Golgi apparatus; Nucleus.
OsVED	0	Thylakoid luminal transfer peptide (0.4687)	Chloroplast
OsZEP1	0	Chloroplast transfer peptide (0.9269)	Chloroplast
OsZEP2	0	Mitochondrial transfer peptide (0.0001)	Chloroplast
OsPasA	0	Signal peptide (0.0104); Mitochondrial transfer peptide (0.0002)	Chloroplast
OsPsaB	0	Mitochondrial transfer peptide (0.0414); Thylakoid luminal transfer peptide (0.0069); Signal peptide (0.0059); Chloroplast transfer peptide (0.0009)	Chloroplast
OsPsbB	0	Mitochondrial transfer peptide (0.0524); Signal peptide (0.0109); Chloroplast transfer peptide (0.0009); Thylakoid luminal transfer peptide (0.0001)	Chloroplast; Plastid.
OsUN	0	Chloroplast transfer peptide (0.9433)	Chloroplast
OsLTP1	0	Signal peptide (1)	Cell wall
OsLTPL2	0	Signal peptide (1)	Cell membrane
OsLTPL2A	0	Signal peptide (1)	Cell wall
OsLTPL2B	0	Signal peptide (1)	Cell wall
OsLTPL3	0	Signal peptide (1)	Cell membrane
OsLTPL4	0	Signal peptide (1)	Cell wall
OsLTPLC4	0	Signal peptide (1)	Cell membrane
OsLTPLC6	0	Signal peptide (1)	Cell membrane
OsLTPL1	149	Signal peptide (1)	Cell membrane
OsMLP	0	Signal peptide (0.0004); Mitochondrial transfer peptide (0.0001)	Cytoplasm
OsPR-10	0	Signal peptide (0.0137); Mitochondrial transfer peptide (0.0008); Chloroplast transfer peptide (0.0001)	Cytoplasm

[a]: GPI Modification Site Prediction website: https://mendel.imp.ac.at/gpi/gpi_server.html;

[b]: TargetP website: https://services.healthtech.dtu.dk/services/TargetP-2.0/;

[c]: Plant-mPLoc website: http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/.

Table S4 The lipid transporter structural assessment by Procheck and ProSA

	Procheck [a]				ProSA [b]
	Most favoured regions (%)	Additional allowed regions (%)	Generously allowed regions (%)	Disallowed regions (%)	Z-Score
OsTIL	89.2	10.8	0.0	0.0	−6.16
OsCHL	70.0	24.1	3.8	2.1	−4.49
OsVDE	78.7	14.8	3.6	2.9	−6.44
OsUN	83.4	13.8	1.7	1.1	−10.28
OsZEP1	84.9	11.8	2.4	0.9	−10.20
OsZEP2	82.6	13.5	2.5	1.4	−5.34
OsLTP1	93.9	6.1	0.0	0.0	−4.61
OsLTP2	93.5	5.2	1.3	0.0	−4.22
OsLTP2A	96.0	4.0	0.0	0.0	−4.73
OsLTP2B	94.9	5.1	0.0	0.0	−4.60
OsLTP3	94.4	5.6	0.0	0.0	−4.06
OsLTP4	96.0	3.0	0.0	0.0	−3.81
OsLTPC4	97.4	2.6	0.0	0.0	−2.61
OsLTPC6	84.4	11.9	2.8	0.9	−5.16
OsLTPL1	86.4	8.8	2.7	2.0	−5.05
OsPsaA	92.4	7.1	0.5	0.0	−7.24
OsPsaB	93.4	6.2	0.2	0.3	−7.79
OsPsbB	93.3	6.7	0.0	0.0	−4.95
OsMLP	95.0	4.3	0.7	0.0	−6.58
OsPR-10	91.9	8.1	0.0	0.0	−5.76

[a]: Procheck website: https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/index.html;

[b]: ProSA-website: https://prosa.services.came.sbg.ac.at/.

Table S5 OPE congeners confirmation and quantitation ions and retention times (RT)

OPEs	Confirmation ion (m/z)	Quantitation ion (m/z)		RT (min)
TEHP	99	113	57	12.565
TMPP	368	367	107	14.075
CDP	340	339	77	13.090
TBEP	57	45	56	12.155
TDCPP	75	99	191	11.600
TCEP	63	249	251	7.280
TEP	99	155	127	3.455

Table S6 Scalar recovery and method detection limit (MDL) of seven different OPEs (n ≥ 3)

OPEs	Recovery (%)		MDL* (ug/g)
	solution	plant tissues	
TEHP	86.1	98.2	0.00308
TMPP	77.7	97.8	0.00342
CDP	78.6	111.3	0.02394
TBEP	129.4	94.5	0.00684
TDCPP	115.8	97.3	0.06738
TCEP	122.5	95.2	0.01163
TEP	66.4	96.5	0.00342

* The MDL was determined for the instrument.

Table S7 Gene ID of 6 lipid transporters and their primers used in qPCR

gene	Gene ID		Primer sequence
18s	4333919	F	GCCGTCCTCTCTCTGTATGC
		R	GGGGACAGTGTGGCTGAC
OsTIL	4329967	F	GGTGGTTCAAGTCGCTCTTC
		R	GCTATACATCGTCGTCCTCTG
OsLTP2	4331362	F	GTCTTGGCGTCATCTCGTCAC
		R	GAACTGACCACCCAAGCACA
OsLTP4	4351314	F	CTGCTTACCTGTTGCTTCGTC
		R	CCCTCGTCCCGTAAAGGAG
OsLTPL1	4332993	F	ACAGTTTGTCGTTGTGATCGTG
		R	TTAATCACGGCAGGGTGCAT
OsMLP	4336091	F	CCTCAAGTACACCGAAGGGGTG
		R	GTACGACACCACCTTCTTCTCG
OsPR-10	4352487	F	GAAGCTCAACCCTGCTGTGG
		R	TGACTTGATAATGTGAGCTGCG

Table S8 Analysis of covariance results of OPEs binding affinities with numbers of benzene rings, carbon in main chain, Halogenated group and log K_{ow}

	Factors	F	p-Value	Partial Eta squared
Aryl-OPEs	Numbers of benzene rings	10.623	< 0.001	0.098
	log K_{ow}	0.393	0.531	0.002
Alky-OPEs	Numbers of carbon in main chain	5.547	< 0.001	0.841
	log K_{ow}	0.589	0.444	0.003
Halogenated alky-OPEs	Numbers of Halogenated group	0.709	0.548	0.011
	log K_{ow}	0.047	0.828	0.000

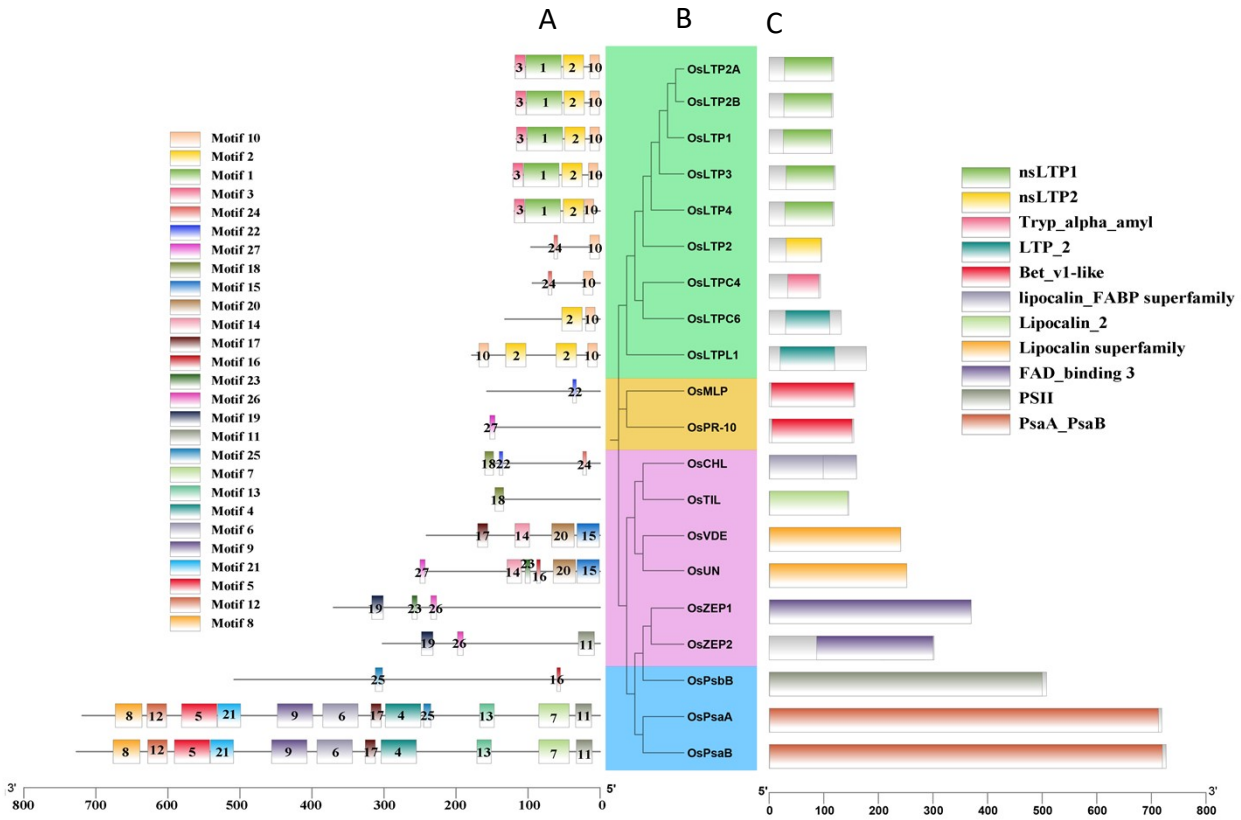
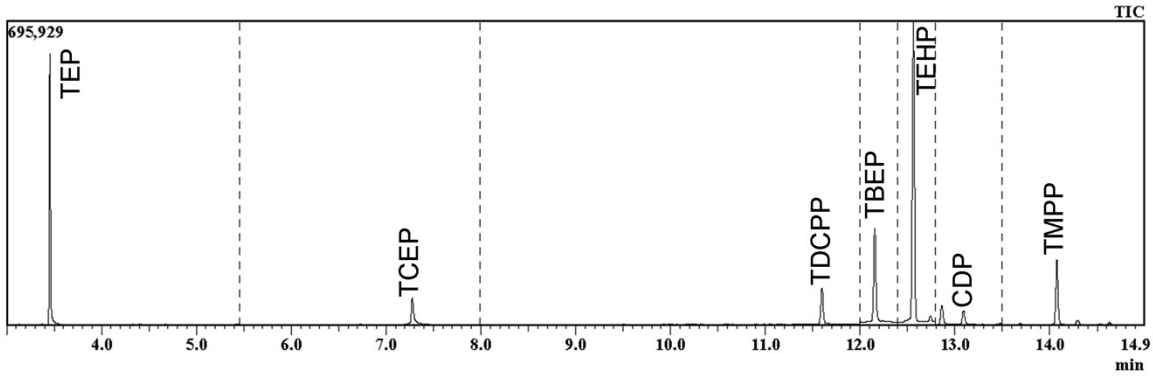


Fig. S1 (A) conserved motifs, (B) Phylogenetic analysis, (C) conserved domains of 20 lipid transporters.

A



B

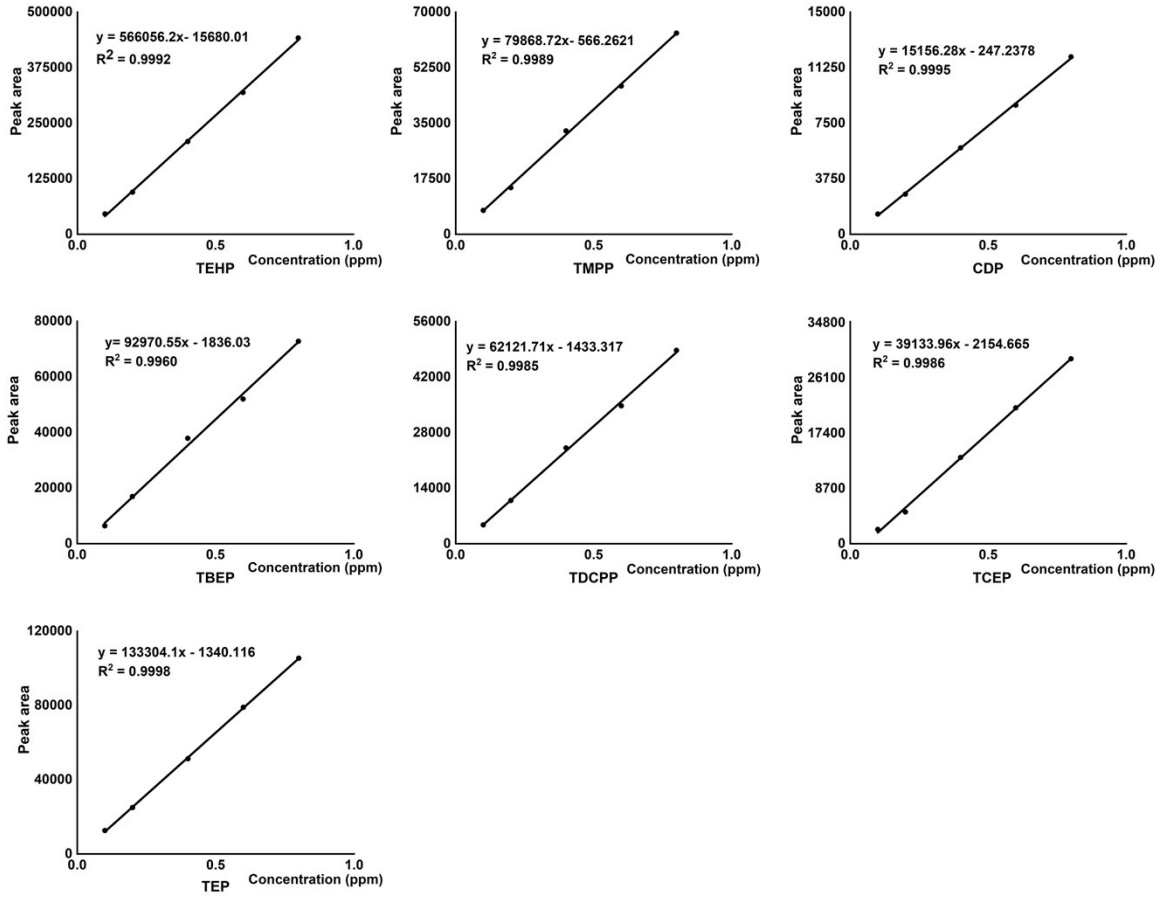


Fig. S2 (A) The chromatogram and (B) standard curve of 7 different OPEs.

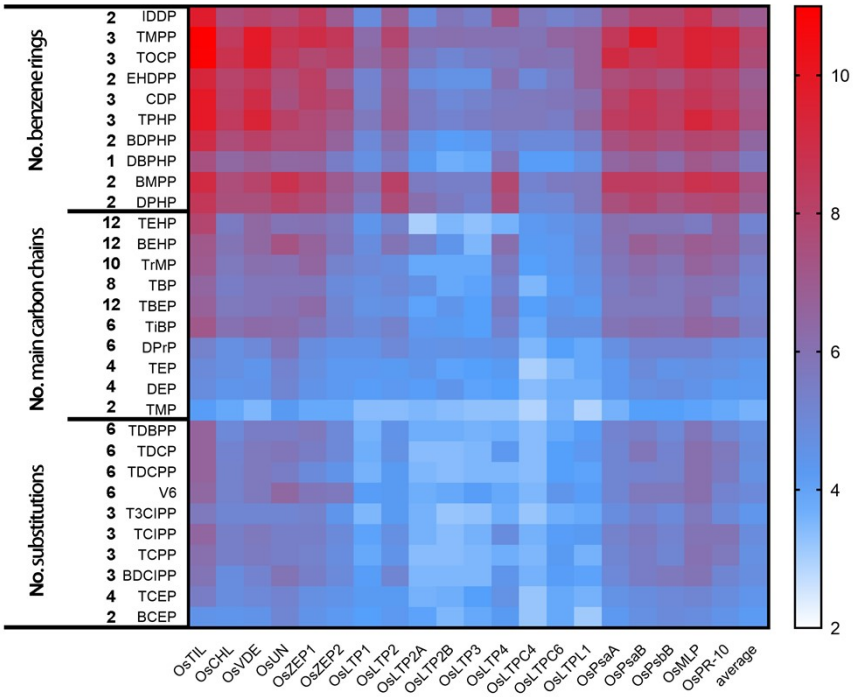


Fig. S3 Binding affinities Heatmap showing 20 lipid transporters to 30 OPEs and average binding affinity of 30 OPEs (−kcal/mol).

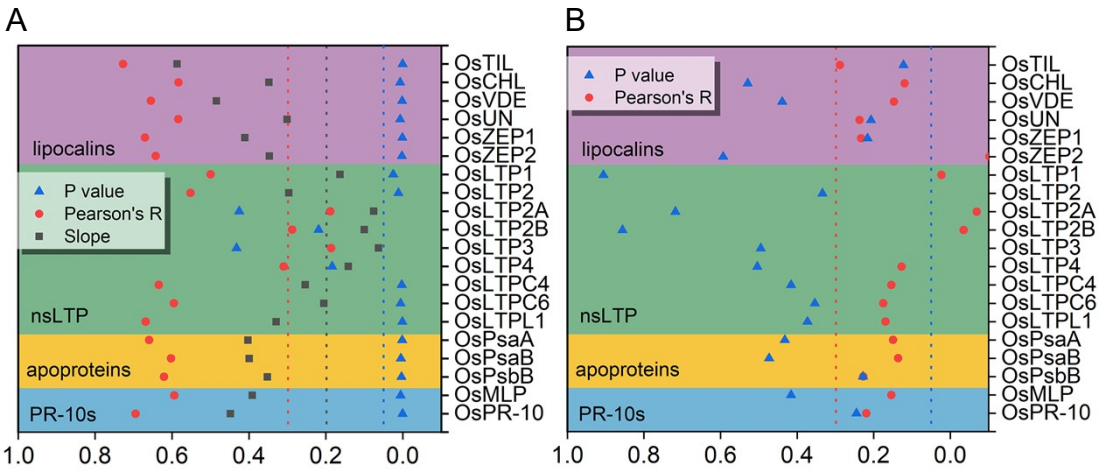


Fig. S4 (A) R value, P value and Slope value of the linear fit between binding affinities (−kcal/mol) of 20 lipid transporters to 30 OPEs with 30 OPEs' log K_{ow}.(B) R value and P value of correlation analysis between the binding affinities (−kcal/mol) of 20 lipid transporters to 30 OPEs with 30 OPEs' molecular weight. The red dashed line indicates the R value of 0.3. The blue dashed line indicates the P value of 0.05. The black dashed line indicates the slope value of 0.2.

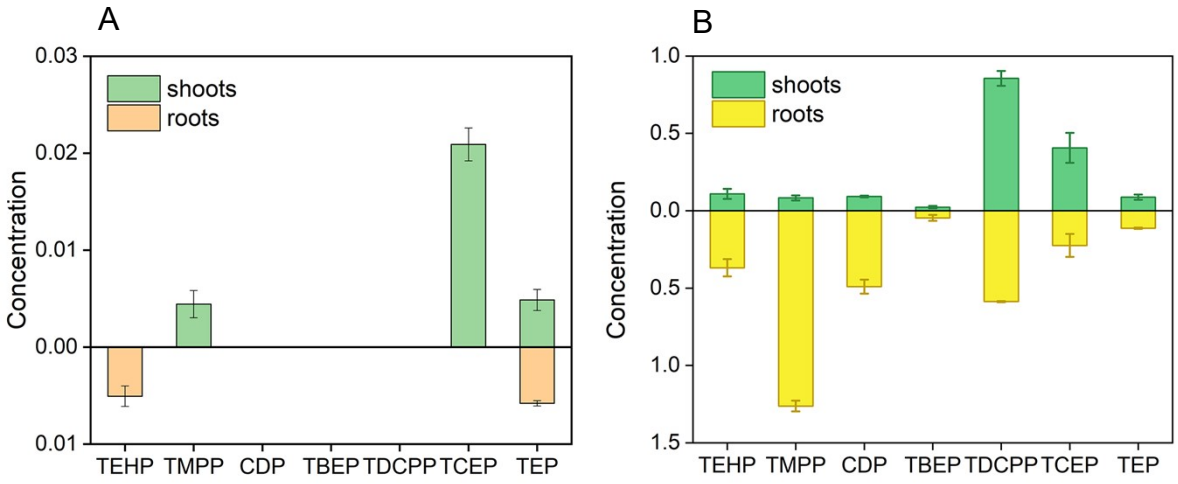


Fig. S5 OPEs concentrations of (A) OPEs-untreated and (B) OPEs-treated of rice seedlings in shoots and roots (μg/g) (n ≥ 3).

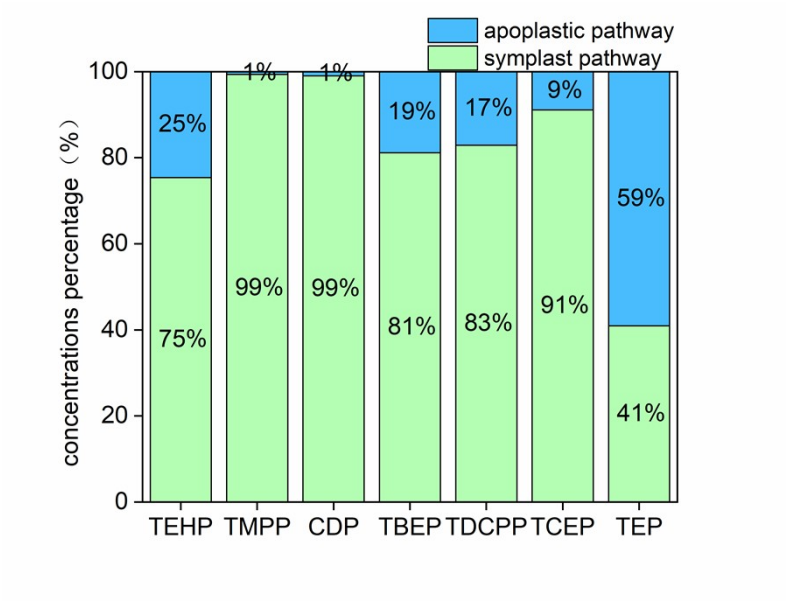


Fig. S6 Apoplastic and symplast distributions of OPEs in rice roots (n ≥ 3).