Supplementary data

Supplementary text

Cloning of *SmAOS2*

Total RNA of *S. moellendorffii* (ca. 120 mg) was extracted using the conventional phenol-chloroform method. Reverse transcription (M-MLV Reverse Transcriptase, Invitrogen, Carlsbad, CA USA) was performed according to the manufacturer's instructions to generate cDNA. The primer set of *SmAOS2*, SmAOS2-F and SmAOS2-R, was used to amplify the open reading frame (ORF) of *SmAOS2*. To afford the ORF of *SmAOS2*, the PCR reaction was performed in 50 µl per tube, containing 10 µl of dNTP mixture (2.0 mM each), 1 µl each from forward and reverse primer (5 µM), 0.5 µl of cDNA, 25 µl of PCR buffer (2 ×), 0.5 µl of KOD FX DNA polymerase (Toyobo, Japan) and 12 µl of Milli-Q water under following conditions; pre-denaturation at 94°C for 2 min followed by 40 cycles of denaturing at 98°C for 10 s, annealing at 56°C for 30 s, and extension at 68°C for 1 min, and then a final extension at 68°C for 10 min. The obtained PCR product was purified and inserted into the pBlueScript SK II (+) vector (Stratagene, USA), which was digested with *EcoRV* to obtain the plasmid of pSK-SmAOS2. DNA sequencing was performed to confirm that the target gene was successfully inserted into the plasmid. The primers used in this study are listed in Supplementary Table S2.

Synthesis of a recombinant SmAOS2 in *E. coli*

The primer set for overexpression of SmAOS2, SmAOS2NdeI-F with *NdeI* site and SmAOS2XhoI-R with *XhoI* site, in *E. coli* was designed according to a *SmAOS2* sequence. PCR was performed in 15 µl of a reaction mixture containing 1.5 µl of dNTP mixture (2.0 mM each dNTP), 0.9 µl of 25 mM $MgSO_4$, 1.5 µl of KOD plus neo buffer (10 ×), 0.9 µl of each primer (SmAOS2NdeI-F and SmAOS2XhoI-R, 5 µM), 1 µl of pSK-SmAOS2 (200 ng/ µl), 0.3 µl of KOD plus neo DNA polymerase (Toyobo, Japan), and 8 µl of Milli-Q water. PCR was conducted with the following conditions: 2 min at 94°C for pre-denaturation; 30 cycles of 98°C for 10 s, 58°C for 30 s, and 68°C for 90 s and then final extension at 68°C for 10 min. The PCR product then was purified and ligated into pET23a vector (Merck, USA) using Ligation Mix (Takara, Japan) according to the standard procedure. The constructed plasmid, pET23a-SmAOS2, was transformed in *E.coli* BL21 (DE3) according to standard procedure and was subsequently grown in LB agar medium supplemented with 100 µg/ml of ampicillin. A single colony was inoculated into 10 ml of LB medium containing 100 µg/ml of ampicillin and then incubated at 37°C for overnight. A 10 ml aliquot was transferred into 1 l of LB medium containing 100 µg/ml of ampicillin and then incubated at 37°C until the OD_{600} reached 0.4; the sample was finally induced by 1 mM of IPTG for overnight at 25°C. The bacterial cells were collected by centrifugation at 5,000 × *g* for 20 min and lysed by ultrasonication in 50 mM phosphate buffer pH 7.4, 0.3 M NaCl, and 20 mM imidazole. Cell debris was removed by centrifugation at 15,000 × *g* for 15 min; then, the supernatant was subjected to Ni-NTA agarose column chromatography (2 ml, GE Healthcare, USA). SmAOS2, fused with a His-tag, was eluted with 50 mM phosphate buffer (pH 7.8) containing 100 mM imidazole and 0.3 M NaCl. The resulting recombinant protein solution was dialyzed with 50 mM Tris-HCl (pH 8.0) containing 20 mM NaCl. The purity was checked by SDS-PAGE (Supplementary Fig. S3). The dialyzed product was concentrated using Amicon Ultra-15 Centrifugal Filter Device 10K (Merck Millipore Ltd., Germany) and then used for evaluation of enzymatic activity. The primers used in this study are listed in Supplementary Table S2.

Cloning of *SmAOC1*

Total RNA and cDNA of *S. moellendorffii* were obtained according the method described above. Full length cDNA of *SmAOC1* (Sm_91887) was obtained with 5'-Full RACE Core Set according to the manufacturer's instructions. Subsequently, the primer set of *SmAOC1*, SmAOC1-F and SmAOC1-R was used to amplify the open reading frame (ORF) of *SmAOC1*. The PCR reaction was performed in 50 µl per tube, containing 10 µl of dNTP mixture (2.0 mM each), 1 µl each from forward and reverse primer (5 µM), 0.5 µl of cDNA, 25 µl of PCR buffer (2 ×), 0.5 µl of KOD FX DNA polymerase (Toyobo, Japan) and 12 µl of Milli-Q water under following conditions: pre-denaturation at 94°C for 2 min followed by 40 cycles of denaturing at 98°C for 10 s, annealing at 55°C for 30 s, and extension at 68°C for 1 min, and then a final extension at 68°C for 10 min. The obtained PCR product was purified and inserted into the pBlueScript SK II (+) vector (Stratagene, USA), which was digested with *EcoRV* to obtain the plasmid of pSK-SmAOC1. DNA sequencing was performed to confirm that the target gene was successfully inserted into the plasmid. The primers used in this study are listed in Supplementary Table S2.

Synthesis of a recombinant SmAOC1 in *E. coli*

ChloroP v 1.1 and TargetP predicted that the fifty amino acids at the N-terminus of SmAOC1 was a chloroplast transit peptide. The primer set for overexpression of *SmAOC1*, SmAOC1SphI-F with *SphI* site and SmAOC1SalI-R with *SalI* site, in *E. coli* was designed according to a *SmAOC1* sequence, which had 150 nucleotides deleted from the codon for the first methionine. PCR was performed in 15 µl of a reaction mixture containing 1.5 µl of dNTP mixture (2.0 mM each dNTP), 0.9 µl of 25 mM $MgSO_4$, 1.5 µl KOD plus neo buffer (10 ×), 0.9 µl of each primer (SmAOC1SphI-F and SmAOC1SalI-R, 5 µM), 1 µl of pSK-SmAOC1 (200 ng/µl), 0.3 µl of KOD plus neo DNA polymerase (Toyobo, Japan), and 8 µl of Milli-Q water. PCR was conducted with the following conditions: 2 min at 94°C for pre-denaturation; 30 cycles of 98°C for 10 s, 60°C for 30 s, and 68°C for 90 s and then final extension at 68°C for 10 min. The PCR product then was purified and ligated into pQE30 vector (Qiagen, USA) using T4 DNA ligase (Takara, Japan) according to the standard procedure. The constructed plasmid, pQE30-SmAOC1, was transformed in *E.coli* M15 according to standard procedure and was subsequently grown in LB agar medium supplemented with 100 µg/ml of ampicillin. A single colony was inoculated into 10 ml of LB medium containing 100 µg/ml of ampicillin and then incubated at 37°C for overnight. A 10 ml aliquot was transferred into 1 l of LB medium containing 100 µg/ml of ampicillin and then incubated at 25°C until the OD_{600} reached 0.5; the sample was finally induced by 0.2 mM of IPTG for 4 h at 25°C. The bacterial cells were collected by centrifugation at 7,000 × *g* for 20 min and lysed by ultrasonication in 50 mM phosphate buffer pH 7.8, 0.3 M NaCl, and 20 mM imidazole. Cell debris was removed by centrifugation at 15,000 × *g* for 15 min; then, the supernatant was subjected to Ni-NTA agarose column chromatography (2 ml, GE Healthcare, USA). SmAOC1, fused with a His-tag, was eluted with 50 mM phosphate buffer (pH 7.8) containing 200 mM imidazole and 0.3 M NaCl. The resulting recombinant protein solution was dialyzed with 50 mM Tris-HCl (pH 8.0) containing 20 mM NaCl. The purity was checked by SDS-PAGE (Supplementary Fig. S3). The dialyzed product was concentrated using Amicon Ultra-15 Centrifugal Filter Device 10K (Merck Millipore Ltd., Germany) and then used for evaluation of enzymatic activity. The primers used in this study are listed in Supplementary Table S2.

Subcellular localization of SmAOC1

To analyze the localization of SmAOC1, PCR was performed using primers, SmAOC1NdeI-F and SmAOC1EcoRV-R, with *NdeI* and *EcoRV* restriction sites, respectively. The PCR reaction mixture contained 1.5 µl of dNTP mixture (2.0 mM each dNTP), 0.9 µl of 25 mM $MgSO_4$, 1.5 µl of KOD plus neo buffer (10 ×), 0.9 µl of each primer (5 µM), 1 µl of pSK-SmAOC1 (200 ng/µl), 0.3 µl of KOD plus neo DNA polymerase (Toyobo, Japan), and an adjusted Milli-Q water level up to a volume of 15 µ1. PCR was conducted for 2 min at 94°C for pre-denaturation; 30 cycles of 98°C for 10 s, 60°C for 30 s, and 68°C for 90 s; final extension was performed at 68°C for 10 min. The PCR product was ligated into the pENTR4 vector using GeneArt Seamless Cloning and Assembly Kit (Invitrogen, USA). The pENTR4-SmAOC1 entry clone was introduced into the pUGWnew5 destination vector by Gateway LR Clonase Mix (Invitrogen, USA) to generate the plasmid encoding 35S::SmAOC1-GFP (pUGWnew5-SmAOC1). The constructed plasmid of 35S::SmAOC1-GFP was transformed into protoplasts of *P. patens*, which were grown in BCDATG medium for 3 days under white fluorescent light using the PEG-mediated transformation technique. Localization of the SmAOC1-GFP fusion protein was observed under a TCS-SP5 confocal laser scanning microscope (Leica, Germany) after 3 days incubation. Images were observed at an excitation of 488 nm and emission of 530 nm for detecting the GFP signal as well as emission over 655 nm for detecting auto-fluorescence from the chloroplasts. The primers used in this study are listed in Supplementary Table S2.

Cloning of *SmOPR1*

Total RNA and cDNA of *S. moellendorffii* were obtained according the method described above. The primer set of *SmOPR1*, SmOPR1-F and SmOPR1-R, was used to amplify the open reading frame (ORF) of *SmOPR1*. To afford the ORF of *SmOPR1*, the PCR reaction was performed in 50 µl per tube, containing 10 µl of dNTP mixture (2.0 mM each), 1 µl each from forward and reverse primer (5 µM), 0.5 µl of cDNA, 25 µl of PCR buffer (2 ×), 0.5 µl of KOD FX DNA polymerase (Toyobo, Japan) and 12 µl of Milli-Q water under following conditions; pre-denaturation at 94°C for 2 min followed by 40 cycles of denaturing at 98°C for 10 s, annealing at 60°C for 30 s, and extension at 68°C for 1 min, and then a final extension at 68°C for 10 min. The obtained PCR product was purified and inserted into the pBlueScript SK II (+) vector (Stratagene, USA), which was digested with *EcoRV* to obtain the plasmid of pSK-SmOPR1. DNA sequencing was performed to confirm that the target gene was successfully inserted into the plasmid. The primers used in this study are listed in Supplementary Table S2.

Synthesis of a recombinant SmOPR1 in *E. coli*

The primer set for overexpression of SmOPR1, SmOPR1EcoRI-F with *EcoRI* site and SmOPR1NotI-R with *NotI* site, in *E. coli* was designed according to a *SmOPR1* sequence. PCR was performed in 15 µl of a reaction mixture containing 1.5 µl of dNTP mixture (2.0 mM each dNTP), 0.9 µl of 25 mM $MgSO_4$, 1.5 µl of KOD plus neo buffer (10 ×), 0.9 µl of each primer (SmOPR1EcoRI-F and SmOPR1NotI-R, 5 µM), 1 µl of pSK-SmOPR1 (200 ng/ µl), 0.3 µl of KOD plus neo DNA polymerase (Toyobo, Japan), and 8 µl of Milli-Q water. PCR was conducted with the following conditions: 2 min at 94°C for pre-denaturation; 30 cycles of 98°C for 10 s, 60°C for 30 s, and 68°C for 90 s and then final extension at 68°C for 10 min. The PCR product then was purified and ligated into pET23a vector (Merck, USA) using Ligation Mix (Takara, Japan) according to the manufacturer's instruction. The constructed plasmid, pET23a-SmOPR1, was transformed in *E.coli* BL21 (DE3) according to standard procedure and was subsequently grown in LB agar medium supplemented with 100 µg/ml of ampicillin. A single colony was inoculated into 10 ml of LB medium containing 100 µg/ml of ampicillin and then incubated at 37°C for overnight. A 10 ml aliquot was transferred into 1 l of LB medium containing 100 µg/ml of ampicillin and then incubated at 25°C until the OD_{600} reached 0.4; the sample was finally induced by 1 mM of IPTG for overnight at 18°C. The bacterial cells were collected by centrifugation at 7,000 × *g* for 20 min and lysed by ultrasonication in 50 mM phosphate buffer pH 7.4, 0.3 M NaCl, and 20 mM imidazole. Cell debris was removed by centrifugation at 15,000 × *g* for 15 min; then, the supernatant was subjected to Ni-NTA agarose column chromatography (2 ml, GE Healthcare, USA). SmOPR1, fused with a His-tag, was eluted with 50 mM phosphate buffer (pH 7.4) containing 200 mM imidazole and 0.3 M NaCl. The resulting recombinant protein solution was dialyzed with 50 mM Tris-HCl (pH 7.8) containing 20 mM NaCl. The purity was checked by SDS-PAGE (Supplementary Fig. S3). The dialyzed product was concentrated using

Amicon Ultra-0.5mL Centrifugal Filter Units 30K and 50K (Merck Millipore Ltd., Germany) and then used for evaluation of enzymatic activity. The primers used in this study are listed in Supplementary Table S2.

Cloning of *SmOPR5*

Total RNA and cDNA of *S. moellendorfii* were obtained according the method described above. The primer set of *SmOPR5*, SmOPR5-F and SmOPR5-R, was used to amplify the open reading frame (ORF) of *SmOPR5*. To afford the ORF of *SmOPR5*, the PCR reaction was performed in 50 µl per tube, containing 10 µl of dNTP mixture (2.0 mM each), 1 µl each from forward and reverse primer (5 µM), 0.5 µl of cDNA, 25 µl of PCR buffer (2 ×), 0.5 µl of KOD FX DNA polymerase (Toyobo, Japan) and 12 µl of Milli-Q water under following conditions; pre-denaturation at 94°C for 2 min followed by 40 cycles of denaturing at 98°C for 10 s, annealing at 60°C for 30 s, and extension at 68°C for 1 min, and then a final extension at 68°C for 10 min. The obtained PCR product was purified and inserted into the pBlueScript SK II (+) vector (Stratagene, USA), which was digested with *EcoRV* to obtain the plasmid of pSK-SmOPR5. DNA sequencing was performed to confirm that the target gene was successfully inserted into the plasmid. The primers used in this study are listed in Supplementary Table S2.

Synthesis of a recombinant SmOPR5 in *E. coli*

The primer set for overexpression of SmOPR5, SmOPR5BamHI-F with *BamHI* site and SmOPR5XhoI-R with *XhoI* site, in *E. coli* was designed according to a *SmOPR5* sequence. PCR was performed in 15 µl of a reaction mixture containing 1.5 µl of dNTP mixture (2.0 mM each dNTP), 0.9 µl of 25 mM $MgSO_4$, 1.5 µl of KOD plus neo buffer (10 ×), 0.9 µl of each primer (SmOPR5BamHI-F and SmOPR5XhoI-R, 5 µM), 1 µl of pSK-SmOPR5 (200 ng/µl), 0.3 µl of KOD plus neo DNA polymerase (Toyobo, Japan), and 8 µl of Milli-Q water. PCR was conducted with the following conditions: 2 min at 94°C for pre-denaturation; 30 cycles of 98°C for 10 s, 60°C for 30 s, and 68°C for 90 s and then final extension at 68°C for 10 min. The PCR product then was purified and ligated into pET23a vector (Merck, USA) using In-Fusion HD Cloning Kit (Takara, Japan) according to the manufacturer's instruction. The constructed plasmid, pET23a-SmOPR5, was transformed in *E.coli* BL21 (DE3) according to standard procedure and was subsequently grown in LB agar medium supplemented with 100 µg/ml of ampicillin. A single colony was inoculated into 10 ml of LB medium containing 100 µg/ml of ampicillin and then incubated at 37°C for overnight. A 10 ml aliquot was transferred into 1 l of LB medium containing 100 µg/ml of ampicillin and then incubated at 25°C until the OD_{600} reached 0.4; the sample was finally induced by 1 mM of IPTG for overnight at 18°C. The bacterial cells were collected by centrifugation at 15,000 × g for 20 min and lysed by ultrasonication in 50 mM phosphate buffer pH 7.4, 0.3 M NaCl, and 20 mM imidazole. Cell debris was removed by centrifugation at 15,000 × g for 15 min; then, the supernatant was subjected to Ni-NTA agarose column chromatography (2 ml, GE Healthcare, USA). SmOPR5, fused with a His-tag, was eluted with 50 mM phosphate buffer (pH 7.4) containing 200 mM imidazole and 0.3 M NaCl. The resulting recombinant protein solution was dialyzed with 50 mM Tris-HCl (pH 7.8) containing 20 mM NaCl. The purity was checked by SDS-PAGE (Supplementary Fig. S3). The dialyzed product was concentrated using Amicon Ultra-0.5ml Centrifugal Filter Units 30K and 50K (Merck Millipore Ltd., Germany) and then used for evaluation of enzymatic activity. The primers used in this study are listed in Supplementary Table S2.

Cloning of *SmJAR1*

Total RNA and cDNA of *S. moellendorfii* were obtained according the method described above. The primer set of *SmJAR1*, SmJAR1-F and SmJAR1-R, was used to amplify the open reading frame (ORF) of *SmJAR1*. To afford the ORF of *SmJAR1*, the PCR reaction was performed in 50 µl per tube, containing 10 µl of dNTP mixture (2.0 mM each), 1 µl each from forward and reverse primer (5 µM), 0.5 µl of cDNA, 25 µl of PCR buffer (2 ×), 0.5 µl of KOD

FX DNA polymerase (Toyobo, Japan) and 12 µl of Milli-Q water under following conditions; pre-denaturation at 94°C for 2 min followed by 40 cycles of denaturing at 98°C for 10 s, annealing at 60°C for 30 s, and extension at 68°C for 1 min, and then a final extension at 68°C for 10 min. The obtained PCR product was purified and inserted into the pBlueScript SK II (+) vector (Stratagene, USA), which was digested with *EcoRV* to obtain the plasmid of pSK-SmJAR1. DNA sequencing was performed to confirm that the target gene was successfully inserted into the plasmid. The primers used in this study are listed in Supplementary Table S2.

Construction of *E. coli* transferred with an overexpression vector of *SmJAR1*

The primer set for overexpression of SmJAR1, SmJAR1BamHI-F with *BamHI* site and SmJAR1XhoI-R with *XhoI* site, in *E. coli* was designed according to a *SmJAR1* sequence. PCR was performed in 15 µl of a reaction mixture containing 1.5 µl of dNTP mixture (2.0 mM each dNTP), 0.9 µl of 25 mM $MgSO_4$, 1.5 µl of KOD plus neo buffer (10 ×), 0.9 µl of each primer (SmJAR1BamHI-F and SmJAR1XhoI-R, 5 µM), 1 µl of pSK-SmJAR1 (200 ng/µl), 0.3 µl of KOD plus neo DNA polymerase (Toyobo, Japan), and 8 µl of Milli-Q water. PCR was conducted with the following conditions: 2 min at 94°C for pre-denaturation; 30 cycles of 98°C for 10 s, 60°C for 30 s, and 68°C for 90 s and then final extension at 68°C for 10 min. The PCR product then was purified and ligated into pET23a vector (Merck, USA) using In-Fusion HD Cloning Kit (Takara, Japan) according to the manufacturer's instruction. The constructed plasmid, pET23a-SmJAR1, was transformed in *E.coli* BL21 (DE3) according to standard procedure. The resultant strain was used for an assay of JA-Ile synthetic activity. The primers used in this study are listed in Supplementary Table S2.

Supplementary tables

Supplementary Table S1. Putative genes of *AOS, AOC*, *OPR*, and *JAR1* in *S. moellendorffii*.

Designation	Locus name	Location	Query (GenBank accession no.)
SmAOS1	271334	scaffold_38	*Arabidopsis* AOS (AED94842)
SmAOS2	177201	scaffold_38	*Arabidopsis* AOS (AED94842)
SmAOS3	228572	scaffold_38	*Arabidopsis* AOS (AED94842)
SmAOC1	91887	scaffold_12	*Arabidopsis* AOC (CAC83764)
*SmOPR1**	270843	scaffold_14	*Arabidopsis* OPR3 (AEC06000)
*SmOPR5**	111662	scaffold_41	*Arabidopsis* OPR3 (AEC06000)
SmJAR1	110439	scaffold_40	*Arabidopsis* JAR1 (AEC10684)

*The numbering of *SmOPRs* was previously described by Li *et al*. (2009).

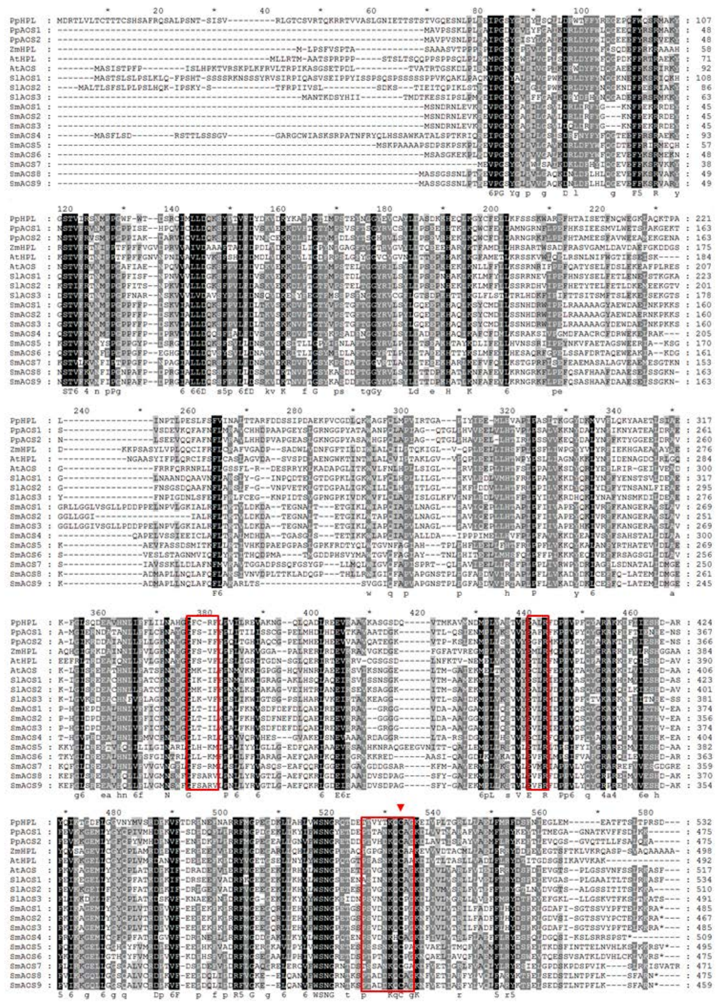
Supplementary Table S2. Oligonucleotide primers.

Primer Name	Sequence 5’ to 3’	Purpose
SmAOC1-RT	GGAGCAACGGACTGC	5’-RACE PCR
SmAOC1_A1	GAGATGTGGCCGTAGTCCC	5’-RACE PCR
SmAOC1_S1	GGATACGTACATGGCGATCA	5’-RACE PCR
SmAOC1_A2	AAGCTGTAGGTGGCCTCGTA	5’-RACE PCR
SmAOC1_S2	CTTCTACTTGGAGGGCATCG	5’-RACE PCR
SmAOC1-F	ATGGCAAGTTCCCTGGCG	ORF SmAOC1
SmAOC1-R	TTAATCTGTGAAGTTTGGAGCAAC	ORF SmAOC1
SmAOC1SphI-F	TAGCATGCTCAGCTGCCATTGTCCC	Hetero-overexpression of SmAOC1
SmAOC1SalI-R	CGGTCGACTTAATCTGTGAAGTTTGGAG	Hetero-overexpression of SmAOC1
SmAOC1NdeI-F	AAAAGCAGGCTCCACCATGGCAAGTTCCCT	Sub-cellular localization of SmAOC1
SmAOC1EcoRV-R	AAGCTGGGTCTAGATTTAATCTGTGAAGTT	Sub-cellular localization of SmAOC1
SmOPR1-F	ATGGATGCGCCCCAGGAGCA	ORF SmOPR1
SmOPR1-R	AGCTATCGTTTTTCTTAATCTTCAAGGAAAGGA	ORF SmOPR1
SmOPR1EcoRI-F	GAATTCATGGATGCGCCCCAGG	Hetero-overexpression SmOPR1
SmOPR1NotI-R	GCGGCCGCATCTTCAAGGAAAGG	Hetero-overexpression SmOPR1
SmOPR5-F	ATGGAAAGCTCATCAAATCCTCTGA	ORF SmOPR5
SmOPR5-R	CTAAAGTTTGCTGGGGTGTTTCTT	ORF SmOPR5
SmOPR5BamHI-F	ATGGGTCGCGGATCCATGGAAAGCTCATCA	Hetero-overexpression SmOPR5
SmOPR5IXhoI-R	GTGGTGGTGCTCGAGAAGTTTGCTGGGGTG	Hetero-overexpression SmOPR5
SmJAR1-F	ATGCCAGGGATTCCATTGAT	ORF SmJAR1
SmJAR1-R	CTACTCTCTCCTCACTCCCG	ORF SmJAR1
SmJAR1BamHI-F	ATGGGTCGCGGATCCATGCCAGGGATTCCA	Hetero-overexpression SmJAR1
SmJAR1IXhoI-R	GTGGTGGTGCTCGAGCTCTCTCCTCACTCC	Hetero-overexpression SmJAR1

Supplementary Table S2. Oligonucleotide primers (continued).

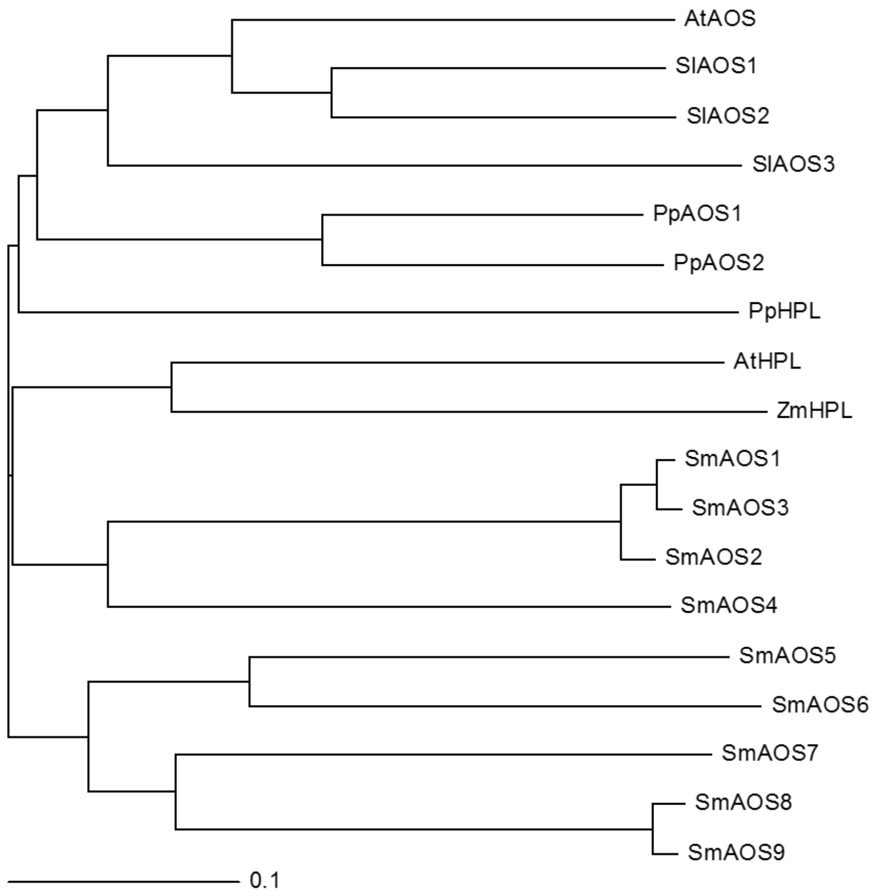
Primer Name	Sequence 5’ to 3’	Purpose
SmAOS1_F	CTTAGCAGCTCACACTTCAC	ORF SmAOS1
SmAOS1_R	GACATACACACTAGTGCATTTC	ORF SmAOS1
SmAOS2_F	CTTAGCAGCTCATACTTCACTTC	ORF SmAOS2
SmAOS2_R	GACAACGAACACAGGTGATTGTG	ORF SmAOS2
SmAOS3_F	CTTAGCAGCTCAAACTTCAC	ORF SmAOS3
SmAOS3_R	GCATTTCATTTCATGCTCGC	ORF SmAOS3
SmAOS1EcoRI-F	CCGAATTCATGAGCAACGACAGGAACCT	Hetero-overexpression SmAOS1
SmAOS1XhoI-R	TGCTCGAGTGCTCGCTTCTTGAGCTCGG	Hetero-overexpression SmAOS1
SmAOS2NdeI-F	TACATATGAGCAACGACAGGAACCT	Hetero-overexpression SmAOS2
SmAOS2XhoI-R	TGCTCGAGTGCTCGCTTCTTGAGCTCGG	Hetero-overexpression SmAOS2
qSmAOC1-F	TTCCCGACCAAGCTCTTCTA	Expression analysis of SmAOC1
qSmAOC1-R	AAGTTTGGAGCAACGGACTG	Expression analysis of SmAOC1
qSmOPR5-F	AGCTGTGCATGACAAAGGTG	Expression analysis of SmOPR5
qSmOPR5-R	GGACTGTCCATCTGGGAAGA	Expression analysis of SmOPR5
qSmJAR1-F	AGTATACCGCCCATGCTGAC	Expression analysis of SmJAR1
qSmJAR1-R	CCATGCAATCACAACACTCC	Expression analysis of SmJAR1
qSmUbi1-F	ATACCATCGGCGATTTGAAG	Reference gene ubiquitin
qSmUbi1-R	CGCTTACAAGGAAAGCACCT	Reference gene ubiquitin
SmActin-F	ACTGGGACGACATGGAGAAG	Reference gene actin
SmActin-R	CGCCTGAATAGCAACGTACA	Reference gene actin

Supplementary figures



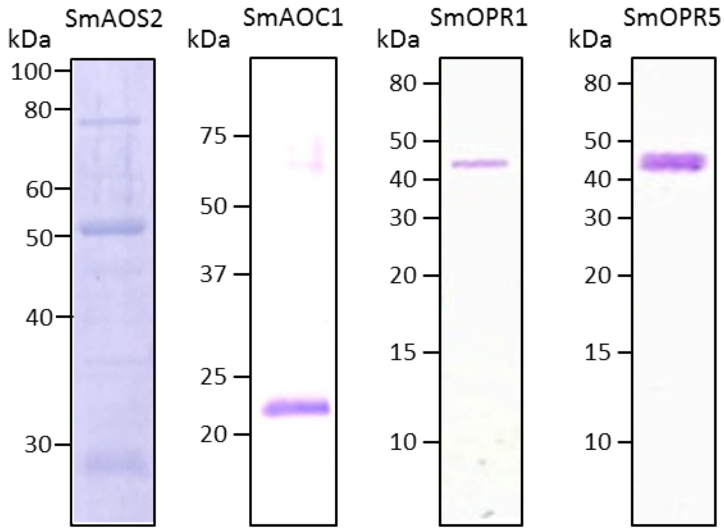
Supplementary Fig. S1. Amino acid sequence alignment of SmAOSs with the previously reported AOSs.

The Clustal Omega was used for the alignment. The conserved cysteine residue for heme-ligand binding is indicated with a red triangle. The I-helix GXXX(F/L), EXLR motif, and PXVXNKQCPG for heme-binding domain are in red box (Koeduka et al. 2015). The reported AOSs: PpAOS1 (*Physcomitrella patens*, CAC86919), PpAOS2 (*Physcomitrella patens*, XP_001759629), SlAOS1 (*Solanum lycopersicum*, CAB88032), SlAOS2 (*Solanum lycopersicum*, AAF67141), SlAOS3 (*Solanum lycopersicum*, AAN76867), AtAOS (*Arabidopsis thaliana*, CAA63266), AtHPL (*A. thaliana* hydroperoxide lyase, AAC69871), ZmHPL(*Zea mays* hydroperoxide lyase, AAS47027), and PpHPL (*P. patens* hydroperoxide lyase, CAC86920). AOS homologues in *Selaginella moellendorffii*: SmAOS1 (SELMODRAFT_271334), SmAOS2 (SELMODRAFT_177201), SmAOS3 (SELMODRAFT_228572), SmAOS4 (SELMODRAFT_133317), SmAOS5 (SELMODRAFT_81998), SmAOS6 (SELMODRAFT_177485), SmAOS7 (SELMODRAFT_92382), SmAOS8 (SELMODRAFT_98717), and SmAOS9 (SELMODRAFT_41357).



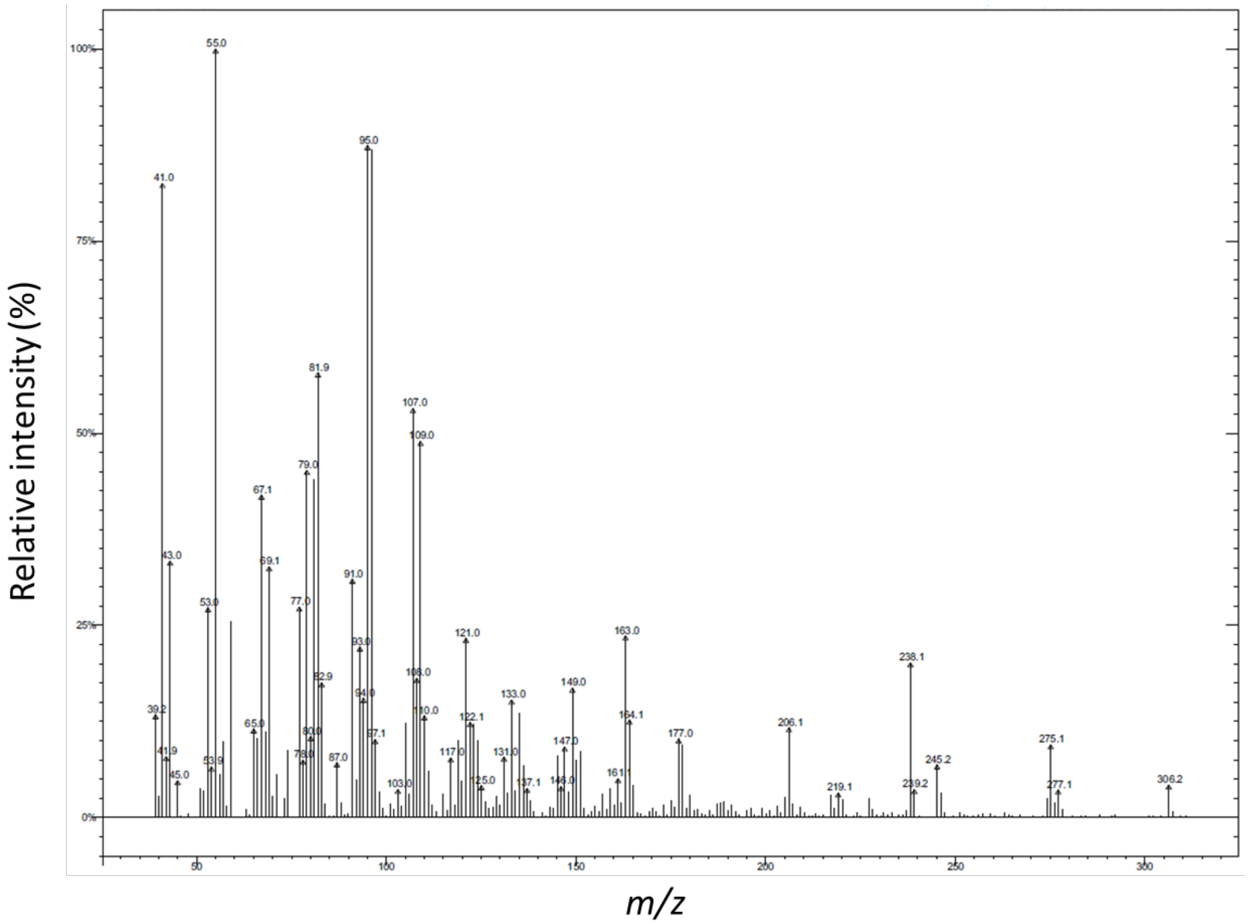
Supplementary Fig. S2. Phylogenetic tree of SmAOSs and previously reported AOSs.

Phylogenetic tree was constructed using TreeView X program based on amino acid sequence alignment. The bars represent evolutionary distance. The reliability of the tree measured by bootstrap analysis with 1,000 replicates. The reported AOSs: PpAOS1 (*Physcomitrella patens*, CAC86919), PpAOS2 (*Physcomitrella patens*, XP_001759629), SlAOS1 (*Solanum lycopersicum*, CAB88032), SlAOS2 (*Solanum lycopersicum*, AAF67141), SlAOS3 (*Solanum lycopersicum*, AAN76867), AtAOS (*Arabidopsis thaliana*, CAA63266), AtHPL (*A. thaliana* hydroperoxide lyase, AAC69871), ZmHPL(*Zea mays* hydroperoxide lyase, AAS47027), and PpHPL (*P. patens* hydroperoxide lyase, CAC86920). AOS homologues in *Selaginella moellendorffii*: SmAOS1 (SELMODRAFT_271334), SmAOS2 (SELMODRAFT_177201), SmAOS3 (SELMODRAFT_228572), SmAOS4 (SELMODRAFT_133317), SmAOS5 (SELMODRAFT_81998), SmAOS6 (SELMODRAFT_177485), SmAOS7 (SELMODRAFT_92382), SmAOS8 (SELMODRAFT_98717), and SmAOS9 (SELMODRAFT_41357).

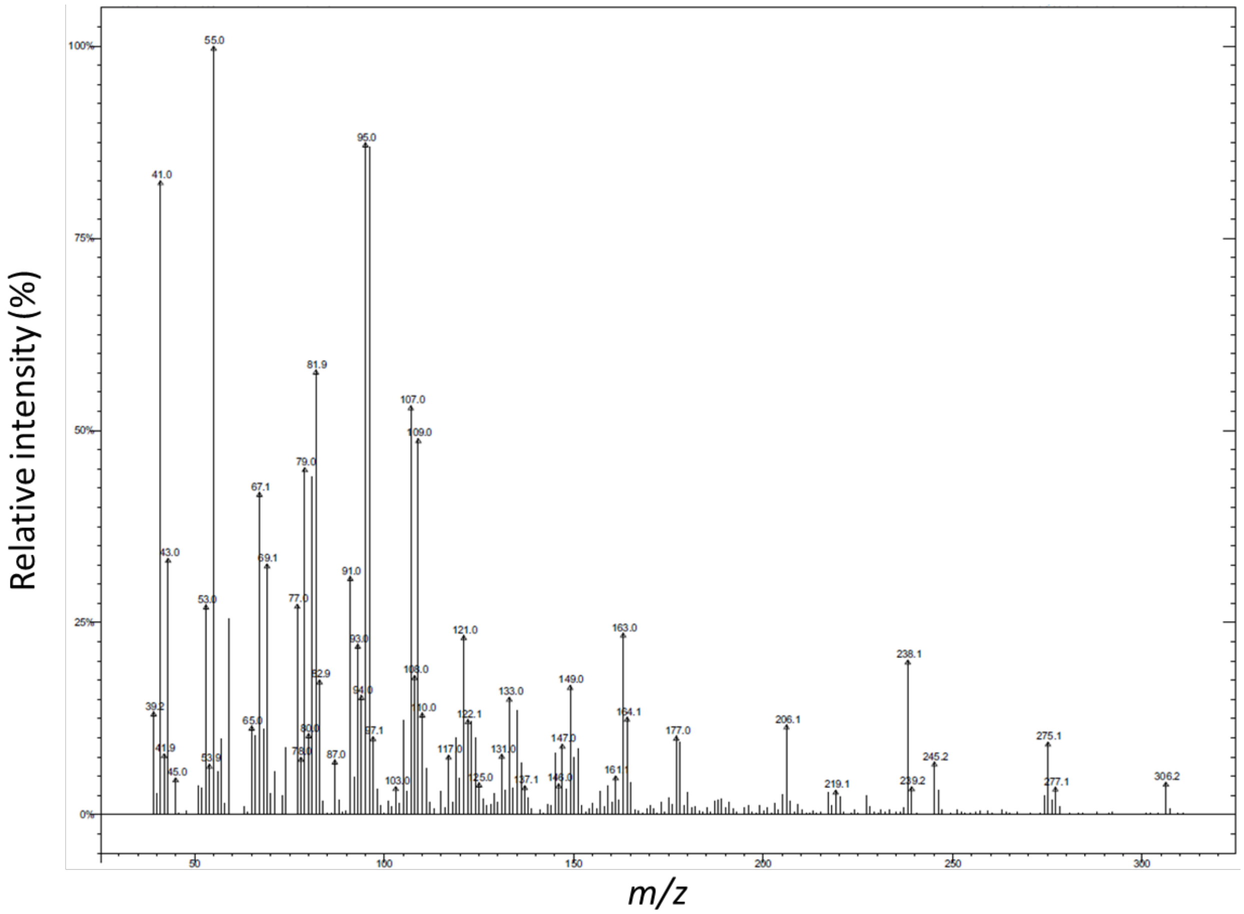


Supplementary Fig. S3. SDS-PAGE analysis of recombinant SmAOS2, SmAOC1, SmOPR1 and SmOPR5.

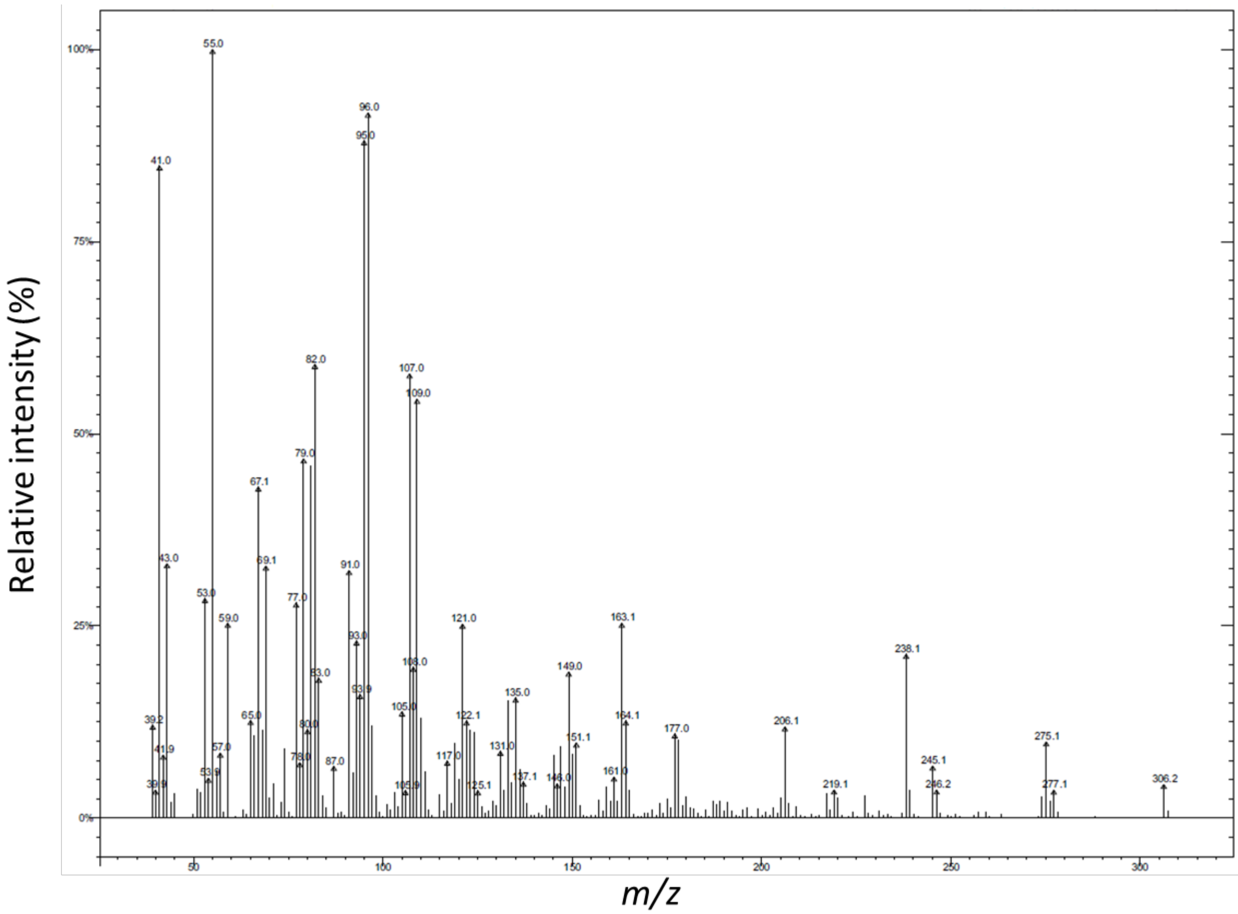
The recombinant proteins fused with His-tag were overexpressed in *E. coli* and were purified using Ni-NTA agarose column chromatography. SmAOC1 was analyzed by 15% SDS-PAGE. SmAOS1, SmOPR1, and SmOPR5 were analyzed by 10% SDS-PAGE, respectively. Proteins were stained by Coomassie Brilliant Blue (CBB).



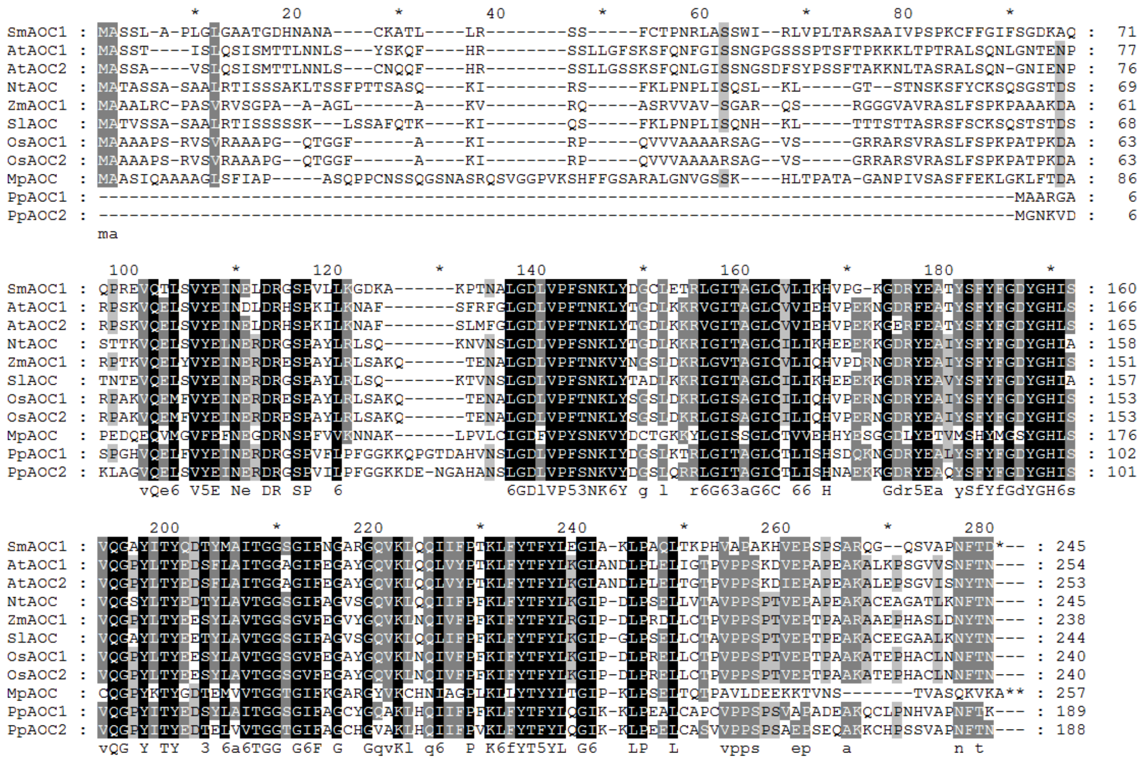
Supplementary Fig. S4. GC-MS spectrum of methyl ester of (+)-*trans*-OPDA.



Supplementary Fig. S5. GC-MS spectrum of methyl ester of (−)-*trans*-OPDA.

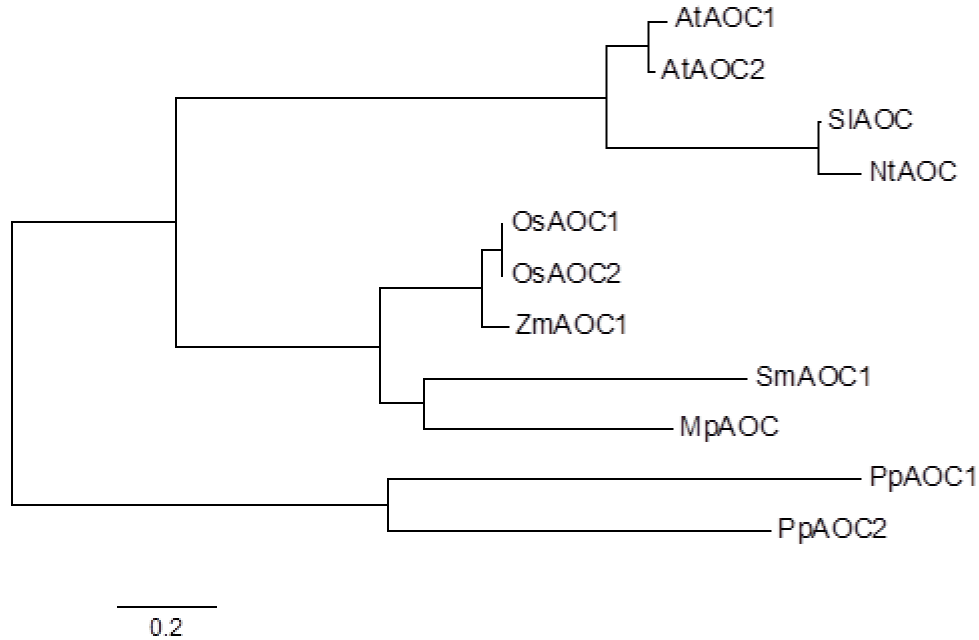


Supplementary Fig. S6. GC-MS spectrum of methyl ester of standard OPDA.



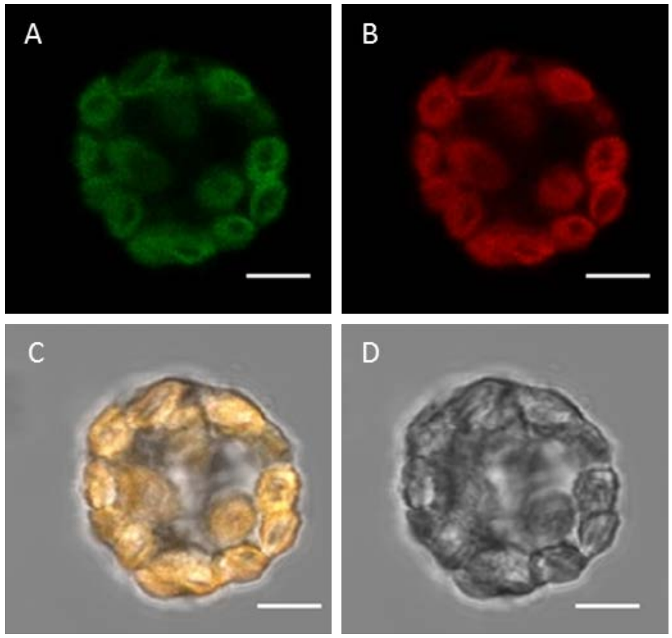
Supplementary Fig. S7. Amino acid sequence alignment of SmAOC1 with the previous reported AOCs.

Amino acid sequences were aligned using Clustal Omega. Identical and similar amino acids are highlighted in black and gray, respectively. The aligned sequences include SmAOC1 (*Selaginella moellendorffii*, Sm_91887), PpAOC1 (*Physcomitrella patens*, CAD48752), PpAOC2 (*Physcomitrella patens*, CAD48753), MpAOC (*Marchantia polymorpha*, BAO93687), AtAOC1 (*Arabidopsis thaliana*, AEE77065.1), AtAOC2 (*Arabidopsis thaliana*, AEE77066.1), ZmAOC (*Zea mays*, NP_001105245), OsAOC1 (*Oryza sativa*, ABV03555), OsAOC2 (*Oryza sativa*, ABV45432), SlAOC (*Solanum lycopersicum*, AAK62358), and NtAOC (*Nicotiana tabacum*, CAC83765).



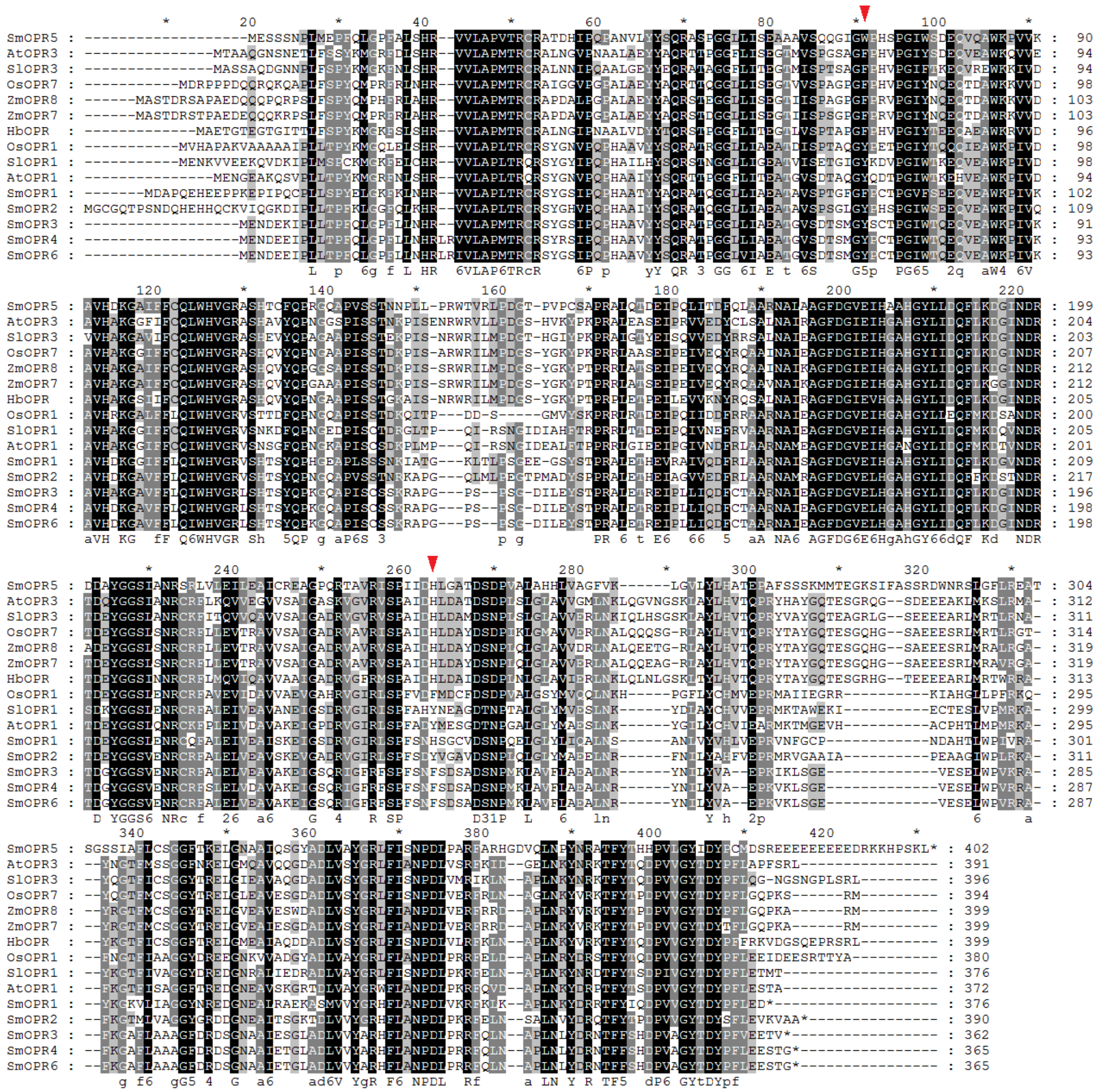
Supplementary Fig. S8. Phylogenetic tree of SmAOC1 and previously reported AOCs.

Phylogenetic tree was constructed using the neighbor-joining method with MEGA 5.2 program based on amino acid sequence alignment. The bars represent evolutionary distance. The reliability of the tree measured by bootstrap analysis with 1,000 replicates. The analysis was performed with following: SmAOC1 (*Selaginella moellendorffii*, Sm_91887), PpAOC1 (*Physcomitrella patens*, CAD48752), PpAOC2 (*Physcomitrella patens*, CAD48753), MpAOC (*Marchantia polymorpha*, BAO93687), AtAOC1 (*Arabidopsis thaliana*, AEE77065.1), AtAOC2 (*Arabidopsis thaliana*, AEE77066.1), ZmAOC (*Zea mays*, NP_001105245), OsAOC1 (*Oryza sativa*, ABV03555), OsAOC2 (*Oryza sativa*, ABV45432), SlAOC (*Solanum lycopersicum*, AAK62358), and NtAOC (*Nicotiana tabacum*, CAC83765).



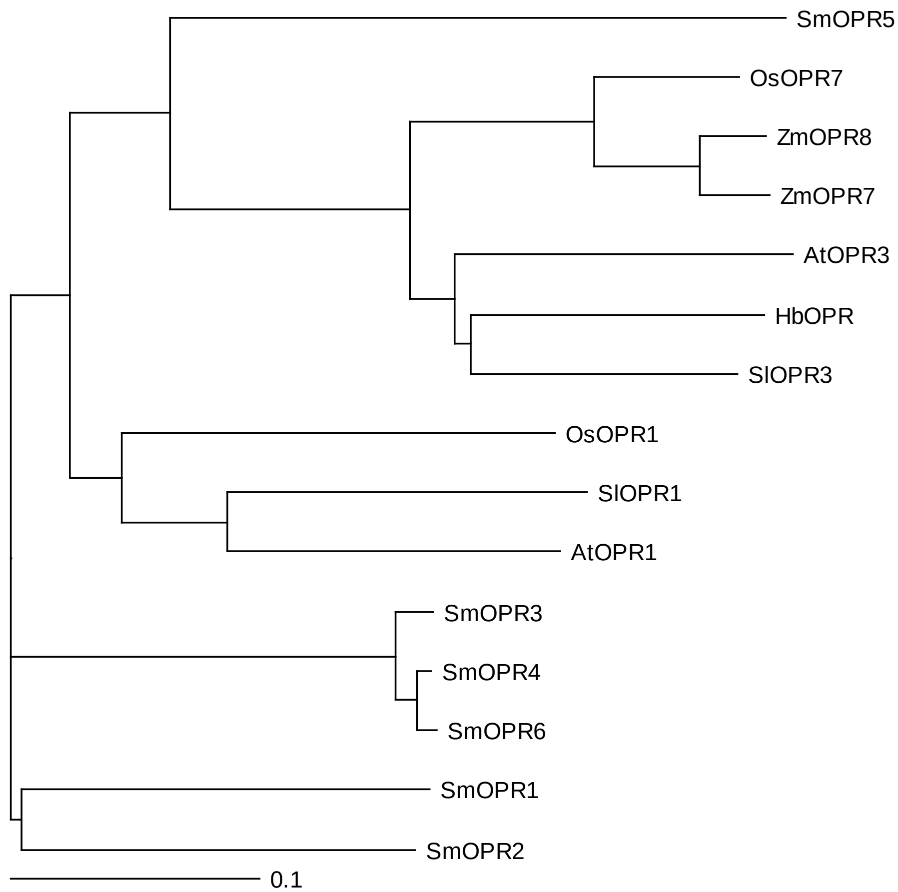
Supplementary Fig. S9. Expression of SmAOC1-GFP fusion protein in the chloroplast of *P. patens* protoplast.

The sub-cellular localization of SmAOC1 was analyzed by constructing the SmAOC1-GFP plasmid, which was introduced into the prepared protoplast of *P. patens* using PEG-mediated transformation method. Images were taken with a confocal laser-scanning microscope with an excitation of 488 nm and emission of 530 nm for detecting GFP and an emission above 655 nm for detecting autofluorescence from chlorophyll. (A) Green fluorescence of SmAOC1-GFP. (B) Red chlorophyll autofluorescence. (C) Merge of the green fluorescence and the red chlorophyll autofluorescence. (D) Bright field. Scale bar: 10 μm.



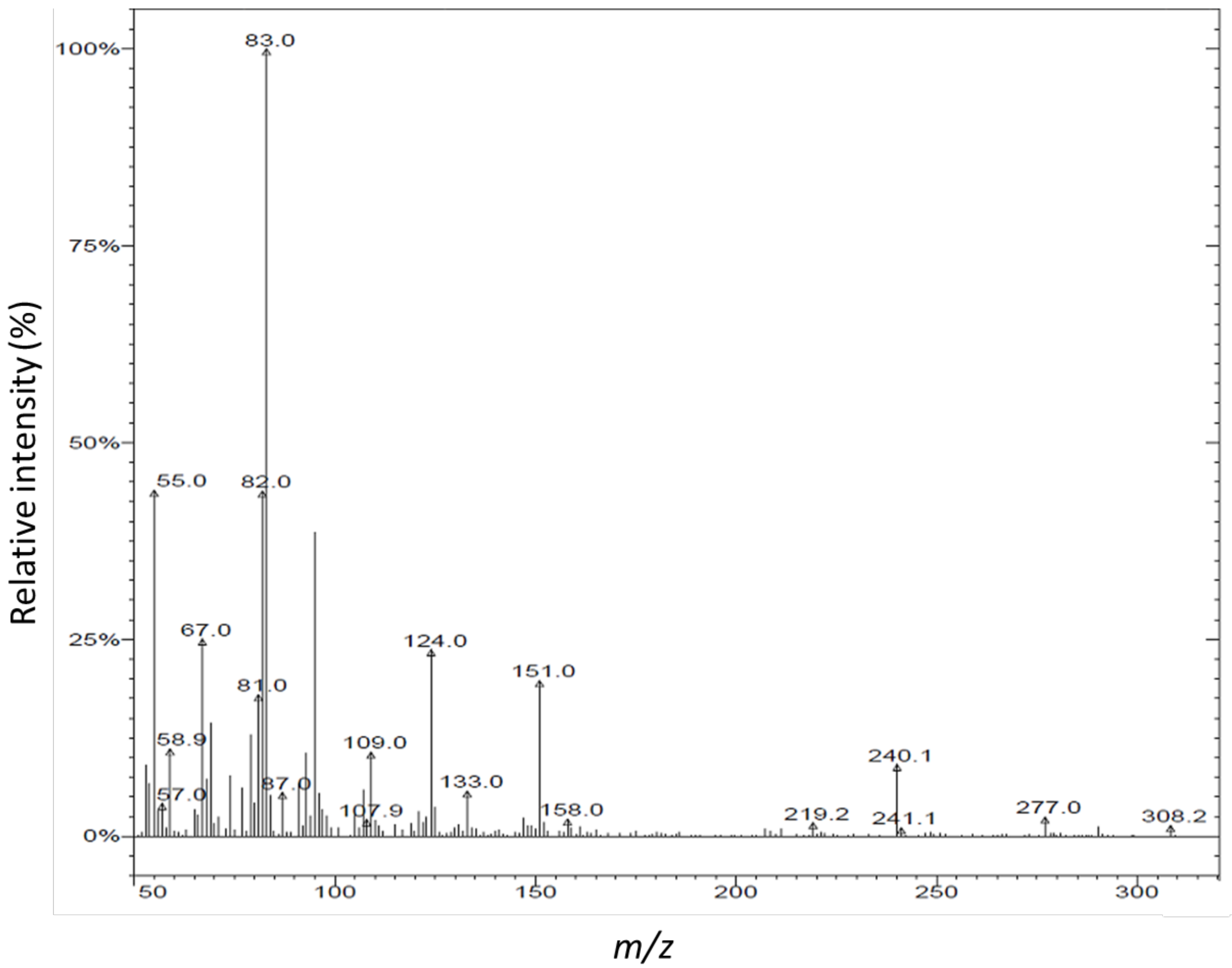
Supplementary Fig. S10. Amino acid sequence alignment of SmOPRs and other known OPRs.

Amino acid sequences were aligned using Clustal Omega. Identical and similar amino acids are highlighted in black and gray, respectively. The two specificity-determining residues are marked by red arrows. The aligned sequences include OPR3-like enzymes (AtOPR3: *Arabidopsis thaliana* OPR3, AEC06000; HbOPR: *Hevea brasiliensis* OPR, AAY27752; OsOPR7: *Oryza sativa* OPR7, Q6Z965; SlOPR3: *Solanum lycopersacum* OPR3, NP_001233873; ZmOPR7: *Zea mays* OPR7, NP_001105910; ZmOPR8, NP_001105833), OPR1-like enzymes (AtOPR1: *Arabidopsis thaliana* OPR1, AEE35875; OsOPR1: *Oryza sativa* OPR1, Q84QK0; SlOPR1: *Solanum lycopersacum* OPR1, NP_001234781), and SmOPRs. The numbering of SmOPRs was previously described by Li *et al*. (2009).

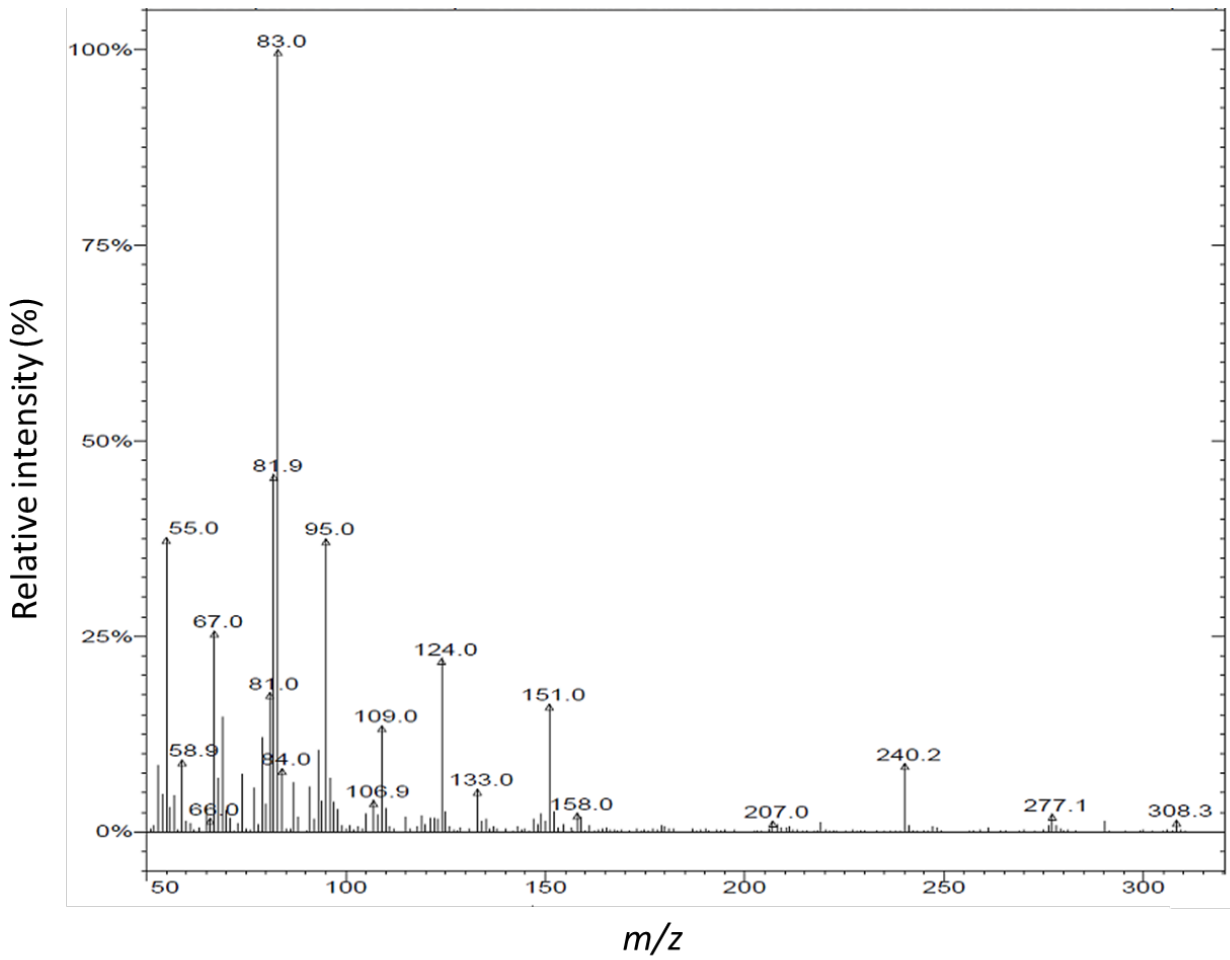


Supplementary Fig. S11. Phylogenetic tree of SmOPRs and previously reported OPRs.

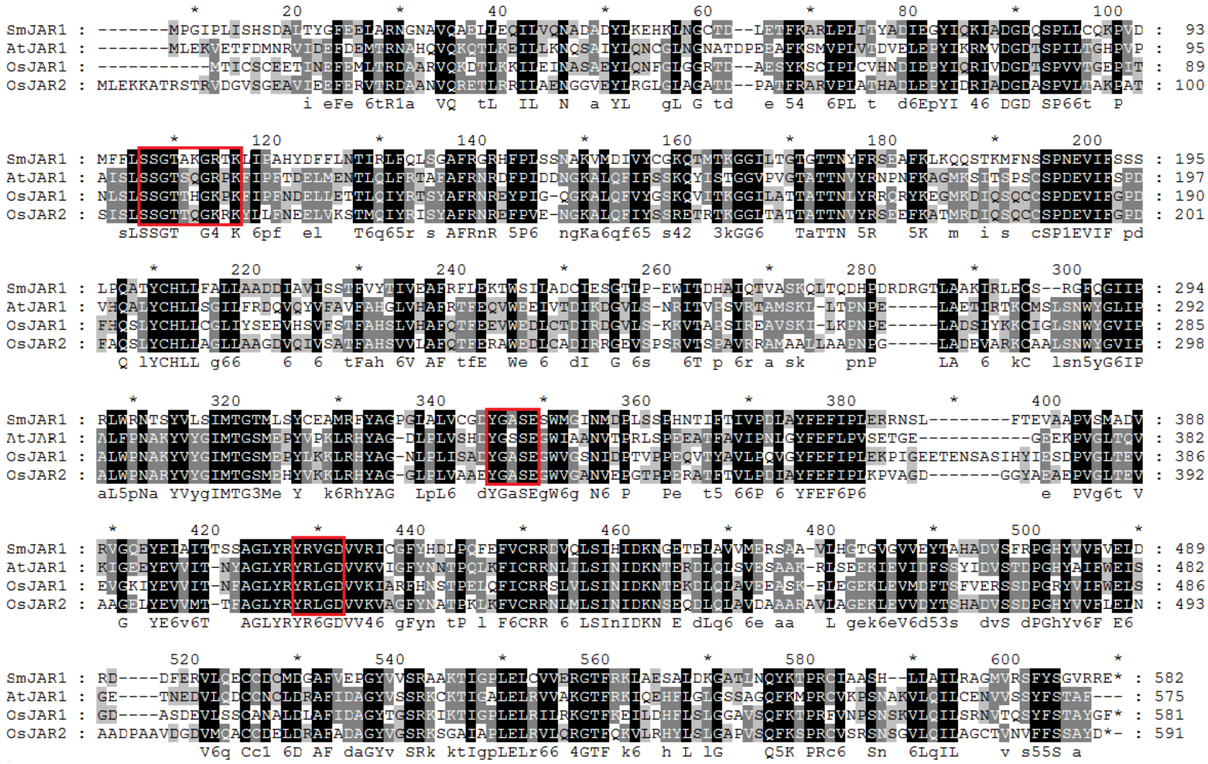
The OPR amino acid sequences were compared using Clustal Omega. The phylogenetic tree was visualized from the resulting alignment using TreeView X program. The analysis was performed with the following: OPR3-like enzymes (AtOPR3: *Arabidopsis thaliana* OPR3, AEC06000; HbOPR: *Hevea brasiliensis* OPR, AAY27752; OsOPR7: *Oryza sativa* OPR7, Q6Z965; SlOPR3: *Solanum lycopersacum* OPR3, NP_001233873; ZmOPR7: *Zea mays* OPR7, NP_001105910; ZmOPR8, NP_001105833), OPR1-like enzymes (AtOPR1: *Arabidopsis thaliana* OPR1, AEE35875; OsOPR1: *Oryza sativa* OPR1, Q84QK0; SlOPR1: S*olanum lycopersacum* OPR1, NP_001234781), and SmOPRs. The numbering of SmOPRs was previously described by Li et al. (2009).



Supplementary Fig. S12. GC-MS spectrum of the peak 1 of Fig. 6.

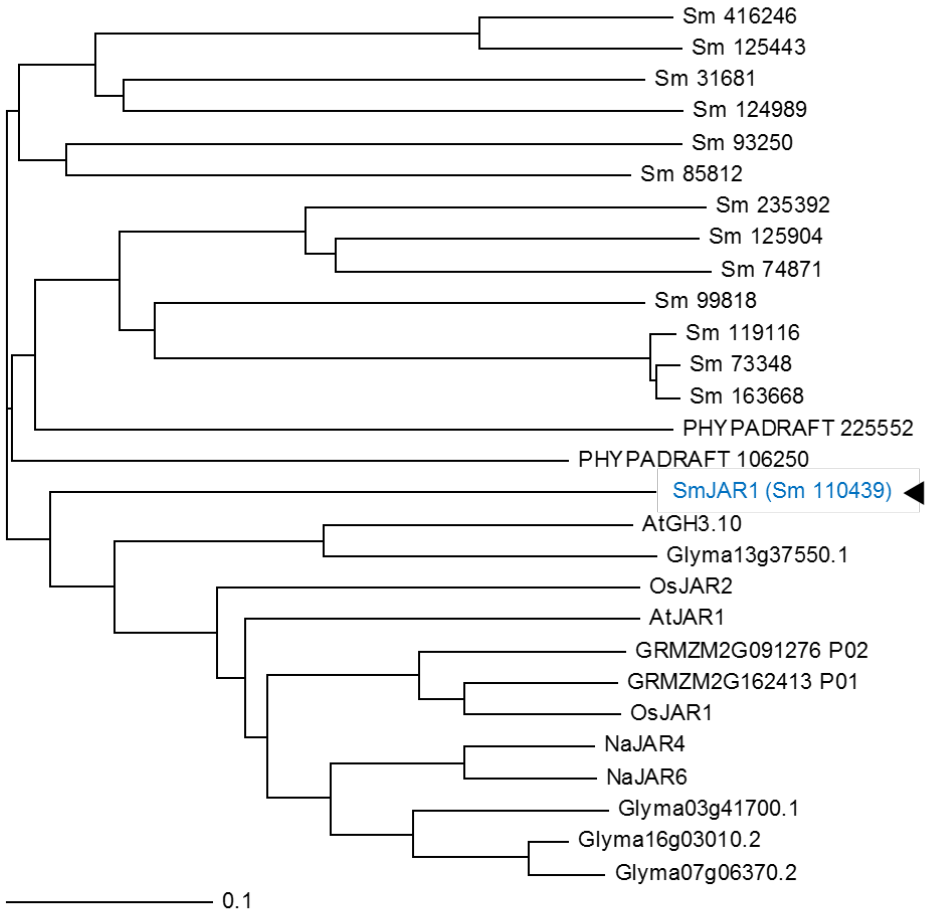


Supplementary Fig. S13. GC-MS spectrum of the standard methyl ester of OPC-8:0.



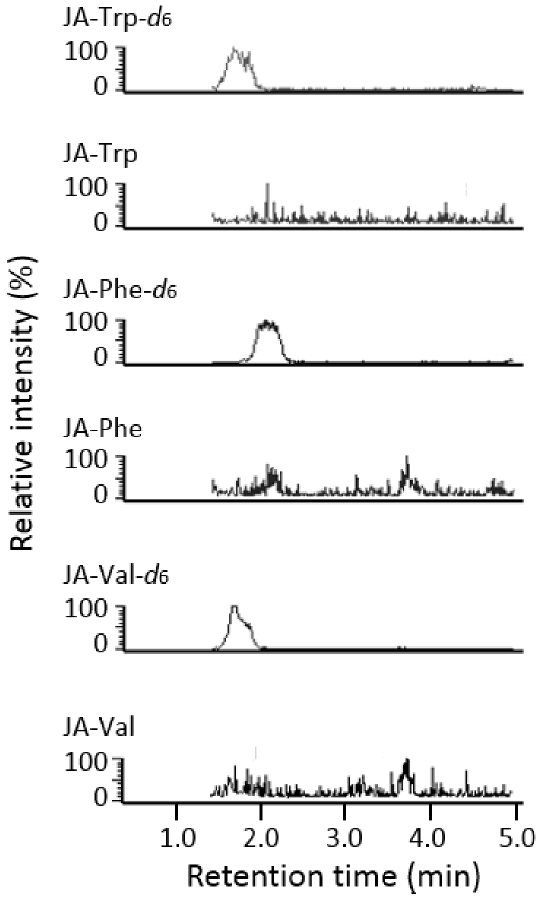
Supplementary Fig. S14. Amino acid sequence alignment of SmJAR1 with three GH3 family members.

Amino acid sequences were aligned using Clustal Omega. Identical and similar amino acids are highlighted in black and gray, respectively. The boxes indicates the three important motifs for enzymatic activity of GH3 proteins. The aligned sequences include AtJAR1 (*Arabidopsis thaliana*, AEC10684), OsJAR1 (*Oryza sativa*, LOC_Os05g50890.1) and OsJAR2 (*Oryza sativa*, LOC_Os01g12160.1).



Supplementary Fig. S15. Phylogenetic tree of JAR1-like proteins in *S. moellendorffii* with other JAR1s.

The tree was generated from amino acid alignment and constructed using Clustal Omega before visualizing with TreeView X program. The analysis was performed with the following: AtJAR1 (*Arabidopsis thaliana*, AEC10684), AtGH3.10 (*Arabidopsis thaliana*, At4g03400), OsJAR1 (*Oryza sativa*, LOC_Os05g50890.1), OsJAR2 (*Oryza sativa*, LOC_Os01g12160.1), Glyma16g03010.2 (*Glycine max*), Glyma07g06370.2 (*Glycine max*), Glyma03g41700.1 (*Glycine max*), Glyma13g37550.1 (*Glycine max*), NaJAR4 (*Nicotiana attenuate*, ABC87760.1), NaJAR6 (*Nicotiana attenuate*, ABC87761.1), GRMZM2G091276_P02 (*Zea mays*), GRMZM2G162413_P01 (*Zea mays*), PHYPADRAFT_106250 (*Phsycomitrella patens*), and PHYPADRAFT_225552 (*Phsycomitrella patens*).

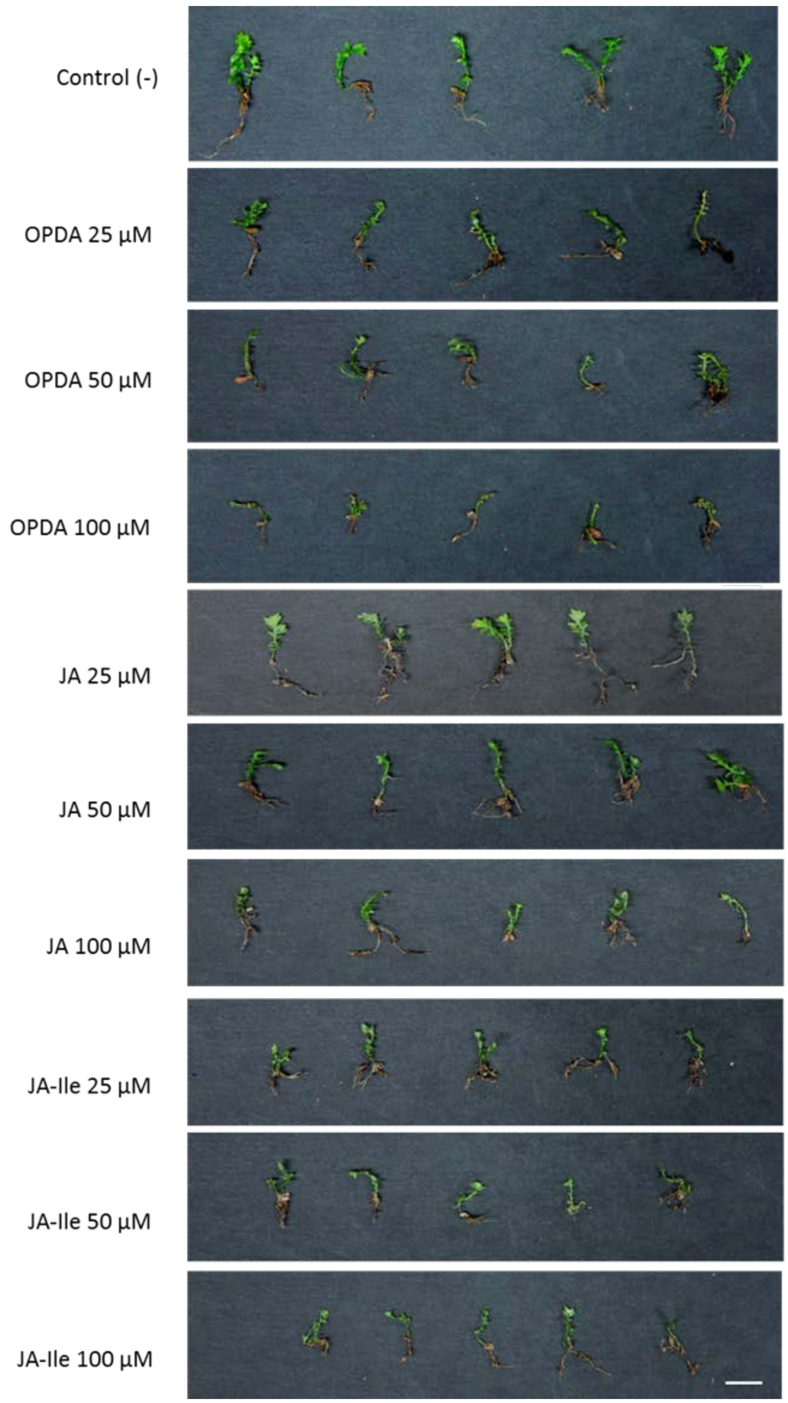


Supplementary Fig. S16. UPLC-MS/MS analysis of conjugates of JA and amino acid (JA-Trp, JA-Phe, and JA-Val) in the culture supernatant of *E. coli* expressing *SmJAR1*.

No obvious peak corresponding to JA-Trp, JA-Phe, and JA-Val was detected in the culture supernatant of *E. coli* expressing *SmJAR1*. Detailed experimental conditions are described in Materials and Methods.

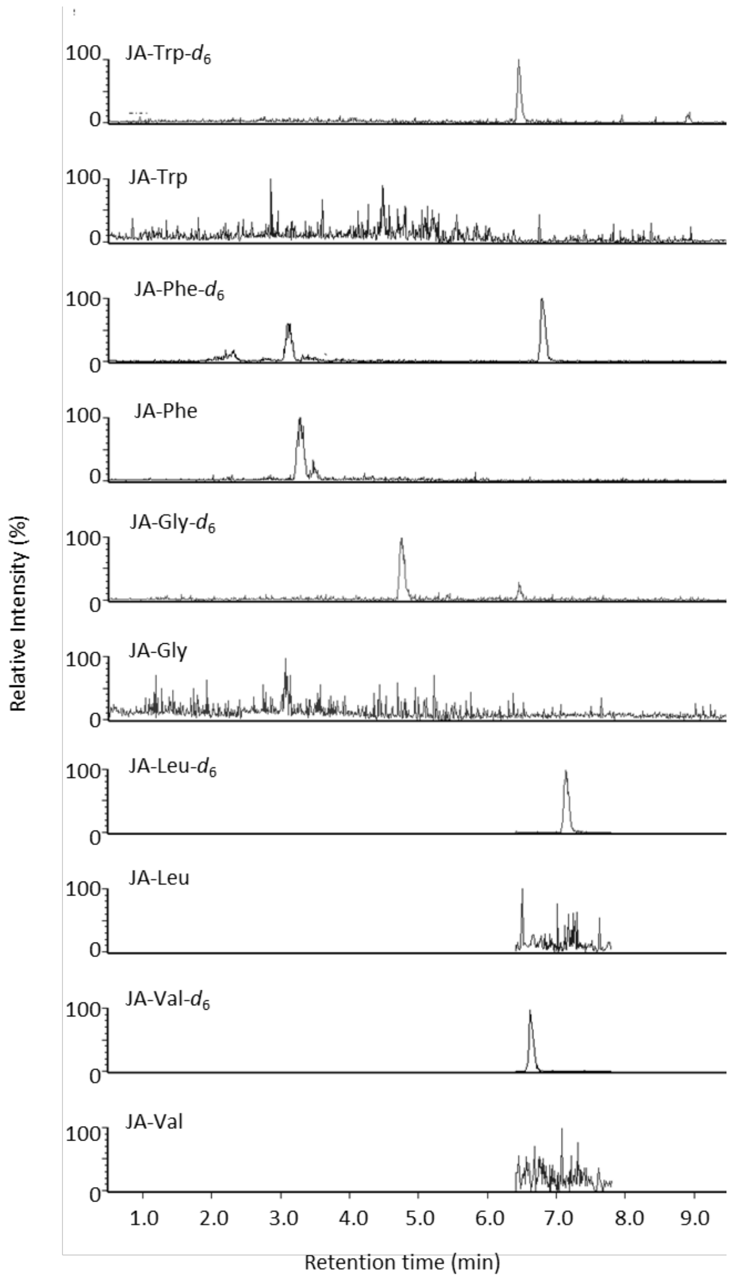
Supplementary Fig. S17. The morphology of a microphyll of *S. moellendorffii*.

The arrow indicates a bulbil.



Supplementary Fig. S18. Growth inhibitory activities of OPDA, JA, and JA-Ile in *S. moellendorffii*.

S. moellendorffii was treated with OPDA, JA, or JA-Ile at a concentrations of 25, 50, and 100 μM. Detailed experimental conditions were described in Materials and Methods. Scale bar: 5 mm.



Supplementary Fig. S19. UPLC-MS/MS analysis of endogenous JA conjugation with other amino acids (Trp, Phe, Gly, Leu, and Val) in wounded *S. moellendorffii*.

No obvious peak corresponding to JA-Trp, JA-Phe, JA-Gly, JA-Leu, and JA-Val was detected in the extract of wounded *S. moellendorffii*.