

Supplementary Materials and Methods

Recombinant expression, purification and in vitro inhibition studies of PPO2

Preparation of auto-induction medium

Single colonies were picked and used to inoculate 1.5 ml of LB or auto-induction media (AIM) containing 50 µg/ml kanamycin. AIM contains the following components: 1% N-Z amine AS (Gibco), 0.5% yeast extract (Gibco), 25 mM Na₂HPO₄ (Merck), 25 mM KH₂PO₄ (Merck), 50 mM NH₄Cl (Merck), 5 mM Na₂SO₄ (Merck), 2 mM MgSO₄ (Merck), 0.5% glycerol (Gerbu), 0.05% glucose (Sigma) and 5.6 mM α-lactose (Sigma). In addition, 0.2x or 1x trace metals were added from a 1000x stock solution containing 0.1 M FeCl₃, 1M CaCl₂, 1M MnCl₂-4H₂O, 1 M ZnSO₄-7H₂O, 0.2 M CoCl₂-6H₂O, 0.1 M CuCl₂-2H₂O, 0.2 M NiCl₂-6H₂O, 0.1 M Na₂MoO₄-2H₂O, and 0.1 M H₃BO₃. LB and TB cultures were induced with 0.1 mM IPTG when the OD₆₀₀ of at least three cultures reached 0.6 – 0.7.

Preparation of substrate

8.1 mg protoporphyrin - IX [CAS-No. 553-12-8] were weighed into a 100 ml three-necked flask. Then 33.5 ml of the solvent (10mM KOH, 20% Ethanol in deionized water) was added and stirred for 30 min in the light. 40.0 g of sodium alum (4-5% Na) [CAS-No. 11110-52-4] from ABCR (AB 109084) were placed in a 100 ml three-necked flask. Then, the protoporphyrin solution was added dropwise. All steps above were performed under nitrogen. Reduction took place for 10min. After reduction the protoporphyrin was filtered in another flask also filled with sodium amalgam. Wash buffer (33.5 ml) is first reduced in the same flask where the protoporphyrin was reduced, then also filtered and added to the flask with protoporphyrin (Wash buffer was prepared using 250mM Tris HCl PH 8.5, 5mM EDTA, 5mM GSH in deionized water). Another reduction took place for 10 minutes. Then the substrate was filtered into flask under nitrogen and immediately aliquoted in 1.5ml eppendorf tubes (800 ml aliquots). 400µl Paraffin was added, and the tubes frozen on dry ice and stored dark at -80°C. The final concentration of the substrate was 216 µM.

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      10      20      30      40      50      60      70      80      90     100
SS  MLIQSITHLSPKLA LPSPLSISAKNYPVAVMGNISERE EPTS AKRVA VVGAGV SGLAAAYK LKSHGL SVTLFE ADSRAGGK LKTVK KDDGF I WDEGAN TMT
S1  .....D.....N.....
S2  .....N.....
S3  .....N.....
R1  .....D.....N.....
R2  .....N.....
R3  .....D.....N.....
R4  .....N.....
R5  .....N.....

     110     120     130     140     150     160     170     180     190     200
SS  ESEAEVSSLIDDLGLREKQLPISQNKRYIARDGLPVLLPSNPAALLTSNFLSAKSKLQIMLEPFLWKKRNATELSDEHVQESVGEFFERHFGKEFVDYV
S1  .....
S2  .....
S3  .....
R1  .....
R2  .....
R3  .....
R4  .....
R5  .....

     210     220     230     240     250     260     270     280     290     300
SS  IDPFVAGTCGGDPQSLSMHHTFPDVWNVEKRFGSVFAGLIQSTLLSKKEKGGGENASIKKPRVRGFSFHHGGMQLVDIMCKQLGEDELKLCQCEVLSLSY
S1  .....
S2  .....
S3  .....
R1  .....
R2  .....
R3  .....
R4  .....
R5  .....

     310     320     330     340     350     360     370     380     390     400
SS  NQKGIPLSLGNWSVSSMSNNISEDQSYDAVVVIAPIRNVKEMKIMKFGNPFSLDFIPEVTYVPLSVMITAFKKDKVKRPLEGFGVLIPSKEQHNGKLTIGT
S1  .....
S2  .....
S3  .....
R1  .....G.....A.....
R2  .....A.....
R3  .....A.....
R4  .....A.....
R5  .....A.....

     410     420     430     440     450     460     470     480     490     500
SS  LFSMMFPDRAPSDMCLFTIFVGGSRNRKLAKASTDELKQIVSSDLQQLGTEDEPSFVNHLFWSNAFPLYGHNYDSVLR AIDKMEKDLPGFFYAGNHKG
S1  .....
S2  .....
S3  .....
R1  .....N.....
R2  .....
R3  .....
R4  .....
R5  .....N.....

     510     520     530
SS  GLSVGKAMASGCKAAELVLSYLD SHLYVKMDEKTA
S1  .....
S2  .....
S3  .....
R1  .....
R2  .....
R3  .....
R4  .....
R5  .....

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Fig. S1: PPO2 amino acid alignments

Full-length amino acid sequence of R1-R5 and S1-S3 from the same population was aligned with *A. palmeri* SS (ATE88443) as a reference. Dots represent identity at that position compare to SS. Amino acid numbers are indicated above the reference sequence.

Amaranthus palmeri (ATE88443.1)
Amaranthus hypochondriacus (ABS72165.1)
Amaranthus tuberculatus (ABD52326.1)
Spinacia oleracea (XP_021854936.1)
Chenopodium quinoa (XP_021775105.1)
Beta vulgaris (XP_010693290.1)
Vitis vinifera (XP_002263321.1)
Ziziphus jujube (XP_015867153.1)
Jatropha curcas (XP_020534796.1)
Prunus avium (XP_021833288.1)
Malus domestica (XP_008341682.1)
Theobroma cacao (EOY02725.1)
Juglans regia (XP_018849245.1)
Carica papaya (XP_021890416.1)
Herrania umbratica (XP_021299062.1)
Momordica charantia (XP_022133942.1)
Gossypium raimondii (KJB76437.1)
Cucumis sativus (XP_011656375.1)
Cajanus cajan (XP_020218703.1)
Cucumis melo (XP_008441360.1)
Gossypium hirsutum (XP_016747921.1)
Nicotiana tabacum (XP_016496870.1)
Gossypium raimondii (KJB76435.1)
Capsicum annuum (XP_016570686.1)
Cajanus cajan (KYP63379.1)
Solanum lycopersicum (NP_001335305.1)
Nicotiana tabacum (XP_016434987.1)
Arachis duranensis (XP_015971359.1)
Solanum pennellii (XP_015070810.1)
Lupinus angustifolius (XP_019458023.1)
Hevea brasiliensis (XP_021645933.1)
Solanum tuberosum (XP_006356026.1)
Brassica napus (XP_009121685.1)
Coffea canephora (CDP16674.1)

PSKEQ-HNGLKTLGTLFSSMMFPDRAPSDMCLFTTFV
 PSKEQ-HNGLKTLGTLFSSMMFPDRAPSDMCLFTTFV
 PSKEQ-HNGLKTLGTLFSSMMFPDRAPSDMCLFTTFV
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 PSNEQ-HNGLKTLGTLFSSMMFPDRAPSDVYLYTTTFV
 PSNEQ-HNGLKTLGTLFSSMMFPDRAFSDMYLYTTTFV
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 PSKEQ-ENGLKTLGTLFSSMMFPDRAPSDLYLYTTTFV
 PSEQ-KHGLKTLGTLFSSMMFPDRAPNNVYLYTTTFV
 PSIEQ-KHGFKTLGTLFSSMMFPDRAPSDLHLYTTTFI
 PSKEQ-QNGLKTLGTLFSSMMFPDRAPNDTYLYTTTFV

Brassica napus (XP_013667641.1)
Eucalyptus grandis (XP_010034660.1)
Nelumbo nucifera (XP_010250782.1)
Brassica napus (CDY57892.1)
Oryza sativa (XP_025880545.1)
Setaria italic (XP_004976030.1)
Panicum hallii (XP_025825969.1)
Sorghum bicolor (XP_002446710.1)
B. distachyon (XP_024311695.1)
Zea mays (NP_001105004.2)
Ananas comosus (XP_020111709.1)
Triticum aestivum (SPT18865.1)
Elaeis guineensis (XP_019708490.1)

Arabidopsis thaliana (NP_196926.2)
Chenopodium quinoa (XP_021730637.1)
Beta vulgaris (XP_010693036.1)
Amaranthus tuberculatus (ABD52324.1)
Vitis vinifera (CAN69262.1)
Citrus clementina (XP_006444625.1)
Populus euphratica (XP_011025274.1)
Populus trichocarpa (XP_002320252.2)
Medicago truncatula (XP_013469143.1)
Gossypium hirsutum (XP_016695806.1)
Brassica napus (XP_013666368.1)
Carica papaya (XP_021897188.1)
Gossypium hirsutum (XP_016710428.1)
Nelumbo nucifera (XP_010240808.1)
Nicotiana tabacum (NP_001313110.1)
Arabidopsis lyrata (XP_020877341.1)
Momordica charantia (XP_022146407.1)
Camelina sativa (XP_010428671.1)
Glycine max (XP_003536005.1)
Solanum lycopersicum (NP_001335308.1)

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PSAEQ-KNGLRTLGLTLFSSMMFPDRAPTELYLYTTTFI
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PTKEQ-KHGFKTLGTLFSSMMFPDRPCSDLHLYTTTFI
PYSKEQHNLKTLGTLFSSMMFPDRAPSDMCLETTTFV
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PSKEQE-NGLKTLGTLFSSMMFPDRAPSDQYLYTTTFI
PFKEQQKHGLKTLGTLFSSMMFPDRAPNDQYLETTTFI
PFKEQQKHGLKTLGTLFSSMMFPDRAPNDQYLYTTTFV

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GQLHPRSQGVETLGTIYSSSLFPGRAPPGRRTLILSYI
GQLHPRSQGVETLGTIYSSSLFPGRAPPGRILILSYI
GQLHPRSQGVETLGTIYSSSLFPGRAPPGRRTLILSYI
GQLHPRSQGVETLGTIYSSSLFPNRAPGRILLNLYI
GQLHPRSQGVETLGTIYSSSLFPNRAPAGRVLLNLYI
GQLHPRSQGVETLGTIYSSSLFPNRAPTRILLNLYI
GQLHPRSQGVETLGTIYSSSLFPNRAPAGRILLNLYI
GQLHPRSQGVQTLGTIYSSSLFPNRAPGRVLLNLYI
GQLHPRSQGIETLGTIYSSSLFPNRAPSGRVLLNLYI
GQLHPRTQKVETLGTIYSSSLFPNRAPGRVLLNLYI
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GQLHPRTQGVETLGTIYSSSLFPNRAPGRVLLNLYI
GQLHPRSQGVETLGTIYSSSLFPNRAPGRVLLNLYI
GQLHPRSQGVETLGTIYSSSLFPNRAPNGRVLLNLYI

<i>Solanum pennellii</i> (XP_015058045.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPNGRVLLLNYI
<i>Capsicum chinense</i> (PHU30347.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPNGRVLLLNYI
<i>Capsicum baccatum</i> (PHT59762.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPNGRVLLLNYI
<i>Oryza sativa</i> (XP_015626054.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPAGRVLLLNYI
<i>Panicum miliaceum</i> (RLM91355.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPAGRVLLLNYI
<i>Setaria italica</i> (XP_004967639.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPAGRVLLLNYI
<i>Zea mays</i> (NP_001105564.2)	GQLHPRSQGVETLGTIYSSSLFPNRAPDGRVLLLNYI
<i>B. distachyon</i> (XP_003567497.2)	GQLHPRSQGVETLGTIYSSSLFPNRAPAGRVLLLNYI
<i>Sorghum bicolor</i> (XP_002455484.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPAGRVLLLNYI
<i>Aegilops tauschii</i> (XP_020151190.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPAGRVLLLNYI
<i>Phoenix dactylifera</i> (XP_008800315.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPPGRVLLLNYI
<i>Ananas comosus</i> (OAY70844.1)	GQLHPRSQGVETLGTIYSSSLFPNRAPPGRVLLLNYI

Fig. S2: Multiple sequence alignment of eukaryotic PPO proteins showing conservation of G399 at the protein level

Representative eukaryotic species aligned using Cobalt analysis (NCBI). Alignment with *A. palmeri* PPO2 is shown on the top line and the region around G399 position is used for comparison. Arrowheads represent 399 position in *A. palmeri* PPO2 protein. Dark purple and light purple box represents conserved glycine for, PPO2 and PPO1 protein, respectively across representative eukaryotic species

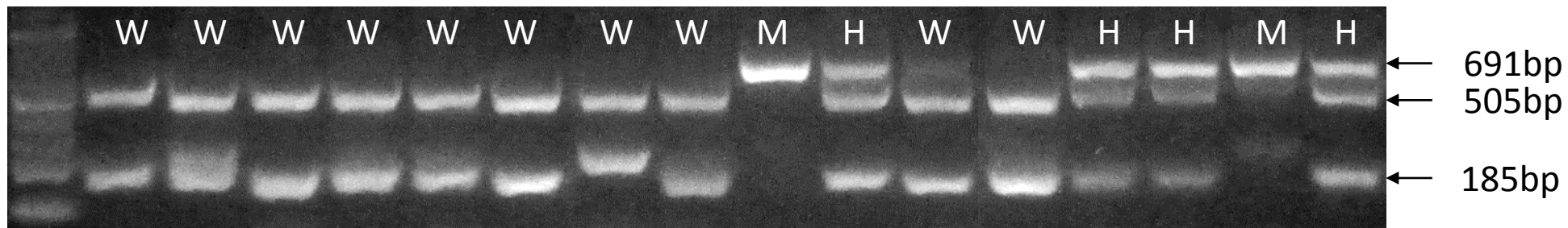


Fig. S3: Representation of detection of G399A mutation on 3 % agarose gel.

Genomic DNA isolated from fomesafen resistant plants was subjected to PCR using *PPO2* gene specific primers flanking mutation site. The resulted PCR product was digested with *RsaI* restriction enzyme to distinguish the WT allele and different state of mutant allele. M refers to presence of mutant allele in homozygous state, H refers to presence of mutant allele in heterozygous state, W refers to wild type allele (absence of mutant allele)

Table S1: Relative activity of purified enzymes

Enzyme	Maximum fluorometric measurements(A) (FU/min)	Amount of each enzyme(B) (ng)	Enzyme activity C=A/B	% Relative activity (C/enzyme activity of WT*100)
Wild-type	1128	25	45	100
G339A	400	300	1.3	3
Δ G210	2172	200	11	24
R128L	1899	100	19	42