

**Supplemental Figure S1. The phylogenetic tree of CYP72A protein in rice, maize and Arabidopsis.**

The phylogenetic tree was generated by the CLUSTAL W algorithm. Black, blue and green characters indicate CYP72A proteins of rice, maize and Arabidopsis, respectively. Rice and maize P450 deduced amino acid sequences were used in RAP-DB (<http://rapdb.dna.affrc.go.jp/>) or Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/index.shtml>) based on the information listed in Cytochrome P450 Homepage (<http://drnelson.uthsc.edu/CytochromeP450.html>). Arabidopsis P450 sequences were used in The *Arabidopsis* Cytochromes P450, Cytochromes b<sub>5</sub>, P450 reductase, β-Glucosidase, and Glycosyltransferase Site ([http://www.p450.kvl.dk/At\\_cyps/types.shtml](http://www.p450.kvl.dk/At_cyps/types.shtml)).

CYP72A32 (Kas) 1 **MV**LG**GW**L**LMWAPASSPTILVAFGLLFGLVLAWQAGLQLHRLWWRPRRLEKALRARGLRGS**  
 CYP72A32 (NB) 1 **MV**LG**GW**L**LMWAPASSPTILVAFGLLFGLVLAWQAGLQLHRLWWRPRRLEKALRARGLRGS**  
 CYP72A33 (Kas) 1 -----**MWAPASSPTILAAFG-LVGLVLAWQAGLQLHRLWWRPRRLEKALRARGLRGS**  
 CYP72A33 (NB) 1 -----**MWAPASSPTILAAFG-LVGLVLAWQAGLQLHRLWWRPRRLEKALRARGLRGS**  
 CYP72A31 (Kas) 1 **MVFRG-WLMWAPAS-APV**LV**VFGLLFGLALVWQAGRL**L**HRLWWRPRRLEKALRARGLRGS**

CYP72A32 (Kas) 61 **SYRFLTGD**L**AEE**S**RRRKEAWARPLPLRCHDIAPRIE**P**FLHDAV**VR**PEQ****HYCKPCITW**L**GP**  
 CYP72A32 (NB) 61 **SYRFLTGD**L**AEE**S**RRRKEAWARPLPLRCHDIAPRIE**P**FLHDAV**VR**PEQ****HYCKPCITW**L**GP**  
 CYP72A33 (Kas) 52 **R****YRFLTGD**L**AEE**S**RRRKEAWARPLPLRCHDIAPR**V**EPFLHGA**V**G**-**VGA**A**HCKP**R**I**T**WFGP**  
 CYP72A33 (NB) 52 **R****YRFLTGD**L**AEE**S**RRRKEAWARPLPLRCHDIAPR**V**EPFLHGA**V**G**-**VGA**A**HCKP**R**I**T**WFGP**  
 CYP72A31 (Kas) 59 **SYRFLTGD**L**AEE**S**RRRKEAWARPLPLRCHDIAPRI**K**PFLHDT**L**G**-**EHG**K**QR**Q**PCITWFGP**

CYP72A32 (Kas) 121 **TPEVHV**TD**PELAKVVM**SN**KFGHFEK****IRFQA**L**SKLLPQGLSYHEGEKWAKHRRILNPAFQL**  
 CYP72A32 (NB) 121 **TPEVHV**TD**PELAKVVM**SN**KFGHFEK****IRFQA**L**SKLLPQGLSYHEGEKWAKHRRILNPAFQL**  
 CYP72A33 (Kas) 111 **TPEVHV**A**DPELARVVLSN**K**F**G**HFEKVS**F**P**E**LSKL**T**PQGLSA**H**E**G**EKWAKHRRILN**P**VFQL**  
 CYP72A33 (NB) 111 **TPEVHV**A**DPELARVVLSN**K**F**G**HFEKVS**F**P**E**LSKL**T**PQGLSA**H**E**G**EKWAKHRRILN**P**VFQL**  
 CYP72A31 (Kas) 118 **TPEV**N**ITDPELAKV**LS**N**K**F**G**H**L**ER**V**R**F**KEVSKLLSQGL**T**YHEGEKW**V**KHRRILNPAFQL**

CYP72A32 (Kas) 181 **EKLKLM**LP**VFSACCEELISR**W**MGAT**IG**SDGSYE**V**DCWPEL**K**SLTGDVISRTAFGSSYLEGR**  
 CYP72A32 (NB) 181 **EKLKLM**LP**VFSACCEELISR**W**MGAT**IG**SDGSYE**V**DCWPEL**K**SLTGDVISRTAFGSSYLEGR**  
 CYP72A33 (Kas) 171 **EKLKLM**LP**VFSACCEELISR**W**MG**S**IGSDGSYE**V**DCWPE**F**KSLTGDVISRTAFGSSYLEGR**  
 CYP72A33 (NB) 171 **EKLKLM**LP**VFSACCEELISR**W**MG**S**IGSDGSYE**V**DCWPE**F**KSLTGDVISRTAFGSSYLEGR**  
 CYP72A31 (Kas) 178 **EKLKLM**LP**A**F**SACCEELISR**W**IGS**I**G**C**DGSYE**V**DCWPEL**K**SLTGDVISRTAFGSSYLEGR**

CYP72A32 (Kas) 241 **RIFELQ**G**ELFERVMKS**V**E**K**IF**P**GYMYLPTENNRKMHQ**N**KETESILRS**M**IGKRMQAMKE**  
 CYP72A32 (NB) 241 **RIFELQ**G**ELFERVMKS**V**E**K**IF**P**GYMYLPTENNRKMHQ**N**KETESILRS**M**IGKRMQAMKE**  
 CYP72A33 (Kas) 231 **RIFELQ**G**ELFERV****IK**S**IQK**F**IF**P**GYMYLPTENNRKMHQ**N**KETESILRGMIGKRMQAMKE**  
 CYP72A33 (NB) 231 **RIFELQ**G**ELFERV****IK**S**IQK**F**IF**P**GYMYLPTENNRKMHQ**N**KETESILRGMIGKRMQAMKE**  
 CYP72A31 (Kas) 238 **R**V**FELQA**E**Q**F**ERAMK**C**Q**K**IS**I**P**G**YMS**L**P**I**ENNRKMHQ**N**KETESILRGMIGKRMQAMKE**

CYP72A32 (Kas) 301 **GESTKDDL**L**GILLES**N**MRH**T**EENS**Q**SSQGLT**I**KDIMEECKLFYFAGADTTSVLLTWT****ILL**  
 CYP72A32 (NB) 301 **GESTKDDL**L**GILLES**N**MRH**T**EENS**Q**SSQGLT**I**KDIMEECKLFYFAGADTTSVLLTWT****ILL**  
 CYP72A33 (Kas) 291 **GESTKDDL**L**GILLES**N**TRHME**V**NGQSN**Q**GLT**I**KDIMEECKLFYFAGADTTSVLLTWTMLL**  
 CYP72A33 (NB) 291 **GESTKDDL**L**GILLES**N**TRHME**V**NGQSN**Q**GLT**I**KDIMEECKLFYFAGADTTSVLLTWTMLL**  
 CYP72A31 (Kas) 298 **GESTKDDL**L**GILLES**N**T**K**HMEENG**Q**SSQGLT**I**KDIMEECKLFYFAGADTTSVLLTWTAMLL**

CYP72A32 (Kas) 361 **LSMHPEWQDRAR**K**E**IL**GLFG**K**NKPEYDGLSH**L**K**I**V**T**MILYEVLRLYP**P**F**I**EL**K**RRTYKEM**  
 CYP72A32 (NB) 361 **LSMHPEWQDRAR**K**E**IL**GLFG**K**NKPEYDGLNN**L**K**I**V**T**MILYEVLRLYP**P**F**I**EL**K**RRTYKEM**  
 CYP72A33 (Kas) 351 **LSMHPEWQDRAREE**IL**GLFG**K**NKPDYDGLSRL**K**I**V**T**M**ILYEVLRLYP**P**F**I**EL**T**RKTYKEM**  
 CYP72A33 (NB) 351 **LSMHPEWQDRAREE**IL**GLFG**K**NKPDYDGLSRL**K**I**V**T**M**ILYEVLRLYP**P**F**I**EL**T**RKTYKEM**  
 CYP72A31 (Kas) 358 **LSMHPEWQDRAREE**IL**GLF**R**K**N**KPDY**F**GLSRL**K**I**V**T**M**ILYEVLRLYP**P**F**I**EL**T**RKTYKEM**

CYP72A32 (Kas) 421 **K**I**GGV**T**YPAGVI**I**N**L**PVLF**I**HHD**L**E**I**W**G**SDVHEFKPERFSEGISKASKDPGAF**L**PFGWGP**  
 CYP72A32 (NB) 421 **K**I**GGV**T**YPAGVI**I**N**L**PVLF**I**HHD**L**K**I**W**G**SDVHEFKPERFSEGISKASKDPGAF**L**PFGWGP**  
 CYP72A33 (Kas) 411 **E**I**GG**I**T**Y**PAGVI**I**N**L**PV**M**F**I**HHD**P**E**I**W**G**SDVHEFKPERFSEGISKASKDPGAF**L**PFGWGP**  
 CYP72A33 (NB) 411 **E**I**GG**I**T**Y**PAGVI**I**N**L**PV**M**F**I**HHD**P**E**I**W**G**SDVHEFKPERFSEGISKASKDPGAF**L**PFGWGP**  
 CYP72A31 (Kas) 418 **E**I**GGV**T**YPAGV**S**IK**I**P**V**LF**I**HHD**P**D**T**W**G**SDVHEFKPERFSEGISKASKDPGAF**L**PFGWGP**

CYP72A32 (Kas)	481	<b>RICIGQNFALLEAKMALCLILQRLEFELAP</b> <b>TYTHAPHTMITLHPMHGAQIK</b> <b>IRAI</b> -----
CYP72A32 (NB)	481	<b>RICIGQNFALLEAKMALCLILQRLEFELAP</b> <b>TYTHAPHTMITLHPMHGAQIK</b> <b>IRAI</b> -----
CYP72A33 (Kas)	471	<b>RICIGQNFALLEAKMALCLILQRLEFELAT</b> <b>SYTHAPHT</b> <b>ITSLHPMHGAQIKV</b> <b>KSYMTISD</b>
CYP72A33 (NB)	471	<b>RICIGQNFALLEAKMALCLILQRLEFELAT</b> <b>SYTHV</b> <b>PHT</b> <b>ITSLHPMHGAQIKV</b> <b>KSYMTISD</b>
CYP72A31 (Kas)	478	<b>RICIGQNFALLEAKMALCLILQRLEFELAP</b> <b>SYTHAPHTM</b> <b>ITLHPMHGAQIKV</b> <b>RAI</b> -----
CYP72A32 (Kas)		-----
CYP72A32 (NB)		-----
CYP72A33 (Kas)	531	YSVFY
CYP72A33 (NB)	531	YSVFY
CYP72A31 (Kas)		-----

**Supplemental Figure S2. Alignment of CYP72A31, 32 and 33 protein in Nipponbare and Kasalath.**

The deduced amino acid sequences of CYP72A32 and 33 in Nipponbare (NB) were taken from RAPDB (<http://rapdb.dna.affrc.go.jp/>) or the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/index.shtml>) based on information listed on the Cytochrome P450 Homepage (<http://drnelson.uthsc.edu/CytochromeP450.html>). The deduced amino acid sequences of CYP72A31, 32 and 33 in Kasalath (Kas) were searched for in the genomic sequence of chromosome 1 of Kasalath based on information in the databases mentioned above. The alignments were generated by the CLUSTAL W algorithm. Black and grey boxes represent identical and similar residues, respectively.

## A

Kasalath	1	<b>ATGGTTTTCAGAGGGTGGTTGATGTGGGCTCCGGCTCAGCGCCAGTCCTCGTGGTGTTC</b>
Koshihikari	1	<b>ATGGTTTTCAGCGGGTGGTTGATGTGGGCTCCGGCTCAGCGCCAGTCCTCGTGGTGTTC</b>
Kasalath	61	<b>GGTCTCCTCTTCGGCTCGCCCTCGTGTGGCAGGCCGGCCGCTGCTTACC GGCTGTGG</b>
Koshihikari	61	<b>GGTCTCCTCTTCGGCTCGCCCTCGCGTGGCAGGCCGGCCGCTGCTTACC GGCTGTGG</b>
Kasalath	121	<b>TGGCGGCCGGCGGGCTGGAGAAGGGCTGCGCGCGCGGGGCTCCGCGGCTCGTCTAC</b>
Koshihikari	121	<b>TGGCGGCCGGCGGGCTGGAGAAGGGCTGCGCGCGCGGGGCTCCGCGGCTCGTCTAC</b>
Kasalath	181	<b>CGCTTCTCACCGGGACCTCGCGGAGGAGAGCCGGCGGAGGAAGGAGGCCTGGGCGAGG</b>
Koshihikari	181	<b>CGCTTCTCACCGGGACCTCGCGGAGGAGAGCCGGCGGAGGAAGGAGGCCTGGGCGAGG</b>
Kasalath	241	<b>CCGCTGCCGCTGCCGTGCCACGACATCGCGCCGCGCATCAAGCCGTTCTCCACGACACC</b>
Koshihikari	241	<b>CCGCTGCCGCTGCCGTGCCACGACATCGCGCCGCGCATCAAGCCGTTCTCCACGACACC</b>
Kasalath	301	<b>CTCG-----CGGAGCACGGCAAGCAGCGGCAGCCGTGCATCACCTGGTTCGGCCCGACG</b>
Koshihikari	301	<b>GTCTGCAGGAGCACAGCACGGCAAGCAGCGGCAGCCGTGCATCACCTGGTTCGGCCCGACG</b>
Kasalath	355	<b>CCGGAGGTGAACATCACCGATCCCAGCTGGCCAAGGTCGTCCTGTCCAACAAGTTCGCGC</b>
Koshihikari	361	<b>CCGGAGGTGAACATCACCGATCCCAGCTGGCCAAGGTCGTCCTGTCCAACAAGTTCGCGC</b>
Kasalath	415	<b>CACCTGGAGAGGGTCAGGTTCAAGGAGGTGTGCAAGCTGCTATCCCAAGGCCCTACCTAC</b>
Koshihikari	421	<b>CACCTGGAGAGGGTCAGGTTCAAGGAGGTGTGCAAGCTGCTATCCCAAGGCCCTACCTAC</b>
Kasalath	475	<b>CACGAGGGCGAGAAGTGGGTCAAGCACAGGAGGATCATCAACCCGGCTTCCAGCTCGAG</b>
Koshihikari	481	<b>CACGAGGGCGAGAAGTGGGTCAAGCACAGGAGGATCATCAACCCGGCTTCCAGCTCGAG</b>
Kasalath	535	<b>AAGCTCAAGCTCATGCTGCCAGCGTTTTCTGCATGCTGCCAGGAAGTATAGCAGGTGG</b>
Koshihikari	541	<b>AAGCTCAAGCTCATGCTGCCAGCGTTTTCTGCATGCTGCCAGGAAGTATAGCAGGTGG</b>
Kasalath	595	<b>ATAGGGTCCATTGGCTGTGATGGCTCGTACGAGGTGGATTGCTGGCCGGAGCTCAAGAGC</b>
Koshihikari	601	<b>ATAGGGTCCATTGGCTGTGATGGCTCGTACGAGGTGGATTGCTGGCCGGAGATCAAGAGC</b>
Kasalath	655	<b>CTCACCGGAGATGTCATCTCGCGCACCCGCTTCGGAAGCAGCTATCTTGAAGGAAGAAGG</b>
Koshihikari	661	<b>CTCACCGGAGATGTCATCTCGCGCACCCGCTTCGGAAGCAGCTATCTTGAAGGAAGAAGG</b>
Kasalath	715	<b>GTCTTCGAGCTGCAGGCCGAGCAATTTGAGCGCGCAATGAAATGCATGCAGAAGATTCC</b>
Koshihikari	721	<b>GTCTTCGAGCTGCAGGCCGAGCAATTTGAGCGCGCAATGAAATGCATGCAGAAGATTCC</b>
Kasalath	775	<b>ATCCCGGTTACATGTCTTTGCCTATTGAGAACAACCCGAAGATGCATCAAATAAATAAA</b>
Koshihikari	781	<b>ATCCCGGTTACATGTCTTTGCCTATTGAGAACAACCCGAAGATGCATCAAATAAATAAA</b>
Kasalath	835	<b>GAGATCGAATCGATTCTAAGAGGTATAATTGGGAAAAAATGCAAGCTATGAAAGAAGGT</b>
Koshihikari	841	<b>GAGATCGAATCGATTCTAAGAGGTATAATTGGGAAAAAATGCAAGCTATGAAAGAAGGT</b>
Kasalath	895	<b>GAAAGCACAAAAGATGATTTACTTGGCATATTGCTTGAGTCAAACACAAAGCACATGGAA</b>
Koshihikari	901	<b>GAAAGCACAAAAGATGATTTACTTGGCATATTGCTTGAGTCAAACACAAAGCACATGGAA</b>
Kasalath	955	<b>GAGAATGGTCAATCAAGCCAGGGTTGACAATCAAGGATATCGTGGAGGAGTCAAGCTG</b>
Koshihikari	961	<b>GAGAATGGTCAATCAAGCCAGGGTTGACAATCAAGGATATCGTGGAGGAGTCAAGCTG</b>

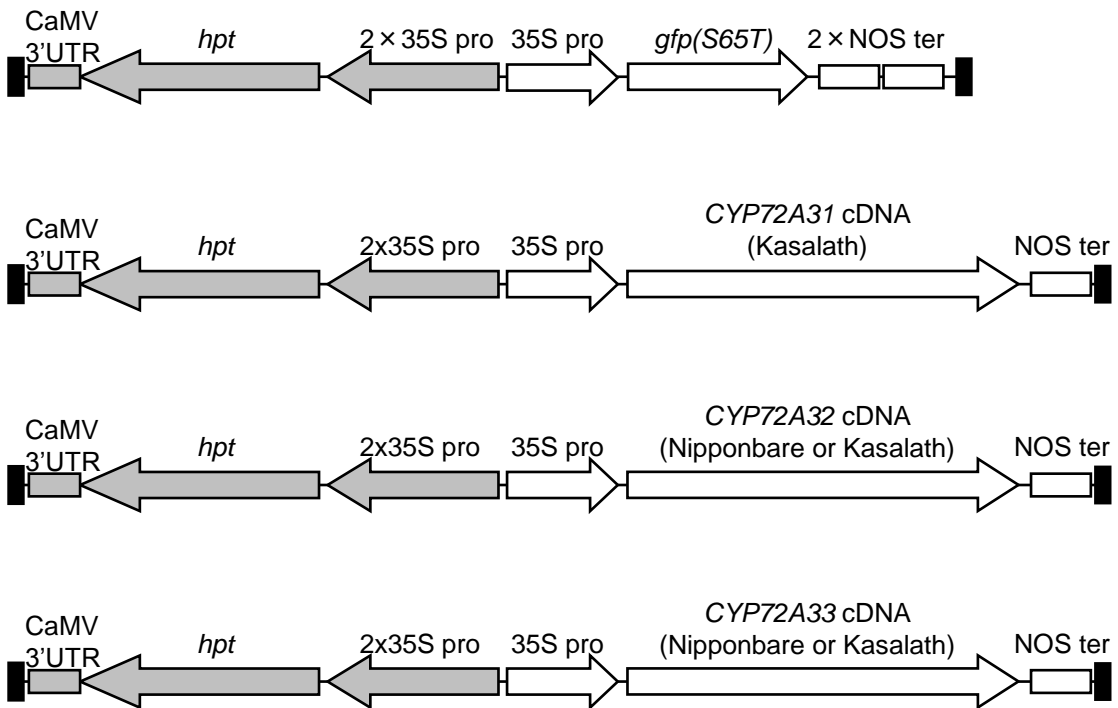
Kasalath	1015	<b>TTCTACTTTGCAGGAGCGGAGACAACATCAGTGC TTCTCACATGGCCATGCTGCTATTA</b>
Koshihikari	1021	<b>TTCTACTTTGCAGGAGCGGAGACAACATCAGTGC TTCTCACATGGCCATGCTGCTATTA</b>
Kasalath	1075	<b>AGCATGCACCCGGAGTGGCAGGACCGTGCAAGGGAAGAGATCC TGGGATTATTTAGGAAG</b>
Koshihikari	1081	<b>AGCATGCACCCGGAGTGGCAGGACCGTGCAAGGGAAGAGATTA TGGGATTATTTAGGAAG</b>
Kasalath	1135	<b>AACAAACCTGACTACGAAGGCTTGAGCCGCCTCAAAATTGTGACGATGATCCTCTACGAG</b>
Koshihikari	1141	<b>AACAAACCTGACTACGAAGGCTTGAGCCGCCTCAAAATTGTGACGATGATCCTCTACGAG</b>
Kasalath	1195	<b>GTTCTTCGGTTGTACCACCGTTCATCGAGATTGGTCCGGAAAACATACAAAGAGATGGAG</b>
Koshihikari	1201	<b>GTTCTTCGGTTGTACCACCGTTCATCGAGATTGGTGGAAAACATCAAAGAGATGGAG</b>
Kasalath	1255	<b>ATAGGAGGAGTCACTTACCCAGCTGGTGTGAGCATTAAAAATCCCCGTGTTGTTTCATCCAC</b>
Koshihikari	1260	<b>ATAG-----</b>
Kasalath	1315	<b>CATGATCCGGACACCTGGGGAAGTGATGTGCATGAGTTCAAACCTGAGAGGTTCTCTGAG</b>
Koshihikari		<b>-----</b>
Kasalath	1375	<b>GGGATCTCTAAGGCGTCTAAGGATCCGGGTGCATTCCCTCCCGTTCCGTTGGGGGCCACGA</b>
Koshihikari		<b>-----</b>
Kasalath	1435	<b>ATCTGCATCGGCCAAAACCTTCGCGCTGCTTGAGGCCAAGATGGCATTGTGCCTGATTCTT</b>
Koshihikari		<b>-----</b>
Kasalath	1495	<b>CAACGCTTGGAGTTTGAGCTTGCGCCATCGTATACTCATGCGCCGCATACTATGGTAACT</b>
Koshihikari		<b>-----</b>
Kasalath	1555	<b>CTGCATCCAATGCACGGTGCACAGATTAAGTTAGAGCTATATGA</b>
Koshihikari		<b>-----</b>

**B**

Koshihikari	1	<b>MVFS</b> <b>GWLMWAPASAPVLVVFGLLFGLAL</b> <b>AWQAGRLLHRLWWRPRRLEKAL</b>
Kasalath	1	<b>MVFR</b> <b>GWLMWAPASAPVLVVFGLLFGLAL</b> <b>VWQAGRLLHRLWWRPRRLEKAL</b>
Koshihikari	51	<b>RARGLRGSSYRFLTGDLAESRRRKEAWARPLPLRCHDIAPRIKPF<sup>→</sup>LHGT</b>
Kasalath	51	<b>RARGLRGSSYRFLTGDLAESRRRKEAWARPLPLRCHDIAPRIKPF<sup>→</sup>LHDT</b>
Koshihikari	101	<b>VVRE</b> <b>QHGKRR</b> <b>QPCITWFGPTPEVNITDPELAKVLSNKF</b> <b>AHLERVRFKEV</b>
Kasalath	101	<b>LG--E</b> <b>HGKQ</b> <b>RQPCITWFGPTPEVNITDPELAKVLSNKF</b> <b>CHLERVRFKEV</b>
Koshihikari	151	<b>SKLLSQGLS</b> <b>YHEGEKVVKHRR</b> <b>IINPAFQLEKCLKMLPAFSACCEELISRW</b>
Kasalath	149	<b>SKLLSQGLT</b> <b>YHEGEKVVKHRR</b> <b>IINPAFQLEKCLKMLPAFSACCEELISRW</b>
Koshihikari	201	<b>IGSIGS</b> <b>DGSYEVDWCPE</b> <b>MKSLTGDVISRTAFGSSYLEGRRVFELQAEQFE</b>
Kasalath	199	<b>IGSIGC</b> <b>DGSYEVDWCPE</b> <b>MKSLTGDVISRTAFGSSYLEGRRVFELQAEQFE</b>
Koshihikari	251	<b>RAMKCMQKIS</b> <b>IPGYMSLPTIENNRKMHQINKEIESILRG</b> <b>IGKRMQAMKEG</b>
Kasalath	249	<b>RAMKCMQKIS</b> <b>IPGYMSLPTIENNRKMHQINKEIESILRG</b> <b>IGKRMQAMKEG</b>
Koshihikari	301	<b>ESTKDDLLGILLESNTKHMEENGQSSQGLT</b> <b>MKDIVEECKLFYFAGAETTS</b>
Kasalath	299	<b>ESTKDDLLGILLESNTKHMEENGQSSQGLT</b> <b>MKDIVEECKLFYFAGAETTS</b>
Koshihikari	351	<b>VLLTWT</b> <b>MLLSMHPEWQDRAREE</b> <b>IMGLFRKNKPDYEGLSRLKIVTMI</b> <b>FYE</b>
Kasalath	349	<b>VLLTWA</b> <b>MLLSMHPEWQDRAREE</b> <b>IMGLFRKNKPDYEGLSRLKIVTMI</b> <b>LYE</b>
Koshihikari	401	<b>VLRLYPPFIE</b> <b>IGWKT</b> <b>SKRWR</b> -----
Kasalath	399	<b>VLRLYPPFIE</b> <b>IGRKT</b> <b>YKEME</b> <b>IGGVTYPAGVSIKIPVLFIHHPDPTWGS</b> <b>SDV</b>
Koshihikari		-----
Kasalath	449	<b>HEFKPERFSEGISKASKDPGAFLP</b> <b>FGWGPRICIG</b> <b>QNFALLEAKMALCLIL</b>
Koshihikari		-----
Kasalath	499	<b>QRLEFELAPSYTHAPHTMVTLHPMHGAQIKVRA</b> <b>I</b>

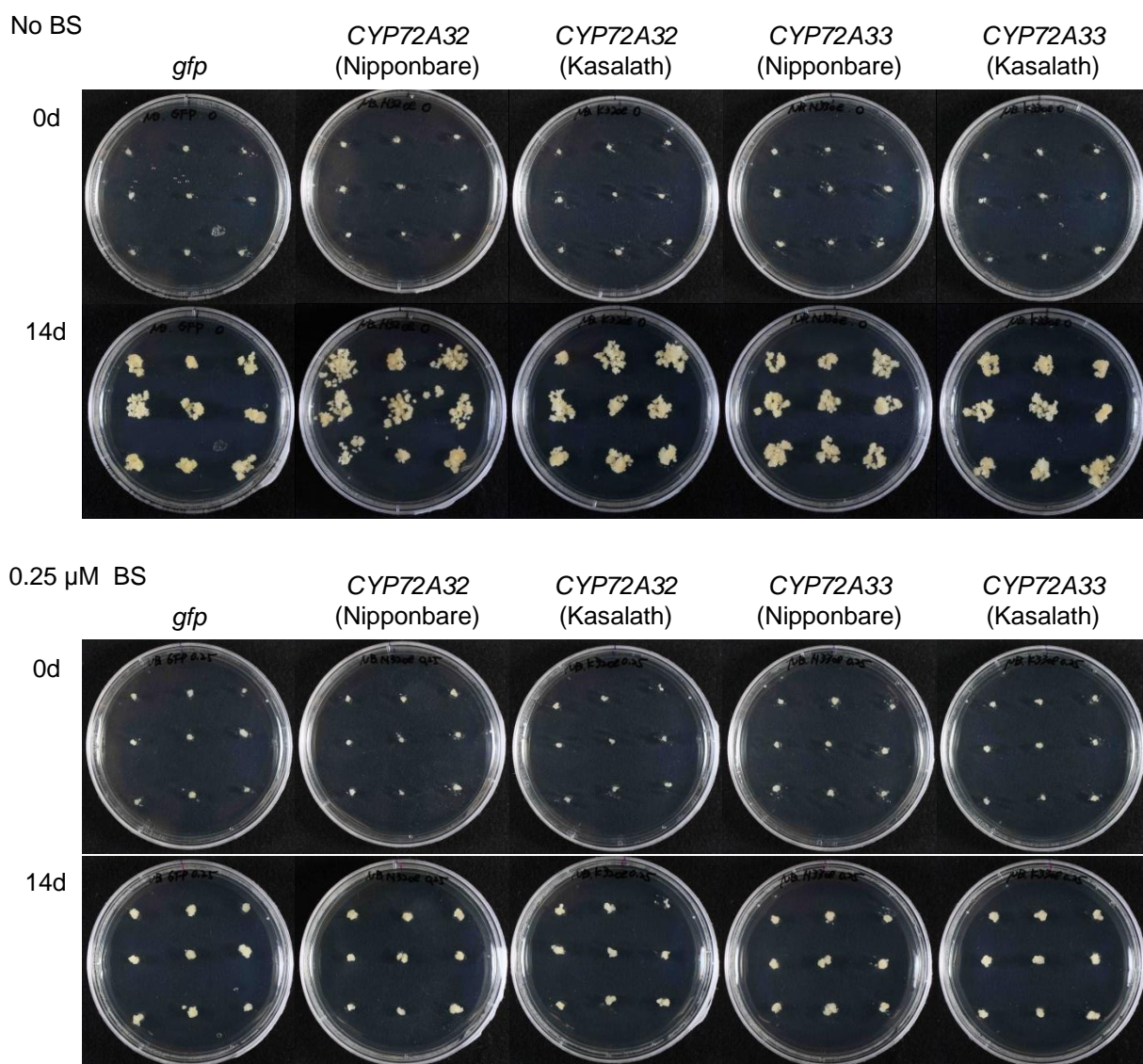
**Supplemental Figure S3. The alignment of CYP72A31 in Koshihikari and Kasalath.**

**A** The cDNA sequences of *CYP72A31* in Koshihikari and Kasalath. The cDNA sequence of *CYP72A31* in Koshihikari was determined by RT-PCR amplified with total RNA extracted from callus as a template. The cDNA sequence of *CYP72A31* in Kasalath was determined by RT-PCR amplified with total RNA extracted from shoots as a template. An pink arrowhead shows -1 frame-shift mutation in Koshihikari. **B** The deduced amino acid sequences of *CYP72A31* in Koshihikari and Kasalath. Yellow and bold character shows the cytochrome P450 cysteine heme-iron ligand signature. Details are same as Supplemental Figure S2.



**Supplemental Figure S4. Structure of *CYP72A31*, 32 and 33 overexpression vectors.**

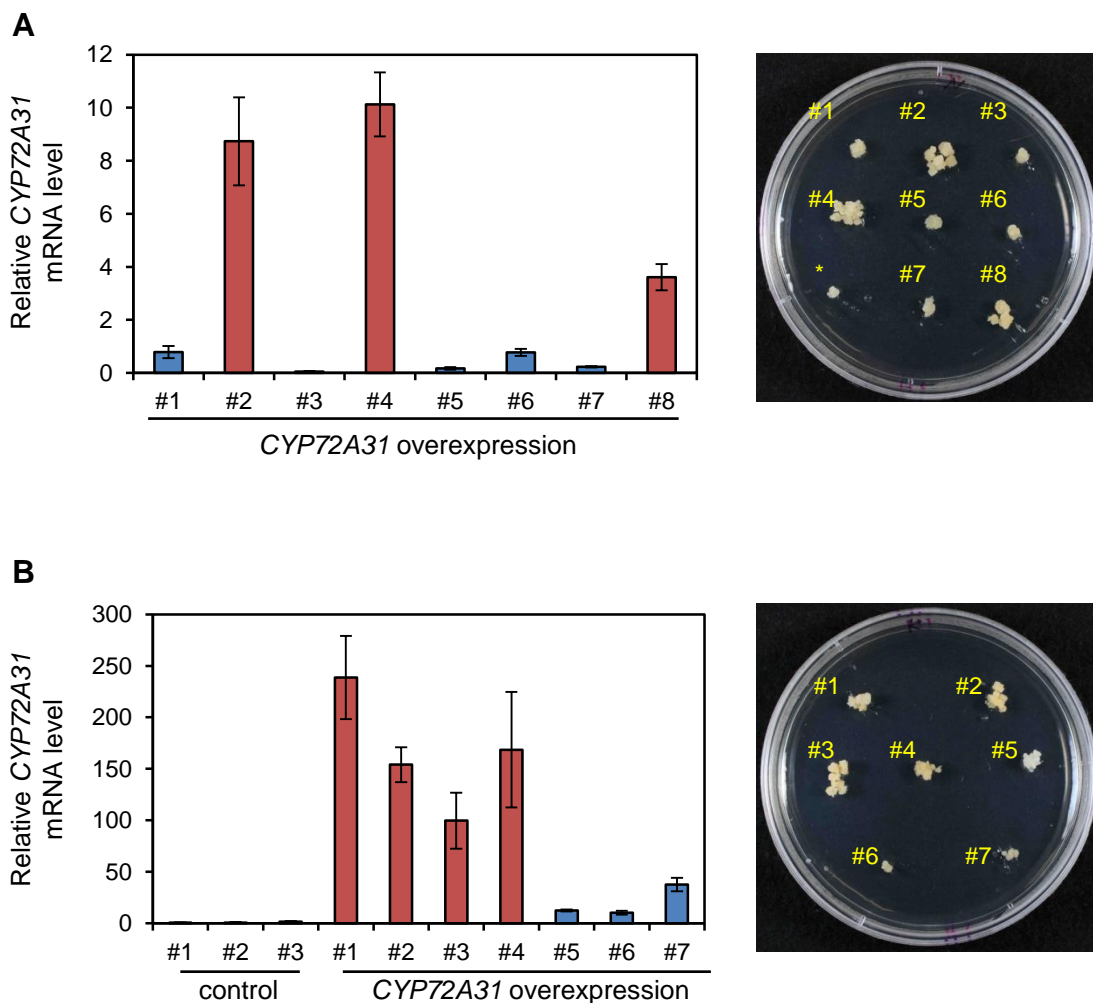
The *gfp*, *CYP72A31*, *CYP72A32* and *CYP72A33* expression cassettes were cloned in the binary vector pCambia1390. These genes are under the control of the CaMV 35S promoter (35S pro) and nopaline synthase terminator (NOS ter); *hpt* expression was directed by a duplicated 35S promoter (2x35Spro) and the CaMV 35S polyA (CaMV 3'UTR). In these vectors, a Kasalath *CYP72A31* cDNA fragment, and Nipponbare and Kasalath *CYP72A32* or *CYP72A33* cDNA fragments were used.



**Supplemental Figure S5. BS sensitivity of overexpression of *CYP72A32* and *CYP72A33* genes in Nipponbare.**

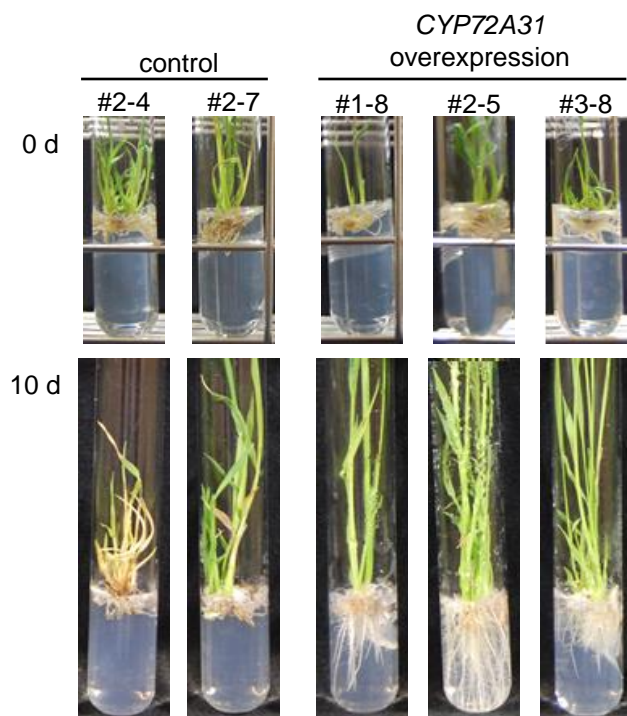
Rice calli in T<sub>0</sub> generation were transferred to fresh medium containing 0.25 μM BS and cultivated for 14 days. Transgenic calli overexpressing *CYP72A32* or *CYP72A33* gene showed BS sensitivity similar as control calli transformed with *gfp* expressing vector.





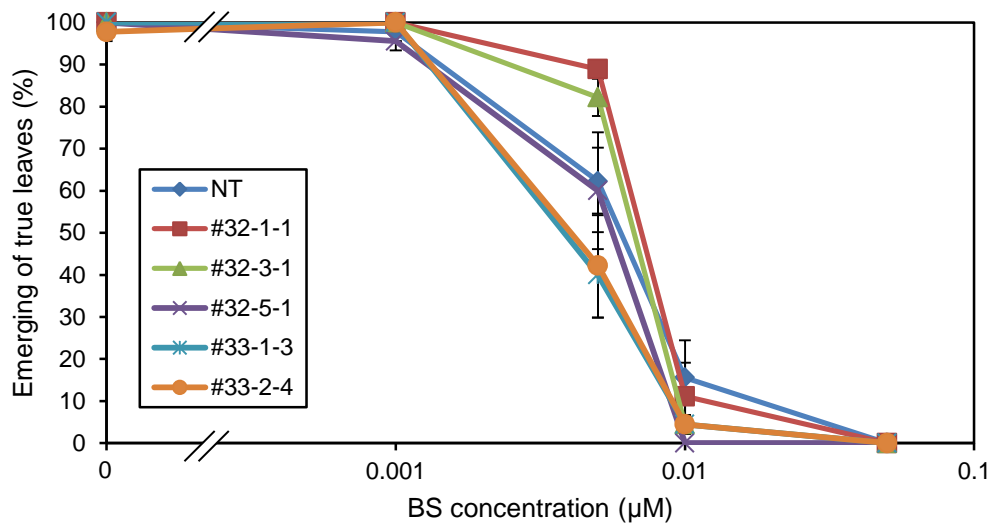
**Supplemental Figure S6. Correlation of *CYP72A31* expression levels and BS tolerance in rice callus.**

BS sensitivity test in *CYP72A31* overexpressing callus of Nipponbare (A) and Kasalath (B). In graphs, red and blue bars indicate the lines that showed tolerant and sensitive to 0.75  $\mu\text{M}$  BS, respectively. The y axis shows relative *CYP72A31* mRNA levels normalized to the *OsActin1* mRNA level as a control. In B, mRNA levels are presented as a ratio of that in the average of control samples (#1-3). The photo shows the calli grown on the medium containing 0.75  $\mu\text{M}$  BS for 14 days. The lines #4 and #6 in A are same as shown in Fig. 4. It is suggested that *CYP72A31* expression level in #6 is sufficient to confer tolerance to 0.25  $\mu\text{M}$  BS, although it is not sufficient to confer tolerance to 0.75  $\mu\text{M}$  BS. The callus line marked an asterisk in A is not tested. We estimated that the line #5 in B was BS sensitive because it was bleached on the medium containing BS.



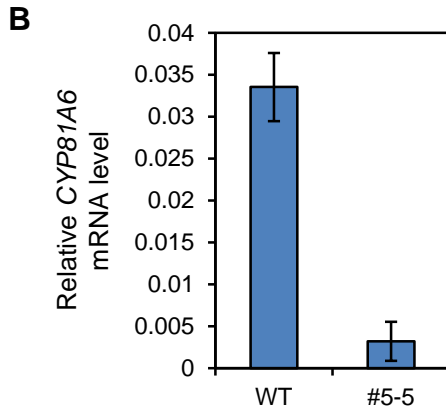
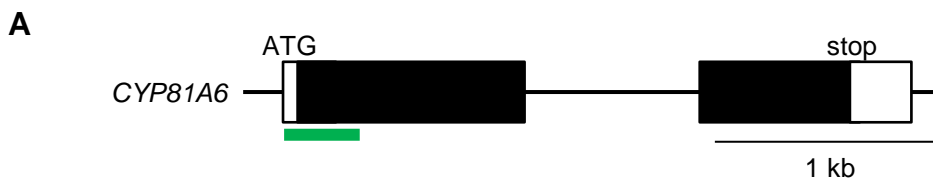
**Supplemental Figure S7. Overexpression of *CYP72A31* gene confers BS tolerance in regenerated seedlings of Nipponbare.**

Regenerated plantlets were transferred to fresh medium containing 1  $\mu$ M BS and cultivated for 10 days. The roots of transgenic plants overexpressing *CYP72A31* gene grew vigorously, although control plants stopped growth.



**Supplemental Figure S8. BS sensitivity of overexpression of *CYP72A32* and *CYP72A33* genes in Arabidopsis.**

Transgenic Arabidopsis seedlings overexpressing *CYP72A32* (#32-1-1, #32-3-1 and #32-5-1) or *CYP72A33* (#33-1-3 and #33-2-4) gene showed BS sensitive similar as wild-type plants. Data show mean  $\pm$  SE of three experiments.



**Supplemental Figure S9. *CYP81A6* knock-down rice plants.**

A. Schematic representation of *CYP81A6* gene (Os03g0760200). Black and white boxes show the putative untranslated region and coding region of *CYP81A6*, respectively. Green bar shows the region used as a 326-bp inverted repeat sequence of *CYP81A6* for RNAi. Bar = 1 kb. B. Relative mRNA levels of *CYP81A6* gene in wild-type (WT) and *CYP81A6* knock-down line (#5-5).

**Supplemental Table S1** Putative QTL for BS tolerance detected through composite interval mapping using 183 BILs (Koshihikari/Kasalath//Koshihikari)

Chr	Marker interval	(Mb) <sup>a</sup>	LOD <sup>b</sup>	a <sup>c</sup>	r <sup>2</sup> <sup>d</sup>
1	C178-C122	15.9-28.6	17.3	-0.4	37.4

<sup>a</sup> According to IRGSP build 5.

<sup>b</sup> LOD values >3.3 are shown.

<sup>c</sup> Additive effect of the Kasalath allele.

<sup>d</sup> Percentage of the total phenotypic variance explained.

## Supplemental Table S2 Primers used in this study

### For vector construction

primer name	sequence (5'-3')
CYP72A31 F	ATCAGCAGCATCTTTTCACCCCCTTCT
CYP72A31 R	CAAAGGTACAAATATCACAACCTCACAACCA
CYP72A32 F	GCTTCTTTTCACTTCATTCCCCCTGTTC
CYP72A32 F2	TACTTACAGGtcGAcCACGGCCAGTAGTAG
CYP72A32 R	TCCTATGCCACTTTTTATTCCAAAGGTACAA
CYP72A33 F	CTTCTTTTTCACCTGGTTTTTCGCTTGT
CYP72A33 R	CAAAGGTACAAATGGTATAACCCAACCA
CYP81A6 F	CACCGAGTGCACCAGAGTCACAGAAACACATCACAC
CYP81A6 R	CGGTGAAGCACTCCCTGGCGCAC
M13 Rv	GGAAACAGCTATGACCATGATTACGC

### For real time RT-PCR

primer name	sequence (5'-3')
CYP72A31 RT-F	GAAGAACAACCTGACTACGAAGGCT
CYP72A31 RT-R	CTCCATCTCTTTGTATGTTTTCCGACCAAT
CYP72A32 RT-F	GAAGAACAACCTGAGTACGATGGCT
CYP72A32 RT-R	TTCATCTCCTTGTATGTTCTCCGCTTAAG
CYP72A33 RT-F	GGAAGAATAAACCAGACTATGATGGCC
CYP72A33 RT-R	CTCCATCTCCTTGTATGTTTTTCGAGTAAG
OsAct1 RT-F	AGGCCAATCGTGAGAAGATGACCCA
OsAct1 RT-R	GTGTGGCTGACACCATCACCAGAG
AtEF1 $\alpha$ 4 RT-F	AGGCTGGTATCTCTAAGGATGGTCA
AtEF1 $\alpha$ 4 RT-R	TTGTATCCGACCTTCTTCAGGTATG
CYP81A6 RT-F	TGTTCTTCGCGAGCTCGTCT
CYP81A6 RT-R	TGCTCACGAGAAGCTTATTTGCTAC
CA000683_F	CCCAAGAATGCTAAGCCAAGAG
CA000683_R	TGATAACAGATAGGCCGGTTGAA

### For genotyping

primer name	sequence (5'-3')
*RM9 F	GGCCCTCATCACCTTCGTAGC
*RM9 R	CGTCCTCCCTCTCCCTATCTCC
*RM11288 F	CAATATCAGCCTAGCACAAAGG
*RM11288 R	CTATCCAACCAGCCATTTATCC
41834-50 F	ACTAATTACTGAAACATACAAATAGCACCA
41834-50 R	TTCAACCAAATAAACTTAATGTTTCACA
*RM11302 F	CTTAGGCCACATTTGATGTCC
*RM11302 R	CCCATCGTTTGCTCATATTCC

\*Matsumoto et al. (2005) *Nature* 436:793-800