

**Supplementary Table 1. Group L UGTs included in phylogenetic analysis**

GT	Species	Acc. no.	Activity	Source
UGT74B1	<i>Arabidopsis thaliana</i>	AAC00570	Thiohydroximate glucosyltransferase	1
UGT74C1	<i>Arabidopsis thaliana</i>	AAD32293	-	3
UGT74D1	<i>Arabidopsis thaliana</i>	AEC08580	Jasmonate glucosyltransferase	2
UGT74E1	<i>Arabidopsis thaliana</i>	POC7P7	-	3
UGT74F2	<i>Arabidopsis thaliana</i>	AAB64024	Salicylic acid and anthranilic acid glucosyltransferase	4-6
UGT75B1	<i>Arabidopsis thaliana</i>	AEE27854	Indole-3-acetic acid glucosyltransferase	29
UGT75C1	<i>Arabidopsis thaliana</i>	CAB10189	Anthocyanin-5-O-glucosyltransferase	7
UGT75D1	<i>Arabidopsis thaliana</i>	CAB10333	Flavonoid glucosyltransferase	8
UGT84A1	<i>Arabidopsis thaliana</i>	AEE83609	Hydroxybenzoate glucosyltransferase	4
UGT84B1	<i>Arabidopsis thaliana</i>	AAB87119	Indole-3-acetic acid glucosyltransferase	9
UGT74H5	<i>Avena strigosa</i>	ACD03250	N-methylanthranilate glucosyltransferase	This study
UGT74H6	<i>Avena strigosa</i>	ACD03261	Benzoic acid glucosyltransferase	This study
UGT74H7	<i>Avena strigosa</i>	ACD03246	-	This study
UGT84C2	<i>Avena strigosa</i>	ACD03236	-	This study
UGT75E2	<i>Avena strigosa</i>	ACD03260	-	This study
UGT75E3	<i>Avena strigosa</i>	ACD03251	-	This study
UGT75D1 like6	<i>Brachypodium distachyon</i>	XP_003576359	-	*
UGT75D1 like5	<i>Brachypodium distachyon</i>	XP_003560621	-	*
UGT75D1 like4	<i>Brachypodium distachyon</i>	XP_003571494	-	*
UGT84A2 like	<i>Brachypodium distachyon</i>	XP_003565946	-	*
Cinnamate-GT like2	<i>Brachypodium distachyon</i>	XP_003567004	-	*
Cinnamate-GT like1	<i>Brachypodium distachyon</i>	XP_003571042	-	*
IAA-GT like	<i>Brachypodium distachyon</i>	XP_003559553	-	*
UGT74F1 like1	<i>Brachypodium distachyon</i>	XP_003578449	-	*
UGT74F1 like2	<i>Brachypodium distachyon</i>	XP_003576736	-	*
UGT74F2 like	<i>Brachypodium distachyon</i>	XP_003578448	-	*
UGT84A9a	<i>Brassica napus</i>	CAS03354	Sinapate glucosyltransferase	10
CsGT45	<i>Crocus sativus</i>	ACM66950	Flavonoid glucosyltransferase	11
EpGT-1	<i>Eucalyptus perriniana</i>	BAD90934	Monoterpene glucosyltransferase	12
EpGT-2	<i>Eucalyptus perriniana</i>	BAD90935	Monoterpene glucosyltransferase	12
5GT-A	<i>Eustoma grandiflorum</i>	BAF49285	Anthocyanin 5-O-glucosyltransferase	13
FaGT2	<i>Fragaria x ananassa</i>	Q66PF4	Cinnamate glucosyltransferase	14
5GT7	<i>Gentiana triflora</i>	BAG32255	Anthocyanin 5-O-glucosyltransferase	15
HGT8	<i>Glandularia x hybrida</i>	BAA36423	Anthocyanin 5-O-glucosyltransferase	16
GgSGT	<i>Gomphrena globosa</i>	BAG14302	Sinapate glucosyltransferase	17
ant5GT	<i>Iris hollandica</i>	BAD06874	Anthocyanin 5-O-glucosyltransferase	18
HCA1GT-1	<i>Lobelia erinus</i>	BAF99686	Hydroxycinnamate-O-glucosyltransferase	19
SAGTase	<i>Nicotiana tabacum</i>	AAF61647	Salicylic acid glucosyltransferase	21
Os11g0145200	<i>Oryza sativa</i>	NP_001065729	-	*
Os02g0203300	<i>Oryza sativa</i>	NP_001046237	-	*

Supplementary Table 1 continued...

Os01g0686300	<i>Oryza sativa</i>	NP_001043905	-	*
Os01g0686200	<i>Oryza sativa</i>	NP_001043904	-	*
Os03g0693600	<i>Oryza sativa</i>	NP_001050964	-	*
Os09g0518000	<i>Oryza sativa</i>	NP_001063684	-	*
Os09g0518200	<i>Oryza sativa</i>	NP_001063685	-	*
Pf3R4	<i>Perilla frutescens</i>	BAA36421	Anthocyanin 5-O-glucosyltransferase	16
F7GT	<i>Pyrus comunnis</i>	AAAY27090	Flavonoid 7-O-glucosyltransferase	22
GAGT	<i>Solanum lycopersicum</i>	CAI62049	Gentisic acid xylosyltransferase Triterpene carboxylic acid glucosyltransferase	20
UGT74M1	<i>Vaccaria hispanica</i>	ABK76266	Resveratrol/hydroxycinnamic acid O- glucosyltransferase	24
ResOGT	<i>Vitis labrusca</i>	ABH03018	glucosyltransferase	25
IAGLU	<i>Zea mays</i>	NP_001150551	Indole-3-acetate beta-glucosyltransferase	26, 27
IAGT	<i>Zea mays</i>	NP_001150551	-	26
UGT2	<i>Crocus sativus</i>	AAP94878	Croctin glucosyltransferase	28

\*Predicted by automated computational analysis

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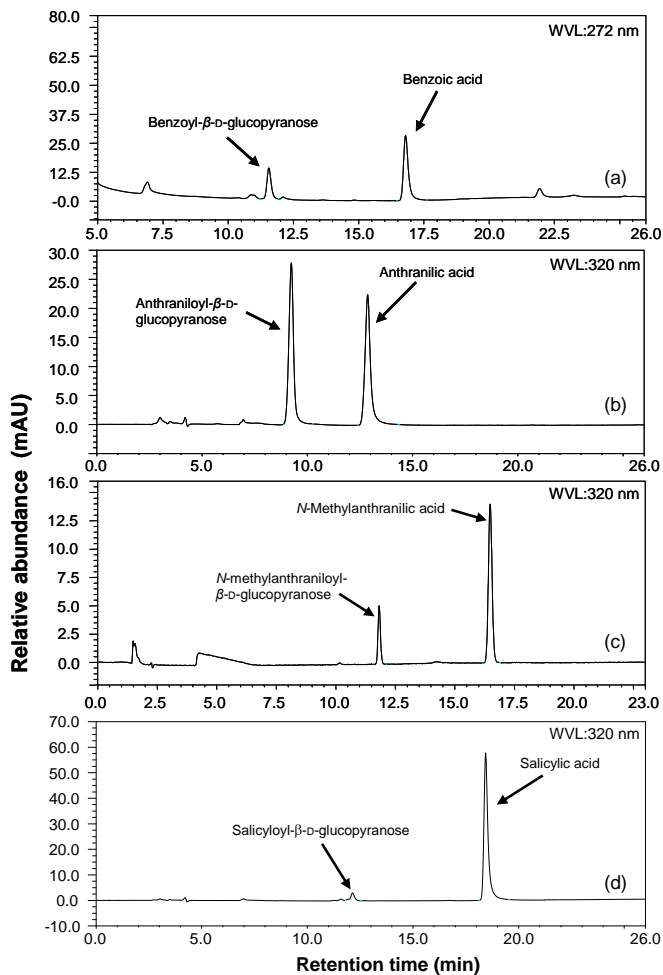
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**Supplementary Table 2. Oligonucleotide primer sequences**

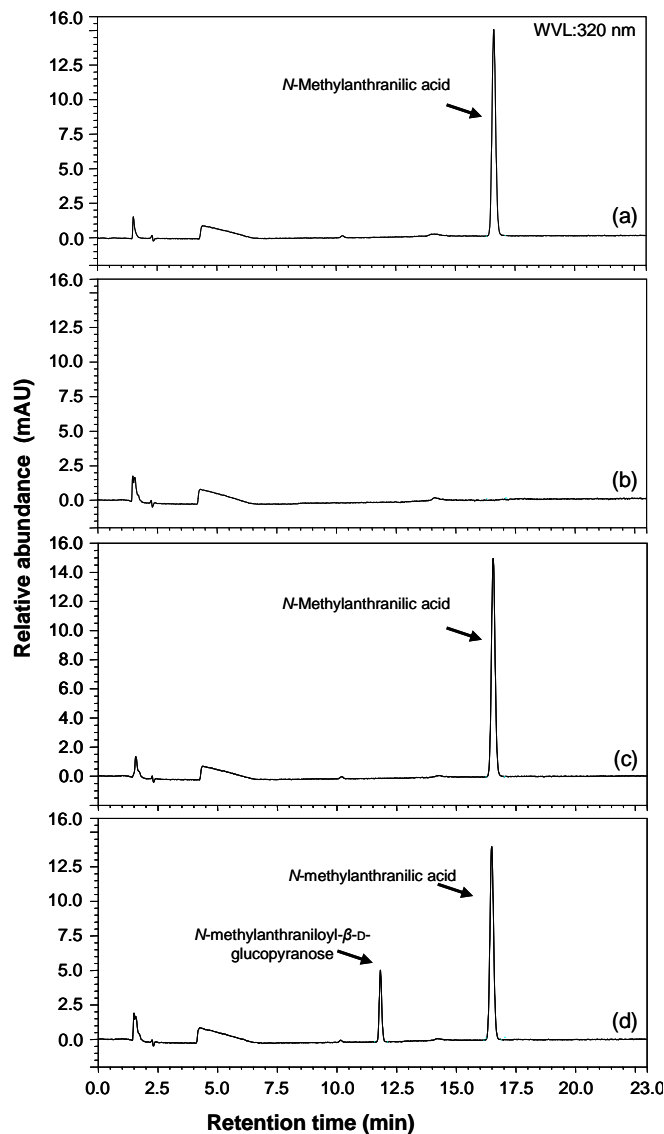
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<b>Primer no.</b>	<b>DNA template</b>	<b>Oligonucleotide primer sequence (5'→3')</b>
<b>For His<sub>6</sub> tags:</b>		
1	<i>UGT74H5</i>	GGAATTCATATGGGGGCTGAGTGGGAG
2	<i>UGT74H5</i>	GGAATTCATATGTCA TGCATCTAACCCAC
3	<i>UGT74H7</i>	GGAATTCATATGGGGGCTGAGCCGGGCCACGTA
4	<i>UGT74H7</i>	GGAATTCATATGCTAGATTTGTTTGGGCTGAGTTG
5	<i>UGT74H6</i>	GGAATTCATATGGGGGCTGAGCCGGGCCACGTA
6	<i>UGT74H6</i>	GGAATTCATATGTCAGATGATTAATTTCTGGGATTTTC
<b>For native protein:</b>		
7	<i>UGT74H5</i>	TTCATATGGGGGCTGAGTGGGAG
8	<i>UGT74H5</i>	AGGAATTCATATGTCA TGCATCTAACCCAC
9	<i>UGT74H7</i>	TTCATATGGGGGCTGAGCCGGGCCACGTA
10	<i>UGT74H7</i>	CTGAATTCATATGCTAGATTTGTTTGGGCTGAGTTG
11	<i>UGT74H6</i>	TACATATGGGGGCTGAGCCGGGCCACGTA
12	<i>UGT74H6</i>	GGAATTCATCAGATGATTAATTTCTGGGATTTTC

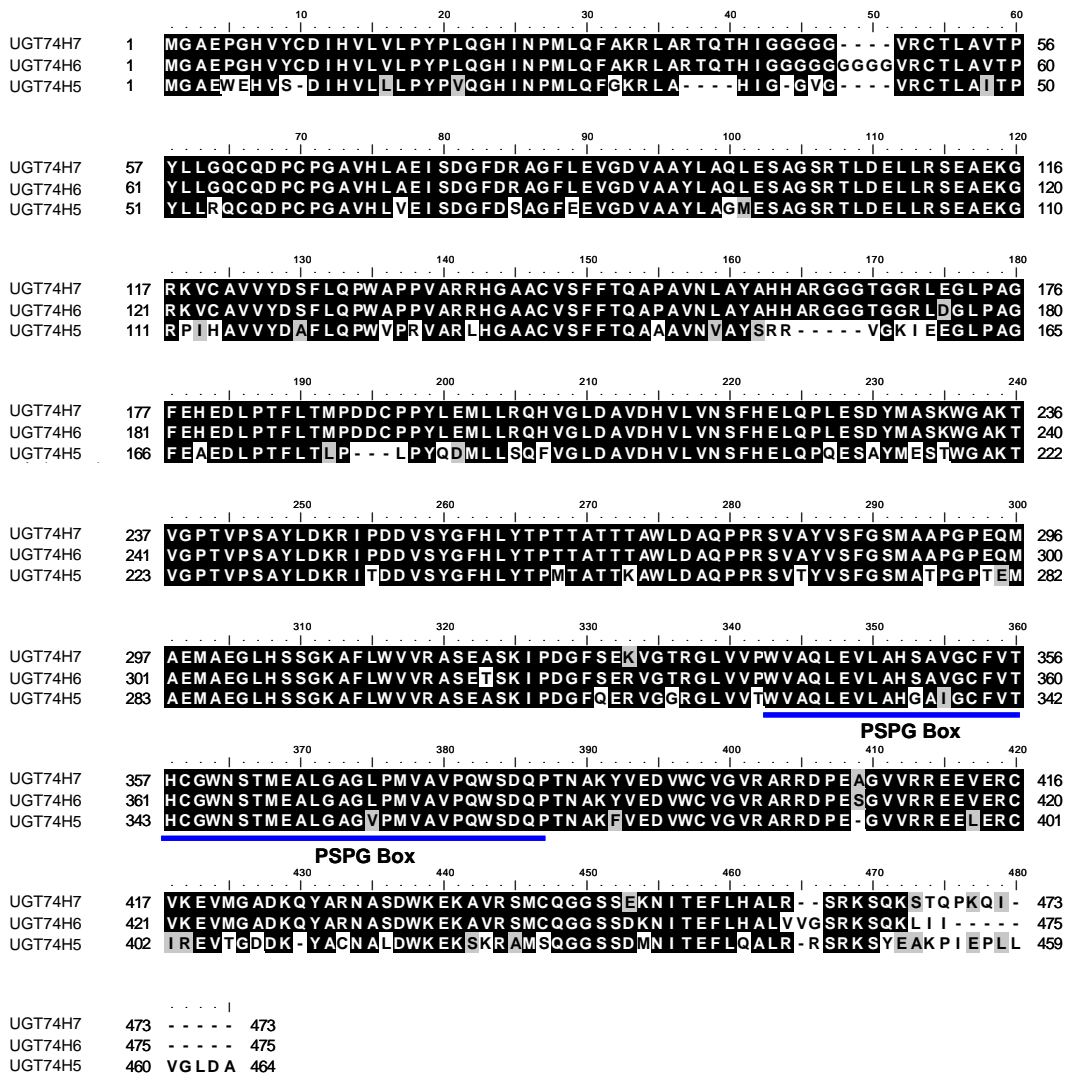
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**Supplementary Figure 1A.** HPLC analysis of glucosylated products following assays with purified recombinant UGT74H5. Reactions were performed in 5 mM UDP-D-glucose, pH 6.8 at 30°C for 30 minutes with 100  $\mu$ M benzoic acid (a), anthranilic acid (b), *N*-methylantranilic acid (c) or salicylic acid (d). Reactions were analysed by reversed phase HPLC using a LUNA 5- $\mu$ m C18 column (150 x 4.6 mm, Phenomenex) with a linear gradient of acetonitrile in water (10-40 % for anthranilate and *N*-methylantranilate; 10-60% for salicylic acid and benzoic acid). The retention times of the glucose conjugates were (a) 11.6 min; (b) 9.2 min; (c) 11.8 min; (d) 12.1 min. Detection wavelengths (WVL) are indicated.

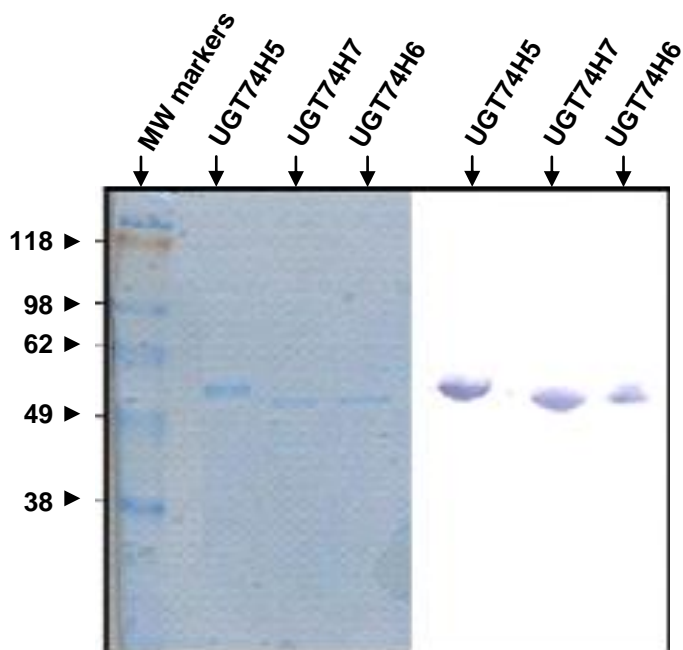


**Supplementary Figure 1B.** HPLC analysis for assays of purified recombinant UGT74H5 with *N*-methylantranilate. Reactions were performed with 0.5 mM UDP-D-glucose, 0.2 mM *N*-methylantranilic acid at pH 7.5, 30°C for 30 minutes. Controls include assays with inactivated enzyme (a), minus *N*-methylantranilic acid (b) and minus UDP-D-glucose (c); (d) reaction with active enzyme. Samples were analysed as for Supplementary Figure 1A. The retention times ( $R_t$ ) of *N*-methylantranilic acid and *N*-methylantraniloyl- $\beta$ -D-glucopyranose were 16.5 min and 11.8 min, respectively. The detection wavelength (WVL) is indicated.

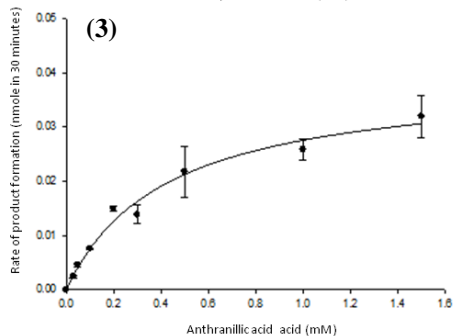
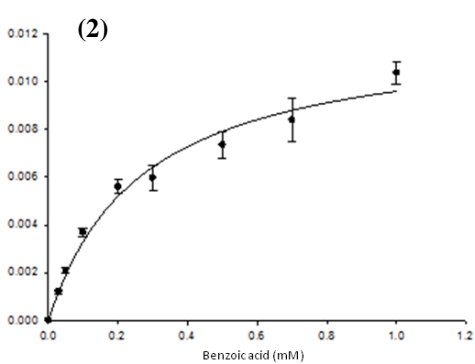
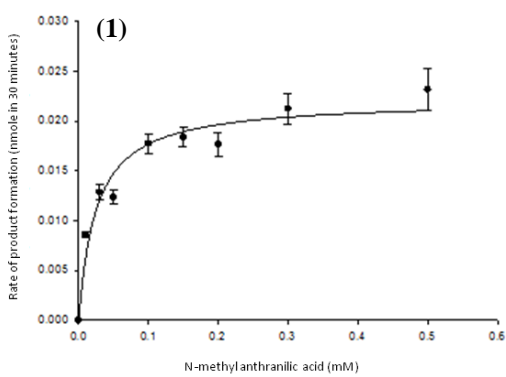


### Supplementary Figure 2.

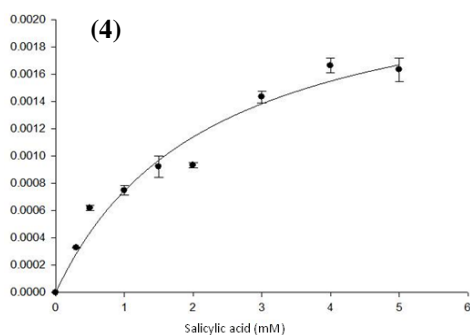
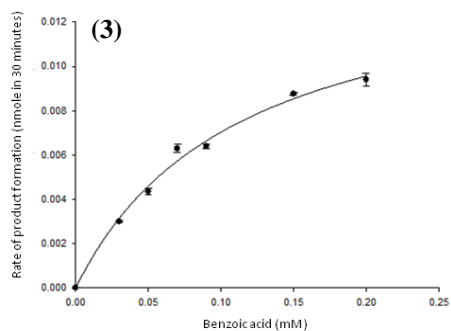
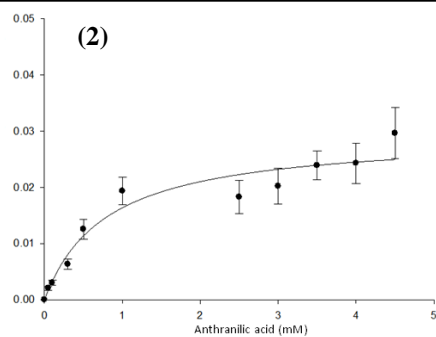
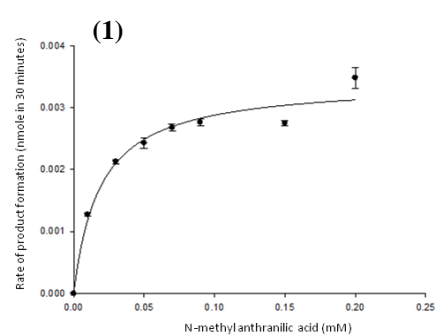
Alignment of amino acid sequences of UGT74H5 and two other closely related GTs expressed in oat roots (UGT74H6 and UGT74H7). Identical amino acids are highlighted in black and similar amino acids in grey. The PSPG box (a conserved region corresponding to the UDP-D-glucose binding domain; Hughes and Hughes, 1994; *DNA Seq* 5: 41-49) is underlined.



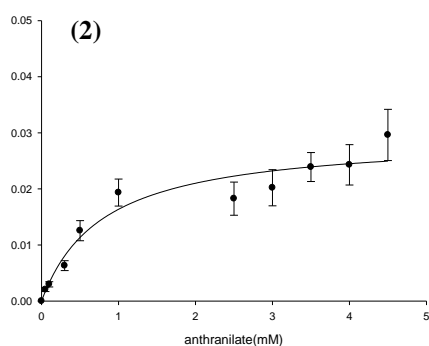
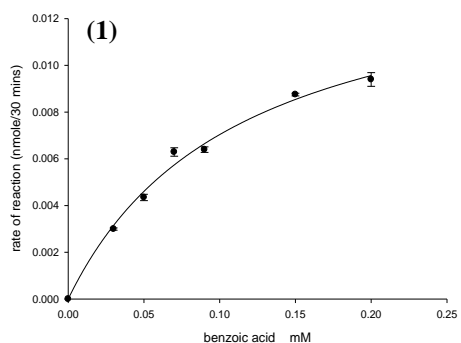
**Supplementary Figure 3.** Analysis of the three purified His<sub>6</sub>-tagged oat glycosyltransferases following expression in *E. coli*. Proteins were run on a 4-12% SDS-PAGE gradient gel and detected by staining with Coomassie brilliant blue (left) or by Western blot analysis using polyclonal antisera raised against UGT74H5 (right).



**Supplementary Figure 4A.** Enzyme kinetics for UGT74H5. Plots of the product formation rates as a function of substrate concentration (1: *N*-methylantranilic acid, 2: benzoic acid, 3: anthranilic acid).



**Supplementary Figure 4B.** Enzyme kinetics for UGT74H6. Plots of the product formation rates as a function of substrate concentration (1: *N*-methylantranilic acid, 2: benzoic acid, 3: anthranilic acid, 4: salicylic acid).



**Supplementary Figure 4C.** Enzyme kinetics for UGT74H7. Plots of the product formation rates as a function of substrate concentration (1: benzoic acid, 2: anthranilic acid).



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      10      20      30      40      50
UGT74H7  1  MGAEPGHVYCDIHVLVLPYP LQGHINPMLQFAKRLAR TQTHIGGGGG ---VRCTLAVTP 56
UGT74H6  1  MGAEPGHVYCDIHVLVLPYP LQGHINPMLQFAKRLAR TQTHIGGGGGGGGGVRCTLAVTP 60
UGT74H5  1  MGAWEHVS-DIHVL L LYPYVQGHINPMLQFGR LA ---HIG-CVG ---VRCTLAITP 50

      70      80      90     100     110     120
UGT74H7  57  YLLGQCQDPCPGAVHLAEISDGFDRAGFLEVGDVAAYLAQLESAGSR TLD ELLRS EAEKG 116
UGT74H6  61  YLLGQCQDPCPGAVHLAEISDGFDRAGFLEVGDVAAYLAQLESAGSR TLD ELLRS EAEKG 120
UGT74H5  51  YLLRQCQDPCPGAVHLV EISDGFDSAGFELEVGDVAAYLAGMESAGSR TLD ELLRS EAEKG 110

      130     140     150     160     170     180
UGT74H7  117 RKVCAVVYDSFLQPWAPPVARRHGAACVSFF TQAPAVN LAYAHHARGGGTGGRL EGLPAG 176
UGT74H6  121 RKVCAVVYDSFLQPWAPPVARRHGAACVSFF TQAPAVN LAYAHHARGGGTGGRL DGLPAG 180
UGT74H5  111 RPIHAVVYDAFLQPWVPRVARLHGAACVSFF TQA AVN VAYSRR ---VGKIEEGLPAG 165

      190     200     210     220     230     240
UGT74H7  177 FEHEDLPTFLTMPDDCPPYLEMLLRQHVG L DAVDHVLVNS FHELQPLESDYMASKWGAKT 236
UGT74H6  181 FEHEDLPTFLTMPDDCPPYLEMLLRQHVG L DAVDHVLVNS FHELQPLESDYMASKWGAKT 240
UGT74H5  166 FEAE DLPTFLTLP ---LPYQDMLLSQFVGLDAVDHVLVNS FHELQPLESAYMES TWGAKT 222

      250     260     270     280     290     300
UGT74H7  237 VGPTVPSAYLDKRIPDDVSYGFHLYTP T TAT TAWLD AOPPRSVAYVSYFGSMAAPGPEQM 296
UGT74H6  241 VGPTVPSAYLDKRIPDDVSYGFHLYTP T TAT TAWLD AOPPRSVAYVSYFGSMAAPGPEQM 300
UGT74H5  223 VGPTVPSAYLDKRITDDVSYGFHLYTPMTATTKAWLD AOPPRSVITVSYFGSMATPGPTEM 282

      310     320     330     340     350     360
UGT74H7  297 AEMA EGLHSSGKAF LWVVRAS EASKIPDGFS EKVGTRGLVVPWVAQLEVL AHS AVGCFVT 356
UGT74H6  301 AEMA EGLHSSGKAF LWVVRAS ETSKIPDGFS ERVGT RGLVVPWVAQLEVL AHS AVGCFVT 360
UGT74H5  283 AEMA EGLHSSGKAFLWVVRAS EASKIPDGFERVGT RGLVVTWVAQLEVL AHS AVGCFVT 342

      370     380     390     400     410     420
UGT74H7  357 HCGWNS TMEALGAGLPMVAVPQWS DQPTNAKYVEDVWC VGVRRARRDPEAGVVRREEVERC 416
UGT74H6  361 HCGWNS TMEALGAGLPMVAVPQWS DQPTNAKYVEDVWC VGVRRARRDPESGVVRREEVERC 420
UGT74H5  343 HCGWNS TMEALGAGVPMVAVPQWS DQPTNAK FVEDVWC VGVRRARRDPE -GVVRREEVERC 401

      430     440     450     460     470     480
UGT74H7  417 VKEVMGADKOYARNASDWKEKAVRSMCQGS S EKNITEFLHALR - -SRKSOKS TQPKQI - 473
UGT74H6  421 VKEVMGADKOYARNASDWKEKAVRSMCQGS SDKNITEFLHALVVGSRKSOKLI I - - - - 475
UGT74H5  402 IREVTGDDK -YACNALDWKEKSKRAMSQGS SDMNITEFLQALR -RSRKS YEAKPIEPLL 459

      . . . . |
UGT74H7  473 - - - - - 473
UGT74H6  475 - - - - - 475
UGT74H5  460 VGLDA 464

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**Supplementary Figure 5.** LC-MS/MS analysis of trypsin-digested protein from oat roots (excised following SDS-PAGE) reveals protein fragments corresponding to UGT74H5 (shown in red).