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Supplemental Material to:

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Arabidopsis thaliana glucuronosyltransferase in family GT14

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Supplemental materials

Materials and methods

DNA cloning

Cloning of *At5g39990* (*AtGlcAT14A*) has been described previously[1] At3g24040 was cloned from *At3g24040* cDNA obtained from Nottingham Arabidopsis Stock Centre (http://arabidopsis.info/) using a forward primer containing the Gateway attB1 site (italics), FLAG tag (double underline) and a *At3g24040* coding sequence encoding amino acid from 30- (single underline): 5'-*ACAAGTTTGTACAAAAAAGCAGGCTTC*ATGGATTACAAGGACGACGACGACAAGTCTTCCCAGTACTCTTCATCTTC-3' and a reverse primer containing the attB1 site (italics) and an *At3g24040* coding sequence including a stop codon (underline)**:** 5'-*ACCACTTTGTACAAGAAAGCTGGGT*TTATTCAGGTACGCATTGGTGCAAT-3'. The PCR product was moved to a Gateway entry vector, pDONR223, by a BP clonase reaction (Life Technologies). The entry clone was further moved to the modified pPicZalphaA vector encoding the N-terminal FLAG tag [1] by a LR reaction (Life Technologies). Other Arabidopsis GT14 isoforms were codon-optimized for an expression in yeast and DNA-synthesized as soluble constructs, including the Gateway attL sites on both sides (GenScript Ltd., Hong Kong). The synthesized DNA was moved to the modified pPicZalphaA as described above.

Gene	Transmembrane domain	Gene synthesized	Calculated MW
At4g27480	-	31-421	44708.9
At4g03340	36-58	59-448	45741.9
At3g03690	7-26	27-378	41935.3
At1g71070	7-29	30-395	42738.4
At3g24040	12-29	30-417[a]	44989.7
At3g15350	12-34	35-424	45867.4
At1g03520	37-59	60-447	45661.6
At1g53100	24-46	47-423	44044.4
At5g15050	22-44	45-434	45599.9
At2g37585	12-34	35-384	41778.9

Above is the summary of (i) the sites of the predicted transmembrane domain in the full-length cDNA (indicated by the sites of amino acids); (ii) the corresponding amino acid sequences synthesized after codon optimization; and (iii) the predicted molecular weight (MW) for each recombinant protein as a fusion protein with a FLAG tag. [a]*At3g24040* was not codon-optimized but cloned manually from Arabidopsis cDNA as described above.

Preparation of recombinant GT14 isoforms

Transformation of the expression vectors to the *P. pastoris* KM71 strain, growth condition, protein purification using anti-FLAG antibody, and the Western blot analysis followed the methods described in Knoch et al.[1]. Estimation of the level of proteins was performed by the analysis of the Western blot images using ImageJ software[2].

Enzyme assay

The enzyme assay basically followed the method described in [1]: in brief, the assay was performed in the presence of 0.1 mM UDP-^{14}[C]-GlcA (containing 277.5 Bq of UDP-^{14}[C]-GlcA), 20 mM McIlvaine buffer, pH 5.0, 5 µl of affinity-purified recombinant proteins collected on anti-FLAG-agarose, and either the $GAGP_8$ or β-1,3-galactan acceptor [3]. Incubation was performed at 22°C for 16 h, and either the ^{14}[C]-GlcA incorporated products were collected in 70% (v/v) ethanol and subsequently treated with specific hydrolases and by size exclusion chromatography or the products were precipitated in the presence of 0.25 µl of 10 mg/ml horseradish peroxidase and 0.28 µl of 0.3% H_2O_2. The size exclusion chromatography was performed using Superdex Peptide HR 10/30 (GE Healthcare) and eluted by 50 mM ammonium formate (flow rate: 0.4 mL/min, 2 min/fraction). The [^{14}C]-sugars were analyzed by scintillation counting.

References

1. Knoch E, Dilokpimol A, Tryfona T, Poulsen CP, Xiong G, Harholt J, Petersen BL, Ulvskov P, Hadi MZ, Kotake T, et al. A β-glucuronosyltransferase from Arabidopsis thaliana involved in biosynthesis of type II arabinogalactan has a role in cell elongation during seedling growth. Plant J 2013; 76:1016–29.

2. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012; 9:671–5.

3. Geshi N, Johansen JN, Dilokpimol A, Rolland A, Belcram K, Verger S, Kotake T, Tsumuraya Y, Kaneko S, Tryfona T, et al. A galactosyltransferase acting on arabinogalactan protein glycans is essential for embryo development in Arabidopsis. Plant J 2013; 76:128–37.