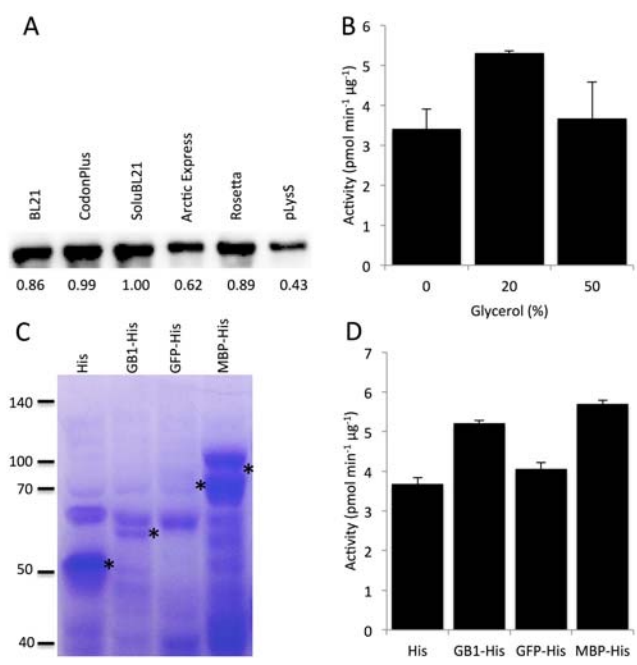
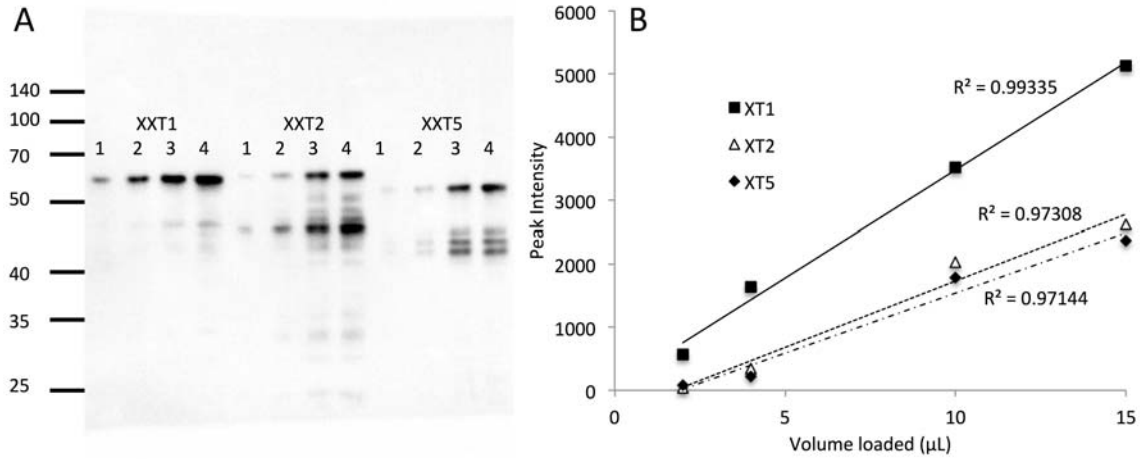


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 2 Supplemental Figure S1. Predicted topology of xyloglucan xylosyltransferases
 3 according to the Universal Protein Resource (<http://www.uniprot.org>). A. Diagram of
 4 xyloglycated glucan subunit showing nomenclature used. B. Positions of the predicted
 5 transmembrane domain (TM) and catalytic domain based on primary amino acid
 6 sequence. The section between the TM and the catalytic domain corresponds to the stem
 7 region of the protein. Red lines indicate the predicted glycosylation sites (Asn-X-
 8 Ser/Thr). C. Position of the His-Tag, solubility tag, and cDNA of recombinant proteins.
 9 D. Sequence alignment of XXT1, XXT2, XXT5, and PBVC-1 GT (Zhang et al., 2007).
 10 Red boxes indicate the positions of mutated amino acids. Sequence alignment was
 11 created using Clustal Omega.



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17 Supplementary Figure S2. Optimization of expression conditions for XXT2. A. Western
 18 blot of purified His-GB1-XXT2 expressed in various cell lines. Numbers below the
 19 bands indicate the relative intensity of the bands as analyzed with ImageJ. B. Effect of
 20 glycerol on His-GB1-XXT2 activity. All assay reactions contain equal amounts of total
 21 protein. C. Coomassie stained SDS-PAGE gel of XXT2 expressed with various
 22 solubility tags. All constructs were expressed in SoluBL21 cells and were purified with
 23 Ni-NTA columns. Proteins were loaded in equal volumes. XXT2 protein with the
 24 various tags is shown with asterisks. Expected sizes are His-XXT2: 49.5 kDa; His-GB1-
 25 XXT2: 56.9 kDa; His-GFP-XXT2: 76.4 kDa; His-MBP-XXT2: 91.2 kDa. D. Enzyme
 26 activity of purified XXT2 fused with various tags. All reactions contained an equal
 27 volume of the elution fraction collected from Ni-NTA resin. Products of the enzyme
 28 assays in B and D were analyzed by HPAEC, quantified by peak integration, and are
 29 presented as pmol xylose transferred per minute per μg of XXT2. All enzyme assays
 30 were performed in duplicate.



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32 Supplemental Figure S3. Quantitative western of XXT1, XXT2, and XXT5. A. Western

33 blot of XXT1, XXT2, and XXT5. Concentrated protein was diluted to the appropriate

34 level for detection on western blot, XXT1 was diluted 70-fold, XXT2 was diluted 32-

35 fold, and XXT5 was diluted 20-fold. All diluted protein samples were loaded at four

36 volumes, 2, 4, 10, and 15 μL. B. Band intensity was plotted against volume loaded on

37 the SDS-PAGE gel. Band intensity of full-length proteins was determined using ImageJ.

38 The solid line indicates XXT1 line, the dashed line indicates XXT2 , and the dashed line

39 with large spaces indicates XXT5.

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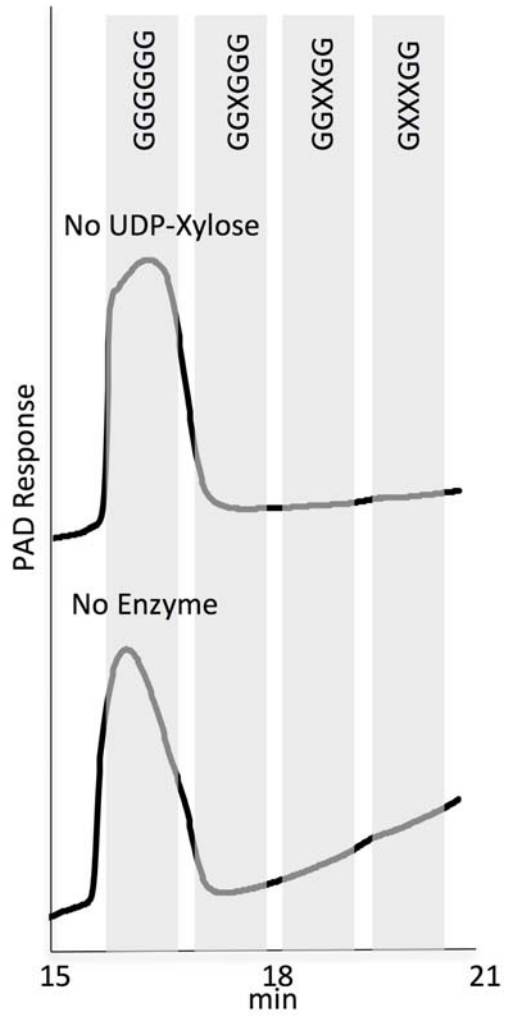
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50 Supplemental Figure S4. Negative Controls of XXT2 activity assay. Negative control
 51 reactions were done as described in Material and Methods with either no UDP-xylose or
 52 no XXT2 in the activity assay mixture.

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Gene	Direction	Organism	Sequence
XXT1	forward	Bacterial	ATA GGA TCC GAG AAC CTG TAC TTT CAG GGC ACG CCG GAG AAA GAT ATC
XXT1	reverse	Bacterial	ATA GTC GAC TCA CGT CGT CGT CG
XXT2	forward	Bacterial	ATA GGA TCC GAG AAC CTG TAC TTT CAG GGC AAA TTC GGA ACT CCG GAG
XXT2	reverse	Bacterial	GCA CTC GAG TCA AAC TTG ATT GGT
XXT5	forward	Bacterial	ATA AGA TCT GAG AAC CTG TAC TTT CAG GGC AAC CTA GGA AGC TCA AGC GCC GAT
XXT5	reverse	Bacterial	ATA CTC GAG CTA GTT CTG TGG TTT GGT
XXT5 D228A:D230A	forward	Bacterial	GATCTGGTGGATGGCTAGTGCTGCTTTGTTCCT
XXT5 D228A:D230A	reverse	Bacterial	AGTGAACAAAGCAGCACTAGCCATCCACCAGATC
XXT5	forward	Plant	AATAAGCTTATGGGTCAAGATGGTTC
XXT5	reverse	Plant	ACCATATGCTAGTTCTGTGGTTTGGTTCCAC
XXT5 D228A:D230A	forward	Plant	GTTGATGTTGTCTCATCCAGAAGTTGAGTGGATCTGGTGGATGGCTAGTGCTGCTTTGTTC
XXT5 D228A:D230A	reverse	Plant	CCACCAGATCCACTCAACTTCTGGATGAGACAACATCAAC

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60 Supplemental Table S1. Forward and reverse primers used for the amplification of the
61 XXTs and the truncations of XXT2. Gene shows the XXT that the primer was used for.
62 Direction shows the direction the primer will amplify. Organism shows the expression
63 system.

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