#### SUPPLEMENTAL DATA

Supplemental Table I. Sequence and annealing temperatures of the primers used in this work.

**Supplemental Figure 1.** Sequence of  $\varphi Atxyl2$  in ecotypes Wassilewskija and Columbia. Translations in the three reading frames of potential Wassilewskija coding regions are shown between the aligned sequences. The conserved frame is highlighted in grey. Inactivating mutations or insertions in both ecotypes are shown in red, while differences between ecotypes are highlighted in black. Reading frames created by insertions and leading to premature stop codons are also highlighted in black.

Supplemental Figure 2. MALDI-TOF/TOF spectra of oligosaccharides accumulated in Atxyl1-2 growth medium and reference samples. A. MALDI-TOF/TOF spectrum of commercial XXXG. Structure is shown in upper left corner. Only fragments resulting from breaks of glycosidic bonds have been labeled with their size and losses (Pent for pentose and Hex for hexose). Fragmentation of glycosidic bonds results in Y or B ions, depending if the charge is retained at the reducing or nonreducing end (Domon and Costello, 1988). The expected sizes of these fragments are indicated in the structure. Other fragments are combinations of both kinds of fragmentation. B. MALDI-TOF/TOF spectrum of the peak at m/z 1085 detected in liquid growth media of 7-day-old Atxyl1-2 seedlings. C. Calibration curve of XXXG. Maltoheptaose at 50 µM final concentration was added to triplicated samples of each calibration point and the ratio of the peak areas at m/z 1085 (XXXG) and 1175 (maltoheptaose) was calculated. D. MALDI-TOF/TOF spectrum of commercial XLLG. Structure is shown in upper left corner. Triangles mark Y and B ions resulting from fragmentation between the central glucose residues. E. MALDI-TOF/TOF spectrum of the peak at m/z 1247 detected in liquid growth media of 7-day-old Atxyl1-2 seedlings. Expected ions resulting from fragmentation between the central glucose residues are shown for both XXLG and XLXG. The 659 ion is much more abundant than the 773 ion (grey triangles) suggesting that XLXG is, at most, a minor component. It might not even be present since several double and triple fragmentations can produce 773 ions from XXLG (one is shown by dotted lines). This type of fragmentation is represented by the 935 peak in the XLLG spectrum.

**Supplemental Figure 3.** MALDI-TOF/TOF spectrum of the peak at m/z 1247 in enzyme-accessible xyloglucan from *Atxyl1-2* stems. Ions resulting from fragmentation of glycosidic bonds are labeled with their size and losses. Expected ions resulting from fragmentation between the central glucose residues are shown for both XXLG and XLXG. The 659 ion is much more abundant than the 773 ion (grey triangles) suggesting that XLXG is, at most, a minor component.

**Supplemental Figure 4.** Silique development in *Atxyl1-1*. A. Silique length from anthesis to 10 days after. Error bars are standard deviations. Two asterisks indicate non-significant differences, one asterisk is for differences significant with a 95% confidence, all other differences are significant with a 99.5% confidence. B. Silique width in *Atxyl1-2*. Data are from the same siliques as in A and symbols are used in the same way.

### REFERENCES

**Domon B, Costello CE** (1988) A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. Glycoconj J **5**: 397-409

Supplemental Table I. Sequence and annealing temperatures of the primers used in this work.

Purpose	Name	Sequence	Annealing
Screening for <i>Atxyl1-1</i>	MUT1	TTTCTCCATATTGACCATCATACTCATTG	65°C
	JL-202	CATTTTATAATAACGCTGCGGACATCTAC	
Absence of insertion Atxyl1-1	XYL1-1L	TCTTGGACACTACTGCTTGAGTCAT	58ºC
	XYL1-1R	TGGGATGATCCTCCTTACAAGATTA	
Presence of insertion <i>Atxyl1-1</i>	XYL1-1R	TGGGATGATCCTCCTTACAAGATTA	58ºC
	WIS-LB	TTTATAATAACGCTGCGGACATCTAC	
Absence of insertion Atxyl1-2	XYL1-2L	TTTATGGTTCGCATCCGATGTA	58ºC
	XYL1-2R	CAAGAACCGTGGTCCATGTCTA	
Presence of insertion <i>Atxyl1-2</i>	XYL1-2L	TTTATGGTTCGCATCCGATGTA	58ºC
	GABI-LB	CCATATTGACCATCATACTCATTGC	
Cloning AtXYL2	XYL2L1	TGCTAAACAAAATCAATGGCTTC	58ºC
	XYL2R1	TCTCTCTTTTGGGGACAATTT	
Promoter AtXYL1	PRXYL1L1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGATTT TAACGGCCGTATGTGTG	58°C for 10 cycles, 68°C for 25
	PRXYL1R1	GGGGACCACTTTGTACAAGAAAGCTGGGTGGAG AGAGAGATGGAGGGGTTT	

Wassilewskija CAATGGCTTCTTGTCTTTCTCTTGTTGCTATTATCCTCTGCTTCTCATCTCCCAGTGTTCCAACGCCATCGGC G F L S F S S C C Y Y P L L L I S P V F Q R H R Q W L L V F L F L L L L S S A S H L S S V P T P S A MASCLSLLVAIILCFSSLQCSNAIG Columbia  ${\tt caatggcttcttgtcttcttcttcttgttgctattatcctctgcttctcatctcccagtgttccaacgccatcggc}$ 

- 91 AAAGGCTACCGTCTGATCTCCATGGAGAAGTCTCCCGATGATGGCAGCTTCATTGGCTATCTCCAAGTGAAGCAGAGTAACAAAATCTAC R L P S D L H G E V S R \* W Q L H W L S P S E A E \* Q N L R K A T V \* S P W R S L P M M A A S L A I S K \* S R V T K S T K G Y R L I S M E K S P D D G S F I G Y L Q V K Q S N K I Y AAAGGCTACCGTCTGATCTCCATGGAGAAGTCTCCCGATGATGGCAGCTTCATTGGCTATCTCCAAGTGAAGCAGAGTAACAAAATCTAC
- L \* Y N H P S T L H Q A L I \* P S F D S S S S G S D I T I L R L F I K
  - S

271 cgttttttttttgtagGCACGAGACGGATCATCGCCTCGCGTTCACATCACAGACGCAAAGAAACAACGATGGGAAGTTCCGTACAATC HETDHRLRVHITDAKKQRWEVPYNL ARDGSSPPRSHHRRKETTMGSSVQS TRRIIASAFTSQTQRNNDGKFRTI 

- 361 TCCTCCGCCGGGAACAACCACCGAATGTAATCGGAAAATCAAGAAAATCTCCGGTTACGGTACAAGAAATATCCGGACGTGAGTTT L R R E Q P P N V I G K S R K S P V T V Q E I S G <mark>R</mark> E L I L PPPGTTTECNRKIKKISGYGTRNIRT\*ADF SSAGNNHRM\*SENQENLRLRYKKYPDVS\*F TCCTCCGCCGGGAACAACCACCGAATGTAATCGGAAAATCAAGAAAATCTCCGGTTACGGTACAAGAAATATCCGGACCGGAGCTGATTT
- 451 TAATCTTCACCGTATATCCTTTCAGCTTCGCCGTGAGAAGAAGATCCAACGGCGAGACGATTTTCAACACTAGTAGCTCCGATGAGTCAT IFTV PFSFAV RRRSNGETIFNTSSSDESF N L H R I S F Q L R R E K K I Q R R D D F Q H \* \* L R \* V I \* S S P Y I L S A S P R E E D P T A R R F S T L V A P M S H Т TAATCTTCACCGTAGATCCTTTCAGCTTCGCCGTAAGAAGAAGAAGAACCACCGGCGAGACGATTTTCAACACTAGTAGCTCCGATGAGTCAT
- 541 TCTGGAGAGATGGTGTTCAAGGACCAGTACCTCGAGATCTCAACTTCTTTACCTAAAGACGCGTCTCTATACGGATTTGGTGAAAACTCT W R D G V Q G P V P R D L N F F T \* R R V S I R I W \* K L E R W C S R T S T S R S Q L L Y L K T R L Y T D L V K KLS Т T. S G E M V F K D Q Y L E I S T S L P K D A S L Y G F G E N S TC GGAGAGATGGTGTTCAAGGACCAGTACCTCGAGATCTCAACTTCTTTACCTAAAGACGCGTCTCTATACGGATTTGGTGAAAACTCT
- 631 CAAGCCAATGGAATCAAACTGGTTCCTAATGAGCCATACACTCTCTTCACTGAAGACGTCTCGGCGTTTAATCTCAACACTGATCTTTAC S Q W N Q T G S \* \* A I H S P H \* R R L G V \* S Q H \* S L R K P M E S N W F L M S H T L S S L K T S R R L I S T L I F T Q A N G I K L V P N E P Y T L F T E D V S A F N L N T D L Y caagccaatggaatcaaactggttcctaatgagccatacactctcttcactgaagacgtctcggcgtttaatctcaacactgatctttac
- 721 GGGTCGCATCCGGTTTACATGGATTTGAGAAACGTTAGAGGTAAAGCCTATGCACACTCTGTTCTGCTTCTTAACAGTCATGGTATGGAT V A S G L H G F E K R \* R \* S L C T L C S A S \* Q S W Y G C G R I R F T W I \* E T L E V K P M H T L F C F L T V M V W M G S H P V Y M D L R N V R G K A Y A H S V L L L N S H G M D GGGTCGCATCCGGTTTACATGGATTTGAGAAACGTTAG
- 811 GTGTTTTACAGAGGCGATTCCTTGACGTACAAGGTAATTGGAGGTGTTTTTGATTTCTATTTCTTCGCTGGACCGTCGCCCTCTTAATGTC V L Q R R F L D V Q G N W R C F \* F L F L R W T V A S \* C R C F T E A I P \* R T R \* L E V F L I S I S S L D R R L L M S V F Y R G D S L T Y K V I G G V F D F Y F F A G P S P L N V GTGTTTTACAGAGGCGATTCCTTGACGTACAAGGTAATTGGAGGTGTTTTTGATTTCTATTTCTTCGCTGGACCGTCGCCTCTTAATGTC
- 901 GTTGATCAATACACTTCGTTAATAGGACGGCCAGCGCCAATGCCGTACTGGTCTCTAGqtaatatatctcacttqtcttaqtttaactqt \* S I H F V N R T A S A N A V L V S R J N T L R \* \* D G Q R Q C R T G L \* V D Q Y T S L I G R P A P M P Y W S L G  ${\tt GTTGATCAATACACTTCGTTAATAGGACGGCCAGCGCCAATGCCGTACTGGTCTCTAGgtaatatatctcacttgtcttagtttaactgt$
- 991 aacttatgtggtttcttactaatctttaacctaatttgtgctctagGGTTTCACCAATTTAGATGGGGGTACCGTAATGTATCAGTTGTT VSPI\*MGVP\*CISC\* GFTNLDGGTVMYQLL

F H Q F W G Y R N V S V V aacttatgtggtttcttactaatctttaacctaatttgtgctctagGGTTTCACCAATGTAGATGGGGGTACCGTAATGTATCAGTTGTT

R C G G \* L P K G \* D P S \* C D L E R C \* L H G W L Q G L H K M W W I I T K R L R S L L M \* F G T M L I T W M V T G T S K D V V D N Y Q K A K I P L D V I W N D A D Y M D G Y K D F 

- 1171 ACTTTGGATCTTGTGAATTTTCCTCATGCTAAGCTATTGAGTTTCTTGGATAGAATCCATAAGATGGGAATGAAGTATGTTGTGATAAAA F G S C E F S S C \* A I E F L G \* N P \* D G N E V C C D K R L W I L \* I F L M L S Y R V S W I E S I R W E \* S M L \* \* K T L D L V N F P H A K L L S F L D R I H K M G M K Y V V I K ACTTTGGATCTTGTGAATTTTCCTCATGCTAAGCTATTGAGTTTCTTGGATAGAATCCATAAGATGGGAATGAAGTATGTTGTGTATAAA
- 1261 GATCCTGGTATTGGTGTCAACGCGAGTTACGGTGTATACCAAAGAGGCATGGCTAGTGATGTGTTCATTAAATATGAAGGAAAGCCTTTC S W Y W C Q R E L R C I P K R H G \* \* C V H \* I \* R K A F L I L V L V S T R V T V Y T K E A W L V M C S L N M K E S L S D P G I G V N A S Y G V Y Q R G M A S D V F I K Y E G K P F GATCCTGGTATTGGTGTCAACGCGAGTTACGGTGTATACCAAAGAGGCATGGCTAGTGATGTGTTCATTAAATATGAAGGAAAGCCTTTC
- 1351 TTGGCTCAAGTGTGGCCTGGTCCAGTTACTTCCCTGATTCCTTAACCCGAAAACGGTTTCTTGGTGGGGCGATGAAATTCGACGTTC G S S V A W S S L L P \* F P \* P E N G F L V G R \* N S T F P W L K C G L V Q F T S L I S L T R K R F L G G A M K F D V S L A Q V W P G P V Y F P D F L N P K T V S W W G D E I R R F TTGGCTCAAGTGTGGCCTGGTCCAGTTTACTTCCCTGATTCCTTAACCCGAAAACGGTTTCTTGGTGGGGGCGATGAAATTCGACGTTC
- 1531 CAGTGTCCGAGTGGGGGGGGAGAACCTGGTGTAACGTGTGCTTGGACTGCAAGAATATAACCAATACAAGCTGGGATGA<mark>TCCACCAT</mark>TCCAC V S E W G R T W C N V L L G L Q E Y N Q Y K L G \* S T I P P S V R V G E N L V \* R V A W T A R I \* P I Q A G M I H H S T Q C P S G G E P G V T C C L D C K N I T N T S W D D P F H CAGTGTCCGAGTGGGGGGGGAGAACCTGGTGTAACGTGTGCTTGGGACTGCAAGAATATAACCAATACAAGCTGGGATGA<mark>TCCACCAT</mark>TCCAC
- 1621 CTTACAAGATCAATGCTACTGGACACAAAGCT Y K I N A T G H K A P L G F K T I P T S A Y H Y N G V R E Y L Q D Q C Y W T Q S S S R V Q D Y S H K C L S L Q W C S \* V L T R S M L L D T K L L \* G S R L F P Q V L I T T M V F V S CTTACAAGATCAATGCTACTGGACACAAAGCT

- 1981 ACCGTTGGATTGAAGTTGGTGCGTTTTACCCCTTTTCAAGAGATCACGCTGATTACTACGCTCCAAGAAAAGAGCTTTACCAATGGGGAA R W I E V G A F Y P F S R D H A D Y Y A P R K E L Y Q W G T P L D \* S W C V L P L F K R S R \* L L R S K K R A L P M G N T V G L K L V R F T P F Q E I T L I T T L Q E K S F T N G E ACCGTTGGATTGAAGTTGGTGCGTTTTACCCCTTTTCAAGAGATCACGCTGATTACTACGCTCCAAGAAAAGAGCTTTACCAATGGGGAA
- 2161 GTGCALCGCTAGACCGCTCTTCTCTCTCTCTCCTGAGTTCACTGATGCTACGGTTTGAGCAAACAGTTCTTGCTCGGAAGCAGCT A S I A R P L F F S F P E F T E C Y G L S K Q F L L G S S L C I N R \* T A L L L F P \* V H \* M L R F E Q T V L A R K Q L V H Q S L D R S S S L S L S S L N A T V \* A N S S C S E A A GTGCACATCGCTAGACCGCTCTTCTCTCTCTCTCCTGAGTTCACTGATGCTACGGTTTGAGCAAACAGTTCTTGCTCGGAAGCAGCT
- 2251 TAATGATATCGCCGGTTCTTGAACAAGGTAAAACCCAAGTTGAAGCACTGTTCCCACCAGGTTCTTGGTACCACATGTTTGACATGACTC M I S P V L E Q G K T Q V E A L F P P G S W Y H M F D M T Q N D I A G S \* T R \* N P S \* S T V P T R F L V P H V \* H D S \* \* Y R R F L N K V K P K L K H C S H Q V L G T T C L T \* L TAATGATATCGCCGGTTCTTGAACAAGGTAAAACCCAAGTTGAAGCACTGTTCCCACCAGGTTCTTGGTACCACATGTTTGACATGACTC

- 2341 AGGTCGTTGTTTCAAAGAACGGGAGGCTTTTCACCCTCCCGCTCCGTTTAACGTTGTGAACGTGCATCTTTACCAGAACGCGATACTAC V V V S K N G R L F T L P A P F N V V N V H L Y Q N A I L P G R C F K E R E A F H P P R S V \* R C E R A S L P E R D T T R S L F Q R T G G F S P S P L R L T L \* T C I F T R T R Y Y AGGTCGTTGTTTCAAAGAACGGGAGGCTTTTCACCCTCCCGCTCCGTTTAACGTTGTGAACGTGCATCTTTACCAGAACGCGATACTAC
- R L R E T L S \* \* \* \* A S G D E T W K R E V N V H \* L L R I T P P G N S F L M M M S F R R \* N L E T G S Q R T L T S T H ACGCCTCCGGGAAACTCTTTCTTGATGATGATGAGCTTCCGGAGATGAAACTTGGAAACGGGAAGTCAACGTACATTGACTTCTACGCAT
- 2611 CGGTTGGAAATGAGAGT TGAAGATATGGTCGCCAAGTGAAAGAAGGTCAGTTTGCGTTGAGCCAAGGTTTGGTGATTGAGAAAGTGATTG V G N E S I K I W S Q V K E G Q F A L S Q G L V I E K V I V G W K \* E F E D M V A S E R R S V C V E P R F G D \* E S D C R L E M R V \* R Y G R K \* K K V S L R \* A K V W \* L R K \* L CGGTTGGAAATGAGAGT TGAAGATATGGTCGCAAGTGAAAGAAGGTCAGTTTGCGTTGAGCCAAGGTTTGGTGATTGAGAAAGTGATTG
- 2701 TTTTGGGACTTAAAGGAACATGGAAAGTTTCTGAAATACTTCTTAATGGAAGTTCAATCTCCAATGAGACCAAGACGATTGAGGTAAGCT L G L K G T W K V S E I L L N G S S I S N E T K T I E V S S F G T \* R N M E S F \* N T S \* W K F N L Q \* D Q D D \* G K L F W D L K E H G K F L K Y F L M E V Q S P M R P R R L R \* A TTTTGGGACTTAAAGGAACATGGAAAGTTCTGAAATACTTCTTAATGGAAGTTCAATCTCCAATGAGAACCAAGACGATTGAGGTAAGCT
- 2881 GTAAGGACTTCAACATATCTTGGAAAATGGCTAGTACTAATGTACTATCAATGGCAGGAAATGAGGTTATCGCGCGCtaagaaataaaat K D F N I S W K M A S T N V L S M A G N E V I A R \* G L Q H I L E N G \* Y \* C T I N G R K \* G Y R A L V R T S T Y L G K W L V L M Y Y Q W Q E M R L S R A GTAAGGACTTCAACATATCTTGGAAAATGGCTAGTACTAATGTACTATCAATGGCAGGAAATGAGGTTATCGCGCGCtaagaaataaaat

2971 tgtccccaaaagagagaga

tgtccccaaaagagagaga

Α



Mass (m/z)





