Supplemental data 1

Phosphate signals of spots 1, 3, 4 and 5 were not detected in the apoplastic proteins from the transgenic cells overexpressing NtPAP12, based on two-dimensional electrophoresis and staining with Pro-Q Diamond followed by SYPRO Ruby (**Figure S1**). This phenomenon is similar to that in the case of the addition of the purified purple acid phosphatase to the protein preparations from wild type cells (**Figure 1**). Their protein signals, however, continue to occur in two-dimensional gels after treatment with phosphatase (**Figure 1**) as well as in the case of overexpression of NtPAP12 (**Figure S1**). These results demonstrate that purple acid phosphatase can catalyze the hydrolysis of phosphate esters for the proteins represented by spots 1, 3, 4 and 5 both *in vitro* (treatment with the enzyme) and *in vivo* (overexpression of the enzyme). Overexpressed NtPAP12 was detected in the two-dimensional gel of wall proteins as a tailing spot with a molecular mass of 60 kDa and a pI of 7.3 (**Figure S1**). Although there are some more differences in protein spot patterns between the wild-type and transgenic cells, the differences might be partly due to an increase in secreted PAP together with a decrease and increase in other proteins, and partly due to some other indirect effects of the dephosphorylated four proteins.

Supplemental data 2

We previously demonstrated that the activities of cellulose and callose synthases are enhanced by overexpression of purple acid phosphatase in tobacco cells (Kaida et al., 2009). Although we solubilized plasma membranes from tobacco membrane sheets (Hirai et al., 1998) with a rehydration buffer and subjected them to phosphoproteomic analysis (**Figure S3**), no candidate for the substrate of the phosphatase was found in a predicted molecular size for either cellulose synthase or callose synthase among the plasma membrane proteins.

CesA and *Gsl* gene products are in the 110-120 kDa and 190-220 kDa ranges,

respectively (Bessueille and Bulone, 2008). Levels of phosphate signals in the molecular size range of the synthases were higher for the proteins from transgenic cells with high phosphatase activity than for those from wild-type cells. It is in fact very likely that those proteins, which exhibit high molecular sizes, did not enter the first isoelectric focus gel.

Supplemental method

Preparation of plasma membrane proteins

Plasma membrane proteins were obtained from plasma membrane sheets (Hirai et al., 1998). Tobacco protoplasts were immediately transferred to a coverslip that had been coated with poly-L-lysine (70,000-150,000 molecular size; Sigma-Aldrich, St. Louis, MO, USA), according to the method for plasma membrane sheets (Hirai et al., 1998). These protoplasts were then subjected to lysis in 50 mM PIPES-KOH (pH 6.8) containing 1 mM $MgCl_2$ and 5 mM EGTA. The membrane sheets were washed three times with phosphate-buffered saline (PBS; 20 mM sodium-phosphate, pH 7.0 and 150 mM NaCl), and the membrane proteins were then extracted with a rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 1% dithiothreitol, and 0.5% IPG buffer, pH 3-10; GE Healthcare).

Bessueille L, Bulone V (2008) A survey of cellulose biosynthesis in higher plants. Plant Biotechnol **25:** 315-322

Hirai N, Sonobe S, Hayashi T (1998) *In situ* synthesis of β-glucan microfibrils on tobacco plasma membrane sheets. Proc Natl Acad Sci USA **95:** 15102-15106

Kaida R, Satoh Y, Bulone V, Yamada Y, Kaku T, Hayashi T, Kaneko TS (2009) Activation of β-glucan synthases by wall-bound purple acid phosphatase in tobacco cells. Plant Physiol. **150:** 1822-1830

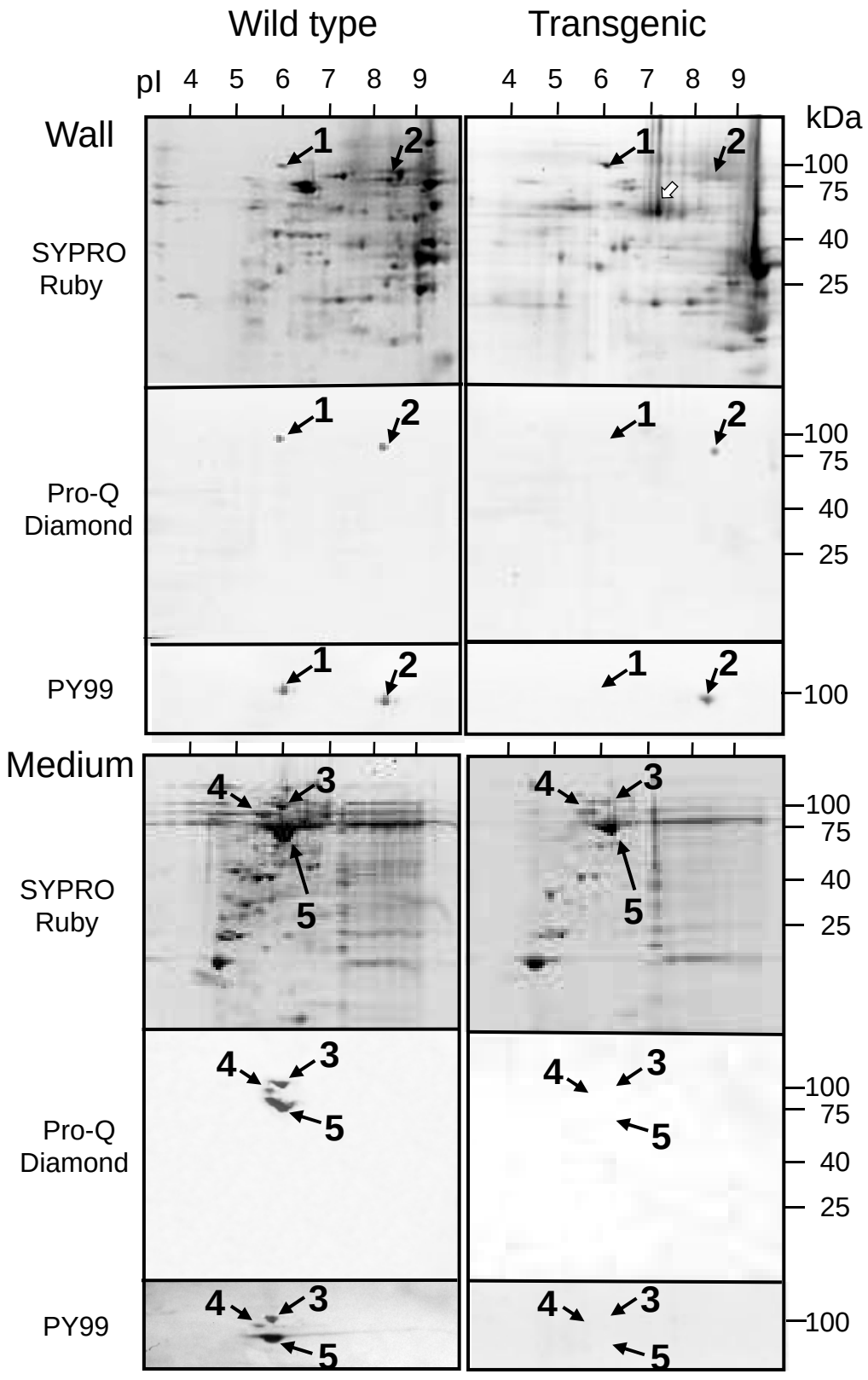

Figure S1. Images of phosphoproteomic two-dimensional analyses of proteins in the cell walls and culture media. The left and right panels show the patterns of proteins extracted from wild type cells and those of proteins extracted from transgenic cells overexpressing NtPAP12, respectively. Proteins were separated according to pH range (pH 3-10) for first-dimensional electrophoresis and by means of SDS-PAGE for second-dimensional electrophoresis. The proteins were stained with SYPRO Ruby, phosphates with Pro-Q Diamond and phosphotyrosines with PY99 antibody. Spots 1-5 are further characterized in Table 1 and Table S1. White arrow indicates overexpressed NtPAP12. The analyses by two-dimensional electrophorysis and staining with Pro-Q Diamond and PY99 antibody were performed three times.

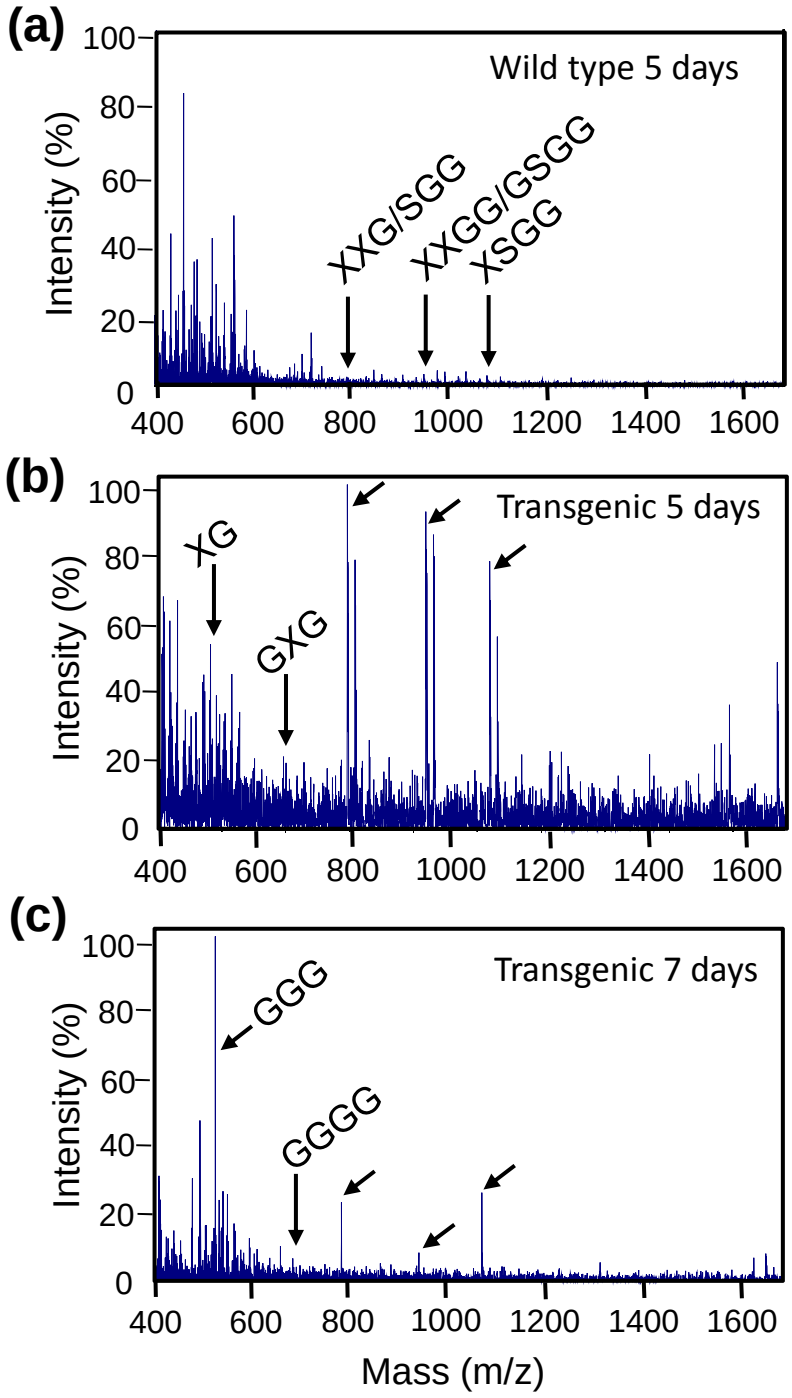

Figure S2. MALDI-TOF mass spectra of oligosaccharides released into the culture medium. The arrows indicated peak m/z values of 495, 527, 659, 689, 791, 953 and 1,085 corresponding to XG, GGG, GXG, GGGG, XXG (SGG), XXGG (GSGG) and XSGG, respectively. (a) Five-day culture of wild type cells. (b) Five-day culture of transgenic cells overexpressing purple acid phosphatase. (c) Seven-day culture of transgenic cells. Spectra were determined by addition of potassium alone, but some spectra were obtained by presence of a mixture of potassium and sodium, because it was difficult to completely remove sodium via dialysis of the culture medium.

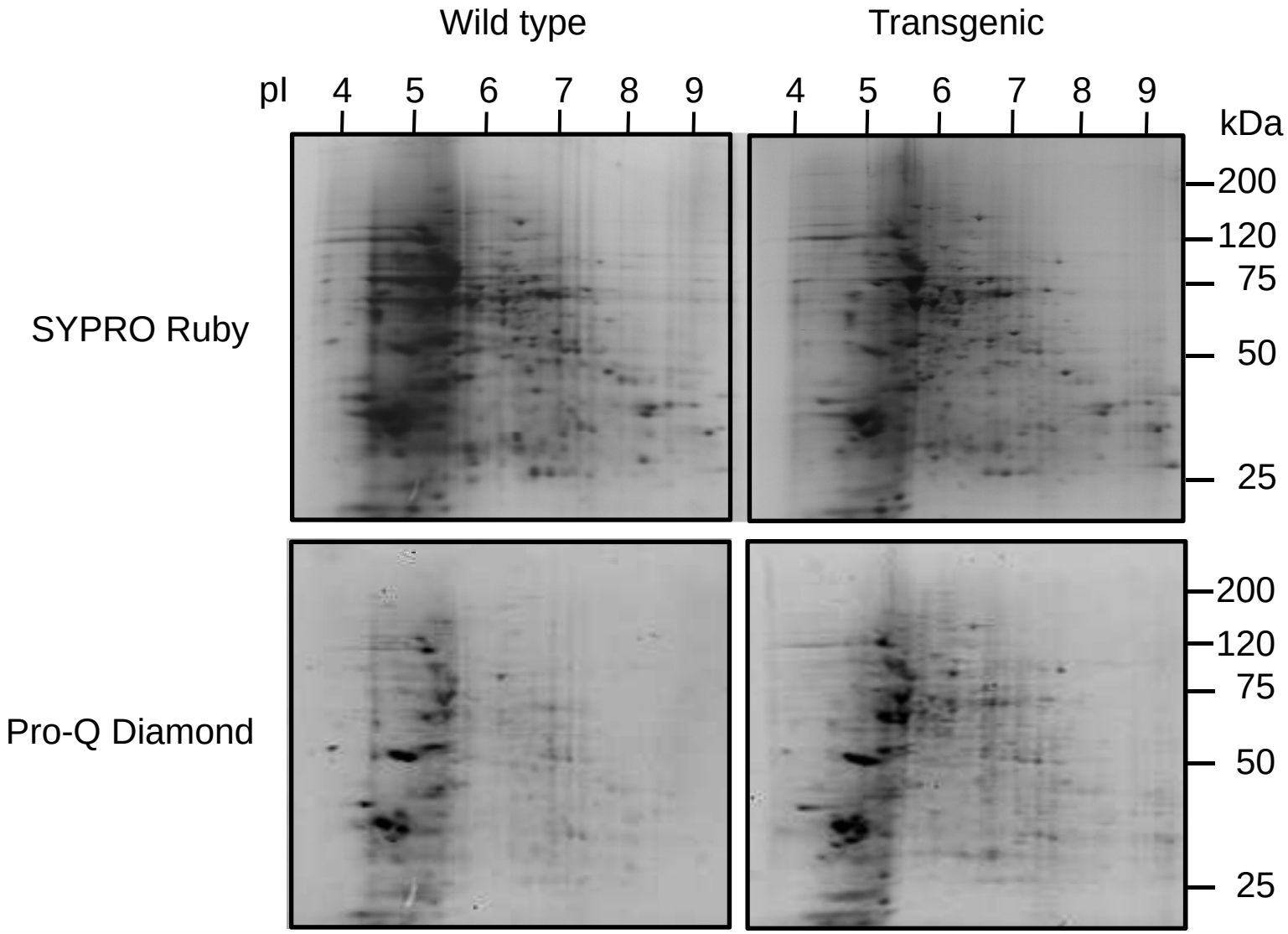

Figure S3. Images of phosphoproteomic two-dimensional analyses of plasma membrane proteins prepared from the wild type tobacco cells and transgenic tobacco cells overexpressing NtPAP12, respectively. Proteins were separated according to pH range (pH 3-10) for first-dimensional electrophoresis and by means of SDS-PAGE for second-dimensional electrophoresis. The proteins were stained with SYPRO Ruby, and the phosphates with Pro-Q Diamond. The analyses by two-dimensional electrophorysis and staining with Pro-Q Diamond were performed three times.

Table S1. Peptide sequences identified by LC-MS/MS analysis.
Each spot number shown here corresponds to that in Figure 1 and Table 1.

Spot	Predicted protein	Matched peptide sequence	Score
1	*Tropaeolum majus*	KDQYLEISTKL	44
	α-Xylosidase	KDASLYGLGENTQPHGIKI	32
		GYKTIATSATHYNGVRE	88
		RDHANYYSPRQ	45
	Solanum tuberosum	KTIATSATHYNGVR	88
	α-Glucosidase		
	Arabidopsis thakiana	KTIATSATHYNGVR	88
	α-Xylosidase		
2	Copper ion binding	ELGMPDGVLINGK	65
3	*Tropaeolum majus*	KDQYLEISTKL	44
	α-Xylosidase	KDASLYGLGENTQPHGIKI	82
		GYKTIATSATHYNGVRE	88
		HSIYGFSETIATH KGLQALEGK	64
		ATFVGSGHYAAHWT GDNK	59
		RDHANYYSPRQ	52
		FLDEDELPEMK	79
5	*Arabidopsis thakiana*	LPMTWYPQSYADK	34
	β-Glucosidase	GPTVFNFGDGLSYSNYK	59
	Lycopersicon esculentum		
	β-Xylosidase		

Mascot scores were for individual peptides ($p < 0.05$).