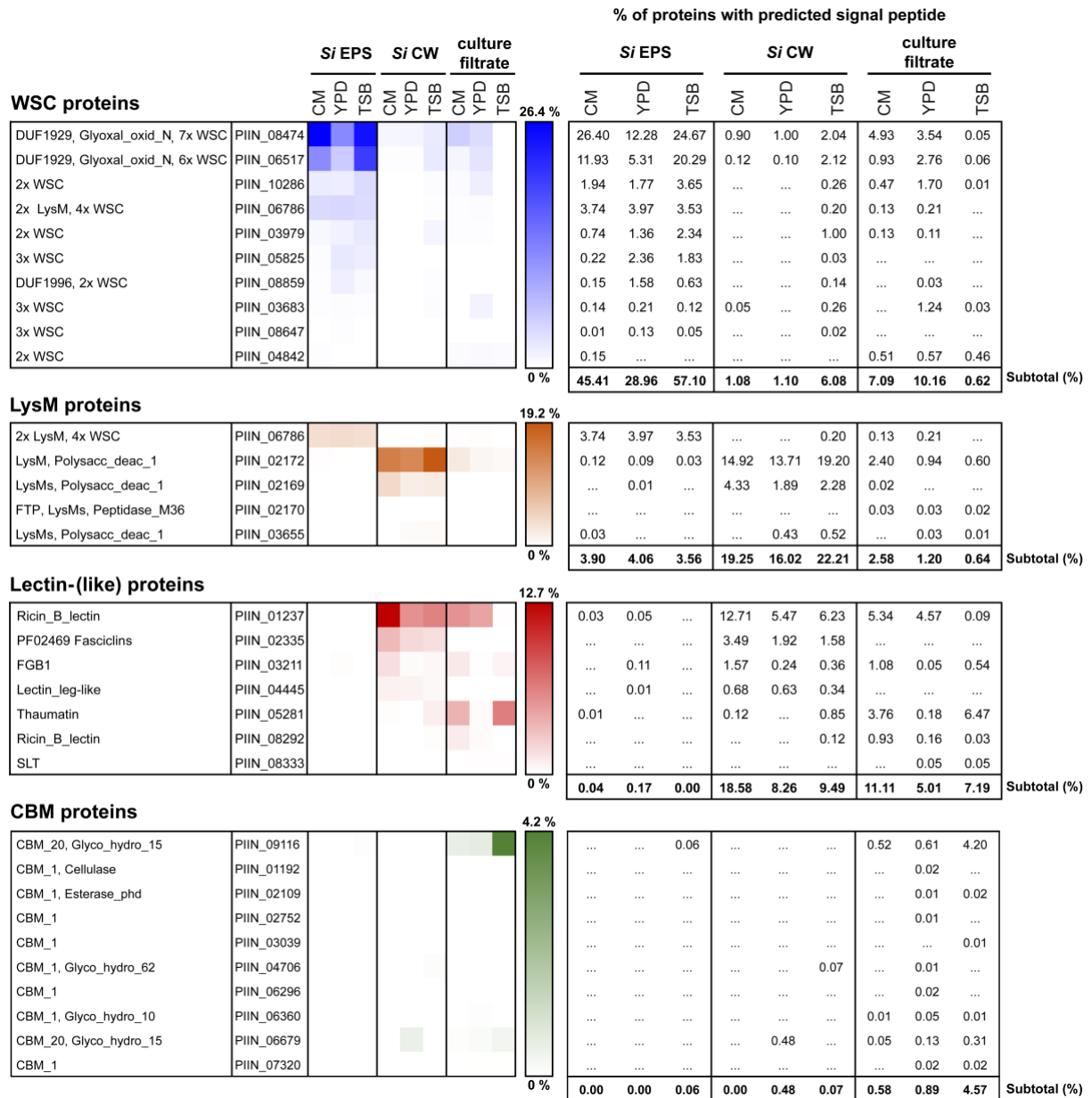




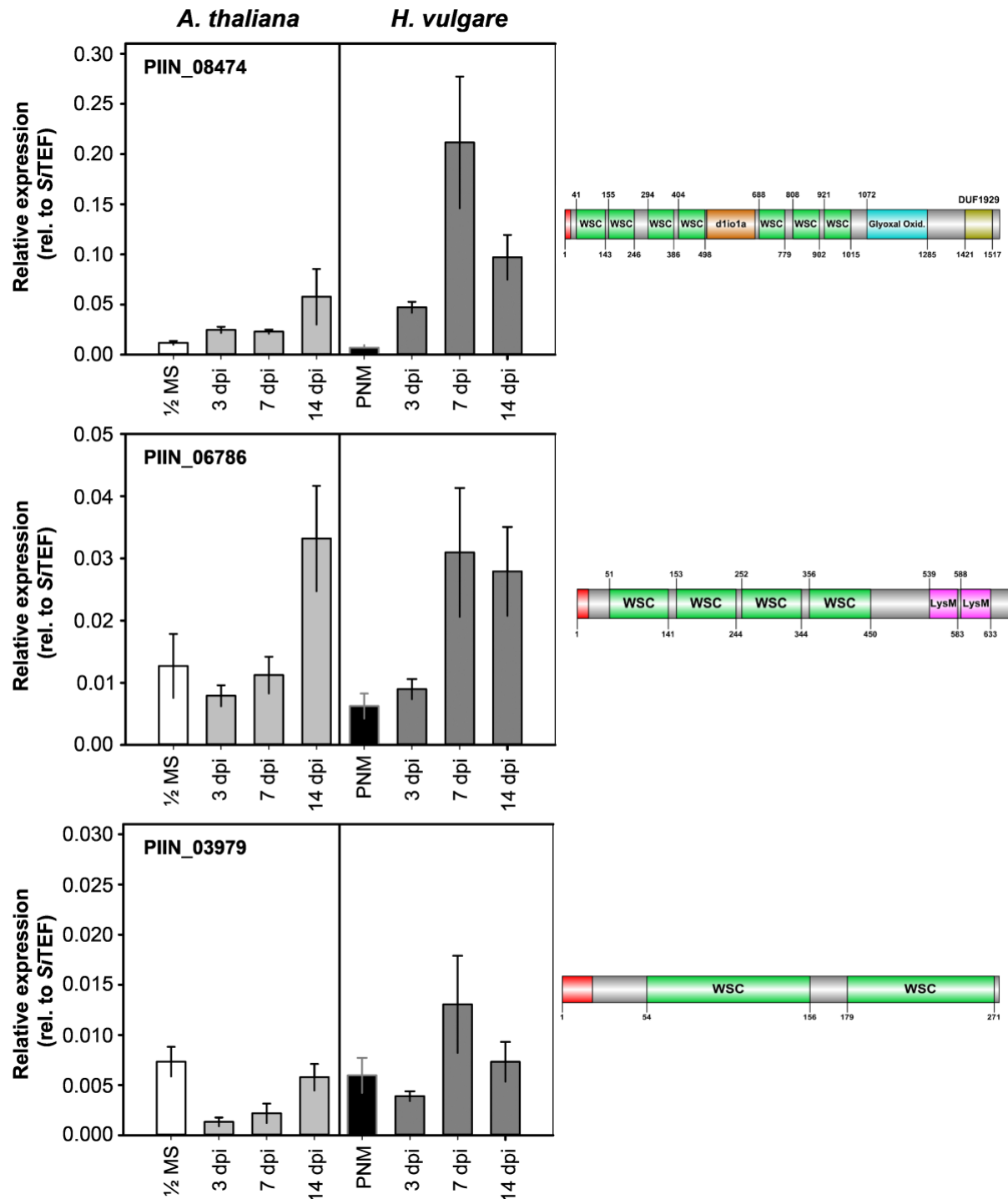
**Soluble
extracellular
polysaccharide
(EPS) matrix**

Supplemental Figure S1. EPS matrix isolated from *S. indica* grown in CM medium using a cryogelation approach. Supports Figure 2. Air bubbles were embedded in the gel-like EPS matrix after stirring to make the EPS matrix visible.



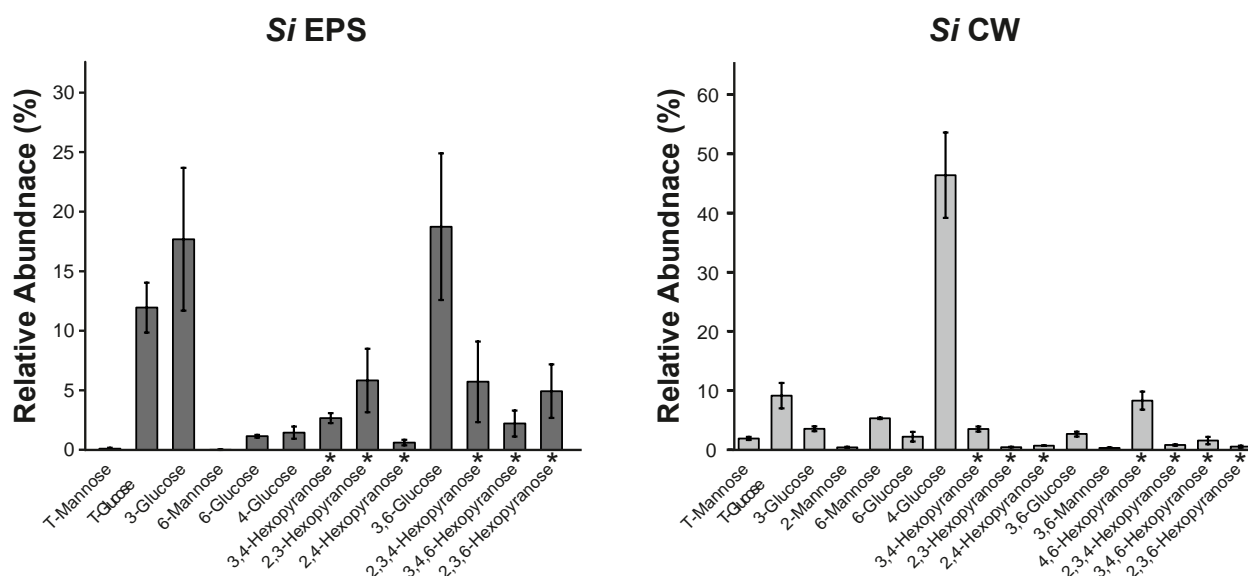
[illegible]

Supplemental Figure S2. Proteome analysis of *S. indica* EPS matrix, CW and culture filtrate. Supports Figure 2. *S. indica* spores were inoculated into three different liquid culture media: CM, YPD and TSB. The EPS matrix and CW proteins were isolated and solubilized by adding SDS-gel loading buffer and boiling at 95°C. The proteins present in the culture filtrate were precipitated using trichloroacetic acid and solubilized by boiling in SDS-gel loading buffer. The proteins were separated using a 10% SDS PAGE gel for 15 minutes and subsequently stained with Coomassie Brilliant Blue. The visible protein band in each sample was cut out and prepared for trypsin digest. The peptides were analyzed using LC-MS. Proteins with a predicted signal peptide were annotated using the protein families database Pfam. The relative abundance was calculated using the label-free quantification intensities values and expressed as a percentage (%). The proteome analysis of the EPS matrix and CW revealed that several WSC domain-containing proteins, including SWSC3 (PIIN_05825), are abundant in the matrix compared to the CW. Additionally, two polysaccharide deacetylases with a LysM domain are enriched in the CW of *S. indica*. This suggests that the detected polysaccharide deacetylases with LysM domain might be involved in chitin remodeling within the CW of *S. indica*. Furthermore, a thaumatin family protein (PIIN_05281) is enriched in the culture filtrate. Thaumatin family proteins have been shown to exhibit antifungal activity through their β -1,3-glucanase activity. It is possible that PIIN_05281 exhibits an antimicrobial function against fungal competitors. Likewise, peptidases and glycosyl hydrolases with fibronectin type III module (Fn3) domains are enriched in the matrix and the culture medium but not in the CW. Fn3 domains are versatile domains in proteins that play a major role in mediating protein–protein interactions. CW: cell wall; EPS: extracellular polysaccharide; Fn3: fibronectin type III module; LysM: lysine motive; *Si*: *Serendipita indica*; WSC: cell wall integrity and stress response component.



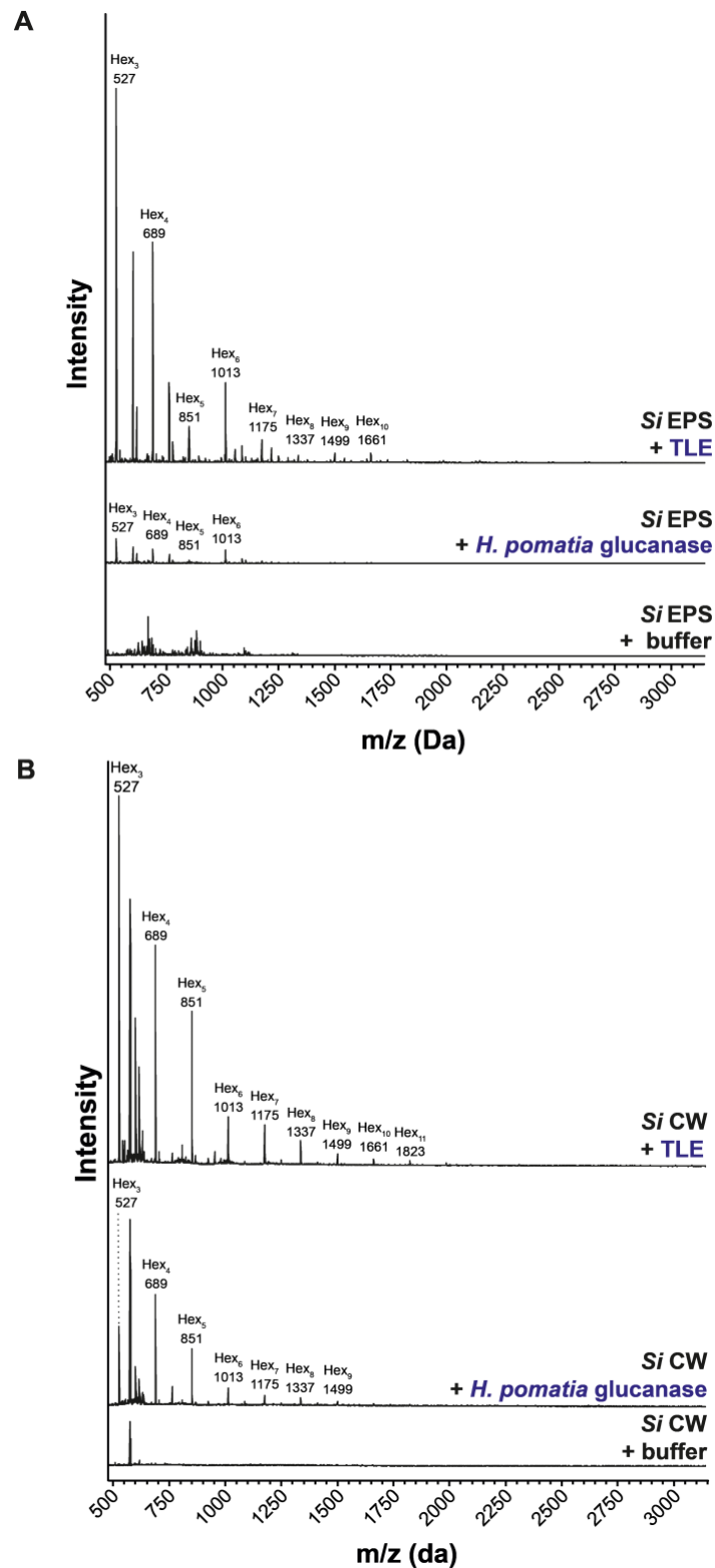
Supplemental Figure S3. Expression analysis of selected genes with WSC domains in *S. indica* during plant colonization at 3, 7 and 14 days post inoculation (dpi). Supports Figure 2. The gene expression of *S. indica* WSC domain-containing proteins during root colonization of barley and Arabidopsis was monitored via quantitative reverse transcription PCR (RT-qPCR) as previously described (Wawra et al., 2019). Average and standard deviation of three independent biological replicates (n=3) are shown. For each biological replicate, roots from several individual

plants were pooled (Arabidopsis: 60 plants; barley: 4 plants). LysM: lysine motive; MS: Murashige-Skoog medium; PNM: plant nutrition medium; WSC: cell wall integrity and stress response component.



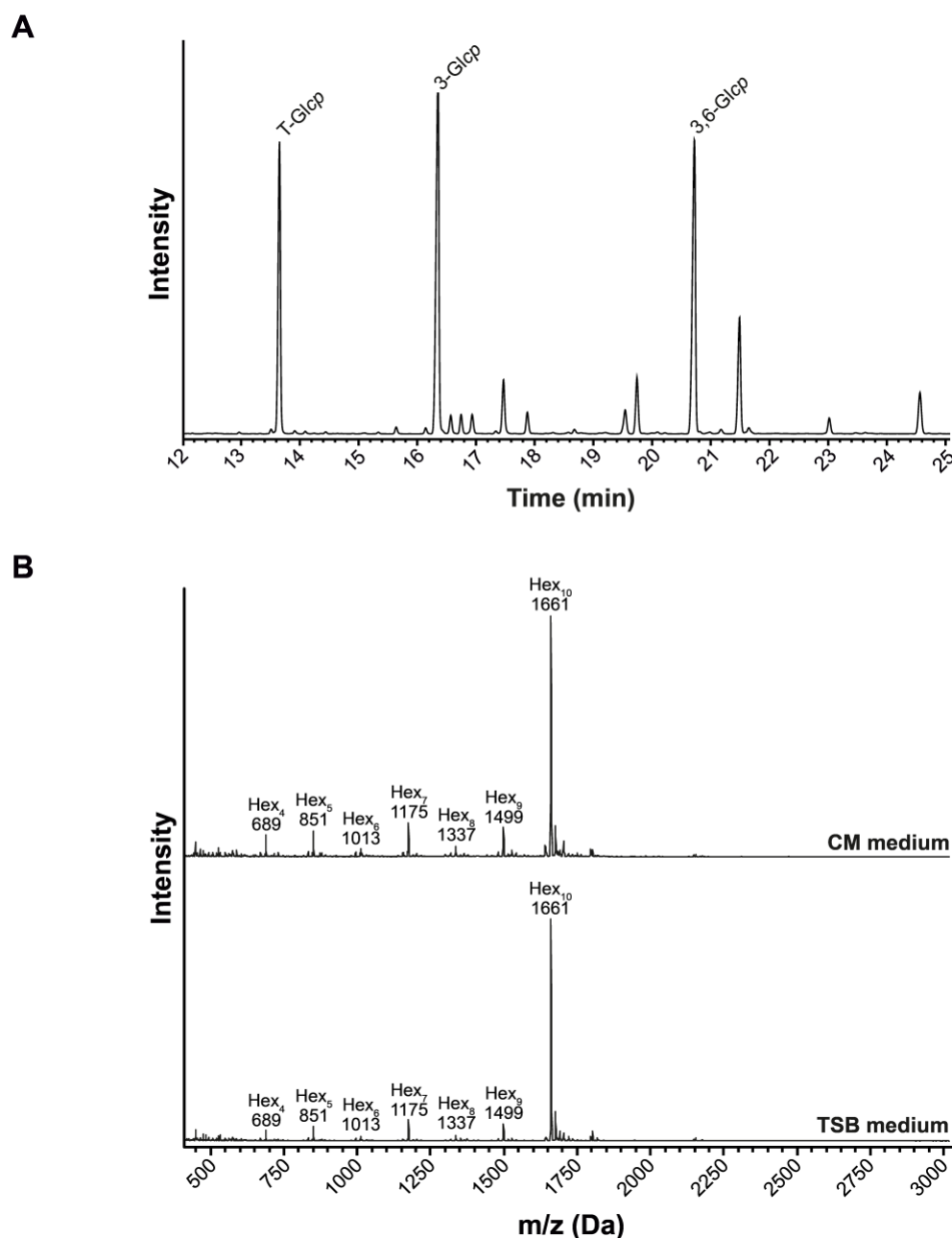
Supplemental Figure S4. Glycosyl linkage analysis of *Si* EPS and *Si* CW.

Supports Figure 2. Ground EPS matrix or the alcohol insoluble residue (CW fraction) isolated from *S. indica* grown in TSB medium containing 1% sucrose was subjected to glycosyl linkage analysis as previously described (Liu et al., 2015). The sugar residues were derivatized into partially methylated alditol acetates and analyzed using GC-MS. The glycosidic linkages were assigned based on the retention time and the mass spectrum available at the publicCCRC spectral database. The annotated glycosyl residues detected in the EPS matrix or CW are expressed as a percentage of the total glycosyl peak areas. The other minor unannotated glycosyl residues are listed in Supplemental Data Set S4 and S5. Average and standard deviation of four independent biological replicates (n=4) are presented. CW: cell wall; EPS: extracellular polysaccharide matrix; *Si*: *Serendipita indica*; *exact sugar moiety unknown; overrepresentation of linkages due to undermethylation cannot be excluded.



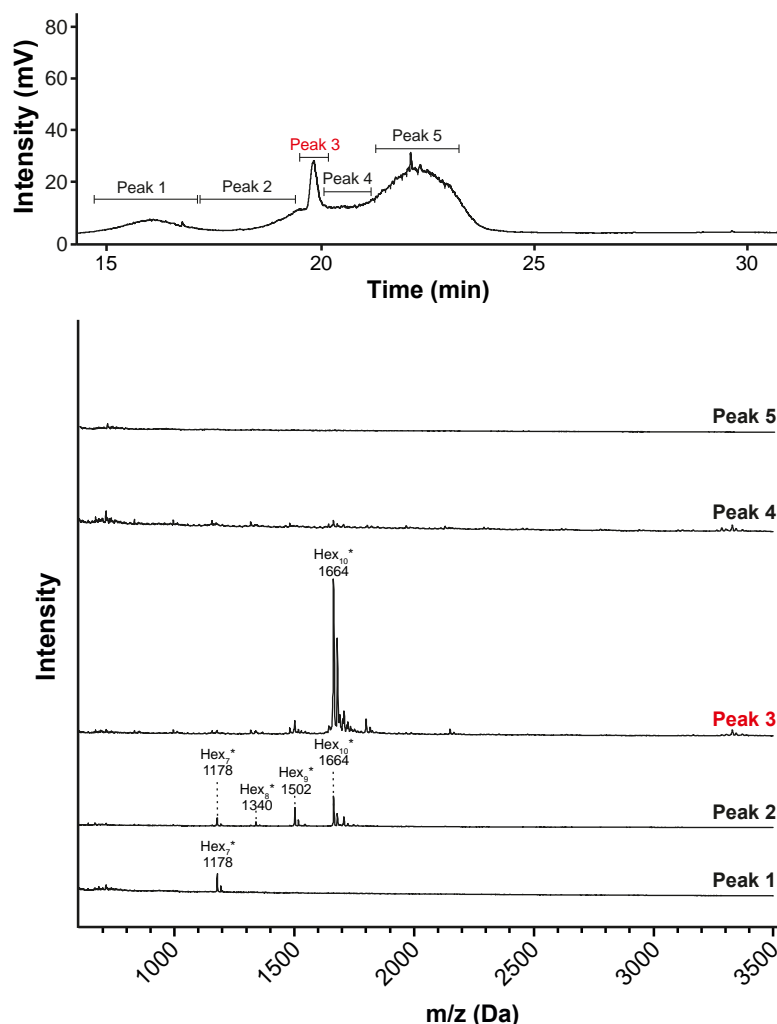
Supplemental Figure S5. Analysis of oligosaccharides released from the EPS or protein-free CW of *S. indica* by the action of β -1,3-glucanases. Supports Figure 3. 1 mg of freeze-dried EPS (A) or CW (B) obtained from *S. indica* was soaked in 2 mM sodium acetate, pH 5.0 (TLE), or 2 mM MES pH 5.0 (*H. pomatia* glucanase) at 70°C

overnight. The soaked material was incubated with TLE or *H. pomatia* β -1,3-glucanase at 40°C for 16 hours. The supernatant fraction from the digested EPS matrix and CW were analyzed by MALDI-TOF. The m/z (M+Na)⁺ Da of oligosaccharides resulting from the digestion of the samples are labeled with their hexose composition. The digestion of *Si* EPS with TLE or *H. pomatia* glucanase was repeated independently two times with a similar result and the digestion of *Si* CW with TLE or *H. pomatia* was performed once. CW: cell wall; EPS: extracellular polysaccharide; Hex_n: oligosaccharides with the indicated hexose composition; *Si*: *Serendipita indica*; TLE: *Trichoderma harzianum* lysing enzymes.



Supplemental Figure S6. Glycosyl linkage and MALDI-TOF analysis of the glucan fraction (β -GD) released from the EPS matrix of *S. indica*. Supports Figure 3. (A) Glycosyl linkage analysis of β -GD released from the EPS matrix of *S. indica*. The experiment was performed with three independent biological replicates of β -GD and the total ion chromatogram (TIC) of one of the replicates is represented. (B) *S. indica* was grown either in liquid CM or TSB medium containing 1% sucrose. β -GD was isolated and subjected to MALDI-TOF mass spectrometry. The ions corresponding to oligosaccharides with varying degree of polymerization are labelled with their hexose composition. The experiment performed with the TSB medium was

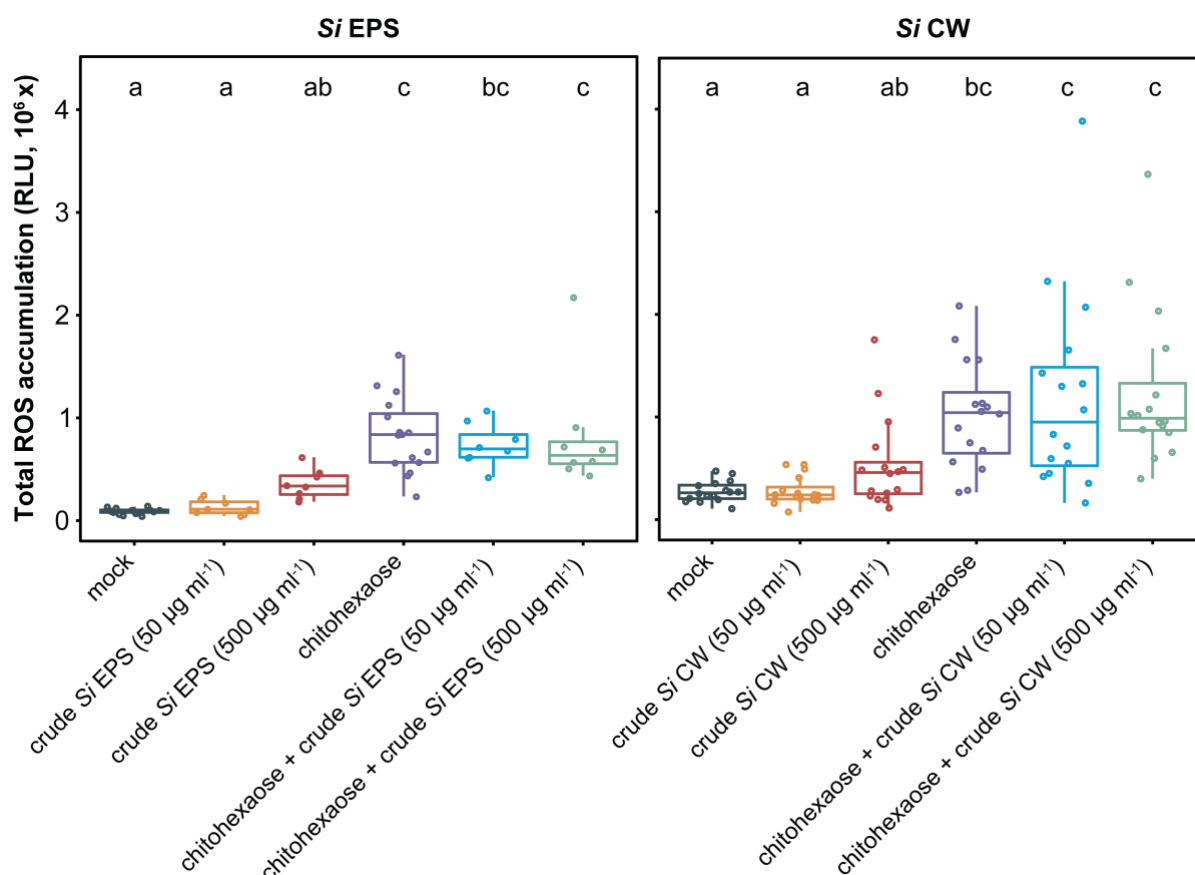
repeated independently more than three times with a similar result and the experiment performed with the CM medium was performed once. CM: complex Hill-Käfer medium; EPS: extracellular polysaccharide; Hex_n: oligosaccharides with the indicated hexose composition; TSB: Tryptic Soy Broth.



Supplemental Figure S7. Purification of the β -GD fragment for ^1H NMR analysis.

Supports Figure 3. 40 mg of the β -GD enriched fraction was reduced using NaBD_4 . The reduced fraction was neutralized with acetic acid, washed with methanol and air-dried. The dried material was dissolved in 2 ml of 6% methanol and 50 μl of the sample was subjected to liquid chromatography using a reverse-phase column (Vydac, Hesperia, CA, USA) connected to an evaporative light scattering detector. The detected peaks were collected and analyzed using MALDI-TOF. The samples were injected multiple times (24x) and peak 3 (shown in red) containing the reduced 1661 Da fragment was pooled and freeze-dried. The freeze-dried material was used for the ^1H NMR analysis. β -GD: β -1,3;1,6-glucan deca-saccharide; Hex_n: oligosaccharides

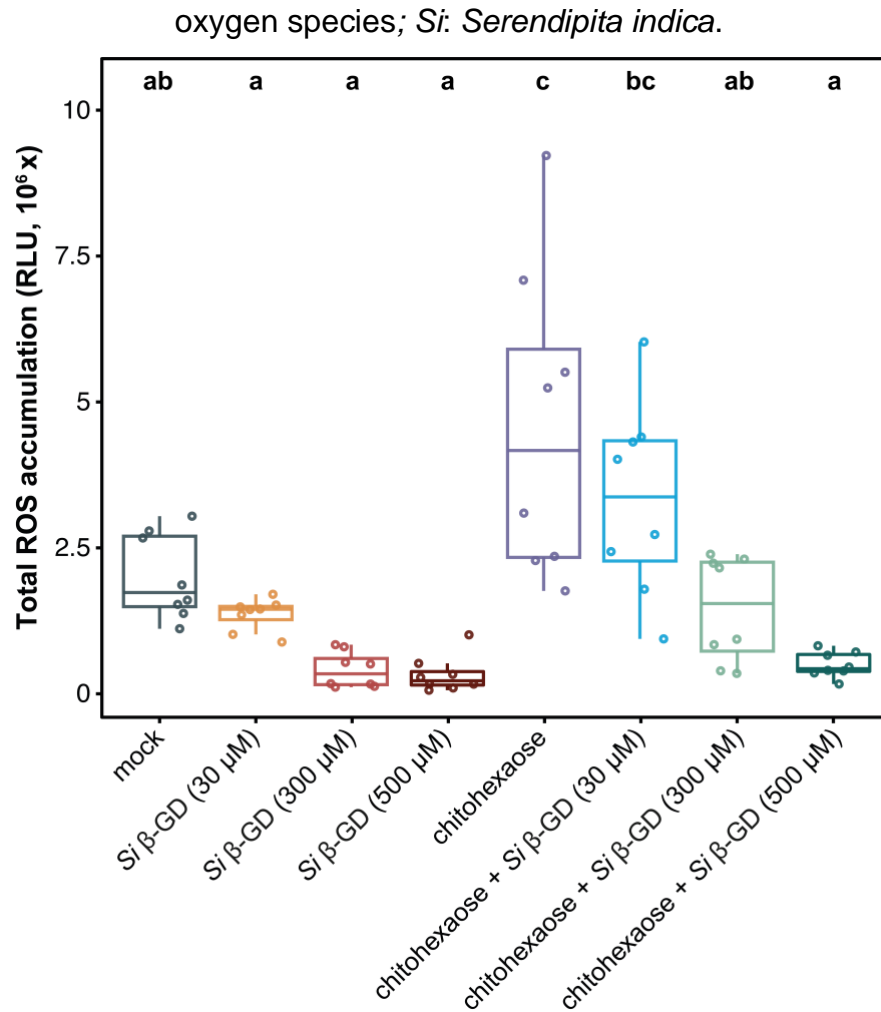
with the indicated hexose composition; * represents the increase by +3 Da in the detected oligosaccharides due to the reduction of the anomeric C1 atom.



Supplemental Figure S8. Mechanically-released fragments from *S. indica* EPS matrix and CW layer do not exhibit ROS scavenging activity. Supports Figure 4.

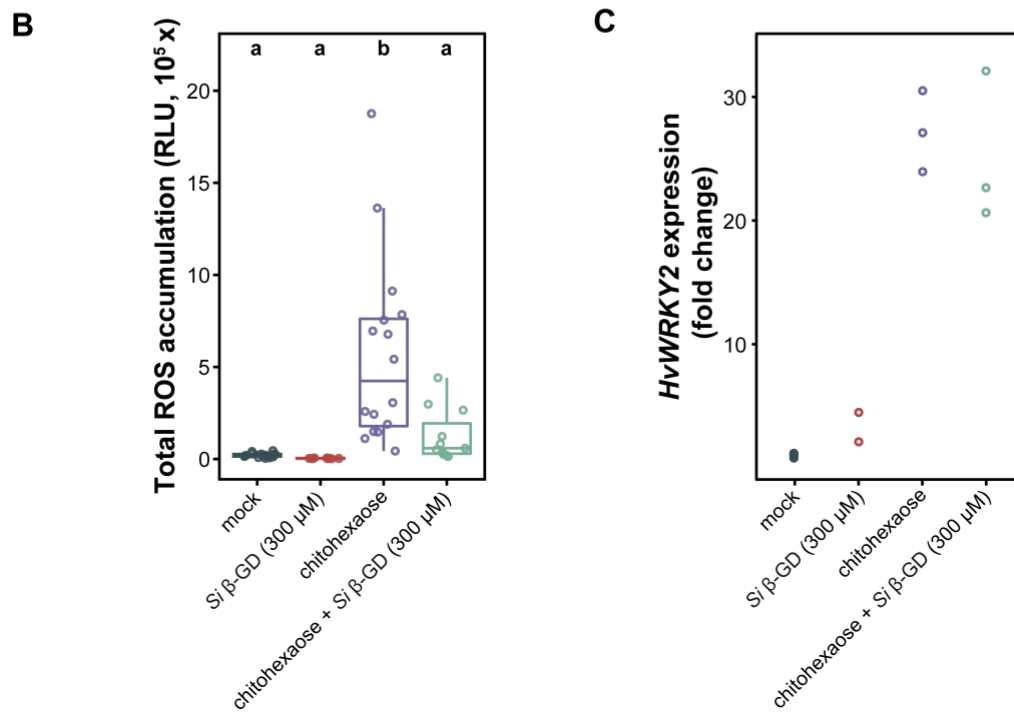
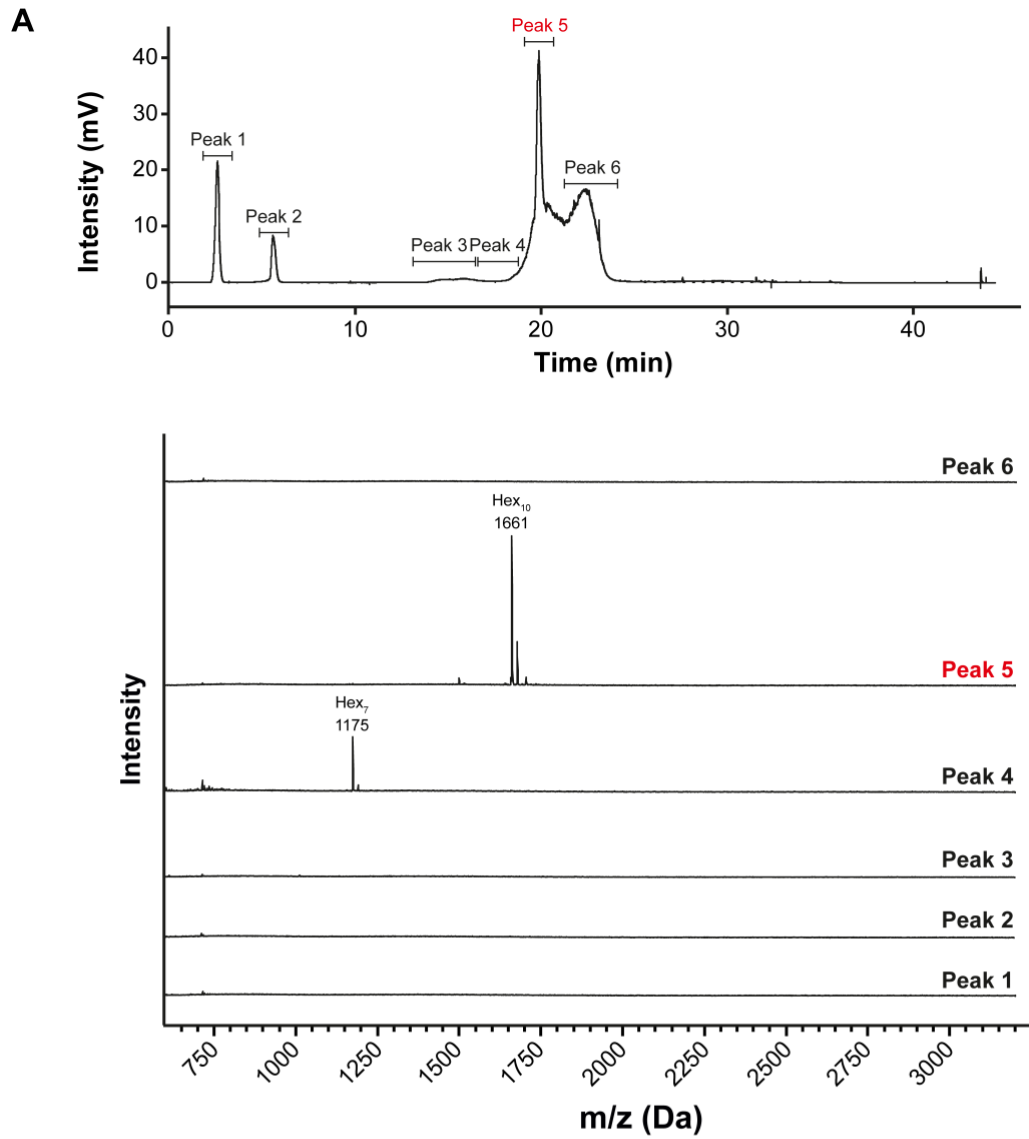
(A) *S. indica* EPS matrix and CW substrates were ground, heat-treated and sonicated prior to application to barley roots. Roots were treated with Milli-Q water (mock, n=16), chitohexaose (25 µM, n=16), crude EPS matrix or CW preparations (n=8-16) and combinations of chitohexaose and EPS matrix or CW preparations (n=8-16). Total cumulative ROS was calculated from a measured time interval of 60 minutes. Each data point in the boxplot represents the integrated value from an individual well (center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range). Experiments were performed two times with similar results.

Different letters indicate statistically significant differences based on a one-way ANOVA and Tukey's post-hoc test (significance threshold: p-value ≤ 0.05). CW: cell wall; EPS: extracellular polysaccharide; RLU: relative light units; ROS: reactive

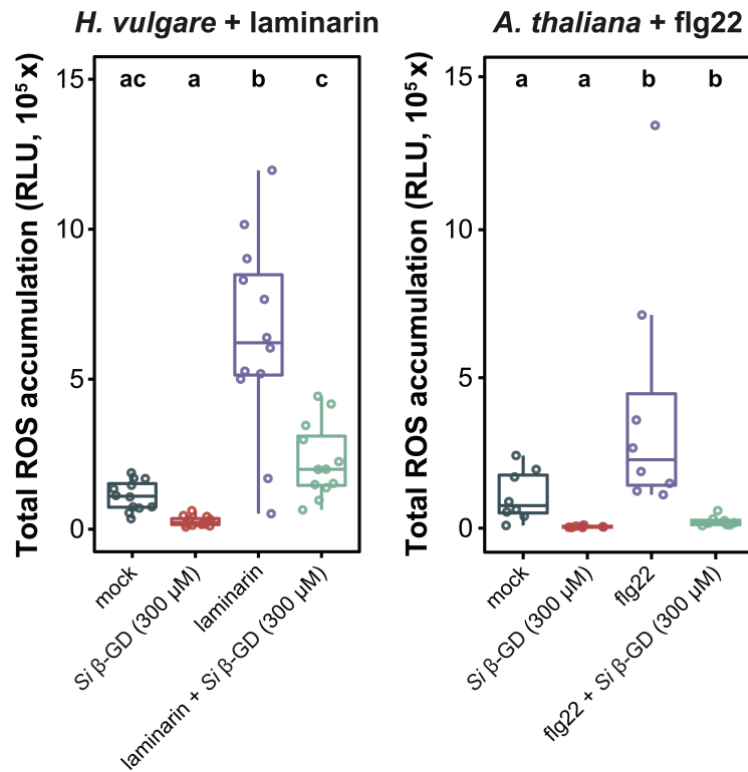


Supplemental Figure S9. Chitohexaose-triggered ROS accumulation is decreased by *S. indica* β-GD treatment in a concentration-dependent manner.

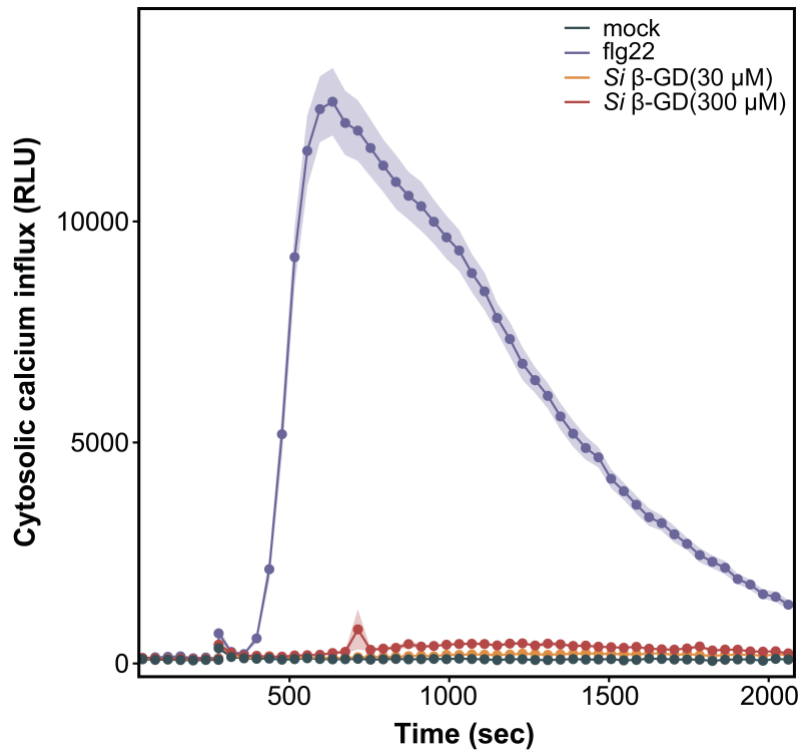
Supports Figure 4. Apoplastic ROS accumulation after treatment of eight-day-old barley roots with 25 μM chitohexaose and/or purified β-GD from *S. indica*. Treatment with Milli-Q water was used as mock control. Boxplots represents total ROS accumulation over the measured time period (center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range). Each data point represents measurement of an individual well containing four root pieces. In total, roots from 16 individual barley plants were used per experiment. This assay represents one out of four experiments with independent β-GD preparations. Letters represent statistically significant differences in expression based on a one-way ANOVA and Tukey's post-hoc test (significance threshold: $p\text{-value} \leq 0.05$).



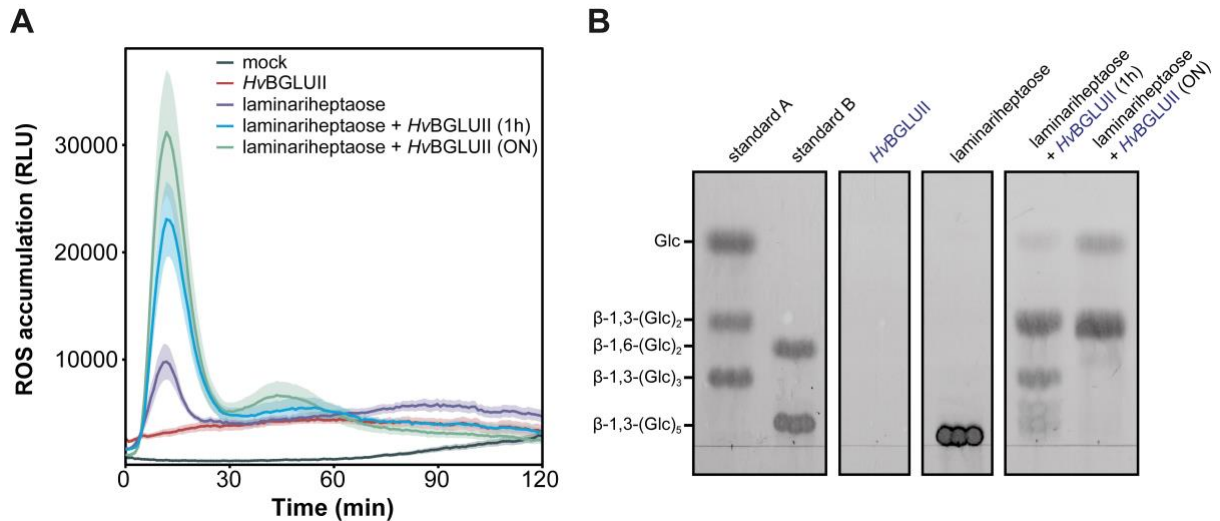
Supplemental Figure S10. Purification of native β -GD and immunogenic characterization. Supports Figure 4. (A) 40 mg of the β -GD enriched fraction was dissolved in 2 ml of 6% methanol and 50 μ l of the sample was subjected to liquid chromatography using a reverse-phase column (Vydac, Hesperia, CA, USA) connected to an evaporative light scattering detector. The detected peaks were collected and analyzed using MALDI-TOF. The samples were injected multiple times (24x) and peak 5 (shown in red) containing the 1661 Da fragment was pooled and freeze-dried. HPLC-purified β -GD fraction was used in the ROS burst assay (B) and the quantitative reverse transcription PCR gene expression analysis (C) was performed using root pieces obtained from eight-day-old barley plants. (B) The following treatments were applied in the ROS burst assay: mock treatment with Milli-Q water (n=16), β -GD (300 μ M, n=8), chitohexaose (25 μ M, n=16) and combined β -GD/chitohexaose treatments (concentrations as in previous treatments, n=11). ROS production was measured for 25 minutes. Each data point in the boxplot represents the integrated value from an individual well (center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range). Different letters indicate statistically significant differences based on a one-way ANOVA and Tukey's post-hoc test (significance threshold: p-value \leq 0.05). (C) Samples for quantitative reverse transcription PCR were collected after 1 hour of treatment. Applied elicitor concentrations were as described above for the ROS burst assay (n=2-3). Gene expression of elicitor-responsive *HvWRKY2* was calculated relative to housekeeping gene expression (*HvUBI*) and normalized to mock treatment. Calculation of statistical significance was not performed due to low sample number. β -GD: β -1,3:1,6-glucan decasaccharide; RLU: relative light units; ROS: reactive oxygen species; *Si*: *Serendipita indica*.



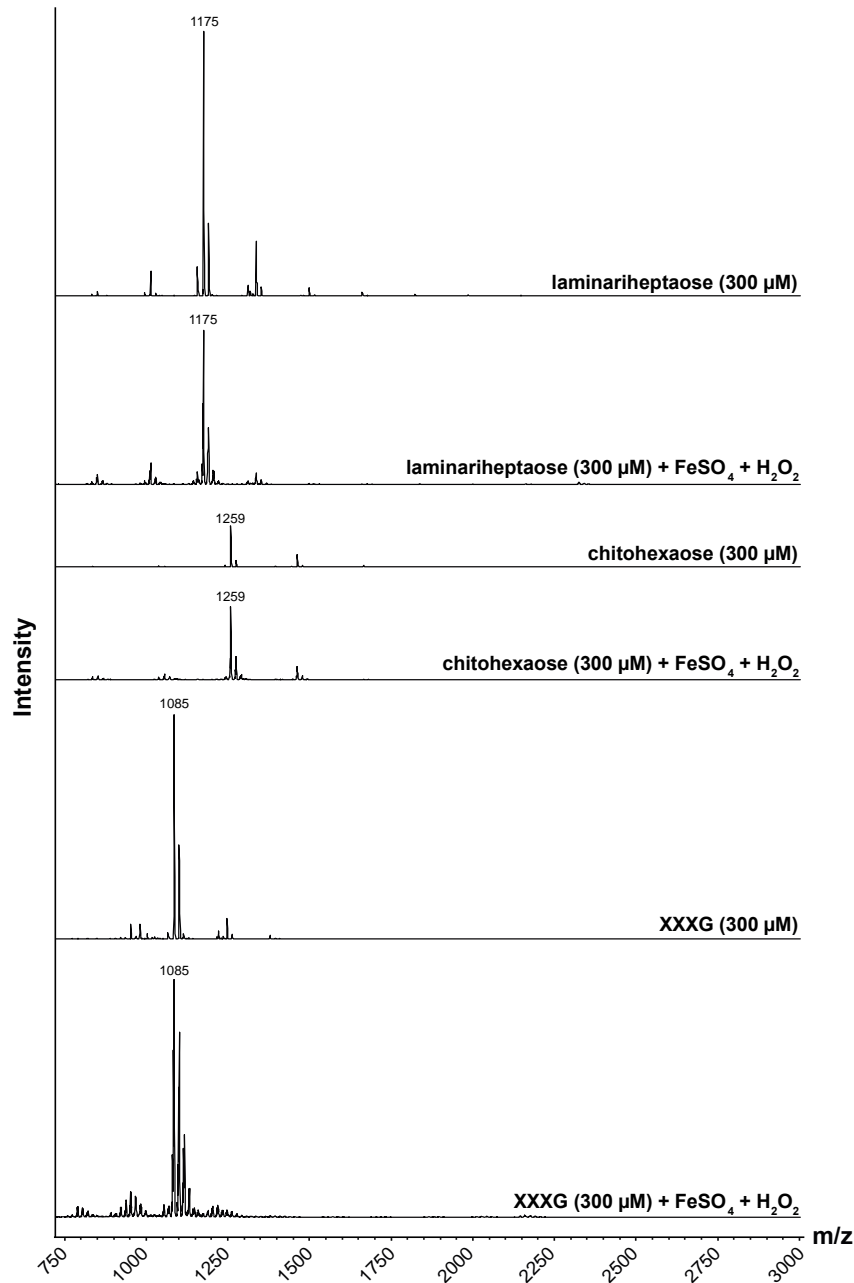
Supplemental Figure S11. Detoxification of apoplastic ROS by *S. indica* β -GD is independent of elicitor treatment and plant species. Supports Figure 4. ROS burst assays were performed with the roots of eight-day-old barley plants ($n=12$, data shown from one out of two independent experiments) and two-week-old *Arabidopsis* seedlings ($n=8$, single experiment). Plants were treated with Milli-Q water (mock), 2 mg/ml laminarin, 100 nM flg22, 300 μ M β -GD and combinations of laminarin or flg22 and β -GD. Each data point in the boxplot represents the integrated value from an individual well (center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range). Different letters indicate statistically significant differences based on a one-way ANOVA and Tukey's post-hoc test (significance threshold: p -value ≤ 0.05). β -GD: β -1,3:1,6-glucan deacasaccharide; RLU: relative light units; ROS: reactive oxygen species; *Si*: *Serendipita indica*.



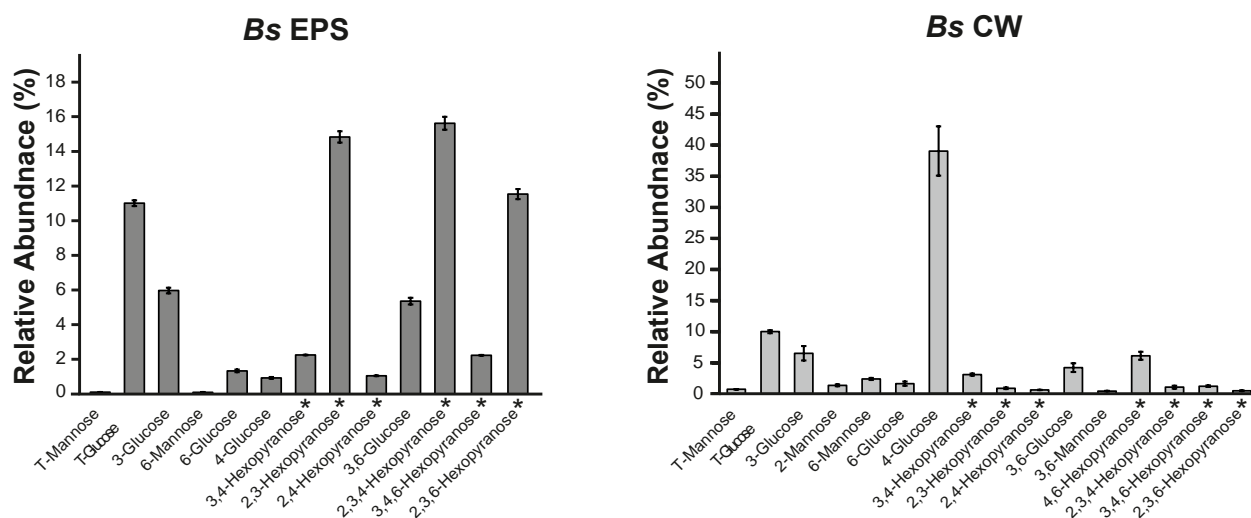
Supplemental Figure S12. *S. indica* β -GD treatment does not trigger cytosolic calcium influx in *A. thaliana* seedlings. Supports Figure 4. Calcium influx assays were performed with two-week-old *A. thaliana* seedlings expressing the calcium reporter protein aequorin in the cytosol. After an initial baseline measurement (5 min), seedlings were treated with Milli-Q water (mock), 100 nM flg22, 300 μ M β -GD and and β -GD. β -GD: β -1,3:1,6-glucan decasaccharide; RLU: relative light units; *Si*: *Serendipita indica*. The experiment was performed once with 16 *A. thaliana* seedlings (n=16) per treatment.



Supplemental Figure S13: Digestion of laminariheptaose with *HvBGLUII* enhances ROS production in barley roots. Supports Figure 4. (A) Before treatment of barley roots, 3 mM laminariheptaose was digested with *HvBGLUII* (10 units in total digestion volume of 200 μ l) for one hour or overnight (ON) at 40°C, 450 rpm. As controls, Milli-Q water with *HvBGLUII* and laminariheptaose without *HvBGLUII* were treated in a similar way. Digestion was stopped by incubation at 90°C (10 minutes, 450 rpm) followed by centrifugation (10 min, 13000xg, room temperature). Supernatants were used in ROS burst assays with the roots of eight-day-old barley plants ($n=8$, three root pieces per replicate). Plants were treated with Milli-Q water (mock), Milli-Q water with *HvBGLUII* or 300 μ M laminariheptaose (digested or non-digested). (B) Digestion of substrates used in the ROS burst assay was analyzed via thin-layer chromatography. Glc: glucose; ON: overnight; RLU: relative light units; ROS: reactive oxygen species. The experiment was repeated 3 times with similar results.

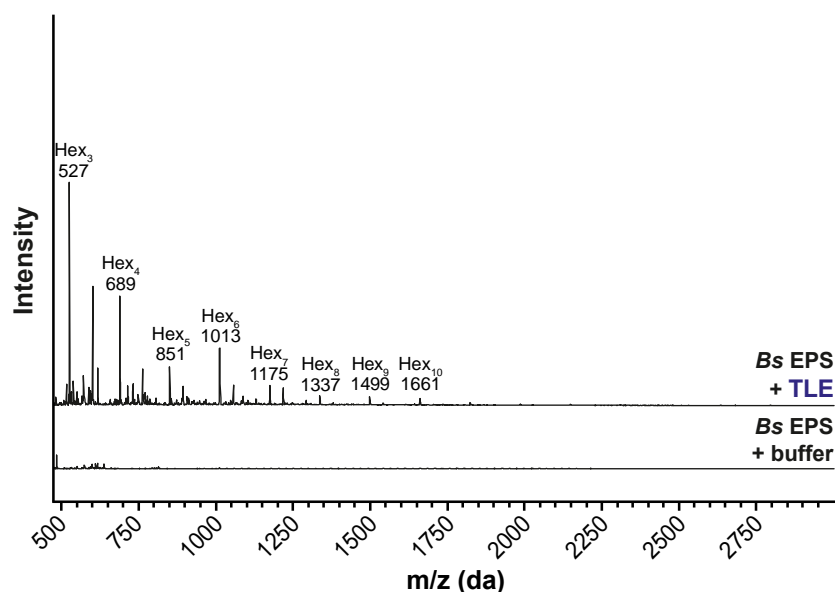


Supplemental Figure S14. Glycan controls are not degraded by hydrogen peroxide during Fenton reaction. Supports Figure 4. Laminariheptaose, chitohexaose and xyloglucan heptasaccharide (XXXG) were tested for oxidative degradation by overnight Fenton reaction (1 mM H_2O_2 , 100 μM FeSO_4) followed by MALDI-TOF analysis. The experiment was repeated independently two times with similar results.



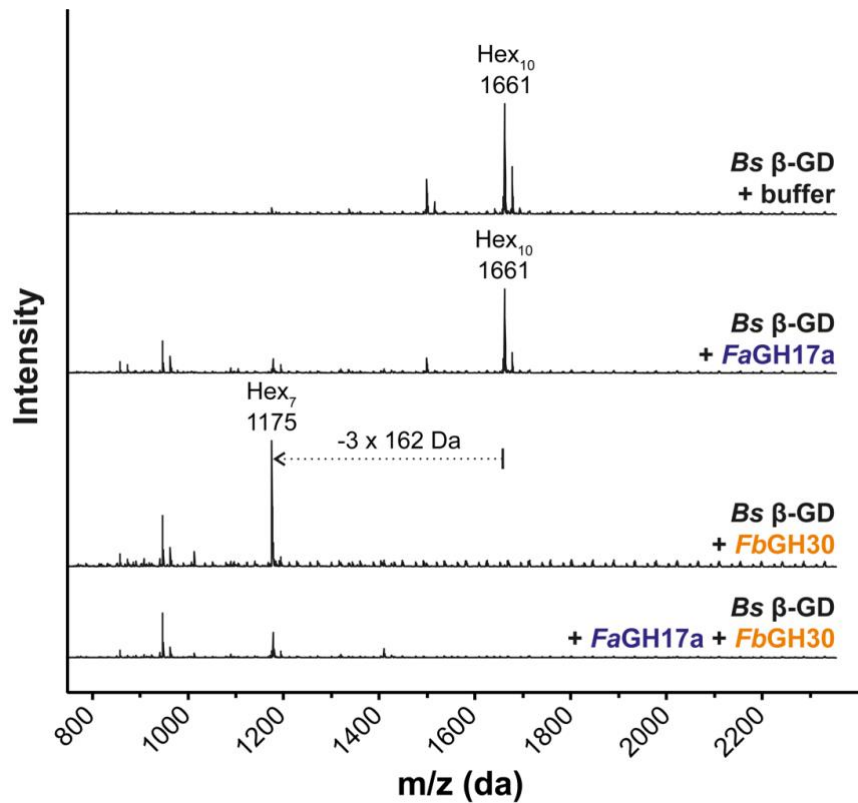
Supplemental Figure S15. Glycosyl linkage analysis of *B. sorokiniana* EPS

matrix and CW. Supports Figure 5. 2 mg of ground EPS matrix or the alcohol insoluble residue (CW fraction) isolated from the cultures of *B. sorokiniana* grown in YPD medium was subjected to glycosyl linkage analysis as previously described (Liu et al., 2015). The sugar residues were derivatized into partially methylated alditol acetates and analyzed using GC-MS. The glycosidic linkages were assigned based on the retention time and the mass spectrum available at CCRC spectral database. The annotated glycosyl residues detected in the EPS matrix or CW are expressed as a percentage of total glycosyl peak areas. The other minor unannotated glycosyl residues can be found in Supplemental Data Set S7 and S8. Average and standard deviation of three independent biological replicates (n=3) are presented. *Bs*: *Bipolaris sorokiniana*; CW: cell wall; EPS: extracellular polysaccharide; *exact sugar moiety unknown; overrepresentation of linkages due to undermethylation cannot be excluded.

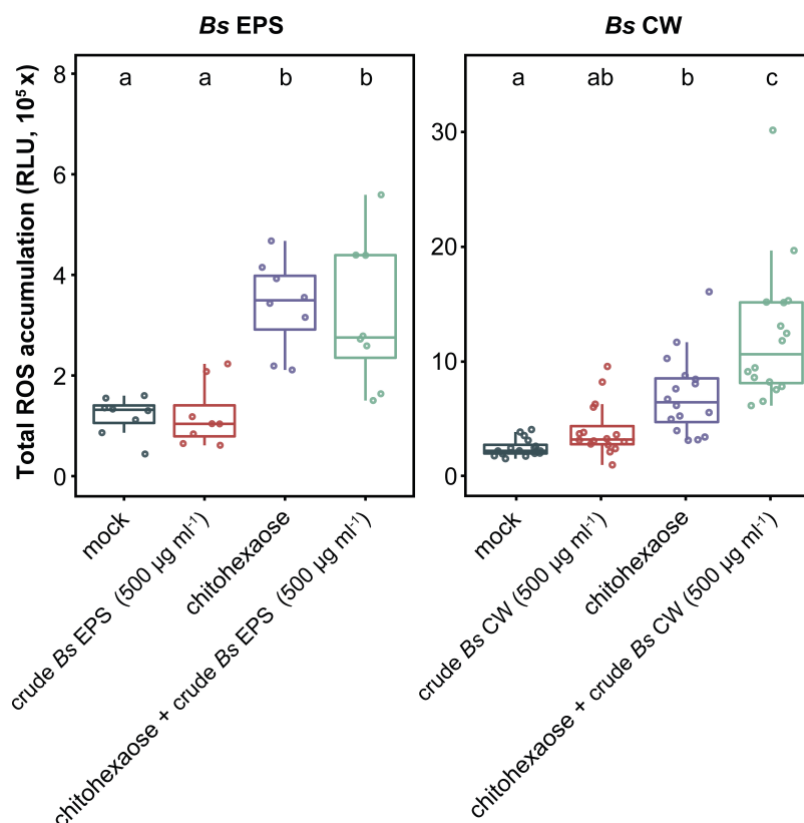


Supplemental Figure S16. Analysis of oligosaccharides released from the EPS of *B. sorokiniana* by the action of *Trichoderma harzianum* lysing enzymes.

Supports Figure 5. 1 mg of freeze-dried EPS obtained from *B. sorokiniana* was soaked in 2 mM sodium acetate, pH 5.0 at 70°C overnight. The soaked material was incubated with TLE at 40°C for 16 hours. The supernatant fraction from the digested EPS matrix was analyzed by MALDI-TOF. The m/z ($M+Na$)⁺ Da of oligosaccharides resulting from the digestion of EPS is indicated along with their hexose composition. The experiment was performed once. *Bs*: *Bipolaris sorokiniana*; EPS: extracellular polysaccharide; Hex_n: oligosaccharides with the indicated hexose composition; TLE: *Trichoderma harzianum* lysing enzymes.



Supplemental Figure S17. The β -GD released from the EPS matrix of *B. sorokiniana* consists of a seven unit β -1,3-linked glucan backbone substituted with three β -1,6-glucosyl residues. Supports Figure 5. β -GD was treated with *FaGH17a* and/or *FbGH30* at 40°C overnight. The digested β -GD fragment was analyzed by MALDI-TOF. The loss of three hexoses ($-3 \times 162 \text{ Da}$) as a result of *FbGH30* (orange) treatment is indicated with a dotted arrow. β -GD: β -1,3;1,6-glucan decasaccharide; *Bs*: *Bipolaris sorokiniana*; EPS: extracellular polysaccharide. The experiment was performed once.



Supplemental Figure S18. Mechanically-released fragments from *B. sorokiniana* EPS matrix or CW do not scavenge ROS. Supports Figure 5. Assays were performed and analyzed as described in Figure S8. Total cumulative ROS was calculated from a measured time interval of 60 minutes. Each data point in the boxplot represents the integrated value from an individual well (center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range). The experiment was performed two times with similar results. *Bs*: *Bipolaris sorokiniana*; CW: cell wall; EPS: extracellular polysaccharide; RLU: relative light units; ROS: reactive oxygen species.

Supplemental References

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- Wawra, S., Fesel, P., Widmer, H., Neumann, U., Lahrmann, U., Becker, S., Hehemann, J.-H., Langen, G., and Zuccaro, A. (2019). FGB1 and WSC3 are in planta-induced β -glucan-binding fungal lectins with different functions. *New Phytologist* **222**, 1493-1506.