

BRIEF COMMUNICATION OPEN ACCESS

Natural Variation in *Brachypodium distachyon* Primarily Links Lignin Reinforcement to *Rhizoctonia solani* Resistance

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Received: 19 June 2026 | **Revised:** 3 August 2026 | **Accepted:** 20 September 2026

Funding: Japan Society for the Promotion of Science, Grant/Award Numbers: JP21H02197, JP24K01759

Rhizoctonia solani is a soil-borne necrotrophic phytopathogenic fungus that employs effector proteins and cell wall-degrading enzymes (CWDEs) to breach plant barriers and colonize diverse crops worldwide (Zheng et al. 2013; Lakshman et al. 2016). However, plant resistance mechanisms remain poorly understood. To develop effective disease control strategies, understanding both pathogen virulence and the plant immune mechanisms that counteract infection is essential. We therefore used the *R. solani*–*Brachypodium distachyon* pathosystem to investigate host defence.

We previously identified *B. distachyon* accessions resistant to leaf infection by *R. solani* AG-1 IA, the causal agent of rice sheath blight (Kouzai et al. 2018, 2020). Resistance in Bd3-1 depended on salicylic acid (SA), and SA pretreatment conferred resistance to AG-1 IA in rice, *B. distachyon*, and barley, suggesting a transient biotrophic phase early in infection (Kouzai et al. 2018, 2020; Mahadevan et al. 2025). By contrast, AG-4 HG-I + II infected both leaves and roots of *B. distachyon* and barley, and its leaf infection was not suppressed by SA pretreatment. Together with the morphological differences in infection hyphal structures, these observations suggest that infection strategies differ among *R. solani* isolates or lineages and cannot necessarily be explained by anastomosis group (AG) classification alone (Mahadevan et al. 2025).

Necrotrophic pathogens typically rely on CWDEs for colonization (Miedes et al. 2014). *R. solani* possesses a diverse repertoire of carbohydrate-active enzymes (CAZymes), including glycoside

hydrolases, glycosyltransferases, polysaccharide lyases, carbohydrate esterases, carbohydrate-binding modules and auxiliary activity enzymes (Zheng et al. 2013). Secretome analysis of an AG-4 isolate identified hundreds of proteins potentially associated with cell wall degradation (Lakshman et al. 2016). Plant cell walls function as physical barriers and immune sensors, and lignin content generally correlates positively with disease resistance (Miedes et al. 2014). Pathogen-induced lignification can strengthen cell walls and improve tolerance to pathogen-derived CWDEs (Miedes et al. 2014).

To further investigate the genetic basis of resistance to the AG-4 isolate, we monitored resistance levels in 153 *B. distachyon* accessions using leaf and root inoculation assays and found that leaf and root resistance traits had evolved independently (Fernanda et al. 2026). To clarify the mechanism underlying leaf resistance in *B. distachyon* against AG-4 HG-I + II, we selected 22 resistant and 6 susceptible accessions and quantified lignin content in inoculated detached leaves at 3 days post-inoculation (dpi). We thereby investigated the relationship between foliar lignin content and resistance of *B. distachyon* accessions to *R. solani* AG-4.

In all six susceptible accessions (Bd21, Koz-3, X4006, Adi-4, BdTR2h and BdTR10m), lignin levels were significantly reduced by up to 91%, despite high basal levels (Figure 1a). This suggests that AG-4 HG-I + II infection causes a substantial net loss of lignin-containing cell wall material in susceptible accessions. In contrast, nine resistant accessions (Gaz-1, Adi-9,

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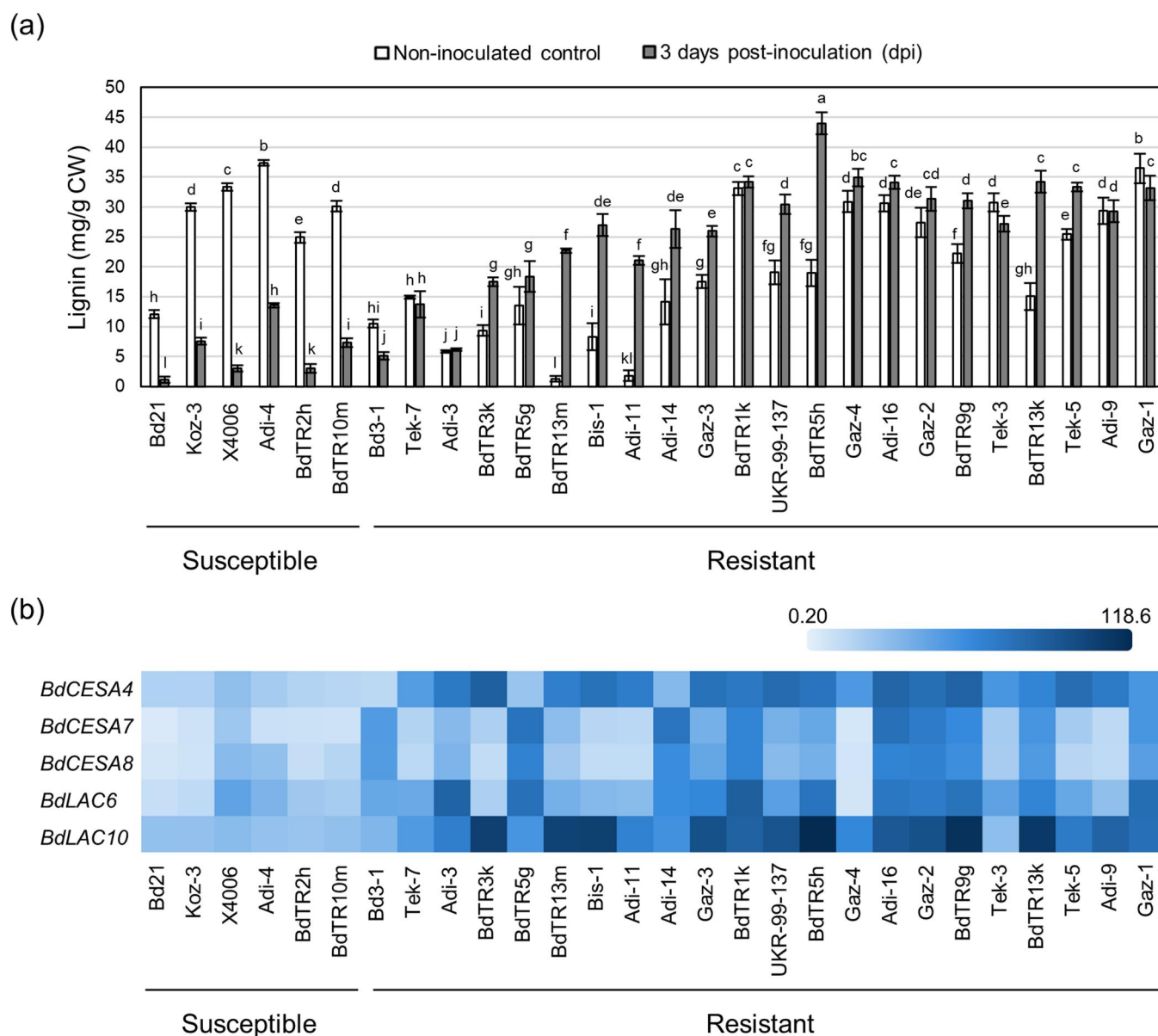


FIGURE 1 | Lignin content and expression of secondary cell wall (SCW) biosynthesis genes in resistant and susceptible *Brachypodium distachyon* accessions in response to *Rhizoctonia solani* AG-4 HG-I + II infection. (a) Lignin content in non-inoculated (white) and inoculated (grey) detached leaves at 3 days post-inoculation. Data are presented as mean values $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences (one-way ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$). (b) Heatmap showing expression patterns of SCW-related genes, including cellulose synthase (*BdCESA*) and laccase (*BdLAC*) genes, during infection. The expression levels at 6, 12, 24 and 48 h post-inoculation are indicated by the colour scale, which represents log_{1p}-transformed expression levels.

Tek-5, Tek-3, BdTR9g, Gaz-2, Adi-16, Gaz-4 and BdTR1k) with high basal lignin maintained or further increased lignin during infection (Figure 1a). Ten resistant accessions (BdTR13k, BdTR5h, UKR-99-137, Gaz-3, Adi-14, Adi-11, Bis-1, BdTR13m, BdTR5g and BdTR3k) with moderate or low basal lignin accumulated more lignin after infection. Thus, 86% of resistant accessions maintained or increased lignin levels. Resistance was therefore associated with the capacity to maintain or reinforce lignin-containing cell walls rather than with basal lignin content alone.

We next measured expression of the secondary cell wall (SCW)-related genes, *BdCESA7* and *BdCESA8*, and *BdLAC6* at 6, 12, 24 and 48 h post-infection (hpi) (Supporting Information S1:

Figure 1). *BdCESA4* and *BdCESA7* contribute to SCW biosynthesis in *B. distachyon*, and loss of either gene results in thinner walls (Handakumbura et al. 2013). Laccases are multicopper oxidases that catalyze oxidative polymerization of monolignols into lignin. We integrated these results with previously published *BdCESA4* and *BdLAC10* expression data (Fernanda et al. 2026), and visualized the profiles as a heatmap (Figure 1b). Each resistant accession induced at least one *CESA* gene and one *LAC* gene at one or more time points, although the responsive family members varied among accessions, indicating diversity in SCW fortification responses.

B. distachyon also induces *CESA* and *LAC* genes in response to AG-1 IA infection (Kouzai et al. 2018). Therefore, cell wall

fortification may represent a common defence mechanism against *R. solani*, even when infection processes differ among isolates or lineages. However, susceptible accessions, such as X4006 and Adi-4, also induced SCW-related genes during AG-4 HG-I + II infection (Supporting Information S1: Figure 2), although their lignin content decreased (Figure 1a). Transcriptional activation alone is therefore insufficient for net lignin reinforcement. In susceptible accessions, fungal degradation or infection-associated damage may outpace host SCW biosynthesis. Factors that protect cell wall polymers or inhibit CWDEs, including inhibitors of polygalacturonases, pectin lyases, pectin methylesterases, endo-xylanase or xyloglucan-specific endoglucanases, may also contribute to resistance.

Notably, two resistant accessions (Adi-3 and Tek-7) maintained relatively low lignin levels after infection, while Bd3-1 showed a decrease; in all three, lignin levels at 3 dpi were comparable to those in susceptible accessions (Figure 1a). These results suggest that their resistance may not primarily rely on SCW reinforcement. Interestingly, these three resistant accessions also induced at least one *CESA* gene and one *LAC* gene during infection (Figure 1 and Supporting Information S1: Figure 1). This indicates that induction of SCW biosynthesis-related genes does not necessarily lead to net lignin reinforcement; fungal degradation may be balanced by, or may exceed, host biosynthetic reinforcement, resulting in unchanged or reduced lignin accumulation. These resistant accessions may employ additional mechanisms, such as CWDE inhibition, localized cell wall remodelling, cell wall integrity signalling, or SCW-independent immune responses, none of which are captured by bulk lignin quantification. Thus, SCW fortification is a major, but not universal, resistance strategy against AG-4 HG-I + II in *B. distachyon*.

In conclusion, high basal lignin may provide a preformed barrier, whereas infection-induced de novo SCW biosynthesis and lignification may further strengthen defence. SCW fortification therefore appears to be a major, although not universal, defence strategy against AG-4 HG-I + II.

Acknowledgements

This research was financially supported by the Japan Society for the Promotion of Science (JSPS), KAKENHI Grant Nos. JP21H02197 and JP24K01759 to Y.N. The authors thank the NARO Genebank, Japan for maintaining and distributing the *R. solani* isolates. R.F. and N.M. were supported by the MEXT scholarship.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available in the manuscript and the [Supporting Information](#).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: Expression of secondary cell wall (SCW) biosynthesis-related genes in resistant *Brachypodium distachyon* accessions in response to *Rhizoctonia solani* AG-4 HG-I + II infection. (a–c) Relative mRNA levels of the cell wall biosynthesis genes *BdCESA7* (a) and *BdCESA8* (b) and the lignin biosynthesis gene *BdLAC6* (c) in detached leaves at 6, 12, 24 and 48 h post-inoculation. Bd21 was used as a susceptible control accession. Data are presented as mean values $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences within each accession, as determined by one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

Figure S2: Expression of secondary cell wall (SCW) biosynthesis-related genes in susceptible *Brachypodium distachyon* accessions in response to *Rhizoctonia solani* AG-4 HG-I + II infection. (a–c) Relative mRNA levels of the cell wall biosynthesis genes *BdCESA7* (a) and *BdCESA8* (b) and the lignin biosynthesis gene *BdLAC6* (c) in detached leaves at 6, 12, 24 and 48 h post-inoculation. Bd21 was used as a susceptible control accession. Data are presented as mean values $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences within each accession, as determined by one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

Table S1: Primers used in this study.

Supporting File: pce70937-sup-0002-Supmat.docx.