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Genetic insights into root disease resistance using the model cereal *Brachypodium distachyon*

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Abstract

Fusarium spp. and *Gaeumannomyces tritici* are fungal root pathogens that cause major yield losses in cereal crops. Management strategies include fungicides, croprotation, and genetic resistance crop applications, but these pathogens continually evolve. *Brachypodium distachyon* (Bd) acts as a valuable model for temperate cereal crops and is useful for studying root-pathogen interactions. The BrachyTAG program is used in this study to disrupt individual genes in the Bd21 accessions using T-DNA mutagenesis, and the sensitivity of the selected lines were tested to the *Fusarium* mycotoxin deoxynivalenol (DON) and the root necrosis was monitored during germination. The results showed that disruption of BdAA (annotated accessions 398, 441, and 319) led to distinct resistance to *G. tritici* and *F. culmorum* infections and a bZIP transcription factor-encoding gene potentially confers broad-spectrum resistance. These pathogen-specific and general defence genes can be used for breeding and gene editing to enhance cereal crop resilience against root diseases.

Keywords Take-all disease, Fusarium root rot, *Brachypodium distachyon*, Broad-spectrum resistance, Fungal disease

Background

Fusarium spp. and *Gaeumannomyces tritici* are among the most economically significant pathogens responsible for root diseases in cereal crops, severely affecting both yield and quality. The economic impact of these root diseases is considerable. Fusarium diseases, particularly Fusarium head blight (FHB), cause billions of dollars in losses annually. China experienced an annual wheat yield loss of 3% between the year of 2000 and 2018 (Chen et al. 2019), with 27% of all disease related yield loss in the US in 2024 being attributed to Fusarium diseases (Anderson et al. 2024). Similarly, *G. tritici* diseases are responsible for high annual losses around the world, with an average wheat loss of 5–20% in the UK (Palma-Guerrero et al. 2021) and accounts for 98% of all root and stem disease related losses in Alberta and Ontario within Canada (Anderson et al. 2024).

Fusarium spp. cause multiple diseases affecting major cereals, including head blight (FHB), root rot (FRR), and crown rot (FCR) (Kazan and Gardiner 2018; Haidoulis and Nicholson 2022). This genus is amongst the most important cereal fungi in the world, including key species like *F. graminearum* and *F. culmorum* (Goncharov et al. 2020). FHB is of particular concern because it results in significant yield losses due to reduced grain quality, primarily through the production of mycotoxins, such as deoxynivalenol (DON) (Xu et al. 2022). DON reduces grain quality and poses health risks (D'Mello et al. 1999), making Fusarium diseases a major agricultural challenge.

The diseases caused by Fusarium affect different areas, and all lead to substantial yield lost. FHB primarily affects

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the wheat spike, reducing grain quality and kernel size (Wegulo et al. 2015), FRR causes necrosis in the roots, leading to weakened seedlings (Beccari et al. 2011), and FCR affects the stem base, limiting water and nutrient flow (Kazan and Gardiner 2018). Fusarium can also remain in the soil through mycelium on debris, causing persistent disease in the field (Pereyra et al. 2004). These diseases contribute to major yield losses in crops like wheat, barley, and oats, highlighting Fusarium as a critical agricultural threat.

Gaeumannomyces tritici, the causal agent of take-all disease, is another fungal root pathogen that is of economic concern in cereal production. This pathogen blocks vascular tissue in the root systems, severely limiting water and nutrient uptake, resulting in symptoms such as stunted growth, premature yellowing of leaves, reduced tiller production, and head bleaching (Guilleroux and Osbourn 2004). Early infection results in empty spikes or smaller seeds, and in extreme cases, plant death, reducing yield quantity and quality (Oliver 2024). Similar to *Fusarium* spp., *G. tritici* survives in soil on infected crop debris, but it is also spread effectively by water movement, which helps the pathogen infect new plants (van Toor et al. 2018).

Efforts to manage root diseases have been limited, but have focused on agronomical strategies, such as crop rotation and fungicide use, and breeding to incorporate disease resistance genes. In wheat, Fusarium resistance in the crown and root is typically achieved through fungicide seed treatments, cultivar resistance, and systemic acquired resistance (Moya-Elizondo and Jacobsen 2016). Take-all disease is mainly managed by crop rotation, disrupting the pathogen's lifecycle, and reducing soil inoculum, with legumes and oilseeds providing better benefits than other cereals as crops to sow before wheat (Lawes et al. 2013).

Despite ongoing efforts, managing Fusarium and take-all diseases remains challenging. Genetic resistance is better studied in Fusarium (particularly FHB), while effective strategies for take-all disease remain limited. Current management relies heavily on crop rotation and agronomic practices, highlighting the urgent need for improved breeding efforts and novel resistance sources (Naqvi et al. 2025). Previous studies have shown that resistance to root pathogens, particularly reductions in necrosis severity, correlates strongly with improved yield outcomes in cereals (Beccari et al. 2011; Palma-Guerrero et al. 2021). Identifying resistance genes in the target crop is useful but not always feasible; however, the related species can offer transferable defences (Liu et al. 2013).

Cereal crops provide the majority of the world's food, with temperate cereals (including wheat, oats, barley, and rye) - belonging to the Triticeae tribe within the Pooideae subfamily - being essential for global food security (Hensel et al. 2011). Given the difficulties in studying root diseases directly in wheat and barley, alternative model species are essential for advancing disease resistance research. While rice (*Oryza sativa*) has historically served as a model for monocot species, its adaptation to semi-aquatic environments and distant phylogenetic relationship to temperate cereals limit its utility (Das and Uchimiya 2002). In contrast, *Brachypodium distachyon* (Bd) offers a closer genetic relationship to wheat and barley, providing a more relevant system for studying root-pathogen interactions and resistance mechanisms (Catalan et al. 2014). Bd has a small, manageable genome, a short life cycle, and self-fertility, making it an ideal candidate for studying plant-pathogen interactions (Draper et al. 2001). The Bd genome, sequenced in 2009, revealed over 25,500 protein-coding genes, with 77–84% similarity to rice and sorghum, making Bd a valuable model for studying disease resistance (Huo et al. 2009).

Bd is susceptible to a wide range of pathogens, including *Fusarium* species, and exhibits symptoms similar to those in wheat, such as small brown spots, lesion spread, and spike bleaching (Peraldi et al. 2011). The similarity in infection patterns makes Bd a reliable model for studying cereal diseases, offering insights into the genetic mechanisms of pathogen resistance. Bd enables discovery of resistance genes that can inform cereal crop improvement. While some differences exist - such as the absence of wheat's *Lr34* gene (Catalan et al. 2014) - its close relation to temperate cereals makes it a useful model for studying root disease resistance.

The BrachyTAG program was established to enhance research on pathogen resistance in Bd. A collaboration between the International Brachypodium Tagging Consortium and the USDA-ARS utilises T-DNA mutagenesis to generate Bd mutant lines, offering a powerful tool for identifying disease resistance genes (Thole et al. 2011). T-DNA mutagenesis is particularly advantageous due to its low copy number and stable transgene expression compared to other transformation techniques (Gelvin 2017). The BrachyTAG program has created extensive collections of single-insertion mutants, enabling researchers to identify genes that contribute to Bd's resistance against pathogens like *Fusarium* spp. These findings have the potential to inform breeding programs aimed at improving disease resistance in economically important cereals. The current study examines ten Bd mutant accessions from the BrachyTAG program, selecting based on predicted gene functions that may influence fungal root disease resistance, with the goal of identifying genes that confer resistance to two major root pathogens. In this study, several Bd mutants exhibited altered responses to *Fusarium* spp. and *G. tritici*, revealing

candidate genes potentially involved in root disease resistance. These findings provide new insights into the genetic basis of cereal root defence and offer targets for future crop improvement efforts.

Results

Mutation effect on necrotic lesion size

To identify candidate genes involved in root disease resistance, we selected ten *Brachypodium distachyon* T-DNA mutant accessions from the BrachyTAG collection. These were chosen based on predicted gene annotations associated with plant immunity, such as transcription factors, kinases, and leucine-rich repeat (LRR) proteins, which are commonly involved in defence signalling. Each mutant line was compared to its corresponding near-isogenic line (NIL), which lacked the T-DNA insertion but shared the same genetic background, identified via Green Fluorescent Protein (GFP) segregation.

Screening was performed by applying a fungal slurry of either *F. culmorum* or *G. tritici* to the root tip of young seedlings. Lesion development was monitored at multiple timepoints post-inoculation, and the length of necrotic tissue extending from the inoculation site (Additional file 1: Figure S1a) was measured using image analysis.

Of the ten accessions tested, two of them (BdAA 222 and 319) showed significant increases in lesion size following *F. culmorum* infection (p-values < 0.043), with mutants displaying 18.6% and 20.7% longer necrotic lesions, respectively, compared to NILs (Fig. 1). In response to *G. tritici*, three accessions exhibited significant differences in lesion size (p-values < 0.024), including BdAA 319, which also responded to *F. culmorum*.

BdAA 319 was the only accession to show significant differences between the mutant and NIL for both pathogens, with an increase in induced necrosis in the mutant with *G. tritici*, and the lesion was 53% larger compared to the NIL. The two other accessions that showed significant differences, BdAA 398 and 441, were the only lines that showed significantly smaller lesions in the mutants (Fig. 1), in which the reductions in lesion lengths were by 7.2% and 12.4%, respectively.

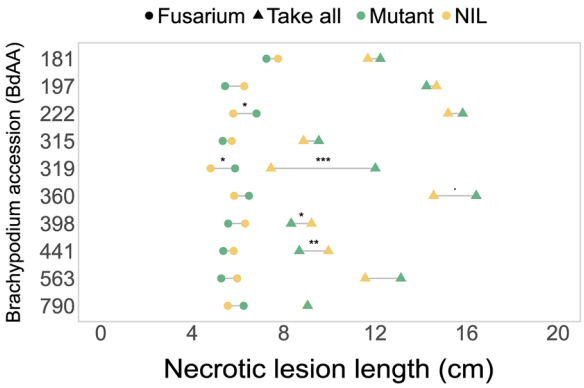

Fig. 1 The average length of necrotic lesion induced by either *F. culmorum* (circle) or *G. tritici* (triangle) in *Brachypodium* accessions that have undergone different T-DNA mutagenesis events, comparing the difference in length between the induced mutant (green) and the near isogenic line (yellow). Significant differences are only shown between the mutant and NIL for each accession and each disease, determined using a linear mixed-effects model followed by Tukey post hoc pairwise comparisons, the denotations are '·' for $p < 0.1$ (non-significant but notable), '*' for $p < 0.05$, '*' for $p < 0.01$, and '***' for $p < 0.001$.

Change in necrotic lesion growth over time

Analysis of infection patterns revealed that more accessions showed significant differences between the mutant and NIL populations in *F. culmorum*-induced necrosis, with six accessions (p-values < 0.035), compared to *G. tritici*-induced necrosis, which showed significant differences in only two accessions (p-values < 0.032).

BdAA 197 showed (Fig. 2) that whilst the mutation started with higher infection levels early on, the progression of the *F. culmorum*-induced necrosis over time was much slower with the mutation (p = 0.0155). BdAA 441 showed a similar, but comparatively delayed, infection pattern – with the final day showing a decreased *F. culmorum*-induced necrosis in the mutation vs the NIL ($p = 0.0007$).

BdAA 563 showed reduced disease in the mutant lines at the beginning and the end of the experiment, but similar levels in the middle (p = 0.030). BdAA 315 and 398 showed that there was less *F. culmorum*-induced necrosis in the mutant at the beginning of the experiment, but this equalised by the end of the experiment (p-values = 0.022 and 0.035, respectively).

BdAA 360 showed similar levels of *F. culmorum*-induced necrosis early on, but the progression of the disease in the mutant line was much faster ($p < 0.0001$). Similar patterns were seen in BdAA 222 in the *G. tritici*-induced necrosis ($p = 0.031$).

Of all the accessions showing a significantly different pattern over time between the NIL and the mutation, only BdAA 319 showed a significant difference in the overall infection as well. The pattern changes in the *G. tritici*-induced necrosis of BdAA showed a steep increase in the infection rate of the mutant line in the later stages of the experiment ($p = 0.015$).

The two accessions (BdAA 222 and 319) that showed different *G. tritici* infection patterns over time were the only two lines that showed differences in the overall *F. culmorum*-induced necrosis. Two of the six lines (BdAA 398 and 441) that showed differences in the

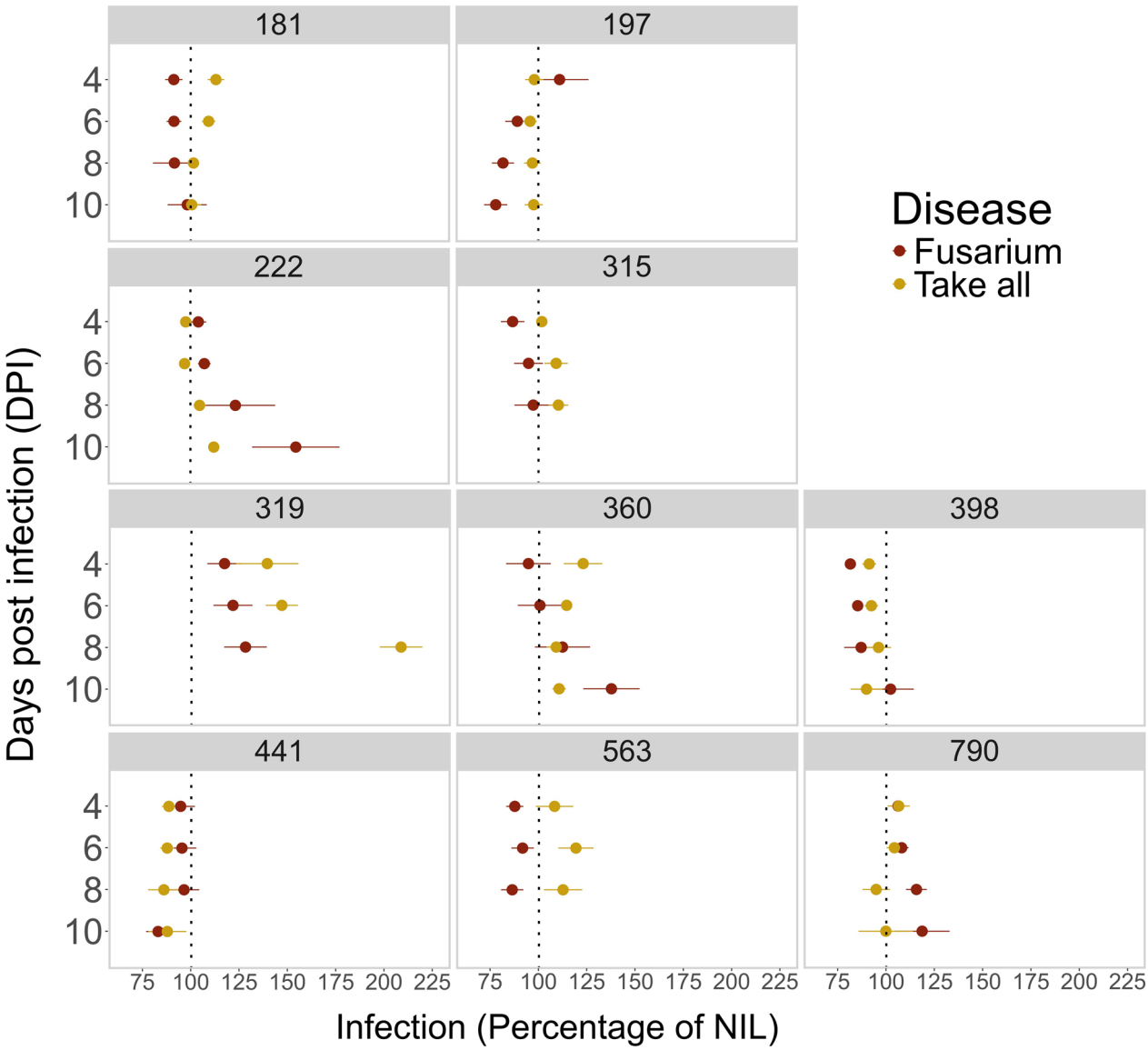

Fig. 2 The necrosis length in *Brachypodium* accessions that have undergone different T-DNA mutagenesis events, as a percentage of the near isogenic lines, over multiple time point (in days post inoculation) in each BrachyTAG mutation accession – induced by either *F. culmorum* (red) or *G. tritici* (ochre).

overall *G. tritici*-induced necrosis showed different *F. culmorum* infection patterns.

Most accessions showed similar patterns (with varying significance levels); however, accession BdAA 360, and to a lesser extent BdAA 181, showed indications of opposite patterns between the infection patterns of *G. tritici* and *F. culmorum* (Fig. 2).

Connection with predicted function

Of all the ten accessions selected for their possible connections to fungal root disease, eight showed possible changes in infection rate and patterns (Table 1), with only two showing no difference (BdAA 181 and 790).

Among the five mutants with predicted kinase and/or LRR functions, three affected the *F. culmorum* infection pattern, and the kinases positively or neutrally affected this pattern (positively meaning a trend towards lower disease levels), but the LRR affected negatively. The kinase positively affecting the Fusarium pattern (BdAA 441) also caused a reduction in the total levels on *G. tritici* - induced necrosis. The other two lines - a kinase and an LRR-kinase - showed no significant differences.

Of the three transcription factors, two were shown to affect the *F. culmorum* infection pattern, with one of those being positively. The third transcription factor mutant (BdAA 319) exhibited the clearest effect on root disease, with both *F. culmorum* and *G. tritici* induced necrosis being increased, as well as the take-all infection pattern being negatively affected.

The WRKY mutant showed similar patterns to some of the kinases, affecting Fusarium infection pattern, and increasing the total levels on *G. tritici* - induced necrosis. The Zinc finger mutant showed the opposite pattern, affecting take-all infection pattern, and increasing the total levels on *F. culmorum* - induced necrosis.

Exploratory assay of DON sensitivity during germination

An exploratory DON germination assay was conducted on four of the previously tested BdAA accessions to evaluate potential links between mycotoxin sensitivity and root disease phenotypes. Of these, two accessions, BdAA 222 and 360, showed significantly different responses between the mutant and the NIL.

Although limited in replication, the assay provided preliminary insight into DON sensitivity. BdAA 222 showed a difference in germination duration ($p = 0.01$), with the NIL exhibiting a 6.6% decrease in germination time and the mutant showing a 6.5% increase (Fig. 3a). Notably, under control conditions, the mutant line germinated approximately 13.4% more slowly than the NIL. BdAA 360 showed a significant difference in germination success ($p < 0.05$); the NIL showed a large decrease in germination in response to DON, whereas the mutant line was less affected (Fig. 3b). These results should be interpreted with caution and considered hypothesis-generating until further validated.

Discussion

Control of disease in cereal crops is of critical concern in maintaining crop yields, especially in the light of climate change causing shifts in global disease (Lahlali et al. 2024). Root disease is of particular concern due to the understudied nature, and the critical role of the system, causing large amounts of economic damage (Poole et al. 2015). One strategy in the defence against root diseases in cereals is through the incorporation of defence genes (Qalavand et al. 2023), however, pathogens can overcome this resistance (Hafeez et al. 2021), thus necessitating the need for identification of novel genes in reducing the pathogens success. As the genes currently in the breeding material of temperate cereals are thus vulnerable to shifts in pathogen races, it is critical that we search for diverse sources of resistance genes that can be used in pre-breeding efforts (Babu et al. 2020). *B. distachyon*, as a model for temperate cereals that can be grown rapidly in laboratory conditions, allows for quick and successful assessment of resistance genes for different pathogens (Wang et al. 2020; Wu et al. 2022). The current study used *B.*

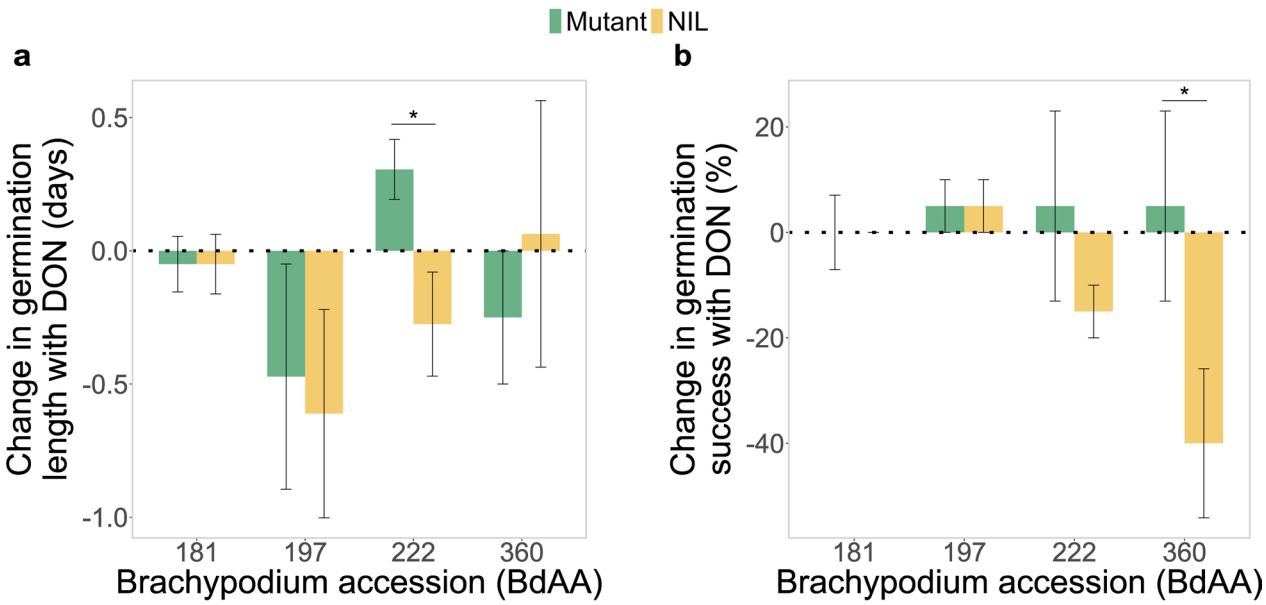

Fig. 3 The change in the number of days until germination **a** or the germination rate **b** with the addition of the mycotoxin deoxynivalenol (DON) for the mutant (green) and NIL (yellow) for four of the BrachyTAG accessions. Significant differences, calculated using Welch's *t*-test, are only shown between the mutant and NIL for each accession, the denotations are '*' for $p < 0.05$. As the assay was conducted without full biological replication, results are presented as preliminary and exploratory.

distachyon with T-DNA insertions, characterised by Thole et al. (2010), to study the impact of targeted genes on root disease resistance for *F. culmorum* and *G. tritici*.

The phenotypic differences observed between mutant lines and NILs suggest that some of the targeted genes play a role in the plant's defence arsenal. To understand the functional implications of the mutation, it is essential to examine how the mutation responds to different diseases. The phenotypic differences observed between mutant lines and NILs suggest that some of the targeted genes play a role in the plant's defence mechanisms. To understand the functional implications of the mutation, it is essential to examine how the mutation responds to different diseases. The increased lesion size observed in both BdAA 222 and BdAA 319 under a Fusarium infection indicates that the mutated genes in these lines contribute to resistance against Fusarium. However, the additional susceptibility of BdAA 319 to take-all suggests that the gene disrupted in this line has a broader role in disease resistance. This difference in response highlights the different aspects of plant immunity, where some genes may provide general protection while others confer pathogen-specific resistance. The changed infection progression patterns - where some mutants initially exhibited higher infection levels that later stabilised or even decreased - could indicate differences in the timing or amplitude of defence activation. These observations are consistent with previous studies in cereals, where mutations in genes encoding signalling molecules or transcription factors have been shown to change the response pattern of the pathogen (Blyth et al. 2023; Zhang et al. 2023; Hua et al. 2025). The changes in lesion size and infection dynamics in our study suggest that these genes are involved in the defence responses and may act at different stages of the plant immune response. While many of these mutations contribute to defence responses, not all are viable candidates for breeding, highlighting the need to examine their specific molecular mechanisms to understand their broader implications.

The genes that were identified in this study that are involved in disease response suppression include BdAA 398 and 441. BdAA 398 holds a mutation in a gene predicted to encode a transcription factor with a WRKY domain, which is key in regulating defence-related genes (Eulgem and Somssich 2007). However, the mutant lines with the reduced function of this gene showed reduced take-all necrosis - but not Fusarium root rot, suggesting that this particular WRKY transcription factor is involved in the suppression on the disease response, as found in other cereals (Yu et al. 2023). This disruption highlights the critical role of transcriptional regulation in disease resistance and the potential impact of WRKY proteins on pathogen defence. BdAA 441 carries a mutation in a gene predicted to encode a serine/threonine protein kinase with a Curculin-like lectin domain, part of the Bulb-type lectin domain superfamily. In other systems, similar proteins regulate pathogen recognition and cell death (Hwang and Hwang 2011; Guidarelli et al. 2014). Our results indicate that this gene is also required for the activation of cell death in Bd, as its disruption led to reduced necrosis, particularly in response to take-all disease. A smaller reduction in necrosis was observed during Fusarium infection, possibly because Fusarium remains in a biotrophic phase longer, which does not trigger strong cell death responses. These findings suggest that proper function of this gene is needed for the activation of the cell death response, and that this is a more critical trigger with some pathogens, affecting how necrosis spreads during pathogen attack. This is critical for the suppression of necrotic pathogens (or pathogens in the necrotic phase), but could cause the increase of other non-necrotic diseases due to reduction in spread (McCombe et al. 2022). Other Bd lines with disruptions in genes encoding for protein kinases, however, do not show any clear effect on the disease progression.

The genes involved in disease response promotion include BdAA 222, 319, and 360. BdAA 222 harbours a disruption in a gene predicted to regulate DNA-templated transcription via a zinc-finger domain. Zinc-finger protein transcription factors have been demonstrated in multiple studies to be important in plant immunity, acting as transcriptional regulators to promote defence responses (Noman et al. 2019; Cui et al. 2021). The increased Fusarium root rot necrosis, but lack of changes in take-all disease, suggests that this gene plays a role in pathogen-specific host response. Its function may be required for activating defence pathways that are particularly effective against Fusarium, or hemibiotrophs in general. This aligns with findings in other plant systems, where certain zinc-finger transcription factors enhance immunity against specific fungal pathogens with distinct infection strategies (Gupta et al. 2012). Additionally, the reduced function of this gene resulted in an increased germination time with DON, indicating its involvement in defending against mycotoxins. We noticed that the DON germination assay was conducted only once per accession with internal technical replication and lacked the independent biological repeats. As such, the observed differences should be interpreted as exploratory. We have intentionally framed these findings as hypothesis-generating, and further replication will be essential to confirm the functional role of these genes in DON sensitivity. The orthologues of the predicted protein from the disrupted gene are, however, only found in the more distantly related Sorghum. Thus, whilst they

could be a target in Sorghum, we do not know how they will react in this more distinct background. This gene could, however, be a target of integration through genetic modification (Hafeez et al. 2021). BdAA 319, which is the only line to show increased necrosis for both Fusarium root rot and take-all disease when disrupted, encodes a predicted transcription factor containing a basic leucine zipper (bZIP) domain. The bZIP transcription have been linked with both the stress and immune responses, with a number of studies showing their role in pathogen defence (Jakoby et al. 2002; Alves et al. 2013). The role in broad-spectrum resistance observed in BdAA 319 suggests that the gene acts in the broad-spectrum immunity pathways, like the hormone-mediated defence such as those using jasmonic acid or salicylic acid (Bian et al. 2020; Li et al. 2023). These findings are particularly important, as genes involved in broad-spectrum resistance are highly valuable in breeding programs, reducing the risk of resistance breakdown due to pathogen evolution (Li et al. 2020). This can be directly used in a number of breeding programs as orthologues of this gene are found in temperate cereals – such as wheat and barley, wild relatives of temperate cereals – such as wild einkorn and Tausch's goatgrass, as well as other more distantly related cereals such as sorghum and foxtail millet. BdAA 360, which showed increased necrosis length in the later stages of Fusarium root rot, carries a mutation in a gene encoding a leucine-rich repeat receptor-like kinase (LRR-RLK). LRR-RLKs act as pattern recognition receptors (PRRs) or co-receptors in pathogen perception and thus are important in plant immune signalling (Tang et al. 2017; Chakraborty et al. 2019). Whilst in the initial infection by Fusarium, the disrupted mutant showed no difference from the NIL, but in the latter stages there was a large increase, suggesting that this gene plays a role in restricting colonisation rather than in the immediate defence response to infection. As LRR-RLK's play a central role in multiple areas of the defence response – recognition signalling, defence mechanism activation, and developmental control (Afzal et al. 2008) – this aligns with the findings here, where the gene disrupted in BdAA 360 has a role in the later stages. The similarity of domains in the protein with ribonuclease inhibitor superfamily also raises the possibility of RNA stability regulation as a contributing factor in immune signalling (Gusho et al. 2020). Our data suggests that, whilst this gene may not be crucial for early-stage defence, it becomes necessary for containing the pathogen in later stages of colonisation, a mechanism that could be exploited to enhance durable resistance in cereals. As orthologues of this gene in other cereal crops have only very limited homology, it is promising for incorporating into breeding programs through genetic modification (Hafeez et al. 2021).

Although orthologues of several candidate genes were identified in temperate cereals such as wheat and barley, their functional roles in disease resistance remain to be experimentally confirmed. Future studies will be needed to assess the conservation of these gene functions in crop systems through transformation or mutation analysis.

Conclusions

The results from this study provide a snapshot into the genetic basis of disease resistance in *B. distachyon*, highlighting the role of specific transcription factors and kinases in the plant defence response to root pathogens. By characterising mutant lines with disruptions in genes encoding WRKY, bZIP, zinc-finger, and LRR-RLK domains, we have demonstrated how these factors contribute to resistance against *F. culmorum* and/or *G. tritici* in the root systems. Our findings emphasise the complexity of the plant defence response, with some genes being pathogen-specific, whilst others being broad-spectrum. The critical genes identified in this study include a transcription factor with a bZIP domain involved in broad-spectrum resistance – with orthologues found in multiple cereals, and two other genes (a transcription factor and a kinase) involved in Fusarium specific resistance (including defence against mycotoxins) that could be candidates for inclusion via genetic modification. Additionally, two genes (a transcription factor and a kinase) were identified as suppressors of defence responses against *G. tritici*, or necrotrophic pathogens in general.

This study highlights the importance of using molecular genetics with applied plant pathology to identify resistance to root pathogens for future crop development. However, a long-term assessment of these genes impact on the pathogen's progress, the plants health, and the final yield will be vital in assessing their usefulness in agricultural systems. As climate change will alter the disease pressures in cereal crops, particularly in regard to soil pathogens, translating these findings into breeding strategies will be crucial for safeguarding global crop yield, ensuring sustainable agricultural production.

Methods

Plant material

T-DNA accessions of *B. distachyon*, generated by Agrobacterium-mediated transformation (Thole et al. 2010), were provided as first generation mutant (T_1) seeds (BdAA (annotated accessions) 181, 197, 222, 315, 319, 398, 441, 563, and 790) , with a tenth provided as T_2 seed (BdAA 360). All were provided from the primary tillers, with the exception of BdAA 197, which was obtained from secondary tillers. All T-DNA-tagged accessions

carry the reporter gene encoding Green Fluorescent Protein (GFP). Seeds had their husks manually removed before being germinated between damp filter paper in aluminium-covered Petri dishes before incubating at 4°C for five (±1) days. They were then grown at 22°C under a light/dark (16h /8h) photoperiod for four (±1) days. GFP expression was assessed using a microscope (Nikon Eclipse 800) equipped with a FITC filter set (excitation 465–495 nm; dichroic mirror 505 nm; barrier filter 515–555 nm). Embryos lacking GFP (GFP negative) were classified as near-isogenic lines (NILs) and served as controls, whereas GFP-positive embryos were recorded as carrying the mutant allele.

The seedlings were then transplanted to pots containing a 1:1 (w/w) mixture of John Innes Loam Compost Number 2 and a peat-sand blend, and cultivated in a Climate Chamber (Snijders Scientific Model MC1000HE-EVD) at 22°C, 70% relative humidity, and a light/dark (20h/4h) photoperiod until seed set (approximately 12–14 weeks). Initially, seedlings were covered for 3 days, and cover removal coincided with the application of a nematode treatment a biocontrol of various insect pests (Koppert Biological Systems Steinernema feltiae Entonem). Plants were then allowed to dry for a minimum of 1 week prior to harvest. Homozygous (GFP-fixed) lines were identified by screening 20 germinated seeds per accession for 100% GFP fluorescence (versus the expected 3:1 ratio in heterozygotes) and then transplanted with corresponding NILs for seed generation.

Fungal inoculation

F. culmorum cultures (Fu42; JIC collection) and *G. tritici* cultures (GgT5; JIC collection) were grown on Potato Dextrose Agar (PDA) plates (*G. tritici* plates supplemented with 50 µg/mL streptomycin and 50 µg/mL carbenicillin), sealed with Parafilm and incubated at 20 °C under a light/dark (16h/8h) photoperiod until mycelial growth fully covered the plate (10–14 days).

For each accession, the mutant lines and NILs were germinated as described above for one day before being transferred to a 100 mm square petri dishes containing 45 mL of 0.8% agar with a layer chromatography paper (3 mm) on top of the agar. Four replicated dishes were prepared, each replicate containing six mutant and six NILs for each accession. This was run for each diseases, and run twice (totalling to eight replicates) for accessions BdAA 398, 441, 790, and 197 (the later with *F. culmorum* only).

Root inoculation occurred by blending the fungal containing PDA plates with 1-1.5 mL of distilled water per plate to make a fungal slurry. The resulting slurry was applied to the root tip (covering ~3 mm) and incubated until signs of infection, before removing the slurry with a wet brush. Photographs were taken following the disease progression at 4-, 6-, 8-, and 10-days post inoculation (DPI) using a light camera (Olympus Camedia C-750 Ultra Zoom). Lesion lengths were quantified using ImageJ with a ruler reference (Schneider et al. 2012), by measuring the length of necrotic tissue extending from the inoculation site (Additional file 1: Figure S1a).

DON sensitivity assay

Deoxynivalenol (DON) sensitivity assays were performed on accessions BdAA 181, 197, 222, and 360. Petri dishes were prepared using 25 mL of 0.8% agar, eight with 2 ppm DON and eight without, covered with filter paper. A 40 × 40 mm grid (divided into four 20 × 20 mm quadrants) were drawn over each dish (Additional file 1: Figure S1b). For each accession, two plates of each DON and control were arranged, with 10 seeds from NIL and 10 seeds from the mutant line distributed over 2 quadrants per plate. Plates were sealed with Parafilm and incubated at 4°C in darkness. Germination (defined as complete rupture of the endosperm) was monitored daily for 5 days using a light microscope (Heerbrugg Wild M3Z, 6 V, 20 W).

Statistical analysis

Models were made with the statistical program 'R' (R Core Team 2013) and the packages lme4 (Bates et al. 2015), and emmeans (Lenth 2022). A linear mixed-effects model was used to analyse the length of the necrosis in the fungal inoculation experiments, for each BdAA accession and disease, with Genotype (Mutant and NIL) and DPI as factors. A correlation structure (corAR1) was applied to account for repeated measurements (DPI as a numeric value), with a random effects structure for plant within box for the experiment. A linear mixed-effects model was also used for each accession to analyse both the average days until germination and the total germination rate (per dish) for the DON assay, with genotype (Mutant and NIL) and application (control vs. DON) as fixed effects and dish as a random effect. Each model was fitted using a Restricted Maximum Likelihood (REML), before an ANOVA was run. Finally, Tukey post hoc pairwise comparisons were conducted to assess differences in genotype within each disease or application category.

The DON assay data for the germination length and rate was then summarised to calculate the mean germination rate and its associated standard error (SE) for each combination of BdAA, application, and genotype. The change caused by DON was calculated as the difference in length or rate between DON treatment and control. The SE for this change was calculated as the square root

of the combined standard error of the difference in germination rates between the DON and control treatments - calculated by summing the squared standard errors of both treatments. The statistical significance of this difference between the NIL and mutant was calculated for each line using a Welch's *t*-test with a corresponding *p*-value computed using a two-tailed test with an approximation for infinite degrees of freedom.

Data visualisation was done using 'R' packages ggplot2 (Wickham 2016), ggpubr (Kassambara 2023), and rmisc (Hope 2013).

Identification of orthologues

The data on the position of each T-DNA insertion was provided with the BrachyTAG seed, using the Plant Ensembl Genomes resource (Yates et al. 2021) to identify the domains in the affected protein and its orthologues in other cereals. The ten mutant lines analysed in this study were associated with disruptions in genes predicted to encode proteins with various biological functions. The predicted functions, molecular domains, orthologous genes, and mutation positions for each disrupted gene are detailed below (detailed information about the mutation is provided in Additional file 2: Table S1).

BdAA 181 is predicted to encode a protein involved in cellular protein modification via phosphorylation, with molecular functions including protein kinase activity, leucine-rich repeat (LRR) domain presence, ATP binding, and transferase activity. Orthologous genes with 75% sequence similarity are found in *Oryza* species, with similar matches in *Sorghum bicolor*, *Zea mays*, and *Hordeum vulgare*. The mutation is located approximately 60 nucleotides (nt) upstream of the start codon, potentially affecting transcription.

BdAA 197 is predicted to encode a protein involved in the regulation of DNA-templated transcription, with DNA-binding activity. Orthologous genes with 84% sequence similarity are found in *Triticum aestivum*, with additional homologs in *Oryza* species (77–79%) and *Zea mays* (67–70%). The mutation is located 166 nt downstream of the start codon, 2 nt before the end of exon 1.

BdAA 222 encodes a protein predicted to regulate DNA-templated transcription via a zinc-finger domain, with associated molecular functions of DNA binding and zinc ion binding. Orthologous genes with 75% sequence similarity are found in *Sorghum* species, along with similar matches in *Oryza* species. The mutation is located approximately 382 nt upstream of the start codon, likely influencing transcription.

BdAA 315 is predicted to be a member of the YABBY gene family, encoding a transcriptional regulator associated with lateral organ development. The protein contains a YABBY domain, and orthologous genes with 89% sequence similarity are identified in *Triticum aestivum*, with additional homologs in *Hordeum vulgare* and *Aegilops tauschii* (86%) and *Oryza* species (79%). The mutation is located 29 nt upstream of the start codon, potentially affecting transcription.

BdAA 319 encodes a predicted transcription factor involved in DNA-templated transcription regulation, possessing a basic leucine zipper (bZIP) domain. Orthologous genes with 70–75% sequence similarity are found in *Triticum aestivum*, *Triticum urartu*, *Hordeum vulgare*, *Sorghum bicolor*, *Aegilops tauschii*, and *Setaria italica*. The mutation is located 49 nt upstream of the start codon, potentially altering transcriptional activity.

BdAA 360 encodes a leucine-rich repeat receptor-like protein kinase (LRR-RLK), with a portion of the protein sharing homology with a ribonuclease inhibitor superfamily. Weak orthologues (above 50% similarity) are identified in *Oryza brachyantha* and *Leersia perrieri*. The mutation is positioned 375 nt upstream of the start codon, potentially affecting transcription regulation.

BdAA 398 is predicted to encode a transcription factor involved in DNA-templated transcription regulation, containing a WRKY DNA-binding domain. This domain is characterised by a conserved WRKYGQK sequence at the N-terminal and a zinc-finger-like motif. Orthologous genes with 91% similarity are identified in *Triticum aestivum*, with additional homologs in *Oryza* species (83–85%) and *Hordeum vulgare*. The mutation is located 365 nt upstream of the start codon, likely influencing transcription (Table 1).

BdAA 441 encodes a predicted serine/threonine-specific protein kinase with additional domains, including an Apple-like PAN-2 domain involved in protein binding and a bulb-type lectin domain (Curculin-like) associated with pathogen defence responses. Orthologous genes with 72–75% similarity are found in *Triticum aestivum*, *Hordeum vulgare*, *Aegilops tauschii*, and *Triticum urartu*. The mutation is located 82 nt downstream of the start codon, within exon 1.

BdAA 563 encodes a protein kinase predicted to be involved in phosphate transfer, containing a serine/threonine-specific protein kinase domain and leucine-rich repeats (LRRs). A portion of the protein also shares homology with a ribonuclease inhibitor superfamily. Orthologous genes with 80–81% similarity are identified in *Hordeum vulgare*, *Aegilops tauschii*, *Triticum aestivum*, and *Triticum urartu*. The mutation is positioned 181 nt upstream of the start codon, affecting transcription.

BdAA 790 encodes a protein with a serine/threonine-specific protein kinase domain and an epidermal

Table 1 *P*-values indicating the significance of allele effects and allele-by-time interactions in the necrotic response to *F. culmorum* and *G. tritici* infection across *Brachypodium* accessions. Cells are marked to indicate the direction of the significantly different mutant phenotype compared to the near-isogenic line (NIL): significantly reduced necrosis (*****), significantly increased necrosis ($_{\times}$), and significant but a non-directional difference (~).

Accession information			Fusarium		Take-all	
BdAA	**Chromosome**	**Predicted gene category**	**Allele**	**Allele × Time**	**Allele**	**Allele × Time**
181	Bd5	LRR-kinase	0.3587	0.8116	0.5535	0.5548
197	Bd5	Transcription factor	0.143	0.0155*	0.4842	0.2743
222	Bd2	Zinc-finger	$0.042_{\times}$	0.0579	0.4189	$0.0311_{\times}$
315	Bd5	Transcription factor	0.5816	0.0214~	0.275	0.2592
319	Bd2	Transcription factor	$0.0278_{\times}$	0.1527	$0.0001_{\times}$	$0.0148_{\times}$
360	Bd2	LRR	0.3053	$0.0001_{\times}$	0.0518	0.6228
398	Bd1	WRKY	0.244	0.0346~	0.0238*	0.483
441	Bd2	Kinase	0.1599	0.0007*	0.0003*	0.4365
563	Bd3	Kinase	0.1293	0.0295~	0.0993	0.1237
790	Bd5	Kinase	0.1111	0.3394	0.6821	0.1417

growth factor (EGF)-like calcium-binding domain, which may facilitate protein-protein interactions. Orthologous genes with 94% similarity are identified in *Triticum aestivum*, with additional homologs in *Hordeum vulgare* and *Triticum urartu* (84–87%). The mutation is located 1349 nt downstream of the start codon, within exon 3 - 908 nt before the exon terminus.

Abbreviations

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BdAA	Brachypodium distachyon annotated accession
bZIP	Basic leucine zipper
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
DPI	Days post inoculation
EGF	Epidermal growth factor
FCR	Fusarium crown rot
FHB	Fusarium head blight
FITC	Fluorescein isothiocyanate
FRR	Fusarium root rot
GFP	Green fluorescent protein
JIC	John Innes Centre
LRR	Leucine-rich repeat
LRR-RLK	LRR receptor-like kinase
NIL	Near-isogenic line
PDA	Potato Dextrose Agar
PRR	Pattern recognition receptor
REML	Restricted maximum likelihood
SE	Standard error
T-DNA	Transfer DNA (from Agrobacterium)
USDA-ARS	United States Department of Agriculture - Agricultural Research Service

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-025-00385-7.

Additional file 1: Figure S1. Representative images of the phenotyping assays used to evaluate root disease progression and DON sensitivity in *B. distachyon*, using accession BdAA 222 as an example.

Additional file 2: Table S1. Detailed information on T-DNA insertion sites in *Brachypodium* accessions, including flanking sequence tag (FST) identifiers, chromosomal positions, affected genes, relative insertion locations within the genes, and predicted functional annotations from EnsemblPlants.

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Author contributions
JC: Methodology, Formal analysis, Investigation, Writing—Original Draft, Visualisation; AP: Conceptualisation, Writing—Review and Editing, Supervision, Project administration.

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The work presented in this manuscript did not involve the use of human or animal subjects.

Consent to publication
The work presented in this manuscript did not involve the use of human or animal subjects.

Competing interests
The authors declare no competing interests.

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