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Xiang, Zikai

Nakayashiki, Hitoshi

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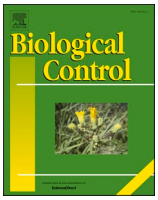


Corynascella humicola, a root endophytic fungus from crabgrass, confers growth promotion and induced systemic resistance in gramineous plants

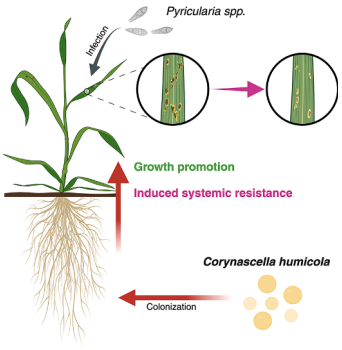
Zikai Xiang, Hitoshi Nakayashiki, Kenichi Ikeda *

Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan

HIGHLIGHTS

- *Corynascella humicola*, isolated as an endophytic fungus from the roots of crabgrass.
- *C. humicola* had a plant growth promoting effect against gramineous plants, and this effect was pronounced when no fertilizer was applied, expecting the use of chemical fertilizer would be reduced.
- *C. humicola* also conferred induced systemic resistance against *Pyricularia oryzae*, and this effect was observed even when fertilizer was applied, suggesting that a mechanism different from PGP is involved.

GRAPHICAL ABSTRACT



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ABSTRACT

Root-habiting fungi have great potential for plant growth promotion (PGP) and induced systemic resistance (ISR) against pathogens. In the present study, we isolated a potentially beneficial fungus, *Corynascella humicola* CHDR1-1, from crabgrass roots. Root colonization increased over time when barley, rice, and wheat seeds were planted in the soil containing CHDR1-1 mycelial aggregates. The PGP effect has been observed in barley and wheat, particularly under fertilizer-free conditions. CHDR1-1 conferred ISR against *Pyricularia oryzae* in all tested gramineous plants, i.e., barley, rice, and wheat. Most pathogenesis-related genes were expressed at higher levels in the CHDR1-1-treated group than in the untreated group after pathogen inoculation. Utilizing filamentous fungi with these characteristics may reduce the reliance of agricultural systems on chemical fertilizers and pesticides.

1. Introduction

The production and development of global agriculture increasingly emphasize agricultural microbial technology to address the growing severity of environmental issues. Plant growth-promoting microorganisms (PGPM) have attracted considerable attention due to their potential economic and ecological benefits (Bhattacharyya and Jha, 2012; Rana et al., 2020). Many soil microbiota function as PGPMs, enhancing crop

* Corresponding author at: Laboratory of Cell Function and Structure, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan.
E-mail address: ikeken@phoenix.kobe-u.ac.jp (K. Ikeda).

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growth, reducing dependence on fertilizers, and improving biotic/abiotic stress resistance (Rana et al., 2020; Yaghoubian et al., 2022). Plant growth-promoting fungi (PGPF) are crucial components of PGPMs and have garnered significant interest in mycology. PGPFs can exert a range of beneficial effects on plants in a highly efficient and eco-friendly manner (Murali et al., 2021; Gowtham et al., 2024). Cucumbers treated with *Periconia macrospinosa* and *Aspergillus elegans* exhibited a nearly two-fold increase in shoot length, along with a significant increase in both shoot weight and chlorophyll content (Sidhoum et al., 2024). *Penicillium olsonii* enhances the growth of tobacco plants and increases leaf area while improving salt tolerance by boosting proline concentration and catalase and superoxide dismutase activities (Tarroum et al., 2022). Moreover, many PGPFs promote plant growth and induce plant resistance and related defense mechanism proteins to help plants resist the invasion of other pathogens. *Fusarium equiseti* and *Phoma* sp. can mitigate damage to pepper from root rot and wilt pathogens (El-Kazzaz et al., 2022). *Aspergillus niger* and *Penicillium oxalicum* protect tomato plants from *Fusarium* wilt (Attia et al., 2022). *Talaromyces verruculosum* improves both growth and disease resistance in muskmelons by acting as a source of bioactive oligosaccharides (Mahadevaswamy et al., 2023). Most PGPFs benefit plants as non-pathogenic endophytes; however, various factors, such as phosphorus, affect the efficiency of this symbiotic relationship (Cao et al., 2024). Changes in environmental factors can transform symbiotic fungi into pathogens. Phosphate reportedly affect the expression of the transcription factor CtBOT6, which regulates the transition of the fungus from a symbiotic to a pathogenic lifestyle, in *Colletotrichum* (Hiruma et al., 2023; Ujimatsu et al., 2024).

An increasing number of PGPFs that act on dicotyledonous plants (e.g., cucumbers, peppers, and tomatoes) have been identified and developed (Jogaiah et al., 2013; Sidhoum et al., 2024). However, PGPFs remain relatively underexplored in gramineous crops, a primary global food production source. Therefore, the exploring and identifying potential PGPF candidates for gramineous crops are important yet challenging issues. *Piriformospora indica* and *Trichoderma* spp. have been reported to exhibit PGPF activity in barley and wheat (Waller et al., 2005; Saadaoui et al., 2023). Another well-known PGPF, *Chaetomium globosum* (Chaetomiaceae), has been shown to promote the growth and yield of crops such as wheat and corn while serving as an effective biocontrol fungus that enhances plant defense responses against various pathogens (Dhingra et al., 2003; Jiang et al., 2017; Elshahawy and Khattab, 2022; Feng et al., 2023). Notably, most fungi in the family Chaetomiaceae are cellulolytic saprobes with significant economic potential because of their ability to produce industrially relevant enzymes such as cellulases and peroxidases (Doveri, 2018; Vivi et al., 2019; Wang et al., 2022). Regarding the excellent performance of *C. globosum* in enhancing crop growth, productivity, and biocontrol, some strains in the Chaetomiaceae family that are closely related to *C. globosum* are expected to have similar characteristics and have the potential to become new PGPF or biocontrol agents.

We previously isolated *Pyricularia grisea* from the roots of crabgrass (*Digitaria* sp.) and revealed a novel life cycle in the fungal rhizosphere (Xiang et al., 2025). In this study, we isolated several fungi—including *Corynascella humicola*, which belongs to the Chaetomiaceae family—from crabgrass roots. *C. humicola* was initially isolated from soil in the United States and formed a distinct clade phylogenetically close to *C. globosum* (Von Arx, 1975; Noumeur et al., 2020; Wang et al., 2022). However, their interactions with plants remain poorly understood. Amplicon sequencing analysis also confirmed its frequent presence in crabgrass roots, suggesting that it behaves as an endophytic fungus. Therefore, we expected *C. humicola* to behave similarly to gramineous crops. In the present study, *C. humicola* was investigated for its potential as a novel PGPF in gramineous crops. We evaluated its ability to enhance shoot growth under nutrient-deficient conditions and to trigger early defense responses in wheat and barley, which resulted in reduced blast symptoms caused by *Pyricularia oryzae*.

2. Materials and methods

2.1. Fungal isolation

Root samples (2–4 mm) were cut and surface-sterilized by immersing the roots in 80 % EtOH for 30 s, and then in 2 % NaClO solution for 30 s. The samples were then immersed in sterile distilled water (SDW) for 1 min, and this was repeated twice. Sterilized roots were incubated on isolation agar medium at 25 °C in the dark for 5 days. The colonies were transferred to fresh medium.

2.2. DNA extraction, PCR, and sequencing

A portion of the mycelia of the isolated fungal colony grown on potato dextrose agar (PDA) medium (2.4 % BD DIFCO potato dextrose broth and 1.5 % agar powder) was picked with a toothpick and placed in a 1.5 mL tube. Fungal DNA was extracted using the Kaneka Easy DNA Extraction Kit version 2 (KANEKA CORPORATION, Osaka, Japan), following the manufacturer's instructions. The rDNA-ITS region was amplified with–ITS1-ITS4 primers (Table S1), and the PCR products were directly sequenced with the same primer set used for PCR using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). Sequencing was performed using the ABI 3130 Genetic Analyzer (Thermo Fisher Scientific) at Kobe University.

2.3. Amplicon sequencing

Three to five different roots were randomly selected and cut into 1 cm pieces. DNA extraction and amplicon sequencing methods were performed as described by Xiang et al. (2025).

2.4. Phylogenetic analysis

We conducted a long-read genomic analysis of the highly purified genomic DNA of CHDR1-1 using NucleoBond HMW DNA (Takara Bio Inc., Ohtsu, Japan). PacBio DNA sequencing and AUGUSTUS gene prediction were performed by the Bioengineering Lab. Co. Ltd. (Sagamihara, Japan). The nucleotide sequences of rDNA-ITS, beta-tubulin, and RNA polymerase subunit II (RPB2) are registered in DDBJ (accession numbers LC832712, LC832813, and LC832714, respectively). A genealogical concordance phylogenetic species recognition (GCPSR) phylogenetic tree of multiple strains belonging to the Chaetomiaceae family (reference genes are listed in Table S2) was constructed using the neighbor-joining method with 1000 bootstrap replications (Hirata et al., 2007).

2.5. Plant materials

Seeds were placed in culture dishes (with filter paper), soaked in water, and allowed to germinate overnight. Barley (*Hordeum vulgare* L. cv. Nigrate) and wheat (*Triticum aestivum* L. cv. Norin4) were grown in vermiculite (NITTAI.Co., LTD.) or Akadama soil (Hirota Shoten Co. Ltd., Kanuma, Japan) in a plant growth chamber maintained at 23 °C. Fertilization was performed by top-watering 5 mL of a 1000-fold diluted solution of Hyponex (Hyponex Japan Co., Ltd., Osaka, Japan) every 2 days. Crabgrass (*Digitaria ciliaris*) was grown in vermiculite in a plant growth chamber maintained at 28 °C. The rice (*Oryza sativa* L. var. CO39) was grown in vermiculite (for upland conditions) and potting soil (for flooded conditions) (Kumiai Ube Baido No. 2, MC Ferticom, Tokyo, Japan) in a plant growth chamber maintained at 26 °C. Under flooded conditions, the bottom of the pot was sealed, and the rice plants were grown in a flooded environment. The artificial light and dark cycle in the plant growth chamber lasted for 12 h. Three plants were selected to represent different cultivation environments (barley and wheat in drylands and rice in paddy fields) to evaluate the colonization ability and PGP effects of CHDR1-1. In addition, the resistance of barley (broadly

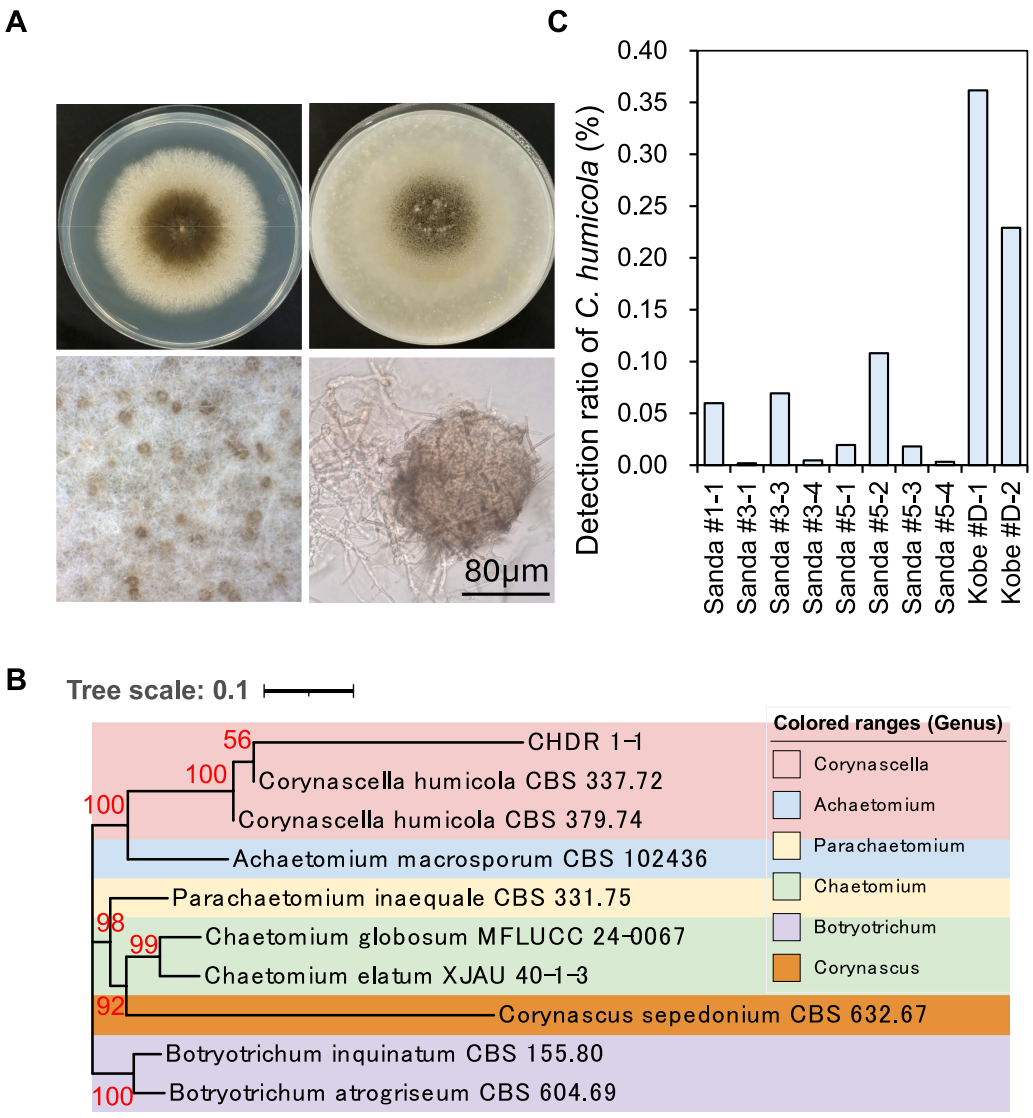

Fig. 1. Characterization of CHDR1-1. (A) The mycelial phenotypes of CHDR1-1 in PDA and oatmeal media after 14 days of incubation and the microscopic photo of the ascomata. Scale bars = 80 μ m. (B) The GCPSR phylogenetic tree of CHDR1-1. The gene accession numbers used in this study are listed in Table S2. The bootstrap values are indicated at each node. (C) The proportional abundance of CHDR1-1 in fungal abundance in crabgrass roots collected from the fields (Sanda City and Kobe University) using amplicon sequencing.

susceptible to multiple *Pyricularia* isolates), wheat, and rice (susceptible only to host-specific isolates) to CHDR1-1 was compared in pathogenicity assays.

2.6. Fungal treatment in soil

CHDR1-1 was cultured in CM liquid medium (0.3 % yeast extract, 0.3 % casamino acid, and 0.5 % sucrose) for 5–7 days and then filtered using a Kimwipe S-200 (Cresia Corp.) to recover CHDR1-1 mycelia (remove as much liquid as possible). Each rectangular pot was filled with soil to a depth of approximately 3 cm, and 0.3–0.5 g of fresh mycelium was added and mixed thoroughly. Then, 6–8 seeds that had germinated for 1 day were placed in each pot and covered with soil.

2.7. Fungal biomass estimation in roots

Root samples were collected at 7, 14, and 21 days after germination (DAG), and DNA was extracted after washing three times with SDW, following the method of Xiang et al. (2022). A CHDR1-1-specific primer set, Ch-sp, was designed based on the rDNA-ITS sequence of CHDR1-1. A standard curve was generated by using a series of diluted CHDR1-1 genomic DNA samples. CHDR1-1 biomass was calculated based on DNA concentration. Plant housekeeping gene primers *HvActin, TaCDCP,* and *OsActin* were used to confirm successful DNA extraction.

2.8. Inoculation methods

2.8.1. Root inoculation with the CHDR1-1

CHDR1-1 was grown on PDA medium for 7 days (25 °C) and mycelial plugs (approximately 2 cm^2) were used to directly inoculate the roots of the plants. Seeds were placed on filter paper in culture dishes and grown in the dark for 3–4 days. Roots of 3–4 cm in length were used in the experiments. The detailed procedures were performed as described by Xiang et al. (2022).

2.8.2. Leaf inoculation with Pyricularia isolates

Leaves from barley, rice, and wheat (14 DAG) were used for inoculation. For rice leaf inoculation, 50 mL of 1.5 % (w/v) ammonium sulfate was added to the pots at 7 DAG. For preparation of spore suspension, *Pyricularia* isolates (an *Oryza* (Guy11), a *Setaria* (GFSI1-7-2), a *Triticum*

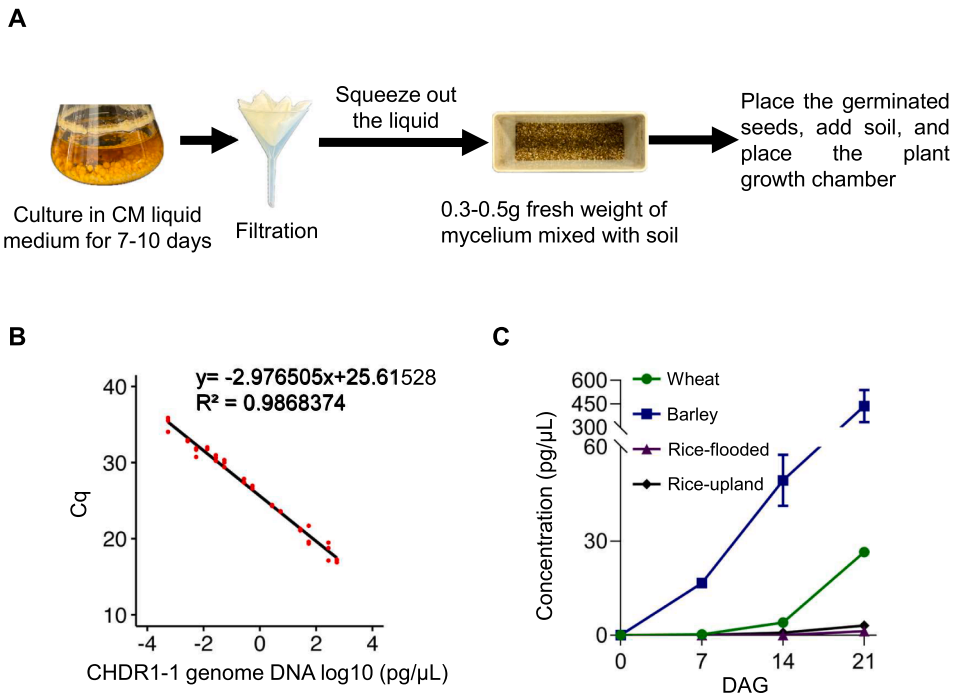

Fig. 2. Evaluation of the root colonization ability of CHDR1-1 on barley, rice, and wheat. (A) Diagram of the process of mixing CHDR1-1 with soil and cultivating plants. (B) Standard curve of the amplification (Cq value) of CH-sp primer from the dilution series of the genomic DNA of CHDR1-1 genomic DNA by qPCR. The Cq values were plotted against the log10-transformed DNA concentrations to calculate the regression equation. (C) The biomass of CHDR1-1 in plant roots (green circles: wheat, blue squares: barley, purple triangles: flooded rice-field condition, black diamonds: upland rice-field condition) after 0, 7, 14, and 21 days of CHDR1-1 treatment. The bars represent standard deviations calculated from three biological replications.

(Br48) pathotypes of *P. oryzae*, and a *Digitaria* (Dig4-1) pathotype of *P. grisea*) were cultivated in oatmeal agar medium for 7 days (25 °C). Aerial hyphae were removed and placed under black light blue (BLB) lamps for 5–7 days to form spores. SDW was added to the surface of the colonies and rubbed with a cotton stick. The spore suspension was then filtered through a KimWipe S-200 filter (Cresia Corp.). The spore suspension concentration was adjusted to 1×10^5 spores/mL and supplemented with 0.01 % Tween-20. Plant leaves were sprayed or drop-inoculated with a spore suspension, and the inoculated plants were incubated under humid conditions in the dark for 24 h and then transferred to a plant growth chamber. For drop inoculation, the lesion area was quantified using ImageJ, while the general disease severity was assessed using a grading scale for spray inoculation. The disease index was determined as follows: 6 (disease symptom of wild type 5 days after inoculation (DAI)), 5 (70 % of index 6), 4 (50 % of index 6), 3 (30 % of index 6), 2 (20 % of index 6), 1 (10 % of index 6), 0 (no symptoms).

2.9. Gene expression analysis

Plant tissue samples were frozen and ground in liquid nitrogen, and total RNA was extracted using Sepasol RNA I Super G (Nacalai Tesque Inc., Kyoto, Japan). The extracted total RNA was stored at −80 °C. Total RNA (1 μL) was reverse-transcribed to obtain cDNA using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Tokyo, Japan). The quantitative PCR (qPCR) analysis was conducted using GeneAce SYBR qPCR Mix α Low ROX (NIPPON GENE CO., LTD., Tokyo, Japan) on a Dice Real-Time System thermal cycler (model TP850; Takara). The reaction volume (20 μL) contained 0.2 μM of each primer and 1 μL of cDNA. Primers used in this study are listed in Supplementary Table S1. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Three biological replicates were analyzed independently.

2.10. Antifungal activity

CHDR1-1 cells were plated in the center of a Petri dish containing CM agar. After 4 days, mycelial plugs of *Pyricularia* isolates (Guy11, Br48, and Dig4-1) were planted in the surrounding areas. The growth inhibition rate was calculated after 5 days as the ratio of the distance that the hyphae extended from the center of *P. oryzae*/*P. grisea* toward CHDR1-1 to the extent that the hyphae extended in the opposite direction.

2.11. Data analysis

Statistical analyses were performed using IBM SPSS Statistics (v. 28.0). An independent sample *t*-test, assuming equal variance, was conducted to compare the means between the two groups for all analyses. The assumption of equal variance was confirmed using Levene's test. A two-tailed P-value was calculated, and differences were considered statistically significant at $P < 0.05$. Multiple comparisons of ΔΔCt values (from qPCR) were performed using the Tukey–Kramer test ($P < 0.05$) for the gene expression analysis in *Pyricularia* isolate inoculation experiments.

3. Results

3.1. The isolation and distribution of C. humicola in plant roots

Three fungal isolates were incidentally obtained during our previous attempt to isolate the *Pyricularia* fungus from crabgrass roots collected from the paddy field ridge in Sanda city (Kogaki, Sanda, Hyogo Prefecture, Japan; 34°58′58.2″N 135°16′02.9″E) (Xiang et al., 2025). These isolates were identified as *Corynescella humicola*, *Pseudothielavia terricola* (PtDR-1), and *Talaromyces (Penicillium) pinophilus* (TaDR-6) based on their rDNA-ITS sequences (Fig. S1A). Barley roots were treated with each fungus, and shoot growth was assessed to examine the plant growth promotion (PGP) activity of these fungi. The *C. humicola* isolate

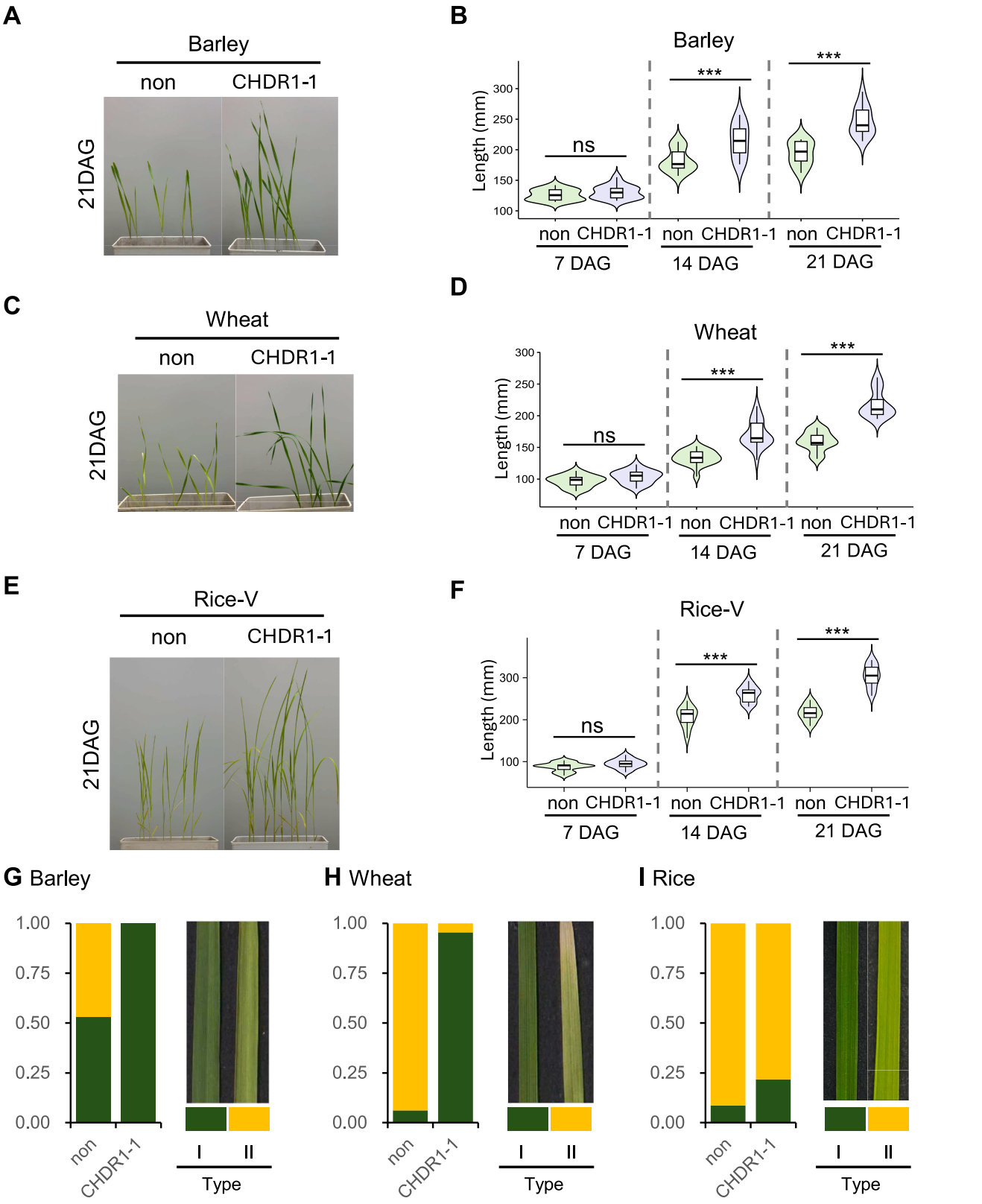

Fig. 3. Effects of CHDR1-1 treatment on shoot growth and leaf health of barley, wheat, and rice. The representative photos and the violin plots of the shoot length, and the effect of CHDR1-1 on leaf health of barley (A, B, and G), wheat (C, D, and H), and rice (E, F, and I) 7, 14, and 21 DAG without fertilizer. The data was an average of at least 10 plants. The asterisks indicate significant differences between the treatments using *t*-tests (ns; not significantly different, ***; $P < 0.001$). (G-I) Leaf health classification based on visual phenotype at 21 DAG. Type I indicates dark green, healthy leaves; Type II indicates light green or yellowing leaves. Bar graphs represent the proportion of each leaf type for barley (G), wheat (H), and rice (I) treated with or without CHDR1-1.

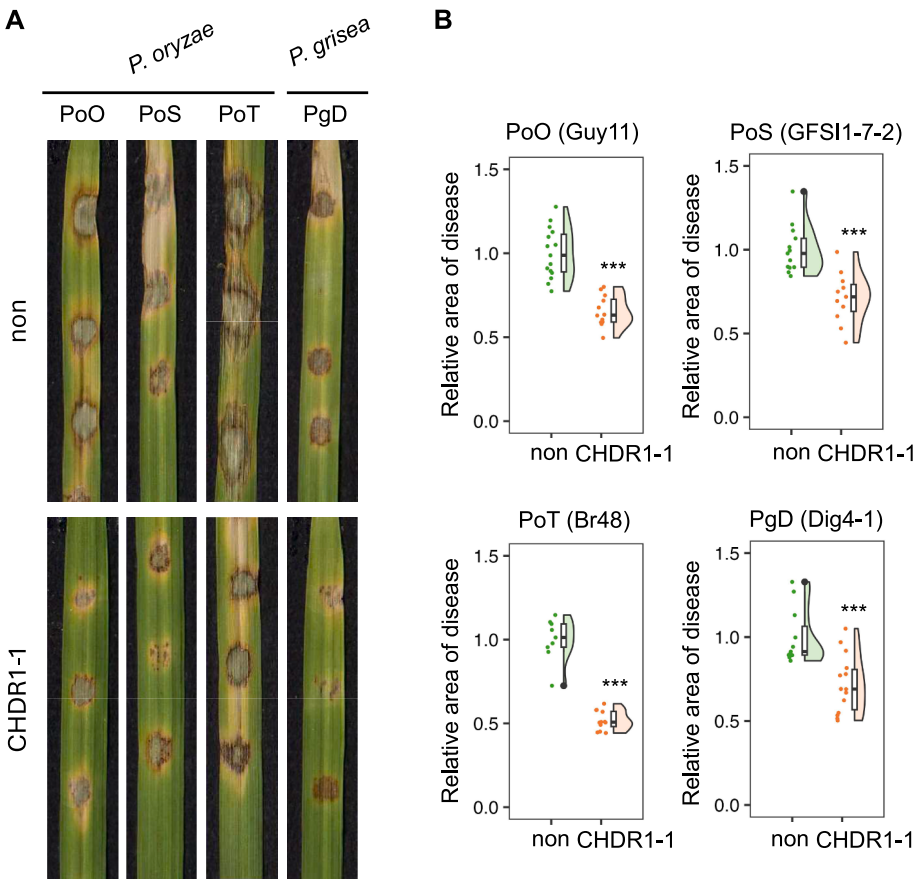

Fig. 4. Effects of CHDR1-1 on induced resistance against *Pyricularia* isolates on the barley leaves. (A) Disease symptoms on the barley leaves drop-inoculated with different *Pyricularia* isolates (*Oryza* (Guy11), *Setaria* (GFSI1-7-2), *Triticum* (Br48) pathotypes of *P. oryzae*, and *Digitaria* (Dig4-1) pathotype of *P. grisea*) at 6 DAI. (B) Quantification of disease severity based on the relative lesion area. The combined half-violin and half-dot plots are shown based on the lesion size from at least nine lesions. The asterisks indicate significant differences between treatments using *t*-tests (***; $P < 0.001$).

designated as CHDR1-1 (*C. humicola* from *Digitaria* roots, sample Sanda1-1 in Xiang et al. (2025)) exhibited the highest PGPF activity (Fig. S1B and C), and was selected for further characterization (this fungal strain was deposited in the NARO Genebank project, National Agriculture and Food Research Organization, Japan, accession number MAFF 248080).

CHDR1-1 formed ascomata (Fig. 1A), which are characteristic of the Chaetomiaceae family. We performed a long-read genome sequencing analysis using CHDR1-1. A phylogenetic tree of CHDR1-1 with other typical strains belonging to the family Chaetomiaceae was constructed using GCPSR with the rDNA-ITS, beta-tubulin, and RPB2 genes. The GCPSR phylogenetic tree showed that CHDR1-1 belonged to *C. humicola* (Fig. 1B).

We extracted DNA from crabgrass roots in paddy fields in Sanda and an experimental field at Kobe University and performed amplicon sequencing to analyze the root-inhibiting fungal community (Xiang et al., 2025). *C. humicola* was widely distributed in the roots of wild crabgrass, with fungal abundance rates ranging from 0.172 to 36.170 % (Fig. 1C).

3.2. *CHDR1-1 proliferates in the roots of gramineous plants without inhibiting root growth*

Mycelial blocks of the CHDR1-1 on PDA medium were placed on the roots of crabgrass (the original host) and three other typical crops (i.e., barley, rice, and wheat) for inoculation. All roots inoculated with CHDR1-1 exhibited browning without root growth inhibition (Fig. S2A). The expression of PR genes (*HvPR-1a, HvPR-10,* and *HvMyc2* in barley; *OsPR-1a, OsPR-10 (PBZ1)*, and *OsMyc2* in rice; and *TaPR-1, TaPR-10*, and *TaMyc2* in wheat) was induced in CHDR1-1-treated roots (Fig. S2B–D).

Mycelial aggregates were mixed with the soil, and germinated seeds were planted (Fig. 2A) to assess whether CHDR1-1 effectively colonized and proliferated plant roots in the soil. We evaluated the amount of genomic DNA in CHDR1-1 roots using qPCR. The CHDR1-1-specific primer set, CH-sp, was designed based on polymorphisms in the rDNA-ITS sequence (Table S1). A standard curve for qPCR was created from the data of the dilution series of genomic DNA of CHDR1-1 with the CH-sp primer set (Fig. 2B). Root samples were collected at 7, 14, and 21 DAG, and DNA was extracted after washing the roots three times with SDW. For rice, experiments were conducted under both flooded and upland conditions. The biomass of CHDR1-1 in the barley, rice, and wheat roots increased significantly over time (Fig. 2C). However, the relative abundance of CHDR1-1 in the roots varied greatly among the plant species, being the highest in barley and the lowest in rice (Fig. 2C).

3.3. *Effects of CHDR1-1 on plant growth*

Shoot length was measured to evaluate the effects of root colonization by CHDR1-1 on plant growth. Without fertilizer, CHDR1-1 treatment increased shoot length and reduced leaf yellowing in barley, wheat, and rice (Fig. 3) but had no effect on crabgrass (Fig. S3). In this study, we used nutrient-poor vermiculite as the soil. Similar results were obtained using Akadama soil, which is also nutrient-poor (Fig. S4). No PGP effect was observed when the CM medium was used alone (data not shown).

As nutrient conditions primarily affected the interactions between fungi and plants, we evaluated the PGP effects of CHDR1-1 under

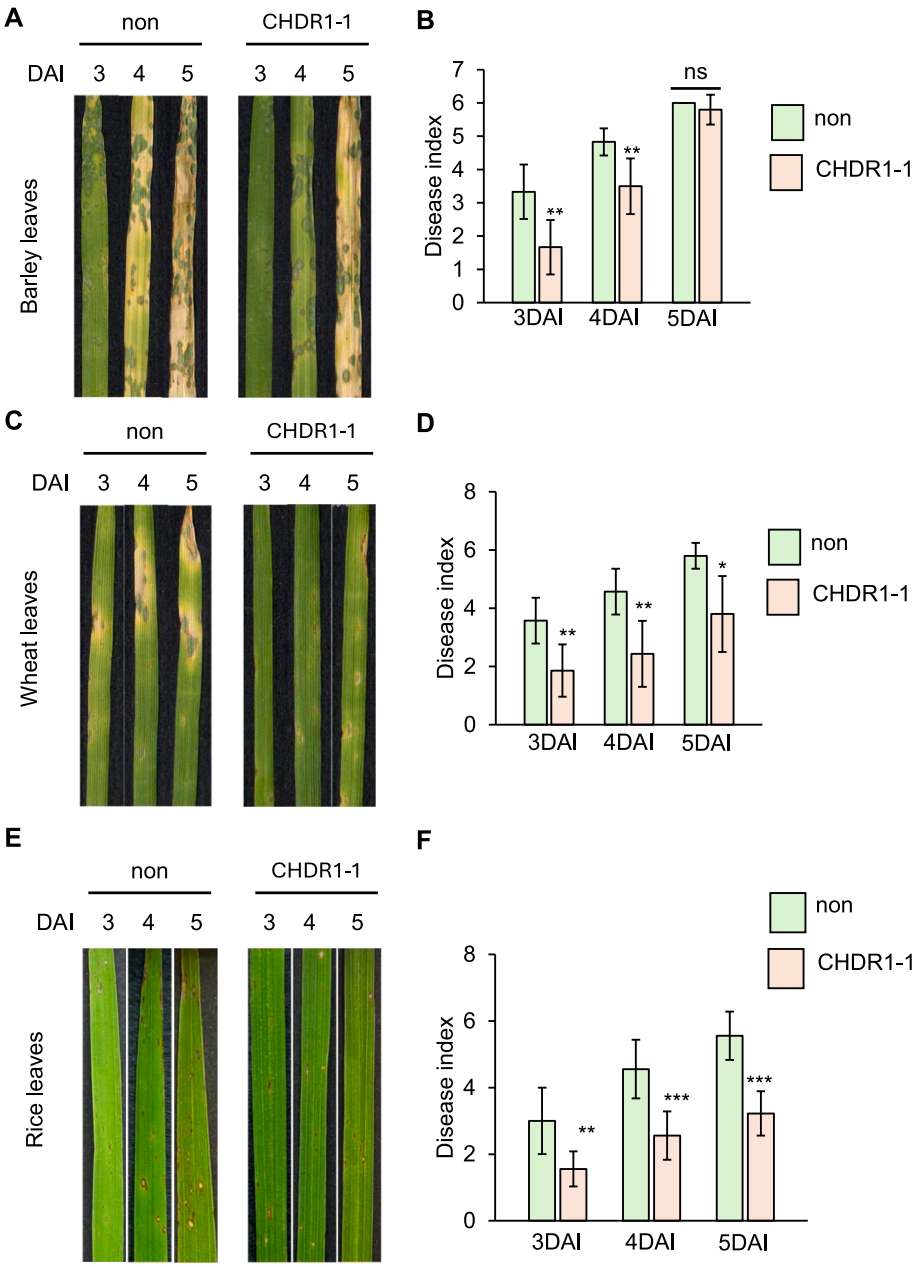

Fig. 5. Inoculation tests of *Pyricularia* isolates on barley, wheat, and rice leaves treatment with or without CHDR1-1. Disease symptoms on the barley (A and B), wheat (C and D), and rice (E and F) leaves spray-inoculated with *Triticum* pathotype Br48 (on barley and wheat) or *Oryza* pathotype Guy11 (on rice). The bars represent standard deviations calculated from five plants. The asterisks indicate significant differences between the treatments using *t*-tests (ns; not significantly different, *; $P < 0.05$, **; $P < 0.01$, ***; $P < 0.001$).

fertilizer-supplemented conditions using barley and wheat. No differences in shoot growth were observed between CHDR1-1-treated and untreated plants in the early stages (up to 14 DAG); however, differences became apparent after 21 DAG (Fig. S5A–D). Under these conditions, the leaves remained healthy and green at 21 DAG, even without the CHDR1-1 treatment (Fig. S5E and F). Rice was also cultivated using potting soil containing nutrients to raise seedlings. No differences in shoot growth were observed between the CHDR1-1-treated and untreated plants (Fig. S6).

3.4. Effect of CHDR1-1 on lesion formation caused by P. oryzae

We evaluated the effects of CHDR1-1 on lesion formation caused by *P. oryzae* and *P. grisea* in barley. Barley is a universal host for various *Pyricularia* pathotypes, including *Oryza*, *Setaria*, and *Triticum* pathotypes in *P. oryzae* and the *Digitaria* pathotype in *P. grisea*, which causes brown necrotic lesions on leaves (Xiang et al., 2022). The lesion areas on CHDR1-1-treated plants were significantly smaller in the drop inoculation experiments using barley leaves (14 DAG) than those on the untreated plants for all pathotypes tested (Fig. 4).

We conducted spray inoculation tests on barley and wheat with *P. oryzae* Triticum pathotype Br48 to investigate the role of CHDR1-1 in induced systemic resistance (ISR) in more detail. The disease index was lower in barley and wheat treated with CHDR1-1 than in the untreated plants (Fig. 5A–D). However, the effects of ISR on barley diminished 5 DAI (Fig. 5A and B). We also performed inoculation experiments using rice and *Oryza* pathotype Guy11 under flooding conditions using potting soil. Despite the low colonization rates in rice (Fig. 2C), ISR against *P. oryzae* infection was observed in CHDR1-1-treated rice (Fig. 5E and F). Furthermore, we assessed ISR induced by CHDR1-1 under fertilized

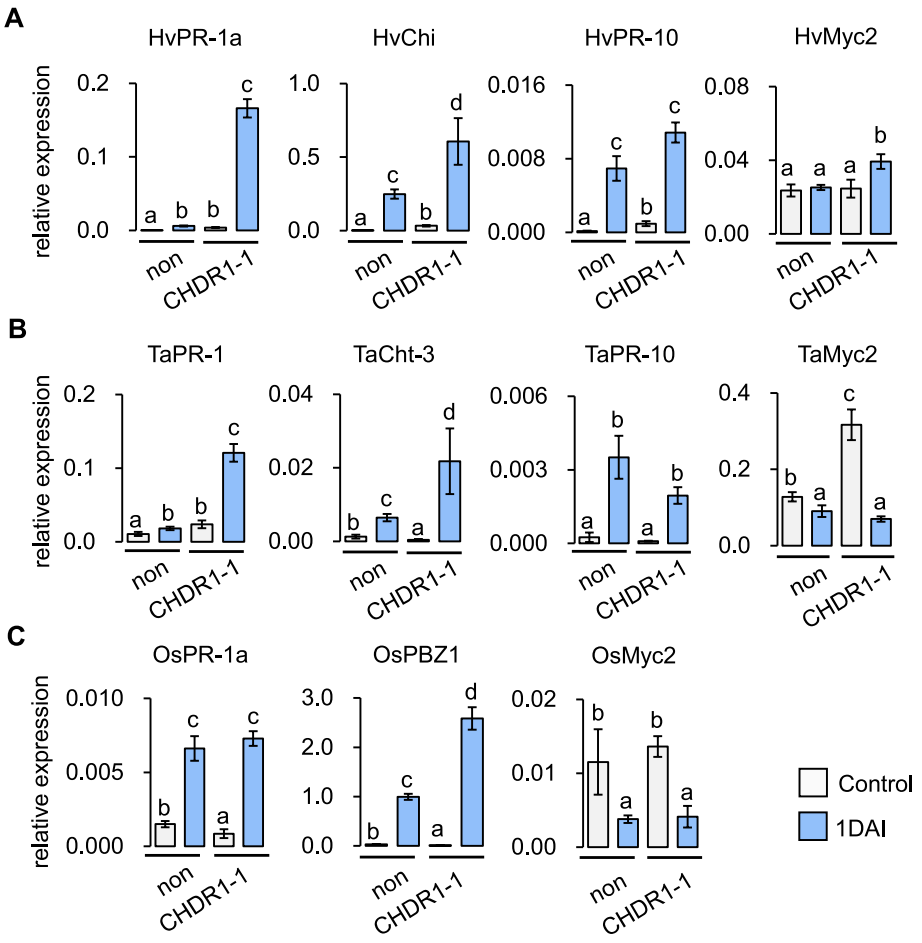

Fig. 6. Relative expressions of pathogenesis-related and Myc genes in the leaves of barley, wheat, and rice inoculated with *Pyricularia* isolates. Expression patterns of PR and Myc genes in the leaves of barley (A), wheat (B), and rice (C) determined using RT-qPCR. The housekeeping genes *TaCDCP* (EU267938.1), *HvActin* (AY145451.1), and *OsActin* (Os11g0163100), respectively, were used as the internal standards. The bars represent standard deviations calculated from at least three biological replications. The different alphabets indicate significant differences between treatments using Tukey–Kramer tests ($P < 0.05$).

conditions. The results indicated that the ISR levels under fertilized conditions were similar to those under non-fertilized conditions in both barley and wheat (Fig. S7).

The gene expression analysis indicated that the SA-dependent pathogenesis-related genes (PR genes) were induced after *P. oryzae* inoculation in all inoculation experiments (Fig. 6). In particular, the expression of PR-1 (*HvPR-1a* and *TaPR-1*), chitinase (*HvChi* and *TaCht-3*), and *OsPR-10* (*PBZ1*) was significantly upregulated in the leaves inoculated with CHDR1-1 (Fig. 6). PR-1 encodes a secreted protein that is frequently used as a marker of systemically acquired resistance (SAR) (van Loon et al., 2006). Chitinases contribute to the defense by directly degrading fungal cell walls (Hou et al., 2014). *OsPR-10* (*PBZ1*) is highly responsive to pathogen challenges in rice and is associated with localized cell death during hypersensitivity responses (Kim et al., 2004; Hagiwara et al., 2020). Notably, the PR-10 (*HvPR-10* and *TaPR-10*) gene expression patterns were inconsistent between barley and wheat (Fig. 6A and B). The JA-dependent genes *OsMyc2* and *TaMyc2* in rice and wheat (Liu et al., 2016; Ogawa et al., 2017) were down-regulated upon pathogen inoculation (Fig. 6B and C). Furthermore, the expression level of *TaMyc2* was highest in the CHDR1-1 treatment without pathogen challenge (Fig. 6B).

Although the expression of *OsPR-10 (PBZ1)* in the CHDR1-1 treatment in rice was significantly higher than that in non-treated plants, no difference was observed in the expression of *OsPR-1* between plants with and without CHDR1-1 treatment (Fig. 6C).

The direct antifungal effects of CHDR1-1 were investigated using confrontation culture experiments. The results showed that CHDR1-1 inhibited the growth of *Pyricularia* isolates on CM medium (Fig. S8), suggesting that CHDR1-1 produces some antifungal substance.

4. Discussion

In this study, we demonstrated that *C. humicola* strain CHDR1-1 positively affects plants. CHDR1-1 exhibited PGP effects and helped maintain host plant health in upland crops, such as barley and wheat, especially under nutrient-deficient conditions. In addition, CHDR1-1 has been shown to trigger ISR in wheat, barley, and rice.

PGPF colonize diverse plant sites, including root cells, intercellular spaces of roots, and the rhizosphere (Hossain and Sultana, 2020). *C. humicola* was ubiquitously detected in crabgrass roots. While members of the Chaetomiaceae family are widely distributed in soil environments as saprobes, they can colonize roots and function as endophytes. For example, *C. globosum*, another member of the family Chaetomiaceae, functions as an endophyte that enhances plant growth and disease resistance (Zhai et al., 2018; Elshahawy and Khattab, 2022).

The ability of *C. humicola* to colonize roots varies depending on the genus of the gramineous plant. The poor colonization of rice roots compared to that of barley and wheat may be due to the structure of the roots, which develop aerechyma to prepare for flooding. Flooding or waterlogging, as extreme high-water environments, significantly alter the composition and diversity of rhizosphere microorganisms (Martínez-Arias et al., 2022; García et al., 2024).

PGPFs promote plant growth through multiple mechanisms, including nutrient mineralization and fungal phytohormone production

(Hossain and Sultana, 2020; Rana et al., 2020). Our results revealed that CHDR1-1 exhibited PGP effects on barley, wheat, and rice, and these effects were more pronounced without supplemental fertilization. However, no such effects were observed in the original crabgrass hosts. Because crabgrass seedlings are smaller than the other plants tested, fungal colonization of the roots may negatively affect their growth. *Mortierella* sp. are generally considered PGPFs that promote the growth of various plants; however, certain species such as *Mortierella* sp. MXBP304 inhibits crabgrass growth (Ozimek and Hanaka, 2020; Du et al., 2024).

In the present study, colonization by CHDR1-1 helped maintain green leaves in host plants, even under limited fertilizer conditions. Nitrogen deficiency typically causes leaves to turn pale or yellow, reducing photosynthetic capacity and adversely affecting crop growth and yield (Zhao et al., 2005; Luo et al., 2018). These results suggested that CHDR1-1 enhanced the nutrient uptake efficiency of plant roots. Similar effects have been reported in *Acrophialophora jodhpurensis* (Chaetomiaceae family), *C. globosum, Phoma* sp., and *Trichoderma* spp. (Shivanna et al., 2005; Tarafdar and Gharu, 2006; Zhang et al., 2019; Daroodi et al., 2022; Ma et al., 2023). Moreover, *Alternaria alternata* and *Fusarium petersiae* have been shown to increase the nitrogen content of wheat plants significantly (Qiang et al., 2019; Mohamed et al., 2022).

In addition to PGP activity, the pretreatment of roots with CHDR1-1 triggers ISR in the leaves of barley, rice, and wheat plants. This effect did not always coincide with the effects of PGP and was not affected by the presence or absence of fertilizer, suggesting that ISR may be activated by a PGP-independent mechanism. ISR is induced by beneficial microorganisms in the roots, possibly through jasmonic acid (JA) and ethylene (ET) signaling pathways (Pieterse et al., 1998). In contrast, systemic SAR is triggered by pathogens that activate the salicylic acid (SA) signaling pathway (Ryals et al., 1996). Although the ISR and SAR are thought to have distinct regulatory mechanisms, some genes overlap (Cecchini et al., 2015; Cecchini et al., 2019; Vlot et al., 2021).

When CHDR1-1 colonized plant roots, brown lesions appeared, and the expression of various PR genes, increased, suggesting that both SA- and JA/ET-dependent signaling pathways were activated (Cameron et al., 1999; Heil and Bostock, 2002; Pangesti et al., 2013). In contrast, the expression of most PR genes in CHDR1-1-treated leaves was suppressed to low levels prior to pathogen inoculation. After inoculation with *P. oryzae*, the expression levels of PR genes, such as *TaPR-1, TaCht-3, HvPR-1a, HvPR-10, HvChi*, and *OsPR-10 (PBZ1)* were higher in CHDR1-1-treated plants than in non-treated controls. In wheat plants that exhibited high PGP and resistance, the JA-responsive gene *TaMyc2* was induced in CHDR1-1-treated leaves but suppressed after inoculation with *P. oryzae*. These changes in gene expression patterns suggested that CDHR1-1-treated leaves were in a 'priming' state due to ISR (Vlot et al., 2021; Yang et al., 2022). Although CHDR1-1 showed antifungal activity *in vitro*, its protective effect in plants is likely due to systemic signaling initiated in the roots rather than direct inhibition at the infection site.

CHDR1-1 colonized the barley roots most efficiently and exhibited PGP effects while inducing low levels of ISR. This is likely because of the low basal resistance of barley to *P. oryzae*, which is recognized as a common host of this fungus (Hyon et al., 2012). In addition, the intensity of defense responses and CHDR1-1 colonization varied among plant species, which may reflect differences in root architecture or immune sensitivity.

In conclusion, we isolated and characterized the root endophyte *C. humicola* CHDR1-1 from crabgrass. CHDR1-1 consistently colonized the roots of gramineous crops and conferred multiple beneficial effects, including enhanced shoot growth, improved leaf health under nutrient-deficient conditions, and increased resistance to *P. oryzae*. Gene expression analysis indicated that CHDR1-1 exerted a priming effect, and strong PR gene expression was induced following infection with *P. oryzae*. These results demonstrated the potential of CHDR1-1 as a multifunctional bioinoculant for sustainable agriculture.

CRediT authorship contribution statement

Zikai Xiang: Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hitoshi Nakayashiki:** Writing – review & editing, Validation, Data curation, Conceptualization. **Kenichi Ikeda:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2025.105810.

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