# Bioherbicidal activity and host range of *Teratoramularia rumicicola* strain TR4 isolated from *Rumex crispus* in Japan

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**Abstract:**

*Rumex* species, including *R. crispus*, *R. obtusifolius*, and *R. japonicus*, are problematic weeds that reduce forage production and hinder effective land use in Japanese pasture systems. This study aimed to identify a bioherbicide candidate that selectively targets *Rumex* weeds while remaining safe for forage crops. To this end, we isolated a fungal pathogen from a naturally infected *R. crispus* leaf collected in Japan and conducted morphological and molecular identification and host range evaluations. The isolate, designated TR4, was identified as *Teratoramularia rumicicola* based on its morphological characteristics and phylogenetic analysis of concatenated internal transcribed spacer (ITS) and large subunit (LSU) rDNA sequences. Host range studies revealed that TR4 caused visible symptoms and significantly reduced shoot biomass in all three *Rumex* species, while no disease symptoms were observed in any of the five forage crops tested. This study reports *T. rumicicola* for the first time in Japan, extending its known distribution beyond South Korea. The host specificity and liquid culture compatibility of TR4 suggest its potential as a locally adapted microbial herbicide for selective *Rumex* control in pasture systems.

**Keywords:** *Teratoramularia rumicicola*, *Rumex*, bioherbicide, pasture systems, host range

## Introduction

Herbicides have long been used to enable efficient weed control in modern agriculture. However, the repeated use of herbicides has led to the widespread emergence of herbicide-resistant weed biotypes (Heap 2025). Moreover, the discovery of herbicides with novel modes of action has slowed significantly in recent decades (Umetsu and Shirai 2020), increasing the urgency to develop alternative weed management strategies. Integrated Weed Management, which incorporates chemical, mechanical, cultural, and biological approaches, is gaining prominence as a sustainable approach to weed control (Westwood et al. 2018), as it reduces the selection pressure associated with sole dependence on chemical herbicides.

Among the various components of Integrated Weed Management, microbial herbicides, which are applied in large quantities to achieve rapid weed suppression via an inundative approach, have gained renewed attention as alternatives to synthetic herbicides (Duke 2024). They offer distinct advantages, including high crop safety, low toxicity to mammals and nontarget organisms, limited environmental persistence, relatively low development and registration costs, and no reported cases of resistance (Duke et al. 2022). However, their broader adoption has been constrained by challenges such as inconsistent efficacy under diverse field conditions, the need for low-cost mass production, development of formulations that enhance shelf life and field performance, and assurance of biosafety throughout the product lifecycle (Gressel 2024).

*Rumex* species are potential targets for the development of microbial herbicides, particularly in temperate forage systems. *R. obtusifolius* L. and *R. crispus* L. are among the most problematic broadleaf weeds in temperate pasturelands and

arable fields, owing to their perennial growth and ability to compete with forage crops and reduce productivity (Zaller 2004). Notably, *R. obtusifolius* has evolved resistance to acetolactate synthase-inhibiting herbicides, such as florasulam, metsulfuron-methyl, and thifensulfuron-methyl, in France (Heap 2025). In parallel with chemical control efforts, several fungal pathogens, including *Uromyces rumicis*, *Ramularia rubella*, *Venturia rumicis*, and *Armillaria mellea*, have been investigated as biological control agents for *Rumex* species, although their practical use remains limited owing to insufficient efficacy and biosafety concerns (Bond et al. 2007). These limitations highlight the need to identify novel microbial candidates with greater potential for practical applications.

In this study, we focused on Japan, where *R. obtusifolius*, *R. crispus*, and *R. japonicus* Houtt. are widely distributed in pastures and disturbed habitats. While the former two are introduced species and *R. japonicus* is native, all three are recognized as invasive weeds and are listed as "alien species of concern" by the Ministry of the Environment (Ministry of the Environment, Japan 2015). In addition, *R. obtusifolius* and *R. japonicus* are designated as “prohibited weed species” by the Japan Grassland Agriculture and Forage Seed Association because of their significant impact on forage production, and their contamination is not tolerated in seed certification (Japan Grassland Agriculture and Forage Seed Association 2019). These facts underscore the serious weed problems posed by *Rumex* species in Japan; however, the available chemical herbicides for their control remain limited, such as thifensulfuron-methyl. Considering the global development of herbicide resistance in *Rumex* species, alternative control measures, such as microbial herbicides, are urgently needed. However, to our knowledge, no studies have been published on

84 microbial herbicides targeting *Rumex* weeds in Japan.

85 To address this gap, we aimed to isolate and identify a fungal pathogen with
86 bioherbicidal activity against *Rumex* species of relevance to forage systems in Japan.
87 A candidate strain was obtained from a naturally infected *R. crispus* leaf.
88 Morphological and molecular analyses were conducted to determine its taxonomic
89 identity, and host range tests were performed to evaluate its potential as a selective
90 microbial herbicide.

91

92 **Materials and Methods**

93 **Fungal Isolation**

94 A symptomatic leaf of *Rumex crispus* was collected from a roadside plant in Tsukuba
95 City, Ibaraki Prefecture, Japan on 17$^{th}$ November 2024. The leaf was detached and
96 incubated at 25 °C under moist and light conditions for 12 days until conidial
97 structures emerged from the lesions. Emerging conidia were collected and streaked
98 onto potato dextrose agar (PDA, BD Difco$^{TM}$/BBL$^{TM}$) amended with lactic acid for
99 five days for single-conidium isolation. The plates were incubated at 25 °C for fungal
100 growth. One isolate, designated as TR4, was selected as the representative isolate for
101 subsequent experiments. The isolate TR4 was deposited as MAFF 248116 in the
102 NARO Genebank (National Agriculture and Food Research Organization, Japan).

103

104 **Morphological Observations**

105 TR4 was transferred to the center of PDA plates (90 mm in diameter) with a small
106 piece of mycelium and incubated at 25 °C in the dark. After one month, colony
107 characteristics, including growth rate, surface texture, pigmentation on both the

surface and reverse sides, and margin shape were recorded. Conidial suspensions were prepared by flooding the colony surface with sterile distilled water, and mounted on sterile distilled water. Conidial length and septation were observed using a light microscope (BH-2, Olympus, Japan). Morphological characteristics were compared with reference to the published descriptions of *Teratoramularia rumicicola* S.I.R. Videira, H.D. Shin & P.W. Crous and *Teratoramularia rumicis* Kushwaha, S.K. Verma, S. Yadav & Raghv. Singh, known foliar pathogens of *Rumex* species (Videira et al. 2016; Verma et al. 2021).

**DNA Extraction**

TR4 was cultured on PDA plates at 25°C in the dark for six weeks. The mycelia were harvested and immediately frozen in liquid nitrogen. The frozen material was pulverized using a bead homogenizer (MB456KU(S), Yasui Kikai, Japan) at 2000 rpm for 30 seconds. Genomic DNA was extracted using the NucleoSpin Soil Kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. DNA quality and concentration were assessed using a NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA).

**PCR Amplification and Sequencing**

PCR amplification was performed for two genomic regions: the internal transcribed spacer (ITS) region using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990), and the large subunit ribosomal RNA (LSU) region using primers LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR7 (5'-TACTACCACCAAGATCT-3') (Vilgalys and Hester 1990). These

132 primer sets were selected with reference to Verma et al. (2021) and Choi et al. (2025),
133 who employed ITS and LSU sequences to discriminate *T. rumicicola* and *T. rumicis*.
134 Each 10 μL PCR reaction contained 5.0 μL of 2× PCR buffer, 2.0 μL of 2
135 mM dNTPs, 0.3 μL of each primer (10 μM), 0.2 μL of KOD FX Neo DNA polymerase
136 (Toyobo, Japan), 1.2 μL of sterile distilled water, and 1 μL of the genomic DNA. The
137 thermal cycling conditions were as follows: initial denaturation at 94°C for 2
138 minutes; 35 cycles of 98°C for 10 seconds, 58°C for 15 seconds, and 68°C for 30
139 seconds; and a final extension at 68°C for 5 minutes. Successful amplification was
140 confirmed by electrophoresis on a 1.5% agarose gel stained with SYBR Green (Takara
141 Bio, Japan).
142 The PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific,
143 USA) and submitted to Eurofins Genomics (Tokyo, Japan) for Sanger sequencing.
144 Chromatograms were manually assembled and edited using CodonCode Aligner
145 version 12.0.1. The resulting consensus sequences were analyzed using the BLASTn
146 algorithm on the NCBI website to determine their closest taxonomic affiliation. The
147 sequences were deposited in GenBank under accessions PX069134 (ITS) and
148 PX069135 (LSU).
149
150 **Phylogenetic Analysis**
151 ITS and LSU sequences obtained in this study were concatenated and included in the
152 phylogenetic analysis. Reference sequences of *T. rumicicola*, *T. rumicis*, *T. infinita*,
153 *T. kirschneriana*, and *T. persicariae* were retrieved from GenBank. The reference
154 sequences and their GenBank accessions are listed in Supplementary Table 1.
155 Multiple sequence alignment was conducted using MUSCLE in MEGA version 11

156 (Tamura, Stecher, and Kumar 2021), and ambiguously aligned and highly variable
157 terminal regions were manually trimmed. The final concatenated alignment of ITS
158 and LSU sequences consisted of 1,191 bp.

159 Maximum likelihood analysis was performed using RAxML v8.2.4 under the
160 GTR+GAMMA model with 1000 rapid bootstrap replicates (Stamatakis 2014). The
161 tree was rooted with *Staninwardia suttonii* CPC 13055 and visualized using
162 Interactive Tree of Life (Letunic and Bork 2024). Bootstrap values ≥70% were
163 displayed.

164

165 **Plant Materials and Growth Conditions**

166 Seeds of the weed species were obtained by purchase (*R. crispus* and *R. obtusifolius*,
167 Espec Mic Co., Ltd., Japan) or by field collection in Kyoto City, Kyoto Prefecture
168 (*R. japonicus*). Seeds of the forage species were obtained from commercial suppliers:
169 *Dactylis glomerata* and *Lolium multiflorum* from Snow Brand Seed Co., Ltd. (Japan),
170 *Phleum pratense* and *Trifolium repens* from Takii & Co., Ltd. (Japan), and *Medicago*
171 *sativa* from Kaneko Seeds Co., Ltd. (Japan).

172 All seeds were pre-germinated on moistened filter paper in Petri dishes at
173 25°C/15°C under a 12 h light/12 h dark photoperiod in a growth chamber. After
174 several days, the germinated seedlings were transplanted into plastic pots (φ = 5 cm)
175 filled with a 1:1 (v/v) mixture of vermiculite and akadama soil (Japanese granular
176 clay soil) and grown under the same conditions. One seedling was grown per pot and
177 watered as needed. Inoculations were performed at the 1–2 leaf stage, approximately
178 2 weeks after sowing for forage species and 3 weeks for weed species.

179

180 **Inoculum Preparation and Application Conditions**

181 For preculture, a 6-mm-diameter agar disc of isolate TR4 grown on PDA was excised
182 and inoculated into 50 mL of 0.5% YM liquid medium, consisting of 0.5% yeast
183 extract and 0.5% malt extract (both from Solabia Biokar Diagnostics, France), and
184 0.5% D-(+)-glucose (Nacalai Tesque, Japan), in a 200 mL Erlenmeyer flask. The
185 culture was incubated at 25 °C for 3-4 days with rotary shaking at 130 rpm using a
186 rotary shaker (NR-20, TAITEC Corporation, Japan). Subsequently, 1.5 mL of the
187 preculture was transferred into 150 mL of 0.5% YM liquid medium in a 500 mL
188 Sakaguchi flask. The culture was incubated at 25 °C for 7 days with reciprocal
189 shaking at 130 rpm in a shaking water bath incubator (BT200, Yamato Scientific Co.,
190 Ltd., Japan).

191 The culture broth was filtered through two layers of cotton gauze (High
192 Gauze White, Iwatsuki Co., Ltd., Japan) to remove the large mycelial fragments. The
193 resulting suspension was adjusted with YM liquid medium to a final concentration of
194 $3.0 \times 10^5$ propagules/mL for weed species and $1.0 \times 10^6$ propagules/mL for forage
195 species using a hemocytometer. The term "propagules" refers to both conidia and
196 hyphal fragments, although their proportions were not determined in this study. The
197 suspension was applied to *R. crispus*, *R. japonicus*, *R. obtusifolius*, *D. glomerata*, *L.*
198 *multiflorum*, *P. pratense*, *M. sativa*, and *T. repens* at the 1-2 leaf stage using a hand-
199 held sprayer (Daiya Spray, Furupla Co., Ltd., Japan) at an application volume
200 equivalent to 4000 L/ha. YM liquid medium was used as a non-treated control.
201 Following treatment, plants were enclosed in moistened polyethylene bags at 25°C
202 in the dark for 48 hours. They were transferred to growth conditions at 25 °C under
203 a 12 h light/12 h dark photoperiod, while remaining enclosed in moistened

204 polyethylene bags.

205

206 **Biological Evaluation**

207 Leaf-level disease incidence was assessed in all test species 14 days after treatment
208 (DAT) and again at 21 DAT for *Rumex* species. Leaf-level disease incidence was
209 calculated as the number of leaves exhibiting visible symptoms divided by the total
210 number of leaves. Leaves were considered symptomatic when white conidiomata,
211 characteristic of *T. rumicicola* (Choi et al. 2025), was observed on the surface.
212 Cotyledons that withered naturally without visible fungal colonization, which were
213 observed in control plants, were excluded from both the symptom count and total leaf
214 number to ensure that only pathogen-induced symptoms were recorded. Surface
215 symptoms on infected *Rumex* leaves were documented using a digital microscope
216 (VHX-970F, KEYENCE, Japan). In addition, the aboveground parts of *Rumex* species
217 were harvested at 21 DAT and immediately weighed to determine shoot fresh weight.
218 To confirm pathogen re-isolation as part of Koch's postulates, conidia were collected
219 from symptomatic *Rumex* leaves at 21 DAT using a sterile platinum loop and re-
220 inoculated onto PDA plates. After several days of incubation at 25 °C, colony and
221 conidial morphologies were examined and compared with those of the original isolate.

222

223 **Statistical Analysis**

224 All bioassay results are presented as the mean ± standard error from at least three
225 biological replicates. Statistical analyses were performed using the Mann–Whitney
226 U test for leaf-level disease incidence, and Student's *t*-test for shoot biomass after
227 confirming homogeneity of variances by an F-test in R (version 4.5.0). Each bioassay

228 was independently repeated at least twice to ensure its reproducibility.

229

230 **Results and Discussion**

231 **Isolation of the Fungal Pathogen**

232 A symptomatic leaf of *Rumex crispus* was collected from a roadside plant in Tsukuba,
233 Japan. After incubation under moist conditions at 25 °C for 12 days, lesions with dark
234 purple to brown margins and white fungal growth were observed on the leaf surface
235 (Figure 1a, b). Microscopic observation revealed the presence of twiggy
236 conidiophores and cylindrical conidia (Figure 1c). Conidia were transferred to lactic
237 acid-amended PDA for isolation, and one isolate, designated as TR4, was selected for
238 further study.

239

240 **Morphological Characterization**

241 Colonies on PDA incubated at 25 °C in the dark grew very slowly, reaching
242 approximately 10 mm in diameter after one month (Figure 2). They were subcircular
243 with moderately elevated, uneven surfaces and coarse, wrinkled textures. The surface
244 appeared dark, with white zones at the center and margin. The surrounding medium
245 was faintly stained red due to pigment diffusion, particularly toward the colony center.
246 On the reverse, colonies showed a dark center and pale edge. Microscopic observation
247 revealed hyaline, cylindrical conidia, mostly aseptate with occasional 1-septate forms,
248 measuring 5.5–21.0 × 1.7–2.9 μm ($n$ = 30), with slightly rounded ends (Figure 3).
249 These features closely resembled those of the ex-type strain CPC 14653 of
250 *Teratoramularia rumicicola* (Videira et al. 2016). According to Verma et al. (2021),
251 *T. rumicis*, the species most closely related to *T. rumicicola*, produces longer conidia

252 with a higher frequency of septation (0–2 septa) than *T. rumicicola*. This
253 morphological distinction supports the identification of TR4 as *T. rumicicola*.
254
255 **BLAST and Phylogenetic Analysis**
256 BLASTn analysis of the ITS sequence of TR4 showed 100% identity to three *T.*
257 *rumicicola* accessions (OL711641, PP301328, and PP301329), as well as to the ex-
258 type strain of *T. rumicis* (NR_174642). The ex-type strain of *T. rumicicola*
259 (NR_154517) also showed a very high similarity, with 99.58% identity (469/471 bp).
260 These results indicate that the ITS region alone lacks sufficient resolution to
261 distinguish *T. rumicicola* from *T. rumicis*, highlighting the importance of additional
262 markers for accurate species identification.
263 The LSU sequence of TR4 showed 98.6% identity (697/707 bp) to the ex-
264 type strain of *T. rumicis* (MW276098), whereas 100% identity to the ex-type strain
265 and two other *T. rumicicola* accessions (KX287254, KX287255, and KX287256). Two
266 additional *T. rumicicola* accessions (PP301336 and PP301337) exhibited 99.86%
267 identity (722/723 bp). These results demonstrate that LSU provides a higher
268 resolution than ITS for distinguishing *T. rumicicola* from *T. rumicis* and supports the
269 assignment of TR4 to *T. rumicicola*.
270 A phylogenetic tree was constructed based on concatenated ITS and LSU
271 sequences using the maximum-likelihood method. The resulting tree resolved four
272 well-supported clades corresponding to *T. kirschneriana*, *T. infinita*, *T. persicariae*,
273 and a combined clade containing both *T. rumicis* and *T. rumicicola*. TR4 clustered in
274 the same clade as the ex-type strains of both *T. rumicicola* and *T. rumicis*, along with
275 other *T. rumicicola* reference strains, with a bootstrap support of 100%. Although

both type strains were included within the same clade, the *T. rumicis* ex-type strain was positioned on a distinctly long branch, suggesting substantial divergence from TR4 and the *T. rumicicola* group. Previous studies by Verma et al. (2021) and Choi et al. (2025) reported *T. rumicicola* and *T. rumicis* as forming separate clades based on ITS–LSU concatenated analyses. The observed differences between our results and those of previous studies may, in part, be due to methodological choices, including alignment, trimming, or tree-building protocols. Nonetheless, the close phylogenetic proximity to the *T. rumicicola* group, distinct from the more divergent *T. rumicis* branch, is consistent with the identification of TR4 as *T. rumicicola*.

**Taxonomic Assignment of TR4**

The colony characteristics and conidial morphology of TR4 closely resembled those of the *T. rumicicola* ex-type strain (CPC 14653). Molecular analyses further reinforced this identification, as TR4 exhibited complete identity in the LSU region and high similarity in the ITS region to the *T. rumicicola* ex-type strain, with phylogenetic placement among *T. rumicicola* reference strains. In addition to morphological and sequence-based evidence, the known geographic distribution supports this assignment. *T. rumicicola* has been isolated from multiple sites in South Korea, whereas *T. rumicis* is represented by a single isolate from India (Videira et al. 2016; Verma et al. 2021; Choi et al. 2025). Therefore, the Japanese origin of TR4 aligns more closely with that of *T. rumicicola*. Currently, only ITS and LSU sequences are available in GenBank for *T. rumicis*, and no additional gene regions (e.g., RPB2 and TEF1) have been reported. This lack of multi-locus data limits the potential for more robust species delimitations. Nonetheless, the integration of

300 morphological, molecular, phylogenetic, and biogeographical evidence strongly
301 supports the identification of TR4 as *T. rumicicola*.
302
303 **Host Range and Specificity**
304 The leaf-level disease incidence at 14 DAT was 85.3 ± 6.5% in *R. obtusifolius*,
305 74.0 ± 7.3% in *R. crispus*, and 70.0 ± 4.2% in *R. japonicus*, all significantly higher
306 than that in the control ($p < 0.01$, Mann–Whitney U test; Table 1). These values
307 remained consistently high at 21 DAT. Infected leaves exhibited foliar necrosis and
308 abundant white fungal growth, which are characteristic of *T. rumicicola* infections
309 (Figure 5, S1, and S2). Shoot biomass at 21 DAT was significantly reduced in all
310 *Rumex* species tested. The biomass of *R. crispus* and *R. japonicus* declined to
311 40.7 ± 6.5% and 45.2 ± 6.7% of the control, respectively, whereas *R. obtusifolius*
312 retained a relatively higher biomass at 60.8 ± 7.8% ($p < 0.01$, Student's *t*-test; Table
313 1). These results indicate a moderate but consistent suppression of shoot growth
314 across species, with a slightly lower susceptibility in *R. obtusifolius*. Furthermore,
315 conidia collected from symptomatic *Rumex* leaves inoculated with TR4 produced
316 colonies and conidia that were morphologically identical to the original isolate. These
317 observations fulfill Koch's postulates and confirm the causal role of TR4 in symptom
318 development.
319 In contrast, the leaf-level disease incidence at 14 DAT was 0% in all five
320 tested forage species. The primary diagnostic symptom was not observed on any of
321 the leaf surfaces (Figure 6). These findings demonstrate that TR4 exhibits host
322 specificity against *Rumex* weeds without affecting forage species, highlighting its
323 potential as a selective bioherbicide in pasture systems.

**Significance and Implications of the Present Study**

This study documents the first occurrence of *T. rumicicola* in Japan, thus extending its known geographic range beyond South Korea. Since its establishment in 2016, the genus *Teratoramularia* has remained poorly studied and *T. rumicicola* has been reported in only two publications, both based on South Korean isolates (Videira et al. 2016; Choi et al. 2025). This newly reported Japanese isolate provides valuable taxonomic and biogeographical insights. The consistency between morphological and molecular features further strengthens the framework for species identification within this understudied genus.

In addition to its original host *R. crispus*, TR4 exhibited pathogenicity toward the invasive alien species *R. obtusifolius* and the native weed *R. japonicus*, all of which are considered major weeds in Japanese pasture systems. In contrast, no pathogenic effects were observed in the five tested forage species, including grasses and legumes. These results from the preliminary screening provide the first experimental evidence that *T. rumicicola* strain TR4 may possess a host range and specificity desirable for bioherbicidal use. However, further studies are needed to evaluate its weed control efficacy under field conditions and confirm its safety toward a broader range of forage and crop species.

Although not directly examined in this study, TR4's ability to grow in liquid culture suggests the potential for large-scale production. In contrast to obligate pathogens such as *Uromyces rumicis*, TR4 may offer a more practical platform for product development in terms of commercialization. Moreover, as a strain isolated locally in Japan, TR4 may exhibit greater ecological compatibility with the domestic

environment and could be more amenable to regulatory approval as a regionally adapted microbial control agent. Further investigation is needed to optimize mass-production systems and assess the natural distribution and ecological behavior of *T. rumicicola* in Japan.

Collectively, this study contributes to the taxonomic understanding of the little-studied genus *Teratoramularia* and the early stage evaluation of *T. rumicicola* strain TR4 as a candidate microbial herbicide. By documenting its pathogenicity and host specificity, particularly in relation to *Rumex* weeds and non-target forage species, this study establishes a foundation for future development and deployment in weed management in pasture systems.

## Conclusion

This study reports the first isolation of *Teratoramularia rumicicola* in Japan and demonstrates its pathogenicity toward *Rumex crispus*, *R. obtusifolius*, and *R. japonicus*. No pathogenic effects were observed in the tested forage crops. TR4's ability to grow in liquid culture suggests its potential for scalable production, laying the groundwork for future development as a regionally adapted microbial herbicide.

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## Author Contributions

M.I. conceived and led the study, performed most of the experiments, and wrote the manuscript. T.S. contributed to fungal isolation and provided expertise in inoculum preparation and fungal identification. All authors contributed to the critical revision of the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

## Disclosure Statement

The authors declare no conflicts of interest.

## Data Availability Statement

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

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**Figure Legends**

**Figure 1. Isolation of TR4 from a naturally infected leaf of *Rumex crispus*.**

(a) Symptomatic leaf after 12 days of incubation at 25°C under moist and light conditions. (b) Lesion surface observed under a stereomicroscope, with visible white conidiophores and conidia. (c) Light microscopy image of conidia collected from the lesion (×400), showing cylindrical morphology (phase contrast microscopy).

**Figure 2. Colony Morphology of Isolate TR4 on PDA.**

Colony appearance of TR4 grown on potato dextrose agar (PDA) after one month of incubation at 25 °C in the dark: (a) surface view; (b) reverse view. Scale bars = 10 mm

**Figure 3. Microscopic Morphology of TR4 Conidia.**

Microscopic morphology of TR4 conidia observed under a light microscope. The conidia were mostly aseptate with occasional 1-septate forms, cylindrical, and hyaline. Scale bars = 10 µm

**Figure 4. Phylogenetic Placement of Isolate TR4 Based on Concatenated ITS and LSU Sequences.**

Maximum likelihood tree constructed from concatenated ITS and LSU sequences using the GTR+GAMMA model. Bootstrap values ≥70% (1000 replicates) are shown at the nodes. TR4 is indicated in red font, and ex-type strains are indicated in bold font. Related *Teratoramularia* species were retrieved from GenBank. *Staninwardia suttonii* was used

468 as the outgroup.

469 **Figure 5. Pathogenicity of TR4 Against *Rumex* Species.**

470 Foliar appearance 21 days after inoculation. (a–c) Untreated control plants of *R. crispus*,
471 *R. japonicus*, and *R. obtusifolius*, respectively. (d–f) Corresponding TR4-inoculated
472 plants, showing visible white fungal growth and necrotic lesions. Scale bars = 10 mm

473 **Figure 6. Lack of Pathogenicity of TR4 against Forage Species.**

474 Foliar appearance 14 days after inoculation. (a) *Lolium multiflorum*, (b) *Dactylis*
475 *glomerata*, (c) *Phleum pratense*, (d) *Medicago sativa*, and (e) *Trifolium repens* following
476 inoculation with TR4. No symptoms were observed in any of the species. Scale bars =
477 10 mm

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479

480 **Table 1. Leaf-level Disease Incidence and Shoot Biomass of *Rumex* Weeds Treated**

481 **with TR4.**

482 Values represent the mean ± SE ($n$ = 4–5). Statistical differences from the control were

483 determined using the Mann–Whitney U test or Student's $t$-test ($p < 0.01$).

484

| | Leaf-level Disease Incidence (%) | | Shoot Biomass (% of control) |
|---|---|---|---|
| **Weeds** | **14 DAT** | **21 DAT** | **21 DAT** |
| *Rumex crispus* | 74.0 ± 7.3 †† | 72.0 ± 4.9 †† | 40.7 ± 6.5 ** |
| *Rumex japonicus* | 70.0 ± 4.2 †† | 69.7 ± 3.5 †† | 45.2 ± 6.7 ** |
| *Rumex obtusifolius* | 85.3 ± 6.5 †† | 83.0 ± 4.4 †† | 60.8 ± 7.8 ** |

††$p < 0.01$ vs. control; Mann–Whitney U test, $n$ = 4-5

**$p < 0.01$ vs. control; Student's $t$-test, $n$ = 4-5

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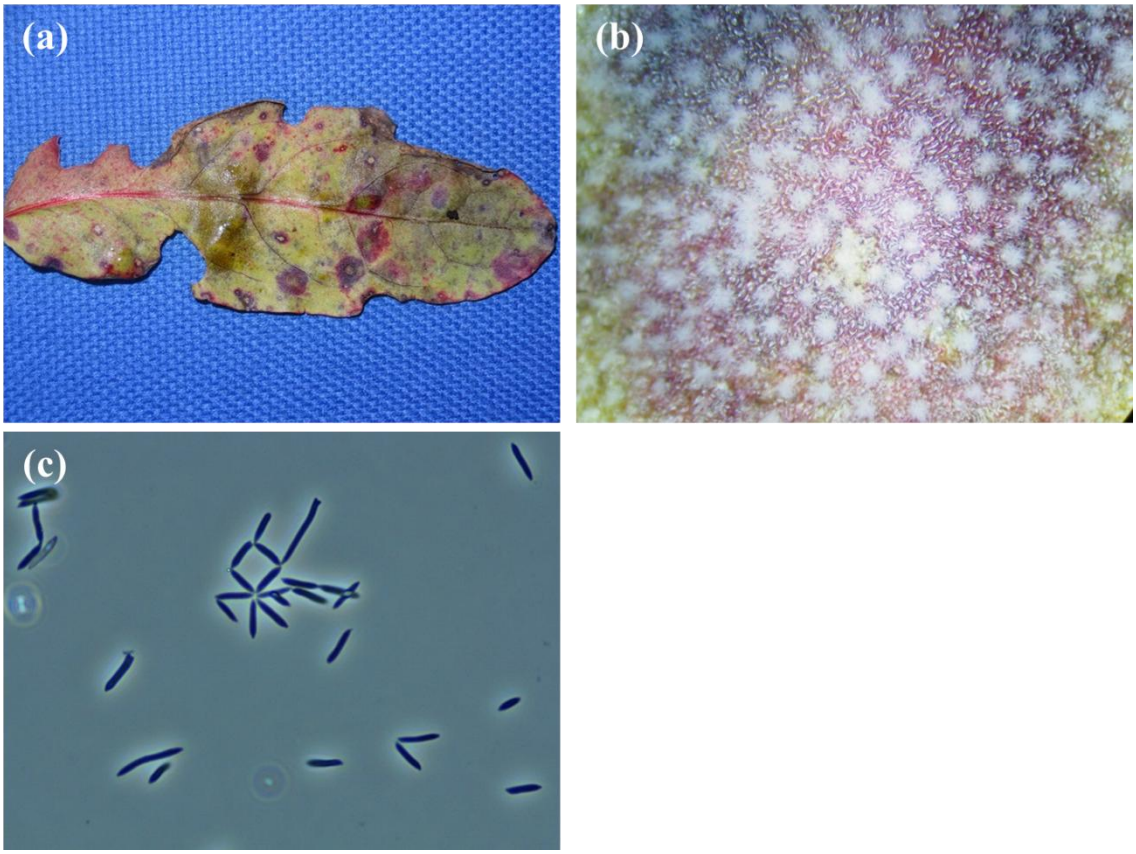


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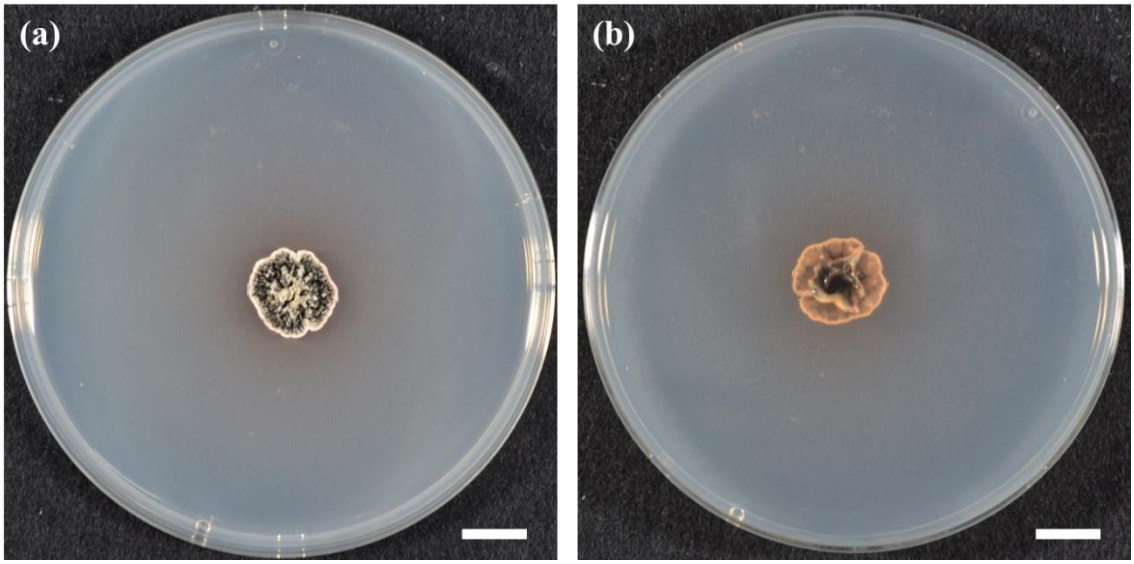
(a) Symptomatic leaf after 12 days of incubation at 25°C under moist and light conditions. (b) Lesion surface observed under a stereomicroscope, with visible white conidiophores and conidia. (c) Light microscopy image of conidia collected from the lesion (×400), showing cylindrical morphology (phase contrast microscopy).

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Colony appearance of TR4 grown on potato dextrose agar (PDA) after one month of incubation at 25 °C in the dark: (a) surface view; (b) reverse view. Scale bars = 10 mm

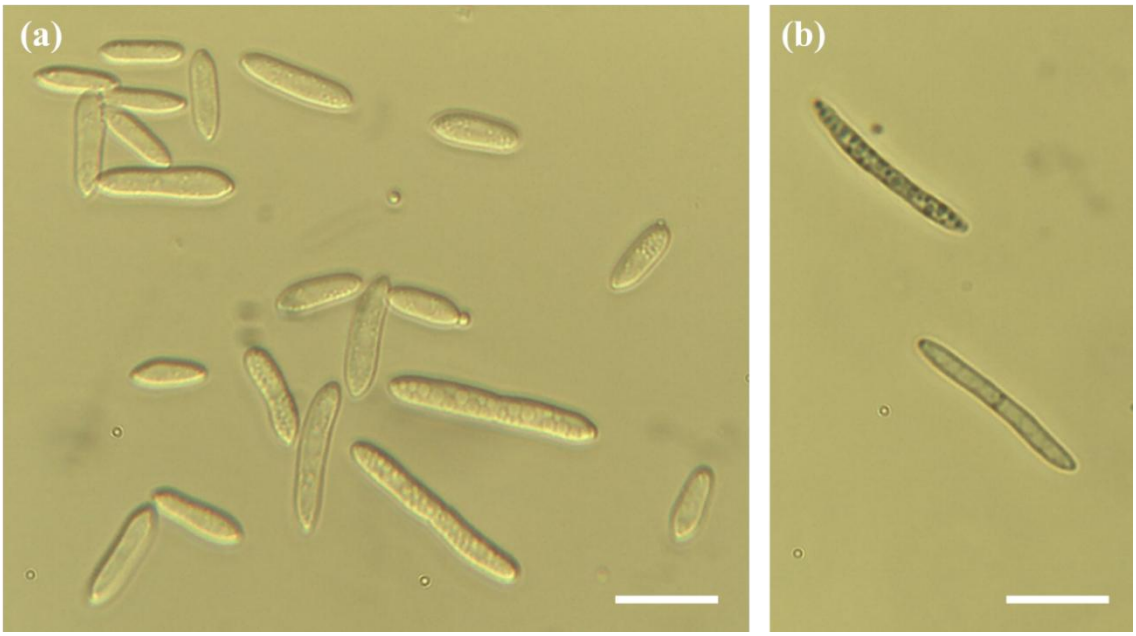



**Figure 3. Microscopic Morphology of TR4 Conidia.**

Microscopic morphology of TR4 conidia observed under a light microscope. The conidia were mostly aseptate with occasional 1-septate forms, cylindrical, and hyaline. Scale bars = 10 µm

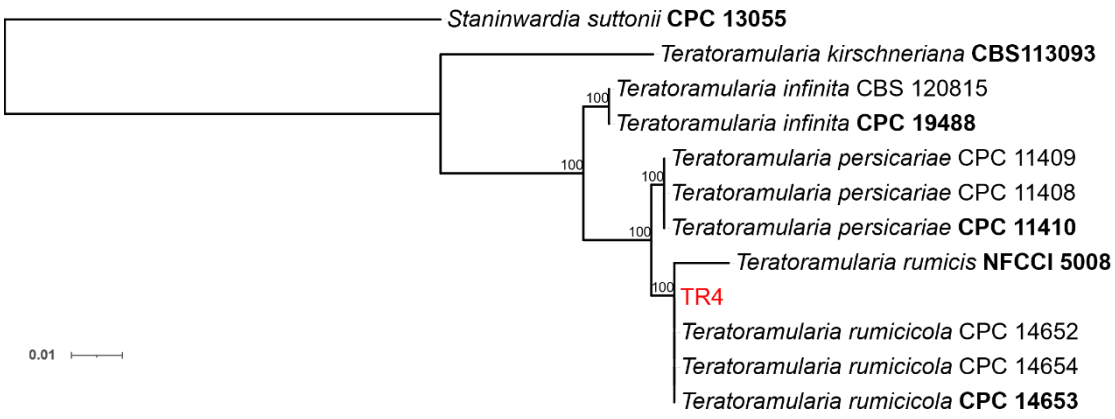



**Figure 4. Phylogenetic Placement of Isolate TR4 Based on Concatenated ITS and LSU Sequences.**

Maximum likelihood tree constructed from concatenated ITS and LSU sequences using the GTR+GAMMA model. Bootstrap values ≥70% (1000 replicates) are shown at the nodes. TR4 is indicated in red font, and ex-type strains are indicated in bold font. Related *Teratoramularia* species were retrieved from GenBank. *Staninwardia suttonii* was used as the outgroup.

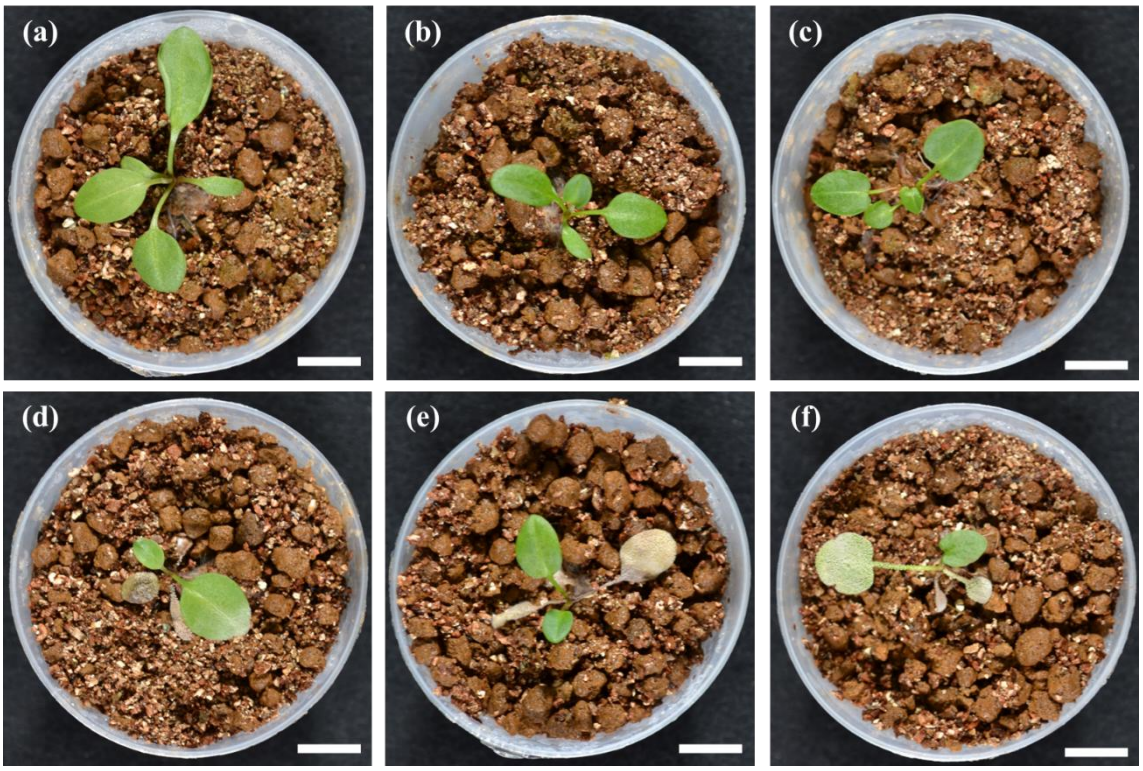



**Figure 5. Pathogenicity of TR4 Against *Rumex* Species.**

Foliar appearance 21 days after inoculation. (a–c) Untreated control plants of *R. crispus*, *R. japonicus*, and *R. obtusifolius*, respectively. (d–f) Corresponding TR4-inoculated plants, showing visible white fungal growth and necrotic lesions. Scale bars = 10 mm

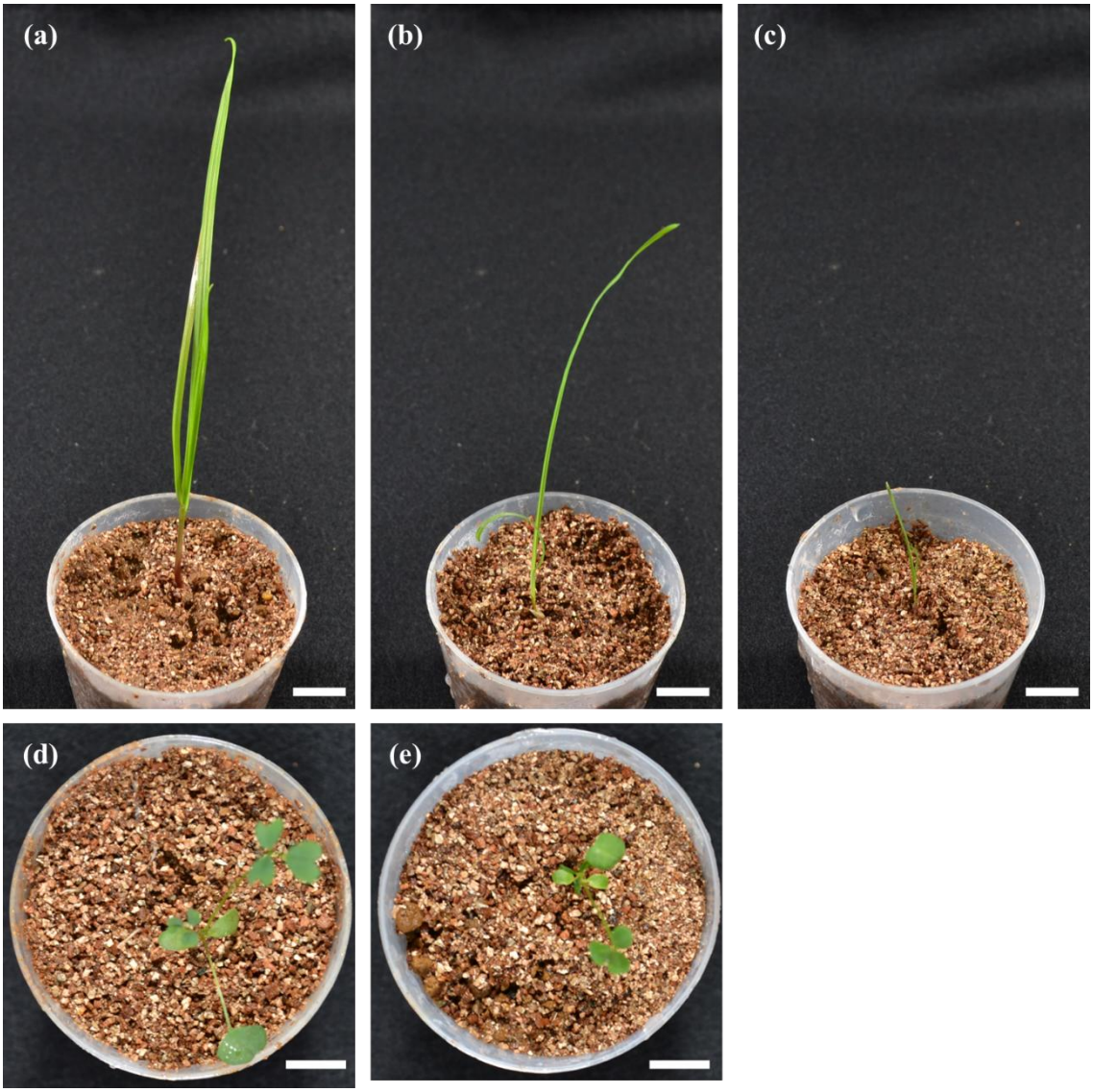


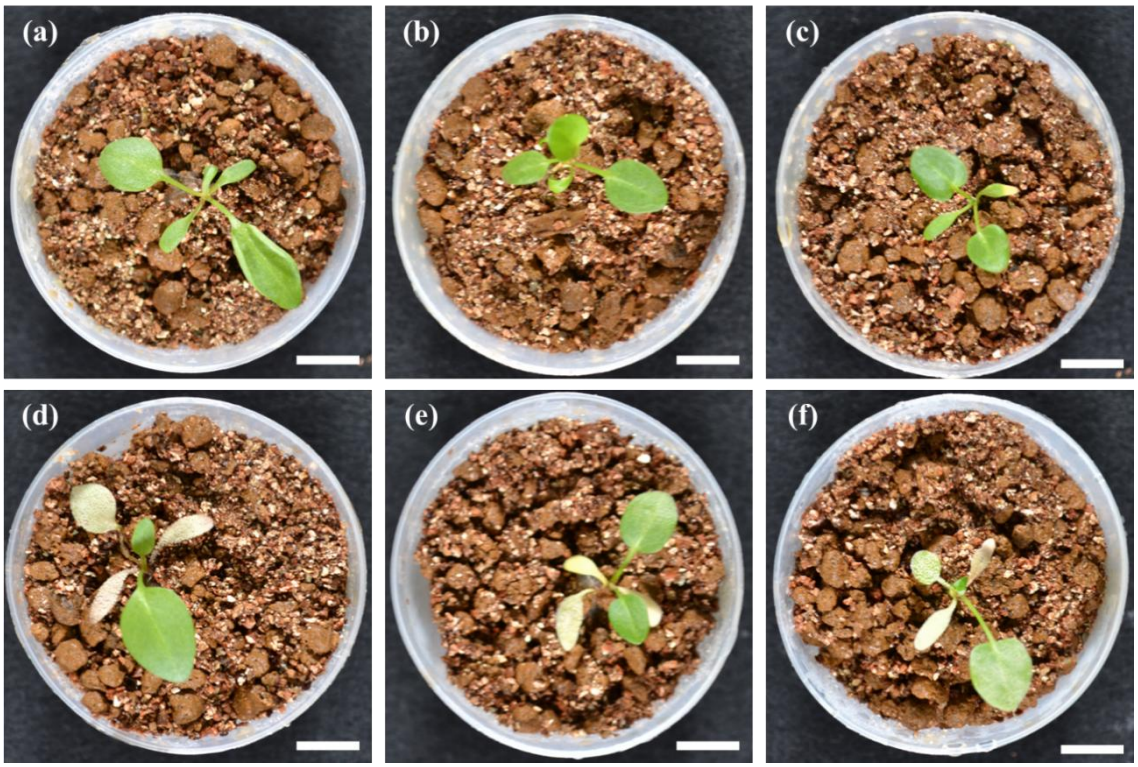

**Figure 6. Lack of Pathogenicity of TR4 against Forage Species.**

Foliar appearance 14 days after inoculation. (a) *Lolium multiflorum*, (b) *Dactylis glomerata*, (c) *Phleum pratense*, (d) *Medicago sativa*, and (e) *Trifolium repens* following inoculation with TR4. No symptoms were observed in any of the species. Scale bars = 10 mm

**Supplementary Table 1. GenBank accessions of ITS and LSU sequences used for the phylogenetic analysis in this study.**

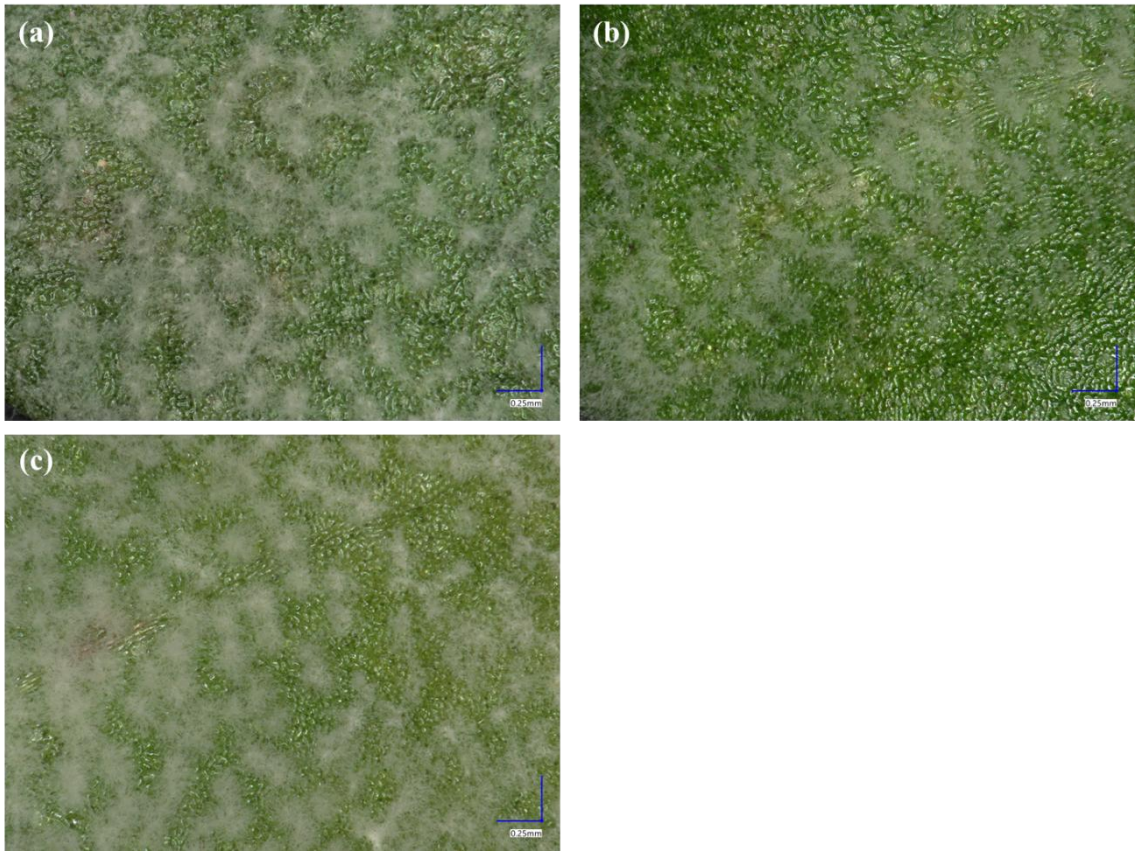
ITS and LSU rDNA sequences used in the phylogenetic analysis are listed with GenBank accessions and references. The strain used in this study are shown in bold.

| Species | Culture number | ITS | LSU | References |
|---|---|---|---|---|
| *Teratoramularia infinita* | CBS 120815 | KX287544 | KX287248 | Videira et al. 2016 |
| | CBS 141104; CPC 19488 | KX287545 | KX287249 | Videira et al. 2016 |
| *Teratoramularia kirschneriana* | CBS 113093; RoKi 1144 | GU214669 | GQ852627 | Crous et al. 2009 |
| *Teratoramularia persicariae* | CPC 11408 | KX287546 | KX287250 | Videira et al. 2016 |
| | CPC 11409 | KX287547 | KX287251 | Videira et al. 2016 |
| | CBS 141105; CPC 11410 | KX287548 | KX287252 | Videira et al. 2016 |
| *Teratoramularia rumicis* | NFCCI 5008 | MK967558 | MW276098 | Verma et al. 2021 |
| ***Teratoramularia rumicicola*** | **TR4** | **PX069134** | **PX069135** | **This study** |
| | CPC 14652 | KX287549 | KX287254 | Videira et al. 2016 |
| | CBS 141106; CPC 14653 | KX287550 | KX287255 | Videira et al. 2016 |
| | CPC 14654 | KX287551 | KX287256 | Videira et al. 2016 |
| *Staninwardia suttonii* | CPC 13055 | DQ923535 | DQ923535 | Summerell et al. 2006 |




**Supplementary Figure 1. Pathogenicity of TR4 Against *Rumex* Species at 14 DAT.**

Foliar appearance 14 days after inoculation. (a–c) Untreated control plants of *R. crispus*, *R. japonicus*, and *R. obtusifolius*, respectively. (d–f) Corresponding TR4-inoculated plants showing visible white fungal growth and necrotic lesions. Scale bars = 10 mm




**Supplementary Figure 2. Digital microscope images showing white fungal growth of TR4 on the surface of infected *Rumex* leaves.**

Images represent the typical symptoms observed 21 days after inoculation.

(a) *R. crispus*, (b) *R. japonicus*, (c) *R. obtusifolius*. Scale bars = 0.25 mm